ISSN 1854-0678
www.institut-isi.si

SANITARNO INŽENIRSTVO

INTERNATIONAL JOURNAL OF SANITARY ENGINEERING RESEARCH

volume 6 | number 1 | december, 2012

Editorial

This issue of the *International Journal of Sanitary Engineering Research* contains papers which are mostly dealing with bacteria and food safety. A. Ovca at al studied the cross bacteria contamination of the mobile phone. Nowadays almost every person including child and student uses mobile phone. Nobody knows in what environment and to what influences the mobile phones are exposed. The results of the presented study showed that about 60 % of 35 mobile phones exceeded 100 RLU according to ATP measurements. In 90% of 90 swabs taken from mobile phones, more than 5 CFU/100 cm^2 were determined. In addition to total aerobic mesophilic microorganisms (90 %), bacteria of the genera *Staphylococcus* (65 %) and the *Enterobacteriaceae* family (39 %) were most often identified.

T. Janković at al investigated and studied the aggregation ability of three potential probiotic strains of *Lactobacillus plantarum* and Coaggregation with different food–borne pathogens: *Salmonella enterica* serotype Typhimurium, *Listeria monocytogenes* EGD strain and enterohaemorrhagic *Escherichia coli* (EHEC). Read the results of the autoaggregation and coaggregation, which should be an important feature in the selection of probiotic bacteria.

In the another original scientific paper M. Kučan at al studied the use of lactic acid bacteria as probiotics. You can find the results of the study with the possibility of adhesion and colonization of *Lactobacillus plantarum* strain S1 to gastrointestinal system of mice. They additional examined the influence of *L. plantarum* S1 on composition of intestinal microflora of mice.

Everybody knows that water is one of the most important resouce for our existence. On the area where is no underqround water or rivers available the only water source is rainwater. The rain water is used for living from early beginning to the present days expecially on the islands, deserts, montains etc. There are some risk that the rain water in our reservoirs can be contaminated by the *Legionella*. The research results indicate that both *Legionella* species can survive in the rainwater environment which means the raiwater could represent a reservoir for *Legionella*.

If we talk about food, the food chain in last 20 years changed dramatically. Because of the open market, you can find all kind of food from every place of the world. From some reasons this means probably cheaper food but also low quality. We have to be aware that the contamination of this food is real fact. In the other hand domestic production of the healthy food is disappearing. The multinational companies are taking over the markets and force their low quality food products. In the paper *Flows of raw materials and food safety of products of Slovenian manufacturers after EU entry* is evident how this process works in Slovenia where almost all big EU multinational companies dealing with food are present. What concernes is the fact that domestic food producers trust EU companies regarding raw materials and food quality. The history teaches that where the profit is the main driving force is not worth to be trusted.

Sincerely,

Janez Petek
Editor-in-Chief

SANITARNO INŽENIRSTVO

INTERNATIONAL JOURNAL OF SANITARY ENGINEERING RESEARCH

Izdaja – Published by:
INŠTITUT ZA SANITARNO INŽENIRSTVO
INSTITUTE OF FOOD SAFETY AND ENVIRONMENTAL HEALTH

ZBORNICA SANITARNIH INŽENIRJEV SLOVENIJE
THE CHAMBER OF SANITARY ENGINEERS OF SLOVENIA

NASLOV REDAKCIJE IN UPRAVE / Address of the Editorial Board and Administration

Zaloška cesta 155, SI-1000 Ljubljana, Slovenija
Tel: (+386)-1-5468-393, Fax: (+386)-1-5468-394
E-mail: info@institut-isi.si
www.institut-isi.si

Content

Original scientific paper

Students' mobile phones – how clean are they?

Andrej **OVCA**[1*], Barbara **REDNAK**[2], Karmen **TORKAR**[1], Mojca **JEVŠNIK**[1], Martin **BAUER**[1]

ABSTRACT

Mobile phones can act as a potential microbiological threat, serving as vehicles to transfer contamination from place to place. The aim of this study was therefore *i)* to detect the contamination rate of mobile phone surfaces with microorganisms and organic matter using ATP bioluminescence tests; *ii)* to identify and to quantify the microorganisms present on mobile phones' surfaces owned by different groups of students, divided according to their course of study; *iii)* to examine the success of different methods for the elimination of microorganisms from mobile phone surfaces. About 60 % of 35 mobile phones exceeded 100 RLU according to ATP measurements. In 90 % of 90 swabs taken from mobile phones, more than 5 CFU/100cm^2 were determined. In addition to total aerobic mesophilic microorganisms (90 %), bacteria of the genera *Staphylococcus* (65 %) and the *Enterobacteriaceae* family (39 %) were most often identified. Among all tested procedures for the elimination of microorganisms from surfaces, a putty containing a special antibacterial compound proved to be the most effective. The results show that mobile phones can be considered to be a factor of microorganism cross-contamination.

Key words: mobile phone, students, microbiological examination

Received: 18. 6. 2012.
Accepted: 18. 7. 2012.

[1] University of Ljubljana
Faculty of Health Sciences
Department of Sanitary Engineering
Zdravstvena pot 5
SI-1000 Ljubljana

[2] Tomšičeva 5
SI-2380 Slovenj Gradec

[*] *Corresponding author*
Andrej Ovca
University of Ljubljana
Faculty of Health Sciences
Department of Sanitary Engineering
Zdravstvena pot 5
SI-1000 Ljubljana, Slovenia
Phone: +386 1 300 11 82
Fax: +386 1 300 11 19
E-mail: andrej.ovca@zf.uni-lj.si

INTRODUCTION

Interactions between microorganisms and contact material surfaces play an important role in biology and different technologies, including the food, pharmacy and service industries [1]. Different materials are in contact with different types of microorganisms and their forms, which can harm human health because of their pathogenic properties [2]. Bacteria readily adhere to wet surfaces and form organised colonies of cells enclosed in an extracellular polysaccharide (EPS) matrix that facilitates adhesion to surfaces and each other [3]. Materials have different characteristics for adhering and loading for various contaminants, including bacteria, yeasts, fungal and bacterial spores, and viruses. Due to adhesion, they serve as vehicles to transfer contamination vectors from place to place. If the contact materials allow microbes to survive, the probability of transferring contamination to the next recipient is consequently very high, which has strong impact on the safety and quality of final products or service [4].

Mobile phones have become an integral part of modern telecommunications. In many countries, more than half of the population uses a mobile phone. According to the recent estimation by International Telecommunication Union (ITU) there were more than 4.6 billion mobile phone subscriptions around the world at the end of year 2009, and this number surged to 5.3 billion in next year [5]. In Slovenia, the mobile telephones were used by more than 2.1 million users at the end of 2010 (1 % and 3 % more than over one and two years ago respectively), which is more than number of inhabitants, and continues to increase [6].

It is important to be aware of the new health risks that new products and new behaviours can introduce. With the emergence of the mobile phone, telephony has completely permeated public space, with people talking on the phone in most public places, such as buses, swimming pools, streets, shopping centres, gyms etc. [7]. Mobile phones have become part of so-called emotional technology and are an indispensable accessory, both professionally and privately, used frequently in environments of high bacteria presence [8]. Users are in an emotional relationship with their phone and feel connected with them, which is a consequence of personalised mobile devices and services [9].

Not much research has been done on the microbiological status of mobile phone surfaces. Mostly studies conducted in hospital environments can be found, but studies among the general population are rare. Research on the microbiological status of mobile phone surfaces in food industry could not be found. This is in spite of the fact that work positions where usage of mobile phone is unavoidable can be identified in both hospitals and the food industry. Different studies reviewed by Brady et al. [10] examining mobile phones owned by health care workers report presence of pathogenic bacteria (up to 15 % of all cases). It is alarming that mobile phones have been found to harbour a variety of multidrug-resistant pathogens. According to Cuttler et al. [11], in the general population, one of six mobile phones in Britain is contaminated

It is important to be aware of the new health risks that new products and new behaviours can introduce.

Not much research has been done on the microbiological status of mobile phone surfaces. Mostly studies conducted in hospital environments can be found, but studies among the general population are rare.

with faecal matter. As presented by Khivsara et al. [12], mobile phone contamination and hand contamination suggest cross contamination. One should be aware that mobile phones can act as a potential "Trojan horses", through which unclean device is introduced into the clean working operation.

The purpose of this study was therefore *i)* to estimate the contamination rate of mobile phones with an ATP bioluminescence test; *ii)* to identify and to quantify the microorganisms present on mobile phones' surfaces owned by three different groups of students divided according to their course of study; and *iii)* to investigate the success of different methods for eliminating microorganisms from mobile phone surfaces. Students were chosen as a population because mobile phones are very popular among them. Regarding their course of study, students of health and of food science were chosen, because personal hygiene is an important part of their study. Both groups are also foreseen as working in hygienically more sensitive environments in comparison to students of computer science, who were chosen as a control group without that special knowledge.

Although lower microbiological quality of phone surfaces was expected, the results show that mobile phones can be considered to be a factor of microorganism cross-contamination.

To detect the contamination rate of mobile phone surfaces with microorganisms and organic substances, a preliminary investigation was conducted on 35 mobile phones owned by students of health sciences.

MATERIALS AND METHODS

Sampling

To detect the contamination rate of mobile phone surfaces with microorganisms and organic substances, a preliminary investigation was conducted on 35 mobile phones owned by students of health sciences. The phones were examined using the rapid ATP bioluminescence test. Special Ultrasnap swabs (with luciferase enzyme) were taken from both front and back surfaces.

In order to determine the microbiological quality, 90 standard swabs were taken from the phone surfaces, from three sub-groups (30 swabs per sub-group) of students, according to their course of study (health science, food science or computer science).

The effectiveness of various cleaning procedures for mobile phones was tested by collecting classical swabs from 30 randomly collected mobile phones. The swab was at first taken from one half of the mobile phone. The second half of the surface was then treated with one of the cleaning or disinfection agents: 70 % alcohol, dry paper towels or a putty containing a special antibacterial compound (Cyber Clean, Joker AG/AS, Switzerland) and swabbed afterwards. The total bacterial count was estimated with standard microbiological methods.

ATP bioluminescence method

ATP bioluminescence measurements were carried out according to manufacturers' instructions (Ultrasnap™, Hygiena, Germany). The biolu-

minescence was measured with a System Sure II Luminimeter (Hygiena, Germany). The method is based on the determination of adenosine triphosphate (ATP) by means of luminescence measurement during enzymatic oxidation of luciferin by luciferase. The emitted radiation coming from the swab was measured by a luminometer and is expressed in Relative Light Units (RLU). The results are directly related to the amount of ATP on the surface of the swab and consequently to the amount of organic matter and microbiological contamination remaining on the examined surface [13]. After sampling, the Ultrasnap™ swabs were activated by breaking the tops of the containers to release the luciferaze enzyme. After 15 seconds, the emitted light was measured by luminimeter [14].

Microbiological examination

The samples were examined with standard classical microbiological tests for reliable numbers of aerobic mesophilic microorganisms, coliform microorganisms, the representatives of genera *Enterococcus*, *Staphylococcus*, *Bacillus* and fungi. After the sampling, the swabs were transferred into the tubes with 5 mL of sterile saline solution and mixed using Vortex. 1 mL of the suspension was transferred into a petri dish and mixed with melted medium. The total bacterial count at 30 °C was enumerated on PCA agar (Merck, Germany), according to the EN ISO 4833 standard [15].

For the enumeration of enterococci in swabs, KF Streptococcus agar with a TTC supplement (Merck, Germany) was used according to the ISO 7899-2 standard [16] and the manufacturer's instructions [17].

For the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species), the Manitol Salt Phenol Red Agar (Merck, Germany) and Baird Parker with RPF supplement agar (Biolife, Italy) were used [18]. The isolates were additionally identified by using API Staph biochemical tests (bioMerieux, France) and API WEB Programme V4.0. The number of yeasts and moulds in samples the yeast-extract-glucose-chloramphenicol agar (YGC) (Merck, Germany) was used.

Yeast and mould colonies growing on the plates were counted after five days of incubation at 25 °C [19].

After activating the bacterial spores with thermisation at 80 °C for 10 minutes, the number of members from the genus *Bacillus* was determined on *B. cereus* selective medium MYP (Merck, Germany) The plates with the samples were incubated at 30 °C for 24 to 48 hours. Colony morphology, cell morphological and physiological characteristics were determined using conventional procedures [20].

For the determination of the number of *Enterobacteriaceae* and presence of presumptive *E. coli*, the DEV ENDO Agar (Merck, Germany) was prepared, mixed with the sample and incubated at 37 °C for 24 hours [17].

Haemolytic activity of *Staphylococcus* and *Bacillus* isolates was determined on blood agar (Brain Heart Infusion Medium, Merck, Germany,

with defibrinated sheep blood) prepared by Institute for Microbiology and Immunology, Slovenia.

All bacterial isolates from the selection media were selected by microscopic examination according gram staining, oxidase and catalase tests.

STATISTICAL ANALYSES

Mathematical-statistical data processing was performed using Microsoft Office Excel 2010 and IBM SPSS Statistics 20.

RESULTS AND DISCUSSION

Preliminary investigation with ATP Bioluminescence

The bioluminescence method is in accordance with European Directive 93/43/EEC for the intended use of rapid methods in hygiene control, and has the widest application in the food industry, restaurants, hospitals and other facilities where rapid determination of the degree of contamination is necessary. The mean RLU value of all 35 analysed mobile phones was 158.3, with a wide range between 6 and 572 RLU. In eight cases, luminometer response was above 200 RLU and two cases even above 500 RLU. High luminometer response indicates the presence of ATP-containing living cells, meaning ATP from bacteria, yeast, and mould as well as ATP from any organic residue on the investigated surface. Although there is strong correlation between ATP and microbial cells, according to the manufacturers' instructions and some other authors, the RLU units cannot be transferred into colony-forming units (CFU) [13, 21, 22].

All bacterial isolates from the selection media were selected by microscopic examination according gram staining, oxidase and catalase tests.

Microbiological examination

Several authors have studied the microbiological colonisation of mobile phones [23, 24] particularly among healthcare workers. Studies that explore the microbial colonisation of mobile phones in the general population are much rarer. During the microbiological examination of the swab samples in 81 (90 %) of 90 cases, microorganisms were detected in 97 %, 93 % and 80 % of 30 tested samples for students of health, food and computer science courses of study, respectively. With these results, the assumption of Srikanth et al. [25] is confirmed, i.e. that the contamination of mobile phones in the everyday environment is possible. However, the differences regarding study course were not statistically significant for any of identified group of microorganisms ($p > 0.05$). The most commonly represented groups were *Enterobacteriaceae* family and genus *Staphylococcus* (Fig. 1). *Enterococcus spp.* was found only in samples of food science students, where *Bacillus* species and fungi were also more frequently identified in comparison to other two sub-groups (**Figure 1**). Relatively low average values of total aerobic mesophilic microorganisms in comparison to normal human hand skin colonisation ranging from 3.9 × 104 to 4.6 × 106 CFU/cm^2 [26] were revealed. However, as reported by Pittet et al. [27], fingertip

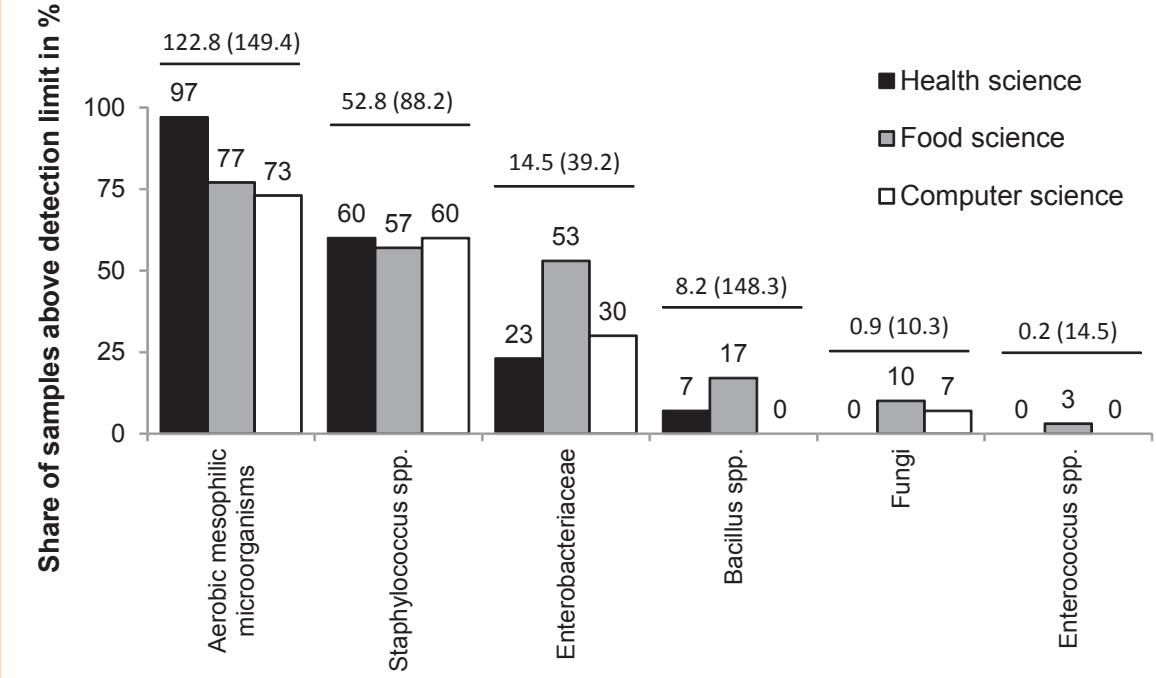


Figure 1.
Percentage distribution of individual group of microorganisms on the surface of mobile phones among students of individual courses of study. The numbers above the horizontal line represent average values (CFU/100 cm^2) of all samples and samples above detection limit only (in brackets), for each group of microorganisms regardless of the course of study.

contamination ranges from 0 to 300 CFU, when sampled by agar contact methods. Nevertheless, higher values were expected, especially for the *Staphylococcus spp.* as part of human skin resident flora and *Enterococcous spp.* as an indicator of faecal contamination, considering that almost 50 % of users of public toilets in Slovenia [28] do not wash their hands after using the toilet. Similar situations were also observed in Australia [29] and USA [30]. In general, resident flora is less likely to be associated with infections, but may cause infections in sterile body cavities, in the eyes, or on non-intact skin [31]. The *Enterobacteriaceae* is a large family that includes many of the pathogens, such as *Salmonella spp.*, *Escherichia coli*, *Yersinia pestis*, and *Shigella spp.* [32]. A similar pattern of microorganism distribution was also identified by Ulger et al. [33], who investigated the level of contamination of mobile phones with nosocomial pathogens. They also identified *Staphylococcus spp.*, *S. aureus*, *Enterococcus spp.* and fungi.

In **Table 1**, Pearson correlation coefficients between total aerobic mesophilic microorganisms and other isolated groups of microorganisms are presented. It can be concluded that number of total aerobic mesophilic microorganisms is highly synergistic with *Staphylococcus spp.* (correlation coefficient > 0.8) among health and food science students. Surprisingly, this correlation is not present among computer science students, indicating a more heterogeneous group regarding microbiological colonisation of their mobile phones. The same situation can be observed also in the case of *Enterobacteriaceae* although with lower synergy.

More detailed species of *Staphylococcus* were investigated further, to explore how many of the potentially infectious species are present. Among genus of Staphylococcus, species *S. warneri* (40 %), *S. epider-*

Table 1.
Pearson correlation coefficients between number of aerobic mesophilic microorganisms and other isolated groups of microorganisms.

	Total aerobic mesophilic microorganisms		
	Health science	**Food science**	**Computer science**
Enterococcus spp.	0.000	0.387	0.000
Staphylococcus spp.	**0.872**	**0.819**	0.172
Enterobacteriaceae	**0.678**	**0.592**	0.118
Bacillus spp.	0.000	- 0.029	- 0.054
Fungi	0.044	0.052	0.000

midis (30 %), *S. capitis* (10 %), *S. sciuri* (10 %), *S. xylosus* (5 %) and *S. aureus* (5 %) were identified. *S. aureus* was the only representative of coagulase-positive staphylococci, while all the other species belong to the group of coagulase-negative staphylococci. *S. epidermidis*, *S. warneri* along with *S. aureus* are part of human skin resident and transient flora [35] but can cause sudden illness in people, especially those with compromised immune system. They are also agents of nosocomial infections [35]. People are natural reservoirs for *S. aureus*, a frequent cause of infections in both the community and hospitals, although asymptomatic colonisation is far more common than infection. It is estimated that 20 % of people are long-term carriers of *S. aureus* [35]. Although *S. epidermidis* and S. *warneri* are not usually pathogenic, people with compromised immune systems are often at risk for developing an infection. These infections can be either nosocomial or community acquired [36].

People are natural reservoirs for *S. aureus*, a frequent cause of infections in both the community and hospitals, although asymptomatic colonisation is far more common than infection.

The gender ratio among all students whose mobile phones were analysed was 48 (54 %) women against 42 (46 %) men. In the group of health science students, females were dominant (90 %), in contrast to the group of computer science students which, with the exception of one female student, was entirely male. The group of food science students consisted of 67 % females and 33 % males. With an independent t-test, the average amount of microorganisms regarding gender was analysed. The relationship between gender and the microbiological status of mobile phones has proven to be statistically significant ($p = 0.047$) in case of *Staphylococcus spp*. The data analysis (**Figure 2**) confirmed that on the mobile phones of female users are significantly more colonised with this genus compared with males. One of the reasons for this discrepancy could be the generally known more frequent use of facial cosmetics by females. In spite of added preservatives, cosmetic products, especially those with high water content, are subject to constant and variable microbial contamination from the domestic environment, consumers' hands and body fluids, from the moment of opening until the moment of discharge. Due to their ability to proliferate on many different substrates, genera like *Pseudomonas* and *Staphylococcus* are often found in contaminated cosmetics [37]. However, this situation should be further investigated, considering also to the time and the way mobiles phone are mainly used (for texting or talking).

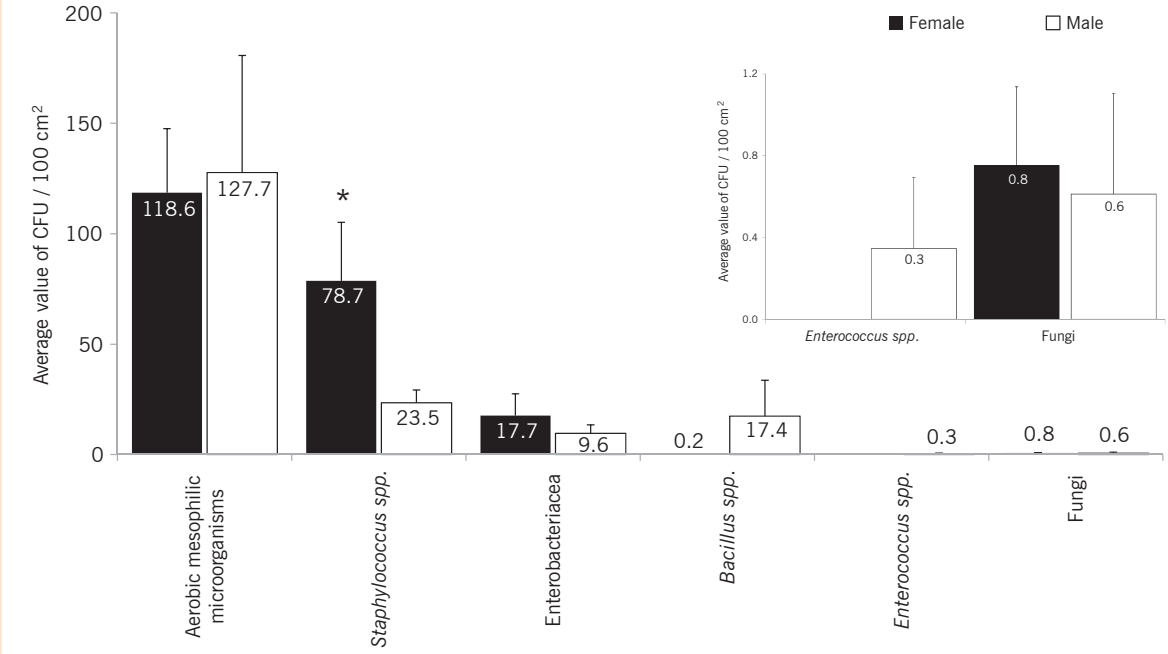


Figure 2.
The average values (CFU/100 cm²) of individual group of microorganisms according to user gender. The statistically significant difference ($p < 0.05$) is marked with an asterisk (*).

Whether the shape of the mobile phone (block, slider or flip and touchscreen) affects its microbiological status, was also investigated. The most common type was touchscreen (44.4 %) flowed by the block shape (40.0 %) and slider or flip (both at 15.6 %; hereinafter considered as one group). The difference in average values regarding total aerobic mesophilic microorganisms was the most obvious, although statistically not significant ($p > 0.05$), with values of 150.9, 105.2 and 74.4 CFU/100 cm² for block, touchscreen and flip/slider, respectively. The higher average values for older style mobile phones in comparison to the touchscreen are most probably the consequence of the shape and the fact that in spite of relatively similar material for mobile phone shells, on touchscreens fingerprints hinder clear views of the screen and owners "clean" them more often. As presented further, microorganisms can be eliminated from the mobile phone surface simply with paper towels and friction.

Removal of microorganisms from the mobile phones' surfaces

Most gram-positive bacteria, such as *Enterococcus spp.*, *S. aureus* and especially bacterial spores, can survive on dry surfaces for several months. Furthermore, many gram-negative species, such as *Acinetobacter spp.*, *E. coli*, and *Shigella spp.*, are able to spend months on dry surfaces [38]. Manufacturers of mobile phones describe in general how users should maintain their phones. The instructions for proper maintenance and cleaning provided by mobile phone manufacturers [39, 40] do not give any specific recommendations regarding cleaning itself. They just warn against the use of corrosive chemicals, cleaning solvents or strong detergents. Considering the mobile phone shape, it comes into the contact with exposed surfaces of the body (mouth, nose, ears) during each phone call. Mobile phones are usually also not a subject of standardised cleaning, and there is also a lack of professional recom-

The instructions for proper maintenance and cleaning provided by mobile phone manufacturers do not give any specific recommendations regarding cleaning itself.

mendations on how to clean/maintain the mobile phones to meet hygiene standards in everyday life, or in different working environments if their use is unavoidable.

Some recent studies [8, 33, 40] conducted in a hospital environment confirmed that the transfer of microorganisms from the hands of health workers on the mobile phone surface and vice versa. If we translate this into the general population where, according to the Co-operative Group report [40], 32 % of people use their mobile phone when they use the toilet and consider the fact that almost half of the people do not wash their hands after using it [11, 28, 29, 30], one needs to be aware that the transfer of potentially pathogenic microorganisms on the mobile phone surfaces is not exceptional. According to Cuttler et al. [11], 16 % of hands and 16 % of phones were found to harbour bacteria of a faecal origin, where those who had bacteria on their hands were more likely to have bacteria on their phone as well. In such situations, hand washing is the simplest and also the most effective measure to prevent the spread of agents responsible for communicable diseases.

In **Table 2**, the efficiency (based on reduction rate of total aerobic mesophilic microorganisms) of three different procedures for the elimination of microorganisms is presented. Paper towels were chosen to test whether elimination with physical force can be achieved. Antibacterial

Table 2.
Comparison of elimination efficiency for aerobic mesophilic microorganisms using paper towels, antibacterial putty and 70 % ethanol.

Sample number	Used method	CFU / swab before intervention	CFU / swab after intervention	Elimination efficiency (%)
1	Paper towels	12	3	75
2		12	0	100
3		212	65	69
4		15	2	87
5		11	2	82
6		178	1	99
7		29	4	86
8	Antibacterial putty	48	3	94
9		15	0	100
10		740	1	99
11		39	0	100
12		252	1	99
13		22	0	100
14		419	7	98
15	70% Ethanol	110	1	99
16		64	1	98
17		12	3	75
18		92	21	77
19		27	3	89
20		10	0	100

putty was chosen as a product meant especially for the cleaning of electronic devices, for which manufacturer claims that in one minute of contact time bacteria such as *E. coli*, *P. aeruginosa*, *S. aureus*, and fungi can be inactivated. Ethanol (70 %) was chosen as the disinfecting agent that should inactivate all the bacteria present on the surface. The antimicrobial activity of alcohols results from their ability to denature proteins. Alcohol solutions containing 60–80 % alcohol are most effective, with higher concentrations being less potent, as a consequence that proteins are not denatured in the absence of water [43]. Alcohols have an excellent in vitro germicidal activity against gram-positive and gram-negative vegetative bacteria (including multidrug-resistant pathogens such as MRSA), and a variety of fungi [43, 44]. However, they have virtually no activity against bacterial spores or protozoan oocysts, and very poor activity against some non-enveloped (non-lipophilic) viruses [44]. Alcohols are also not good cleansing agents, and their use is not recommended when visible dirt is present on the surface [43].

