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Editorial

Four years have passed and in front of you is the Volume 4, No. 2 issue of *International Journal of Sanitary Engineering Research*. The fact is that we together: readers, papers contributors, the editorial board, the advisory board and finally editors can be proud to ensure regular publishing of the journal. The first, the most important goal is now achieved. But we must not to stop. The real endeavor has been just started and we all together are responsible:

- to improve the quality of papers and the whole journal;
- to increase the number of the original scientific papers published in the journal;
- to increase number of the readers and subscribers;
- to enhance the transfer of the knowledge from the universities and institutes to industry and at the same time the transfer of know-how from industry back to research institutions.

Now I am asking you to take the active role in this process. Universities and institutes, publish your research results in our journal, take the active part in the rising quality of the papers. Industry, use those results in your processes, products in order to improve quality of your processes, products or even at developing new products. This journal should be the link between those actors and tool for transferring knowledge and know-how.

This issue contains the two original scientific papers. One of them, titled *Chemical Analysis and the River Mura Water Quality* is the results of the several years sampling and analysing the water of the river Mura by the two independent laboratories, one from Slovenia and the other from Austria. This border river between two countries was heavily polluted in the past. The results of the research showed that quality of the water has been increasing. The content of the ammonia, nitrite, nitrate and orthophosphate are decreasing. The main reason of the water quality improving is the increased environmental awareness of the industry, rational plant protection products on agriculture land near river and stronger environmental legislation in the both countries.

The researchers from University of Ljubljana, Faculty of Health Sciences, the Department of Sanitary Engineering studied eating habits of the Slovene students. They focused primarily on the analysis of the frequency of the consumption of fruit, vegetables, vitamin and mineral supplements. The results are applicable to the universities restaurants and for the students themselves and for the further research in this area.

The paper in Slovenian language deals with the topic regarding bio-film generation on various surfaces which are in contact with food and water. Those biofilms cause reproduction and introduction the micro-organisms into the food and water. The special attention is given to the methods of the removing the biofilms. The author describes the physical, chemical, biological and enzymatic methods. This area is particularly interesting for industry and it is worth to be detailed investigated and results presented to our readers.

Sincerely,

Janez Petek
Editor-in-Chief

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Chemical Analysis and the River Mura Water Quality

Bojana **KRAJNC GALUNDER**, Mitja **KOLAR***

ABSTRACT

The environment contains several water sources such as brooks, rivers and lakes, that indirectly or directly contribute to human wellbeing and a variety of water ecosystems. Rivers are, unfortunately, still the main domestic and industrial waste-water transmission source, creating both the permanent and largest pollution source. From among other significant pollution sources, outflows from agricultural areas, also have an influence on climatic changes. Rivers are regarding pollution easily accessible, therefore it is very important to supervise water-pollution, the quality of river-basins' waters, and to assess water quality according to time and space influences.

This paper presents the physical and chemical parameters of the River Mura's water for the period 1996 to 2009. Samples of water were taken at four diverse measuring places; analyses being performed in two independent laboratories. The parameters that characterized the 336 samples were: water temperature, pH, electrical conductivity, dissolved oxygen, oxygen saturation, oxygen deficiency, biochemical and chemical oxygen demand in filtered and non-filtered samples, total and carbonate hardness, suspended substance share, AOX, TOC and phosphate, ammonium, nitrite, nitrate, calcium, magnesium, sodium, potassium, chloride, sulphate and phosphate ions contents.

The seasonal and space influences of pollution on the River Muras' were researched using linear regression.. Special attention was devoted to the monitoring of those individually measured parameters that most reflected river's quality. Monitoring of the River Mura's water pollution and the results from particular measured parameters, can also be used when searching for pollution causes and when planning preventive measures for protective purpose.

KEY WORDS:

River, Water quality, Pollution, Physical-chemical parameters, Linear regression.

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INTRODUCTION

1.1 The River Mura

A river-based landscape is often an area within, people live and which is constantly being changed by its inhabitants. This process has been evolving over millennia, as great rivers are sources for sustaining life. These sustainable activities have more recently begun to destroy the rivers, and the natural dynamics and diversities of the many life-forms associated with them. However, the lower-flow of the River Mura has managed to preserve many rare parts of its landscape. Conservation of the river's flow, has in addition to exceptional ecological functions and the role of huge water restraint, providing better flood-safety, had a decisive influence on underground water-quality and the supply of clean drinking water.

Rivers can generally be classified as either alluvial, bedrock, or some mix of the two. Alluvial rivers have channels and flooded-plains that are self-formed in non-consolidated or weakly-consolidated sediments. Bedrock rivers form when the river breaks through the newer sediments and into the underlying bedrock.

Rivers are used as a source of water, for obtaining food, for transportation and defense purposes, as a source of hydropower for driving machinery, for bathing, and as a means of waste-disposal. They can provide a rich source of fish and other edible aquatic life, and are a major source of fresh water, which can be used for both drinking and irrigation.

The flora and fauna of rivers use the aquatic habitats available, from torrential waterfalls through to lowland mires. The organisms in the riparian zone respond to changes in river-channel locations and flow-patterns. The basic idea is that the river can be described as a system that is continuously changing along its length in regard to physical parameters, the availability of food particles, and the composition of the ecosystem.

The chemistry of rivers is complex and depends on inputs from the atmosphere, the geology through which it travels, and inputs from humans' activities. The chemistry of the water has a large impact on the ecology of that water for both plants and animals, and also affects any usage that may be made of the river's water. Understanding and characterizing river water chemistry requires a well-designed and managed programme of sampling and analysis.

Like many other Aquatic ecosystems, rivers are also under increasing threat of pollution.

The River Mura, 444 km in length, rises in Austria (1898 m above sea-level) and as a border river between Croatia and Hungary, before it flows into the River Drava. The size of its basin is 14,304 km². More than half of its surface is in Austria. The Slovenian section of the basin is 1,393 km² in area, the Croatian 987 km², and Hungary 1911 km². The average fall of the river is 0.21 %. The average fall in Slovenia is 0.1 % and less than 0.06 % at the border between Croatia and Hungary.

These sustainable activities have more recently begun to destroy the rivers, and the natural dynamics and diversities of the many life-forms associated with them.

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The average flow-distribution over the year mainly depends on any snowfall and the duration of the snow-blanket in Austria.

Due to the needs for flood protection and the exploiting of energy potential, the Mura in Austria (315 km) is mainly regulated, high water dams have been built, and 31 hydroelectric power plants are situated on it.

The main riverbed is otherwise balanced and deepened, but within high water dams there are still preserved

The River Mura can be divided according to the regions through which it flows:

- Mountain section (source - Bruck) over 216 km (46 %) within a catchment area of 4,700 km² (34 %),
- Transitional section (Bruck - Mureck) over 119 km (26 %) within a catchment area of 5,070 km² (36 %),
- Lowland section (Mureck - Legrad) over 130 km (28 %) within a catchment area of 4,255 km² (30 %).

Its major left tributaries are Murica and Ledava and right tributaries Ščavnica and Trnava which were, under regulation, redirected from the Drava into the Mura.

The average flow-distribution over the year mainly depends on any snowfall and the duration of the snow-blanket in Austria. Above-average flows occur in Spring, (March-May), whilst the late Autumn and Winter months have low-flows.

In the Autumn the volume of water increases in the channel due to rain, and during that period the river occasionally floods the hills, very occasionally embankment which protects the cultivated areas. Flooding is a natural part of a river's cycle. The majority of river channels' erosion and the erosion and deposition on the associated flooded-plains occur during the flooding stage. In many developed areas, human activity has changed the formations of river's-channel, altering different magnitudes and frequencies after flooding. In many cases human activities regarding rivers and flooded-plains have dramatically increased the risk of flooding. Due to the needs for flood protection and the exploiting of energy potential, the Mura in Austria (315 km) is mainly regulated, high water dams have been built, and 31 hydroelectric power plants are situated on it. In the Summer the level of water decreases considerably due to high temperatures and strong evaporation, and in the Winter due to low rainfall.

Despite the relative slow current of the Mura, it does flow much faster than it once did, which also deepens it. Regulations in Slovenia have been introduced gradually and less systematically than in Austria. In the past on River Mura and its subsidiaries several hydro-technical facilities were built (regulated channels, shore protection, transverse steering structures) but in this section there are no hydroelectric installations. Before the regulations, only about 40 % of water went under the main river-bed, now almost all the water runs there and, due to its stronger flow it loosens more gravel away. The main riverbed is otherwise balanced and deepened, but within high water dams there are still preserved (many former branches and legacies of the river). The middle and lower parts of the River Mura, which begin at the border between Slovenia and Austria, some natural morphological and ecological characteristics of the river area have been preserved.

The region from the left bank of the River Mura, in the northeastern part of Slovenia is called Prekmurje and together with Prlekija on the

right bank, forms Pomurje. There is no doubt that River Mura gives life to the whole of Pomurje, especially to flooded forests, gravel pits, animals, plants, as well as people.

In order to protect settlements and agricultural land from flooding in the flat part of the river's basin and in order to facilitate construction and intensive farming, the River Mura and its three major tributaries were continuously regulated in the past. The consequences of urbanization, primarily dispersed basin settlement, and a large proportion of agricultural land has caused excessive nutritive substances (nitrogen and phosphorus compounds) in the water, and a significant hydro-morphological burden on most water bodies. Organic pollutants can also be present in the surface water. The hydro morphological burden on the surface water of the River Muras' basin and the burdening of the environment with heavy metals, organic matter and nitrogen and phosphorus compounds that plants don't take-up had an influence on the quality and quantity of groundwater in aquifers. Therefore from the environmental point of view, lowland alluvial forests, old overgrown channels, river islands, gravel deposits, and the erosive areas in river channels, are extremely important because they not only represent a habitat for existing endangered species of animals and plants, but also provide long-term infiltration of surface water in the soil, the allocation of high water over more surface area, and the chemical-intensive processes, (micro) biological degradation of nitrogen and phosphorus compounds, together with other dangerous chemical substances in the water. Reduction of flood risks and human health, the environment, economic activities and cultural heritage, safer care for residents when drinking water, ensuring the biodiversity of the River Mura area and its wetlands and river basins are, thus, the most important objectives of water management [1-6].

1.2 Linear regression

Linear regression demonstrates the relationship between two variables by fitting a linear equation to observed data. One variable is considered to be an explanatory-variable, and the other a dependent variable.

Before attempting to fit a linear model to observed data, it should first be determined whether or not there is a general relationship between the variables of interest. A scatter plot can be a helpful tool in determining the strength of the relationship between two variables. A valuable numerical measure of the association between two variables is the correlation coefficient, which is a value between -1 and 1, indicating the strength of the observed data's association for the two variables.

The most common method for fitting a regression line is the method of "least-squares". This method calculates the best-fitting line for the observed data by minimizing the sum of the squares of the vertical deviations from each data point to the line (if a point lies on the fitted line exactly, then its vertical deviation is 0). Because the deviations are first squared, then summed, there are no cancellations between positive and negative values.

The consequences of urbanization, primarily dispersed basin settlement, and a large proportion of agricultural land has caused excessive nutritive substances (nitrogen and phosphorus compounds) in the water, and a significant hydro-morphological burden on most water bodies.

