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Editorial

"An ounce of prevention is worth a pound of cure"

Dear readers of International Journal of Sanitary Engineering Research (IJSER). In front of you is volume number 12. The author of the quote above is Benjamin Franklin and I would like to draw your attention and put this quote into the context of environmental health hazards. Why? Because it is always difficult to demonstrate and make people aware how many illness or deaths have been prevented with prevention. One of the reasons for this is the fact that environmental health hazards are ubiquitous, affecting all aspects of our life. Our environment in general consists of physical, chemical and biological factors. We are always in interactive relationships with our environment and as we affect our environment, our environment affects us. To reduce the adverse impact of environmental hazards on human health we need to understand the hazard origin and routes of exposure as well as pathways through which the specific hazard affects peoples' health. In the current volume we present five papers. Two of them are dealing with hazards in food and another two with hazards in water. Additionally, we present one more paper whose authors investigated the quality of the health-related data.

Marić et al., and Fox et al. investigated food related hazards. Marić et al. investigated the presence of organochlorine pesticides in different food samples of plant origin, available in area of Rijeka (Croatia). Their research demonstrates the presence of organochlorinated pesticides in the tested food samples is below the maximum permissible concentration, presenting minimal risk to human health. Fox et al. on the other side explored the awareness, understanding and practices of allergen management in selected food business in the North West of England. Their findings revealed a significant gap between the level of confidence expressed by food business owners and their practices exposing customers to a significant level of risk.

Kelava et al. and Godič Torkar & Oder investigated water related microbiological hazards. Kelava et al. analysed the five-year trend of bacteriological contamination of specific water supply system. They found out that due to faecal contamination investigated water supply system is not safe for human health. They concluded that without basic sanitary-technical maintenance of the water supply system and chlorination, identified microbiological hazards cannot be maintained at acceptable level. Godič Torkar & Oder studied the influence of temperature, disinfectant and presence of water softener on the bacterial growth of *Legionella pneumophila*. They concluded based on their laboratory experiments that the temperature of 55 °C, even after a long time of incubation, does not eliminate all bacteria. They have also proven that incomplete or improper disinfection of drinking water may cause an intensive reproduction of bacteria. Additionally, they demonstrated that sodium polyphosphate in a water softening product caused an intensive multiplication of the bacteria. At last but not at least Stević et al. investigated the quality of cause-of-death data. Their objective was to determine percentage of the "garbage codes" (GCs) coded as the underlying cause of cancer death.

Despite the papers published in this volume we should not forget that environmental health hazards may be encountered at home, work, or in the community via several pathways and routes of exposure. In addition we should not ignore global environmental conditions which may also adversely affect human health. Therefore, I am looking forward to new manuscripts submitted for the next volume of International Journal of Sanitary Engineering Research.

Sincerely,

Andrej **OVCA**, Editor-in-Chief

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Contents

Original scientific article

The organochlorine pesticides in food samples

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ABSTRACT
Nowadays the concept of food is changed, because food is no longer considered as a source of energy alone, but must provide a sense of pleasure and satisfaction with eating meals. But the unwillingness to develop awareness and raise the concept of food to a higher level, we have to emphasize and warn of the food safety related to chemical hazards. The presence of pesticides in food has a detrimental impact on human health and we tested the presence of organochlorine pesticides in eleven food samples of plant origin, randomly selected in one supermarket in Rijeka. During the sample analysis, the methods of extraction, reextraction, degradation, purification, GPC and GC-ECD were used. Our data indicated the presence of pesticides in the tested food samples, but the level is below the maximum permissible concentration (MPC). According to the results it can be concluded that the analyzed food samples did not have harmful effect on human health.

Key words: food of plant origin, organochlorine pesticides, health care, GPC, GC – ECD

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INTRODUCTION

The topic that nowadays occupies a great deal of attention in the everyday life of people is food safety. In many European countries food samples contain pesticide residues within legal limits, but continuous monitoring is necessary. Part of the health safety assessment of food is the remains of pesticides whose detection is more precise and accurate today, which allows us to constantly develop technology [1]. Food safety dimensions are: food availability, food access, utilization and stability [2]. Pesticide residues are the result of the use of pesticides in plant protection in veterinary medicine and biocidal products. They include active substances, degradation products or reactants of active substances or their metabolites previously used or currently used in plant protection products. The levels of pesticide residues depend on the amount of pesticide applied, the physicochemical properties of pesticides, the number of applications and the agricultural culture to which it applies [3]. Given that excessive use of pesticides results in their accumulation in the environment as well as in treated herbs, pesticide residue monitoring is carried out. Organochlorine pesticides, pesticides easily enter the food chain due to their lipophilic nature and accumulate in egg yolks, fatty tissue and liver of different animals [4]. Symptoms of acute poisoning with organochlorine pesticides include nausea, vomiting, tremor, dizziness, fatigue, headache, cramping and lethargy, while signs of chronic poisoning are anemia, anorexia, weight loss, skin rash, memory loss, vision problems, insomnia, nervousness , anxiety and muscular weakness [4]. On the other hand, pesticides are essential for food production and human survival, as the speed of life and growth of the world population results in the needs of larger quantities of food. That is why the use of pesticides must be under constant and strict control of science [5]. Since pesticides are applied in agriculture and livestock farming, food of plant and animal origin may contain pesticide residues. If food pesticides do not break down, there is a buildup of a toxic compound in food or a phenomenon of residues of pesticides which are often not destroyed by food preparation [6]. There are three major groups of organochlorine insecticides (Table 1.)

The levels of pesticide residues depend on the amount of pesticide applied, the physicochemical properties of pesticides, the number of applications and the agricultural culture to which it applies.

Table 1. Organochlorine insecticides

Groups of organochlorine insecticides	Types of organochlorine insecticides
Dichlorodiphenylethane	DDT, DDD, DDE, Rotary, Pertane, Methoxychlor, Methochlor, Dikofol
Chlorinated cyclodienes	Endosulfan, Clordan, Endrin, Dieldrin, Aldrin, Heptachlor
Chlorinated benzene and cyclohexanes	Klordekon, HCB, HCH, Mireks, Lindan, Toksafen

One of the ways to combat the excessive use of pesticides today is organic production where pesticide use is prohibited.

In the Republic of Croatia, pesticides are controlled by the Pesticide Sustainable Use Policy (OG 014/2014). It has defined the distribution and sales system, handling and sustainable use of pesticides. It also wants to raise levels of pesticide awareness to reduce the negative effects and risk of pesticide use [7]. One of the ways to combat the excessive use of pesticides today is organic production where pesticide use is prohibited. However, given the fact that pesticides are overwhelmed and found in the air, soil and water, the question is if we really have the possibility of such production [8].

The aim of this paper was to investigate the amount of pesticide by reference methods that provide reliable results in randomly selected foods and to determine whether there is presence of pesticides in quantities greater than MPC as well as their harmfulness to human health.

METHODS

All the samples were collected by the same trained professional person. The food samples were taken from 11 different owners, but every sample has also been taken in three independent pattern. This investigation was a part of regular/state monitoring in the Department of Public Health of Primorsko-Goranska County. All samples were transported by special transport car with refrigerator in which there is a system of constant measurement of the temperature of the cooling chamber. Within this study 11 samples of food of plant origin were analyzed.

They are marked with numbers from 1 to 11 in the following order (Figures 1 and 2).

1. Choco Muesli (K plus)
2. Apple Chips (Velis)
3. Slow chips (K plus)
4. Rice (K plus)
5. Sweet paprika – minced spice pepper (K plus)
6. Wheat white flour – smooth (millet)
7. Swamp Juice Soup (Knorr)
8. Apples and concentrated fruit juice nectar (TO)
9. Pineapple Compounds (K plus)
10. Apple Golden Delis (Moslavina fruit d.o.o.)
11. Pepperoni (Pepperoni Rossi)

These 11 food samples were analyzed for the following pesticides (Table 2).

Figure 1. Mix dry samples.
Sample 1. – Choco Muesli; sample 2. – Apple chips; sample 3. – Slow chips; sample 4. – Rice; sample 5. – Sweet pepper – ground spice pepper; sample 6. – Wheat white flour – smooth; sample 7. – Mushroom soup with raspberries.

Figure 2. Mix wet samples.
Sample 8. – Apples and concentrated fruit juice nectar; sample 9. – Pineapple compote; sample 10. – Apple Gold Delis; sample 11 – Paprika red.

Table 2. Pesticides analyzed in the samples

Organochlorine pesticides				Other pesticides containing chlorine	
No.	Name of pesticide	No.	Name of pesticide	No.	Name of pesticide
1.	alpha – HCH	12.	4,4` – DDE	1.	PCIB
2.	beta – HCH	13.	2,4` – DDT	2.	HCB
3.	linden	14.	endrin	3.	pentachlorophenol
4.	delta – HCH	15.	endosulfanII	4.	vinclozolin
5.	heptachlor	16.	4,4` – DDD	5.	alachlor
6.	aldrin	17.	endrin aldehyde	6.	isodrin
7.	heptachlorepoxide	18.	endosulfan sulfate	7.	captan
8.	gamma – chlordane	19.	4,4` – DDT	8.	tolyfluanid
9.	endosulfan I	20.	endrin ketone	9.	iprodione
10.	alpha – chlordane	21.	methoxychlor		
11.	dieldrin				

The methods used during sample analysis are: extraction, reextraction, degreasing, purification, GPC and GC – ECD. Pesticides were determined according to the norms:

1. HRN EN 12393-1: 2013 (EN 12393-1: 2013) "Food of plant origin – Multi-lingering methods for determination of residues of pesticides by gas chromatography – Part 1: General considerations".

2: Methods for extraction and purification "(EN 12393-1: 2013)" Food of plant origin – Multi-lingering methods for the determination of residues of pesticides using GC or LC-MS / MS".

3. HRN EN 12393-1: 2013 (EN 12393-3: 2013) "Plant origin foods – Multi-lingual methods for the determination of residues of pesticides using GC or LC-MS / MS – Part 3: Determination and Certification" [9].

RESULTS

This paper examines the presence of organochlorine pesticides in 11 randomly selected samples. 21 organochlorine pesticides have been determined and 9 are not organoclorinate but have chlorine. The quantification limit is a parameter in quantitative analysis. It represents the smallest amount of an analyte in a sample that is quantified with precision and accuracy and is determined when the level of analyte concentration is low [10].

Sample 1 – Choco Muesli (K plus) in Table 3.

Table 3. Display results obtained by GC-ECD method in sample 1. organochlorine pesticides (ND-non detected)

Name	Time [Min]	Quantity [mg/kg]	Area [μV. Sec]	Area [μV. SMin]	Height [μV]	Ret. time Offset [Min]	Quantification limits [mg/kg]
alfa-HCH	7.97	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
beta-HCH	8.91	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
lindan	9.20	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
delta-HCH	10.11	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
heptaklor	12.28	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
aldrin	13.89	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
heptaklorepoksid	15.89	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
gama-klordan	17.11	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
endosulfan I	17.71	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
alfa-klordan	17.92	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
dieldrin	19.09	0.0017	241533.5	4025.6	32658.7	0.01	0,004
4,4'-DDE	19.32	0.0001	15892.8	0264.9	2969.4	0.01	0,004
2,4'-DDT	19.71	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endrin	20.15	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endosulfan II	20.68	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
4,4'-DDD	21.49	0.0006	63350.5	1055.8	9803.1	-0.03	0,004
endrin aldehid	21.69	0.0006	52765.5	879.4	8730.0	-0.04	0,004
endosulfan sulfat	23.03	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
4,4'-DDT	23.32	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endrin keton	25.41	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
metoksiklor	26.94	N.D.	N.D.	N.D.	N.D.	N.D.	0,020
Total	-	**0.0031**	**61867367.7**	**1031122.8**	**2770873.8**		

Table 4. Display results obtained by GC-ECD method in sample 1. Other pesticides containing chlorine (ND-non detected)

Name	Time [Min]	Quantity [mg/kg]	Area [μV. Sec]	Area [μV. SMin]	Height [μV]	Ret. time Offset [Min]	Quantification limits [mg/kg]
PCIB	5.28	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
HCB	8.26	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
pentaklorfenol	9.13	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
vinelozolin	12.15	0.0003	18324.8	305.4	3929.0	0.05	0,002
alaklor	12.46	N.D.	N.D.	N.D.	N.D.	N.D.	0,005
izodrin	15.25	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
captan	16.28	0.0012	8108.0	135.1	2539.0	0.02	0,020
tolilfluanid	16.34	N.D.	N.D.	N.D.	N.D.	N.D.	0,020
iprodion	26.14	N.D.	N.D.	N.D.	N.D.	N.D.	0,010
Total	-	**0.0016**	**61222567.3**	**1020376.1**	**2505895.0**		

Sample 2 – Apple Chips (Velis)

Table 5. Display results obtained by GC-ECD method in sample 2. Organochlorine pesticides (ND-non detected)

Name	Time [Min]	Quantity [mg/kg]	Area [μV. Sec]	Area [μV. SMin]	Height [μV]	Ret. time Offset [Min]	Quantification limits [mg/kg]
alfa-HCH	7.96	0.0001	13294.5	221.6	1517.8	-0.01	0,002
beta-HCH	8.91	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
lindan	9.20	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
delta-HCH	10.11	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
heptaklor	12.28	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
aldrin	13.92	0.0014	213494.8	3558.2	19941.9	0.03	0,002
heptaklorepoksid	15.89	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
gama-klordan	17.11	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
endosulfan I	17.71	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
alfa-klordan	17.92	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
dieldrin	19.08	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
4,4'-DDE	19.31	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
2,4'-DDT	19.71	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endrin	20.15	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endosulfan II	20.72	0.0024	0.0024	255938.4	4265.6	50953.5	0,004
4,4'-DDD	21.52	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endrin aldehid	21.73	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endosulfan sulfat	23.03	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
4,4'-DDT	23.32	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endrin keton	25.41	0.0002	14584.8	243.1	3677.1	0.00	0,004
metoksiklor	26.94	N.D.	N.D.	N.D.	N.D.	N.D.	0,020
Total	-	**0.0040**	**61867367.7**	**1031122.8**	**2770873.8**		

Table 6. Display results obtained by GC-ECD method in sample 2. Other pesticides containing chlorine (ND-non detected)

Name	Time [Min]	Quantity [mg/kg]	Area [μV. Sec]	Area [μV. SMin]	Height [μV]	Ret. time Offset [Min]	Quantification limits [mg/kg]
PCIB	5.28	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
HCB	8.26	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
pentaklorfenol	9.13	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
vinelozolin	12.10	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
alaklor	12.46	N.D.	N.D.	N.D.	N.D.	N.D.	0,005
izodrin	15.25	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
captan	16.26	N.D.	N.D.	N.D.	N.D.	N.D.	0,020
tolilfluanid	16.34	N.D.	N.D.	N.D.	N.D.	N.D.	0,020
iprodion	26.14	N.D.	N.D.	N.D.	N.D.	N.D.	0,010
Total	-	**0.0000**	**11172514.5**	**186208.6**	**1913786.0**		

Sample 3 – Slow Chips (K plus)

Table 7. Display results obtained by GC-ECD method in sample 3. Organochlorine pesticides (ND-non detected)

Name	Time [Min]	Quantity [mg/kg]	Area [μV. Sec]	Area [μV. SMin]	Height [μV]	Ret. time Offset [Min]	Quantification limits [mg/kg]
alfa-HCH	7.97	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
beta-HCH	8.91	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
lindan	9.20	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
delta-HCH	10.11	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
heptaklor	12.28	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
aldrin	13.89	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
heptaklorepoksid	15.89	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
gama-klordan	17.11	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
endosulfan I	17.71	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
alfa-klordan	17.92	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
dieldrin	19.09	0.0002	9753.7	162.6	3727.2	0.01	0,004
4,4'-DDE	19.34	0.0004	22587.3	376.5	4810.2	0.03	0,004
2,4'-DDT	19.71	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endrin	20.15	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endosulfan II	20.68	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
4,4'-DDD	21.52	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endrin aldehid	21.73	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endosulfan sulfat	23.03	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
4,4'-DDT	23.32	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
endrin keton	25.41	N.D.	N.D.	N.D.	N.D.	N.D.	0,004
metoksiklor	26.94	N.D.	N.D.	N.D.	N.D.	N.D.	0,020
Total	-	**0.0006**	**58137688.9**	**968961.5**	**1690362.6**		

Table 8. Display results obtained by GC-ECD method in sample 3. Other pesticides containing chlorine (ND-non detected)

Name	Time [Min]	Quantity [mg/kg]	Area [μV. Sec]	Area [μV. SMin]	Height [μV]	Ret. time Offset [Min]	Quantification limits [mg/kg]
PCIB	5.28	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
HCB	8.26	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
pentaklorfenol	9.13	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
vinelozolin	12.10	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
alaklor	12.46	N.D.	N.D.	N.D.	N.D.	N.D.	0,005
izodrin	15.25	N.D.	N.D.	N.D.	N.D.	N.D.	0,002
captan	16.26	N.D.	N.D.	N.D.	N.D.	N.D.	0,020
tolilfluanid	16.34	N.D.	N.D.	N.D.	N.D.	N.D.	0,020
iprodion	26.14	N.D.	N.D.	N.D.	N.D.	N.D.	0,010
Total	-	0.0000	58985425.2	983090.4	1445137.7		

The values that deviate but do not exceed MPC are 2, 4'-DDT, 4, 4'-DDT and vinclozolin. Derived values (2, 4'-DDT and 4, 4`-DDT) were found in samples 4 and 6 (sample 4 – Rice, sample 6 – Wheat white flour – smooth) while the vinclozoline elevated value was found in the sample 8 (Apple nectar and concentrated fruit juice). Therefore, the calibration directions (Figures 3, 4) and their values of computer curves are shown.