Each procedure was tested on 10 randomly collected mobile phones (N=30). Samples for which initial colonisation (before intervention) was below 10 CFU/ swab were not included in the calculation to avoid the risk of mislead information, considering the high differences when calculating percentages on small absolute numbers. The average elimination rate was 85.4 %, 89.7 %, and 98.6 % for paper towels, ethanol and antibacterial putty respectively. Although (or because of) the highest (219.3 CFU/swab) mobile phone average colonisation was cleaned with antibacterial putty, this procedure was most effective. The results also show that significant amount of microorganism can be removed with "dry cleaning" where only paper towel and physical force are applied. The lower efficiency of ethanol in comparison to antibacterial putty could be the consequence of several factors, such as the cleaning procedure itself, antibacterial putty physical properties, mobile surface properties and contact time.

Alcohols are also not good cleansing agents, and their use is not recommended when visible dirt is present on the surface.

During the microbiological examination, we found that mobile phones of female users are significantly more colonised.

CONCLUSIONS

Preliminary testing with ATP bioluminescence gave evidence that a significant amount of organic material is present on mobile phone surfaces. It is important to be aware that ATP bioluminescence is a fast and sensitive screening technique for hygiene control, especially for control of cleaning efficiency, although this method cannot replace standard microbiological examination, which is also able to identify the bacterial species present on surfaces. While the observed situation indicates poor hygiene awareness of mobile phone users regarding cleaning of their electronic accessories, more specific microbiological investigation was further employed. During the microbiological examination, we found that beside aerobic mesophilic micororgnisms, the most common group of microorganisms are representatives of the genus *Staphylococcus* (also part of resident human skin flora), and that mobile phones of female users are significantly more colonised. Parallel swabbing of users hands and/or ears should be taken to obtain insight regarding what is the most common

It should be noted that in addition to proper cleaning of mobile phones, prohibiting or restricting their use at the workplace in hygiene-sensitive work processes is generally more logical and, from the hygienic point of view, more effective measure.

source of this microorganism on mobile phone surfaces. Testing the success of different methods for the elimination of microorganisms from mobile phone surfaces gave surprising but not conclusive results in favour of antimicrobial putty. However, further investigation with more samples should be performed to confirm these results. The efficiency of alcohol in comparison to other antimicrobial agents should also be tested on different types of surfaces. Today's mobile phones are important devices for professional and social lives of their users. Every mobile phone is in principle and mostly controlled by hand; therefore, personal hygiene and hand hygiene are important measures in preventing the transmission of microorganisms from our hands to the different surfaces and vice versa. It should be noted that in addition to proper cleaning of mobile phones, prohibiting or restricting their use at the workplace in hygiene-sensitive work processes is generally more logical and, from the hygienic point of view, more effective measure. People, whose behaviour in their working environment and private life is not always in accordance with good hygiene practice, often present a hidden microbiological risk factor. While the usage of mobile phones has greatly increased in recent years, there is an emerging need to supplement the principles and guidelines of good hygiene practice with rules for the proper handling of mobile phones in hygiene-sensitive work processes.

REFERENCES

[1] Raspor P, Jevšnik M. Good nutritional practice from producer to consumer. Crit Rev Food Sci. 2008; 48: 276-292.

[2] Raspor P. Total food chain safety: how good practices can contribute? Trends Food Sci Tech. 2008; 19: 405-412.

[3] Vickery K, Pajkos A, Cossart Y. Removal of biofilm from endoscopes: Evaluation of detergent efficiency. Am J Infect Control. 2004; 32(3): 170-176.

[4] Vesterlund S, Paltta J, Karp M, Ouwehand A.C. Measurement of bacterial adhesion—in vitro evaluation of different methods. J Microbiol Meth. 2005; 60: 225-233.

[5] ITU. ITU sees 5 billion mobile subscriptions globally in 2010. Strong global mobile cellular growth predicted across all regions and all major markets: http://www.itu.int/newsroom/press_releases/2010/06.html (18. 8. 2011).

[6] Statistični urad republike Slovenije – SURS. Nekateri kazalniki razvoja elektronskih komunikacijskih storitev, Slovenija, 2010 – končni podatki: http://www.stat.si/novica_prikazi.aspx?id=4236 (11. 2. 2012).

[7] Pušnik M. Telefonska razmerja: O razvoju telefona in premenah družbenih interakcij. V: Mobilni telefon in transformacija vsakdana. Ljubljana: Fakulteta za družbene vede. 2009; 40: 48.

[8] Akinyemi KO, Atapu AD, Adetona OO, Coker AO. The potential role of mobile phones in the spread of bacterial infection. J Infect Dev Ctries. 2009; 3(8): 628-632.

[9] Lassen A. Affective technologies – emotions and mobile phones: http://ucm.academia.edu/AmparoLas %C3 %A9n/Papers/461823/Affective_Technologies._Emotions_and_Mobile_Phones (15. 1. 2012).

[10] Brady RR, Hunt AC, Visvanathan A, Rodrigues MA, Graham C, Rae C, Kalima P, Paterson HM, Gibb AP. Mobile phone technology and hospitalized patients: a cross-sectional surveillance study of bacterial colonization, and patient opinions and behaviours. Clin Microbiol Infect. 2011; 17: 830-5.

[11] Cuttler RR, Bowman L, Mkrtchyan H, Russell C.A., Curtis V. Survey of hand and mobile phone contamination across the UK: http://www.lshtm.ac.uk/newsevents/news/2011/global_handwashing_day_2011.html (28. 3. 2012).

[12] Khivsara A, Sushma TV, Dahashree B. Typing of Staphylococcus aureus from mobile phones and clinical samples. Curr Sci. 2006; 90: 910-912.

[13] Grbalová S, Večerek V, Tremlová B, Chloupek P, Pišteková V. Comparison of hygiene and sanitation levels by bioluminiscence method at different units produvcing components for instatnt soups. Czech J Food Sci. 2003; 21(4): 129-136.

[14] Directions for use of Ultrasnap ATP swab with Hygiena ATP hygiene monitoring system, Hygiena, Watford, UK, INS0039, Rev A.

[15] EN ISO 4833. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of micro organisms – Colony-count technique at 30 °C. International Organization for Standardization, Genova, Switzerland. 2003; 1-9.

[16] ISO 7899-2. Water Quality. Detection and enumeration of intestinal enterococci. Part 2. membrane filtration method. Genova, Switzerland. 2000: 1-7.

[17] Merck Microbiological Manual, 12 th Edition, Merck KGaA, Darmstadt, Germany. 2006; 91: 125.

[18] SIST EN ISO 6888-2. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) Part 2: Technique using rabbit plasma fibrinogen agar medium. International Organization for Standardization, Genova, Switzerland. 1999: 1-7.

[19] ISO 6611 (E)/IDF 94. Milk and milk products-Enumeration of colony-forming units of yeasts and/or moulds – Colony-count technique at 25 °C, International Organization for Standardization, Genova, Switzerland. 2004: 1-6.

[20] ISO 7932. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of presumptive *Bacillus cereus* – Colony-count technique at 30 °C, pp 7, International Standard Organization Genova, Switzerland. 2004: 1-6.

[21] Marriot NG, Gravani BR. Principles of food sanitation. Fifth edition, Springer Science Business Media. ZDA. 2006: 1–15, 25–69, 83–98.

[22] Exploding the myths about ATP hygiene monitoring. http://www.hygiena.net/docs/atp_myths.pdf/ (14. 1. 2011).

[23] Chawla K, Mukhopadhayay C, Gurung B, Bhate P, Bairy I. Bacterial "Cell" Phones: Do cell phones carry potential pathogens? OJHAS. 2009; 1(8): 1-5.

[24] Trivedi HR, Desai KJ, Trivedi LP, Malek SS, Javdekar TB. Role of Mobile Phone in Spreading Hospital Acquired Infection: A Study in Different Group of Health Care Workers. NJIRM. 2011; 2(3): 61-66.

[25] Srikanth P, Rajaram E, Sudharsanam S, Lakshman A, Sangappan Mariappan US, Jagannathan K. Mobile phones: emerging treat for infection control. JIP. 11 (3): 87-90.

[26] Adams BG, Marrie TJ. Hand carriage of aerobic gram-negative rods may not be transient. J Hyg (London). 1982; 89: 33-46.

[27] Pittet D, Dharan S, Touveneau S, Sauvan V, Perneger TV. Bacterial contamination of the hands of hospital staff during routine patient care. Arch Intern Med. 1999; 159: 821-826.

[28] Jereb G. Likar K. Umazane roke – grožnja našemu zdravju. V: Zbornik radova Međunarodni stručno – znanstveni simpozij Sanitarno inženjerstvo – Sanitary engineering, Opatija, 9. do 11. 3. 2006. Rijeka: Hrvatska udruga za sanitarno inženjerstvo. HUSI, 2006.

[29] Buchtmann L. Hand washing understanding and behaviour by Australian consumers http://www.foodsafety.asn.au/publications/articlesandsurveys/handwashingsurvey.cfm (16. 5. 2012).

[30] Another US airport travel hazard – dirty hands http://www.eurekalert.org/pub_releases/2003-09/asfm-aua091103.php (16. 5. 2012).

[31] Lark RL, VanderHyde K, Deeb GM, Dietrich S, Massey JP, Chenoweth C. An outbreak of coagulase-negative staphylococcal surgical – site infections following aortic valve replacement. Infect Cont Hosp Ep. 2001; 22: 618-623.

[32] Brenner DJ, Krieg NR, Staley JT, Garrity GM, Boone DR, De Vos P, Goodfellow M, Rainey FA, Schleifer KH. Bergey's Manual® of Systematic Bacteriology, Volume Two The Proteobacteria, Part B The Gammaproteobacteria Online: http://www.springerlink.com/content/978-0-387-24144-9#section=682029&page=170&locus=30 (28. 3. 2012).

[33] Ulger F, Esen S, Dilek A, Yanik K, Gunaydin M, Lebleicioglu H. Are we aware how contaminated our mobile phones with nosocomial pathogens? ACMA. 2009; 8: 7-10.

[34] Kluytmans J, van Belkum A, Verbrugh H. "Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks". Clin Microbiol Rev. 1997; 10 (3): 505-520.

[35] Marolt-Gomišček M, Radšel-Medvešček A. Infekcijske bolezni. Ljubljana: Tangram. 2002; 136: 267.

[36] Queck SY, Otto M. "Staphylococcus epidermidis and other Coagulase-Negative Staphylococci". V: Staphylococcus: Molecular Genetics. Caister Academic Press. Norfolk, UK: Caister Academic Press. 2008; 227-230.

[37] Lundov MD, Moesby L, Zachariae C, Johansen JD. Contamination versus preservation of cosmetics: a review on legislation, usage, infections, and contact allergy. Contact Dermatitis. 2009; 60: 70-78.

[38] Wendt C, Wiesenthal B, Dietz E, Rüden H. Survival of Vancomycin-Resistant and Vancomycin-Susceptible. J Clin Microbiol. 1998; 36(12): 3734-3736.

[39] Nokia 9300 (2005). Users Guide: http://nds1.nokia.com/phones/files/guides/Nokia_9300_UG_sl.pdf (25. 8. 2011).

[40] Samsung Galaxy. Users Guide: http://admin.gsmaparati.com/upload/documents/7456241734325886.pdf (25. 8. 2011).

[41] Yusha"u M, Bello M, Sule H. Isolation of bacteria and fungi from personal and public mobile cellphones: A case study of Bayero University, Kano. IJBHS. 2010; 6 (1): 97-102.

[42] The Co-operative Group. Mobile phone hygiene risk to millions of people in the UK: http://www.co-operative.coop/corporate/Press/Press-releases/Pharmacy/Mobile-phone-hygiene-risk-to-millions-of-people-in-the-UK/ (15. 8. 2011).

[43] Larson EL, Morton HE. Alcohols. In: Block SS, ed. Disinfection, sterilization and preservation, 4th ed. Philadelphia, Lea & Febiger. 1991; 191-203.

[44] Kampf G., Jarosch R., Rüden H. Limited effectiveness of chlorhexidine-based hand disinfectants against methicillin-resistant Staphylococcus aureus (MRSA). J Hosp Infect. 1998; 38: 297-303.

Original scientific paper

Aggregation ability of potential probiotic *Lactobacillus plantarum* strains

Tamara **JANKOVIĆ**[1*], Jadranka **FRECE**[2], Maja **ABRAM**[1], Ivana **GOBIN**[1]

ABSTRACT

Aggregation is the process of reversible gathering of bacterial cells belonging to the same bacterial strain (autoaggregation) or two different bacterial strains (coaggregation). Autoaggregation ability of probiotic bacteria correlates with adhesion, which is a prerequisite for colonization and protection of gastrointestinal tract, while coaggregation provides close interaction with pathogenic bacteria.

In this experiment the aggregation ability of three potential probiotic strains of *Lactobacillus plantarum* were investigated. Coaggregation with different food–borne pathogens: *Salmonella enterica* serotype Typhimurium, *Listeria monocytogenes* EGD strain and enterohaemorrhagic *Escherichia coli* (EHEC) was also studied.

The results showed that all *Lactobacillus* strains when cultivated in broth had better autoaggregation and coaggregation abilities then those cultivated on agar. After 24 hours almost 80 % of *Lactobacillus* aggregated. All lactobacilli coaggregated similarly with selected food-borne pathogens.

All three strains of *L. plantarum* possess the ability to autoaggregate and coaggregate, which is an important feature in the selection of probiotic bacteria.

Key words: aggregation, *Lactobacillus plantarum,* probiotics

Received: 4. 3. 2012.
Accepted: 23. 6. 2012.

[1] University of Rijeka
School of Medicine
Department of Microbiology and Parasitology
Braće Branchetta 20
51000 Rijeka, Croatia

[2] University of Zagreb
Faculty of Food Technology and Biotechnology
Laboratory for General Microbiology and Food Microbiology
Pierottijeva 6
10000 Zagreb, Croatia

[*] *Corresponding author*
Tamara Janković
University of Rijeka
School of Medicine
Department of Microbiology and Parasitology
Braće Branchetta 20
51000 Rijeka, Croatia
E-mail: tamara.jankovic@medri.hr

INTRODUCTION

Probiotics are defined as live microorganisms which when administered in adequate quantity confer health benefits to the host [1]. Lactic acid bacteria from the genera *Lactobacillus* and *Bifidobacterium* are commonly used as probiotics [2]. However, probiotic properties are characteristics of each strain, not the genus or even a species. Potential probiotic strain must meet numerous criteria before its commercial usage. Criteria of utmost importance in the selection of probiotic candidates are the ability to aggregate and to adhere to epithelial cells, because they are prerequisite for colonization of probiotic strains.

Aggregation is the process of reversible accumulation of cells, causing them to spontaneously precipitate in the medium in which they are suspended [3,4,5]. There are two different types of aggregation: autoaggregation and coaggregation. Autoaggregation is clumping of bacteria which belong to the same strain, while coaggregation is the result of cell-to-cell recognition between two different bacterial strains. Autoaggregation of probiotic strains has been correlated with adhesion to intestinal epithelial cells, known to be a prerequisite for colonization and enhanced persistence in the gastrointestinal system. Coaggregation abilities may form a barrier that prevents colonization by pathogenic microorganisms [6].

The objective of this experiment was to investigate the autoaggregation abilities of three selected strains of *L. plantarum* as well as their capability to coaggregate with different food-borne bacteria.

It is known that *Lactobacillus* spp. interfere with pathogens by different mechanisms, like production of antimicrobial compounds such as lactic acid, hydrogen peroxide, bacteriocine like substances etc. [7,8,9]. Lactic acid bacteria can prevent adhesion of pathogenic bacteria by competition for bonding places on intestinal epithelial cells, and consequently reduce pathogen colonization and prevent infection [2,3,10]. The objective of this experiment was to investigate the autoaggregation abilities of three selected strains of *L. plantarum* as well as their capability to coaggregate with different food-borne bacteria. Our results indicated the capability of all three *L. plantarum* strains to autoaggregate and coaggregate with selected food-borne pathogens.

METHODS

Bacterial strains and growth conditions

We used three different food derived (isolated from whey – S1, homemade cow cheese – A, and homemade sheep cheese – B) strains of *L. plantarum* obtained from Faculty of Food Technology and Biotechnology, University of Zagreb. Coaggregation abilities of *Lactobacillus* strains were tested with selected food-borne pathogens: *Salmonella enterica* serotype Typhimurium, *Listeria monocytogenes* EGD strain and enterohaemorrhagic *Escherichia coli* (EHEC) from culture collection of the Department of Microbiology and Parasitology, University of Rijeka. All tested bacteria were stored at -80 °C in 10 % glycerol broth. Lactobacilli were grown on de Man, Rogosa and Sharpe (MRS) agar or broth (Biolife, Italy) in microaerophilic atmosphere (5 % CO_2) at 37 °C for 48

h. *S.* Typhimurium, *L.* monocytogenes and EHEC were grown on sheep blood agar and incubated aerobically at 37 °C for 18-20 h. Pathogens were grown on different broths: *S.* Typhimurium and EHEC were grown on Lysogeny broth (LB) and *L.* monocytogenes on Brain heart infusion broth (BHI), aerobically at 37 °C for 18-20 h.

Aggregation assays

Autoaggregation and coaggregation assays were performed for bacteria grown in broth and on agar plates. Bacterial cells from agar plates were harvested and suspension was made in sterile Phosphate Buffer Saline (PBS). Bacteria grown in broth were harvested by centrifugation at 3000 rotation per minute (rpm) for 5 min, then washed and resuspended in PBS to give a final optical density of 1 (about 1×10^9 CFU/mL) at 600 nm, as measured by a spectrophotometer (Eppendorf, Germany).

For autoaggregation assay, suspension of different lactobacilli (4 mL) was divided in glass test tubes and mixed by vortexing. Absorbance was measured immediately, after 5 h and 24 h. Autoaggregation percentage was determined using the equation [6]

$$(1 - A_t/A_0) \times 100,$$

where A_t represents absorbance at different time points (t= 5 h or 24 h) and A_0 represents absorbance at the beginning of the assay (0 h).

On the 24 hour of autoaggregation test, samples of bacterial suspension of all three strains *L. plantarum* were taken from the bottom of the glass test tube. Autoaggregation was monitored by light microscopy at 100 times magnification after Gram staining.

For coaggregation assay, bacterial suspension was prepared in the same way as previously described. Equal volumes (2 mL) of probiotic strain and pathogen suspensions were divided in glass test tubes, and mixed by vortexing. Control tubes contained 2 mL of suspension of each bacterial species. Absorbance was measured immediately, after

Table 1.
Coaggregation ability of *L. plantarum* (S1, A, B) strains after cultivation on agar plates (A) and broth (B), with various pathogenic bacteria after 5 and 24 hours. Experiments were repeated at least two times. The results are expressed as per cent of coaggregated bacteria.

	% coaggregation											
	S. Typhimurium				*L. monocytogenes*				EHEC			
	A		B		A		B		A		B	
	5	24	5	24	5	24	5	24	5	24	5	24
L. plantarum S1	5.8	16.3	13.1	38.1	6.5	37.2	12.8	37.8	4.2	16.7	13.7	41.5
L. plantarum A	5.2	21.4	12	40.5	4.6	36.4	16.2	39.7	3.3	15.8	14.7	37.2
L. plantarum B	8.5	24	9.9	30.5	7.4	32.2	9.5	37.4	6.8	22.8	13.3	31.2

5 h and 24 h. The percentage of coaggregation was determined according to Handley *et al*. [11]:

$$\frac{\{\,[(Ax + Ay)/2] - A(x + y)\,\}}{(Ax + Ay/2)} \times 100$$

where A represents absorbance, x and y represent each of the two strains in the control tubes, and (x + y) their mixture.

RESULTS AND DISCUSSION

Aggregation properties are important characteristics of bacterial strains that are used as probiotics [9,12,13]. *In vitro* evaluation of autoaggregation and ability to coaggregate with potential enteric pathogens can be used for preliminary screening and selection of the best probiotic strain. The aggregation rate was measured for three food-derived strains of *L. plantarum* after 5 and 24 hours, and results show that all have better autoaggregation ability after cultivation in broth. After 24 hours of broth cultivation, the autoaggregation rate was at least 80 % (**Figure 1**). Microscopic analysis further confirmed clustering of cells and the presence of aggregates (**Figure 2**). At the same time, after cultivation on agar plates only 30 % bacteria aggregated (**Figure 1**).

The reason for enhanced autoaggregation rate of MRS broth-grown cells could be explained by better growth conditions in liquid than in solid medium. Our results are in agreement with previous reports of Kos *et al*. that broth increased the autoaggregation behaviour of the *Lactobacillus acidophilus* M92 in comparison with agar-grown cells what could be related to cell surface component, because the autoaggregation capability was not lost after washing and suspending the cells in PBS [6].

Coaggregation assay is a reliable method to evaluate the close interaction between lactobacilli and pathogenic bacteria [12,14] in which lactobacilli could release antimicrobial substances in a very close proximity [15]. Food-associated lactobacilli possessing ability to coaggre-

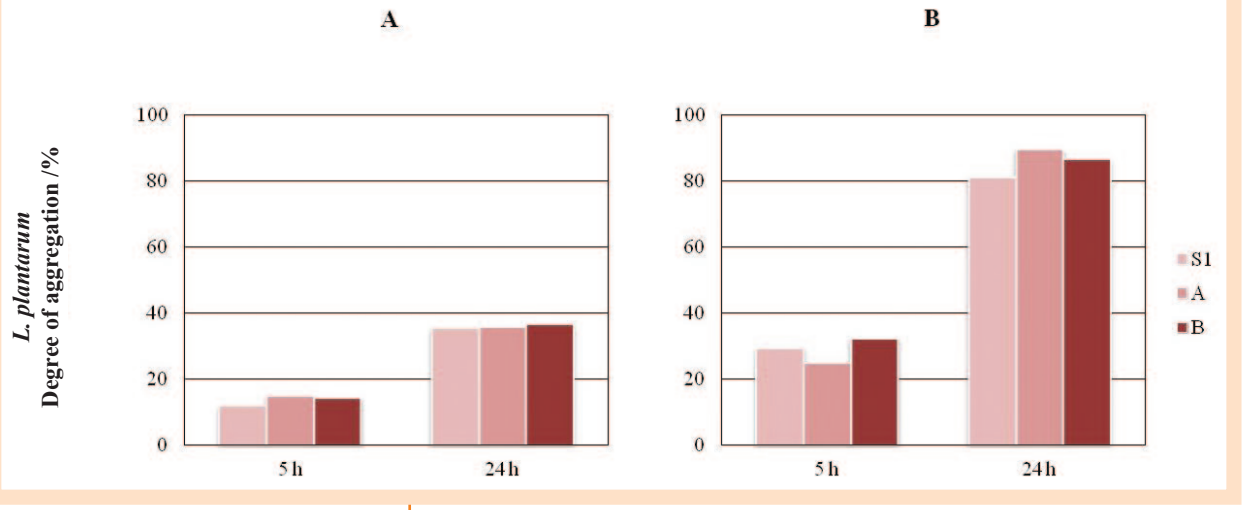


Figure 1.
Autoaggregation ability of *L. plantarum* (S1, A, B) strains after cultivation on agar plates (A) and in broth (B). Experiments were repeated at least two times. The results are expressed as per cent of aggregated bacteria.

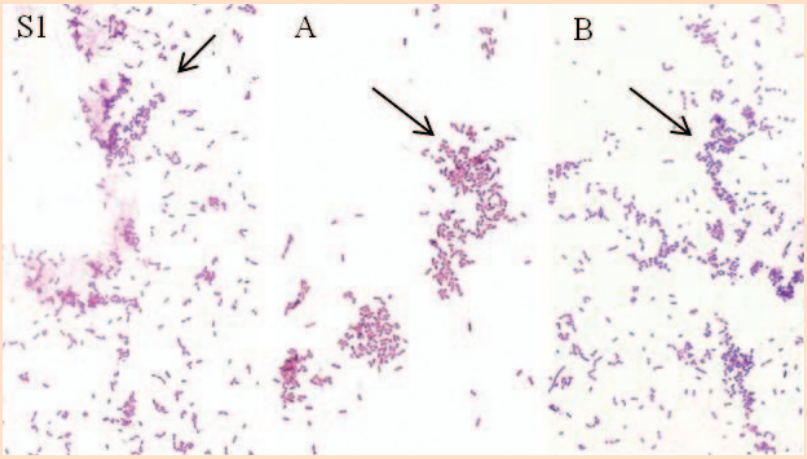


Figure 2.
Autoaggregation of *L. plantarum* (S1, A, B) after 24 hours cultivation in broth. Preparations were stained by Gram. Magnification x 1000.

gate with numerous pathogens are of special interest with regard to potential applications. We have tested coaggregation of three dairy *L. plantarum* probiotic candidates with three food-borne pathogens: *S.* Typhimurium, *L. monocytogenes* and EHEC.

Our results show that all three strains of *L. plantarum* also had better coaggregation ability after cultivation in broth.

L. plantarum S1 showed the best coaggregation result with EHEC, where 41,5 % of bacteria coaggregated, for *L. plantarum* A the best result of coaggregation was 40,5 % with *S.* Typhimurium, and *L. plantarum* B had the best result with *L. mononocytogenes* with 37,4 % of coaggregated bacteria.

In this study, all three *L. plantarum* strains (S1, A and B) showed a high autoaggregation percentage (≥ 80 %) and microscopic clustering of cells which may increase adhesion to intestinal epithelial cells. Also, all three tested strains showed similar degrees of coaggregation with selected food-borne pathogens, and that could allow them to release antimicrobial substances in a very close proximity of pathogenic bacteria.

To conclude, *L. plantarum* strains S1, A and B exhibited desirable autoaggregation and coaggregation abilities as potential probiotic strains.

REFRENCES

[1] FAO/WHO, "Evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Expert consultation report," Food and Agriculture Organization of the United Nations and World Health Organization, Córdoba, Argentina, October 2001.

[2] Frece J *et al*. Synbiotic effect of Lactobacillus helveticus M92 and prebiotics on the intestinal microflora and immune system of mice. J Dairy Res. 2009; 76: 98-104.

[3] Gobin I. The protective role of lactic acid bacteria to systemic Salmonella enterica serotype Typhimurium infection in mice. MSc thesis. Faculty of Food Technology and Biotehnology. University of Zagreb 2011.

[4] Fletcher M. How do bacteria attach to solid surfaces? Microbiol Sci. 1987; 4: 133-136.

[5] Frece J. Synbiotic effect of bacteria: Lactobacillus acidophilus M92, Lactobacillus plantarum L4 and Enterococcus faecium L3. Disertation 2007; Faculty of Food Technology and Biotehnology, University of Zagreb.

[6] Kos B *et al*. Adhesion and aggregation ability of probiotic strain Lactobacillus acidophilus M92. J Appl Microbiol. 2003; 94: 981-987.

[7] McGoarty JA, Reid G. Detection of a Lactobacillus substance that inhibits E.coli. Can J Microbiol. 1988; 34: 974-978.

[8] Reid G *et al*. Lactobacillus inhibitor production against E.coli and coaggregation ability with uropathogens. Can J Microbiol. 1988; 34: 344-351.

[9] Bunjakova D, Kmet V. Aggregation of animal lactobacilli with O157 enterohemorrhagic Escherichia coli. J Vet Med. 2001; 49: 152-154.

[10] Frece J *et al.* Importance of S-layer proteins in probiotic activity of Lactobacillus acidophilus M92. J Appl Microbiol. 2005; 98: 285-292.

[11] Handley SP *et al*. A comparison of the adhesion, coaggregation and cell-surface hydrophobicity properties of fibrillar and fimbriate strains of Streptococcus salivarius. J Gen Microbiol. 1987; 133: 3207-3217.

[12] Collado MC. Indigenous dadih lactic acid bacteria: cell-surface properties and interactions with pathogens. J Food Sci. 2007; 72: M89 – M93.

[13] Kaushik JK. Functional and probiotic attributes of an indigenous isolate of Lactobacillus plantarum. PloS One. 2009; 4: e8099.

[14] Soleimani NA. Antagonistic activity of probiotic lactobacilli against Staphylococcus aureus isolated from bovine mastitis. Afr J Microbiol. 2010; 44: 2169-2173.

[15] Botes M *et al.* Adhesion of the probiotic strains Enterococcus mundtii ST4SA and Lactobacillus plantarum 423 to Caco-2 cells under conditions simulating the intestinal tract, and in the presence of antibiotics and anti-inflammatory medicaments. Arch Microbiol. 2008; 190: 573-584.