From the environmental point of view, lowland alluvial forests, old overgrown channels, river islands, gravel deposits, and the erosive areas in river channels, are extremely important because they provide long-term infiltration of surface water in the soil, the allocation of high water over more surface area, and the chemical-intensive processes, (micro) biological degradation of nitrogen and phosphorus compounds, together with other dangerous chemical substances in the water.

The purpose of this work was to find any correlation between different designated places (sample places) and their variables, obtained using physico-chemical measurements.

Quality River Mura monitoring has been performed continuously for over 30 years, but this contribution only presents the results of the last period between 1996-2009.

After a regression line has been computed for a group of data, a point which lies far from the line (and thus has a large residual value) is known as an "outlier". Such points may represent erroneous data, or may indicate a poorly-fitting regression line. The reason for this distinction is that these points may have a significant impact on the slope of the regression line [7].

River Mura water analysis, which took into consideration the physical and chemical characteristics, was sampled at four designated places. Data collected within the period 1996 to 2009 was first discussed. The purpose of this work was to find any correlation between different designated places (sample places) and their variables, obtained using physico-chemical measurements. These parameters were used to form a model, which was a basis for the separation and classification of diverse water samples over different time-periods.

Chemometric techniques can be used for the classification and comparison of diverse samples. Linear regression, helps to interpret these complex database, which result from long-term monitoring programmes of water quality and when determining several parameters to be used for the presentation of monitoring effectiveness. During the last period several chemometric techniques were used for fresh water characterization and when estimating, groundwater and sea water. The application of different statistical and chemometric techniques is one of the more applied instruments for presenting representative important information from extensive databases.

Quality River Mura monitoring has been performed continuously for over 30 years, but this contribution only presents the results of the last period between 1996-2009. The measurements were performed weekly between October – April, on the same day and the same time, at precisely determined place. Samples were not collected, during the summer months namely, the samples would not be representative due to increased flow. Additional sampling was performed twice a year at four different places along the river-stream. The first sample was taken in the middle of the river in Šentilj. The other three sampling places were 10 km further along the river-stream, in the middle and on both banks (left side in Austria – Bad Radkesburg and the right side in Slovenia – Gornja Radgona). Measurements were performed and exchanged over equal periods by two laboratories: Laboratory for Analytical Chemistry and Industrial Analysis at Faculty of Chemistry and Chemical Engineering, University of Maribor and Amt der Steiermarkischen Landesregierung in Graz, Austria. The intention was to compare the results and both laboratories performed samples contemporarily, twice a year [8-13].

2. EXPERIMENTAL PART

2.1 Samples and sample analyses

For sampling and sample transportation we used the standard sample ISO method [ISO 5667-11:1992 (E)]. [14] Water samples were taken half a meter under the surface in polyethylene containers. All plastic

and glass containers used for the samples and analyses were previously washed off with distilled water. Filtration was accomplished through glass-filters. All measurements were carried out on the same days, as the samples were taken.

2.2 Instruments and reagents

A Dionex CD 20 ion chromatograph a Perkin Elmer 552 UV-VIS spectrophotometers and a Cary 1E Varian were used for the determining the ions specified below. A WTW LF 537 conductivity meter an OXI 96 WTW oximeter and an Orion 920 mV/pH meter were used for conductivity, oxygen, and pH measurements. All the used reagents were analytically graded. The detailed measurement procedures are described in the literature [14-29].

2.3 Analytical methods

For the definition of AOX we applied [ISO 9562:1989], [16] for COD [ISO 6060:1989], [17] and for BOD₅ [ISO 5815:1989].[18] The suspended solids were determined by filtration through glass-fibre filters (ISO/DIS 11923). [19] Conductivity and pH were measured using the standard methods [ISO 7888 1985; ISO 10523 1994]. [20] Ammonium and nitrate were determined spectrophotometrically. Reaction with salicylate and hypochlorite ions in the presence of sodium nitrosopentacyanoferrate was used for ammonium determination, [ISO 7150/1 1984]; [21] and 2,6-dimethylphenol was used as a reagent for determining nitrate [ISO 7890/1 1986] [22]. Most anions were determined by the ISO standard method using ion chromatography [ISO 10304-1 1996] [23]. Nitrite was determined with NO₂ ion reaction with sulphanilamide where the results intensively coloured the diazonium salt, [24] meanwhile, orthophosphate was determined using ammonium molybdate method [25]. Sodium and potassium were determined using flame-emission spectrometry [ISO 9964-3 1993]. [26] Calcium, magnesium and sulphate were determined using EDTA titrations [ISO 6058 1984; ISO 6059 1984; SIST ISO 9280 1996] [27,28].

Oxidation to carbon dioxide by the addition of an appropriate oxidant was applied for the determination of TOC and DOC (ISO 8245:1999) [29].

Additional sampling was performed twice a year at four different places along the river-stream.

Measurements were performed and exchanged over equal periods by two laboratories.

2.4 Data analysis

336 samples were characterized within following physical-chemical variables.

Table 1:
Physical-chemical variables.

Num.	Code		Num.	Code	
1.	V2	Filtered solids (mg/L)	17.	V34	Phosphate (mg/L)
2.	V3	Absorbable organic halogens ($\mu\text{g/L}$)	18.	V36	Phosphates in unfiltered samples (mg/L)
3.	V4	Alkaine (mmol/L)	19.	V37	Phosphates in filtered samples (mg/L)
4.	V6	Ammonia (mg/L)	20.	V40	pH
5.	V14	Calcium (mg/L)	21.	V43	Biochemical oxygen demand after 5 days (BOD5) in filtered samples (mg/L)
6.	V15	Chloride (mg/L)	22.	V44	Biochemical oxygen demand after 5 days (BOD5) in unfiltered samples(mg/L)
7.	V18	Electrical conductivity (mS/m)	23.	V45	Chemical oxygen demand (COD) in unfiltered samples (mg/L)
8.	V19	Dissolved organic carbon (mg/L)	24.	V46	Chemical oxygen demand (COD) in filtered samples (mg/L)
9.	V20	Air temperature ($^{\circ}\text{C}$)	25.	V47	Dissolved oxygen (mg/L)
10.	V22	Total hardness ($^{\circ}\text{dH}$)	26.	V48	Oxygen surplus (mg/L)
11.	V23	Potassium (mg/L)	27.	V49	Oxygen saturation (mg/L)
12.	V24	Carbonate hardness ($^{\circ}\text{dH}$)	28.	V50	Oxygen saturation (%)
13.	V28	Magnesium (mg/L)	29.	V51	m-value (pH up to 4.3)
14.	V30	Sodium (mg/L)	30.	V55	Sulphate (mg/L)
15.	V32	Nitrite (mg/L)	31.	V56	Water temperature ($^{\circ}\text{C}$)
16.	V33	Nitrate (mg/L)	32.	V59	Suspended solids (mg/L)

3. RESULTS AND DISCUSSION

When monitoring the water quality of the river, special attention was paid to those parameters that had maximal influence, namely AOX, nitrate, nitrite, phosphate, and ammonium.

Figure 1 present, the non-linear time dependence for AOX variable from 1996 to 2009. The AOX content had already been monitored in the period 1990 to 1996 [8]. After 2006, the AOX content decreased below the limit value, 20 mg Cl/L. Reduction grounding on the already-mentioned reduction of paper industry efflux, which had the greatest impact on the parameters' high-values. A further course of AOX values is difficult to foresee, but the collected data suggests that the values could decrease in the future.

The bulk of phosphorus in the water is a consequence of human activities such as erosion, ablation of fertilizer, and the effusion of industrial and municipal wastewaters. Phosphorus in the water occurs predominantly in the form of phosphates. The most common forms are ortho-

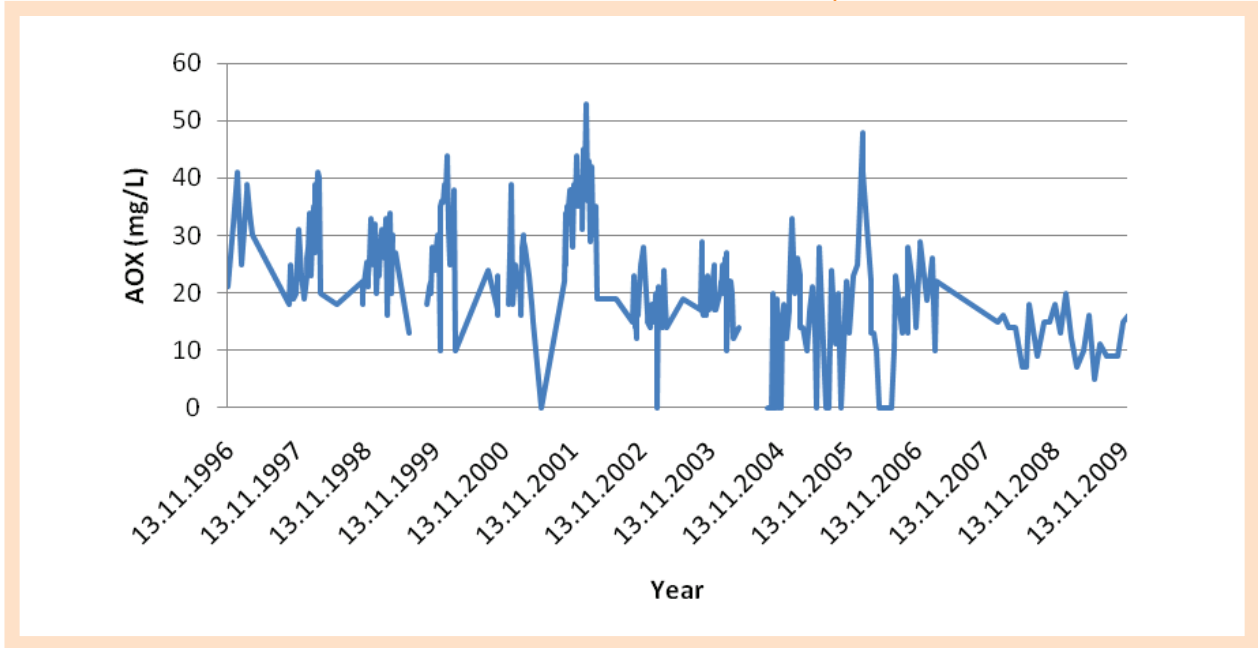
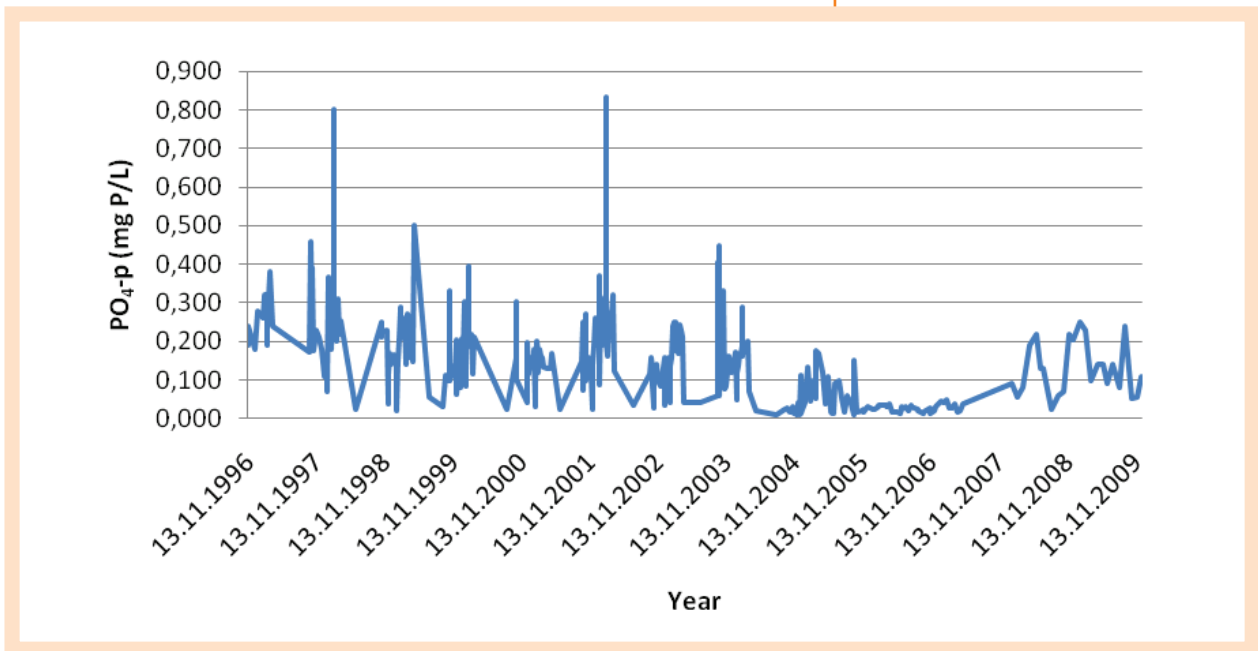