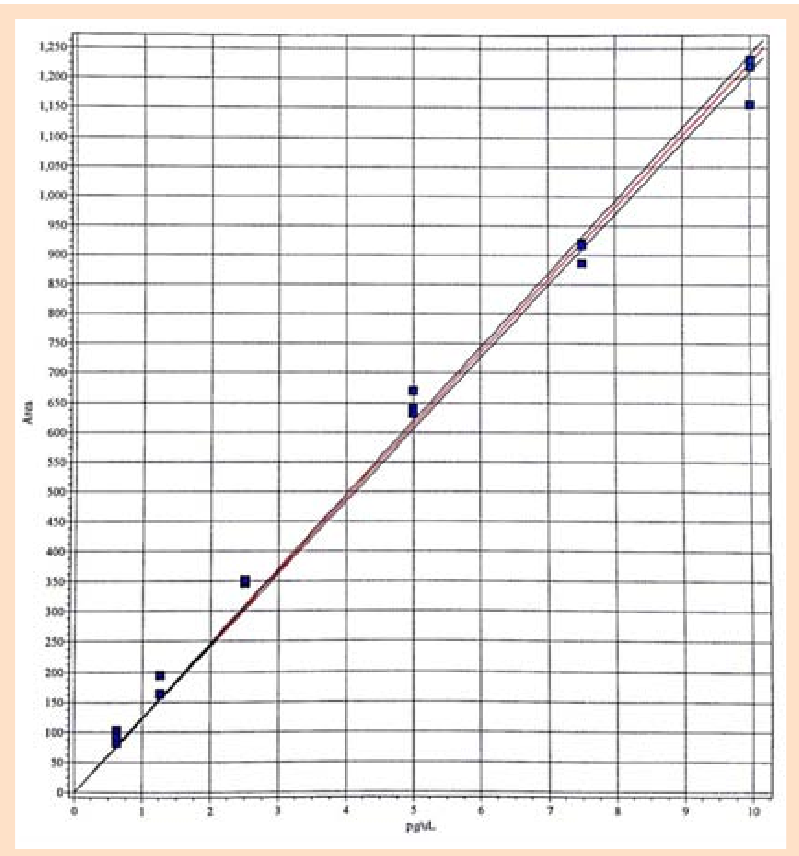


Figure 3.
The dependence of the concentration c (pg μL) on the surface of the sample point 2.4 & gt; DDT.

Figure 4.
Graphic representation of the concentration dependence of c (pg/μL) on the surface of the sample point 4,4-DDT.

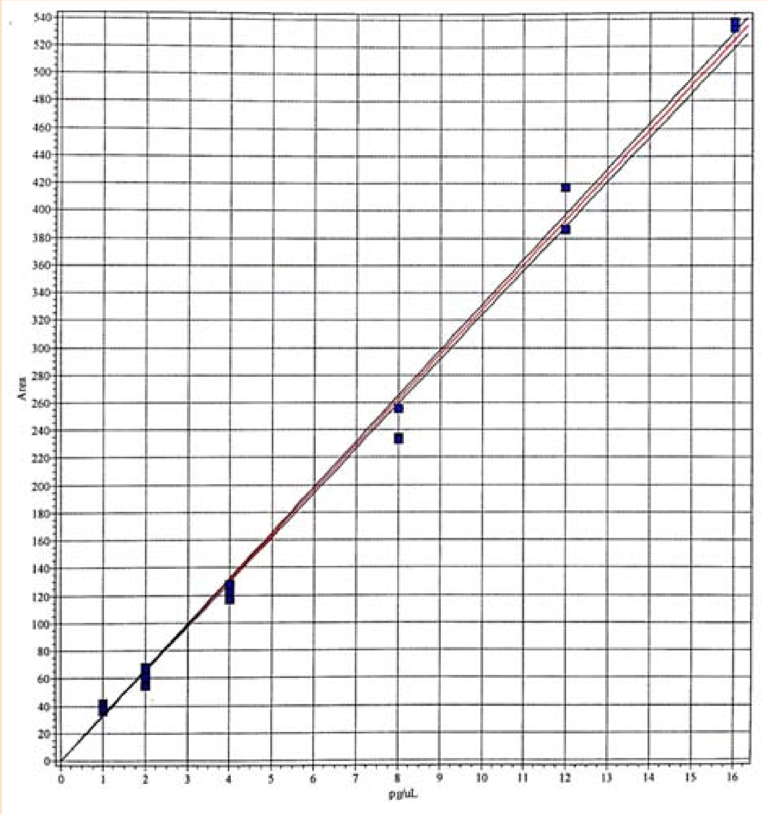
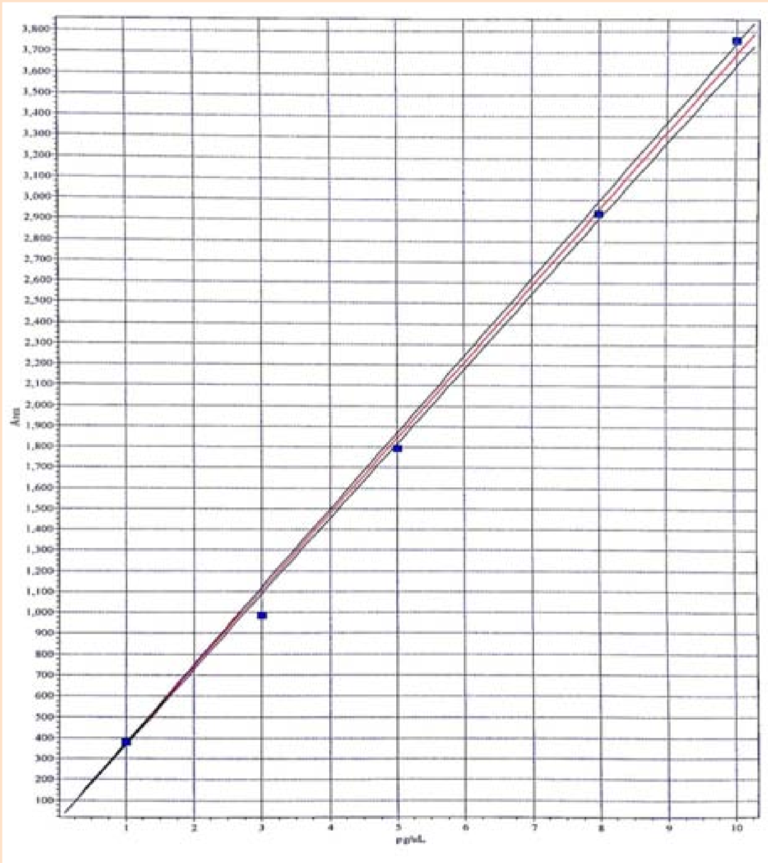


Figure 5.
A graph of the concentration dependence of c (pg/μL) on the surface of the sample point vinclozoline.



Next to the line is the equation of the line and the coefficient of correlation for each direction. The tracks are created in the Galaxy Chromatography Data system version 1.9.3.2. For all types of DDT (p, p – DDT, o, p – DDT, p, p – DDE and p, p – TDE) MDK for flour is 0.05. The same applies to rice. MPC vinklozolina with apples is 0.01.

When sample 4 and 6 were analyzed (sample 4. – Rice, sample 6. – Wheat white flour – smooth), the 2.4'-DDT was elevated as shown in the diagram (Figure 3), whose y is 122.52573 x and the value R is 0.9939.

When we analyzed samples 4 and 6 (sample 4 – Rice, pattern 6 – Wheat white flour – smooth), an elevated value of 4,4 μ-DDT was obtained as shown in diagram (Figure 4) whose y is 32.76046 x and the value R is 0.9944.

When sample 8 was analyzed (apple nectar and concentrated fruit juice), a vinclozoline elevated value was obtained as shown in the diagram (Figure 5) whose y is 368.65825 x and the value R is 0.9969.

For all types of DDT (p, p – DDT, o, p – DDT, p, p – DDE and p, p – TDE) MDK for flour is 0.05. The same applies to rice. MDK vinklozolina with apples is 0.01 [11].

DISCUSSION

Only 3 samples of the 11 analyzed, had a mild deviation (mild increase 27.27%), but below MPC. In samples 4 and 6 (sample 4. – Rice, sample 6. – Wheat white flour – smooth) there can be seen a slight increase of 2.4`-DDT and 4.4`-DDT. Since both pesticides have been increased in both samples, it is assumed that this is a possible impurity or an error in the analysis. Of course, because of the longtime of DDT's dissolution, it is possible that it is a real result. In the results in sample 8 (apple nectar and concentrated fruit juice), vinclozolin pesticides increased. MPC values were not exceeded. A more comprehensive picture of pesticide diffusion and their control can be given to us “Annual reports on the implementation of monitoring programs for pesticide residues in and on plant-based products” [12].

The 2013, report states that out of 335 analyzed samples, none of them contained residues above MPC [1, 12]. Similar results are also found in the 2014 report, when 378 samples were analyzed [3]. In the reports it can be seen that the average in the EU country from 2013 to 2014 increased by 1%. In 2013, the EU average was 1.6%, while the EU average in the EU in 2014 was 2.6% [11]. It was emphasized the use of resources that were allowed for use but not for the products they were found to be. Such an example is stated in the 2014 report, where the strawberry was found to be an active substance chlorpyrifos which has no license for its use [3]. They also mention the finding of active pyrazophos (on tomatoes) that is banned in Croatia and the EU. Pesticide residues, as stated in both reports, are most commonly present in orange and strawberries, while pesticide residues in wheat flour, baby food and bread are not found or are at the limit of quantification [3, 12].

Pesticide residues are most commonly present in orange and strawberries, while pesticide residues in wheat flour, baby food and bread are not found or are at the limit of quantification

On the basis of this data it can be estimated that consumer exposure in the Republic of Croatia is extremely low because none of the samples listed in the reports have passed the MDK or the samples analyzed for the purpose of this paper. It should also be taken into account that MDK values are set several times lower than concentrations of pesticides that are harmful to the health of consumers themselves [3, 12].

CONCLUSIONS

The use of pesticides is a worldwide problem and today's hygiene could not be imagine without them. Pesticides are a necessary today, but their use must be strictly controlled. The presence of DDT and vinclozoline is elevated but within the allowed limits. We can conclude that pesticide concentrations do not exceed the permissible concentration in a single sample and can not cause food poisoning. After the food test was carried out on randomly selected samples in the Public Health Institute's labs of Primorsko-goranska County, it was found that the food was safe and had no adverse effect on humans and their health.

REFERENCES

[1] Sabljak, I., Brlek, V., Grubelić, M. i sur. (2013) *Ostaci pesticida u poljoprivrednim proizvodima tijekom 2013. godine.* Stručni rad. Zagreb: Euroinspekt Croatiakontrola d.o.o.

[2] Zubak, J. (2015). Ljudska sigurnost: sigurnost hrane i glad kao dimenzije međunarodne sigurnosti. *Polemos: časopis za interdisciplinarna istraživanja rata i mira*, XVIII(36), 163-179. Preuzeto s http://hrcak.srce.hr/156742

[3] Uprava kvalitete hrane i fitosanitarne politike (2015) *Godišnje izviješće oprovedbi nacionalnog programa praćenja (monitoringa) ostataka pesticida u i na hrani za razdoblje 2014. godine.* Zagreb: Ministarstvo poljoprivrede.

[4] M. Đokić, M., Bilandžić, N., Briški, F. (2012) Postupci uklanjanja pesticida iz okoliša. *Kemija u industriji.* 61 (7-8), 341–348.

[5] Pavlek V. (1987) Proizvodnja hrane i pesticidi. *Agronomski glasnik*. (2-3), 92-100.

[6] Puntarić, D., Muškulin M., Bošnir J. i sur. (2012) *Zdravstvena ekologija*. Zagreb: Medicinska naklada, 113-126.

[7] Narodne novine (2014) *Zakon o održivoj uporabi pesticida* (NN 014/2014). Zagreb: Narodne novine d.d.

[8] Dokić, M., Bilandžić, N., Briški, F. (2012). Postupci uklanjanja pesticida iz okoliša. *Kemija u industriji: Časopis kemičara i kemijskih inženjera Hrvatske*, 61(7-8), 341-348. Preuzeto s http://hrcak.srce.hr/83941

[9] Hrvatski zavod za norme (2013) *Oglasnik za normativne dokumente 7/2013 – Rezultat hrvatske normizacije.* Hrvatski zavod za norme.

[10] Belupo. Dostupno na: http://www.belupo.com/Default.aspx?sid=6941, pristupljeno 17.05.2017.

[11] EU – Pesticides database. Dostupno na: http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN, pristupljeno 20.05.2017.

[12] Uprava kvalitete hrane i fitosanitarne politike (2014) *Godišnje izviješće oprovedbi nacionalnog programa praćenja (monitoringa) ostataka pesticida u i na hrani za razdoblje 2013. godine.* Zagreb: Ministarstvo poljoprivrede.

Original scientific article

A Matter of trust: A quantitative study to explore allergen awareness and compliance in takeaway food businesses in the Borough of Knowsley

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ABSTRACT

With the number of people suffering from food allergies increasing globally and food allergies accounting for more hospital admissions than food borne diseases, food allergens pose a significant public health threat.

In December 2014, the European Union (EU) introduced legislation which aimed to ensure that customers with food allergens could make informed choices and safely consume food, without the risk of a potentially life-threatening reaction.

Using a questionnaire and allergen audit (designed to provide a consistent and standardised means of measuring food safety practices within food businesses), the aim of the research was to explore the awareness, understanding and practices of 21 randomly selected food business in the Borough of Knowsley, located in the North West of England.

The findings revealed a significant gap between the level of confidence expressed by food business owners and their practices and understanding. Whilst all (n=21) felt confident in providing a safe meal and 90% (n=18) were aware of the need display allergen information, none of the food businesses owners demonstrated a high level of allergen control in their premises and 43% (n=9) did not display any allergen information within their premises. In addition, the research established that there appeared to be no direct link between the levels of food hygiene found in a food business and the awareness and practice of the food business owner regarding food allergens.

This gap leaves customers exposed to a significant level of risk, as it appears that the confidence food business owners have in producing a safe meal is misplaced.

Key words: food allergens, food businesses, risk, allergen audit

The prevalence of food allergies is reported to be rising in many countries, with many studies estimating that food allergies affect 1-2% of adults and 5-8% of children.

The World Health Organisation (WHO) reports that there are more than 70 foods that have been reported as causing food allergen reactions.

INTRODUCTION

Since December 2014, specific EU legislation has required food businesses (FB) to provide information to customers about food allergens, yet concerns still exist as to how effectively this legislation is being implemented.

The importance of ensuring customers are provided with accurate information about potentially life-threatening allergens that may be contained within food cannot be underestimated. A number of deaths in the United Kingdom (UK) have been linked to FBs providing allergen-contaminated food, sometimes despite requests from the customer concerning their allergen status. In one case, the wilful negligence shown by a food business operator (FBO) regarding allergens resulted in his prosecution and conviction for manslaughter after the death of a customer.

The prevalence of food allergies is reported to be rising in many countries [1], with many studies estimating that food allergies affect 1-2% of adults and 5-8% of children [2, 3, 4, 5]. Hospital admissions for children in the UK alone, due to food allergens, have increased by 700% since 1990 [6] and almost twice as many people are hospitalised per year due to allergic reactions to food than food borne diseases [7] although there appears to be no single reason for this.

According to Wang and Sampson [8] food allergies can be defined as adverse immune mediated reactions to specific food proteins that can have a rapid onset time and can sometimes be serious and lead to a life-threatening anaphylactic reactions. Table 1 identifies the area where the reaction could take place and the symptoms that may be experienced.

Food allergies are not the same as food intolerances, which may be caused by difficulties in digesting certain substances such as lactose. With a food intolerance, no allergic reaction occurs, and symptoms develop several hours after consuming the food. In general, it requires a larger amount of food to trigger food intolerances than for food allergies and whilst undoubtedly unpleasant, food intolerances are never life threatening, unlike food allergies.

Table 1. Area of reaction and symptom [9]

Area	Symptom
Skin	Itching, swelling and redness
Gastrointestinal Tract	Pain, nausea, vomiting, diarrhoea, itching and swelling of the oral cavity
Respiratory Tract	Itching and swelling of the nose and throat, asthma
Eyes	Itching and swelling
Cardio-vascular system	Chest pain, abnormal heart rhythm, low blood pressure causing fainting and loss of consciousness

The World Health Organisation (WHO) reports that there are more than 70 foods that have been reported as causing food allergen reactions, however the EU has identified 14 major allergens, as detailed in box 1.