Original scientific paper

Testing the adhesion and colonization ability of *Lactobacillus plantarum* strain S1 to the mice intestinal epithelium

Martina **KUČAN**[1*], Ivana **GOBIN**[1], Ksenija **MARKOV**[2], Diana **JURČIĆ MOMČILOVIĆ**[1], Jadranka **FRECE**[2]

ABSTRACT

Intestinal diseases are often the consequence of a myriad factors which disturb the complex ecosystem of the gastrointestinal system. For that reason, great attention is dedicated to the use of lactic acid bacteria as probiotics. When choosing the strains for probiotic use, one of the important criteria is the ability of adhesion and binding to the intestinal epithelium. Therefore, the aim of this study was to examine the possibility of adhesion and colonization of *Lactobacillus plantarum* strain S1 to gastrointestinal system of mice. At the same time the influence of *L. plantarum* S1 on composition of intestinal microflora of mice was examined. To test the *in vitro* adhesion properties, bacteria were added to freshly prepared tissue of the BALB/c mice small intestine. Mice were fed with *L. plantarum* strain S1 for 5 consecutive days. The result showed that *L. plantarum* strain S1 have good adhesion ability, *in vitro* and *in vivo*. The examined strain of *L. plantarum* successfully colonize the gastrointestinal system of mice and it showed a positive effect on the intestinal microflora, reducing the number of enterobacteria and clostridia.

In conclusion, *L. plantarum* strain S1 shows good properties of adhesion and colonization of the gastrointestinal system of mice and for that reasons could be used as a probiotic strain.

Key words: intestinal microflora, lactic acid bacteria, adhesion, colonization

Received: 12. 3. 2012.
Accepted: 17. 5. 2012.

[1] University of Rijeka
School of Medicine
Department of Microbiology and Parasitology
Braće Branchetta 20
51000 Rijeka, Croatia

[2] University of Zagreb
Faculty of Food Technology and Biotechnology
Laboratory for General Microbiology and Food Microbiology
Pierottijeva 6
10000 Zagreb, Croatia

[*] *Corresponding author*
Martina Kučan
Braće Branchetta 20
51 000 Rijeka, Croatia
Phone: 0038551651145
Fax: 0038551651177
Mobile phone: 00385917824364
E-mail: martina.kucan@gmail.com

INTRODUCTION

Lactic acid bacteria (LAB) are gram-positive bacteria that ferment carbohydrates and higher alcohols in lactic acid. [1]. LAB are part of the natural microflora of the gastrointestinal system of humans and animals and rarely showed pathogenic properties [2, 3]. Among LAB many *Lactobacillus* strains have been characterised as probiotics. Probiotics are live microorganisms thought to be beneficial to the host organism [4]. The recent definition of probiotics says that these are live microorganisms which, consumed in high numbers (less than 10^9 CFU per day), express health effects beyond their usual well-known nutritional value [5, 6].

The long tradition of using LAB with no adverse effects on human health, has provided a GRAS status (Generally regarded as Safe) by U.S. FDA (United States Food and Drug Administration) or QPS status (Qualified presumption of Safety) by European legislation Union [4, 6].

They provide a beneficial effect on health without interference with the gastrointestinal microbial flora [5]. Characteristic of LAB is the formation different compounds and organic acids, hydrogen peroxide, diacetyl, CO_2, and bacteriocins, which secrete into the environment in which they grow [7, 8, 9]. These substances prevent the growth of undesirable microbial populations to humans [7].

To achieve the desired effect on health probiotic LAB must be able to survive and colonize the gastrointestinal (GI) system of the host during enough long period. The main barrier to survival of potential probiotic strains in the GI system are the low pH of the gaster, lysozyme, bile salts and digestive enzymes such as pepsin and pancreatic enzymes. Bacterial strains for probiotic use should satisfy many criteria: general, technical and functional criteria. Selection of potential probiotic strains is based on *in vitro* studies as a prerequisite to determine a probiotic properties *in vivo* [4, 10].

The aim of this study was to examine the probiotic properties of *L. plantarum* strain S1 *in vitro* and *in vivo*. Also, the abillity of adhesion and colonization of the GI system of mice was examined.

MATERIALS AND METHODS

Bacteria

The potentially probiotic properties of *L. plantarum* strain S1, isolated from whey were tested. This strain of *L. plantarum* was obtained from the Laboratory of Microbiology and Food Microbiology, Faculty of Food Technology and Biotehnology, University of Zagreb. Bacteria were stored in MRS (Man-Rogosa-Sharpe) medium containing 30 % (v/v) glycerol at -70 °C.

Mice

Eight to twelve weeks old BALB/c mice were used. Animals were obtained from the Central Animal Facility of the Medical Faculty, Universi-

ty of Rijeka. Each experimental group consisted of three mice, housed in cage. Mice had continual access to water and were fed ad libitum. All experimental procedures were carried out according to the standards set in "The International Guiding Principles for Biomedical Reaserch Involving Animals" from "The Council of International Organisations of Medical Science".

Mice feeding and feces sampling

Bacterial cells were centrifuged at 3500 rpmi for 5 minutes, washed three times and resuspended in sterile 0.8 % NaCl to final concetration of $1x10^{11}$ CFU/ml. The number of bacteria was determined spectrophotometrically. Mice were fed with 100 μL of this bacterial suspension.

Survival of *L. plantarum* strain S1, during transit trought GI system, was determined in one gram dry weight faceal samples, which were individually colleted 2 h after first feeding. Faceal samples were homogenized in 1 mL sterile 0.8 % NaCl and serially diluted before plating on selective media: VRBG agar (Violet Red Bile Glucose) for *Enterobacteriaceae* counts, MRS agar for Total LAB count and Sulphite agar for sulphite-reducing clostridia counts.

In vitro adhesion test

The fresh tissue of the small intestine of healthy BALB/c mice were used. Tissue samples were kept 30 min in phosphate buffer (pH = 7.2), at 4 °C. After washing three times in phosphate buffer, tissue samples were added in the prepared bacterial suspension (~10^8 CFU/ml) and incubated at 37 °C for 30 min. Next, the tissue was fixed in 10 % formalin, dehydrated through graded ethnol (70 % to 95 % to 100 %), embedded in paraffin, and cutted at a sections of 5 μm. Sections were stained by Brawn and Brenn staining. Gram-positive bacteria are colored blue, Gram-negative red or pink, core of tissue cells are red, and other tissue elements are yellow.

In vivo adhesion test and survival in GI system

Mice were fed *per os* with *L. plantarum* strain S1 with a daily dose of $1x10^{11}$ CFU/100 μL bacterial suspension in 0.8 % NaCl. The control group was fed with 100 μL sterile 0.8 % NaCl. After five days feeding procedure ended, and on the day 5 and day 21 adhesion and colonisation ability was determined in homogenates of small and large intestine of BALB/c mice. First time point was chosen to test whether *L. plantarum* strain S1 is able to survive, adhere and colonize the GI system of mice. The second time point was chosen in order to investigate whether *L. plantarum* S1 retained in the intestine of mice and if it has their impact on intestinal microflora.

Bacterial staining in intestine tissue

Fifth day after first feeding the small intestine tissue was fixed in 10 % formalin, dehydrated through graded ethanol, embedded in paraffin, and cutted at a sections of 5 μm. Sections were stained by Brawn and Brenn

staining. Gram-positive bacteria are colored blue, Gram-negative red or pink, core of tissue cells are red, and other tissue elements are yellow.

Statistical analysis

All data points in each experiment were obtained in duplicate, and statistical calculation were made in GraphPad Prism version 4.

RESULTS AND DISCUSSION

When selecting strains of lactic acid bacteria for probiotic use one of the most important criteria is the ability to bind to the intestinal epithelium. Complex interactions involved in adhesion were not yet fully clarified. The adhesion to the surface of intestinal epithelial cells is prerequisite for the colonization of probiotic bacteria in the GI system. This feature allows long-term effects of probiotic bacteria on intestinal microflora and the host immune system [4]. To test the ability of adhesion of *L. plantarum* strain S1, bacteria was added into freshly prepared tissue of the small intestine. The results show that *L. plantarum* strain S1 successfully adhere to intestinal epithelial cells of mice *in vitro.* (**Figure 1**). Perdigo et al. showed that adhesion to epithelial cells and mucus mediates colonisation of the GI system by lactobacilli and may be prerequisite for competitive exclusion of enteropathogenic bacteria and immunomodulation of the host [11].

To detemined if the examined strain passed and survived through the GI system, two hours after feeding, in feces the total number of lactic acid bacteria (TLAB) was determined. In the feces of mice fed with *L. plantarum* strain S1 more lactic acid bacteria than the control group of mice was detected (**Figure 2**).

If a bacterial population is constantly present in the intestine, without periodic oral intake, it is considered that it colonized GI system. For this reason, *in vivo* tests in the GI system of BALB/c mice have been made.

Mice were fed five days in a row with *L. plantarum* strain S1 and on 5th and 21st day after first feeding TLAB, E and SRC in small and large intestine was determined.

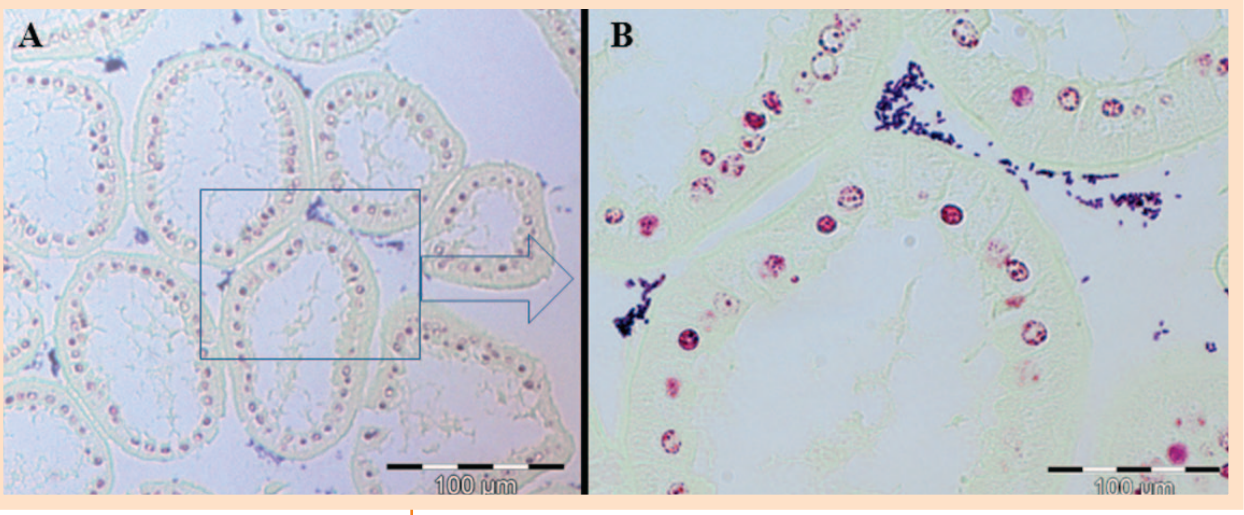


Figure 1.
Adhesion of *L. plantarum* strain S1 to the intestinal epithelium of BALB/c mice *in vitro*. Sections were stained by Brawn and Brenn staining and were analyzed under (A) 200x and (B) 600x magnification. Bacteria cells were stained blue.

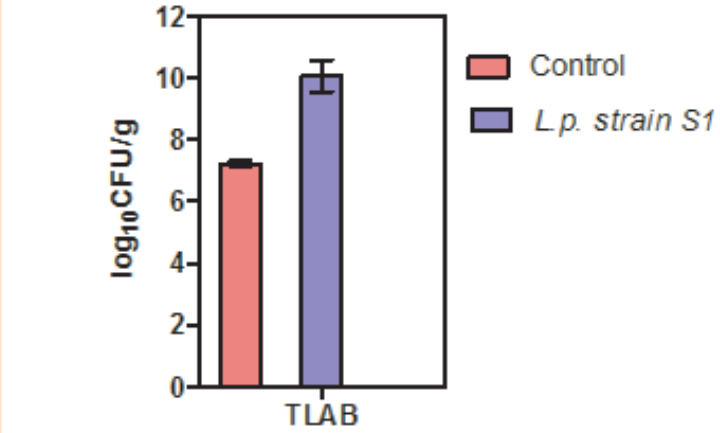


Figure 2.
The number of total lactic bacteria (TLAB) in the mice feces 2 hours after first feeding with *L. plantarum* strain S1. Control mice were fed with sterile saline. TLAB were detected on MRS-agar. Values are mean ± standard deviations of results from two separate experiments.

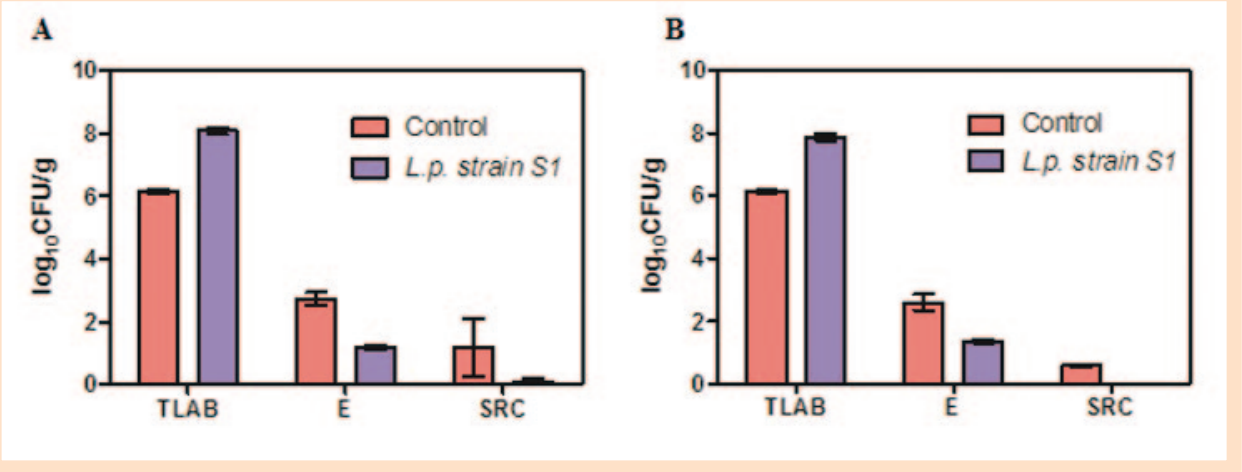


Figure 3.
The number of bacteria in the small intestine 5th day (A) and 21th day (B) after first feeding with *L. plantarum* strain S1. Control mice were fed with sterile saline. Total lactic acid bacteria (TLAB) on MRS-agar; *Enterobacteriaceae* (E) on Violet red bile glucose agar; sulphite-reducing clostridia (SRC) on Sulfite agar were detected. Values are mean ± standard deviations of results from two separate experiments.

The results show that in mice, fed with *L. plantarum* strain S1, was the higher number of lactic acid bacteria, compared to the control group. Also noticeable was the reduced number of enterobacteria and sulphite-reducing clostridia in the same group of mice (**Figure 3**). This results suggested that the tested strain successfully colonize the GI system of mice. Microscopic preparation intestine of mice showing clusters of blue stained bacilli in the intestinal content and on the surface epithelium (**Figure 4**).

It is still not fully explained the mechanism of probiotic effect. Possible mechanisms through which lactic acid bacteria have a protective or therapeutic effects are: competition for nutrients, competition for binding sites in the gastrointestinal system, production of antibacterial substances, modifications of metabolic processes in the gastrointestinal system and immune modulation [1, 3, 9].

Our results showed that *L. plantarum* strain S1 successfully survive the conditions in the GI system of mice and has the ability of adhesion and colonization of the colon and small intestine. Also, the tested strain has positive effect on the microbial flora because it reduces the presence of potentially adverse intestinal microflora populations.

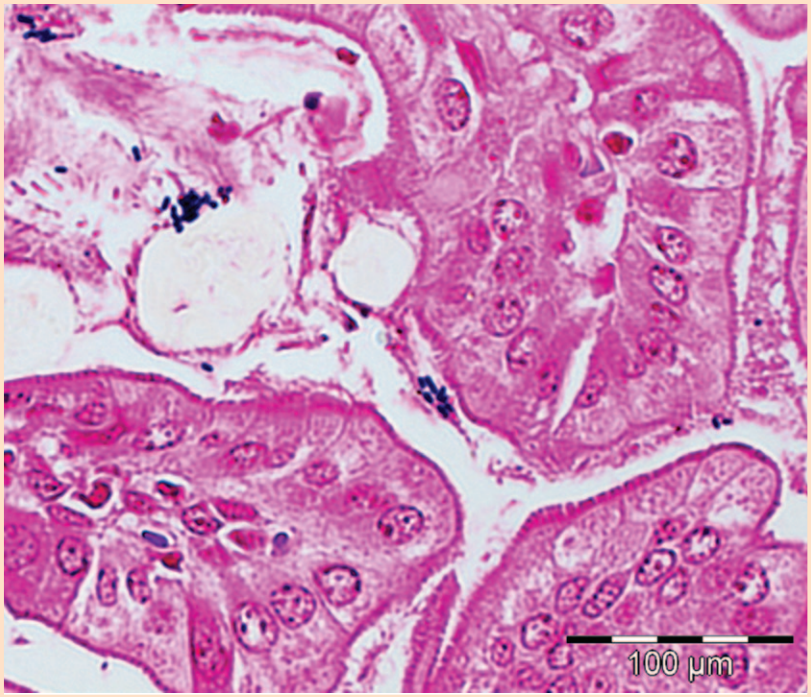


Figure 4. Representative pictures of *L. plantarum* strain S1 in the small intestine of BALB/c mice 5 days after first feeding. Slides were stained by Brawn and Brenn staining and were analyzed under 600x magnification. Clusters of blue stained bacilli in the intestinal content and on the surface epithelium were stain blue (arrow).

REFERENCES

[1] Kos B, et al. Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. Journal of Applied Microbiology. 2003; 94, 981-987.

[2] Frece J, et al. Karakterizacija bakterijskog soja *Lactobacillus plantarum* 1K izoliranog iz "slavonskog kulena" kao probiotičke funkcionalne starter kulture. Meso Vol. XII. 2010; 201-216.

[3] Šušković, J. et al. Mehanizam probiotičkog djelovanja bakterija mliječne kiseline. Mljekarstvo 47. 1997; 57-73.

[4] Tamime A. Y, et al. Probiotički fermentirani mliječni proizvodi. Mljekarstvo 53 (2). 2003; 111-134.

[5] Samac D, et al. Laktobacili u higijeni hrane. Meso Vol. XI. 2009; 37-39.

[6] Frece J. et al. Synbiotic effect of *Lactobacillus helveticus* M92 and prebiotics on the intestinal microflora and immune system of mice. J Dairy Res 76. 2009; 98-104.

[7] Zdolec N, et al. Inhibicijsko djelovanje bakterija mliječne kiseline izoliranih iz svježeg kravljeg sira. Mljekarstvo 57 (1). 2007; 5-13.

[8] Brkić B. et al. Antibakterijska aktivnost različitih sojeva bakterije *Lactobacillus plantarum. Prehrambena tehnologija i biotehnologija* rev. 31 (2-3). 1993; 77-81.

[9] Beganović J, et al. Functionality of the S-layer protein from the probiotic strain Lactobacillus helveticus M92. *Antonie van Leeuwenhoek.* 2011; 100: 43-53.

[10] Šušković J. et al. Sinbiotička svojstva *Lactobacillus acidophilus* M92. Mljekarstvo 53 (2). 2003; 83-110.

[11] Perdigo'n G. et al. Interaction of bifidobacteria with the gut and their influence in the immune function. Biocell 27: 2003; 1-9.

Original scientific paper

The survival of *Legionella* in rainwater

Mia **POTOČNJAK**[1*], Matija **ŠIROKA**[1], Danica **REBIĆ**[1], Ivana **GOBIN**[1]

ABSTRACT

Legionella is an environmental bacteria and a significant worldwide public health problem. These bacteria can survive a long period of time in distilled water, and more than a year in tap water. Due to these characteristics we were interested to explore how long it will survive in rainwater and if heavy bacterial contamination or bacterial microbiota would affect it's survival. Rainwater is a good alternative source of water in households and is used for watering gardens, washing yards and as bathing water. The rainwater from ten different tanks was analyzed and the results showed that in 90 % of samples the number of microorganisms is elevated. Two rainwater samples were chosen according to the amount of microbiota and survival of *L. pneumophila* and *L. longbeachae* was monitored. The results showed that both types of *Legionella* have been able to survive for 30 days in the rainwater samples but haven't been able to replicate. The rainwater microbiota restricted the survival of both *Legionella* strains although the effect was more pronounced in a case of *L. longbeachae.* These results indicate that both *Legionella* species can survive in the rainwater environment which means the rainwater could represent a reservoir for *Legionella*.

Key words: *Legionella*, rainwater, survival

Received: 12. 2. 2012.
Accepted: 16. 5. 2012.

[1] University of Rijeka
School of Medicine
Department of microbiology and parasitology
Braće Branchetta 20
51000 Rijeka, Croatia

[*] *Coresponding author*
Mia Potočnjak, univ. bacc. sanit. ing.
University of Rijeka
School of Medicine
Department of microbiology and parasitology
Braće Branchetta 20
51000 Rijeka, Croatia
Phone: +3850915742873
E-mail: miapotocnjak@gmail.com

INTRODUCTION

The genus *Legionella* belonging to the family *Legionellaceae* currently has at least 52 species comprising more than 70 distinct serogroups. Most *Legionella* species are found in aquatic environments, the exception is *L. longbeachae*, the only soil-dwelling pathogenic *Legionella* species [1]. *Legionella* are ubiquitous in natural aquatic environments and are capable to survive in waters with varied temperatures, pH levels, nutrient and oxygen contents. Also, *Legionella* species have the ability to successfully colonize man-made water handling and storage systems, which often provide ideal conditions of nutrition and temperature for their proliferation. Their widespread survival in water can be attributed to their relationships with other microorganisms. Symbiotic existence with algae and other bacteria, particularly biofilms, increases the availability of nutrients. They are also able to infect protozoas and subsequently reproduce within these organisms.

Although *L. pneumophila* is responsible for the vast majority of legionellosis worldwide some of non-pneumophila species like *L. longbeachae* are also capable of causing disease [1]. *L. longbeachae* is responsible for up to half of legionellosis in Australia and Thailand while in Europe and USA it causes sporadic cases [2].

Although *L. pneumophila* is responsible for the vast majority of legionellosis worldwide some of non-pneumophila species like *L. longbeachae* are also capable of causing disease.

Rainwater is traditionally collected in Primorsko-goranska County in water tanks, which are, by the appearance of water supply, increasingly used as an alternative source rather than primary sources of drinking water [3]. Previous study of microbiological quality of rainwater showed that 66 % of samples have heavy bacterial contamination while 44 % of samples had fecal contamination. The lack of cleaning and maintenance were the main reasons for that condition.

The objective of the present study was to test if the presence of heavy bacterial contamination in rainwater could create ecosystem favorable to *Legionella* survival and multiplication. The survival of two types of *Legionella*, *L. pneumophila* and *L. longbeachae* in two samples of rainwater were examined.

MATERIALS AND METHODS

Bacterial inoculum

Two types of *Legionella* were used in the experiment: *Legionella longbeache* NSW150, *Legionella pneumophila* strains Corby and 130b. Bacteria were stored at -80°C in glycerol broth (10 % glycerol). *Legionella* were used directly from the buffered charcoal yeast extract agar plates (BCYE-α, Oxoid). The number of bacteria in the inoculum was measured spectrophotometically (600 nm), OD (Optical density) was set to 1 what corresponded to a concentration of 1.0 x 10^9CFU (Colony Forming Units). The number of viable bacteria in the inoculums was confirmed by enumeration of the bacteria on BCYE agar. Bacteria cells were transferred into 10 mL of filter-sterilized and non-sterilized rainwater samples to a final concentration of approximately 1.0 x 10^6 CFU.

Rainwater samples

Ten samples of rain water (**Table 1**) were collected from water tanks from different region of Primorsko-goranska County. To test the presence of bacterial microbiota in rainwater samples the total aerobic bacteria at 22 °C, 37 °C and 44 °C on Peptone yeast extract glucose agar were determined. To test the presence of indicators of fecal contaminations, total coliform bacteria on Les Endo agar; fecal coliform bacteria on mFC agar; intestinal enterococci on KEA agar; sulphite-reducing clostridia on Sulfite agar were detected by membrane filtration. For survival studies two rainwater samples were selected according to the presence of bacterial contamination and analysis were conducted in non-sterile and sterile samples. The rainwater samples were sterilized by repeated filtration through a 0.2-μm-pore-size filter.

Table 1.
Microbiological quality of 10 rainwater samples.

Samples	Fecal coliform	Fecal enterococci	Total coliform	Total aerobic bacteria on 22 °C	Total aerobic bacteria on 37 °C	Total aerobic bacteria on 44 °C
	cfu/100 mL			cfu/mL		
1	0	0	57	96	164	6
2	0	0	69	100	40	2
3	5	11	12	259	140	0
4	11	17	Uncountable	1200	992	27
5	0	0	1	23	55	0
6	0	1	0	106	260	0
7	0	0	0	0	0	0
8	0	0	14	0	53	0
9	0	0	13	6	1	0
10	0	0	12	1	5	0

The limit for FC, FE, TC were 0 CFU/100 mL; TAB on 22 °C 100 cfu/mL; TAB on 37 °C 20 cfu/mL

Quantification of viable *Legionella* in water samples

At various time points after inoculation, a 100-μL aliquot was aseptically removed from each of the 10-mL experimental cultures. All plate counts were done in duplicate using BCYE agar plates with antibiotic (Vancomycin (0.001 g/L) and Colistin (0.0015 g/L). Results were expressed as the number of CFU per mL.

Statistical analysis of data

All data points in each experiment were obtained in triplicate, and statistical calculations were made with GraphPad Prism version 4.00 for Windows. *P* values were calculated using the unpaired *t* test, and results were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

Rainwater is collected all around the world as good alternative source of water. It is collected by gutters on the roof and stored in appropriate water tanks [4,5]. Factors that contribute to the bacterial contamination

of rainwater are inadequate cleaning and maintenance of tanks, the stagnation of water in the tanks and the water temperature in the hot parts of the year [5].

In this study the microbiological quality of rainwater from 10 different rainwater tanks in Primorsko-goranska County were analyzed (Table 1). Our results showed that in 70 % rainwater samples the number of bacteria cultivated at 37 °C was elevated. In three samples the fecal contamination was detected while in 8 samples total coliform bacteria were detected.

This result is consistent with previous results which showed that due to inadequate cleaning and maintenance of rainwater tanks the heavy bacterial contaminations of rainwater is common. For example, 41 % of rainwater samples tested by Sazakli *et al.* in Kefalonia, Greece, were positive for *E. coli* [6]. In studies from 2006, in Brazil 50 % of the samples fecal contamination was detected and 98 % of samples had elevated fecal enterococci. The recent study in Primorsko-goranska County also showed that 73 % of the rainwater samples had heavy bacterial contamination [10].

As for many other purposes rainwater is often used for watering gardens during which aerosols could be created and that presents potential health risks.

As for many other purposes rainwater is often used for watering gardens during which aerosols could be created and that presents potential health risks. It is well known that human inhalation of contaminated aerosols leads to *Legionella* infections and disease outbreaks. *L. pneumophila* was linked to Legionnaires' disease, respectively, amongst users of the rainwater as a potable water supply in the US Virgin Islands [7]. The investigation from New Zealand showed that roof-collected rainwater systems in a temperate climate can provide a suitable reservoir for the survival and proliferation of *Legionella* and may have subsequently caused cases of Legionnaires' disease [5].

So, the purpose of this study was to determine whether *L. pneumophila* and *L. longbeachae* are able to survive and multiply in rainwater and if the presence of bacterial contamination has an impact on their survival.

Two rainwater samples were chosen for survival study according to the presence of bacterial contamination (Table 1). Rainwater from sample 4 showed heavy bacterial contamination with high number of fecal coliforms and fecal enterococci, while in sample 5 small amount of bacterial microbiota were present and indicator of fecal contamination were not detected.

Since the water is a natural habitat of *L. pneumophila* two different strains of this species: *L. pneumophila* strain 130B and strain Corby were tested.

Since the water is a natural habitat of *L. pneumophila* two different strains of this species: *L. pneumophila* strain 130B and strain Corby were tested. The starting dose of bacteria was 10^6 CFU/ml, and the samples were incubated at room temperature. The results showed that after the inoculation the number of bacteria was constant for 5 days. After that the decline in the number of bacteria occurs in unfiltered samples and after 30 day of incubation number of bacteria decreased for 3 logarithmic units. In filtered samples there were no significant changes in bacterial number during 30 days. There were no differences in survival between two *L. pneumophila* strains and comparison be-

tween unfiltered samples showed no difference in the survival of *L. pneumophila* strain 130b (Figure 1 A), while Corby strains survived better in the 4th sample (Figure 1 B).

Although the natural environment of *L. longbeachae* is moist soil, it is not yet known exactly how these bacteria come into the ground and what is the exact route of transmission [8,9]. Therefore, the survival of *L. longbeachae* was tested in the filtered and unfiltered samples of rainwater. The results showed that *Legionella* also survived all 30 days in rainwater samples. The number of bacteria in filtered water samples is constant throughout the study period, while in the unfiltered samples the number of bacteria was significantly reduced after the second day of incubation. On the 30th day of incubation 10^2 cfu/ mL *L. longbeachae*

The results showed that *Legionella* also survived all 30 days in rainwater samples.