Figure 1:
AOX concentrations (mg/L) versus time.

phosphate, condensed phosphates, and organic phosphates. An increased flow of phosphorus in the water causes increased primary production of algae and their decomposition as secondary pollution. Therefore, the determination of phosphorus in the water is important in order to evaluate the potential biological productivities of surface waters.

From 1996 to 2009, the critical value for orthophosphate was placed at concentration of 0.5 mg P/L. In 1998 and 2002 at two locations the value exceeded 0.8 mg/L (Figure 2). From 2005 the concentrations were surprisingly low, but by 2008 they began to increase again. The quantities of industrial and municipal discharges and water-wash of mineral fertilizers had a significant impact on orthophosphate content oscillation.

Figure 2:
Orthophosphate concentration (mg P/L) versus time.



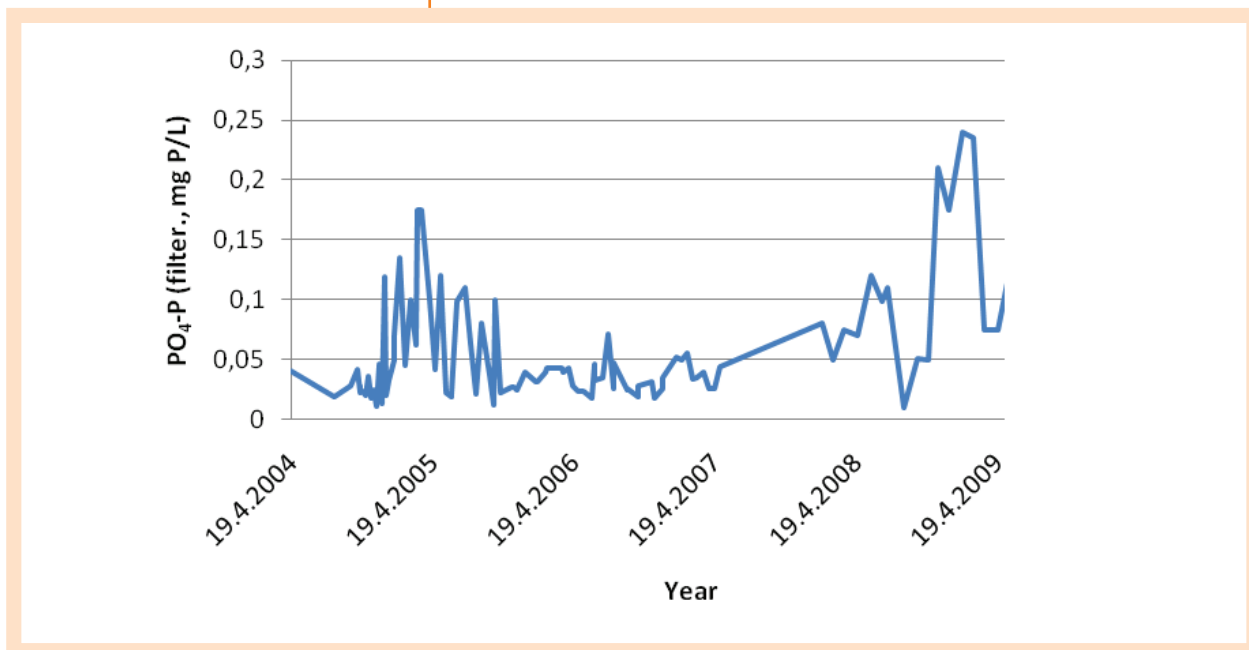
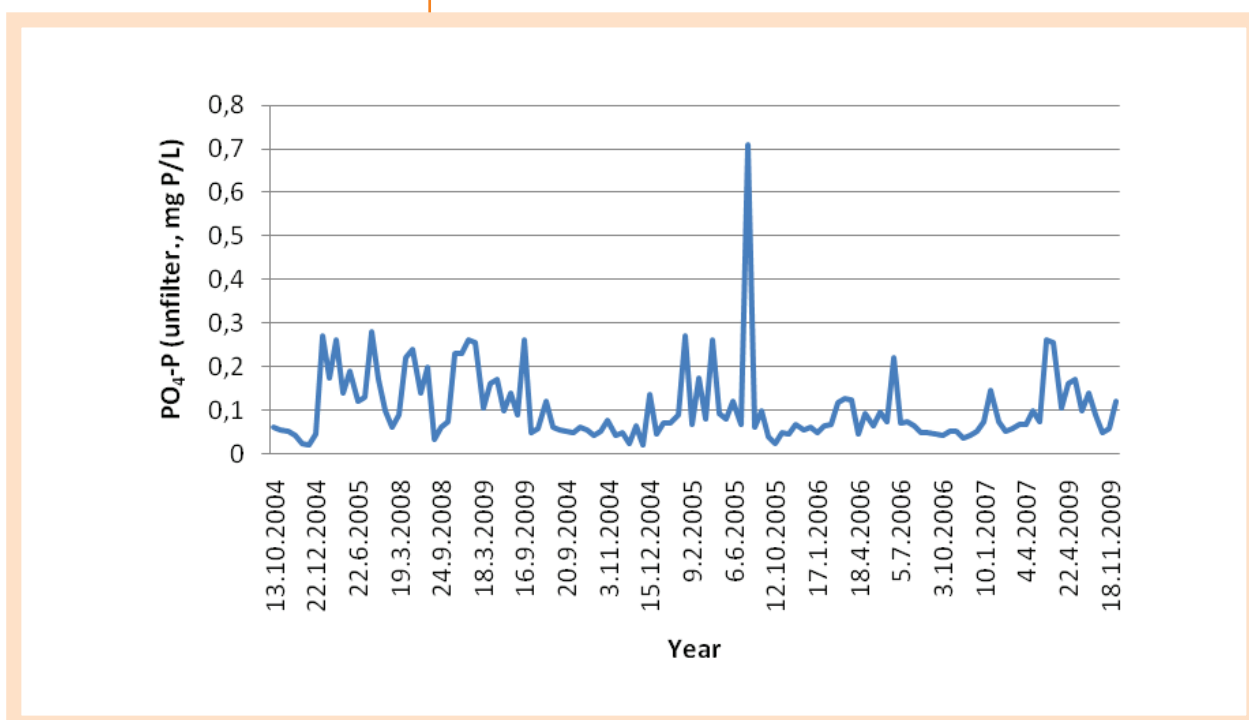


Figure 3: Total phosphorus concentration in the filtered samples versus time (2004-2009).

An concentrations of total phosphorus in the filtered samples had been under monitoring since 2004. Figure 3 present a deviation for the period 2004 to 2005 and the year 2009. As already-mentioned, increased content of total phosphorus in the filtered samples is also a result of agricultural land abluton and the discharges of industrial and municipal wastewater. Similar deviation (maximal value over 0.7 mg P/L) can be observed in 2005 for the concentration of total phosphorus in unfiltered samples – Figure 4.

Figure 4: Total phosphorus concentration in unfiltered samples versus time (2004-2009).

Nitrate and nitrite are present naturally in water and are widespread in the environment. Microbial action in soil or water decomposes wastes



containing organic nitrogen into ammonia, which is then oxidized to nitrite and nitrate. Because nitrite is easily oxidized to nitrate, nitrate is the compound predominantly found in ground and surface waters. All sources of nitrogen are potential sources of nitrate. In water, especially groundwater, these sources include decaying plant or animal material, agricultural fertilizers, manure, domestic sewage, industrial wastewaters, precipitation, or geological formations containing soluble nitrogen compounds. The nitrate concentration in groundwater and surface water is normally low, but can reach high levels from agricultural runoffs, or from contamination by human or animal wastes.

Nitrate is less toxic than the other forms of nitrogen in the aquatic environment, such as nitrite or ammonia. There is growing evidence, though, that nitrate can have a harmful impact on the development of early life stages in aquatic organisms, by reducing the oxygen-carrying capacity of the blood, or by disrupting the ability to maintain a proper balance of salts.