Box 1: List of EU food allergens [10]

Cereals containing gluten namely wheat (such as spelt and Khorasan wheat), rye, barley
Crustaceans and products thereof (for example prawns, lobster, crabs and crayfish)
Egg and products thereof
Fish and products thereof
Peanuts and products thereof
Soybeans and products thereof
Milk and products thereof (including lactose)
Nuts (namely almond, hazelnut, walnut, cashew, pecan nut, Brazil nut, pistachio nut and Macadamia nut (Queensland nut)
Celery and products thereof
Mustard and products thereof
Sesame seeds and products thereof
Sulphur dioxide and/ or sulphites at concentrations of more than 10mg/kg or 10mg/L (litre)
Lupin and products thereof
Molluscs and products thereof (for example mussels, clams, oysters, scallops, snails and squid)

The detriments to the quality of life for allergic consumers and their families has been well documented [11, 12, 13]. Although there are no boundaries on the groups of people food allergies effect, Gowland, [14] recognises that teenagers and people in their early twenties are particularly at risk, as they are beginning to make independent food choices and they are ill equipped in food preparation and have had little experience in dealing with the risk allergens pose. In addition, the vulnerability of this group is exacerbated by the struggle for social acceptance and that due to peer pressure; this group is more likely to engaging in risky behaviour particularly when they are out with friends [15].

Teenagers and people in their early twenties are particularly at risk, as they are beginning to make independent food choices and they are ill equipped in food preparation and have had little experience in dealing with the risk allergens pose.

From December 2014, all FBs have been required to provide information about allergenic ingredients used in foods sold or provided by them. This legal duties placed upon FBs are based on EU Food Information for Consumers Regulation (No.1167/2011) (EU FIC) and Commission Delegated Regulation (EU) No. 78/2014 amending Annex II of 1167/2011. This requirement states that food must be safe, authentic and properly labelled and the responsibility for this falls to the FBO; however, there is an expectation of some regulatory oversight. This is where the enforcement regarding allergens becomes complex and the issue is raised as to whether allergens are a food safety issue or a food standards matter. Allergen enforcement has taken two separate tracks, food safety, e.g. hygiene and contamination and food standards, e.g. labelling, authenticity and fraud [16].

For individuals who experience food allergies, avoidance is often the only solution as accidental allergen ingestion is potentially life threatening for many [17]. The conveying of information regarding allergens then becomes vitality important as eating away from the home has become more of a norm. This presents a particular problem for consumers with

For individuals who experience food allergies, avoidance is often the only solution as accidental allergen ingestion is potentially life threatening for many.

food allergies as the lack of information can lead to exposure of risk and/or unnecessary restrictions. In the UK Bailey et al, [18] reported that of 90 restaurant staff surveyed, 81% reported being confident in providing a safe meal for a food allergic customer yet 38% believed that consuming a small amount of an allergen was safe; Common et al, [19] found that out of 40 UK restaurant staff questioned, all respondents were comfortable and 65% were very comfortable in providing a safe meal, yet 25% believed that cooking food would prevent it causing an allergy – this would seem to justify the concerns that allergic consumers would have in eating away from the home.

Even after the introduction of the legislation, the UK's Royal Society for Public Health [20] established that takeaway food businesses were struggling to meet the new legal requirements: 66% of all takeaways failed to provide the legally required information on how customers can find the 14 allergens; over 50% were unable to state whether their food contained an allergen and 80% were not in possession of records stating whether allergens were present in ingredients.

The studies indicates there is a significant gap in the knowledge and practice of FBs around the provision of allergen safe food to customers. Therefore the aim of this research is to explore the understanding and practices of FBs in relation to food allergens.

The research was undertaken in the Borough of Knowsley which is located in the North West of England. The Borough is typical of a mid-sized urban district in the UK, although it is ranked 2nd in terms of deprivation in the UK. The Environmental Health team for the Borough of Knowsley are responsible for the enforcement of 750 takeaway FBs, which provide a range of cuisines to the local residents and visitors.

METHODS

Research Design

The research itself consisted of two different research elements. The first element of the research aimed to assess the knowledge and attitudes of FBOs surrounding allergens utilising questionnaires. The second element of the research was designed to explore the practises in place within the business and an allergen audit was undertaken.

Sampling

Using pragmatic sampling, from the list of 750 FBs every 25th FB was selected in order to achieve a sample size of 30 businesses. However, given the time and resources limitation for the survey only 21 FBs took part in both the questionnaire and audit elements of the research.

Questionnaires

The questionnaire was specifically developed by the authors for the purpose of this research considering the Food Standards Agency's guidance on food allergens. The questionnaire was designed to assess the knowledge and attitudes of FBOs and took on the style of a descriptive survey, which employed both closed questions and those based on a Likert scale. The questionnaire probed the FBOs level of knowledge regarding the requirements of the legislation and how clear/easy to understand these legislative requirements were. To gain insight into the knowledge FBO's have in being able to identify allergens they were provided with a grid of ingredients and asked to identify any that they believed should be highlighted as an allergen. The grid contained a list of 28 ingredients, of which 17 were classed as allergens. This included all 14 specified allergens plus three other commonly used items that contained one or more allergens (these items were: mixed nut powder; pasta and almond powder). Finally, the questionnaire explored how confident FBOs were in providing a safe meal and the factors that could cause issues for allergen sufferers.

Audit

The audit focused on three main areas:

- The display of information
- Allergen control in storage, kitchen, production and service areas
- Confidence in management (how well the guidance was understood by the FBOs)

In order to allow for a standard and consistent measure for these three areas, an allergen descriptor matrix was developed by the authors for this specific research study. (see box 2). The combined scores for each area of the audit then generated an overall allergen score for each premises. As each area has a highest score of four, the maximum overall score a FB could achieve would be 12. This approach was based around the current food hygiene intervention-rating scheme, which is a risk assessment to determine the frequency of interventions for FBs, and can be found in Annex 5 of the UK's Food Law Code of Practice. Although not its primary purpose, the audit also verifies the answers given in the questionnaire.

Box 2: Allergen Descriptor Matrix

Display of information	
score	**Guidance on scoring criteria**
4	High standard of compliance – clear and informative information displayed on the premises and on the takeaway menu providing the customer with all the necessary information
3	Information is displayed regarding allergens in some areas of the food business but not necessarily both on the premises and on the takeaway menu
2	The need for allergen information is recognised with 'please ask for more information' type signage.
1	Total non -compliance with statutory obligations – no, or very little information displayed on the premises and/or on the menu

Allergen control in storage, kitchen, production and service areas	
score	**Guidance on scoring criteria**
4	High standard of control – allergen ingredients are clearly labelled and separated avoiding the risk of cross contamination. There is documented control with regards to the purchasing of allergen ingredients
3	There is good evidence of labelling and separation to prevent cross contamination
2	The business makes some attempt to control allergens by basic separation and some labelling
1	There is total non-compliance with the Food Standards Agency guidance. Labelling is poor and cross contamination of allergens is evident

Confidence in management	
Score	**Guidance on scoring criteria**
4	FBO is knowledgeable and has an excellent understanding of allergens and legal requirements. They can confidently discuss processes in place to ensure compliance
3	FBO has good knowledge surrounding allergen requirements and the impact this has on the business
2	The FBO has some understanding, however there are obvious gaps in knowledge and understanding
1	There is no, or very little knowledge or understanding by the FBO of allergens, the requirements around them or the impact this has on the business

Data Collection

As this research was undertaken as part of the BSc (Hons) Environmental Health degree programme, prior to collection of any data, ethical approval was obtained from Liverpool John Moores University. The questionnaires were administered face to face with the FBOs and the audits undertaken at the same time. Visits to the food premises were carried out during weekdays and evenings in January/February 2018. The FBs were aware that the research was taking place, as they had previously been contacted directly by the Environmental Health team. However, none of the visit were pre-arranged and the researcher arrived unannounced at each of the premises.

Data Analysis

Data from the questionnaires and audits were analysed using Microsoft Excel. The descriptive statistics are used, as well as Pearson's' correlation co-efficient

RESULTS AND DISCUSSION

Results

Questionnaire Findings

There is a legal requirement under the legislation for FBs to display information regarding allergens in their businesses. When asked, 90% (n=18) of FBOs were aware of this requirement, with only 10% (n=2) of all FBO's providing the answer as no (total n=20).

The FBOs were then asked if the legislation relating to allergens was clear and easy to understand. 85% (n= 17) agreed or strongly agreed

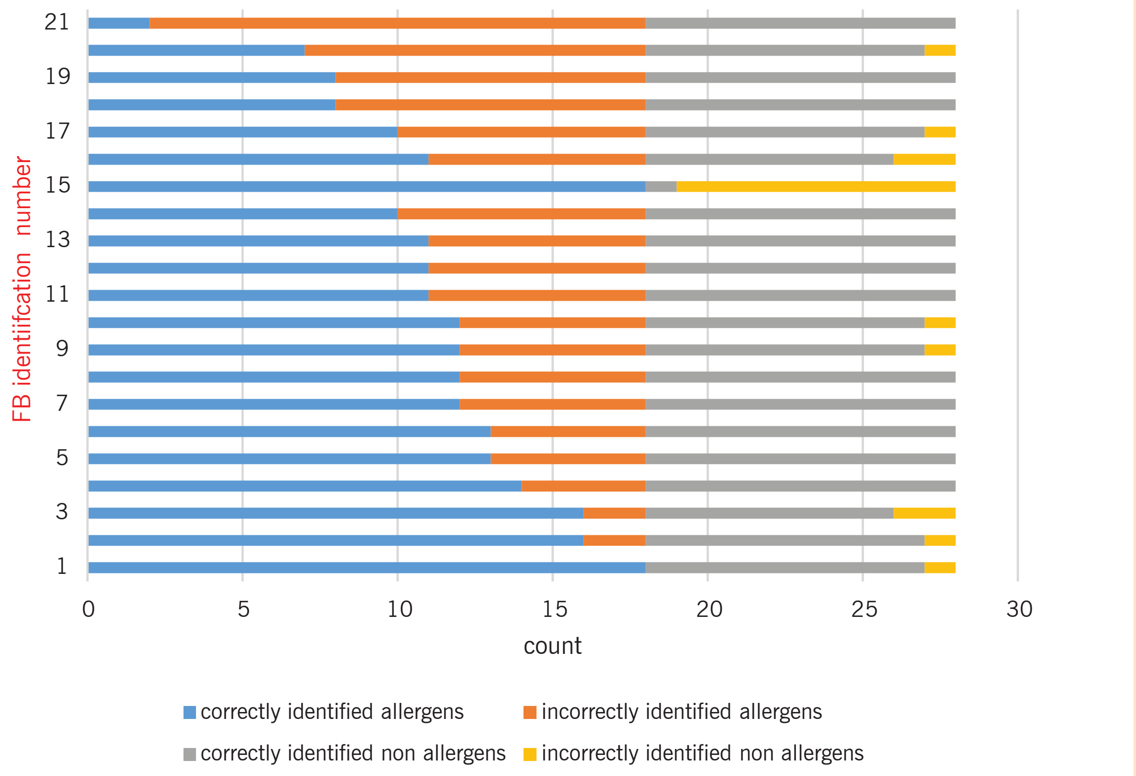


Figure 1.
Allergens and non-allergens correctly and incorrectly identified

that the requirements under the legislation are clear and easy to understand, with 15% (n=3) disagreeing (total n= 20).

Figure 1 shows the number of correctly identified allergens; incorrectly identified allergens; correctly identified non-allergens and incorrectly identified non-allergens. Of particular note is the one FBO who identified all 18 allergens correctly, but-also identified nine incorrectly.

When asked how confident they were in preparing a safe meal 67% (n=14) of FBOs stated they were very confident and 33% (n=7) said they were confident. None of the FBOs stated they were unconfident or very unconfident. (total n=21)

The FBOs were then asked a series of questions relating to the management of allergens and their impact.

Table 2 demonstrates that 23% (n=5) of FBOs thought that when eaten in small amounts, food allergens would be safe for allergen suffers to eat, whereas 77% (n=16) of FBOs did not (total n=21).

Table 2. FBO perceptions towards specific aspects of allergen safety management

	Strongly disagree		Disagree		Agree		Strongly agree		Total	
	No. FBs	% FBs	No. FBs	% FBs	No. FBs	% FBs	No. FBs	% FBs	No. FBs	% FBs
Safe to eat small amount of allergens	10	48%	6	29%	3	14%	2	9%	21	100%
Allergens destroyed by cooking	13	62%	6	29%	2	9%	0	0%	21	100%
Importance of cross contamination	0	0%	1	5%	10	48%	10	48%	21	100%

In addition Table 2 demonstrates that 9% (n=2) of FBOs thought that cooking would destroy food allergens, whereas 81% (n=19) of FBOs did not (total n=21).

Finally, Table 2 highlights that FBOs understand the importance of cross contamination, with 95% (n=20) agreeing that it can be a contributing factor to a dish becoming contaminated with and allergen and only 5% (n=1) disagreeing (total n=21)

Audit findings

The second element of the research was the audit of the premises and this part of the research was designed to explore the actual practises of the business. The highest overall score that each FB could obtain was 12 and Figure 2 shows the range of scores achieved by the FBs.

This data was then broken down further to determine the score achieved in each area of the audit. This is detailed in Figure 3.

Figure 2.
Overall allergen scores achieved

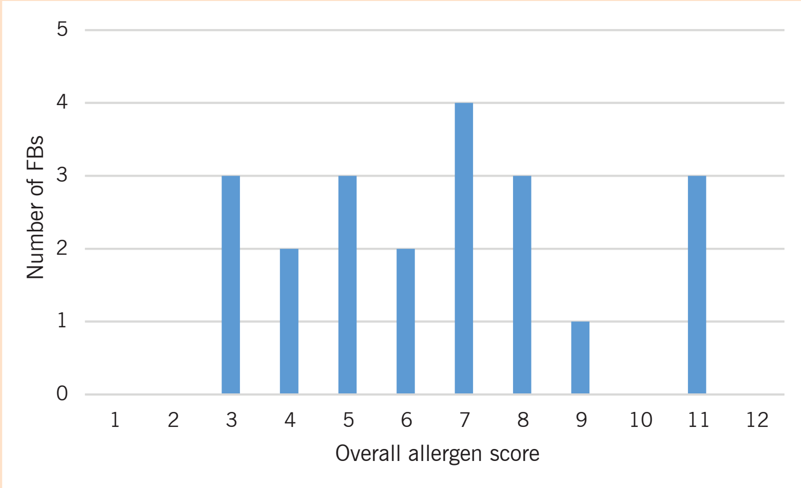
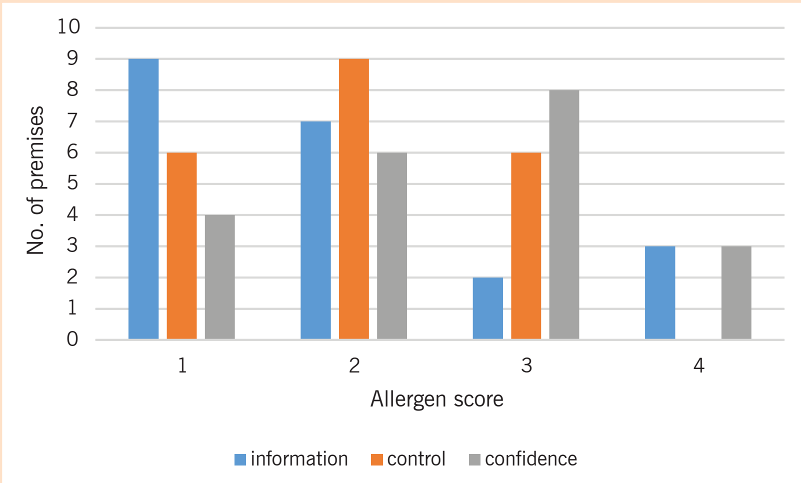


Figure 3.
Allergen scoring for all areas of the Allergen Audit



The score ranges from 1 (total non-compliance with guidance/ cross contamination is inevitable/ no or very little knowledge by the FBO surrounding allergens and the impact of them) to 4 (where a high standard of compliance was observed/allergens are clearly labelled to avoid cross contamination and there are documented controls with regards to purchasing/excellent knowledge and the FBO can confidently discuss processes in place to ensure compliance).

As Figure 3 shows, none of the premises visited receive the highest score available for allergen control and only 25% of FBs received the highest scores for display of information and confidence in management.

All the FBs involved in the research were registered with the local authority and as such, all (except one, which was a new business and was awaiting inspection) had a current Food Hygiene Rating System (FHRS) score. The FHRS generates a score based upon three elements: current level of compliance (hygiene); current level of compliance (structural) and confidence in management/control procedures. The FHRS score ranges from 0 (urgent improvement necessary) to 5 (very good).