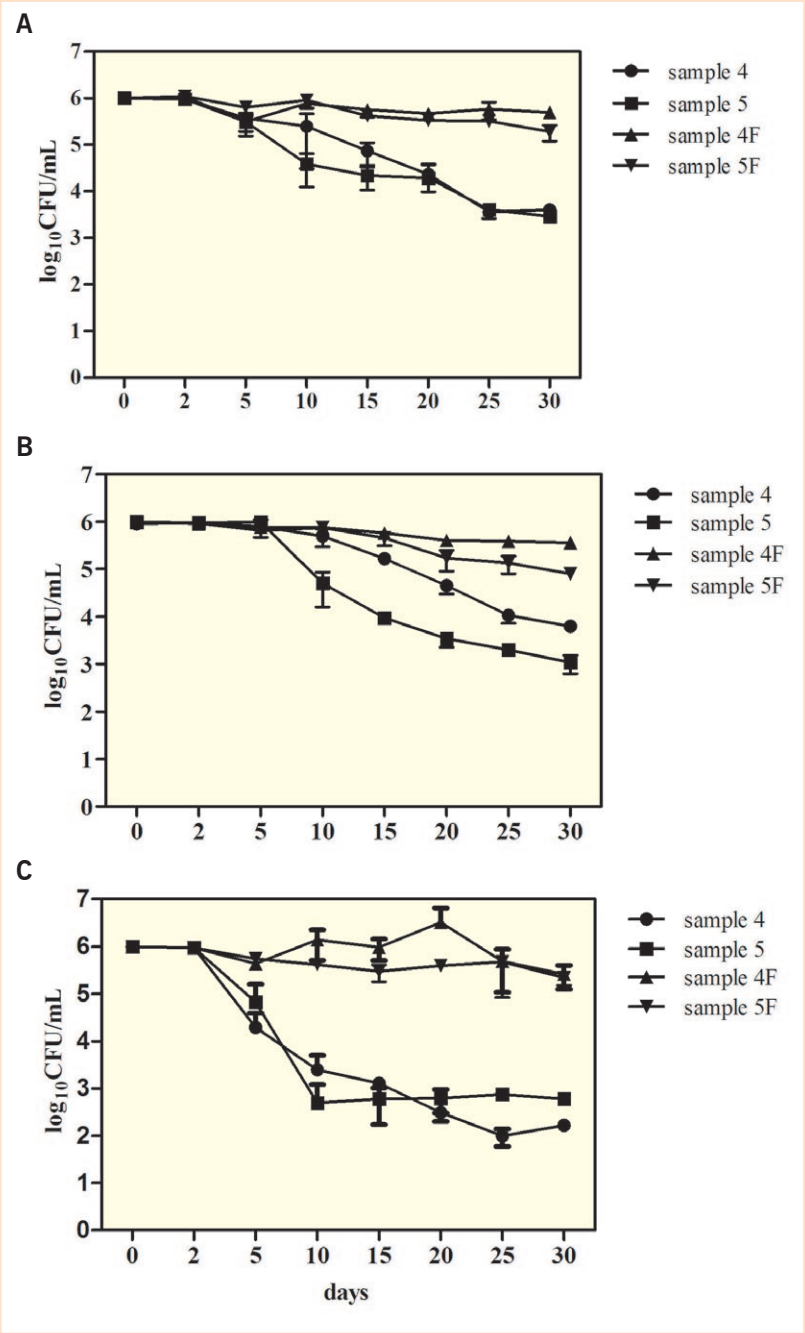


Figure 1.
Survival of *L. pneumophila* strain 130b (A), strain Corby (B) and *L. longbeachae* strain NSW150 (C) in two rainwater samples. Plate counts were done at various time points to determine viable cells remaining. The data are the means of duplicate experiments and the error bars indicate standard deviations.

To conclude, both *Legionella* strains were not able to multiply but they did survive in rainwater for 30 day.

was detected (Figure 1 C). The results showed that heavy bacterial contamination of rainwater restricted the survival of *L. longbeachae.* It seems that this *Legionella* species is not adapted to the rainwater microbiota. This hypothesis was recently confirmed and analysis of the *L. longbeachae* genome has demonstrated that *L. longbeachae* is highly adapted to the soil environment [11, 12].

To conclude, both *Legionella* strains were not able to multiply, but they did survive in rainwater for 30 day. *L. pneumophila* survives better in rainwater with a complex bacterial flora in comparison to *L. longbeachae*. All tested bacteria survive better in filtered samples so the presence of bacterial contamination affects their survival in rainwater. Further research into the ecology of *Legionella* species in rainwater systems is needed.

REFERENCES

[1] Gobin I, *et al.* Infections caused by nonpneumophila species of *Legionella*. Reviews in Medical Microbiology. 2009; 10: 1-11.

[2] Gobin I, *et al.* Experimental *Legionella longbeachae* infection in intratracheally inoculated mice. 2009. J Med Microbiol. 2009; 58: 723-730.

[3] Kovačević N, et al. Microbiological analysis of rainwater in Primorskogoranska County. Proceeding of 5th Croatianwater. 2011. May 18-21; Opatija. Zagreb: Hrvatske vode. 177-183.

[4] Lye DJ. Microbiology of rain water cistern systems: a review. Journal of Environmental Science and Health. 1992; 27: 2123-2166.

[5] Simmons G, *et al.* Legionnaires' disease outbreak: A water blaster and roof-collected rainwater systems. Water res. 2008; 42: 1449-1458.

[6] Sazakli E, Alexopoulos A and Leotsinidis M. Rain water harvesting, quality assessment and utilization in Kefalonia, Greece. Water Res. 2007; 41: 2039-2047.

[7] Schlech WF, *et al.* Legionnaires'disease in the Carribean. An outbreak associated with a resort hotel. Archives of Internal Medicine. 1985; 145: 2076-2079.

[8] O'Connor BA, Carman J, Eckert, K Tucker G, Givney R, Cameron S.Does using potting mix make you sick? Epidemiol Infect. 2007; 135: 34-39.

[9] Steele TW, Lanser J, Sangster N. Isolation of *Legionella longbeachae* serogroup 1 from potting mixes. Appl Environ Microbiol. 1990; 56: 49-53.

[10] May S, and Prado RTA. Experimental evaluation of rain water quality for non-potable applications in the city of São Paulo, Brazil. Urban Water Journal. 2006; 3:145-151.

[11] Cazalet C, *et al.* Analysis of the *Legionella longbeachae* genome and transcriptome uncovers unique strategies to cause Legionnaires' disease. PLoS Genet. 2010; 6:e1000851.

[12] Kozak NA, *et al.* Virulence factors encoded by *Legionella longbeachae* identified on the basis of the genome sequence analysis of clinical isolate D-4968. J Bacteriol. 2010; 192: 1030-1044.

Professional paper

Flows of raw materials and food safety of products of Slovenian manufacturers after EU entry

Romana **TOMŠE**[1*], Vesna **ČANČER**[2], Peter **RASPOR**[3]

ABSTRACT

In food production, the choice of suppliers and raw materials is very important and plays a key role in providing quality, appropriate healthy food. Selection of new suppliers and the planned verification of existing suppliers is also the key point in the HACCP system (Hazard Analysis Critical Control Point, which means the risk analysis and critical control points). The aim of our survey was to analyse the level of food safety in Slovenia before and after Slovenia joined the EU. The aim of our survey was to determine whether joining the EU had an impact on choice of food suppliers and control of the raw materials used in production of foods. The common thread of research was a comparison of answers, for periods before and after Slovenia joined the EU, gained from the survey of the Slovenian food producers.

The study has shown that after joining the EU there were some replacements of suppliers, as well as changes in control of suppliers, but the changes were smaller than expected. The analysis of the responses from the survey shows that the Slovenian manufacturers mostly trust domestic suppliers of raw materials. After Slovenia's accession to the EU, the use of raw materials from other EU countries, as well as the control over suppliers, has increased. After Slovenia's accession to the EU, the percentage of those Slovenian producers who control Slovenian supplier under looser criteria, has slightly increased, while the percentage of those Slovenian producers who control EU supplier under looser criteria has significantly increased. The survey results show partial distrust of free movement of goods. Producers on average partly agree that they equally trust the manufacturers in all EU member states.

Key words: foods, food producers, comparison before and after EU entry, control of foods, survey of food producers.

Received: 8. 5. 2012.
Accepted: 26. 5. 2012.

[1] University of Nova Gorica
Graduate studies of Environmental Sciences
Vipavska 13, Rožna Dolina
SI-5000 Nova Gorica, Slovenia

[2] University of Maribor
Faculty of Economics and Business
Razlagova 14
SI-2000 Maribor, Slovenia

[3] University of Ljubljana
Biotechnical Faculty
Department of Food Science and Technology
Jamnikarjeva 101
SI-1000 Ljubljana, Slovenia

[*] *Corresponding author*
Romana Tomše
University of Nova Gorica
Graduate studies of Environmental Sciences
Vipavska 13, Rožna Dolina
SI-5000 Nova Gorica, Slovenia
Phone:+386 51 378 571
E-mail: romana.tomse@gmail.com

POVZETEK

V proizvodnji živil je izbira dobaviteljev in s tem samih surovin zelo pomembna in ima ključno vlogo pri zagotavljanju kvalitetne, zdravstveno ustrezne hrane. Izbira novih dobaviteljev in načrtovano preverjanje že obstoječih dobaviteljev sta ključni točki tudi v HACCP sistemu (angleška kratica Hazard Analysis Critical Control Point, kar pomeni analiza tveganja in določanje kritičnih kontrolnih točk). Namen naše raziskave je bil analizirati izbiro dobaviteljev in nadzor nad surovinami slovenskih proizvajalcev pred in po vstopu Slovenije v EU. Cilj raziskave je bil ugotoviti, ali je vstop Slovenije v EU vplival na izbiro dobaviteljev in nadzor nad surovinami, ki se uporabljajo v proizvodnji živil. Rdeča nit raziskave je bila primerjava odgovorov, pridobljenih z anketiranjem slovenskih proizvajalcev hrane, za obdobje pred in po vstopu Slovenije v EU.

Raziskava je pokazala, da je po vstopu v EU prišlo do zamenjave nekaterih dobaviteljev, kakor tudi do sprememb pri samem nadzoru dobaviteljev, vendar so bile spremembe manjše, kot smo pričakovali. Analiza odgovorov iz raziskave je pokazala, da slovenski proizvajalci najbolj zaupajo domačim dobaviteljem in surovinam. Po vstopu Slovenije v EU se je povečala uporaba surovin iz drugih držav EU, prav tako tudi nadzor nad dobavitelji. Po vstopu se je nekoliko povečal odstotek tistih slovenskih proizvajalcev, ki obvladujejo slovenske dobavitelja po ohlapnejših kriterijih in ter tistih slovenskih proizvajalcev, ki nadzorujejo EU dobavitelja po ohlapnejših kriterijih. Rezultati ankete prikazujejo delno nezaupanje v prosti pretok blaga. Proizvajalci se v povprečju delno strinjajo, da enako zaupajo proizvajalcem v vseh državah članicah EU.

Ključne besede: živila, proizvajalci živil, primerjava pred in po vstopu v EU, nadzor nad živili, anketiranje proizvajalcev živil.

INTRODUCTION

Issues of flows of raw materials and foodstuffs and their safety represent a challenge for each country, which is in accordance with the Regulation (EC) No. 178/2002 laying down the general principles and requirements of food law, establishment of the European Food Safety Authority and laying down procedures concerning food safety [1]. In April 2011, Slovenian food producers were surveyed. A study of the production of Slovenian food producers before (1999-2004) and after (2005-2010) EU accession was conducted in order to determine the state of food manufacturers in Slovenia. On the basis of producer database, producers were chosen, according to their engagement in the production of food before and after joining the EU.

Understanding the flow of raw materials and products may indicate the potential hazard of the supply of the population with safe food. Thus, it is necessary to have a detailed knowledge of the purchase of raw materials, of the control of suppliers and of the opinion of Slovenian manufacturers and importers of food. According to the political and financial flows, it was expected that many food manufacturers replaced the raw material suppliers since Slovenia joined the EU. Amended legislation also offers various options of the safety management of food chains which is raising doubt that the producers loosened these systems and that after Slovenia joined the EU, suppliers from the EU began to be checked at lower criteria and less often than suppliers from the third countries. The purpose of this work is to examine how such thinking and speculation holds true and how the food flows run along the food chain.

Understanding the flow of raw materials and products may indicate the potential hazard of the supply of the population with safe food.

METHODS

The purchasing of raw materials, control of suppliers and the opinion of Slovenian manufacturers and importers of food, have been interested so the two six-year periods before and after joining the EU have been compared, namely from 1999 to 2010. Of 169 sent questionnaires, 76 responses have been received, representing 45 % of all sent questionnaires.

The hypothesis: “Many food producers, after Slovenia joined the EU, changed suppliers of raw materials” and “After the entry of Slovenia into the EU food producers examine their suppliers from the EU at lower criteria and less often than suppliers from third countries” have been tested.

For this purpose, a questionnaire that consists of several parts has been created. The application and purpose, a description of the composition of the questionnaire and instructions for completion are stated in the introductory part. The questionnaire itself is divided into four parts: A, B, C and D. Part A covers the production before joining the EU, part B covers the production after joining the EU and part C provides opinion and trust of the companies in raw materials and products from the EU. Parts A and B are divided into control of raw materials and control of

suppliers. Part D is designed in a way so that companies can enter the data of customer complaints, withdrawal and recall of food by periods. Purchases of raw materials and control of suppliers before and after Slovenia joined the EU and opinion of the companies are formed on the basis of an agreement scale, which is the five-step interval Likert scale with 1 representing "I strongly disagree", 2 "I mostly disagree", 3 "I partly agree", 4 "I mostly agree" and 5 representing "I totally agree".

Hypothesis were tested by comparing the means of responses, standard deviations of individual statements, and by statistical processing of responses using the Statistical Package for the Social Sciences (SPSS) software. Firstly, the Kolmogorov-Smirnov and Shapiro-Wilk tests have been performed to determine whether normal distribution can be adjusted to empirical data. The One-Sample Kolmogorov-Smirnov Test procedure compares the observed cumulative distribution function for a variable with a specified theoretical distribution, which may be normal, uniform, Poisson, or exponential. The Kolmogorov-Smirnov *Z* is computed from the largest difference (in absolute value) between the observed and theoretical cumulative distribution functions.

Hypothesis were tested by comparing the means of responses, standard deviations of individual statements, and by statistical processing of responses using the Statistical Package for the Social Sciences (SPSS) software.

On the basis of the results of these one-sample non-parametric tests, the nonparametric Wilcoxon Signed Ranks Test for two related samples has been chosen to test the hypotheses.

The sign test computes the differences between the two variables for all cases and classifies the differences as either positive, negative, or tied. If the two variables are similarly distributed, the number of positive and negative differences will not differ significantly. The Wilcoxon signed-rank test considers information about both the sign of the differences and the magnitude of the differences between pairs. The Wilcoxon signed-ranks method tests the null hypothesis that two related medians are the same and compare a single median against a known value or paired medians from the same (or matched) sample.

The mean is the central tendency of a collection of numbers taken as the sum of the numbers divided by the size of the collection.

Standard deviation shows how much variation or "dispersion" exists from the average (mean, or expected value). The low standard deviation indicates that the data points tend to be very close to the mean, whereas high standard deviation indicates that the data points are spread out over a large range of values.

RESULTS

Production before joining the EU

In this section the results of a survey of part A: Production before entering the EU from 1999 to 2004 have been presented. All results are gained from our own research.

The **Table 1** shows that the respondents on average partly or mostly agree with the statement that before Slovenia joined the EU the raw

materials of Slovenian origin were used, they partly agree that they used imported raw materials and also partly agree that they used materials of EU origin. They used raw materials of Slovenian origin, as well as raw materials of EU origin and imported raw materials from third countries. On average they mostly disagree that they used raw materials of Slovenian origin, raw materials of EU origin and raw materials from third countries in the same proportion: on average, they mostly agree that they gave advantage to the Slovenian raw materials (Mean: 3.95±1.142), but they did not give priority to raw materials from EU. Manufacturers, on average, mostly self-produced foods (Mean: 4.66±0.684), only a few, in addition to their own production, also imported final foods (Mean: 2.20±1.479) [2].

Table 1.
Mean and standard deviation of answers on purchase of raw materials before entering the EU.

	Number of answers	Mean	Standard deviation
A1. We used raw material of Slovenian origin.	76	3.45	1.237
A2. We used imported raw material.	76	2.93	1.170
A3. We used raw material from EU members.	76	3.03	1.070
A4. We used materials that were of Slovenian origin, originating from EU countries and imported raw materials from other countries in about the same proportion.	76	2.74	1.170
A5. Priority was given to raw materials, which are of Slovenian origin and to Slovenian suppliers.	76	3.95	1.142
A6. Priority was given to raw materials of EU origin and to EU suppliers.	76	2.39	1.072
A7. We have been producing final food products from raw materials.	76	4.66	0.684
A8. Final products were also imported.	76	2.20	1.479

Analysis of individual statements in the dimension of purchase of raw materials before joining the EU showed that respondents answered most dispersedly to the statement, “Final products were also imported”, with the standard deviation of 1.479, and least dispersedly to the argument that they produced final products by themselves; “We have been producing final food products from raw materials” (0.684). For arguments regarding the use of raw materials of different origins, the respondents answered the most dispersed to the statement, “We used raw materials of Slovenian origin” (1.237), and least dispersedly to the argument “We used raw materials from the EU members” (1.070). The argument, “Priority was given to raw materials, which are of Slovenian origin and to Slovenian suppliers” showed standard deviation of 1.142, and the argument “Priority was given to raw materials of EU origin and EU suppliers” showed standard deviation of 1.072 (**Table 1**) [2].

From the responses to arguments concerning the control of suppliers before entering the EU it can be made out, that HACCP (Hazard Analysis Critical Control Point) system has been in the process of promoting and enforcement [3]. On average, manufacturers partly agree that they had an established HACCP system in the period from 1999 to 2004 (**Table 2**).

On average they partly most agree that suppliers were checked before the first delivery, but they have not been verified on the content of contaminants. Also, on average, they partly agree that suppliers were regularly and periodically checked, but not regularly checked on the content of contaminants. On average, they mostly agree that all suppliers were checked under the same criteria and that Slovenian and EU suppliers were not checked under looser criteria. There is a small difference among the verification of Slovenian and EU suppliers. Slovenian suppliers had been checked less strictly (Mean: 2±1.244) than EU suppliers (Mean: 1.67±1.025). Manufacturers, on average, mostly agree that they had very few consumers' complaints, few non-compliant products and few withdrawals/recalls of food. They partially agree that very few foods were analysed (**Table 2**) [2].

Table 2.
Mean and standard deviation of answers on control of suppliers before entering the EU.

	Number of answers	Mean	Standard deviation
A9. We had set up the HACCP system.	76	3.49	1.194
A10. Suppliers were always verified before the first delivery.	76	3.58	1.146
A11. Before the first delivery, suppliers were always verified on the content of environmental contaminants.	76	2.49	1.291
A12. Suppliers were regularly periodically checked.	76	3.28	1.162
A13. Suppliers were regularly periodically checked on the content of environmental contaminants.	76	2.47	1.238
A14. All suppliers were examined under the same criteria.	76	3.82	1.241
A15. Slovenian suppliers were checked under "looser" criteria than suppliers from imports, because they are "domestic".	76	2.00	1.244
A16. EU suppliers were checked under "looser" criteria than other suppliers from imports, because of European goods.	76	1.67	1.025
A17. We had very few customers' complaints.	76	3.92	0.963
A18. We had very few non-compliant products.	76	4.16	0.713
A19. We had very few withdrawals/recalls of food.	76	4.58	0.497
A20. Very few final products were analysed.	76	3.16	1.386

The replies concerning HACCP system to the statement "We had set up the HACCP system" with the standard deviation of 1.194, were also dispersed.

Analysis of individual statements in the dimension of control of suppliers before entering the EU showed that respondents most dispersedly answered to the argument "Very few final products were analysed" with the standard deviation of 1.386, and least dispersedly to the argument "We had very few withdrawals/recalls of food" with standard deviation of 0.497. They also answered very dispersedly to the statements: "Before the first delivery, suppliers were always verified on the content of environmental contaminants" (1.291), "Slovenian suppliers were checked under "looser" criteria than suppliers from imports, because they are *domestic*" (1.244), "All suppliers were examined under the same criteria" (1.241) and "Suppliers were regularly periodically checked on the content of environmental contaminants" (1.238). The replies concerning HACCP system to the statement "We had set up the HACCP system" with the standard deviation of 1.194, were also dispersed. In addition to the argument: "We had very few withdrawals/recalls of food" the answers to the statements "We had very few non-compliant products" (0.713) and "We had very few customers'

complaints" (0.963) were much more unified. It can be concluded that they had quite different approaches to the implementation of the HACCP system and control of their suppliers, but still they all had very few customer complaints, non-compliant products and very few withdrawals and recalls of food (**Table 2**) [2].

Production after joining the EU

After joining the EU, the producers still mostly favour Slovenian raw materials (Mean: 3.87±1.124). Raw materials of Slovenian origin and EU origin are used in approximately equal proportions. According to the period before accession to the EU slightly more producers import final products (before joining the EU; Mean: 2.20±1.479, after joining the EU; Mean: 2.67±1.491) (**Table 3**). Analysis of individual statements on purchase of raw materials before entry into the EU showed that respondents most dispersedly answered to the statement, "We also import final products" with the standard deviation of 1.491, and least dispersedly to the statement "We are producing final products from raw materials" with a standard deviation of 0.683 (**Table 3**) [2].

Table 3.
Mean and standard deviation of answers on purchase of raw materials after joining the EU.

	Number of answers	Mean	Standard deviation
B1. We use raw materials that are of Slovenian origin.	76	3.42	0.942
B2. We use imported raw materials (from third countries).	76	2.32	1.122
B3. We use raw materials from other EU countries.	76	3.59	0.982
B4. We use raw materials that are of Slovenian origin, originating from other EU countries and imported raw materials in approximately equal proportions.	76	2.55	1.012
B5. We give priority to raw materials, which are of Slovenian origin and to Slovenian suppliers.	76	3.87	1.124
B6. We give priority to raw materials, which are originating from other EU members and EU suppliers.	76	3.07	1.024
B7. We are producing final products from raw materials	76	4.50	0.683
B8. We also import final products.	76	2.67	1.491
B9. Slovenia's entry into the EU has influenced our choice of supplier and raw materials. After joining the EU, we changed raw material supplier(s).	76	2.79	1.320
B10. After Slovenia joined the EU we selected supplier(s) within the EU.	76	2.89	1.281

Regarding control of the suppliers after joining the EU, producers on average mostly agree that the HACCP system is very effective, and that they review the suppliers before the first delivery and regularly periodically. On average manufacturers partly agree that suppliers are also checked on the content of environmental contaminants (**Table 4**). On average, they mostly agree that all the suppliers are checked under the same conditions and that there are few complaints of customers and non-compliant products. Because of entry into the EU, on average, they mostly disagree with the statement that their suppliers are more rarely periodically checked. On average, entry in the EU mostly did not have effect on the number of customer's complaints, on the number of non-compliant products and on the number of withdrawals/recalls. Com-

monly, no more final products are being analyzed than before the entry into the EU (**Table 4**) [2].

Table 4.
Mean and standard deviation of answers on control of suppliers after joining the EU.

	Number of answers	Mean	Standard deviation
B11. A HACCP system is very efficient.	76	4.14	0.761
B12. Suppliers are always checked before the first delivery.	76	4.12	0.894
B13. Suppliers are always checked on the content of environmental contaminants before the first delivery.	76	3.21	1.024
B14. Suppliers are regularly periodically checked.	76	3.88	0.909
B15. Suppliers are also regularly periodically checked on the content of environmental contaminants.	76	3.11	1.066
B16. All suppliers are verified under the same criteria.	76	4.07	0.971
B17. Slovenian suppliers are checked under "looser" criteria than suppliers from imports, due to "domestic" goods.	76	2.12	1.211
B18. EU suppliers are checked under "looser" criteria than suppliers from imports, due to European goods.	76	2.07	1.170
B19. We have very few customers' complaints.	76	4.11	0.723
B20. We have very few non-compliant products.	76	4.26	0.661
B21. We have very few withdrawals/recalls of food.	76	4.50	0.643
B22. Very few final products are analysed.	76	2.53	1.113
B23. Because of entry into the EU, suppliers are rarely periodically checked.	76	1.95	0.815
B24. We have more customer complaints than before entering the EU.	76	2.50	1.361
B25. We have more non-compliant products than before joining the EU.	76	2.33	1.148
B26. We have more withdrawals/recalls of products than before entering the EU.	76	2.11	1.150
B27. More final products are analysed than before joining the EU.	76	2.67	1.518

Analysis of the various arguments concerning the control of suppliers after joining the EU showed that respondents most dispersedly answered to the statement, "More final products are analyzed than before joining the EU" with a standard deviation of 1.518, and least dispersedly to the "We have very few withdrawals/recalls of food" with a standard deviation of 0.643. The following statements: "We have very few non-compliant products" (0.661), "We have very few customers' complaints" (0.723), "A HACCP system is very efficient" (0.761), "Because of entry into the EU, suppliers are rarely periodically checked" (0.815), "Suppliers are always checked before the first delivery" (0.894) and "Suppliers are regularly periodically checked" (0.909) result in low standard deviation (**Table 4**) [2].

Opinion of the producers-confidence in the materials/products of the EU

Replies were received from 76 producers. Manufacturers, on average, mostly do not agree that the safety of their products by entry into the EU is higher and that because of the entry more quality materials are used. On average, they partly agree that they more easily access raw materials of higher quality at the same or lower price. On average, they mostly disagree that due to EU accession they are surer of the quality of

their raw materials and final products. However, on average, they partly agree that after accession to the EU purchases of raw materials and final products from the EU increased while imports of raw materials and final products from third countries decreased. Also, on average, they partly agree that the entry of Slovenia into the EU did not influence the choice of raw materials and suppliers (**Table 5**) [2].

On average, they mostly agree that when selecting raw materials, they take into account both, price and quality (Mean: 4.38), but majority, on average, does not trust raw materials from different EU Member States (Mean: 2.74) equally. On average they partly agree that the scope of the inspection in Slovenia can be reduced at the expense of providing a high level of food safety within the EU, while on average, they mostly agree that the inspection should focus more on raw materials or food of those producers and origins which have had inadequate samples more often. On average they mostly agree, that inspection should be tightened at the import into the EU (**Table 5**) [2].

Analysis of individual statements on the opinion of the food producers in Slovenia showed that respondents answered most dispersedly to the argument "After Slovenia joined the EU, purchases of final food products from the EU have increased" with the standard deviation of 1.421, while least dispersedly to "Inspections in Slovenia should focus more on raw materials/products from those countries that often have inadequate samples" with a standard deviation of 0.667. They also respond very dispersely to the following arguments: "Slovenia's joining to the EU did not affect our choice of raw materials and suppliers" with a standard deviation 1.409, "After Slovenia joined the EU, purchases of raw materials from the EU have increased" with a standard deviation of 1.269, "After joining Slovenia to the EU, imports of final products from third countries have decreased" with a standard deviation of 1.233, and to the statement "After joining Slovenia to the EU, import of raw materials from third countries has decreased" with the standard deviation of 1.225. Less dispersion is found in the following statements: "When given the choice of raw materials, both the quality and the price are considered" with a standard deviation of 0.783, "Inspection at the external border, at the import into the EU, should be tightened" with the standard deviation 0.816, and in the statement "When selecting the preferred raw material, quality has priority regardless of the price" with a standard deviation of 0.883 (**Table 5**) [2].

On average, they mostly agree that when selecting raw materials, they take into account both, price and quality (Mean: 4.38), but majority, on average, does not trust raw materials from different EU Member States (Mean: 2.74) equally.

Table 5.
Mean and standard deviation of answers on business confidence in EU commodities/products.