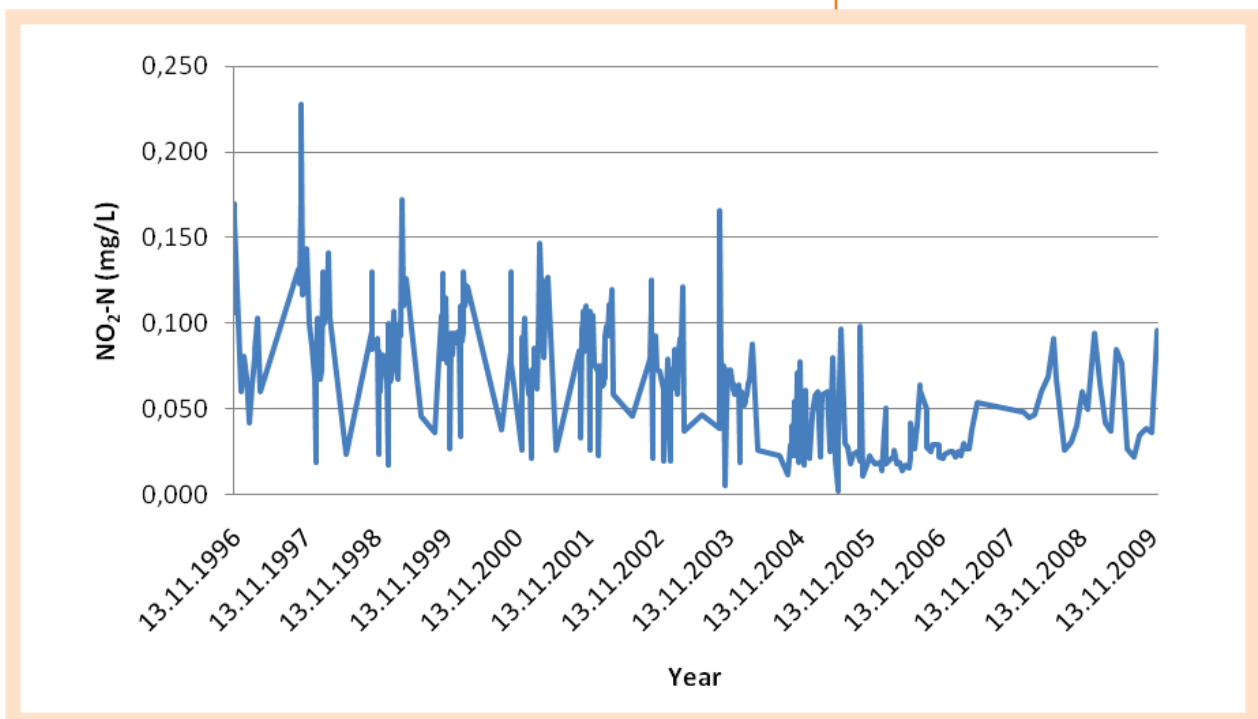
Increased levels of nitrogen in the water, combined with phosphorus, can cause excessive plant and algal growth that depletes oxygen levels, possibly to lethal levels. Some algal blooms also produce toxins that can affect aquatic life or the humans that consume them.

Currently, there are some guidelines for the protection of aquatic life concerning nitrogen compounds in surface water. In order to limit nitrate in the water supply it is necessary to: limit fertilizer use and apply it only at appropriate times, control runoffs and soil erosion, start a compost pile, and recycle yard waste [30,31].

The concentrations of nitrite in the River Mura were, until 2003, constant at up to 0.125 mg/L, maximum values were obtained in 1997, 1999, and 2003 (over 0.15 mg/L) (Figure 5). After 2004 the concen-

There is growing evidence, though, that nitrate can have a harmful impact on the development of early life stages in aquatic organisms, by reducing the oxygen-carrying capacity of the blood, or by disrupting the ability to maintain a proper balance of salts.

Figure 5: Nitrite concentration versus time (1996-2009).



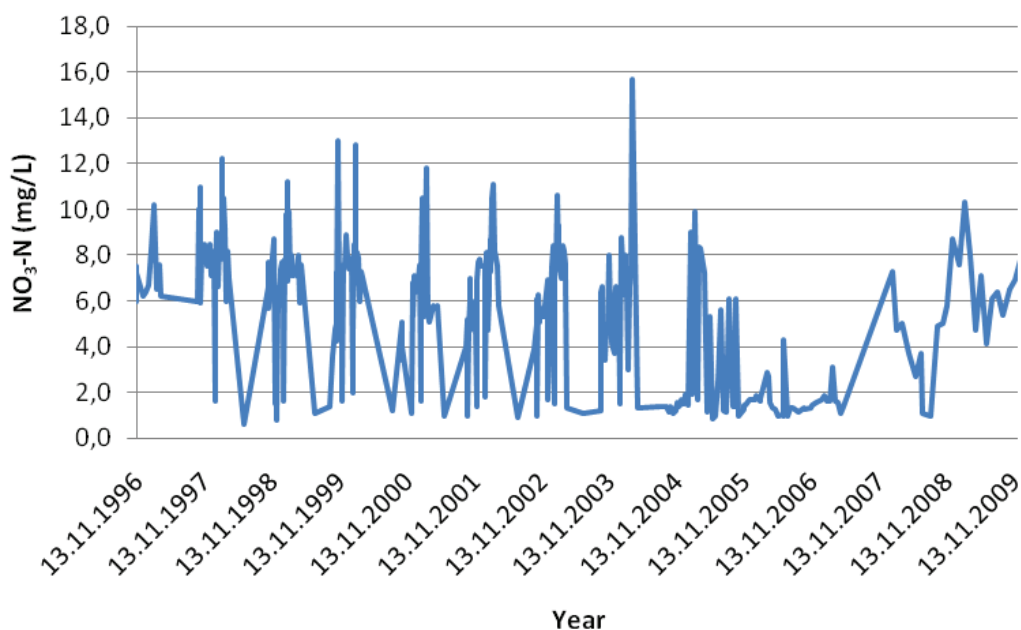


Figure 6:
Nitrate concentration versus time
(1996-2009).

Monitoring nitrite and nitrate content together with ammonia content is essential in order to evaluate nitrogen equilibrium in water.

trations of nitrite started to decrease below 0.10 mg/L and had in 2008 and 2009, reached a value of approximately 0.08 mg/L.

In the River Mura, the concentrations of nitrate have periodically fluctuated (Figure 6), reaching maximum values at up to 16 mg/L. The reduction in nitrate content started in 2005 over a short period, but from 2007 the concentrations increased almost to their primary values. Monitoring nitrite and nitrate content together with ammonia content is essential in order to evaluate nitrogen equilibrium in water.

Ammonia levels in excess of the recommended limits may harm aquatic life. Toxic levels are pH and temperature dependent; toxicity increases as pH, and the temperature decreases. Plants are more tolerant to ammonia than animals, and invertebrates are more tolerant than fish. Ammonia is the typical result of water pollution caused by municipal and industrial effluent and agricultural land ablation. Figure 7 presents the concentrations of ammonia from 1996 to 2009. These concentrations were within the range of 0.5 to 1.0 mg/L until 2003, the maximum values were obtained in 2001 (over 1.2 mg/L). After 2004 the concentrations of ammonia started to decrease below 0.6 mg/L and had, in 2009, reached the value of approximately 0.3 mg/L.

4. CONCLUSION

These parameters that we have studied in detail are among the main indicators of water pollution. Based on the monitoring of complete parameters' trends of the concentrations of ammonia, nitrite, nitrate and orthophosphate from 1996 to 2009, have, without any doubt, definitely decreased. We can give an assurance that the chemical and physical

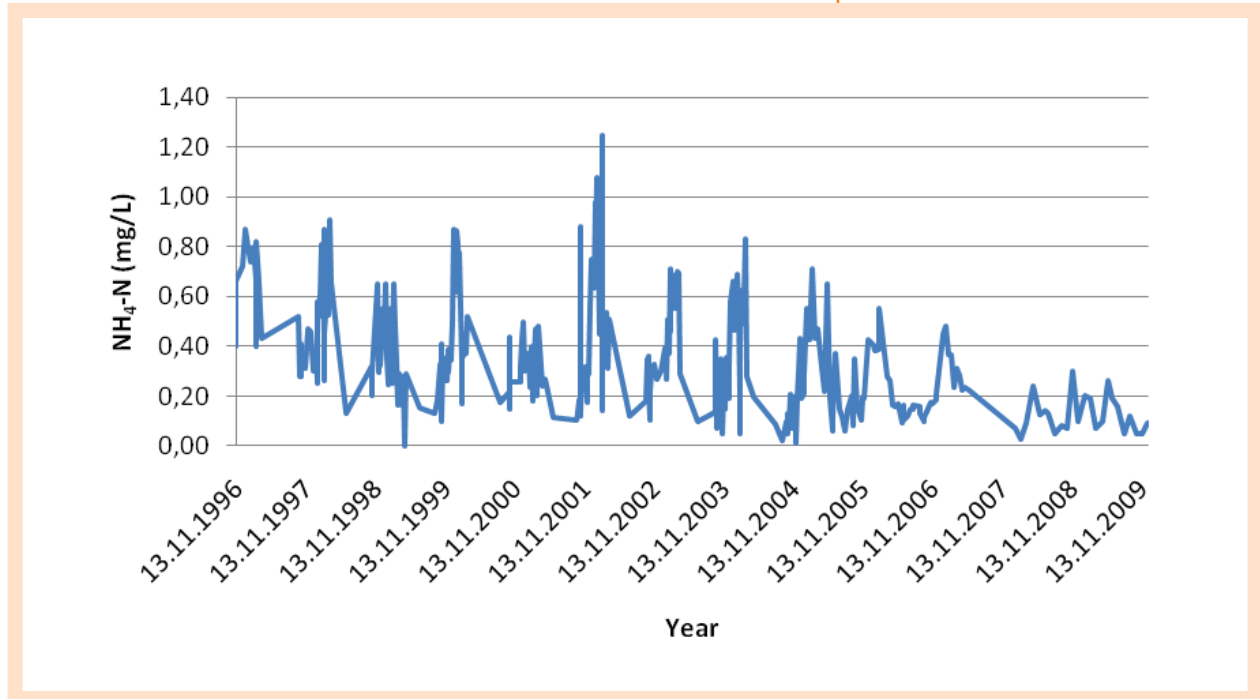


Figure 7:
Ammonium concentration versus time.

state of the River Mura quality, based on the reduction in concentration content, has improved. This significant impact on reduction has increased the awareness of industry when referring discharges into the river and a more rational use of plant protection products on agricultural land.

Compared to the period 1990-1996 AOX values over the period 1996-2009 had stabilized. The content of total phosphorus in the filtered and unfiltered samples had been under monitoring from 2004. Two perceivable deviations, which may be a consequence of the coincident increase when referring to industry and agriculture impact, from the data collected from 2004 to 2009, were observed. In order to determine the trend regarding phosphorus more data needs to be collected, therefore, a monitoring programme is also planned for future years.

Sampling and analyses were performed and exchanged over equal periods between the Slovene and Austrian laboratories; the programme was prepared in advance. Sampling was performed together - twice a year - for comparing measurement capabilities of both laboratories but samples were then analyzed separately. Within the studied period both laboratories performed reliable and comparable results according to the prescribed measurement uncertainties of the methods used.

The performed analyses of River Mura's water samples, have certainly contributed to people's and industry's awareness regarding emissions, because the reduced amount of emissions ensured an improvement in the water quality of the River Mura. Since a number of samples and data-sets using different analytical methods was present, chemometric interpretation of the results is also necessary, and is in preparation.

The performed analyses of River Mura's water samples, have certainly contributed to people's and industry's awareness regarding emissions, because the reduced amount of emissions ensured an improvement in the water quality of the River Mura.

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The Frequency of Fruit and Vegetables Consumption and the Frequency of Vitamin and Mineral Supplements Consumption among Students

Tamara **KAROLI**¹, Barbara **REDNAK**¹, Borut **POLJŠAK**^{1*}

ABSTRACT

Eating habits of Slovenes are bad. The research Eating habits of adult Slovenes [1] showed that Slovenians eat too much unhealthy food and too little fibre, fruit and vegetables. On average a Slovenian adult eats only one piece of fruit per day, whereas 12 % of Slovenians never eat vegetables [1]. In their research from the year 2004, Poljšak et al. similarly come to the conclusion, that half of the participants eat fruit and/or vegetables merely once per day or even less than once. Numerous institutions in cooperation with the government commissions therefore started promoting a diet with more fruit and vegetables. This kind of promotions (PRO GREENS, School fruit scheme) is focusing on the younger population.