To explore any relationship between the FHRS score and the allergen matrix score generated by this research, the FHRS score for each premises was identified. This was then plotted against the allergen matrix score for the premises, as shown in Figure 4.

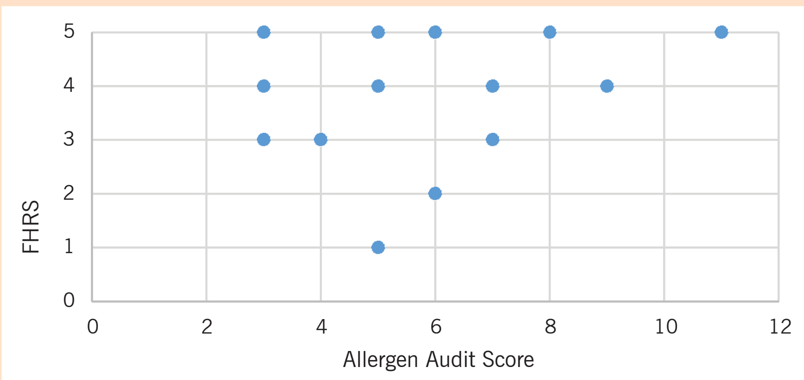


Figure 4.
Scatterplot of Food Hygiene Rating Scores against allergen audit scores

As shown in Figure 4 there does not appear to be a direct relationship between the allergen score and the FHRS score. Whilst the FBs with the highest scoring allergen matrix score did achieve the highest FHRS, so did a FB with one of the lowest allergen matrix scores. Indeed, the three lowest allergen matrix scores were found to be in FBs that were deemed acceptable, according to their FHRS scores.

The Pearson correlation co-efficient (r) for the above scatter plot is moderately positive (+0.40) and has a *p* value of 0.069, which indicates the relationship is not statistically significant.

DISCUSSION

All FBOs that participated in the study felt they were either confident or very confident in providing takeaway meals that were safe to eat for customers with stated food allergies. Yet the results of this study suggest that such a level of confidence may be misplaced, in line with the findings of Bailey et al. [21], Common et al [22] and the Royal Society for Public Health [23].

The vast majority of FBOs claimed to be aware of the legislative requirements to display food allergen information and that in general these requirements are clear and understandable. The allergen audit though, revealed that 33% (n=7) of premises provided only the most basic information and that a further 43% (n=9) had no or very little information on display. This apparent contradiction means that customers who would rely on the information provided by the FBO are being potentially mislead and misinformed as to the presence of allergens in the food provided.

Even if a customer advises an FBO that they suffer from a food allergy, there is still the potential that they are putting themselves at risk. As previously stated, all the FBOs that participated felt they were either confident or very confident in providing takeaway meals that were safe to eat for customers with stated food allergies. The questionnaires and audits would challenge this: 23% (n= 5) believed that it is safe for an allergen sufferer to consume a meal, if only a small amount of the allergen is present; 9% (n=2) assumed that food allergens could be destroyed through the cooking process.

If cross contamination is not effectively controlled in the kitchen then customers are exposed to a significant level of risk.

The risk is that FBOs who hold these beliefs and make these assumptions are endangering the health of their customers; customers who think that by consuming food from establishments display allergen information and informing the FBO of their food allergy, that they have taken appropriate steps to protect themselves from harm.

Overall the FBOs demonstrated a good knowledge of the food allergens themselves and 95% (n=20) were aware that cross contamination was a contributing factor for a dish to contain a food allergen. This knowledge and awareness was not perfect, with none of the FBOs being able to correctly identify all the allergens and non-allergens presented to them, indeed one FBO appeared to simply tick every box. Perhaps of greater concern is that whilst cross contamination was identified as an important factor none of the FBOs achieved the highest mark in the audit for allergen control, with 43% (n=9) just making a minimum effort and 29% (n=6) showing no level of control at all. This is perhaps the key finding for the research. It highlights that regardless of the information provided to the customer and then awareness of the FBOs, if cross contamination is not effectively controlled in the kitchen then customers are exposed to a significant level of risk. The gap between the confidence expressed by FBOs in their ability to provide safe meals and their actual practice poses a clear health risk to customers with food allergies.

In addition, the research found that the level of food hygiene in the FBs was not directly related to their practice and awareness around allergens – although it must be noted that the relatively small sample size could have influenced this value. This could lead to further risk for customers, who may assume that because a FB has a good FHRS score that the FB is able to adequately provide food that is allergen free. It creates a situation where hygienic food is not necessarily safe food – justifying the question as to whether allergens are a food safety issue or a food standards matter.

Limitations

The research utilised small sample of FBs (n=21). It must also be acknowledged that this reported level of confidence may have been generated as a result of the Hawthorne Effect – were participants provide answers they believe that the research is seeking. This is perhaps especially relevant, since the FBOs may have felt a “wrong” answer could have led to formal action against them. However, in order to limit this, it was made clear to the FBOs that the research was part of a University research project and that the researcher was not part of the Environmental Health team.

CONCLUSION

The introduction of legislation seems to have made little difference to the level of compliance found within FBs. It can be suggested that the introduction of it has had little effect on FBs, other than perhaps the cosmetic change of the putting up of a sign, stating that food produced on the premises may contain allergens. This alone does not make the FBO compliant and possibly instils a dangerous sense of false security for customers with food allergies.

Hygienic food is not necessarily safe food.

Those cases that are reported and brought to the attention of enforcement staff, and then afterwards the public, are often the ones that have resulted in fatalities. Yet how many “near misses” occur that are never notified? For any legislation to be effective and to result in behaviour change, it must be adequately enforced and resourced. In the UK, enforcement officers were encouraged to adopt an advisory approach when the legislation commenced, switching to a more formal approach after 12 months. However, the lack of any tools (such as the audit tool developed for this research) to gauge the level of compliance within a FB; the lack of any incident reporting mechanism and the confusion as to whether this is a food safety or food standards issue, leaves customers vulnerable and exposes them to an unacceptable level of risk.

REFERENCES

[1] Perspectives in Public Health (2013) Reactions, Regulations and Risk: protecting consumers with food allergies and intolerances. Vol. 133, No. 6.

[2] Bailey, S., Albardiaz, R., and Smith, H. (2011) Clinical and experimental Allergy. Restaurant staff knowledge of anaphylaxis and dietary care of people with allergies.

[3] Begen, F., Barnett, J., Payne, R., Roy, D., Gowland, H., Lucas, S. (2016) Consumer Preferences for Written and Oral Information about Allergens When Eating Out.

[4] Food Standards Agency (2016) Eating out with a food allergy. A consumer survey carried out by the FSA in partnership with Allergy UK.

[5] World Health Organisation (2003) International Food Safety Authorities Network (IFOSAN). IFOSAN Note No. 3/2006 – Food Allergies.

[6] Gupta, R., Shelik, A., Strachan, D.P., Anderson, H.R. (2007) Time Trends in Allergic Disorders in the UK.

[7] Food.gov.uk. (2017). *Allergen information for loose foods*. [online] Available at: https://www.food.gov.uk/sites/default/files/multimedia/pdfs/publication/loosefoodsleaflet.pdf [Accessed 20 Feb. 2018].

[8] Wang, J., and Sampson, H. (2011) Food Allergy. The Journal of Clinical Investigation.

[9] World Health Organisation (2003) International Food Safety Authorities Network (IFOSAN). IFOSAN Note No. 3/2006 – Food Allergies

[10] Food.gov.uk. (2017). *Allergen information for loose foods*. [online] Available at: https://www.food.gov.uk/sites/default/files/multimedia/pdfs/publication/loosefoodsleaflet.pdf [Accessed 20 Feb. 2018].

[11] Bacal, L.R. (2013) The impact of food allergies on quality of life.

[12] Lieberman, J.A., and Sicherer, S.H. (2011) Quality of life in food allergy.

[13] Watson, W., Woodrow, A., Stadnyk, A. (2013) Persistence of peanut allergen on a table surface.

[14] Gowland, H. (2002) Food allergen avoidance: risk assessment for life.

[15] Food Allergy & Anaphylaxis Connection Team (2017) Risk-Taking and Teens with Food Allergies.

[16] Gowland, H., and Walker, M. (2014) Food allergy, a summary of eight cases in the UK criminal and civil courts: effective last resort for vulnerable consumers?

[17] Begen, F., Barnett, J., Payne, R., Roy, D., Gowland, H., Lucas, S. (2016) Consumer Preferences for Written and Oral Information about Allergens When Eating Out.

[18] Bailey, S., Albardiaz, R., and Smith, H. (2011) Clinical and experimental Allergy. Restaurant staff knowledge of anaphylaxis and dietary care of people with allergies.

[19] Common, I., Corrigan, C., Smith, H., Bailey, S., Harris, S., and Holloway, J. (2013) Journal of Allergy & Therapy, Vol. 4.

[20] Royal Society for Public Health (2015) Allergy sufferers put at risk from fast food takeaways.

[21] Bailey, S., Albardiaz, R., and Smith, H. (2011) Clinical and experimental Allergy. Restaurant staff knowledge of anaphylaxis and dietary care of people with allergies.

[22] Common, I., Corrigan, C., Smith, H., Bailey, S., Harris, S., and Holloway, J. (2013) Journal of Allergy & Therapy, Vol. 4.

[23] Royal Society for Public Health (2015) Allergy sufferers put at risk from fast food takeaways.

Original scientific article

Health safety of water for human consumption in the city of Čabar in the period of 2012-2016

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ABSTRACT

Introduction: The water supply network of the Čabar area (Čabar is a little place in Gorski kotar, mountain part of Croatia) has been carrying bacteriological contamination for many years. The aim of the paper is to present a five-year microbiological profile of drinking water used for supplying the field area. **Methods:** The collected data for Čabar, Gerovo, Prezid and Tršće water sourses were obtained from the Department of Public Health, Primorsko-Goranska County. Data were statistically processed and interpreted for the period from January the 1st 2012. to December the 31 st2016. The number of tested samples is 435 and the analyzed parameters are temperature, color, turbidity, pH, conductivity, KMnO4 consumption, ammonia, coliform bacteria, *Escherichia coli, Enterococci*, colonies at 37 °C and colonies at 27 °C, *Pseudomonas aeruginosa* and free chlorine. **Results:** The highest number of bacteriologically defective samples in the five-year period was in Čabar's water supply with 33% of defective samples compared to the total number of measurements and the Gerovo water supply with 29%. The contaminated samples contain *Escherichia coli* (fecal contamination indicator) and are most present in the Čabar water supply (28%), followed by Gerovo (24%), Tršće (10%) and Prezid (9%). **Conclusion:** Due to fecal contamination, these water supply systems are unsafe for human health because they are the potential causes of the hydric epidemic and need to continue to invest in the protection of sources and sanitary-technical maintenance of the water supply system with regular chlorination.

Key words: fecal contamination, water supply, water for human consumption, health sanitation

INTRODUCTION

Human's need for water, especially for drinking, is constantly increasing due to urbanization, industrialization, population growth and irrational consumption. For the last hundred years, the use of water for human consumption has increased 7 times, mostly due to the needs of industry and food production. Sea and oceans make up 97.5% of the total amount of water on Earth while fresh water accounts for 2.5% [1]. Out of these available fresh water supplies it fell 1.74%. It results that 0.76% of the total mass of fresh water on Earth, which is not all environmentally acceptable for use. Carnations and constant snow cover about 10% of the world's mainland and contain about 70% of the world's quantity of fresh water [1]. The largest amount of available fresh water that can be used for water supply is land-flowing water. Groundwater is the largest resource of drinking water and makes 90% of the available world resources [1]. Millions of people all around the world suffer and may die from diseases caused by water contamination. The largest number of victims are children in developing countries, particularly in Asia and Africa [2]. Because of the large number of diseases and mortality of people due to lack of supply with potable water and non-hygienic conditions, water is considered to be the leading cause of mortality among environmental causes. Precisely because of the great link between health and sanitary water, water management is part of public health [3]. Potable water is considered to be healthy and good if it has good sensory properties, which means it must be free of mute, smell and color . In addition to satisfactory sensory properties it should not contain, in a number that would pose a risk to human health, parasites, microorganisms, as well as their developmental forms. It should not contain harmful substances that may be harmful to human health [3].

Because of the large number of diseases and mortality of people due to lack of supply with potable water and non-hygienic conditions, water is considered to be the leading cause of mortality among environmental causes.

Water intended for human consumption is, according to the Law on Water for Human Consumption (OG 64/15), which has been in its original state or after processing intended for drinking, cooking or other household purposes, irrespective of its origin and irrespective of whether they come from a water tank, tank, or public water supply system. This is also the water used in food-producing industries that are produced, processed or placed on the market of products or substances intended for human consumption unless it has been determined by the competent authority that the water quality can not be affected in its final form, on food safety [4]. Potable water quality, apart from the purity and quality of the water source itself, also depends on the way the water is purified and disinfected, as well as on the quality of the water supply system itself and the water supply network. Healthy water quality depends on the sanitary-technical and hygienic conditions that must be met to ensure that the water used for supplying the population is healthy [5].

The water supply system implies a water supply system for the human consumption, which constitutes a watercourse (arranged and protected), a conditioning device, a pumping station and a water supply system, and a main supply pipeline and a water supply system for transporting water from the waterworks to the consumer [4].

In Primorsko-Goranska County, for the needs of the Ministry of Health, the drinking water is controlled by the Institute of Public Health of Primorsko-Goranska County. The Department that performs control within the Institute is the Department for Control of Drinking Water and Water in Nature. According to the Ministry of Health's instructions only processed water is controlled at the place of consumption. Water samples are mostly taken in public facilities, for example in schools and in public shows. All results obtained by testing are regularly sent to the water supply, sanitary inspection and the Ministry of Health. The results are also publicly available to everyone on the Institute's website: www.zzjzpgz.hr. Gorski kotar occupies the western part of the Republic of Croatia and a third of the land area of the Primorsko-Goranska County. It is naturally very rich in water. Lake Lokvarsko, Lepenica and Bajer are Lake Gorski kotar. The water wealth is crowned by the watercourses of the river Kupa, Čabranka and Dobra. It belongs to the most exquisite parts of the state. Relatively speaking, it is distinguished and characterized by its low population density and its prominent depopulation. Although it belongs to the Primorsko-Goranska County, where there is an excellent water supply system, a number of samples have been observed for a long period of time in analyzes of water for human consumption [6]. The water supply of the Čabarsko area is characterized by a large number of smaller water supply systems, which are concerned with sources of less tolerance. Sanitary-technical maintenance of such systems as well as disinfection is greatly hampered by dispersion and multiplicity. During 2012, the first phase of installation of automatic chlorine in the area of Prezid and Tropet-Parga began. This has greatly improved water quality. The second phase of construction was continued in 2013. It was supposed to be for the area of Mandla-Žagar-Plešac and Gerovo, but only in the territory of Gerovo and Tršće the main part was realized [7, 8].The aim of this paper is to present the status of the water supply system of the Čabar area in the five-year period from January the 1st 2012. to December the 31st 2016. The water supply network of Gorski Kotar and, therefore, the areas of Čabar belonging to it, have for a long time followed a bad picture of health testing of water for human consumption showing the microbiological contamination of the water supplying the area. By reviewing the five-year results, it will be apparent how much the change in the legal regulations as well as the installation of automatic chlorinates have influenced the health of drinking water and how it was in the past five years.

The water supply of the Čabarsko area is characterized by a large number of smaller water supply systems, which are concerned with sources of less tolerance. Sanitary-technical maintenance of such systems as well as disinfection is greatly hampered by dispersion and multiplicity.

MATERIALS AND METHODS

All collected data were analyzed in the Department of Public Health Primorsko-Goranska County. The data obtained are a summary of total measurements during the five-year period from January the 1st 2012 to December the 31st 2016. For the purposes of this paper, the data are systematized in four parts by analyzing microbiological status of samples of the potable water of four water sourses of Čabar area for Čabar, Gerovo, Prezid and Tršće. All water samples were taken at places of consumption, most commonly in public parlors, kindergartens or schools. Samples were taken most frequently once a month by 2013, while from 2014 to 2016 the number of samples was dependent on the amount of water delivered in m^3/day. In the data obtained a total of 1,107 measurement results for Čabar, 1,899 results for Gerovo, 2,210 results for Tršće and 2,218 results for Prezid within the examined five-year period were analyzed. The collected data for Čabar, Gerovo, Prezid and Tršće water sourses were obtained from the Department of Public Health, Primorsko-Goranska County. Data were statistically processed and interpreted for the period from January the 1st 2012. to December the 31st 2016. The number of tested samples is 435 and the analyzed parameters are temperature, color, turbidity, pH, conductivity, KMnO4 consumption, ammonia, coliform bacteria, Escherichia coli, Enterococci, colonies at 37 °C and colonies at 27 °C, *Pseudomonas aeruginosa* and free chlorine.