	Number of answers	Mean	Standard deviation
C1. The safety of our products has increased by entering the EU.	76	2.38	1.107
C2. Joining the EU we use more quality raw materials.	76	2.43	0.929
C3. Easier access to raw materials of higher quality at the same price.	76	3.00	1.033
C4. Easier access to raw materials of higher quality at lower price.	76	2.86	1.092
C5. Because of entry into the EU we are more sure of the quality of raw materials and thus in the final products.	76	2.53	1.101
C6. After Slovenia joined the EU, purchases of raw materials from the EU have increased.	76	3.13	1.269
C7. After Slovenia joined the EU, purchases of final food products from the EU have increased.	76	2.92	1.421
C8. After joining Slovenia to the EU, import of raw materials from third countries has decreased.	76	2.79	1.225
C9. After joining Slovenia to the EU, import of final products from third countries has decreased.	76	2.62	1.233
C10. Slovenia's joining the EU did not affect our choice of raw materials and suppliers.	76	3.04	1.409
C11. When selecting the preferred raw material, quality has priority regardless of the price.	76	3.42	0.883
C12. When selecting the preferred raw material, price has priority irrespective of quality.	76	2.17	0.900
C13. When given the choice of raw materials, both the quality and the price are considered.	76	4.38	0.783
C14. When selecting raw materials we equally trust the manufacturers in all EU Member States.	76	2.74	0.985
C15. The scope of inspection in Slovenia could decrease, since the level of assurance of food safety within the EU is very high.	76	3.01	1.113
C16. Inspections in Slovenia should focus more on raw material/products from those countries that often have inadequate samples.	76	4.36	0.667
C17. Inspection at the external border, at the import into the EU, should be tightened.	76	4.00	0.816

DISCUSSION

Replacement of raw material suppliers

According to the results of the survey, producers, after entering the EU, approached very differently to the replacement of the raw material suppliers. When the results of the responses about purchases of raw materials before and after joining the EU, namely the mean values before and after joining the EU have been compared, the use of raw materials of Slovenian origin after joining the EU decreased by 0.87 % (Δ Mean is 0.03), use of materials from imports decreased by 21 % (Δ Mean is 0.61) while use of materials from the EU increased by 18 % (Δ Mean is 0.56). Priority in the selection of Slovenian producers after accession to the EU decreased by 2 % (Δ Mean is 0.08), but priority in the selection of suppliers from the EU and thereby on average also the raw materials from the EU, after joining the EU, increased by 28 % (Δ Mean is 0.68) (**Table 6**). Differences in responses to statements about raw materials before and after joining the EU are shown in **Figure 1** [2].

Table 6.
Mean and standard deviation of purchase of raw materials before and after joining the EU.

	Mean before	Mean after	Δ Mean (divergence)	Standard deviation (before)	Standard deviation (after)
Use of raw material of Slovenian origin.	3.45	3.42	0.03	1.237	0.942
Use of imported raw material.	2.93	2.32	0.61	1.17	1.122
Use of raw material from EU members.	3.03	3.59	0.56	1.07	0.982
We used materials that were of Slovenian origin, originating from EU countries and imported raw materials from other countries in about the same proportion.	2.74	2.55	0.19	1.17	1.012
Priority to raw materials of Slovenian origin and to Slovenian suppliers.	3.95	3.87	0.08	1.142	1.124
Priority to raw materials of EU origin and to EU suppliers.	2.39	3.07	0.68	1.072	1.024

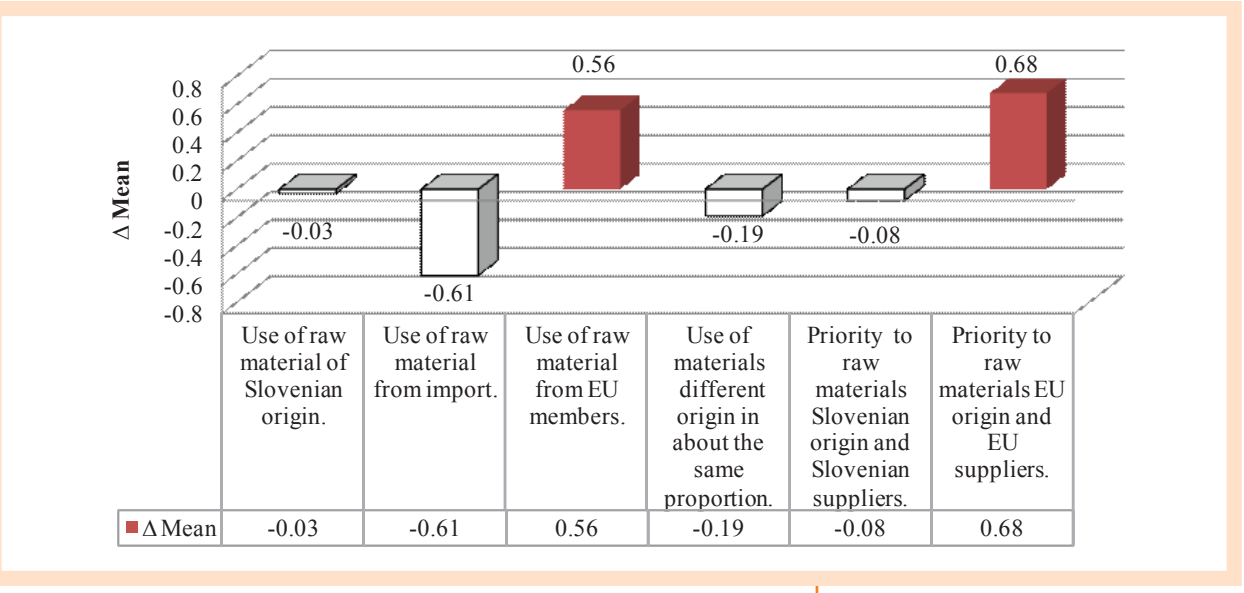


Figure 1.
Difference of mean values of answers of purchase of raw materials before and after joining the EU.

After joining the EU a slight decline in giving priority to Slovenian manufacturers can be detected and significant increase of favouring the EU producers. On the basis of this it is assumed that Slovenian producers which favoured the Slovenian manufacturers partly began to give priority to EU producers, while producers who gave preference to suppliers from the EU, mostly kept giving priority to EU suppliers and raw materials [2].

Table 3 shows that Slovenian producers, on average, partly agree with the statement "Slovenia's entry into the EU has influenced our choice of suppliers and raw materials. After joining the EU, we changed raw material supplier(s)" (Mean is 2.79). Responses to the argument are very dispersed with a standard deviation of 1.320. Also, on average, they partly agree that after Slovenia joined the EU they selected a supplier from the EU (Mean is 2.89). The statement "After Slovenia joined the EU we selected supplier(s) within the EU" was answered with a standard deviation of 1.281 (**Table 3**). According to the results of the survey it may be concluded that the producers, after Slovenia joined the EU have reacted variously and so variously adapted the purchase of raw

On average, they partly agree with the statement "After Slovenia joined the EU, purchases of raw materials from the EU have increased" (Mean is 3.13).

materials. Some have remained with previous suppliers of raw materials, others have changed suppliers [2].

When analyzing the responses of some opinions on the business confidence in the materials and products of EU origin (questions, part C), similar conclusions may be drawn. On average, they partly agree with the statement "After Slovenia joined the EU, purchases of raw materials from the EU have increased" (Mean is 3.13). From the responses it can be concluded that at least part of the producers increased purchases from the EU suppliers or switched their suppliers with suppliers from the EU. They answered quite dispersedly with a standard deviation of 1.269. On average, they partly agree with the statement "After Slovenia joined the EU, purchases of final food products from the EU have increased" (Mean is 2.92). From that it may be concluded that after the accession to the EU purchases of finished products from the EU have increased, but to a lesser extent than the purchase of raw materials originating in the EU. The standard deviation is 1.421, which means that manufacturers have responded very dispersedly. From that it may be concluded that operating of Slovenian producers varies greatly and can not be spoken about common characteristics of all manufacturers. On average they also partly agree (Mean is 3.04) with the statement "Slovenia's accession to the EU did not affect our choice of raw materials and suppliers". The results confirm our conclusion that some producers replaced their suppliers, but others did not and that there is no such thing as the common replacement of suppliers, which would be indicated in all Slovenian suppliers (**Table 5**) [2].

After joining the EU a slight 3 % decline in own food production is perceived (Δ Mean is 0.16) and a 21 % increase (Δ Mean is 0.47) of final product import (**Tables 1 and 3**) [2].

Testing of the hypothesis concerning changing of the suppliers

Hypothesis "Many food producers, after Slovenia joined the EU changed suppliers of raw materials" has been tested using the SPSS programme. Firstly, Kolmogorov-Smirnov test and Shapiro-Wilk tests have been performed to verify whether a normal distribution can be attributed to the data from statements A1 to A6 and B1 to B6. Test results are shown in the **Table 7**. Test results show that the sig. < 0.05, indicating that a normal distribution cannot be attributed to values of the studied variables (arguments A1 to A6 and B1 to B6), so the nonparametric test for two related samples has been chosen to test the above written hypothesis (**Table 8**). In Wilcoxon Signed Rank test the value is statistically significant at sig. <0.05, if its absolute standardized value z is greater than 1.96. On the basis of results it may be concluded that the data from statements can be attributed the statistically significant differences between the compared periods 1999-2004 and 2005-2010 [4, 5].

Table 7.
Tests of normality for statements from A1 to A6 and B1 to B6.

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
A1. We used raw material of Slovenian origin.	0.212	76	0.000	0.891	76	0.000
B1. We use raw materials that are of Slovenian origin.	0.225	76	0.000	0.893	76	0.000
A2. We used imported raw material.	0.220	76	0.000	0.903	76	0.000
B2. We use imported raw materials (from third countries).	0.216	76	0.000	0.867	76	0.000
A3. We used raw material from EU members.	0.280	76	0.000	0.869	76	0.000
B3. We use raw materials from other EU countries.	0.201	76	0.000	0.878	76	0.000
A4. We used materials that were of Slovenian origin, originating from EU countries and imported raw materials from other countries in about the same proportion.	0.170	76	0.000	0.912	76	0.000
B4. We use raw materials that are of Slovenian origin, originating from other EU countries and imported raw materials in approximately equal proportions.	0.223	76	0.000	0.891	76	0.000
A5. Priority was given to raw materials, which are of Slovenian origin and to Slovenian suppliers.	0.230	76	0.000	0.823	76	0.000
B5. We give priority to raw materials, which are of Slovenian origin and to Slovenian suppliers.	0.244	76	0.000	0.842	76	0.000
A6. Priority was given to raw materials of EU origin and to EU suppliers.	0.223	76	0.000	0.892	76	0.000
B6. We give priority to raw materials, which are originating in other EU members and to EU suppliers.	0.211	76	0.000	0.888	76	0.000

a – Lilliefors Significance Correction

df – degrees of freedom

Table 8.
Wilcoxon Signed Ranks Test – Test Statistics(b) for statements from A1 to A6 and B1 to B6.

	*z**	Asymptotic Significance (2-tailed)
We use raw materials that are of Slovenian origin. – We used raw material of Slovenian origin.	-0.119(a)	0.905
We use imported raw materials (from third countries). – We used imported raw materials.	-4.458(a)	0.000
We use raw materials from other EU countries. – We used raw material from EU members.	-4.485(b)	0.000
We use raw materials that are of Slovenian origin, originating from other EU countries and imported raw materials in approximately equal proportions. – We used materials that were of Slovenian origin, originating from EU countries and imported raw materials from other countries in about the same proportion.	-1.414(a)	0.157
We give priority to raw materials, which are of Slovenian origin and Slovenian suppliers. – Priority was given to raw materials, which are of Slovenian origin and to Slovenian suppliers.	-0.634(a)	0.526
We give priority to raw materials, which are originating in other EU members and to EU suppliers. – Priority was given to raw materials of EU origin and to EU suppliers.	-3.993(b)	0.000

The results of nonparametric test for 2 related samples obtained with SPSS programme (**Table 8**) show that the absolute value of the variable z for the use of raw materials of Slovenian origin, the use of raw materials of different origins in the same proportion, and for giving priority to Slovenian raw materials and Slovenian producers, is more than 1.96

According to the results of the survey, after joining the EU more effective control over the suppliers can be observed.

with sig.> 0.05, while for the use of raw materials of Slovenian origin, the use of raw materials of different origins in the same proportion, and for giving priority to Slovenian raw materials and Slovenian producers, absolute value of the variable *z* is less than 1.96 with sig.> 0.05 [2].

Control of suppliers

According to the results of the survey, after joining the EU more effective control over the suppliers can be observed. The results of the responses of control over the suppliers before and after joining the EU, namely the mean values before and after EU accession have been compared and it was found out that, the opinion of producers on the effectiveness of the HACCP system has increased by 19 % (Δ Mean is 0.65), checking of suppliers before the first delivery by 15 % (Δ Mean is 0.54) and verification of suppliers on content of contaminants prior to the first supply increased by 29 % (Δ Mean is 0.72). Periodical checks of suppliers have increased by 18 % (Δ Mean is 0.6) and periodical checks of suppliers on content of environmental contaminants increased by 26 % (Δ Mean is 0.64) (**Table 9**). Before joining the EU, producers answered quite dispersedly, but after joining the EU, the answers are much less dispersed. Reduction of the dispersion occurred due to the introduction and active implementation of the HACCP system. **Figure 2** shows the differences in the answers to the arguments on control of suppliers before and after EU accession [2].

Table 9.
Mean and standard deviation for control of suppliers before and after entering the EU.

	Mean (before)	Mean (after)	Δ Mean (divergence)	Standard deviation (before)	Standard deviation (after)
HACCP system.	3.49	4.14	0.65	1.194	0.761
Verifying of suppliers before the first delivery.	3.58	4.12	0.54	1.146	0.894
Verifying of suppliers before the first delivery on the content of contaminants.	2.49	3.21	0.72	1.291	1.024
Periodical checking of suppliers.	3.28	3.88	0.60	1.162	0.909
Periodical checking of suppliers on the content of contaminants.	2.47	3.11	0.64	1.238	1.066

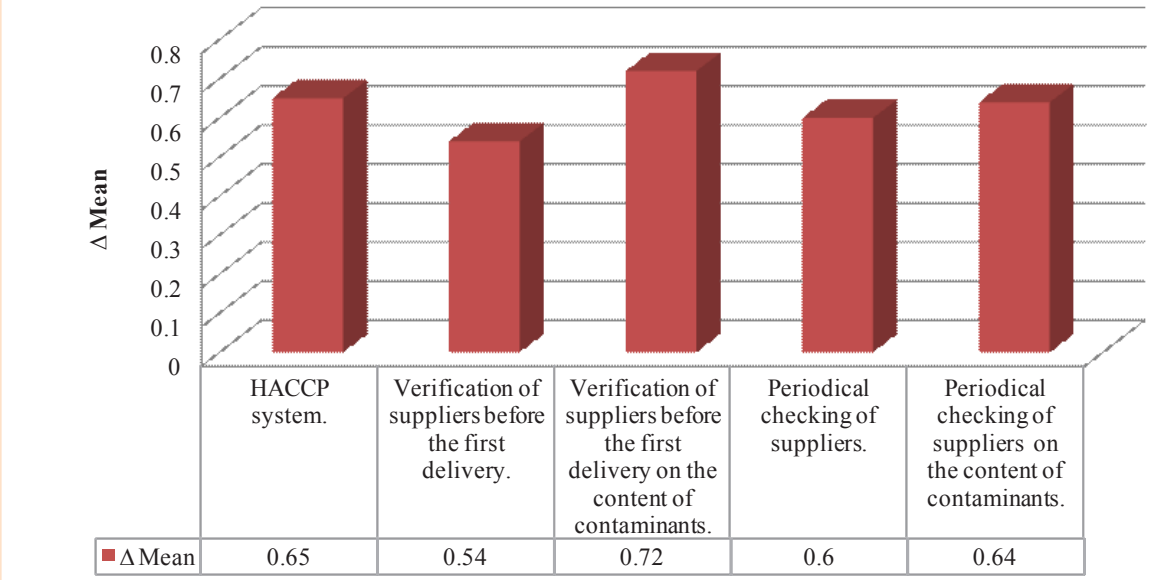


Figure 2.
Difference of mean values of answers for control of suppliers before and after entering the EU.

Before as well as after joining the EU, producers mostly agree that they monitor suppliers under the same criteria. The statement "All suppliers are verified under the same criteria" was on average evaluated with "mostly agree", before (Mean is 3.82) and after EU entry (Mean is 4.07). Before joining the EU the standard deviation was 1.241 and after joining the EU, 0.971, from which may be concluded that after joining the EU producers are more unified in the evaluation of suppliers. Producers, on average, did not agree with the argument "Slovenian suppliers are checked under "looser" criteria than suppliers from imports, due to "domestic" goods" and they before and after joining the EU, selected the statement "mostly disagree". Even in the standard deviation there is no significant difference, from which it may be concluded that the entry of Slovenia into the EU did not influence the selection of Slovenian producers under lower criteria. Also, on average, producers did not agree with the statement "EU suppliers are checked under "looser" criteria than suppliers from import, due to European goods", since they before and after joining the EU, on average, evaluated the statement with "mostly disagree" (before entering the EU, Mean: 1.67, after joining the EU, Mean: 2.07). It is interesting that the agreement with the statement after joining the EU rose by 24 % (Δ Mean is 0.4) and the standard deviation also slightly increased (**Table 10**) [2].

Table 10.
Mean and standard deviation for control of suppliers under "looser" criteria before and after entering the EU.

	Mean (before)	Mean (after)	Δ Mean (divergence)	Standard deviation (before)	Standard deviation (after)
Checking the suppliers under the same criteria.	3.82	4.07	0.25	1.241	0.971
Slovenian suppliers under "looser" criteria.	2.00	2.12	0.12	1.244	1.211
EU suppliers under "looser" criteria.	1.67	2.07	0.40	1.025	1.170

Before joining the EU 62 % of respondents strongly disagree with the statement "EU suppliers are checked on "looser" criteria than suppliers from import, due to European goods", while after joining the EU, only 43 % of producers strongly disagree with the statement. Before joining the EU 9 % of respondents answer "partly agree" and 16 % after joining the EU. Before joining the EU 8 % of respondents "mostly agree" with the statement and 13 % after joining the EU. On the basis of responses we concluded that most producers evaluate all of their suppliers, regardless of origin, under the same criteria, but the percentage of those who evaluate EU suppliers on the "looser" criteria after joining the EU has increased.

Examining the argument "Because of entry into the EU, suppliers are rarely periodically checked", it may be found out that most of the producers (53 %) chose the answer "mostly disagree" (Mean is 1.95), which is consistent with results of previous statements (**Table 4**). It is interesting that the standard deviation is low with 0.815. The answer "strongly disagree" was chosen by 29 % of respondents and answer "mostly disagree" by 53 % of respondents. Partly agree was chosen by 14 % of respondents, but only 4 % of respondents mostly or totally agree with the statement. From the results it may be concluded that entry into the EU did not affect the frequency of periodical checks of suppliers of Slovenian producers [2].

Testing of the hypothesis regarding the control of suppliers

Hypothesis "After the entry of Slovenia into the EU food producers examine their suppliers from the EU under lower criteria and less often than suppliers from third countries" was also tested using the SPSS software. Firstly, we performed the Kolmogorov-Smirnov test and Shapiro-Wilk test to determine whether normal distribution can be attributed to data from statements A9 to A16 and B11 to B18. Test results are shown in **Table 11**. Using the test results it is evident that the sig. is < 0.05, indicating that normal distribution cannot be attributed to values of the studied variables (statements A9 to A16 and B11 to B18), so the nonparametric Wilcoxon Signed Rank test for two related samples has been chosen to test the above written hypothesis (**Table 12**).

Table 11.
Tests of normality for statements from A9 to A16 and B11 to B18.

	Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
We had set up the HACCP system.	0.198	76	0.000	0.886	76	0.000
A HACCP system is very efficient.	0.293	76	0.000	0.775	76	0.000
Suppliers were always verified before the first delivery.	0.206	76	0.000	0.879	76	0.000
Suppliers are always checked before the first delivery.	0.259	76	0.000	0.801	76	0.000
Before the first delivery, suppliers were always verified on the content of environmental contaminants.	0.200	76	0.000	0.860	76	0.000
Suppliers are always checked on the content of environmental contaminants before the first delivery.	0.239	76	0.000	0.901	76	0.000
Suppliers were regularly periodically checked.	0.173	76	0.000	0.908	76	0.000
Suppliers are regularly periodically checked.	0.262	76	0.000	0.844	76	0.000
Suppliers were regularly periodically checked on the content of environmental contaminants.	0.254	76	0.000	0.865	76	0.000
Suppliers are also regularly periodically checked on the content of environmental contaminants.	0.224	76	0.000	0.899	76	0.000
All suppliers were examined under the same criteria.	0.217	76	0.000	0.831	76	0.000
All suppliers are verified under the same criteria.	0.236	76	0.000	0.824	76	0.000
Slovenian suppliers were checked under “looser” criteria than suppliers from import, due to “domestic” goods.	0.289	76	0.000	0.772	76	0.000
Slovenian suppliers are checked under “looser” criteria than suppliers from import, due to “domestic” goods.	0.256	76	0.000	0.826	76	0.000
EU suppliers were checked under “looser” criteria than other suppliers from import, because of European goods.	0.362	76	0.000	0.693	76	0.000
EU suppliers are checked under “looser” criteria than suppliers from import, due to European goods.	0.253	76	0.000	0.819	76	0.000

a – Lilliefors Significance Correction; df – degrees of freedom

Table 12.
Wilcoxon Signed Ranks Test – Test Statistics(b) for statements from A9 to A16 and B11 to B18.

	*z**	Asymptotic Significance (2-tailed)
A HACCP system is very efficient. – We had set up the HACCP system.	-4.348(a)	0.000
Suppliers are always checked before the first delivery. – Suppliers were always verified before the first delivery.	-4.449(a)	0.000
Suppliers are always checked on the content of environmental contaminants before the first delivery. – Before the first delivery, suppliers were always verified on the content of environmental contaminants.	-4.948(a)	0.000
Suppliers are regularly periodically checked. – Suppliers were regularly periodically checked.	-4.341(a)	0.000
Suppliers are also regularly periodically checked on the content of environmental contaminants. – Suppliers were regularly periodically checked on the content of environmental contaminants.	-4.587(a)	0.000
All suppliers are verified under the same criteria. – All suppliers were examined under the same criteria.	-1.813(a)	0.070
Slovenian suppliers are checked under “looser” criteria than suppliers from import, due to “domestic” goods. – Slovenian suppliers were checked under “looser” criteria than suppliers from import, due to “domestic” goods.	-0.863(a)	0.388
EU suppliers are checked under “looser” criteria than suppliers from import, due to European goods. – EU suppliers were checked under “looser” criteria than other suppliers from import, because of European goods.	-3.170(a)	0.002

a – Based on negative ranks; b – Wilcoxon Signed Ranks Test; * standardized test statistic

The analysis results (**Table 12**) showed that the absolute value of the variable *z* for the use of the HACCP system, for the control of suppliers before the first delivery, control of suppliers on the contaminants before the first delivery, periodical checks of suppliers, periodical control of suppliers on the contaminants, and for control of EU suppliers under less restrictive conditions, is greater than 1.96 with sig. < 0.05, so differences of this mean values before and after EU accession are statistically significant. For control of all suppliers under the same conditions and for the control of Slovenian suppliers under less restrictive conditions, the absolute value of the variable *z* is less than 1.96 with sig. > 0.05, so differences of this mean values before and after EU accession are not statistically significant.

Opinion of producers

Considering the responses of part C: Opinion of the producers' confidence in the materials/products from the EU, the following has been established. Producers, on average, mostly disagree with the argument "The safety of our products has increased by entering the EU" (Mean is 2.38), on the basis of which it may be concluded that manufacturers think that due to the entry into the EU the safety of their products has not increased (**Table 5**). The standard deviation is 1.107, which means that they answered quite dispersedly. Only 4 % completely agree with the fact that the safety of their products by entering the EU has increased, 13 % mostly agree, 24 % of them partly agree and 59 % of the producers mostly or strongly disagree with that [2].

On average, producers also mostly disagree with an argument "Joining the EU we use more quality raw materials", (Mean: 2.43).

On average, producers also mostly disagree with an argument "Joining the EU we use more quality raw materials", (Mean: 2.43). According to their opinion, because of entry into the EU, they do not use higher quality materials than before entering the EU. The dispersion of responses is lesser and totals 0.929 (**Table 5**). Only 8 % of manufacturers totally agree and mostly agree with the statement. 46 % of the producers partly agree with the statement, and the remaining 46 % mostly or strongly disagree with the statement. From these responses, it may be concluded that, on average, most producers do not agree that they use more quality materials because of entry into the EU [2].

The statement "Because of entry into the EU we are more sure of the quality of raw materials and thus in the final products" was evaluated with partly agree by 33 % producers and with mostly or strongly disagree by 49 % producers, while 18 % producers assessed the statement with mostly or totally agree. Mean of the statement is 2.53 and standard deviation 1.101 (**Table 5**). From the responses we can conclude that on average producers mostly disagree to partly agree with the statement and that because of entry into the EU itself they are not surer in quality of raw materials and final products [2].

Producers on average partly agree with the statement "After Slovenia joined the EU, purchases of raw materials from the EU have increased" (Mean 3.13). They answered quite dispersed with a standard deviation of 1.269 (**Table 5**). 48 % of producers evaluated the statement with "I

totally agree" and "mostly agree", 18 % of producers partly agree with the argument, and 34 % mostly or strongly disagree. From the responses we conclude that the opinion of manufacturers about purchase of raw materials originating from the EU after accession to the EU varies. Some, after accession to the EU, decided to purchase the raw materials of EU origin, while others stayed with their suppliers [2].

Producers also responded very dispersed to the statement "After Slovenia joined the EU, purchases of final food products from the EU have increased". Mean value is 2.92 and standard deviation 1.421 (**Table 5**). 45 % of manufacturers evaluated the statement with "I totally agree" and "mostly agree", while 14 % of producers partly agree and 41 % of producers mostly or strongly disagree with the statement. We can conclude that the producers, after joining the EU, decided very differently about the purchase of final food products originating from the EU. Some, after the EU entry, began with the purchase of final products from other producers in the EU, the rest still produced foods on their own or still purchased a part of them at the original producers [2].

Producers on average partly agree with the argument "Slovenia's joining the EU did not affect our choice of raw materials and suppliers" (Mean: 3.04). They answered very dispersedly, with a standard deviation of 1.409 (**Table 5**). 42 % of producers answered "I totally agree" and "mostly agree", 18 % of producers answered "partly agree", and about the same number of the producers that agreed with the statement, disagreed with it. 40 % of producers decided for the answer "mostly disagree" and "strongly disagree". On the basis of this data it may be seen that the opinions of Slovenian manufacturers are very different and that not all producers equally and uniformly proceed after EU entry [2].

On the basis of this data it may be seen that the opinions of Slovenian manufacturers are very different and that not all producers equally and uniformly proceed after EU entry.

Answers to the statement "When selecting raw materials we equally trust the manufacturers in all EU Member States" are very interesting. It is evident that Slovenian producers do not equally trust all producers of different EU member states, despite the fact that within the EU the same rules are applied. The mean of these answers is 2.74 with standard deviation of 0.985 (**Table 5**). 20 % of producers answered "I totally agree" and "mostly agree", 41 % of manufacturers answered "partly agree" while the answers "mostly disagree" and "strongly disagree" were chosen by 39 % of the producers [2].

CONCLUSION

On the basis of the results of comparing the producers' responses and mean values, it can be concluded that after Slovenia joined the EU there has been an increased purchase of raw materials from other EU countries, as well as increased preference of suppliers from the EU and thereby also of the raw materials from the EU.

On the basis of the results of the nonparametric test for two related samples (**Chapter** "Testing of the hypothesis regarding the control of suppliers") it can be concluded that the differences between the mean values in the compared periods 1999-2004 and 2005-2010 are not

statistically significant for the use of raw materials of Slovenian origin, for the use of materials of different origins in the same proportion, and for giving priority to Slovenian raw materials and Slovenian producers. However, the differences between the mean values in the compared periods 1999-2004 and 2005-2010 are statistically significant for arguments on the use of raw materials from third countries, on the use of raw materials originating from the EU and for arguments of giving priority to raw materials from the EU. The hypothesis for changing the suppliers based on processing with SPSS software is therefore partially confirmed.

Processing of survey responses and the mean values regarding the control of suppliers showed that Slovenian producers, after joining the EU, control their suppliers better than before entering the EU. The control of the suppliers before the first purchase has improved, as well as periodical control. The control of the suppliers on the content of contaminants has also improved. The credit for this shift may be attributed to effective performance of HACCP system. On the basis of responses it may be concluded that the majority of Slovenian producers all of their suppliers, regardless of origin, verifies under the same conditions. But the finding can not be ignored, that after joining the EU, the number of replies to the statement, that EU suppliers are evaluated under "looser" criteria than suppliers from import, because of European goods, has increased.

After joining the EU, the use of raw material of Slovenian origin slightly decreased and the use of raw materials from other EU countries increased.

On the basis of the results of the nonparametric test for two related samples (**Chapter** "Testing of the hypothesis regarding the control of suppliers") it can be concluded that the differences between the mean values in the compared periods 1999-2004 and 2005-2010 regarding the control of all suppliers under the same conditions and the control of Slovenian suppliers under less strict conditions, are not statistically significant. However, the differences between the means of the compared periods 1999-2004 and 2005-2010 regarding the use of the HACCP system, control of suppliers before the first delivery, control of suppliers on the contaminants before the first delivery, periodical control of suppliers, periodical control of suppliers on contaminants and control of EU suppliers under less strict conditions, are statistically significant. The hypothesis concerning the control of suppliers can therefore be partially confirmed.