Due to the fact that this is a current topic, it was decided to carry out a research among the students of the University of Ljubljana, which would help to find out how often the students eat fruit and vegetables, and how often they consume vitamin and mineral supplements. Regarding available data the observed population does not eat enough fruit and vegetables, and moreover too often takes vitamin and mineral supplements. The results of the research carried out among 100 students show that merely 7 % of the participants eat fruit or vegetables five or more times per day. As many as 40 % of the participants eat fruit and vegetables only once per day. The frequency of taking vitamin and mineral supplements varies. Out of 71 % of students who take vitamin and mineral supplements, 8 % consume supplements regularly on a daily basis.

KEY WORDS:

Nutrition, Research, Fruit and vegetables, Vitamin and mineral Supplement, Student

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INTRODUCTION

Food consumed by an individual has a great impact on health. A healthy diet protects one's health, at the same time improves it and lowers the risk of numerous "contemporary lifestyle diseases" (high blood pressure or hypertension, high cholesterol, cancer, diabetes, etc.). Among nutrients, particularly vitamins and minerals are essential. Human body is incapable of producing them on its own (except for small doses of vitamin D and K, and biotin), therefore they have to be consumed with our food on a daily basis. Most vitamins and minerals are found in fruit and vegetables, and unshelled cereals. According to World Health Organisation (WHO) recommendations every individual should eat fruit and vegetables every day, in at least five meals.

And why is eating fruit and vegetables that important? Both fruit and vegetables contain antioxidants, substances that lower the production of free radicals in the organism [2] and in this way protect body cells from injuries [3].

Contemporary lifestyle and shortage of time are the two major reasons why people increasingly often take vitamin and mineral supplements. According to the data available in literature, approximately 20 % of Americans were taking vitamin supplements in the early 70's of the 20th century, whereas twenty years later the percentage amounted to 40 % [4]. Poljšak et al. [2] carried out a similar research among the Slovenian population, which shows that as many as 12 % of the participants are regularly taking vitamin and mineral supplements. Merely 28,3 % of the participants do not take vitamin and mineral supplements.

Due to the increasing availability of vitamin and mineral supplements, numerous institutions around the world are carrying out a range of research to find out the impact of vitamins on the one hand, and the additional intake of antioxidants on the other. Research findings vary. Up to now, there have been no reliable proofs for the beneficial effect of vitamin supplements. However, the pro-oxidant effect of vitamins C and E with the simultaneous intake of iron has been proved [5,6,7].

Regarding the popularity of the discussed topic, extensive research on the impact of supplements on our health, and the already conducted research, with which Poljšak et al. [2] wanted to research the consumption of food supplements among Slovenian adults, we prepared a model of a pilot study to find out about the supplement consumption among the students of the University of Ljubljana. The selection of the population was not coincidental, since the students belong to the group of young adults. It is said that the world depends on youngsters, therefore the concern for their healthy lifestyle is of utmost importance. It was presumed that the students' daily vitamins and minerals consumption is insufficient and that they too often take additional antioxidants.

Students lead a stressful life due to the fact that this is the time of acquiring education, material wealth, starting a family and a demanding lifestyle. In this period, young people tend to indulge in various pleas-

According to World Health Organisation (WHO) recommendations every individual should eat fruit and vegetables every day, in at least five meals.

Students lead a stressful life due to the fact that this is the time of acquiring education, material wealth, starting a family and a demanding lifestyle. In this period, young people tend to indulge in various pleasures (drinking, smoking), eat unhealthy food, do not practice any sports – all of the mentioned factors result in oxidative stress and illnesses deriving from it.

The obtained results may later be used in a more extensive research on the vitamin and mineral supplement consumption among Slovenian students.

ures (drinking, smoking), eat unhealthy food, do not practice any sports – all of the mentioned factors result in oxidative stress and illnesses deriving from it [2]. Therefore the observed population should be conscientiously eating the recommended daily doses of vitamins and minerals, whereas during the examination periods the doses should be increased. The objective of our research was to determine the rates of vitamin-mineral supplement consumption among the above mentioned population, the kind of supplements they most often use, to find out how often they eat fruit and vegetables and what is their opinion regarding the impact of fruit and vegetables consumption on their health.

The purpose of this paper is to introduce the pilot study results. The obtained results may later be used in a more extensive research on the vitamin and mineral supplement consumption among Slovenian students. The obtained results may also be used as an argument for more frequent and extensive campaigns of the importance of fruit and vegetables consumption among students. The aim is also the argumentation of the removal of unhealthy food from automatic vending machines at faculties and replacing it with a wide choice of seasonal fruit.

METHODS

The study is based on the cross-sectional descriptive method. Interviews were carried out at the site. The study scheme does not allow for the analysis of reasons that triggered the illness or condition. It also does not allow for the analysis of measures taken to contain the illness. Due to the mentioned reasons the study may be labelled as the one of descriptive epidemiologic nature [8]. The primary purpose of the study was not the verification of the presurmise or search for the correlations but the description of the situation. For this reason there was no need to establish the hypothesis. The advantage of such studies is the formation of presumptions that may be tested as part of analytical epidemiologic studies. The study offers data about the condition in a given moment. The population that is exposed to the risk involves all the individuals included in the research [8].

The data were collected with questionnaires designed according to the questionnaires administered by the authors [2] in the research Consumption of vitamin supplements in diet. The questionnaire consisted of 13 questions.

The questionnaire started with the questions that defined the sample characteristics. They were followed by the questions dealing with the individual's attitude to the consumption of vitamin and mineral supplements. The questionnaire concludes with the question dealing with the importance of fruit and vegetables consumption and concern for health.

The questionnaire required marking the chosen statements. Due to the reason that the same questions had been previously used [2], the pilot study that would test the comprehension and optimality of the questionnaire was not carried out.

The interviews took place either in front of or inside the faculties, members of the University of Ljubljana, chosen at random, also on streets, and in students' homes. The participation was voluntary, the questionnaires were filled in by the participants in the presence of the interviewer who was able to clarify any questions. Prior to the interview all the participants were reminded that they may decline the participation, and those who did so, refrained from filling in the questionnaire. 115 individuals were approached, 100 of them agreed and filled in the questionnaires. The most common reasons for the rejection were the lack of time and lack of knowledge about the topic involved. The interviews took place in the period from 22 March 2010 to 16 April 2010.

The questionnaires were processed with the Microsoft Office Excel programme. At the very beginning 5 questionnaires were excluded from the further analysis for they were not filled in according to the instructions.

RESULTS AND DISCUSSION

Results

The results chosen for the graphic illustration refer to age (Figure 1) and gender (Figure 2) structure of the participants, the frequency of fruit and vegetables consumption (Figure 3), vitamin and mineral supplements consumption (Figure 4), and their types (Figure 5), the reasons for consumption (Figure 6) and the opinion of the participants regarding the requirement of the supplement consumption although sufficient fruit (Figure 7) and vegetable intake as well as the requirement of fruit and vegetable consumption although regular intake of the supplements (Figure 8). The remaining findings are included in the discussion. Questions meant to be answered just by the participants who consume vitamin and mineral supplements were not filled in by the participants who do not take them. In case the participants did not respect the instructions, the questionnaire was excluded from the further analysis, as mentioned previously.

Discussion

The results of the research show that 80 % (Figure 3) of the participants eat fruit and/or vegetables every day. 40 % of them eat fruit and/or vegetables two to four times per day, 40 % once per day. Only 7 % of the participants eat fruit and/or vegetables five times per day. Similar results were obtained from the authors of the research [6], in which they wanted to find out how many times per day Slovenians eat fruit and/or vegetables. They found out that only 5 % of the participants eat fruit and/or vegetables five or more times per day. According to World Health Organisation (later on referred to as WHO), one should eat at least five meals per day containing fruit and/or vegetables. Since the recommended norms about the daily amounts of fruit and vegetables are not met, WHO, being aware of the situation, formulated The Global Strategy on Diet, Physical Activity and Health, a very important part of Strategies for Health in the 21st century. It also includes information on

The participation was voluntary, the questionnaires were filled in by the participants in the presence of the interviewer who was able to clarify any questions.

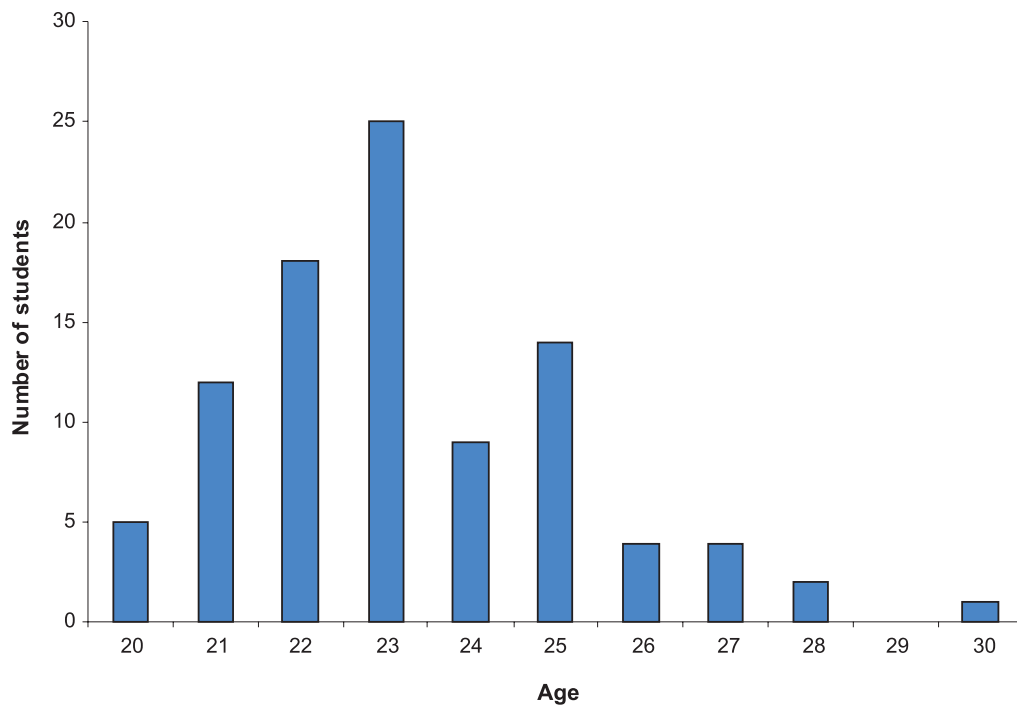


Figure 1: Age.