RESULTS

Table 1 shows the values of indicators obtained by the health check of human health water quality for Čabar watercourse in the period from January the 1st 2012. to December the 31 st2016. For the sake of better visibility, red paint has been shown to show defective number of samples. 64 samples were tested in the Čabar waterway during the five-year period. The water temperature in the five-year period did not exceed the permissible value of 25 °C, which means that the water complied with the prescribed values of the Ordinance. The minimum measured water temperature was 6.5 °C, while the maximum measured temperature was 18.0 °C. The water color was satisfactory and in all measurements it was 0 mg/L Pt/Co, while the maximum permissible concentration was 20 mg/L Pt/Co. pH values are optimal for water for human consumption. The minimum measured value was 7.7, while the maximum measured value was 8.3. The measured values did not exceed the maximum permissible value of 9.5. The conductivity of the samples ranged from 243 μS/cm/20 °C (min.) up to 437 μS/cm/20°C (max.) and it is a characteristic for the water from the river Čabranka. The maximum permissible concentration is 2500 μS/cm/20 °C. Based on this we conclude that the water complied with the prescribed conductivity values. Utility KMNO4 (potassium permanganate), which is an indicator of the presence of organic substances in water consuming oxygen for oxidation, was low and did not exceed the maximum permissible concentration of 5.00 mg/L O_2. The minimum measured value was 0.31 mg/L O_2, while the maximum measured value was 3.54 mg/L O_2. Values for ammonia in water samples were low. The maximum measured value was 0.012 mg/L NH_4, much lower than the maximum permissible concentration of 0.500 mg/L NH4 given in the ordinance. The only microbiological parameter that did not have a defective sample was Pseudomonas aeruginosa but it started to be controlled as an indicator until 2014, so it should be taken into account that these measurements are not the values of this indicator for the period from 2012 to 2013 [9].

Table 1. Results of the health check results of the Čabar water supply from January the 1st 2012 to December the 31st 2016

Indicators	Measuring unit	Total	Min.	Max.	MDK	Incorrect samples
Water temperature	°C	18	6.5	18.0	25.0	0
Water colour	mg/L Pt/Co	61	0	0	20	0
Water turbidity	NTU	61	0.32	18.60	4.00	4
pH value	pH value	61	7.7	8.3	9.5	0
Water conductivity	μS/cm/20 °C	61	243	437	2500	0
Consumption of $KMnO_4$	mg/L O_2	61	0.31	3.54	5.00	0
Ammonium	mg/L NH_4	61	0.000	0.012	0.500	0
Coliform bacteria	Broj/100 mL	64	0	1300		21
Escherichia coli	broj/100 mL	64	0	1300		19
Enterococci	broj/100 mL	64	0	260		17
Colonies at 37°C	broj/1 mL	62	0	560	20	16
Colonies at 22°C	broj/1 mL	62	0	560	100	13
Pseudomonas aeruginosa	broj/100 mL	14	0	0		0
Free chlorine	mg/L	64	0.00	0.86	0.50	6

Table 2. Results of the health examination of the Tršće water supply system from January the 1st 2012 to December the 31st 2016

Indicators	Measuring unit	Total	Min.	Max.	MDK	Incorrect samples
Water temperature	°C	23	9.9	17.3	25.0	0
Water colour	mg/L Pt/Co	134	0	0	20	0
Water turbidity	NTU	134	0.17	5.60	4.00	3
pH value	pH jedinica	134	7.9	8.5	9.5	0
Water conductivity	μS/cm/20 °C	134	166	438	2500	0
Consumption of $KMnO_4$	mg/L O_2	134	0.00	2.10	5.00	0
Ammonium	mg/L NH_4	134	0.000	0.013	0.500	0
Coliform bacteria	Broj/100 mL	134	0	640		14
Escherichia coli	broj/100 mL	134	0	390		14
Enterococci	broj/100 mL	134	0	80		9
Colonies at 37°C	broj/1 mL	125	0	680	20	12
Colonies at 22°C	broj/1 mL	125	0	1000	100	6
Pseudomonas aeruginosa	broj/100 mL	11	0	2		1
Free chlorine	mg/L	134	0.00	4.40	0.50	24

Table 3. Overview of the results of the medical examination of the Prezid water supply system from January the 1st 2012 to December the 31st 2016

Indicators	Measuring unit	Total	Min.	Max.	MDK	Incorrect samples
Water temperature	°C	25	3.3	18.0	25.0	0
Water colour	mg/L Pt/Co	120	0	0	20	0
Water turbidity	NTU	120	0.10	6.20	4.00	3
pH value	pH jedinica	120	7.3	8.4	9.5	0
Water conductivity	µS/cm/20 °C	120	328	442	2500	0
Consumption of $KMnO_4$	mg/L O_2	120	2.00	3.53	5.00	0
Ammonium	mg/L NH_4	120	0.000	0.012	0.500	0
Coliform bacteria	Broj/100 mL	123	0	260		12
Escherichia coli	broj/100 mL	123	0	260		11
Enterococci	broj/100 mL	123	0	300		9
Colonies at 37°C	broj/1 mL	116	0	120	20	7
Colonies at 22°C	broj/1 mL	116	0	560	100	3
Pseudomonas aeruginosa	broj/100 mL	15	0	0		0
Free chlorine	mg/L	123	0.00	4.30	0.50	5

DISCUSSION

Water for human consumption can be influenced by various pollutants as a potential source of hydrocypic epidemic, which is a major threat to the general population. This suggests that great care and supervision of a water-based system depends on many factors, and this task is extremely complex and belongs to the public health field of action [10]. The water network of Čabar area has been carrying bacteriological contamination for many years. This paper analyzes the health and safety of water for the consumption of water supply Čabar, Gerovo, Tršće and Prezid in a five-year period from January the 1st 2012 to December the 31st 2016. All these sites are supplied with water from the Central Water Supply System. The parameters for water temperature (°C), color (mg/L Pt/Co), turbidity (NTU), pH value (pH unit), conductivity (µS/cm/20 °C), $KMnO_4$ (mg / L (number/100 mL), number of colonies at 37 °C and number of colonies at 22 °C (number/1 mL), Pseudomonas aeruginosa (number/100 mL) and free chlorine (mg/L). The water for human consumption in all four water sources met the requirements of the Ordinance for basic physio-chemical parameters. The measured water values for water temperature, color, conductivity, consumption $KMnO_4$ and ammonia did not exceed the maximum permissible concentrations during the period from 01.01.2012 until 31.12.2016. Based on this, we conclude that the water used for supplying the field area was safe in all measurements for the specified parameters. The water was inadequate in 10 measurements. In Čabar 3 watercourse, they are a health defective sample due to the turbidity of the water. In the water supply of Gerovo water turbidity was the cause of malfunction in one measurement. The turbidity exceeded the permissible values in three measurements for the Prezid watercourse as well as for the Tršće watercourse, where there are also only three defective samples of turbidity during a five year period. During the five-year period in four watercourses, water turbot has emerged as a cause of malfunction of water in a small number, therefore it is not a problem for water used for supplying the field area. Water pH values in most measurements were correct and characteristic for the water of this area having a naturally lower pH value. Only in the Gerovo watercourse the pH value of the samples did not meet the requirements of the Ordinance in three measurements in five years [11].

CONCLUSION

Comparing the number of bacteriological inadequate samples with respect to the number of tested samples of the four water sources we can conclude that the water supply Čabar has the largest number of bacteriologically inadequate water samples in the five year period from January the 1st 2012 to December the 31st 2016. Out of a total of 64 tested bacteriologically inadequate samples were found in the Čabar water supply. This means that the water for human consumption of the Čabar water supply was bacteriologically inadequate in 33% of the measurements.

REFERENCES

[1] Mayer, D. (2004) Voda od nastanka do upotrebe. Zagreb, Prosvjeta.

[2] World Health Organization (2000) Global Water Supply and Sanitation Assessment. Geneva.

[3] Vitale, K. i sur. (2010) Uloga sanitarno-inspekcijskog nadzora vode u osiguranju javnozdravstvene zaštite u Republici Hrvatskoj. *Med Jad*. 40(3-4);75–83.

[4] Narodne novine (2015) Zakon o vodi za ljudsku potrošnju. Zagreb: Narodne novine d.d.

[5] Nastavni Zavod za javno zdravstvo Primorsko-goranske županije. Zdravstvena ispravnost vode za piće na području Primorsko-goranske županije u 2016. godini. 2016.

[6] http://www2.pgz.hr/pozivi_skupstina/13-17/skupstina22/TOCKA1-PRILOG2.pdf., pristupljeno 11.08.2017.

[7] Nastavni Zavod za javno zdravstvo Primorsko-goranske županije. Zdravstvena ispravnost vode za piće na području Primorsko-goranske županije u 2013. godini. 2013.

[8] Vukić Lušić, D., Đandara, A. i sur. (2017.) Zdravstvena ispravnost vode za piće u Gorskom kotaru u petogodišnjem razdoblju od 2011. od 2015. *Medicina Fluminensis.* 53;216–224.

[9] Nastavni Zavod za javno zdravstvo Primorsko-goranske županije. Zdravstvena ispravnost vode za piće na području Primorsko-goranske županije u 2014. godini. 2014.

[10] Shiklomanov, I.A., Rodda, J.C. (2003) World water resources at the begining of the TwentyFirst century. Cambrige, Cambrige University press, UNESCO.

[11] Nastavni Zavod za javno zdravstvo Primorsko-goranske županije. Zdravstvena ispravnost vode za piće na području Primorsko-goranske županije u 2015. godini. 2015.

Original scientific article

The influence of temperature, disinfection and water softening of drinking water on the multiplication of *Legionella pneumophila*

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ABSTRACT

The influence of temperature, disinfectant and water softener for drinking water preparation on the bacterial growth of *Legionella pneumophila* was examined. The optimal temperature for bacterial growth was 36 °C. At the incubation temperature of 15 °C the multiplying of bacteria slowed down, as confirmed by the bacteriostatic effect of this temperature. The number of bacteria was after the first 24 h of incubation at 55 °C reduced by more than 6 log in comparison to the culture at 36 °C. After 10 minutes of exposure to the disinfectant dichloroisocyanuric acid, the number of bacteria in the culture decreased for 1.4 log CFU mL^{-1}, followed by an intensive growth immediately after the disinfectant degradation. The number of bacteria was up to 72 hours incubation even higher than in the control sample without a disinfectant, and then the number in both samples equalled at approximately 7.3 log CFU mL^{-1}. Sodium polyphosphate used as a water softener stimulated the bacterial growth. The largest difference in growth was observed after 72 h of incubation, which was 1.9 log CFU mL^{-1} higher in samples with sodium polyphosphate in comparison to those without the softener.

Key words: *Legionella pneumophila;* growth curve; temperature; water softener; disinfectant

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INTRODUCTION

Legionellae are Gram-negative bacteria found in freshwater environments [1]. Problems can occur in buildings where the water temperature is between 25 °C and 42 °C, which are ideal conditions for the reproduction of these bacteria [2]. Many studies have shown that *Legionella pneumophila* may be found in water storage and distribution systems as well as in the recirculating cooling water of air-conditioning systems [3]. There are over 50 different species of *Legionella* and more than half of them can cause Legionnaires' disease and/or Pontiac fever [4, 5]. *L. pneumophila* causes more than 90 % of legionellosis [6, 7]. In 2015 ECDC reported 7034 cases of legionellosis in Europe. *L. pneumophila* serogroup 1 was the most often confirmed pathogen, accounting for 679 of 834 (81%) culture-confirmed cases [8]. The presence of these bacteria in water distribution systems, hospitals, hotels, and spas is a significant problem all around the world [9, 10].

L. pneumophila often survives drinking water preparation and processing procedures and can therefore be found in plumbing systems [11]. First it enters these systems in low numbers and then starts to reproduce rapidly due to favorable temperature conditions [12]. If the presence of *Legionella* bacteria in a facility's drinking water has been confirmed, different procedures for their elimination can be used. The most commonly carried out disinfection procedures use chlorine or heat shock. Heat shock involves flushing the system with water heated to temperature of at least 70 °C for 30 minutes. The water temperature must be 60 °C at the tap most distant from the storage tank [13]. Zhang et al. [14] reported that chlorination was an effective method for killing *L. pneumophila* in water distribution systems, however, the results suggest that the bacteria were not completely removed even after 40 months of constant chlorination. Other preparations for the chemical disinfection of water involve chloramine, chlorine dioxide, bromine, ionization of silver and copper ions, and ozone. Chloramine is more stable and penetrates deeper into biofilms in comparison to chlorine [15]. Ionization of copper and silver ions is an effective method of destroying bacteria [16] but Rohr et al. [17] pointed out that prolonged usage leads to reduced susceptibility of *Legionella* bacteria. In large plumbing systems, hot water is often added to soften water. Water softening is carried out with the intention of avoiding the precipitation of lime scale from the water which is deposited on the equipment, storage tanks, pipes, and taps [18].

Due to the above-described processes and preparation of drinking water in water supply systems, the aim of our study was to verify the influence of different conditions on the multiplication and survival of *Legionella pneumophila*. We studied the effects of various physical and chemical factors (temperature, added disinfectants and water softener) on the survival and reproduction of *Legionella pneumophila*.

The results can be used for further improvement of prevention measures against *Legionella* bacteria that could have more important public health and economic impacts. The limitation of the study is that we did not use different concentrations of softeners and disinfectants.

If the presence of Legionella bacteria in a facility's drinking water has been confirmed, different procedures for their elimination can be used. The most commonly carried out disinfection procedures use chlorine or heat shock.

Zhang et al. reported that chlorination was an effective method for killing *L. pneumophila* in water distribution systems, however, the results suggest that the bacteria were not completely removed even after 40 months of constant chlorination.

METHODS

Bacterial strain

The standard strain *Legionella pneumophila* subsp. *pneumophila* ATCC 33153 (Czech Collection of Microorganisms, Brno, the Czech Republic) was used for studying the growth curve and for determining the impact of temperature, dichloroisocyanuric acid and sodium polyphosphate on bacterial growth. Cultivation took place according to the relevant ISO standard [19].

Bacterial growth

To study the growth curve of the test strain of *L. pneumophila* in different conditions, the method of counting bacterial colonies (CFU mL^{-1}) that grew aerobically after incubation on a solid medium was used [20]. First, bacteria were cultured in test tubes in a liquid medium of Yeast Extract Broth (YEB) (Fluca Analytica, India) with 5 mg L^{-1} of cysteine (Biolife, Italy) broth. During 120 h of incubation at 36 °C in aerobic conditions, 1 mL of overnight culture with a concentration of 5.7 log CFU mL^{-1} was taken from the tubes every 24 h. The serial 10-fold dilutions were prepared in a saline solution and 1 ml of each dilution was mixed with a melted Buffered Charcoal Yeast Extract (BCYE) medium (Biolife, Italy) with cysteine, using the plate count method [20]. After incubation, the colonies growing on the solid medium were counted and the results were expressed as CFU mL^{-1}.

The same procedure was used also for detecting bacterial concentration in the culture exposed to different incubation temperatures, a chlorine disinfectant and a water softener with sodium polyphosphate. The results were calculated and expressed as log CFU mL^{-1} [21].

The influence of temperature on bacterial growth

The growth curve of the bacterial strain was conducted at three different incubation temperatures: 36 °C, which was optimal for bacterial growth, and at the unsuitable temperatures of 15 °C, and 55 °C.

The effect of dichloroisocyanuric acid on bacterial growth

The effect of disinfectants on the growth of the test bacteria was measured by adding dichloroisocyanuric acid. The bacterial culture of *L. pneumophila* were added in YEB with a concentration of 5.7 log CFU mL^{-1}, with added dichloroisocyanuric acid (Oasis, England) with a concentration of 17 mg L^{-1}. This concentration of disinfectant is recommended by the producer of the product (Oasis), as effective for the destruction of micro-organisms in drinking water, so we did not change it. The pH values of bacterial cultures were measured after adding the disinfectant using the Cyberscan pH11 RS232 Meter (Eutech Instruments, Singapore) according to the producer's instructions. Samples were taken after 0 minutes, 10 minutes and then after 24, 48, 72, 96, and 120 h of incubation at 36 °C, to determine the number of bacteria (CFU mL^{-1}). For each selected time, two samples (two parallels) were taken, representing 14 samples in an experiment with added dichloroisocyanuric acid and 14 samples without added dichloroisocyanuric acid. The experiment was repeated three times (42 samples with and 42 without disinfectant), which together represented 84 samples. The bacterial culture was exposed to dichloroisocyanuric acid for 10 minutes. For negative control, the bacteria were grown under the same conditions without exposing them to dichloroisocyanuric acid.

The effect of sodium polyphosphate softener on bacterial growth

The sodium polyphosphate (Microfos SH, TKI Hrastnik, Slovenia) water softener in standard concentrations [22] was added to the broth culture. The softener with a concentration of 5 mg L^{-1} was added to the fresh YEB broth with cysteine and overnight bacterial culture with a concentration of 5.7 log CFU mL^{-1}. We used the product Microfos SH (TKI Hrastnik, Slovenia) in a concentration, as prescribed by the softener manufacturer [23]. Samples were taken to determine the number of bacteria (CFU mL^{-1}) after 0, 24, 48, 72, 96 and 120 h of incubation at 36 °C. Free chlorine in the broth culture was measured with Colorimeter (Chlorine Test Kit, HACH, Floriffaux, Belgium) during incubation. For each selected time, two samples (two parallels) were taken, experiment was repeated three times.