The analysis of the responses from the survey shows that mostly domestic suppliers and raw materials are trusted by the Slovenian manufacturers.

After joining the EU, the use of raw material of Slovenian origin slightly decreased and the use of raw materials from other EU countries increased. After joining the EU, the use of raw materials from import decreased. The analysis of the responses from the survey shows that mostly domestic suppliers and raw materials are trusted by the Slovenian manufacturers. It can be concluded that the imports decreased at the expense of a more simple procedure of the free movement of goods, which is less demanding than the procedure of import. Suppliers within the EU are also familiar with the requirements of the HACCP system and must transparently ensure the quality of their raw materials.

After Slovenia's accession to the EU, the control over suppliers from other EU countries has increased. This is very important for ensuring food safety and competitiveness in the EU market. After Slovenia's accession to the EU, the percentage of those Slovenian producers who control Slovenian supplier under looser criteria has slightly increased, which indicates the confidence in Slovenian suppliers. It is interesting, that the percentage of those Slovenian producers who control EU suppliers under looser criteria has significantly increased. This suggests that suppliers in the EU are trusted by Slovenian producers and that this trust is based on EU requirements for the implementation of HACCP system. This confidence could be turned to great disadvantage in case of unsuitable raw materials.

The survey results also show partial distrust of free movement of goods. Interestingly, the HACCP system is in force in all EU Member States, but producers on average partly agree that they equally trust the manufacturers in all EU States.

Very dispersed responses suggest that the opinions and practices are different, they probably vary even within a single reporting period. It is also likely that the price of raw materials has more influence. Recent research has indicated some interesting findings that indicate the existing deficiencies in the provision of safe food. In order to obtain more detailed information on the status of the Slovenian food industry interviews with the interviewer will continue to be carried out. The questionnaire will also be defined in detail within each period, because a change of practice may occur within the six year period. It will also be interesting to obtain data on those EU Member States which the Slovenian producers trust and have confidence in as well as their reasons why they do so. The detailed questionnaire including the six year period would elucidate to what extent Slovenian producers trust the EU Member States.

Interestingly, the HACCP system is in force in all EU Member States, but producers on average partly agree that they equally trust the manufacturers in all EU States.

REFERENCES

[1] Regulation (EC) No. 178/2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. L31-32002R0178/02: 1-24.

[2] Results of the survey, 2011.

[3] Raspor P. Priročnik za postavljanje in vodenje sistema HACCP. Slovenski institut za kakovost in meroslovje. Biotehniška fakulteta, Oddelek za živilstvo. Ljubljana, 2002: 598.

[4] Bastič M. Metode raziskovanja. Univerza v Mariboru. Ekonomsko poslovna fakulteta Maribor. 2006: 51.

[5] Koprivnik S., Kogovšek T., Gnidovec M. Analize podatkov z SPSS-om 12.0. Fakulteta za družbene vede Ljubljana. 2006: 142.

Professional paper

Threats to local drinking water in the municipality of Ljubljana

Polona **LESJAK**[1*], Barbara **ČENČUR CURK**[2]

ABSTRACT

This article presents the key findings of an analysis of the local water supply in the Municipality of Ljubljana and all changes which affect on local area water quality. The detailed review covers several local water supplies located in the western part of Ljubljana. Inventory of existing land use in the recharge area of individual water supply and a survey of individual reservoir managers were performed in the field. An analysis of the data collected from a laboratory analysis of water samples from local water supplies was performed.

Based on an analysis of the technical conditions of the reservoirs along with the proposed measures for improvement, the statistical processing of the results of the microbiological and physical-chemical analysis of the groundwater samples was carried out. Sampling was monitored from 2005 – 2010. The Water Framework Directive approach DPSIR (*Driving forces, Pressures, State, Responses*), was also used in the study which allows more detailed analysis of local captures and definition of threats. Results of DPSIR approach are not presented in this paper.

The purpose of the research work is to identify threats to existing local reservoirs in the Municipality of Ljubljana regarding the sanitary-technical conditions and the health aspects (water quality impacts on human health) and to define the hydro geological aspects (quantitative and qualitative status of the particular groundwater) and finally to outline measures to improve the situation. The research results show physical and chemical, microbiological and other environmental factors, which influence the quality of each individual reservoir. According to the research data the reservoirs Dolgo Brdo, Vnajnarje Korito and Besnica are the most problematic regarding drinking water quality.

Key words: public and private water supply, local water resource, water capture, drinking water quality

Received: 15. 2. 2012.
Accepted: 27. 8. 2012.

[1] University of Nova Gorica
Vipavska 13, Rožna Dolina
SI-5000 Nova Gorica, Slovenia

[2] Natural Science Engineering and Management
Department of Geology
Aškerčeva 12
SI-1000 Ljubljana, Slovenia

[*] *Corresponding author*
Polona Lesjak
Ljubljanska cesta 37a
SI-1240 Kamnik, Slovenia
Polonca.lesjak@yahoo.com

INTRODUCTION

Provision of safe drinking water is one of the global challenges of the 21st century [1]. Yet, for several decades, about a billion people in developing countries don't have a safe and sustainable water supply. It has been estimated that a minimum of 7.5 litres of water per person per day is required in the home for drinking, preparing food and personal hygiene, the most basic requirements for water; at last 50 litres per person per day is needed to ensure all personal hygiene, food hygiene, domestic cleaning, and laundry needs [2]. The large majority of people in European community have their water supplied by water utilities, some 10 % receive their water from small or very small supplies that are often owned by the consumers themselves. Protecting source water using good management strategies can help communities to reduce the threat of drinking water contamination [3]. Source water protection in a watershed context poses significant challenges for local communities, especially smaller ones [4]. Small and very small water systems are common in Europe. For example in Germany up to 20 % (about 16 million people) have drinking water distributed by small scale water utilities and private wells [5].

The large majority of people in European community have their water supplied by water utilities, some 10 % receive their water from small or very small supplies that are often owned by the consumers themselves.

The local source of drinking water and facilities that provide drinking water (source capture, pumping wells – hereinafter capture) in Ljubljana are secured by three water protection areas: (I) a stringent water protection area with the strictest protection regime; (II) a narrow water protection area with a strict protection regime; and (III) a general water protection area with a temperate protection regime [6, 7, 8].

In the Municipality of Ljubljana 40 local reservoirs are provided. Most local water supply systems are located in the eastern part of Ljubljana (Figure 1). Five are managed by Javno podjetje Vodovod – kanalizacija d.o.o., (hereinafter: J.P.VO-KA), 27 are operated by Municipality of Ljubljana, and eight reservoirs are abandoned or are already connected to the water supply of other communities.

Supply areas are continuously supplied with potable water from one water source, but may also be supplied from two or more; depending on the current water system pressure parameters.

The amount of water consumed per person in Ljubljana in the areas under consideration varies widely and ranges from 150 L to 250 L per person per day.

The amount of water consumed per person in Ljubljana in the areas under consideration varies widely and ranges from 150 L to 250 L per person per day [9]. Since drinking water is considered as a food and is one of the most investigated foodstuffs, the internal control and compliance of the quality of drinking water is conducted continuously parallel to an external control performed by the Ministry of Health. The internal control is carried out in compliance with the HACCP system [10, 11], which allows the risks to be assessed and the entire supply system to be controlled. From the perspective of local communities there is a need for the protection of local water sources, which are now neglected because of larger public water systems.

The working hypothesis stated that the local reservoirs in the eastern part of Ljubljana, which are managed by the Municipality of Ljubljana

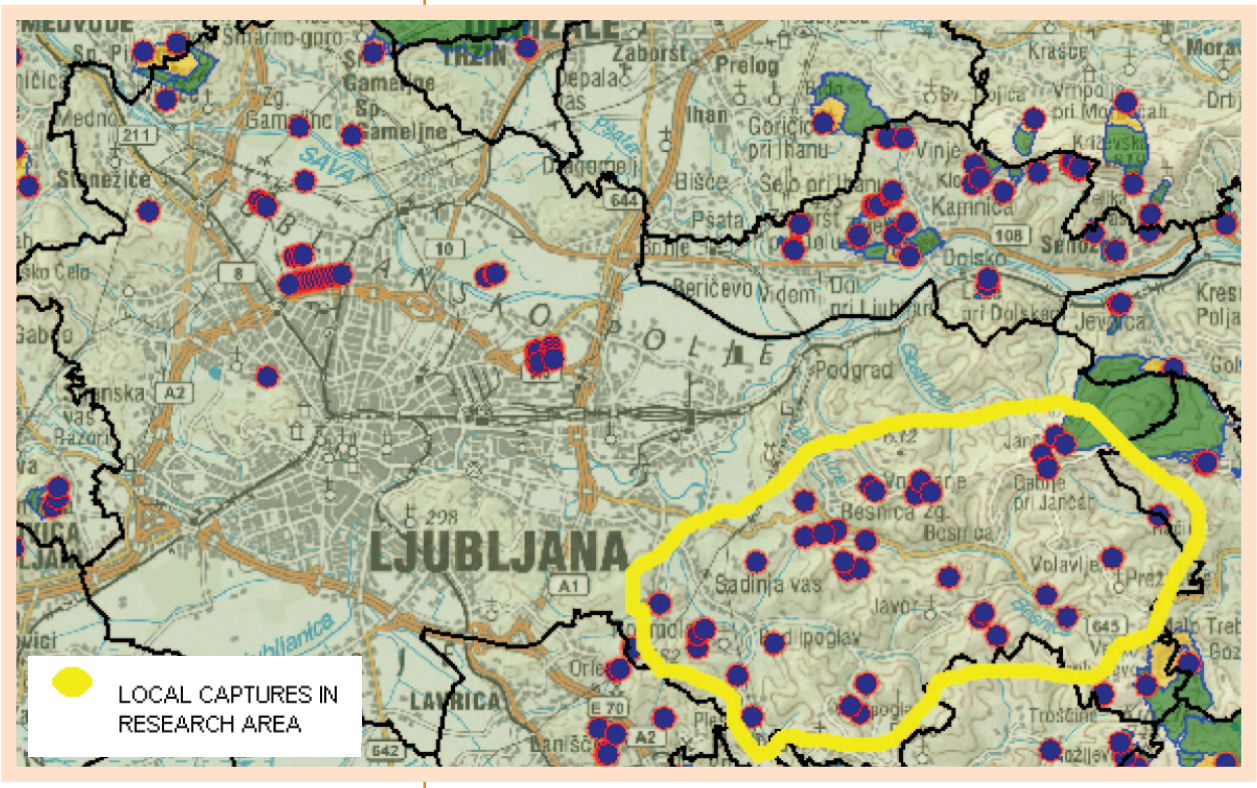


Figure 1.
Map of local water captures for the area of the Municipality of Ljubljana.
(Source: ARSO (http://gis.arso.gov.si/atlasokolja/profile.aspx?id=Atlas_Okolja_AXL@Arso)

(Public Health Institute), are more endangered in terms of achieving quality and volume in comparison with other reservoirs in Ljubljana, which are monitored by the public-sewerage company (J. P. VO-KA). These reservoirs are more endangered due to their access and location. However, the local legislation does not allow inspection control over these reservoirs.

METHODS

Internal control over compliance of drinking water in local water systems is carried out in accordance with the Rules on Drinking Water [12]. It has to follow established procedures based on the HACCP plan [10, 11] which contains the sampling sites, the type of trials and a minimum frequency of the sampling. In the period 2005-2010 there were in average 352 microbiological samples and 144.2 samples for chemical testing. Samples were collected and analysed by the laboratory of the Institute of Public Health.

Data from existing microbiological, physical and chemical analyses of water samples (which were performed by health institutes of Ljubljana, Maribor, Celje and J.P.VO-KA) were statistically analysed [13]. Based on the monitoring program of drinking water [14, 15, 16, 17, 18, 19] in the period 2005-2010 an average of samples were taken once a year for regular and occasionally testing.

Collected existing studies and the background of individual reservoirs were used in preparation of the analysis [20-50]. For each water capture, description of the location, hydro-geological characteristics of catchment area, aquifer type and other parameters were determined.

Data on existing land use were obtained from European CORINE land cover map[1]. Planned land use was gained from spatial master plan of Municipality of Ljubljana. The investigation defined the existing state of the captures including the recharge area from a sanitary – technical point of view. The existing land use and the list of potential pollutants in individual catchments were also established.

The investigation defined the existing state of the captures including the recharge area from a sanitary – technical point of view.

A field survey (opinion poll) included operators who are responsible for maintenance of each individual capture. With field survey several parameters were obtained, such as contaminants across the water protection zones, state reservoirs (age, maintenance, supplies, construction), risk factors, cost control, analysis of land use, etc.

In collaboration with the administrators 35 captures were successfully examined, for 10 captures there were no useful information because they had been connected to the other municipalities. Five local water supplies are operated by the J.P VO-KA, 40 of them are operated by Municipality of Ljubljana [10]. 90 % of captures were successfully examined, 10 % of the reservoirs were not covered in the survey because responsible person refused to participate in the analysis.

The data processing and evaluation were conducted with the help of the inventory data regarding the technical conditions of the captures and proposed measures in order to improve the situation of the local water supplies.

The statistical analysis of the results of the microbiological and physic-chemical analysis of the groundwater samples are shown in the results chapter. Minimum, maximum and average values and trends were determined for each parameter.

All the samples that exceeded the values were discussed. A calculation of all minimal, maximal and average values and expected trends have been made for each local water supply. The numeric data of the most exposed reservoirs has been presented also in the form of graphs, from which the data is visible through the trend curves. The greatest values in the form of graphs (sum, average) have been presented in the Excel Pivot Tables.

The survey covered 81.8 % of all reservoirs, which are managed by the Municipality of Ljubljana. The reservoirs, which are managed by the public sewerage company J.P. VO-KA have not been problematic in terms of water quality [51].

RESULTS

The study of drinking water sample analysis has shown that for some parameters threshold values are often exceeded. These parameters are faecal bacteria's (Escherichia coli, Enterococci), Clostridium perfringens

[1] CORINE (land cover CLC2000) is an important dataset for the implementation of key priority areas of the EU's 6th environment action programme. CLC2000 can show, for instance, where fragmentation of the landscape by roads and other infrastructure is worsening and thus increasing the risk that ecosystems can no longer connect with each other, putting the survival of their flora and fauna in danger.

(including spores), Coliform bacteria counts at 22°C, the number of colonies at 37°C and Manganese (Mn), Iron (Fe) and Desethylatrazine. The focus of this paper is based solely on the analysis and results of these parameters. One factor endangering water supply is heavy rain, which represents (during the flooding in September 2010) another reason for increased pollution.

Physical parameters of drinking water

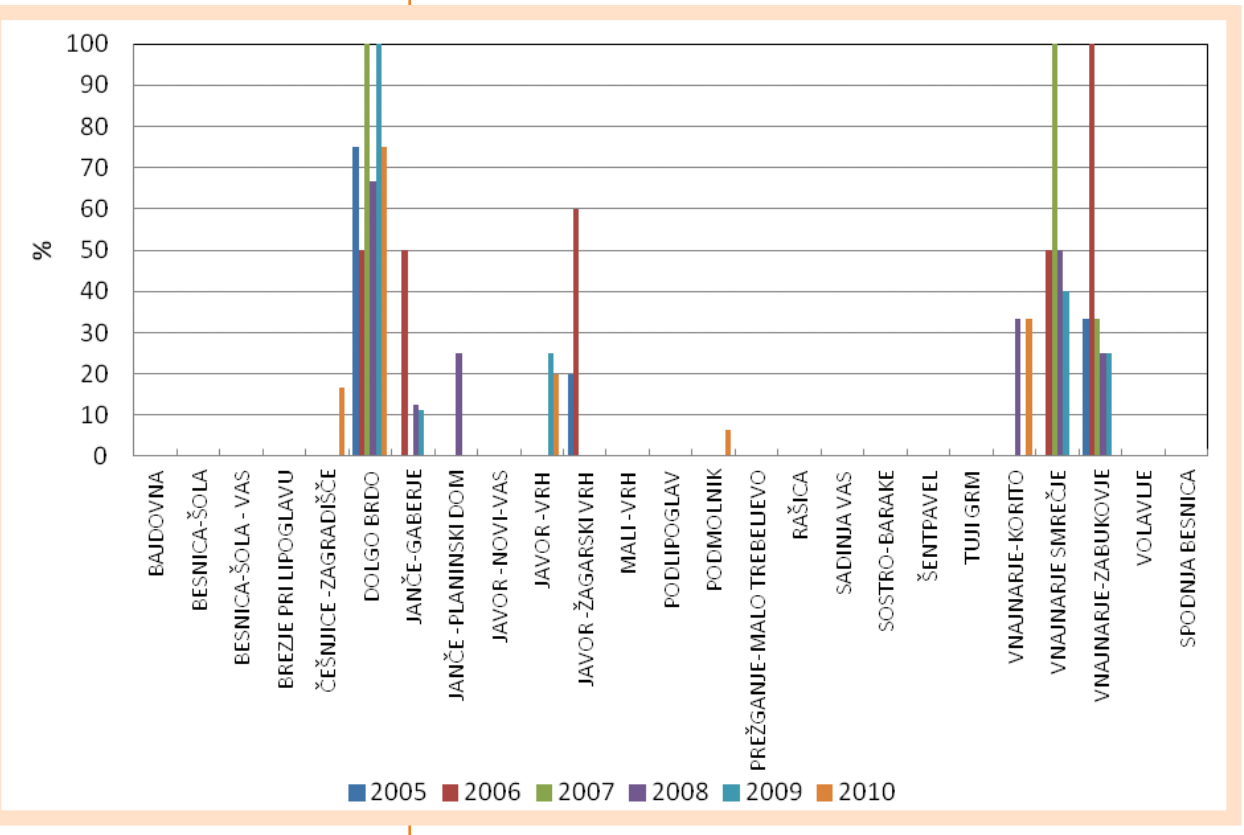
Basic regular physical analyses of drinking water include measurements of following parameters: colour, visible dirt, odour, turbidity, pH and specific electric conductivity.

On the basis of the Rules of the drinking water [12] an annual monitoring program for drinking water has been prepared [14-19], which includes six times a year sampling frequency for regular physical testing, and once a year for occasional one.

The maximum concentration of hydrogen ions (pH below) is in the range 6.5 to 9.5. Analysis of measurements for the period 2005-2010 showed that the pH of excess threshold values samples (75 %) ranging from 5.6 to 6.4, while the remaining 25 % of excess threshold values samples in the alkaline pH of 9.8 to 10.4.

Figure 2 shows the highest proportion of samples (%) with exceeded threshold values for pH for individual local captures (in the period 2005- 2010). There are three prominent captures: Dolgo Brdo, Vnajnarje Smrečje and Vnajnarje Zabukovje. The highest portion samples with poor quality were found in Dolgo Brdo (2007 and 2009). 2007 in Vnajnarje Smrečje and 2006 in Vnajnarje Zabukovje. The portion of in-

Figure 2.
Portion of samples (%) with exceeded threshold values for pH for individual local captures (in the period 2005–2010).



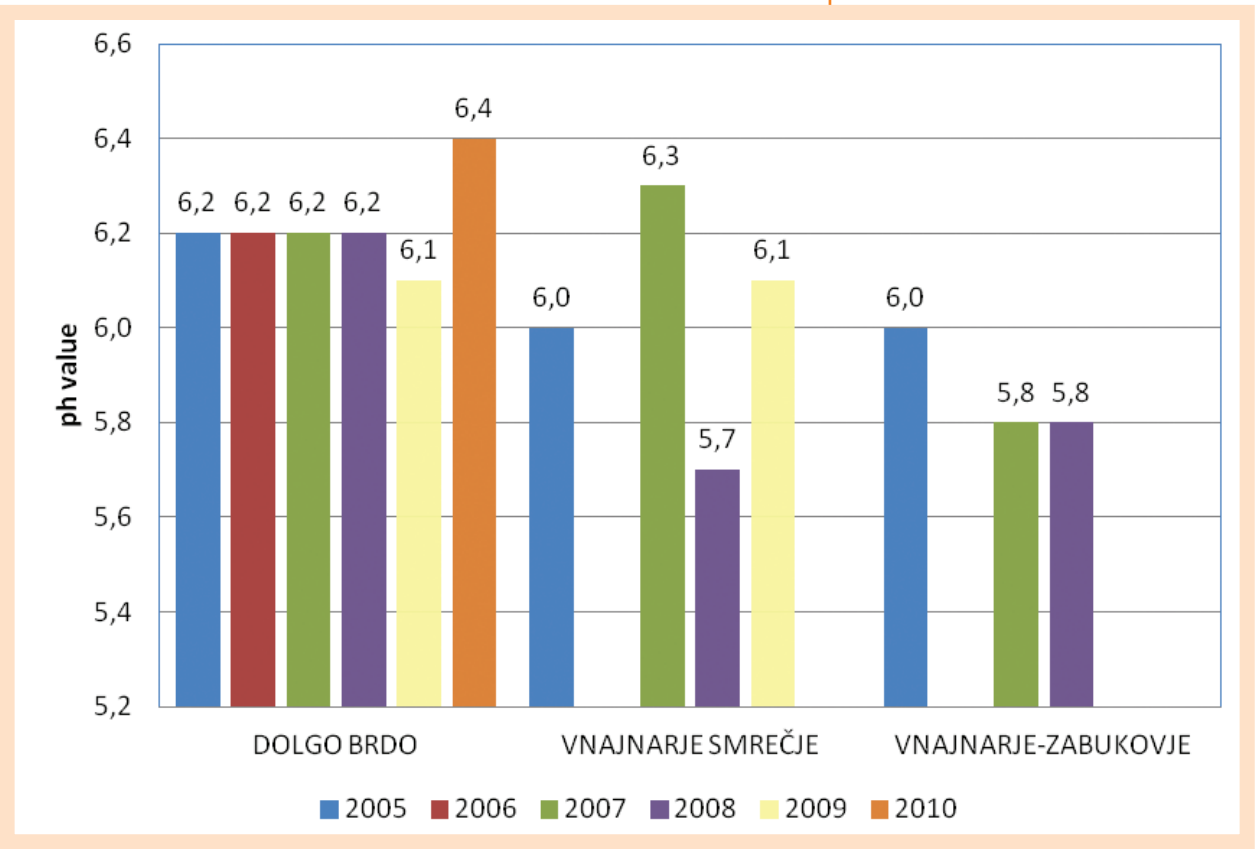


Figure 3.
pH values for individual local water captures (based on all samples with exceeded value included in the period 2005–2010).

ferior quality samples was taken from the entire analysis in which an individual water supply is covered by a water supply capture area and the corresponding well.

The lower pH in the capture is due to pollution and the geological composition of the recharge area. Acidity pH soil affect, soil fertility and susceptibility to pollution and the various uses. Poor content of plant nutrients often coincides with increased acidity of the soil. Soil can be acidic due to non-hydrocarbon composition or due to leaching of nutrients.

Figure 3 shows the yearly average pH of water samples with exceeding threshold values for five years (2005-2010). The highest portion is in the following captures: Dolgo Brdo, Janče Gaberje, Javor Žagarski vrh Vnajnarje, and Vnajnarje – Zabukovje. 100 % share of poor quality samples (of pH) were found in the following local water supplies: Vnajnarje – Smrečje in 2007, Vnajnarje – Zabukovje in 2005, and Dolgo Brdo in 2010. More than 50 % proportion of inferior quality samples were found in the following water supplies: Dolgo Brdo in 2005, 2006, 2007, 2008 and 2010. In 2009 there were no samples outside the limits. Vnajnarje – Smrečje in 2005, 2007, 2008, 2009. In 2006 and 2010 there were no samples outside the limits.

Results of the microbiological analyses

Microbiological parameters include the origin of faecal bacteria: Escherichia coli, Enterococci, Clostridium perfringens (including spores) and indicator bacteria (Coliform bacteria counts at 22 °C, the number of

Colonies at 37 °C). The maximum value for E. coli, Enterococci, Clostridium perfingens coliform is 0 (unit /100 mL). The limits of colonies at 22 °C and number of colonies at 37 °C are less than 100 pro mL.

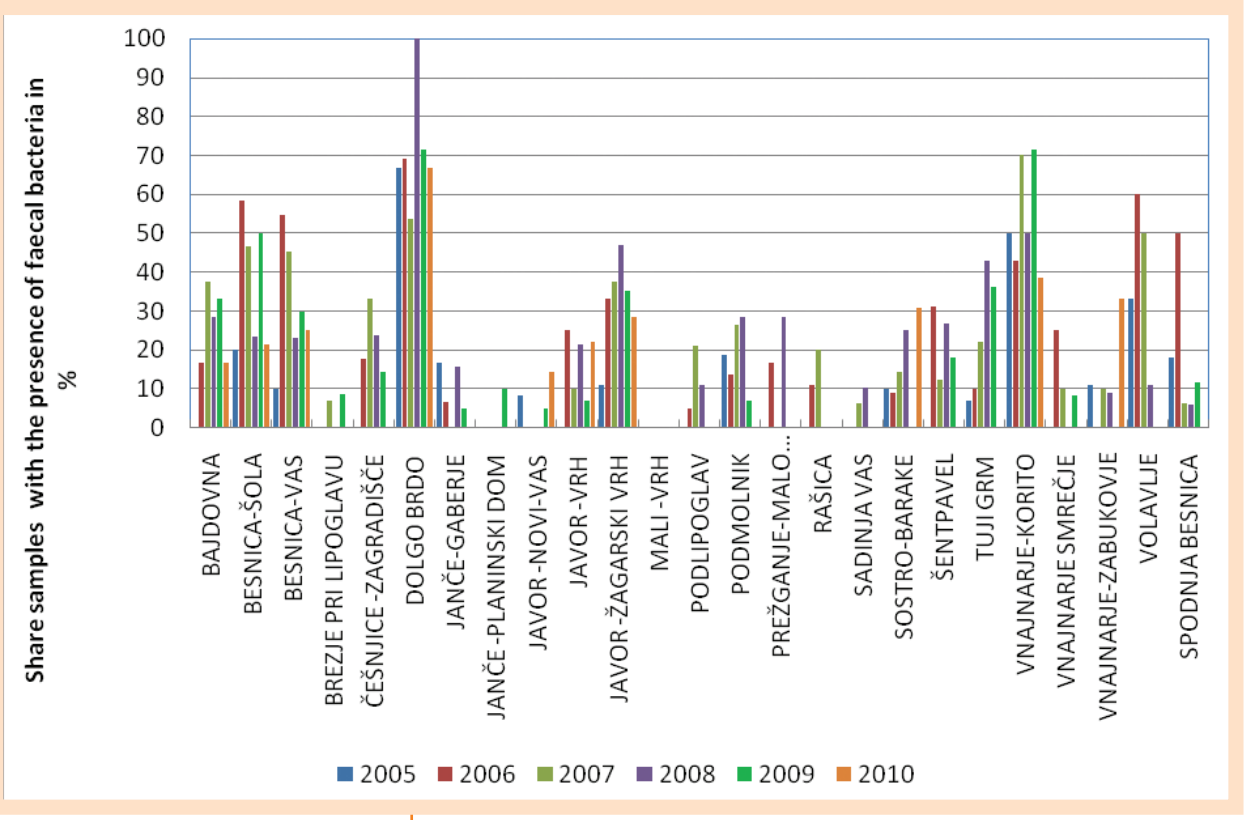
On the basis of internal control system HACCP [12, 13] in the period of 5 years (2005-2010) on average 352 samples per year were taken for microbiological testing by the laboratory of the Institute of Public Health of Ljubljana (ZZV Ljubljana). Annual reports contains only the total number of samples with exceeding parameters and the total number of the faecal bacteria origin.