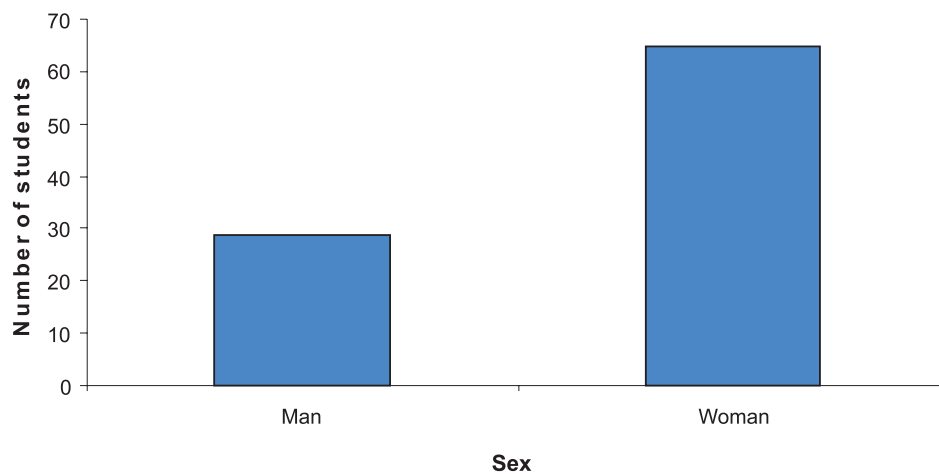


Figure 2: Gender.

healthy diet and it could be included in the group of strategies for essential changes.

Different government institutions functioning on the field of human health and disease prevention are aware of the problem of insufficient fruit and vegetable intake of the slovenian population. With the aim of increasing the frequency of fruit and vegetable intake many measures were taken. Two of them are the programs "PROGREENS" and "Shema šolskega sadja". Both programmes are dedicated to elementary school

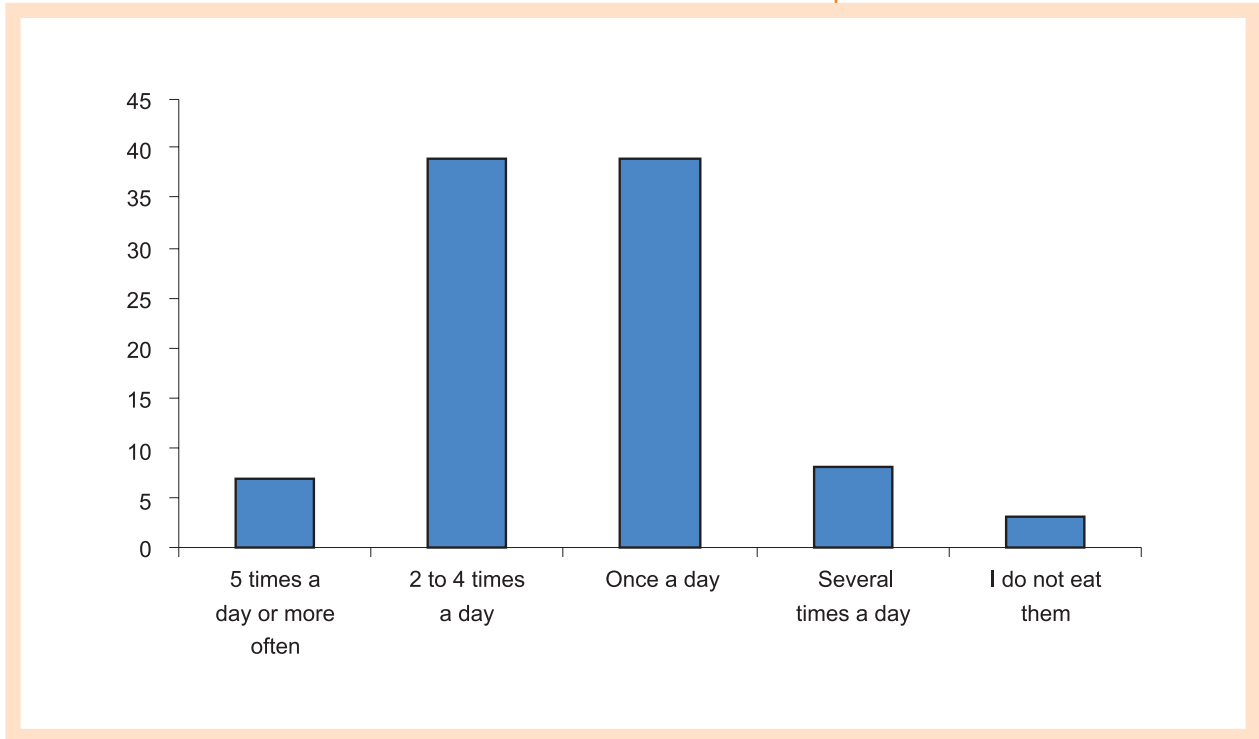


Figure 3:
The frequency of fruit and vegetables consumption ($N=95$).

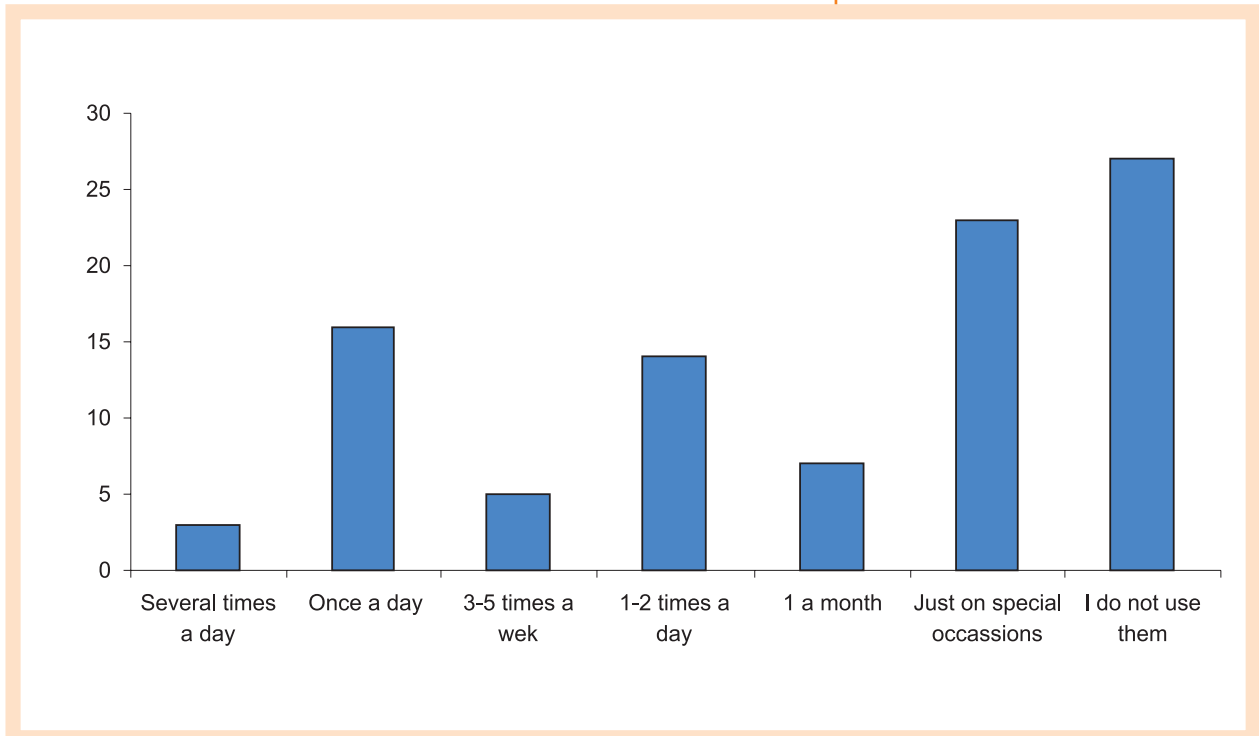


Figure 4:
The frequency of vitamin and mineral supplement consumption ($N=95$).

pupils. Their common goal is to increase fruit and vegetable consumption and thus fortifying the health of target population.

71 % (Figure 4) of the participants take vitamin and mineral supplements. 8 % of participants take supplements on a daily basis, hardly a third of the participants take them a few times per week or per month,

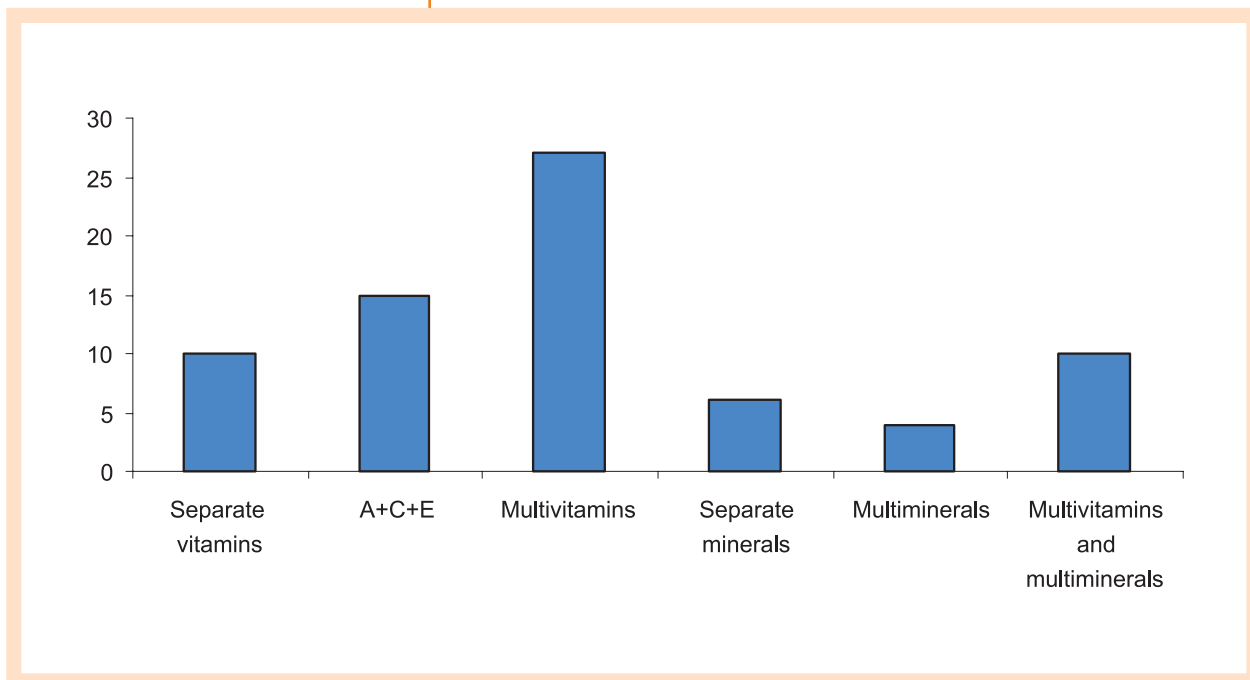


Figure 5: The most commonly used vitamin and mineral supplements ($N=95$).