Statistical analysis

R software version 3.1.3 was used for statistical analysis. The results were analysed using a paired Student t-test. The significance level was set at $p < 0.05$.

RESULTS AND DISCUSSION

The effect of temperature on the growth of *L. pneumophila*

Figure 1 shows the growth curve of *L. pneumophila* at different incubation temperatures. After the first 48 h of incubation at 36 °C, bacteria were in the logarithmic growth phase. From the beginning of the experiment to the stationary phase, the number of bacteria increased by about 3 log CFU mL^{-1}. Their number after 120 h of incubation was 7.4 log CFU mL^{-1}. At the incubation temperature of 15 °C the multiplying of bacteria slowed down, as the number of the initial value of 5.6 log CFU mL^{-1} culture did not significantly change, hence confirming the bacteriostatic effect of those temperatures. Bactericidal activity was defined as a 3 log10 decrease in CFU/ml (99.9 % kill-effect). Bacteriostatic activity was defined as <99.9 % kill of bacterial cells after exposure to an antimicrobial [24]. The reproduction of bacteria at 15 °C was minimal, which has also been confirmed by other studies [3, 25]. The number of bacteria incubated at 55 °C after the first 24 h was reduced by more than 6 log in comparison to the culture at 36 °C. After 48 h, their concentration decreased from 5.7 log CFU mL^{-1} to 0.1 log CFU mL^{-1}. After 72, 96, and 120 h of incubation, the number of colonies that grew on the solid media varied from 0 to 3 CFU mL^{-1}.

The reproduction of bacteria at 15 °C was minimal, which has also been confirmed by other studies.

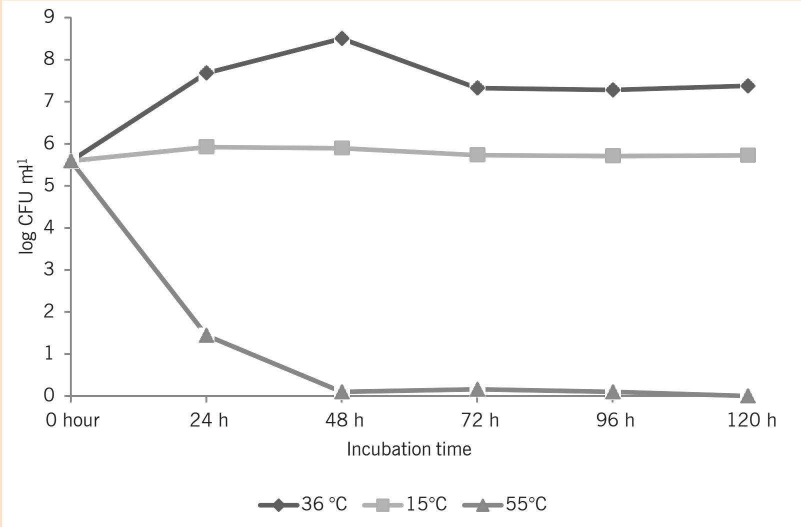

Figure 1.
Growth curve of the bacterium *L. pneumophila* subsp. *pneumophila* ATCC 33152 at three different incubation temperatures.

The bactericidal effect of this temperature was also reported by Cooke [26], who determined that 90 % of *Legionella* cells at a temperature of 50 °C died within 2 h. Serrano-Suarez et al. [27] noted that at a temperature above 55 °C, about 12 % of the population died, as confirmed by Carvalho et al. [28]. Differences in the productivity of the bacteria of *L. pneumophila* in the incubation at 36 °C, 15 °C and 55 °C were statistically significant ($p < 0.05$).

It is clear that the bacteria surviving the disinfection of water replicate more intensely than in the medium without the added dichloroisocyanuric acid.

The effect of dichloroisocyanuric acid disinfectant on the growth of *L. pneumophila*

Among the possible measures for preventing the growth of *Legionella* bacteria in water distribution systems is the use of different disinfectants, especially chlorine [16]. Totaro et al. [29] confirmed in their study the connection between the number of *Legionella* bacteria in water and the concentration of total chlorine and temperature. The growth curve of *L. pneumophila* in a medium with and without the added dichloroisocyanuric acid disinfectant at a concentration of 17 mg L^{-1} was examined. The results showed (Figure 2) that after 10 minutes of exposure to the disinfectant, the number of bacteria decreased from 5.7 log CFU mL^{-1} to 4.3 log CFU mL^{-1} (reduced by 36 %) and the concentration of free chlorine was 0 mg L^{-1}. The change in the pH value of the medium immediately after adding the disinfectant was reduced by only 0.1 grade, from 6.9 to 6.8. Followed by an intensive reproduction of the bacterial number, which had already exceeded the initial value after 24 h of incubation. After 72 h of incubation, the number of bacteria in both samples was about 7.3 log CFU mL^{-1} and did not change much even after 120 h of incubation. The resistance of *Legionella pneumophila* to hydrogen peracetic acid was also confirmed by Farhat et al. (30). Bonadonna et al. [31] determined that the concentration of free chlorine 0.08 mg L^{-1} confirmed the presence of *Legionella* spp. at a level of 400 CFU mL^{-1}. Despite the high concentration of added chlorine, the number of surviving bacteria was high, mainly due to the extremely high concentration of bacteria at the beginning of the experiment, which is not common in most water supply systems.

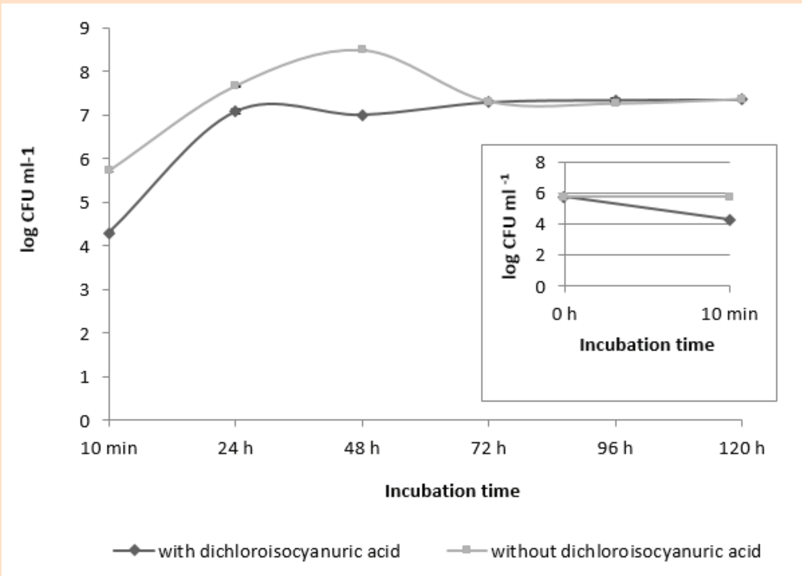

Figure 2.
Growth curve of bacterium *L. pneumophila* subsp. *pneumophila* ATCC 33152 in the presence of dichloroisocyanuric acid, a chlorine concentration of 17 mg L^{-1}.

The number of bacteria in the samples with the addition of a disinfectant increased in the first 24 h of incubation and was only 8 % lower than in the samples that were not exposed to dichloroisocyanuric acid. After 72 h, the number of bacteria was almost the same as in the control. It is clear that the bacteria surviving the disinfection of water replicate more intensely than in the medium without the added dichloroisocyanuric acid. There was a statistically significant difference between the number of bacteria that had been exposed to the disinfectant and the number of bacteria without the disinfectant ($p < 0.05$).

The effect of sodium polyphosphate on the growth of *L. pneumophila*

Figure 3 shows growth curves of *L. pneumophila* in a medium with and without sodium polyphosphate. Water softeners are often used in apartment buildings and large facilities (hospitals, hotels) to prevent calcification in the system [19, 32].

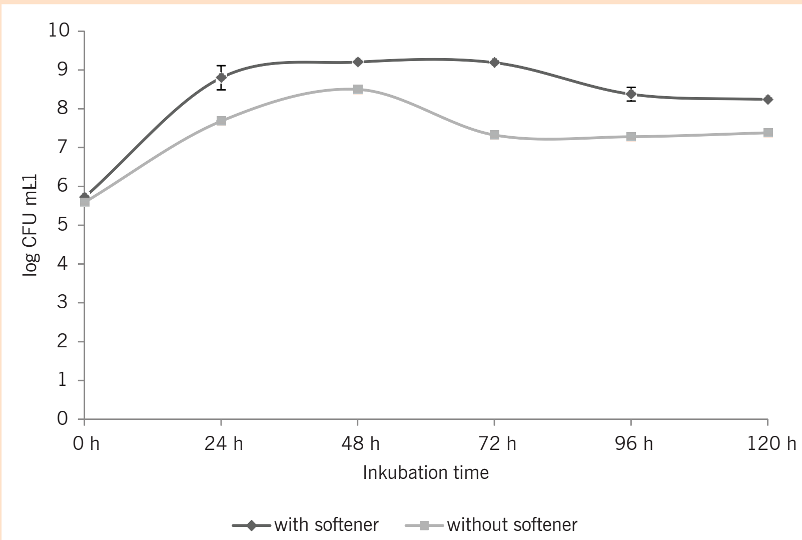

Figure 3.
Growth curve of the bacterium *L. pneumophila* subsp. *pneumophila* ATCC 33152 with adding sodium polyphosphate for water softening in a concentration of 5 mg L^{-1}.

Our results have shown that the incubation temperature of 15 °C has a bacteriostatic effect and a temperature of 55 °C has a bactericidal effect on these bacteria. We concluded that the temperature of 55 °C, even after a long time of incubation, does not completely eliminate all *L. pneumophila* bacteria since we confirmed the presence of bacteria in samples even after 96 h of incubation.

In the current study, sodium polyphosphate was used at a concentration of 5 mg L^{-1} as prescribed by the manufacturer of the product. The pH value of the culture after adding the softener did not change noticeably. Bacteria responded positively to the presence of the softener as their number was much higher at all incubation times. The maximum difference occurred after 72 h of incubation, when the number was higher by about 1.9 log CFU mL^{-1}. After 120 h of incubation, bacteria concentration was reduced to approximately 0.8 log CFU mL^{-1} but was still higher than in the control culture. The shape of the growth curve is similar in both cases, the difference being the number of bacteria in the samples with added sodium polyphosphates, because even after a longer period of incubation the number of bacteria was higher than in the culture without it. The reason for the intensive growth of bacteria might have been the phosphate in the polyphosphate solution as phosphates can also be a nutrient for microorganisms, which Drev et al. also mentions (19). Between the average values, overgrown colonies on the solid medium with sodium polyphosphate showed a statistically significant difference ($p < 0.05$) to the results without the softener.

CONCLUSION

Opinions about the influence of different factors on the reproduction and survival of *Legionella* bacteria in indoor water supply environments vary noticeably. Our results have shown that the incubation temperature of 15 °C has a bacteriostatic effect and a temperature of 55 °C has a bactericidal effect on these bacteria. We concluded that the temperature of 55 °C, even after a long time of incubation, does not completely eliminate all *L. pneumophila* bacteria since we confirmed the presence of bacteria in samples even after 96 h of incubation. We have proven that the bacteria that survived disinfection by water with dichloroisocyanuric acid reproduced as intensively as the bacteria that were not exposed to chlorine. This means that the incomplete or improper disinfection of drinking water in water supply systems may cause an intensive reproduction of bacteria. Sodium polyphosphate in a water softening product caused an intensive multiplication of the bacteria since the reproduction was more intense in the samples with added softener. The process of preventing lime scale build up in water supply systems by adding softeners can thus stimulate the growth of bacteria, which can cause major problems due to the large numbers of bacteria in the drinking water. It should be emphasized that all experiments were carried out under controlled laboratory conditions. Bacteria were grown in liquid medium (yeast extract with added cysteine), which means that we provided them with nutrients for their reproduction. Conditions in plumbing systems are less favourable, so the results would probably be slightly different in the real conditions. In the future, we would like to check the reproduction of bacteria under different temperature conditions, and we are also wondering if other water softeners have a positive effect on the growth of *Legionella* bacteria. This would be very important since we could recommend the use of non-phosphate-based softeners.

REFERENCES

[1] Fliermans CB, Cherry WB, Orrison LH, et al. Ecological distribution of *Legionella pneumophila*. Appl Environ Microb. 1981; 41(1): 9–16.

[2] Tai J, Bencherkroun MN, Ennaji MM, et al. Nosocomial Legionnaires´ Disease: Risque and Prevention. Front Sci. 2012; 2: 62–75.

[3] Surman-Lee S, Fields B, Hornei B, et al. Ecology and environmental sources of *Legionella*. V: Bartram J, Chartier Y, Lee JV, Pond K, Surman-Lee S, eds. *Legionella* and the prevention of legionellosis. Geneva: WHO Press; 2007: 29–38.

[4] Prussin AJ, Schwake DO, Marr LC. Ten questions concerning the aerosolization and transmission of *Legionella* in the built environment. Build. Environ. 2017; 123: 684–95.

[5] Newton HJ, Ang DKY, van Driel IR, et al. Molecular pathogenesis of infections caused by *Legionella pneumophila*. Clin Microbiol Rev. 2010; 23(2): 274–298.

[6] Diederen BMW. *Legionella* spp. and Legionnaires' disease. J Infection. 2008; 56: 1–12.

[7] Harrison TG, Afshar B, Doshi N, et al. Distribution of *Legionella pneumophila* serogoups, monoclonal antibody serogroups and DNA sequence types in recent clinical and environmental isolates from England and Wales (2000-2008). Eur J Clin Microbiol. 2009; 28(7): 781–91.

[8] European centre for Disease Prevention and Control. Surveillance report. Annual Epidemiological Report for 2015. ECDC, Stockholm, 2015: https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/legionnaire258 s-disease-2015.pdf (10.2.2018).

[9] Beauté J. Zucs P, de Jong B. Legionnaires' disease in Europe, 2009-2010. European Legionnaires' Disease Surveillance Network. Euro Surveill. 2013; 18(1): 1–7.

[10] Center for disease control and prevention. Notice to readers: final 2014 reports of nationally notifiable infectious diseases. MMWR Morb Mortal Wkly Rep. 2015; 64: 1019–33.

[11] Lee GF, Jones-Lee A. Public health significance of waterborne pathogens in domestic water supplies and reclaimed water, report to state of California environmental protection agency comparative risk project, Berkeley, CA. 1993.

[12] American Society of Heating, Refrigerating and Air-Conditioning Engineers. Guideline minimizing the risk of legionellosis associated with building water systems. ASHRAE Standard Project Committee, Atlanta 2000; ANS, no. 12-2000.

[13] Canadian Standing Committee on Building and Plumbing Services. Report of the SCBPS: Hot Water Delivery in Building. Ontario, Canada. 2005; 1–28.

[14] Lesnik R, Brettar I, Höfle MG. *Legionella* species diversity and dynamics from surface reservoir to tap water: from cold adaptation to thermophily. ISME J. 2016; 10: 1064–80.

[15] Zhang BZ, Moccani C, Hanrahan J, et al. *Legionella* control by chlorine dioxide in hospital water systems. J Am Water Works Ass. 2009; 101(5): 117–27.

[16] Lin YE, Yu VL, Vidic RD, et al. Discussion of Monochloramine and Legionnaires' disease. J Am Water Works Ass. 2000; 92(10): 88–90.

[17] Lin YE, Stout JE, Yu VL. Controlling *Legionella* in Hospital Drinking Water: An Evidence-Based Review of Disinfection Methods. Infect Cont Hosp Ep. 2011; 32(2): 166–73.

[18] Rohr U, Senger M, Fidelis S, et al. Four Years of Experience with Silver-Copper Ionization for Control of Legionella in a German University Hospital Hot Water Plumbing System. Clin Infect Dis. 1999; 29: 1507–11.

[19] Drev D, Pražnikar Š, Škarja J, et al. Disinfection and removal of biofilm in the internal installations for the cold and the heated water in the

buildings with an increased risk of infections with *Legionella*. V: Roš M. ed. Proceedings Water Days; 2010 Oct 20 and 21; Portorož. Slovenia: Slovenian Society for Water Protection.

[20] ISO 11731:1998. Water quality – Detection and enumeration of *Legionella*.

[21] ISO 8199:2015. Water quality – General guidance on the enumeration of micro-organisms by culture. 2nd ed. 2005, pp. 1–38.

[22] ISO 4833-1:2013. Microbiology of the food chain – Horizontal method for the enumeration of microorganisms. 1st ed. 2013;99. 1–9.

[23] TKI Hrastnik 2006. Technical Sheet, Microfos SH: p. 1.

[24] Giamarellos–Bourboulis EJ, Kentepozidis N, Antonopoulou A, et al. Postantibiotic effect of antimicrobial combinations on multidrug–resistant Pseudomonas aeruginosa. Diagn Microbiol Infect Dis. 2005; 51(2): 113–7.

[25] Garrett TR, Bhakoo M, Zhang Z. Bacterial adhesion and biofilms on surfaces. Prog Nat Sci. 2008; 18: 1049–56.