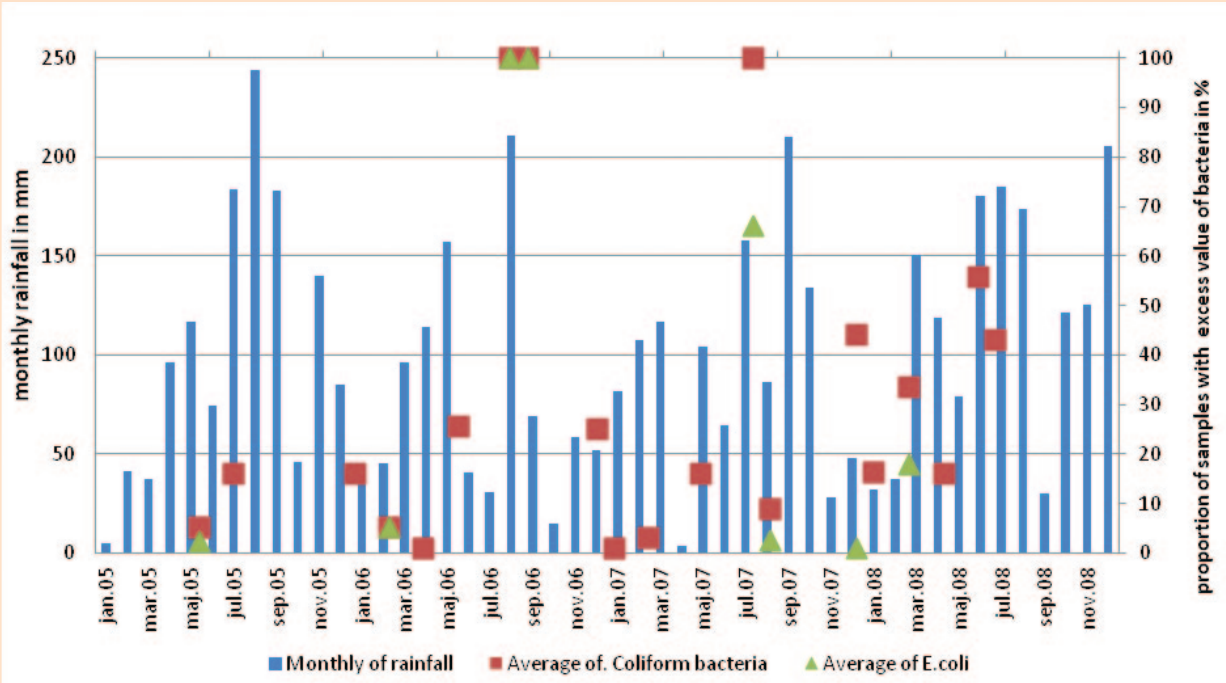
Figure 4 shows the portion (in %) of water samples with the presence of faecal bacteria for individual local water supplies in the period 2005-2010. Water supply Volavlje, Dolgo Brdo and Vnajnarje Korito have the worst water quality regarding presence of bacteria. In 2008 in Dolgo brdo all samples were containing faecal bacteria. The latter were present in 60 % of samples in 2006 in Volavlje and in 70 % or more samples in 2007 and 2009 in Vnajnarje Korito.

The obtained results show the relationship between content of bacteria and amount of rainfall for the most critical captures Besnica vas, Dolgo Brdo and Vnajnarje Korito. In the **Figure 5** presented data were taken and calculated from monthly and annual reports of the Institute of Public health. The data indicate the period in which the samples contained minimal faecal bacteria. The Figure 6 contains monthly data on precipitation over the 10 year period covered in the reports of rainfall compiled in the nearest station Dobrunje [52].

Highest concentration of water samples with presence of faecal bacteria in the Besnica vas local captures were in August – September 2006

Figure 4.
The portion (in %) of water samples with the presence of faecal bacteria for local water supplies in the period 2005–2010.



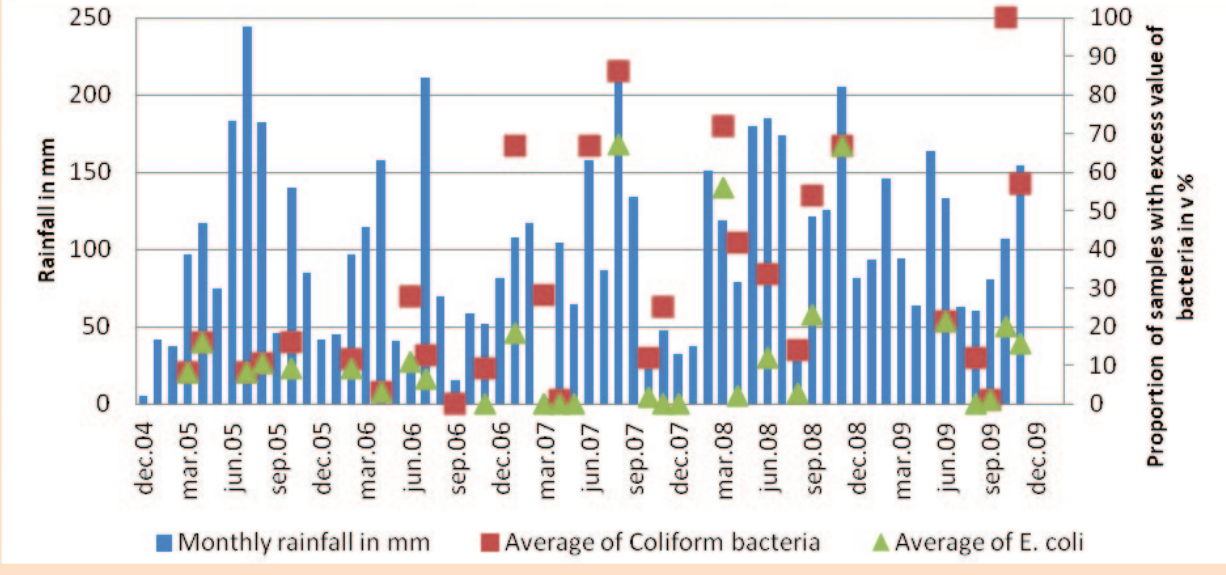


(100 %) and October 2007 (66 % and 100 %). Increase of faecal bacteria (in summer and autumn) is reflected in the period of drought or in the period of increased rainfall in autumn. The climate factor is the main reason for the increased level of pollution of the Besnica vas reservoir.

Figure 6 shows the highest percentage of samples with faecal bacteria in Dolgo brdo (80 % and more) during the period from January 2007 to December 2009 (Coliform bacteria), and for E. coli in the period from July 2007 to November 2009. Given the rainfall patterns for the period July to October 2007, rainfall can be a reason for the microbiological pollution. In 2010 there was no excess value of bacteria.

Figure 5.
Share of water samples (in %) with the presence of faecal bacteria and monthly amount of rainfall for the catchment of the Besnica vas capture.

Figure 6.
Share of samples with excess value of bacteria in % and monthly amount of rainfall for the capture of the Dolgo Brdo.



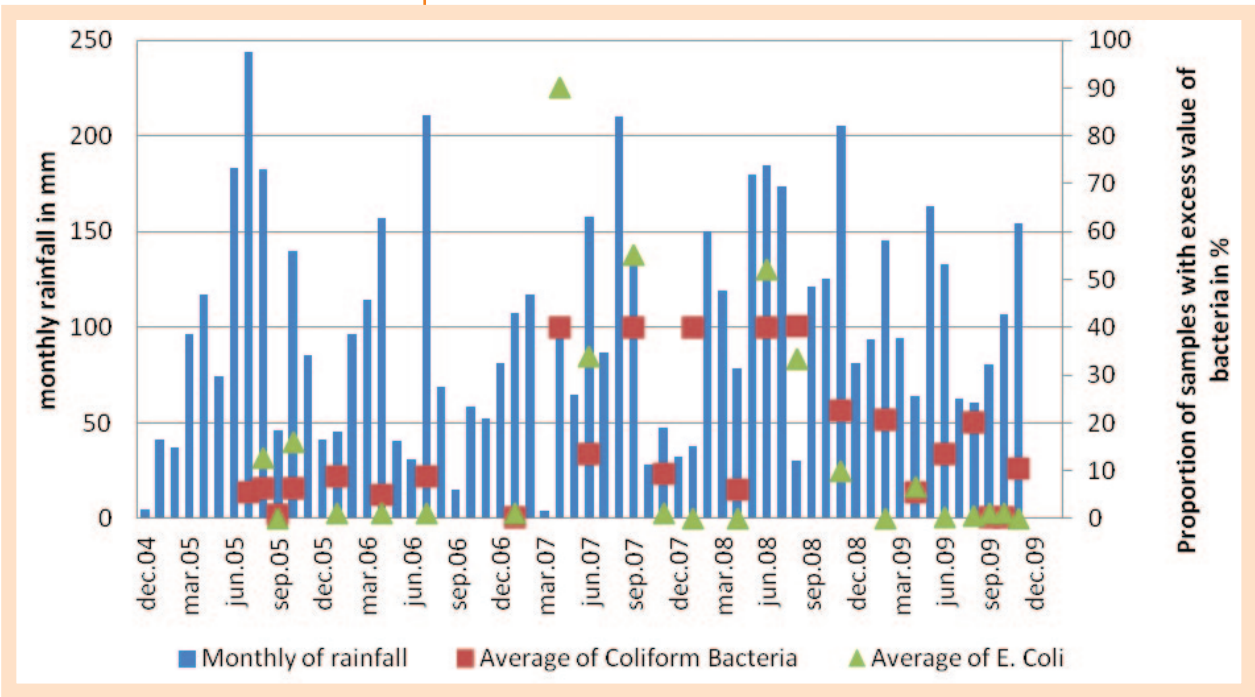


Figure 7.
Share of samples with excess value of bacteria in % and monthly amount of rainfall for the capture of the Vnajnarje Korito.

Figure 7 shows average bacteria content (E. coli and Coliform bacteria) in the capture of Vnajnarje Korito in comparison with monthly rainfall amount. The largest increase in Coliform bacteria for the Vnajnarje Korito is evident for the periods May – October 2007 as well as in February, June and September 2008. Increase in E. coli in this waterworks can be seen also in May 2007. The biggest amount of rainfall is observed in July 2005, August 2006 and December 2008. It is not possible to find the correlation between the ccurrence of the number of bacteria and the increased rainfall. On the basis of the examination of the terrain, the survey and overview of the reports it is established that there are other prevailing factors present (poor construction of the reservoir, fertilizing meadows).

Results of the chemical parameters analysis

Basic regular chemical analyses of drinking water include following parameters: TOC, ammonium, nitrite and occasional investigations nitrate. Detailed chemical analyses include the general physical parameters and the chemical parameters (smell, taste, colour, conductivity, pH, nitrate, etc...), metals and non-metals (aluminium, boron, quicksilver, etc.), pesticides and metabolites (atrazine, desethylatrazine, etc.), polycyclic aromatic hydrocarbons (benzo (b) fluoranthene, etc.), trihalomethanes (trichloromethane, etc...), volatile halogenated aliphatic hydrocarbons (1,1,2-trichloroethene, etc.) and volatile aromatic hydrocarbons (benzene, etc.).

Statutory limit values of the parameters are: Manganese 50 μg/L, Iron 200 μg/L, Desethyl atrazine 0,10 μg/L. Other chemical parameters that are identified in drinking water are metals and non–metals (aluminium, boron, quicksilver, etc.), pesticides and metabolites (atrazine, desethylatrazine, etc.), polycyclic aromatic hydrocarbons (benzo (b) fluoranthene, etc.), trihalomethanes (trichloromethane, etc.), volatile halogenated

aliphatic hydrocarbons (1,1,2-trichloroethene, etc.) and volatile aromatic hydrocarbons (benzene, etc.).

Other parameters are recorded in the period of 5 years. Only the exceeded values of the following parameters are shown. The taste was unacceptable in Janče Gabrje, Janče planinski dom and Dolgo brdo in year 2008. Exceeded threshold values for Desethylatrazine; in Podmolnik (0,12 μg/L in 2005). Exceeded threshold values for Manganese; Vrtina pri Jančah (500 μg/L – 1.100 μg/L) and Janče planinski dom (375 μg/L – 1.525 μg/L).

For the period of five years only those parameters and samples were studied, which have exceeded values, while all the rest of data is not included in the analysis.

Survey of the local captures

Local water systems are mostly located in the Sostro district in the eastern part of Ljubljana. In collaboration with the administrators 35 captures were successfully examined as interview survey; for 10 reservoirs there were no useful information, because they had been connected to the other municipalities or are present in databases. Five local water supplies are operated by the J.P VO-KA, 40 of them are operated by Municipality of Ljubljana. 90 % of captures were successfully examined, 10 % of the reservoirs were not covered in the survey because responsible persons for particular capture refused to participate in the survey.

After analysis of reports, which are made on the basis of archival material [13, 51] for the 2005–2010 period, it is evident that most reservoirs continually have not meet the minimum sanitary and technical conditions required by Rules of Drinking Water [12]. Some water sources have already been replaced by the new wells [20-50]. Public services of supplying drinking water in all settled areas of the Municipality of Ljubljana performed by J.P VO-KA (except in areas above an altitude of 1,500 m a.s.l., and settlement areas with fewer than 50 residents with an annual average water supply less than 10 m^3 per day). Therefore a review of individual local captures was performed in order to obtain data on the number of people receiving supply or water consumers, covering an inventory of facilities, the maintenance of these facilities, and description of the constructed distribution network including water connections. The survey took place over the period from spring to autumn 2010.

Questionnaire was prepared for the local managers of captures and the results of surveying reflect their statements. Data are displayed regarding estimated risk, which is in the range from one to five or ten, from lowest to the highest values.

Figure 8 shows threats to water sources from one to ten. It can be seen that 18 % of water supplies are identified as having the highest risk on the scale and only 2 % are the least threatened.

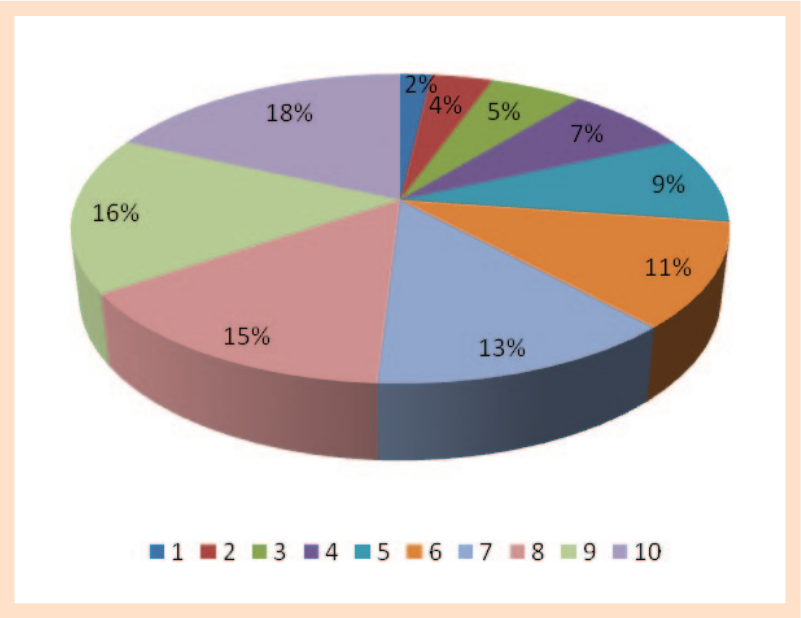


Figure 8.
Threats to water sources (**Legend**: 1... lowest risk, 10... highest risk).

Figure 9 shows that in the most captures (85 %) presence of illegal activities in recharge area affecting the environment were not detected. An 11 % share is represented by catchments areas, where in the close vicinity there is an illegal building (or a built residential facility located in the immediate vicinity of the inner water protection zone).

During the interview, municipal waste (plastic, furniture parts and pieces of iron) was observed in the area of three captures and municipal waste (plastic, furniture parts, pieces of iron, etc.)

Figure 10 shows that 77 % of the surveyed capture managers said that also municipal waste (e.g. household appliances tyres, furniture, and plastic waste) were observed near their reservoirs.

The next question was about the efficiency and economics of internal control (in terms of maintenance, cleaning, etc.) by the local committees of responsible persons.

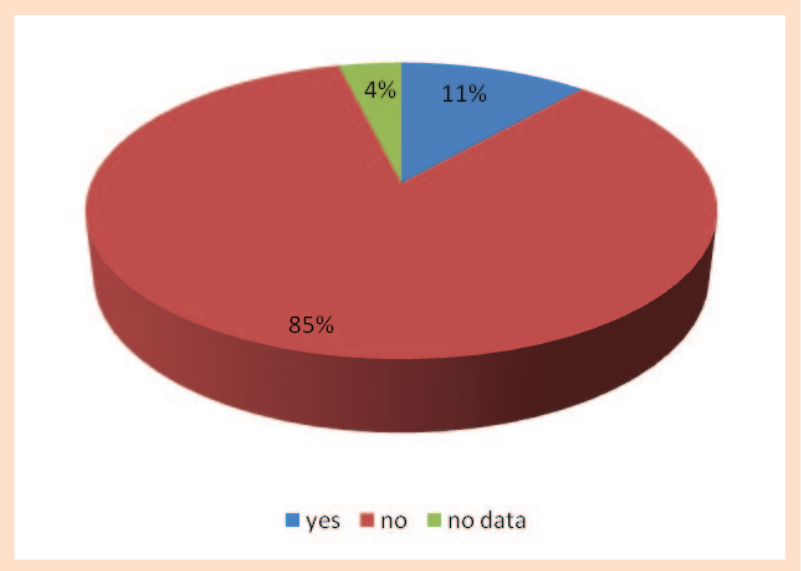


Figure 9.
Presence of illegal construction in the vicinity of the captures.

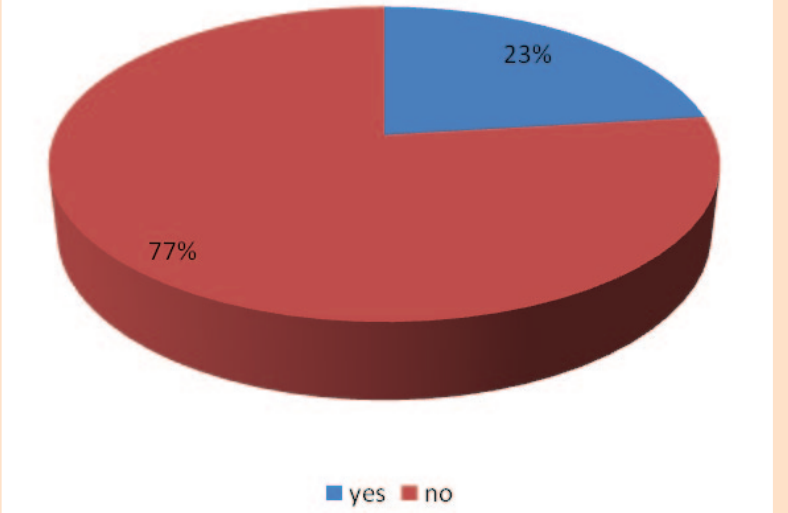


Figure 10.
The presence of municipal waste in the vicinity of capture.

Figure 11 shows how economically the local water reservoirs are controlled by trustees. 7 % of those in charge believe it is sensible and economical to continue to monitor and ensure the capture, while the higher proportion of administrators – trustees (33 %) say that the control is not effective due to obstruction of access, location, and weather conditions.

Figure 11 shows how reasonable is transition to the new capture or borehole. A larger proportion of administrators – trustees said that transition to the new capture or borehole is justified. From the situation in the field and the responses of members of local committees, 7 % believe that the connection was not meaningful. The reason for the transition is the current financial opportunity rather than capture problem.

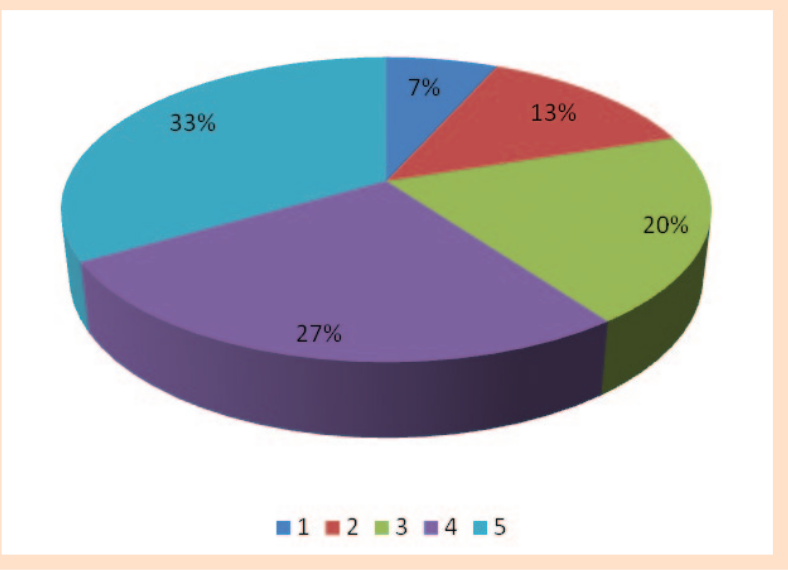


Figure 11.
Cost control of water reservoirs by the managers
(**Legend:** 1 is not economical 5 is high economical).

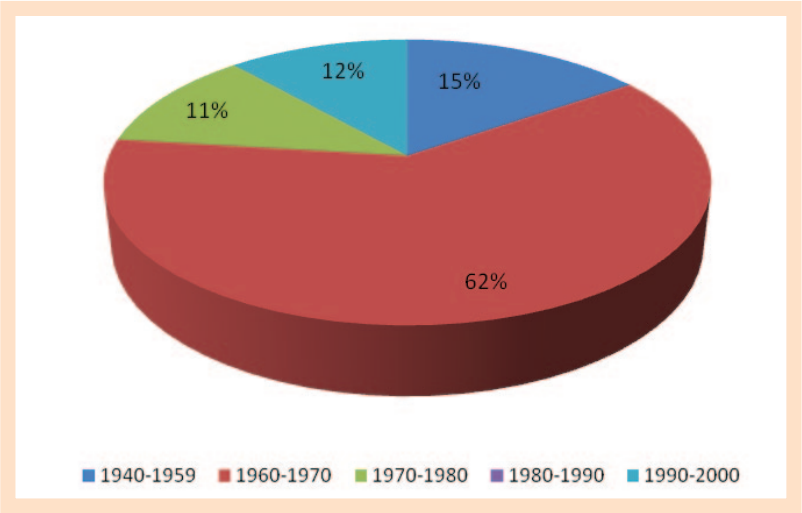


Figure 12.
Local capture of buildings divided by age.

Figure 12 shows that according to the managers' responses, 70 % of reservoirs were built between 1960 and 1970, while the smallest proportion (13 %) were built between 1990 and 2000.

The persons in charge of water supply committees were asked to answer when the capture was restored. **Figure 13** shows the statements of person in charge: half of the reservoirs were restored in 1990 and 2000, 22 % in 2007 and 18 % in 2005.

According to the trustees, the majority of captures are made of concrete (84 %), more than 15 % of the reservoirs are being constructed from other materials (rocks, plastic, bricks), **Figure 14.**

Figure 15 shows the need to look towards nature for a greater share of the risk factors of contamination. Thus, global changes, which are also reflected in the climate (droughts, floods, sudden temperature drops and increases inconsistent with the season) are the dominant causes of water pollution in the catchments area.

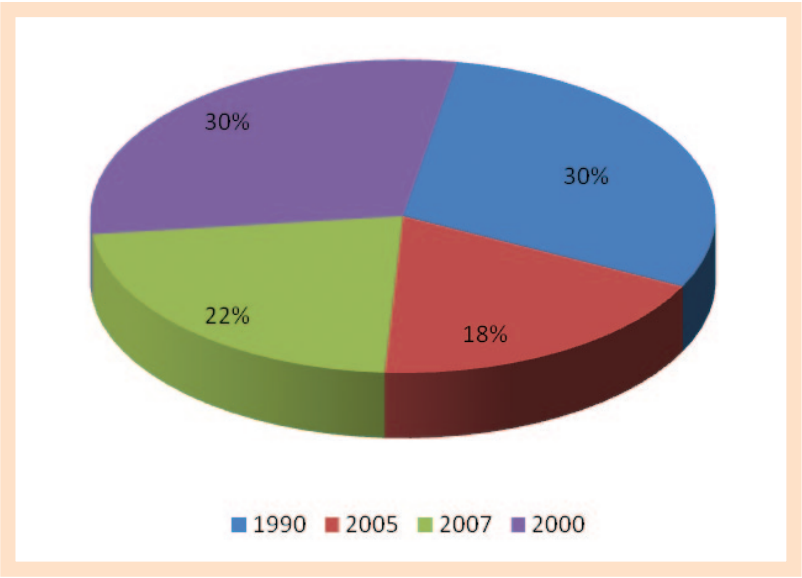


Figure 13.
Local captures by year renovated.

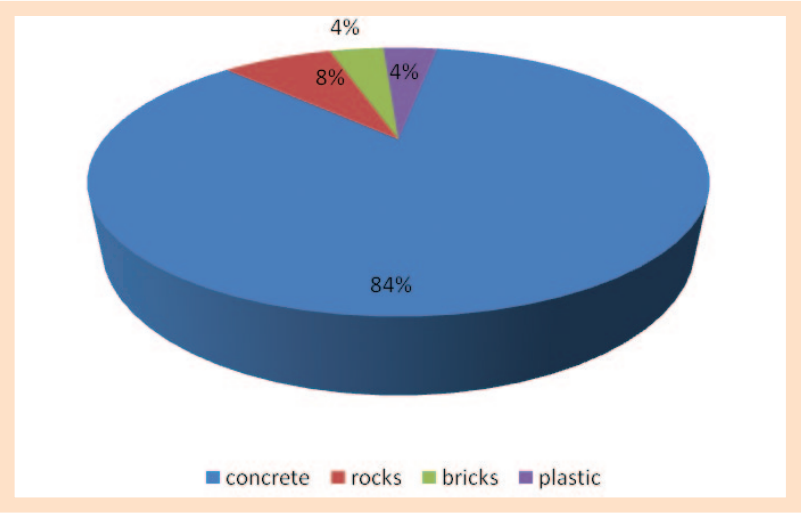


Figure 14.
Local capture by type of material.

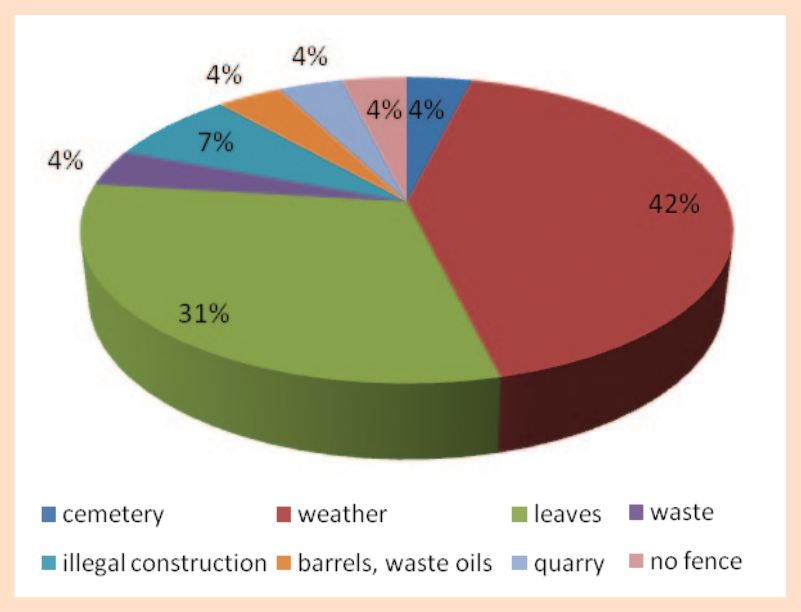


Figure 15.
Risk factors with regard to the subject of risk.

DISCUSSION

Based on the findings of the interview survey of local water supplies and their managers and drinking water quality monitoring, one can state that most of the local water supply is not healthy, mainly due to polluted groundwater in the catchments area.

Microbiological parameters

By considering the situation in the field and reports of past years, it can be found that water in most local water supplies (captures) many times exceed threshold for microbiological parameters. In 2005 there were 17 captures (or 62.9 % of all examined captures) which contained exceeded threshold. In 2006, there were 22 reservoirs (or 81.4 % of all examined reservoirs), in 2008 24 reservoirs or 88.8 % of all examined reservoirs, which contained exceeded threshold. In 2009 there were 17 reservoirs with exceeded thresholds or 62.9 % of all examined reservoirs.

The analysis undertaken can lead to the following hypothetical view that the local water supplies areas under Municipality of Ljubljana management are more vulnerable in terms of achieving qualitative and quantitative conditions such as bigger capture (water works) in the Municipality of Ljubljana and its surroundings. Local water supplies are more vulnerable, becouse of the lack of the certain water protection areas with legal restrictions on land use in these areas.

The catchments areas of local water supply are generally exposed to heavy pollution from agricultural land, inappropriate wastewater discharge from villages and hamlets, unregulated polluted storm water drainage from roads, illegal dumps and uncontrolled water discharges of hazardous substances into the soil and buildings which have appeared in the immediate vicinity of water protection zones.

Physical and chemical parameters

Local water supplies are more vulnerable, becouse of the lack of the certain water protection areas with legal restrictions on land use in these areas.

The physical and chemical parameters of drinking water sample tests also show that water is polluted in several captures.

In 2005, they found six samples with pH exceedances of the limit (of the 27 examined reservoirs). In 2006 they found 4 samples and seven samples they found in 2007, in 2008 they found 10 samples, and 5 samples they found in 2009. The taste was unacceptable in four cases in year 2008. Manganese was exceeded once in 2006 five times in year 2007 and six times in 2008. Desetylatrazine was exceeded once in 2005. On the given results of physico-chemical examinations of drinking water can be concluded that the drinking water in these 5 years in general is less polluted contrary to microbiological examinations.

For some areas, where water is captured near the surface, after abundant rainfall the water is particularly heavily physical and chemical contaminated, creating a possibility of waterborne diseases and epidemics.