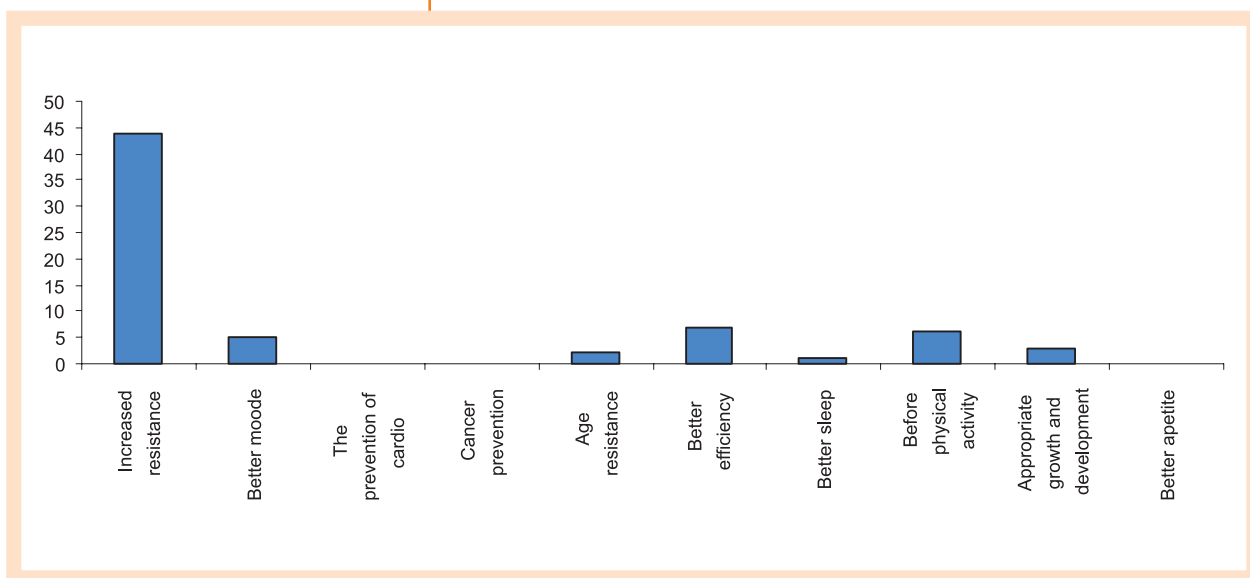


Figure 6: Reasons for vitamin and mineral supplement consumption ($N=95$).

whereas the majority of the participants take them under special circumstances only. The findings of the research The consumption of vitamin and mineral supplements as a part of one's diet [6] show that 12 % of the participants take supplements on a daily basis, 38 % of the participants only in rare cases. The findings of the research Slovenian public opinion from 1994 [9] show that 8,4 % of the participants take supplements on a daily basis, 29,9 % take them rarely and 47,4 % never take them. According to the results of the research Slovenian public opinion [9], from 1996, 9,1 % of the participants take supplements every day, 28,9 % take them rarely, and 47,2 % never take supplements. In the research carried out among nurses in the year 2002

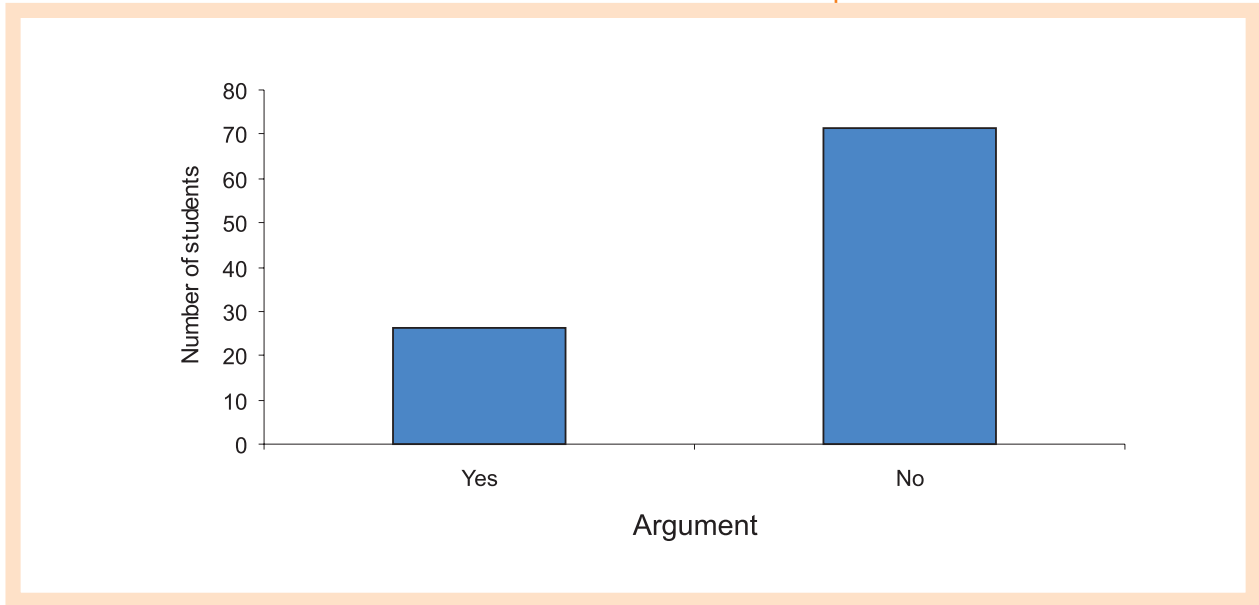


Figure 7: The requirement of the supplement consumption although sufficient fruit and vegetable intake is obtained (N=95).

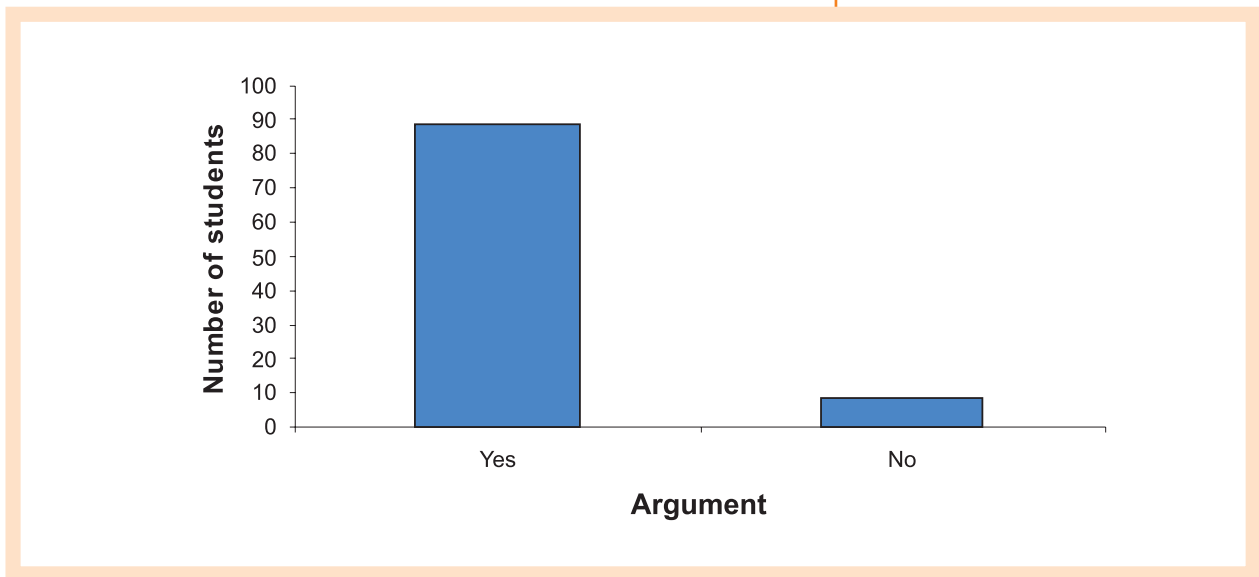


Figure 8: The requirement of the fruit and vegetable consumption although vitamin supplement is regularly used (N=95).

[10] the authors found out that 36,9 % of the participants take supplements once per month or even more seldom, and merely 6,2 % of the participants take them on a daily basis. It can be drawn the conclusion from the listed research that the consumption of vitamin and mineral supplements is the lowest among health services employees, and among our chosen population.

In our research, the participants' opinion on the sensibility vitamin and mineral supplements consumption turned out to be pretty unanimous. The results show that less than 75 % of the participants do not see any point in taking vitamin and mineral supplements, in case one regularly

Fruit and vegetable contains many different antioxidants and specific antioxidant interact in a synergistic way by regeneration of each other, e.g. vitamin E can regenerate vitamin C and glutathione can regenerate vitamin C.

The consumption of vitamin supplements as a part of one's diet.

Regarding the reason for the consumption of vitamin and mineral supplements, prevails the opinion that vitamin and mineral supplements increase the resistance of the organism.

eats fruit and/or vegetables (Figure 8). As much as 95 % of participants as well believe that in case one regularly eats fruit and/or vegetables there is no need for supplements (Figure 7). Similar results were obtained in the research The use of vitamin and mineral supplements as part of one's diet [11], in which 72,3 % of participants turned out to believe that there is no point in taking supplements if one regularly eats fruit and/or vegetables, 86 % of the participants think that in case of regular consumption of fruit and vegetables supplements are not necessary. Furlan [11] and Pokorn [12,13] come to the conclusion that mineral and vitamin supplements are not necessary in preventive medicine, they can even be harmful. They write that a balanced diet is still the most optimal choice for the prevention of some chronic diseases. Many studies confirmed the fact that vitamin intake is better from the food compared to synthetic compounds. Fruits, grains and vegetables contain multiple components that might exert protective effects against disease. It could be any, or any combination of those factors that is a true protective agent. High plasma ascorbate levels or high ascorbate intake could simply be a marker of a good diet rather than a true protective factor [14]. Fruit and vegetable contains many different antioxidants and specific antioxidant interact in a synergistic way by regeneration of each other [14], e.g. vitamin E can regenerate vitamin C and glutathione can regenerate vitamin C [14,15]. If these compounds are not present (e.g. in a food supplement) such regeneration cannot occur and pro-oxidative reaction can begin [14,15]. Such situation may occur when a single vitamin supplement is taken [15].

The research showed that the participants most often take multivitamin supplements (approximately 50 %) (Figure 5). Less than a quarter of them take multivitamin-multimineral supplements or a single-vitamin supplement, less than a sixth of the participants most often take supplements of vitamins A+C+E (Figure 5). Similar conclusions are drawn in the research carried out among the students of the University of Ohio, where the most popular supplements are the multivitamin ones, followed by vitamin C supplements and the ones with calcium [16]. Somewhat different are the results obtained by the authors of the research The consumption of vitamin supplements as a part of one's diet [2]. Their observed population most often take multivitamin supplements (28,7%), and supplements of vitamins A+C+E (28,3%). Similar to these findings are the ones obtained from the American national research, where most often used vitamin and mineral supplements are likewise multivitamin supplements, followed by a very similar percentage of those taking the supplements of vitamin E and calcium [17]. Regarding the reason for the consumption of vitamin and mineral supplements, prevails the opinion that vitamin and mineral supplements increase the resistance of the organism (Figure 6). A small percentage of the participants state as a reason for the consumption of supplements improved productivity and concentration, higher spirits and better physical performance (Figure 6). The participants' idea about the consumption of supplements increasing the resistance of the organism is actually not precise, since it is well known that the natural vitamins'

efficiency by far exceeds that of the synthetic vitamins. Eating a lot of fruit and vegetables is therefore more sensible than taking supplements. For example, the synthetic vitamin C contains only the ascorbic acid, whereas the natural vitamin C found in rose hip contains bioflavonoids and vitamin C complex [19].