[26] Cook RPD. Hazards of water. J Hosp Infect. 2004; 57(4): 290–3.

[27] Serrano-Suárez A, Dellundé J, Salvadó H, et al. Microbial and physico-chemical parameters associated with *Legionella* contamination in hot water recirculation systems. Environ Sci Pollut R. 2013; 20: 5534–44.

[28] Carvalho FR, Nastasi FR, Gamba RC, et al. Occurrence and diversity of *Legionellaceae* in polar lakes of the Antarctic peninsula. Cur Microbiol. 2008; 57(4): 294–300.

[29] Totaro M, Valentini P, Costa AL, et al. Presence of *Legionella* spp. in hot water networks of different Italian residential buildings: A three-year survey. Int J Environ Res Public Health. 2017; 14(11): 1296–305.

[30] Farhat M, Moletta-Denata M, Frere J, et al. Effects of disinfection on *Legionella* spp., eukarya, and biofilms in a hot water system. Appl Environ Microb. 2012; 78(19): 6850–8.

[31] Bonadonna L, Briancesco R, Della Libera S, et al. Microbial characterizat-ion of water and biofilms in drinking water distribution systems at sport facilities. Eur J Public Health. 2009; 17(2): 99–102.

[32] Jereb G, Poljšak B, Eržen I. Contribution of drinking water softeners to daily phosphate intake in slovenia. Int J Environ Res Public Health. 2017; 14(10): 1186–96.

Original scientific article

Trend analysis of "garbage codes" as the underlying cause of cancer death: Joinpoint Analysis

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ABSTRACT

Introduction: The quality of cause-of-death data is commonly assessed by determining the percentage of unspecified, ill-defined or "garbage" causes of death. The objective of the paper was to determine percentage of the "garbage codes" (GCs) coded as the underlying cause of cancer death. **Material and methods:** The data base of all deceased from the Nišava District was retrospective analyzed. The observed period was 2000-2016. Joinpoint regression analyses was performed. **Results:** The total number of deceased was 19598 (11247 men and 8351 women) and the total percentage of GCs was 6.2%. The significantly decreasing trend of GCs by 5.6% yearly ($p<0.05$) between 2000 and 2006 and by 2.9% per year ($p<0.05$) between the 2006 and 2016. In men, the statistically significantly decreasing trend of GCs by 6.1% per year ($p<0.001$) from 2000 up to 2013 was determine. In women, significant decreasing trend by 7.5% yearly ($p<0.005$) was registered from 2008 to 2016. There were statistically significant decreasing trends of GCs both in deceased under 65 ($p<0.001$) and above 65 years of age ($p<0.05$). The significant decreasing trend of GCs by 6.2% yearly ($p<0.01$) for deceased in HSIs, was observed from 2000 to 2009. From 2004 to 2016 there was the significant decreasing trend of GCs by 4.6% yearly ($p<0.01$) among deceased out of HSIs. **Conclusion:** The significantly decreasing trend of GCs was recorded from 2000 to 2016 and the change in trend was in 2006. Continuous education of the staff and control of data quality of medical death certificates are needed.

Key words: cancer, mortality, garbage codes, trend analysis

INTRODUCTION

The routine cancer mortality statistics provide data which are widely used for identifying public health problems and developing health care strategies [1, 2]. Because of the varied uses of these data, it is important that mortality data be reliable and accurate [3].

The quality of cause-of-death data is commonly assessed by determining the percentage of unspecified or ill-defined [4] or “garbage” causes of death [5]. Murray and Lopez introduced the term “garbage coding” for the practice of assigning deaths to causes that are useless for public health analysis [6].

This term “garbage codes” refers to all ill-defined or incomplete diagnoses consisted of codes from the 10th International Classification of Disease (ICD-10) for underlying causes of death. “Garbage codes” don't indicate the specific cause of death and they don’t have any value for public health because they can’t be the underlying cause of death [7, 8] and the appearance of “garbage codes” limits the public health utility of cause of death data [9].

This term “garbage codes” refers to all ill-defined or incomplete diagnoses consisted of codes from the 10th International Classification of Disease (ICD-10) for underlying causes of death.

According to the ICD-10 the underlying cause of death is: “(a) the disease or injury which initiated the train of morbid events leading directly to death, or (b) the circumstances of the accident or violence which produced the fatal injury” [10]. Despite this clear ICD definition of underlying cause of death it is very often difficult to apply it in everyday practice [12, 13].

Mathers et al. (2005) classified Serbia and Montenegro in the group of countries with medium mortality quality data and that means that “the completeness of data was 70-90% or ill-defined codes appear on 10-20% registrations or completeness >90% and ill-defined codes appear on <10% of all registrations” [14].

The system for vital registration and medical certification of deaths in Serbia is characterized by almost 100% coverage of civil registration of the underlying causes of death. Serbia implemented the International Classification of Disease-the tenth revision in 1997, and since then, all medical death certificates issued in the country must be filled in with the original three digit codes – ICD-10 codes.

This system of producing mortality data in Serbia has been decentralized 2006. The reporting of cancer in the Republic of Serbia is obligatory by the law [15]. Data about incidence and mortality of the cancer are collected by hospital-based and population-based cancer registries. The following data are entered into the population cancer register of Central Serbia: descriptive characteristics of new cases or of deceased, possible appearance of multiple primary tumors, date of determination of current illness, diagnostic methods, tumor characteristics (primary and secondary anatomy localization, histology type, stage), outcome of the disease, as well as data on the health institution reporting the cancer [16].

Mortality data collection in Serbia is based on filling in the medical death certifications by physicians-certifiers [17] and the law allows only a physician to fill in the medical death certifications [18] using the codes of ICD-10.

The objective of the paper was to assess the quality of cancer mortality data by determining and analyzing the percentage of "garbage codes" on the medical death certificates coded as the underlying cause of death in the Nišava District, Serbia.

MATERIAL AND METODS

Descriptive study was done. The observed period was 2000 to 2016. The percentage of diagnoses C76 and C80 on the MDCs was analyzed as Naghavi et al. [9] and Mathers and al. suggested [14].

The diagnosis C97 is imprecise too, but it was not taken for the analysis, because since 2013 it cannot be taken as the underlying death cause according to the Instructions to the Physicians-Certifiers in Serbia [17].

Data base of the medical death certifications of the Institute for Public Health in Niš and data base of the Statistical Office of the Republic of Serbia were used in this study. These data bases consisted of deceased from cancer who were residents of the Nišava District. The observed period was from 2000 to 2016. The deceased were retrospective analyzed according to their gender, age and the place of death.

Deceased according to age were divided into two groups: under 65 years of age and above 65 years of age. The notified place of death was divided into: a) the deceased in the hospital stationary institution and b) the deceased out of the hospital stationary institution.

The underlying causes of death from the medical death certificates were accepted as accurate because as such they are published by the official state statistics according to the deceased's place of living and in Serbia doesn't exist the ''gold standard''.

Characteristics of the Nišava District population

According to the vital statistics the population of the Nišava District is very old [19] and it is getting older. According to the data from 2015, there were in average 20.5% persons older than 65 years of age.

According to the vital statistics the population of the Nišava District is very old and it is getting older. According to the data from 2015, there were in average 20.5% persons older than 65 years of age.

The rate of general mortality in the territory of Nišava District ranged 14.3/100 000 inhabitans in 2013 to 15.0 in 2014 and 2015. An increasing trend of general mortality was recorded. Depopulation tendencies of the population with negative growth and negative natural increase were recorded. It is well-known that aging is risk factor for cancer.

Statistical analysis

Trend of the percentage of the "garbage codes" with corresponding 95% confidence intervals (CI) were calculated by performing Joinpoint regression analyses [20]. For regression analyses, Joinpoint Regression software, version 4.2.0.2. was used (available through the Surveillance Research Program of the US National Cancer Institute Statistical Methodology and Application Branch). The trend was considered to be significant increasing (positive change) or decreasing (negative change) when (negative change) when the p-value was below 0.05 ($p < 0.05$).

In Joinpoint analysis, the best fitting points, called 'joinpoints', are chosen where the rate changes significantly. The analysis starts with the minimum number of joinpoints (e.g. zero joinpoints, which is straight line), and tests whether one or more joinpoints (up to three) are significant and must be added to the model. Each significant joinpoint that indicates a change in the slope (if any) is retained in the final model. To describe linear trends by period, the estimated annual percent change (APC) is then computed for each of those trends by fitting [21].

Joinpoint regression model analyses rates, proportions, or any other measure that can be considered (e.g., counts) over time in order to (i) identify the possible time point(s) at which any given trend changes, that is the joinpoint(s), and to (ii) estimate the regression function with joinpoint(s) previously identified. Specific interests were to whether two regression mean functions were parallel (test of parallelism) [22].

Results

In the 17th years observed period the total number of deceased in the Nišava District was 19598 (11247 men and 8351 women). The total number of "garbage codes" was 1217 (6.2%). The percentage of "garbage codes" ranged from 8.5% (2000) to 4.0% (2006).

In men perecentage of "garbage codes" ranged from 8.6% (2004) to 2.5% (2006), and in women ranged from 10.7% (2009) to 4.7 (2016).

The Joinpoint analysis showed statistically significant decreasing trend of "garbage codes" by 5.6% yearly (95% Confidence intervals [CI] =-9.7% to -1.3%; t=-2.632; p=0.0138; p<0.05) in the period 2000-2006 (Table 1, Figure 1).

In the period after the 2006 up to 2016 Joinpoint analysis determined statistically significant decreasing of "garbage codes" by 2.9% yearly (95% CI= -5.2% to -1.7%; t=-2.630; p=0.0139; p<0.05), (Table 1, Figure 1). According to the test of parallelism trends were similar both for men and for women. The Joinpoint analysis showed the change in trend in 2006. The total percentage of the "garbage codes"was the lowest in 2006 (Table 1).

Joinpoint analysis identified gender specific trends of "garbage codes". Men and women significantly differed for the trend in persistence. When parallelism was tested, considering the period from 2000 to 2016 trends were similar (in parallel) (p>0.05) to men and women with a change in 2006.

In men, the Joinpoint analysis showed a statistically significantly decreasing trend of "garbage codesby 6.1% per year (95% CI=-7.3% to -4.8%; t=-10.13; p=0.000; p<0.001) from 2000 up to 2013. The increase of "garbage codes" during 2013-2016 was not statistically significant (p>0.05).

In women, based on the Joinpoint analysis, trend of "garbage codes" decreased from 2000 up to 2008 but not statistically significant (p>0.05). The Joinpoint analysis showed a change in trend for women in 2008. Trend of "garbage codes"statistically significant decreasing by 7.5% yearly (95% CI= -11.9% to -2.9%; t=-3,47; p=0.0046; p<0.005) in the period 2008-2016.

Table 1. The percentage of garbage codes (ICD-10, C76-C80) coded as the underlying cause of death (ICD-10, C00-D48), by gender, in the Nišava District from 2000 to 2016

Year	Total n*/N†	GCs %	Males n*/N†	GCs %	Females n*/N†	GCs %
2000	85/1004	8.5	38/563	6.7	47/441	10.7
2001	83/999	8.3	47/579	8.1	36/420	8.6
2002	82/992	8.3	45/570	7.9	37/422	8.8
2003	80/951	8.4	35/526	6.7	45/425	10.6
2004	80/979	8.2	48/556	8.6	32/423	7.6
2005	80/1083	7.4	40/616	6.5	40/467	8.6
2006	41/1031	4.0	15/593	2.5	26/438	5.9
2007	59/1176	5.0	30/689	4.4	29/487	6.0
2008	80/1156	6.9	37/665	5.6	43/491	8.8
2009	79/1255	6.3	36/709	5.1	43/546	7.9
2010	78/1238	6.3	38/698	5.4	40/540	7.4
2011	74/1254	5.9	43/751	5.7	31/503	6.2
2012	66/1372	4.8	37/794	4.7	29/578	5.0
2013	54/1218	4.4	26/692	3.8	28/526	5.3
2014	70/1325	5.3	32/750	4.3	38/575	6.6
2015	64/1268	5.0	35/733	4.8	29/535	5.4
2016	62/1297	4.8	37/763	4.8	25/534	4.7

*n= the number deceased with 'garbage codes', †N= the number of persons who died from cancer (C00-D48, ICD-10)

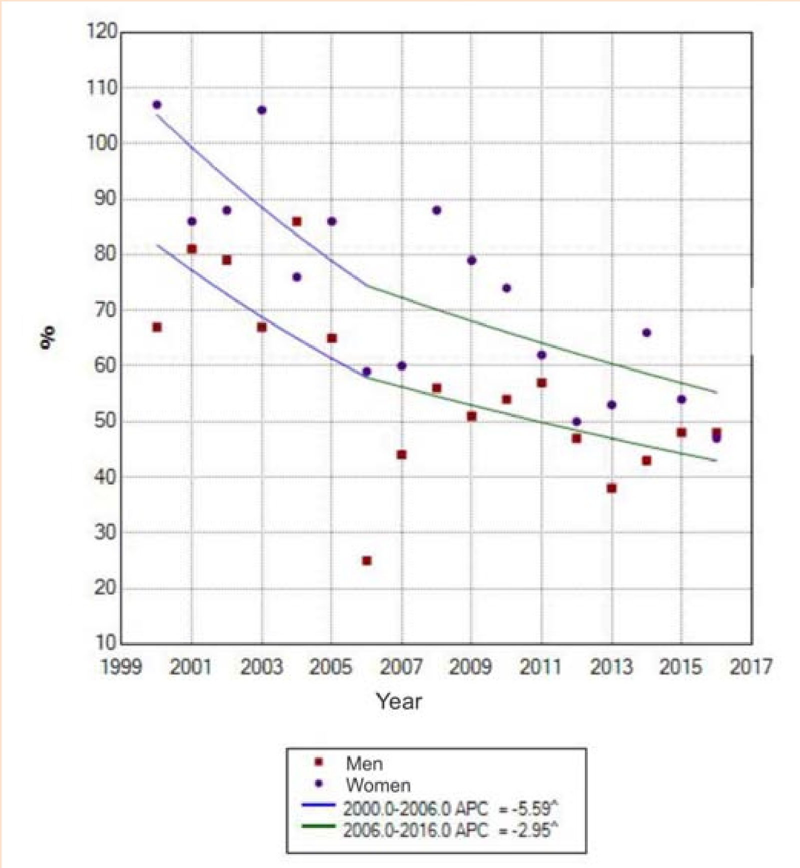


Figure 1.
The trend of garbage codes coded as the underlying cause of death in the cancer mortality data, by gender from 2000 to 2016 in the Nišava District

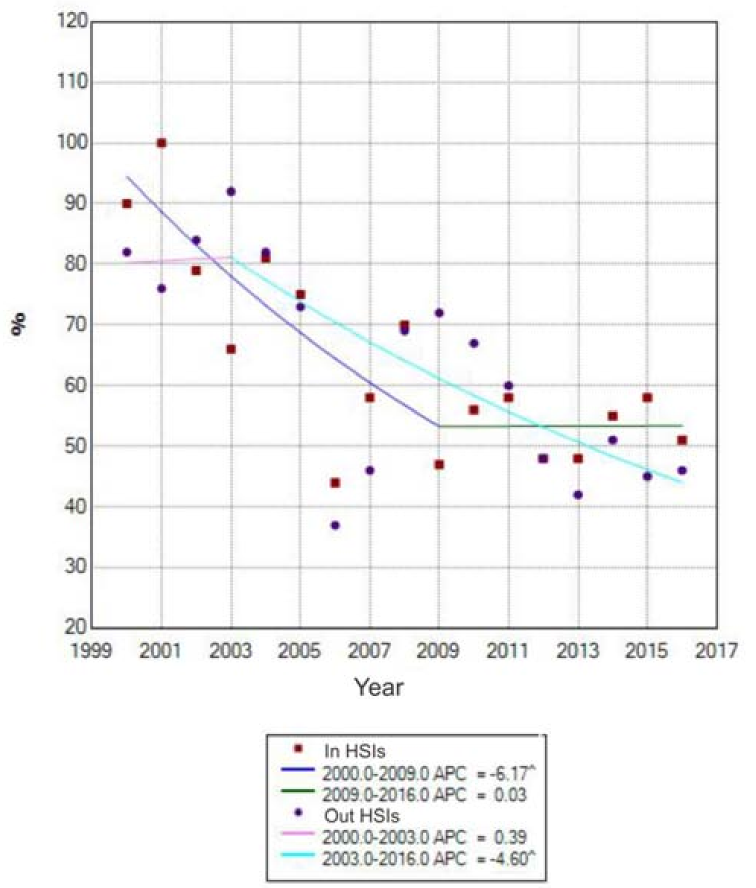
Table 2. The percentage of cancers coded as the underlying cause of death with (C00-D48, ICD-10) in the hospital stationary institutions and among deceased out of the hospital stationary institutions, by age, in the period 2000-2016 in the Nišava District

Year	HSIs‡	GCs	Out of HSIs‡	GCs	<65	GCs	65+	GCs
	n*/N†	%	n*/N†	%	n*/N†	%	n*/N†	%
2000	29/324	9.0	56/680	8.2	39/417	9.4	46/587	7.8
2001	30/301	10.0	53/698	7.6	29/385	7.5	54/614	8.8
2002	22/277	7.9	60/715	8.4	32/406	7.9	50/586	8.5
2003	19/288	6.6	61/663	9.2	33/369	8.9	47/502	9.4
2004	28/346	8.1	52/633	8.2	36/384	9.4	44/595	7.4
2005	29/389	7.5	51/694	7.3	26/450	5.8	54/633	8.5
2006	16/363	4.4	25/668	3.7	15/411	3.6	26/620	4.2
2007	24/417	5.8	35/759	4.6	24/468	5.1	35/708	4.9
2008	25/358	7.0	55/798	6.9	24/468	5.1	56/688	8.1
2009	21/448	4.7	58/807	7.2	23/476	4.8	56/779	7.2
2010	24/432	5.6	54/806	6.7	33/480	6.9	45/758	5.9
2011	27/465	5.8	47/789	6.0	25/488	5.1	49/766	6.4
2012	25/519	4.8	41/853	4.8	30/579	5.2	36/793	4.5
2013	22/455	4.8	32/763	4.2	20/450	4.4	34/768	4.4
2014	30/542	5.5	40/783	5.1	30/510	5.9	40/815	4.9
2015	30/514	5.8	34/754	4.5	20/444	4.5	44/824	5.3
2016	27/534	5.1	35/763	4.6	21/433	4.8	41/864	4.7

*n = the number deceased with 'garbage codes', †N= the number of persons who died from cancer (C00-D48, ICD-10)

‡HSIs = hospital stationary institutions (state and private hospital institutions)

Figure 2.
Trend of GCs in deceased in the hospital stationary institutions and among deceased out of the hospital stationary institutions in the period 2000-2016 in the Nišava District



The total number of deceased in HSIs was 6982 and the total number of “garbage codes” was 428 (6.1%). The percentage of “garbage codes” ranged from 10.0% (2001) to 4.4% (2006). Every tenth diagnosis of cancer as the underlying cause of death in HSIs was coded by “garbage codes” (Table 2).