For some areas, where water is captured near the surface, after abundant rainfall the water is particularly heavily physical and chemical contaminated, creating a possibility of waterborne diseases and epidemics.

The managers of local water supplies acting in water committees in local communities or individuals lack the necessary professional qualifications and perform this function on a voluntary basis.

In recent years the Municipality of Ljubljana has been systematically resolving the complex water supply problems of local water utilities through drilling new wells.

In a regular plan especially restoration and renovation works are carried out on local water distribution systems, which do not have safe drinking water. With renovation works physical and chemical water pollution in reservoirs is prevented in the long term. In some places, the quantity of water has increased.

In 2002 the municipality began to regulate the supply and renovation of local reservoirs in order to finally resolve the problem of drinking water supply.

In the area of the aquifer have been (based on hydrogeological studies) specific micro-location of deep boreholes. They can cover a sufficient quantity of quality drinking water for more local water supply systems where the water shortage or drought is of poor quality.

In the eastern part of the Municipality of Ljubljana, deep wells have been drilled at these locations, providing max capacity of 25 L/s of safe drinking water. The water from these wells will be, according to the building development programme for the restoration of local water utilities, in the near future replaced by water which is inappropriate from a sanitary-hygienic viewpoint.

Intensive agriculture, dysfunctional septic tanks and cesspits, unclean craft activities and ecological disasters (the floods in 2011) in the protection zones of water resources have caused chemical or microbial risks which, even with treatment and rehabilitation processes, cannot be removed or reduced to an acceptable level.

Field survey

Based on the surveys and interviews with the managers of some reservoirs the transition to new wells has not been justified since, by adhering to the rules of preventive environmental protection (uncontrolled pouring slurry zones, unprotected water source, water protection of local, illegal building activity in the immediate vicinity of the local capture), people can on the long term protect the catchments area.

In the eastern part of the Municipality of Ljubljana, deep wells have been drilled at these locations, providing max capacity of 25 L/s of safe drinking water.

In spring and autumn of 2010 terrain examinations of actual reservoirs in presence of the representative of Public Health Institute and interviews with reservoir caretakers were carried out.

In collaboration with the caretakers of the water distribution systems 90 % of the reservoirs have been examined, while 10 % were left unexamined due to non collaboration of caretakers. On the basis of terrain examinations, interviews and examination of reports for the period 2005–2010 it has been established that reservoirs do not meet the minimal sanitary and technical standards required by legislation. Besides obtaining data on the number of people supplied with water, the examination of each local reservoir was included the inventory of the state of facilities, the maintenance of these facilities and the description of the reservoir.

According to the state of terrain and documentation examination some old reservoirs are better replaced by new borehole [20 - 50] which is adequate to health and reliable in terms of quantity.

According to the state of terrain and documentation examination some old reservoirs are better replaced by new borehole which is adequate to health and reliable in terms of quantity.

Further investigations needed to improve the situation

In cases of such threatened water resources, abandoning of supply and connecting to another, more appropriate water source has been proposed. To establish alternative water sources hydro-geological and sanitary-hygiene research within a broad area of research has also drilled deep wells containing significant amounts of quality drinking water. In the near future this water supply will replace what is now supplied by reservoirs threatened by inappropriate water quality or a shortfall in times of drought.

If some locations were not dominated with capital interests, public awareness-raising activities could be carefully planned to ensure the hy-

If some locations were not dominated with capital interests, public awareness-raising activities could be carefully planned to ensure the hydro geological characteristics of individual areas can be easily maintained to sustain individual catchments for several years.

dro geological characteristics of individual areas can be easily maintained to sustain individual catchments for several years.

Of course, often some drinking water from water catchments does not meet the criteria specified in the Rules of Drinking Water [12] and it is therefore necessary to reasonably provide water resources for devices for drinking water by replacing existing water sources with new, wholesome and reliable quantities of water sources.

CONCLUSIONS

This analysis has provided clear evidence for the main factors contributing to the contamination of private water supplies.

The climate (droughts, floods, sudden temperature drop and increases which are not consistent with the seasons) are the dominant causes of water pollution in the capture area.

Protecting source water using good management strategies can help communities to reduce the threat of drinking water contamination

The transfer of powers from the local community (the control and sanctioning) will be represent additional protection of local coverage.

Small water supplies are often vulnerable to contamination. In many rural contexts, there is often a lack of integrated approaches regarding water source protection; sanitary protection of drinking- water sources is frequently inadequate.

Small water supplies are often vulnerable to contamination. In many rural contexts, there is often a lack of integrated approaches regarding water source protection; sanitary protection of drinking- water sources is frequently inadequate.

Small water supplies have relatively greater capital costs for technical installations, and per unit costs of materials and construction are also generally bigger.

There is often a lack of financial mechanisms to cover the local cost for monitoring, maintenance and operation.

Due to the larger geographical spread covered by small-scale water supplies and sometimes their remoteness and isolation, operators do not have easy access to information, expert assistance and technical support; there is also a low level of networking in scientific and professional communities.

REFERENCES

[1] Ivey J. L., De Loë, Kreutzwiser, Ferreyra. C. An institutional perspective on local capacity for source water protection. Guelph Water Management Group, Department of Geography, University of Guelph, Guelph, Ont. Canada NIG2WI. March 2006.

[2] Hunter Paul R., MacDonald Alan M., Carter C. Richard. Water Supply and Health. Plos Medicine. November 2010.

[3] Richardson Hopi Yip, Nichols Gordon, Lane Chris, Lake Lain R., Hunter Paul R. Microbiological surveillance of private water supplies in England - The impact of environmental and climate factors on water quality. School of medicine. May 2009.

[4] Timmer D.K., De Loe R.C., Kreutzwiser. Source water protection in the Annapolis Valley, Nova Scotia: Lessons for building local capacity. Land Use Policy, Canada. May 2005.

[5] Exner M., Hartemann, Hunter R Paul, Levi Yves, Loret Jean-Francis, Mathys Werner, Villesot Daniel, Wilhelm Michael. Consensus report: E. Coli 0104:H4 (HUSECO4) and the potential threat to European water supplies. International Journal of Hygiene and Environmental Health. August 2011.

[6] Odlok o varstvu virov pitne vode (Uradni list RS št. 71/93).

[7] Odlok o varstvu lokalnih virov pitne vode (Uradni list RS št. 78/2000).

[8] Uredba o vodovarstvenem območju za vodno telo vodonosnikov Ljubljanskega barja in okolice Ljubljana (Uradni list RS št. 115/2007).

[9] RS Ministrstvo za okolje in prostor 2011. Operativni program oskrbe z pitno vodo 2011.

[10] Potočnik V., Vudrag M. HACCP sistem: namen in uporaba. Zavod za zdravstveno varstvo. 2001.

[11] Zavod za zdravstveno varstvo Ljubljana. Notranji nadzor na osnovah HACCP. 2010.

[12] Pravilnik o pitni vodi (Uradni list RS št. 92/2004, 35/2004, 26/2006, 92/2006, 25/2009).

[13] Arhivski podatki ZZV LJ o sistematičnem spremljanju zdravstvene ustreznosti pitne vode na lokalnih vodovodih Mestne občine Ljubljana za obdobje zadnjih deset let (letna in druga poročila o zdravstveni ustreznosti pitne vode in varnosti oskrbe, izvidi laboratorijskih preiskav vzorcev pitne vode).

[14] Ministrstvo za zdravje RS. Program monitoringa pitne vode. Ljubljana 2005.

[15] Ministrstvo za zdravje RS. Program monitoringa pitne vode. Ljubljana. 2006.

[16] Ministrstvo za zdravje RS. Program monitoringa pitne vode. Ljubljana. 2007.

[17] Ministrstvo za zdravje RS. Program monitoringa pitne vode. Ljubljana. 2008.

[18] Ministrstvo za zdravje RS. Program monitoringa pitne vode. Ljubljana. 2009.

[19] Ministrstvo za zdravje RS. Program monitoringa pitne vode. Ljubljana. 2010.

[20] Mencej Z., J. Rogelj, GZL. Predlog varovanja pitne vode v vrtini J-3/89 pri Javorju. 1989.

[21] Mencej Z. Pomembnejši vodonosniki v karbonatnih kamninah na vzhodnem območju Mestne občine Ljubljana, II. faza. Hidroconsulting, d.o.o. 1997.

[22] Hidroconsulting d.o.o. Varovanje pitne vode zajetja “Pečovje” pri Prežganju, predlog sanacijskih ukrepov v varstvenih pasovih. April 1992.

[23] Mencej Z. Poročilo o rezultatih kaptažne vrtine PM-1/98 v Podmolniku in Strokovne podlage za zavarovanje zajetja pitne vode z vrtino PM 1/98 v Podmolniku. Hidroconsulting. 1998.

[24] Kmetec M. Poročilo o rezultatih kaptažne vrtine ŠP-1/98 v Šentpavlu in strokovne podlage za zavarovanje zajetja pitne vode z vrtino ŠP-1/98 v Šentpavlu, Hidroconsulting, d.o.o. 1998.

[25] Mencej Z., Hidroconsulting, d.o.o. Zajetja pitne vode in perspektivni vodni viri v občini Moste-Polje. 1993.

[26] Mencej Z., Hidroconsulting, d.o.o. Rezultati raziskav za zajem dodatnih količin pitne vode za vodovod Lipoglav in varovanje zajetja pitne vode z vrtino L-1/94. 1994.

[27] Mencej Z. Zajetja pitne vode in perspektivni vodni viri v občini Moste-Polje (Z. Mencej, Hidroconsulting, d.o.o. 1993.

[28] Mencej Z., Hidroconsulting, d.o.o. Pomembnejši vodonosniki v karbonatnih kamninah na vzhodnem območju Mestne občine Ljubljana, II. faza. 1997.

[29] Kmetec M., Hidroconsulting, d.o.o. Poročilo o rezultatih kaptažne vrtine ŠP-1/98 v Šentpavlu in strokovne podlage za zavarovanje zajetja pitne vode z vrtino ŠP-1/98 v Šentpavlu. 1998.

[30] Mencej Z., Hidroconsulting. Poročilo o rezultatih kaptažne vrtine PM-1/98 v Podmolniku in strokovne podlage za zavarovanje zajetja pitne vode z vrtino PM-1/98 v Podmolniku. 1998.

[31] Mencej Z. Zajetje pitne vode z vrtino J-1/02 pri Jančah, Hidroconsulting, d.o.o. 2002.

[32] Mencej Z. Poročilo o rezultatih kaptažne vrtine J- 4/02 za vodovod Javor, 2002, Poročilo o rezultatih kaptažne vrtine J-4/02 za vodovod Javor. Avgust 2002.

[33] Hidroconsulting d.o.o. Poročilo o rezultatih kaptažne vrtine J-1/02 pri Jančah in strokovne podlage za zavarovanje vodnega vira zajetje z vrtino J-1/02. 2002.

[34] Mencej Z., Hidroconsulting, d.o.o. Zajetje pitne vode z vrtino J-1/02 pri Jančah Možnosti oskrbe krajev in naselij v Krajevni skupnosti Besnica s kvalitetno pitno vodo. 2002.

[35] Hidroconsulting d.o.o. Poročilo o rezultatih kaptažne vrtine SB-1/03 pri spodnji Besnici in strokovne podlage za zavarovanje vodnega vira "Zajetje z vrtino SB-1/03". 2003.

[36] Mencej Z., Hidroconsulting, d.o.o. "Zajetje z vrtino RB-1/03" za oskrbo Ravnega Brda s pitno vodo in strokovne podlage za zavarovanje zajetja. 2003.

[37] Mencej Z., I. Buser, Hidroconsulting, d.o.o. Črpalni poskus na zajetju z vrtino ŽV-1/01 na Žagarskem vrhu. 2004.

[38] Mencej Z., Hidroconsulting, d.o.o. Vzdrževalna in sanacijska dela na lokalnih vodovodih Mestne občine Ljubljana – Poročilo o izvedenih delih v letu 2004. 2004.

[39] Mencej Z., Hidroconsulting, d.o.o. Sanacija zajetja pitne vode za vodovod Tuji Grm. 2004.

[40] Mencej Z., Hidroconsulting, d.o.o. Poročilo o rezultatih kaptažne vrtine SB-1/03 pri Spodnji Besnici in strokovne podlage za zavarovanje vodnega vira "Zajetje z vrtino SB-1/03". 2004.

[41] Mencej Z. Vzdrževalna in sanacijska dela na lokalnih vodovodih Mestne občine Ljubljana – Poročilo o izvedenih delih v letu 2004. Hidroconsulting, d.o.o. 2004.

[42] Mencej Z. Sanacija zajetja pitne vode za vodovod Tuji Grm, Hidroconsulting, d.o.o. 2004.

[43] Hidroconsulting d.o.o. Strokovne podlage za izvedbo postopka za dodelitev vodnega dovoljenja za izkoriščanje podzemne vode iz vrtine ZGB-1/05, Hidrogeološko poročilo za vrtino ZGB-1/05 v Zgornji Besnici. Marec 2005.

[44] Hidroconsulting d.o.o. Strokovne podlage za pridobitev vodnega dovoljenja za oskrbo s pitno vodo iz nadomestnega vodnega vira, zajetje z vrtino D-1/05 pri Dolinarju (Hidrogeološki del). Marec 2005.

[45] Mencej Z., Hidroconsulting, d.o.o. Poročilo o izvedenem čiščenju vrtine S-1/92 in predlogi načina obratovanja vodovoda Sadinja vas. 2005.

[46] Mencej Z. Strokovne podlage za pridobitev vodnega dovoljenja za oskrbo s pitno vodo iz vrtine D-1/05 pri Dolinarju za vodovod Podmolnik – Hidrogeološki del, Hidroconsulting, d.o.o. 2005.

[47] Hidroconsulting d.o.o. Strokovne podlage za izvedbo postopka za dodelitev vodnega dovoljenja za izkoriščanje podzemne vode iz vrtine ZGB-1/05, Hidrogeološko poročilo za vrtino ZGB-1/05 v Zgornji Besnici. Marec 2005.

[48] Hidroconsulting d.o.o. Strokovne podlage za pridobitev vodnega dovoljenja za oskrbo s pitno vodo iz nadomestnega vodnega vira "Zajetje z vrtino D-1/05" pri Dolinarju (Hidrogeološki del). Marec 2005.

[49] Mencej Z. Poročilo o rezultatih kaptažne vrtine SB-1/03 pri Spodnji Besnici in strokovne podlage za zavarovanje vodnega vira "Zajetje z vrtino SB-1/03" Hidroconsulting, d.o.o. 2004.

[50] Mencej Z. Poročilo o izvedenem čiščenju vrtine S-1/92 in predlogi načina obratovanja vodovoda Sadinja vas, Hidroconsulting, d.o.o. 2005.

[51] Inštitut za varovanje zdravja RS. Poročila o preizkušanju pitne vode, obdobje 2005-2010: http://www.jhl.si/vo-ka/informacije/kaksno-vodo-pijemo/lokalni-vodovodni-sistemi. 2010 (1.12.2012).

[52] Podatkovne baze Atlasa okolje http://meteo.arso.gov.si/met/sl/app/webmet/ (1.12.2011).

SANITARNO INŽENIRSTVO

3. MEDNARODNI STROKOVNI IN ZNANSTVENI SIMPOZIJ/ 3rd INTERNATIONAL SYMPOSIUM IN SANITARY ENGINEERING

Mag. Andrej **OVCA**, dipl. san. inž.

ABSTRACT

3rd international symposium in sanitary engineering was held in Opatija, Croatia from 10th to 12th of November 2011. It proved to be an important platform for discussion and exchange of scientific achievements, ideas and information in the field of sanitary engineering by experts and scholars from several countries. 5 invited lectures, 33 thematic lectures and 18 posters were presented during the symposium. The symposium was dealing with challenges on 8 different areas: Importance of sanitary engineering in public health, environmental hygiene, water resources management, waste water and solid waste, new technologies and health, prevention of infectious diseases and hospital hygiene, sanitation and pest control, food quality and food safety. During general discussion a standpoint was formed that a need for an expert like sanitary engineer is increasing, considering current and also emerging public health and environmental health challenges highlighted during the symposium.

Key words: symposium, sanitary engineering, discussion and exchange of scientific achievements

V prvi polovici novembra (10.–12. 11. 2011) je v Opatiji na Hrvaškem potekal že tretji, lahko rečemo tradicionalni mednarodni strokovni in znanstveni simpozij na področju sanitarnega inženirstva. Simpozij so organizirali Hrvatska udruga za sanitarno inženjerstvo – Komora sanitarnih inženjera i tehničara (Hrvaška), Inštitut za sanitarno inženirstvo, Ljubljana (Slovenija), Nastavni zavod za javno zdravstvo Primorsko-goranske županije, Rijeka (Hrvaška), Medicinski fakultet, Sveučilište u Rijeci (Hrvaška) ter Udruga laboratorijskih i sanitarnih tehničara (BiH). Simpozij je po uvodnem predavanju prof. dr. Franja Plavšića, ki je obravnaval rakotvorne snovi v hrani, je v okviru 5 vabljenih in 33 tematskih predavanj ter 18 posterjev, analiziral probleme na kar osmih vsebinskih področjih.

Pomen sanitarnega inženirstva za javno zdravje je bil izpostavljen v zgodovinskem orisu sanitarnega inženirstva v Evropi, v predstavitvi kompetenc sanitarnih inženirjev na področju zdravstvene ustreznosti pitne vode in pomenu sanitarnega inženirja v živilski industriji ter na področju ocenjevanja in obvladovanja tveganj. V okviru **higiene človekovega okolja** je bila obravnavana problematika legionele s poudarkom na preventivnih ukrepih in ukrepih za njeno odstranjevanje, problematika prašnih usedlin kot mo-

Univerza v Ljubljani
Zdravstvena fakulteta
Oddelek za sanitarno inženirstvo
Zdravstvena pot 5
1000 Ljubljana
E-mail: andrej.ovca@zf.uni-lj.si

tnje za izpostavljeno prebivalstvo in problematika oksidativnega stresa kot skupnega imenovalca večine okoljskih onesnaževal. Na področju **čiščenja odpadnih voda** je bila predstavljena učinkovitost novega sistema za čiščenje ladijskih balastnih voda, temelječem na kombinaciji različnih tehnologij: filtracije, kavitacije in hidrociklona. V sklopu **upravljanja z vodnimi viri** je bil poleg problematike posameznih vodooskrbnih sistemov pri zagotavljanju zdravstveno ustrezne pitne vode predstavljen tudi model za simulacijo in ugotavljanje širjenja onesnaženja v vodooskrbnem sistemu. Na področju **novih tehnologij in njihovem vplivu na zdravje** se je po predstavitvi številnih možnosti aplikacije nanotehnologije razvila pestra razprava, predvsem v delu, ki zajema tveganja za okolje in zdravje ljudi. V okviru **preprečevanja nalezljivih bolezni in bolnišnične higiene** so bili, poleg raziskav mikroflore oz. mikrobiote v bolnišničnem okolju, obravnavani tudi ukrepi varstva pri delu z nevarnimi kemikalijami in predstavljena je bila analiza ergonomskih položajev pedagoškega osebja v vrtcih. Avtorji predstavitev v sklopu zagotavljanja **kakovosti in varnosti živil** so, poleg zakonodajnega okvira, obravnavali različna tveganja (aflatoksini, biološka aktivnost nekaterih živil in tveganja za kronične bolezni), ki izhajajo iz nekakovostne in ne varne hrane kot posledica nepravilnega ravnanja tako v živilski industriji kot tudi v domačem okolju. Na področju **čiščenja in obvladovanja škodljivcev** je bilo analizirano mikrobiološko tveganje, ki ga predstavljajo ščurki v živilski dejavnosti, predstavljene pa so bile tudi prednosti in omejitve ob aplikaciji elektrooksigenirane vode za dezinfekcijo površin.

V odmorih med posameznimi sekcijami so si udeleženci simpozija lahko ogledali pester nabor posterjev, v okviru katerih so bili predstavljeni rezultati preliminarnih raziskav na področju delovanja posameznih mikroorganizmov in interakcij med njimi, uvajanja novih tehnologij čiščenja ter interpretacija podatkov spremljanja parametrov v okolju in zdravju populacije. V okviru posterske sekcije je potekalo tudi ocenjevanje s strani mednarodne žirije. Priznanje in nagrado za najboljši poster je, na podlagi aktualnosti tematike za področje sanitarnega inženirstva, uporabljene metodologije, interpretacije rezultatov, jasnosti predstavitve in estetske podobe, prejelo delo z naslovom »Učinek učnih delavnic na področju higiene rok med osnovnošolci / Hand hygiene among pupils – how special workshops can contribute?«. Delo je plod sodelovanja avtorjev mag. Gregorja Jereba in mag. Andreja Ovce (Oddelek za sanitarno inženirstvo Zdravstvene fakultete Univerze v Ljubljani) ter dr. Iztoka Tomažiča (Katedra za biološko didaktiko Biotehniške fakultete Univerze v Ljubljani) v okviru aplikativnega projekta, podprtega s strani Mestne občine Ljubljana.

V okviru splošne razprave se je izoblikovalo stališče o vse večji potrebi in nujnosti profila kot je sanitarni inženir, za reševanje aktualnih javnozdravstvenih in okoljskih problemov, ki so bili izpostavljeni v okviru simpozija; to stališče je podprla večina udeležencev. Pomemben poudarek simpozija je tudi visoka podpora obstoječemu poimenovanju same stroke. Udeleženci so se soglasno strinjali, da bi, upoštevajoč dolgoletno tradicijo in uveljavljenost tako stroke kot samega profila, spremembe v smeri preimenovanja lahko povzročile nepopravljivo škodo samemu ugledu in prepoznavnosti sanitarnega inženirstva.

INSTRUCTIONS FOR AUTHORS

Scope

Sanitarno Inženirstvo/International Journal of Sanitary Engineering Research presents broad interdisciplinary information on the practice and status of research in environmental, food and occupational hygiene, epidemiology, the environmental engineering science, systems engineering, and sanitation. Papers focus on design, development of health engineering methods, management, governmental policies, and societal impacts of drink water, wastewater collection and treatment; the fate and transport of contaminants on watersheds, in surface waters, in groundwater, in the soil, and in the atmosphere; environmental biology, microbiology, chemistry, fluid mechanics, and physical processes that control natural concentrations and dispersion of wastes in the air, water, and soil; non-point source pollution on water-sheds, in streams, in groundwater, in lakes, and in estuaries and coastal areas; treatment, management, and control of hazardous wastes; control and monitoring of air pollution and acid deposition; air-shed management; and design and management of solid waste professional obligations facilities; food technology, management of food quality and food safety. A balanced contribution from consultants, sanitary engineers, and researchers is sought on engineering solutions and responsibilities.

Submission of Articles

All manuscript for Sanitarno Inženirstvo/International Journal of Sanitary Engineering Research should be submitted to:

Inštitut za sanitarno inženirstvo
Institute of Food Safety and Environment Health

Zaloška cesta 155
SI - 1000 Ljubljana
Slovenia
E-mail: info@institut-isi.si.

Your article will be submitted to the review process. All correspondence, including notification of the Editor's decision and request for revision, takes place by e-mail.

Submission of a paper implies that it has not been published previously, that it is not under consideration for publication elsewhere, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, without the written permission of the publisher. Authors are solely responsible for the factual accuracy of their papers.

Types of Contributions

Original research articles: Research articles of 6,000-10,000 words (10 – 18 manuscript pages) in length, with tables, illustrations and references, in which hypotheses are tested and results. Research articles report on significant and innovative achievements, approaches and should exhibit a high level of originality.

Technical articles: Technical articles at least of 6,000 words (to 12 manuscript pages) in length, with tables, illustrations and references. Technical articles should re-

port on significant and innovative achievements of an already described innovation, experiences, state of the art technologies and know-how that are not based on new experiments and research.

Governmental initiatives: Reports on new or existing policies, governmental initiatives and programmes, have up to 2,000 words.

Educational Initiatives: Reports on research activities, education and training and new courses/approaches at academic institutions, training centres, initiatives of transfering knowledge, e-learning etc. The report should have 1.000 - 3.000 words (2 - 3 manuscript pages).

Conference reports: Reports on domestic and international conferences in particular interest to the readers of the Sanitarno Inženirstvo/International Journal of Sanitary Engineering Research. The length of the article should reach up to 2.000 words.

Book reviews, Software reviews: Reviews on new books and software relevant to the scope of the Journal of Sanitary Engineering, of approximately 500 – 1,000 words.

Calendar of Events: Forthcoming meetings, workshops and conferences of relevance to scope of the Sanitarno Inženirstvo/International Journal of Sanitary Engineering Research. The note should provide information on the date, title and venue, contact addresses for further contacts and the web page information if available.

Manuscript preparation

Cover letter: Cover letter should contain:

1. Title of the paper (without any abbreviations).
2. Full name(s) of the author(s).
3. Affiliation and addresses of the author(s).
4. Mailing address of the corresponding author (address, phone and fax number, e-mail).
5. A statement that the article is original, the manuscript or any part of it (except abstract) has not been sent to any other publisher or it is not in consideration for publication anywhere in any language.
6. A statement, that all authors read the article and agree with the content.
7. Written permission of the publisher for the use of tables, figures and any other part of the material in original form used in the article.
8. A statement of the ethnical principals used during the experiments with animals (if any).

A copy of the cover letter must be signed by the corresponding author and sent to the editor by regular mail.

General: Manuscript must be prepared with Word for Windows, double-spaced with wide margin 25 mm. 12 pt Times New Roman font is recommended. Correct spelling and grammar are the sole responsibility of the author(s). Articles should be written in a concise and succinct manner. Research papers should have the following structure: 1. Title, 2. Authors and affiliations, 3. Abstract (max. 200 words), 4. Keywords (max. 6 words), 5. Introduction, 6. Methods, 7. Results and discussion, 8. Acknowledgements (if any), 10. References, 11. Vitae, 12. Figure captions and 13. Tables.

Technical and reviews articles should have a similar structure, abstracts should not exceed 150 words. All articles should be in English, except news, reviews and reports what can be in Slovenian, Croatian or Serbian. Chapters should be arranged according to the standards ISO 2145 and ISO 690. Abbreviations must be explained at the first mention in the text.

First page of the article should contain the title of the article (max. 10 words), author(s)' name(s), institution and address. The corresponding author should be identified with an asterisk and footnote (the text in the footnote: to whom all correspondence should be addressed and added tel. and fax numbers, and e-mail). Second page should contain the abstract and keywords. An abstract of approximately 100 words should have the following structure: aims, scope and conclusions of the article.

Symbols and Units: Authors should follow the ISO 31 and IUPAC recommendations. Please note that all symbols should be written in italic; superscript and subscript are written normal.

Tables, figures and illustrations: Tables, figures and illustrations should be numbered consecutively and given a suitable caption, and each table, figure, and illustration should be given on a separate page and file. No vertical rules for tables should be used. Images should be of sufficient quality for the printed version (300 dpi minimum). Figures should be at good enough resolution (in EPS, JPEG, or TIFF format) to be assessed by the referees.

References: References should be numbered, and ordered sequentially as they appear in the text, methods, tables, figure captions. When numbered in the text, reference numbers should be in brackets, following the punctuation marks. If the cited reference has more then 3 authors, add the mark et al after the third author. References should be given in the following form:

[1] Bhatt Siddharta M. Energy audit case studies II - Air conditioning (cooling) systems. Appl Th Eng. 2000; 20: 297-307.

[2] American college of physicians. Clinical Ecology. An Int Med 1989; 111:168-78.

[3] Vivian VL, ed. Child abuse and neglect: a medical community response. Proceedings of the first AMA national conference on child abuse and neglect. 1984 Mar 30-31; Chicago. Chicago: American Medical Assotiation, 1985.

[4] Mansfieid LW. How the nurse learns which imbalance is present. V: Moidel HC, Sorensen GE, Giblin EC, Kaufman MA, eds. Nursing care of the patient with medical-surgical disorders. New York: Mc Grow-Hill, 1971: 153-60.

[5] Evaluation of the European Agency for Safety and Health at Work: http://osha.europa.eu/publications/other/20010315/index_1.htm (20. 12. 2006).

Letna naročnina za člane Zbornice ZSTI Slovenije
je vključena v članarino.
Za nečlane in pravne osebe znaša letna naročnina 140 €,
za tujino 160 € vključno s poštnino.

Annual subscription: 160 € including postage.

Na podlagi Zakona o davku na dodano vrednost sodi revija
Sanitarno inženirstvo /
International Journal of Sanitary Engineering Research
med proizvode, od katerih se obračunava
DDV po stopnji 8,5%.

Sanitarno inženirstvo /
International Journal of Sanitary Engineering Research
izhaja 1-2 letno v 800 izvodih.

***Sanitarno inženirstvo / International Journal of Sanitary Engineering Research* appears 1-2 per year in 800 copies.**

Graphic Design & Layout: Tempora, Rijeka, HR