Mortality trends determined by the Joinpoint analysis differs for deceased in HSIs and those who died out of the HSIs. Based on the Joinpoint analysis there was statistically significant decreasing trend of “garbage codes”by 6.2% yearly (95% CI=-9.8% to -2.4%; t=3.683; p=0.003; p<0.01) for deceased in HSIs, from 2000 to 2009. After the 2009 trend of percentage of “garbage codes” has been stagnated (p>0.05). Parallelism is excluded (p = 0.0129; p <0.05). The trends do not behave similarly (parallel) to the place of death.

According to the Joinpoint analysis among deceased out of the HSIs there were two time periods. The first was from 2000 to 2003. The percentage of “garbage codes” varied and it was not statistically significant (p>0.05). The Joinpoint analysis showed the change in trend in 2003. From 2004 to 2016 there was a statistically significant reduction in percentage of “garbage codes” in the cancer mortality by 4.6% yearly (95 % CI= -7.7% to -1.4%; t=-3.139; p=0.008; p<0.01).

In deceased under the 65 years of age Joinpoint analysis determined the statistically significant decreasing percentage of “garbage codes” was from 2000 to 2006 by 5.6% yearly (95%CI=-10.0% to -0.8%; t=-2.4; p=0.023; p<0.05). There was significant decreasing trend of “garbage codes” from the 2006 up to 2016 by 3.6% yearly (95% CI= -5.0% to -2.1%; t=-5.1; p<0.001).

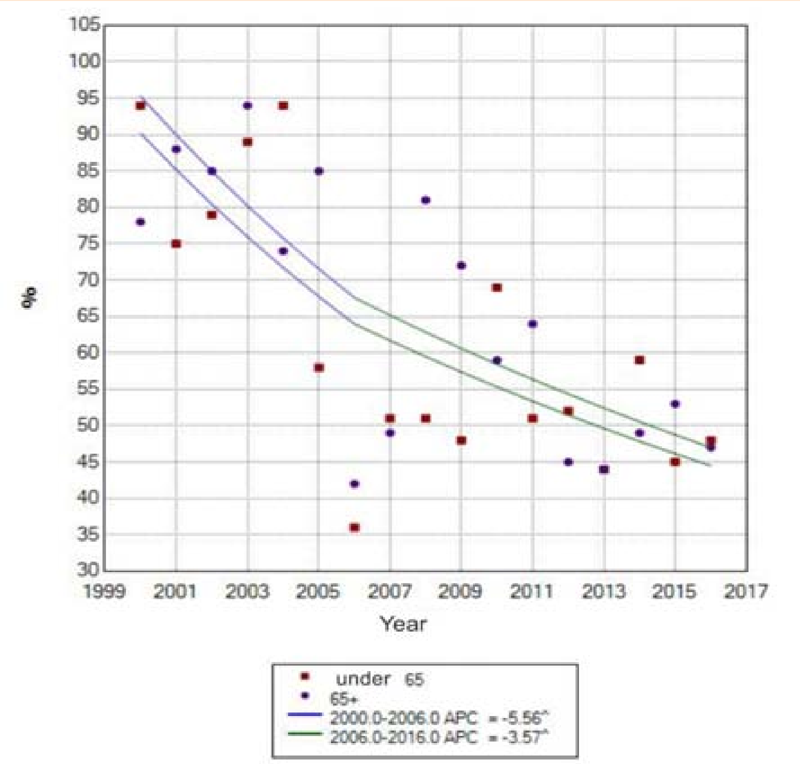


Figure 3.
Trend of percentage of garbage codes coded as underlying cause of death in deceased under 65 years of age and in deceased over the 65 years of age in the period 2000-2016 in the Nišava District

The Joinpoint analysis showed statistically significant decreasing percentage of “garbage codes” in cancer mortality data from 2000 to 2006.

Parallelism can’t be excluded (p>0.05). In other words, trends behave similarly (in parallel) for deceased under than 65 and the older than 65. The year of a change was 2006.

According to the Joinpoint analysis there were statistically significant decreasing frequency of “garbage codes” in deceased under 65 years of age (t=2.0; p=0.019; p<0.05) from 2000 to 2006. After the 2006 up to 2016 there was statistically significant decreasing trend (t=5.369; p=0.0000; p<0.001).

In deceased older than 65 years of age the Joinpoint analysis didn’t determine the year when change in trend has been occurred. There was a statistically significant decreasing trend of “garbage codes” in deceased over 65 years of age by 3.7% per year (95%CI=-4.0% to -3.4%; t=-26.34; p=0.000; p<0.001).

DISCUSSION

It is important to register every death that occurred but it is also important to determine the exact cause of death. In all European countries death certification is mandatory as it is in the Republic of Serbia. The diagnosis on the medical death certificate doesn’t always reflects the real underlying cause of death [23].

Cancer can be misattributed as the underlying cause of death on medical death certificates [1]. In some countries, up to 50% of cancer deaths are coded to C80, the non-specific category with no indication given of the primary site [2]. It is well known that the cause of death can’t be always determined. Autopsy is often considered as the “gold standard”, but autopsies are infrequently undertaken [11].

Our study described trends of “garbage codes” coded as underlying cause of death on the medical death certifications for the population of the Nišava District in the 17-years. The Joinpoint analysis showed statistically significant decreasing percentage of “garbage codes” in cancer mortality data from 2000 to 2006. According to the Joinpoint analysis the change of trend occurred in 2006 when the total percentage of the “garbage codes” was the lowest. The significant decreasing trend of “garbage codes” in cancer mortality statistics has been recorded in the whole observed period.

Since 2006, the cooperation has been started between the physician of the Centre for biostatistics and informatics of the Institute for Public Health Niš and epidemiologists from the same Institute that take care of the population cancer register. There were less percentage of “garbage codes” and the quality of cancer mortality data has been higher.

Mortality trends determined by the Joinpoint analysis differs for deceased in HSIs and those who died out of the HSIs. Based on Joinpoint analysis the percentage of “garbage codes” decreased in the whole mortality data had a very high proportion of “garbage codes” [12, 20, 27, 28]. It is the same in the hospitals in the Nišava District despite that the physicians have to certify the underlying cause of death when a death occurs in a hospital by the law [15]. Generally in every day practice the nurse not the physician or even community health worker certifies the cause of death. That is one of the reasons of poor death certification practice in the stationary health institutions in the Nišava Dstrict.

Decreasing trend of “garbage codes” was observed in deceased out of HSIs in 13 years of all 17-observed years. There was not statistically significant difference in the distribution of “garbage codes” on the medical certifications of cancer death between physicians of secondary and tertiary HSIs and coroners who set the diagnosis of death out of HISs.

According to the literature in many developing countries more than half of all deaths occur outside of hospitals and 'gold standard' for the underlying cause of death doesn’t exist [26, 27]. The most deaths that occur outside of the HISs are assigned to ill-defined causes [2, 5, 28] and the percentage of “garbage codes” are higher on the medical death certifications filled in by coroner.

A higher proportion of “garbage codes” in the older age groups has noticed by other authors [12, 27, 28]. As for the age group of 60 years and over also found a higher proportion of “garbage codes” deaths at higher ages [24, 29]. These results may be due to the greater occurrence of comorbidities in the elderly, which make it difficult to establish the correct underlying cause that led to death [29, 30].

In years of the law changing, introduction of the new recommendations for coding and the change of the personnel, there were more cases of “garbage codes” as the underlying cause of death. An exceptionally beneficial effect on the quality of data was achieved by the cooperation with epidemiologists that were in charge of the population cancer register, as well as frequent educations of clinical doctors and medical personnel.

CONCLUSION

The significantly decreasing trend of “garbage codes” coded as underlying cause of cancer death was recorded from 2000 to 2016. Decreasing trend of “garbage codes” was observed in both deceased men and women, and in both deceased under the 65 years of age and in deceased above 65 years of age. The percentage of “garbage codes” decreased in the whole observed period in deceased in the stationary hospital institutions. Continuous education of the staff and control of data quality of medical death certificates are needed.

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REFERENCES

[1] Turner LE, Metcalfe C, Donovan LD, Noble S, Sterne ACJ, Lane AJ et al. Contemporary accuracy of death certificates for coding prostate cancer as a cause of death: Is reliance on death certification good enough? A comparison with blinded review by an independent cause of death evaluation committee. British Journal of Cancer 2016;115:90–4.

[2] Danilova I, Shkolnikov MVJ, Danov AD, Meslé F, Vallin J. Identifying potential differences in cause of-death coding practices across Russian regions. Population Health Metrics. 2016 14:8. [doi: 10.1186/s12963-016-0078-0.]

[3] Jemal A, Ward E, Anderson RN, Thun MJ. Influence of Rules From the Tenth Revision of the International Classification of Diseases on U.S. Cancer Mortality Trends. J Natl Cancer Inst. 2003;95(22):1727–8. [doi: 10.1093/jnci/djg116.]

[4] World Health Organization. Improving the quality and use of birth, death and cause-of-death information: guidance for a standards-based review of country practices. World Health Organization. 2010. At: http://www.who.int/iris/handle/10665/44274/.

[5] Ishitani HL, Teixeira AR, Abreu XMD, Paixão MML, França BE. Quality of mortality statistics' information: garbage codes as causes of death in Belo Horizonte, 2011-2013. Rev. bras. epidemiol. Vol. 20 supl. 1. São Paulo May 2017, doi: http://dx.doi.org/10.1590/1980-5497201700050004.

[6] Murray CJL, Lopez AD. The global burden of disease: a comprehensive assessment of mortality and disability from diseases, injuries, and risk factors in 1990 and projected to 2020. Cambridge, MA: Harvard School of Public Health/World Health Organization/World Bank; 1996.

[7] Mikkelsen L, Phillips DE, AbouZahr C, Setel PW, de Savigny D, Lozano R et al. A global assessment of civil registration and vital statistics systems: monitoring data quality and progress. Lancet. 2015; [doi: 10.1016/S0140-6736 (15) 60171-4.]

[8] Ahern RM, Lozano R, Naghavi M, Foreman K, Gakidou E, Murray CJ. Improving the public health utility of global cardiovascular mortality data: the rise of ischemic heart disease. Popul Health Metr. 2011;9(1):8. [doi: 10.1186/1478-7954-9-8.]

[9] Naghavi M, Makela S, Foreman K, O'Brien J, Pourmalek F, Lozano R. Algorithms for enhancing public health utility of national causes-of-death data Population Health Metrics 2010; 8: 9.

[10] World Health Organization: International Statistical Classification of Diseases and Related Health Problems. Tenth Revision. Volume 2. Second edition. Geneva. At: http://www.who.int/ classifications/icd/en/.

[11] Pederson GA, Ellingsen LC. Data quality in the Causes of Death Registry. Tidsskr Nor Legeforen. 2015; 8(35): 768–70.

[12] Laurenti R, de Mello Jorge MHP, Gotlieb SLD. Underlying cause-of-death mortality statistics: considering the reliability of data. Rev Panam Salud Publica. 2008;23(5):349–356. [doi: 10.1590/S1020-49892008000500007.]

[13] Foreman JK, Naghavi M, Ezzati M. Improving the usefulness of US mortality data: new methods for reclassification of underlying cause of death. Population Health Metrics. 2016: 14:14, [doi: 10.1186/s12963-016-0082-4.]

[14] Mathers DC, Ma Fat D, Inoue M, Rao C, Lopey DA. Counting the dead and what they died from: Bullten of the World Health Organization. 2005;83:171-7.Mahapatra P, Shibuya K, Lopez DA, Coullare F, Notzom FC, Rao C, Szreter S, MoVE writing group. Civil registration systems and vital statistics: successes and missed opportunities. Lancet. 2007; [DOI: 10.1016/S0140-6736 (07) 61308-7.]

[15] The Healthcare law ("Official Gazette of the Republic of Serbia No.106/15).

[16] Incidence and mortality of cancer in Serbia 2015. Institute of Public Health of the Republic Serbia, Belgrade 2016, Report No. 2016, Belgrade, Serbia.

[17] The Rulebook about the procedure of the approvals on dead and development conformation ("Official Gazette of the Republic of Serbia No. 25/2011).

[18] Law on Health Documentation and Records in the Field of Health (Offical Gazete No. 123/2014,106/20151nd 105/2017).

[19] Vital events in Serbia,2016. Republic of Serbia, Statistical Office of the Republic of Serbia, Communication, No. 172-LXVII, 30.06.2017. Population statistics, ISSN 0353-955, SN40, SRB172SN40 300617.

[20] Rea F, Pagan E, Compagnoni MM, Cantarutti A, PietroPugni P, Bagnardi V et al. Joinpoint regression analysis with time-on-study as time-scale. Application to three Italian population-based cohort studies. Epidemiology Biostatistics and Public Health – 2017;14 (3): e12616-1-12616-7, [DOI: 10.2427/12616].

[21] Sutra S, Chirawatkul A, Leelapanmetha P, Sirisuwan S, Thepsuthammarat K. Evaluation of causes-of-death: which statistics should we rely on, hospital deaths or vital statistics?J Med Assoc Thai. 2012;95(Suppl 7): 262–73.

[22] Ilić M, Vlajinac H, Marinković J, Kocev N. Pancreatic cancer mortality in Serbia from 1991-2010 – a joinpoint analysis 2013. Croat Med J. 2013; 54:369–75.

[23] Goldacre MJ. Cause-specific mortality: understanding uncertain tips of the iceberg. J Epidemiol Community Health. 1993;47:491–6.

[24] Karki RK. Mortality Patterns among Hospital Deaths. Kathmandu Univ Med J (KUMJ). 2016;14(53):65-8.

[25] Hernández B, Ramírez-Villalobos D, Romero M, Gómez S, Atkinson C, Lozano R. Assessing quality of medical death certification: Concordance between gold standard diagnosis and underlying cause of death in selected Mexican hospitals. Popul Health Metr. 2011. [doi: 10.1186/1478-7954-9-38.] At: http://www.pophealthmetrics.com/content/9/1/38.

[26] Cheng TJ, Lin CY, Lu TH, Kawachi I. Reporting of incorrect cause-of-death causal sequence on death certificates in the USA: using hypertension and diabetes as an educational illustration. Postgrad Med J. 2012;88:690–3. doi: 10.1136/postgradmedj-2012-130912.

[27] Johansson AL. Targeting Non-obvious Errors in Death Certificates. Upssala University 2008, Sweden.

[28] Harron KL, Doidge JC, Knight HE, Gilbert RE, Goldstein H, Cromwell DA et al. A guide to evaluating linkage quality for the analysis of linked data. International Journal of Epidemiology. 2017;46:1699–710. doi: 10.1093/ije/dyx177.

[29] GBD 2015 Mortalty and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause specific mortality for 249 causes of death, 1980-2015: A systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016; 388: 1459–544.

[30] Ngo AD, Rao C, Hoa NP, Adair T, Chuc NTK, Mortality patterns in Vietnam, 2006: (findings from a national verbal autopsy survey. BMC Research Notes. 2010; 3(10):78.

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[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).

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