The participants obtained information on the beneficial effects of vitamin and mineral supplements mainly from the media. The same are the findings of the authors of the research [2], in which 40 % of the respondents stated the media (TV, radio, newspaper etc.) as the most important source of information on vitamin and mineral supplements. The results of both examples of research indicate the power of the media and the pharmaceutical industry [2].

The legislation in the area of vitamin and mineral supplements may be considered inappropriate. In the USA markets the supplements containing 10 times of the recommended daily doses may be found. As they are still not treated as medicines, they are not regulated by the Food and Drug Administration monitoring bioabsorption and toxicology medicine tests before they are launched on the market. The European Commission has prepared the regulations in the area of vitamin and mineral supplements that do not fall under the medicine regulations [2]. With the harmonisation of such regulations Slovenia in 2003 first introduced the Regulations on the categorisation of vitamin and mineral supplements taken orally in pharmaceutical form, and included them among medicines [19]. In the year 2008 the regulation was replaced by a new one with one important difference with regard to the criteria of categorisation. According to it the vitamin and mineral supplements taken orally are now considered to be medicines, either when the quantities of vitamins, minerals and oligo elements exceed the quantities quoted in the regulations in Tables 1 and 2, or in case they have medicinal properties and are used also for the prevention of illness [20].

The participants in our research most frequently buy vitamin and mineral supplements in shops. In contrast to this, the participants in the research The consumption of vitamin and mineral supplements as a part of one's diet [2] most often report buying supplements in pharmacy. 90 % of our participants take vitamin and mineral supplements in doses stated by the producers in the instructions according to the recommended supplement intake.

With regard to the assessment of vitamin and mineral impact on health improvement, the five grade scale (with 1 used for a huge impact, and 5 for no impact), 30,4 % participants assessed the impact on the health as huge or considerable. In contrast to this, Poljšak et al. [2] found out that almost 50 % of participants consider the impact of vitamins and minerals on health as huge or considerable. It is concluded that in general the participants acknowledge the importance to vitamins and minerals in their diet, but not sufficiently.

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The restaurants offering subsidised meals to students take them into account to a certain extent, but the recommendations have to be explained to the students as well.

The research shows that students do not consume enough frequent the fruit and vegetables per day.

Fruit and vegetables contain natural vitamins, which cannot be fully replaced with the consumption of synthetic ones.

THE APPLICABILITY OF THE RESULTS

The researchers are aware of the small size of the sample in our research and the fact that the results do not adequately present the total Slovene student population. However, it might be concluded that the obtained results compare to those of the similar studies [2,9,10,21,22] carried out in our country and including larger numbers of participants. The obtained data may presently be used as an argument for the authorities to improve the much needed campaigns for healthy diet among students and the urgent need to change the offer of snacks in vending machines at faculties belonging to the University of Ljubljana.

Although the guidelines for healthy diet of students with menus [23] have been issued, they are not sufficiently used. The restaurants offering subsidised meals to students take them into account to a certain extent, but the recommendations have to be explained to the students as well. The results of the research show the frequency of vitamin and mineral supplements consumption, and the frequency of fruit and/or vegetables consumption. With the help of the guidelines and the obtained data it could be eventually launched the campaign for healthy diet. The guidelines offer advice on fruit and vegetables consumption and the results point at the segments that should be focused on.

It is evident that the obtained data do not lead to clear conclusions, but they nevertheless confirm the fact that it is wise to continue the research.

CONCLUSION

The research shows that students do not consume enough frequent the fruit and vegetables per day. Merely 7 % of the observed participants eat five or more meals of fruit and/or vegetables. The results show that the consumption of vitamin and mineral supplements among the chosen population is moderate, since merely 8 % of the participants take vitamin and mineral supplements on a daily basis, whereas 71 % of the participants only take them in rare cases (for example in case of an illness). The media play a great role in persuading people to start taking supplements.

It is important to inform youngsters about the fact that a healthy person does not need to take any additional antioxidants in case they regularly eat fruit and vegetables, are physically active and lead a healthy lifestyle. When the balance in the organism is disrupted and one falls ill, it is primarily important to eat fruit and vegetables, and not vitamin and mineral supplements. Fruit and vegetables contain natural vitamins, which cannot be fully replaced with the consumption of synthetic ones.

To take vitamin and mineral supplements or eat fruit and vegetables? Does the consumption of vitamin and mineral supplements make sense or not? There is no correct answer to this question. On the one hand there are fruit and vegetables treated with pesticides, fruit is often collected unripe, even if it is known that fruit starts to produce antioxidants to defend itself from solar radiation [2]. On the other hand there is

no credible evidence about additional intake of antioxidants having any positive impact on the organism, yet the pro-oxidative effect of vitamin C and E taken together with iron has been proved [5,6,7]. *What should be done after?*

A healthy grown up individual leading a healthy lifestyle has no need for an additional intake of antioxidants. It is important that one eats a balanced diet containing as much fruit and vegetables as possible, and as little unhealthy food as possible, and stays physically active. This enables us to protect our health on the one hand and improve it on the other.

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Obstojnost biofilmov

Biofilm Stability

Štefan **PINTARIČ**

POVZETEK

Nastajanje biofilmov predstavlja posebne razmere, ki omogočajo mikroorganizmom, da so obstojni na površinah ob običajnih postopkih sanitacije. S tem se povečuje tako možnost kontaminacije površin kot tudi njihovega raznosa v hrano. Lahko pa pride tudi do vnosa mikroorganizmov z biofilmom v organizem ljudi in živali, s čemer povzroči težave pri postoperativnih stanjih ali drugih posegih. V članku predstavljam značilnosti biofilmov z njihovimi lastnostmi, ki so potrebne za pravilen pristop in uspeh sanitacije.

KLJUČNE BESEDE:

Biofilm, Sanitacija, Dezinfekcija

ABSTRACT

Biofilm generation presents special circumstances that allow microorganisms to persist in areas with normal procedures for sanitation. This increases both the risk of contamination of surfaces as well as their costume in food. You can also occur with the introduction of micro-organism in biofilms in humans and animals, thus cause difficulty in postoperative states or other interventions. The article presents the characteristics of biofilms with their properties, which are necessary for the correct approach and the success of sanitation.

KEY WORDS:

Biofilm, Sanitation, Disinfection

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UVOD

Biofilmi so heterogene mikrobne združbe, ki so se uspele prilagoditi različnim življenjskim razmeram. Predvsem njihova mikrobna heterogenost in sposobnost ustvarjanja eksopolisaharidne zaščitne plasti, pripomore k njihovi stabilnosti. Odstranjevanje biofilmov s površin je zaradi njihove obstojnosti na različne vplive še vedno težavna naloga. Čeprav poznamo preko 150 spojin, ki imajo biocidni učinek, le redki izmed njih učinkovito odstranjujejo strukturo biofilmov, k čemur pripomore tudi prisotnost nečistoče. Njihova pogosta prisotnost predstavlja za ljudi in živali nevarnost kontaminacije živil in surovin, saj se postopki sanitacije izvajajo rutinsko. Za uspešno zoperstavljanje organiziranim mikrobnim združbam je pomembno, da poznamo mehanizme nastajanja biofilmov, da se jim lahko zoperstavljamo.

GLAVNE ZNAČILNOSTI BIOFILMOV

Za uspešno zoperstavljanje organiziranim mikrobnim združbam je pomembno, da poznamo mehanizme nastajanja biofilmov, da se jim lahko zoperstavljamo.

Biofilm se lahko v laboratorijskih razmerah dnevno razširi za nekaj milimetrov.

Biofilm so tanke plasti na površinah, sestavljene iz hranilnih snovi in mikroorganizmov. Proces nastajanja biofilma je postopen. Pogosteje zasledimo nastajanje biofilma na površinah, ki jih oblivajo tekočine. Hitrost nastajanja biofilma je večja s povečevanjem koncentracije hranilnih snovi v tekočinah. Pri tem se povečuje nalaganje hranilnih snovi na površinah. Kmalu za tem se zaradi elektrostatičnih privlačnih sil začnejo površinam približevati mikroorganizmi, ki se hitro utrdijo na hranilni površini tudi s pomočjo mikrobnih organelov. Po neločljivi pritrditvi na površini so nekateri mikroorganizmi sposobni ustvariti zaščitne plasti iz eksopolisaharidov, s katerimi se obvarujejo pred škodljivimi vplivi okolice, kar je tudi razlog za otežene postopke čiščenja, dezinfekcije in delovanja antibiotikov. Se pa hitrost nalaganja hranilnih snovi na površini poveča, če je biofilm na površinah že prisoten [1].

Poleg zaščitne vloge imajo eksopolisaharidi pomembno vlogo pri zadrževanju hranilnih snovi, potrebnih za vzdrževanje in širjenje biofilma. Biofilm se lahko v laboratorijskih razmerah dnevno razširi za nekaj milimetrov. Na širitev in velikost biofilma vpliva sestava mikrobne populacije in sožitje mikroorganizmov. V raziskavah ugotavljajo, da so biofilmi z eno vrsto mikroorganizmov površinsko manjši in manj obstojni [1,2].

K nalaganju mikroorganizmov na površino pripomorejo tudi fizikalno-kemijske razmere. S preučevanjem specifičnih mikroorganizmov so ugotovili vpliv pH in temperature na nalaganje na površine. Najugodnejše pH vrednosti za nalaganje *Pseudomonas fragi* so med 7 in 8. Podobno so ugotovili pri *Yersinii enterocolitici* in *Listerii monocytogenes*. Optimalna temperatura za nalaganje *Yersiniae enterocolitice* na površine iz nerjavne pločevine je 21 °C. Pri temperaturi 35 °C kot pri 10 °C je bilo nalaganje manjše. Podobne ugotovitve navajajo v raziskavi, kjer so preučevali vpliv temperature na nalaganje mikroorganizma *Deleya mariana*. Ugotovljena optimalna temperatura za nalaganje na površino je 25 °C. Slabšo adhezijo ugotavljajo pri 19 °C in 37 °C [1].

Čeprav eksopolisaharidna zaščitna plast varuje mikroorganizme pred zunanjimi vplivi, je prepustna za transport hranilnih snovi in kisika iz

okolice. V nasprotni smeri, pa vrača razgradne snovi in ogljikov dioksid. Z raziskavami strukture mikrokolonij v biofilmu so ugotovili obstoj t.i. vodnih kanalčkov, preko katerih se vrši transport hranilnih snovi in plinov v mikrokolonije. Z rastjo biofilma se začnejo spreminjati razmere v njem. Debelitev biofilma otežuje prehajanje kisika, pri čemer se postopoma v spodnjih plasteh ustvarjajo anaerobni pogoji [2].

V procesih razvoja biofilma se deli biofilma lahko oddvojijo od površine in se preselijo na nova mesta. Da se določen del biofilma lahko oddvoji je potreben učinek encimov. Procesom oddvajanja od površine pripomorejo intenzivnost pretoka in vrtinčenje tekočin na površini biofilma, prisotnost kemičnih snovi v tekočini in spremembe na površini materiala, na katerem je biofilm. Sproščeni mikroorganizmi se s pomočjo tekočinskega toka preselijo na novo lokacijo, kjer se začnejo novi procesi nastajanja biofilma [3,4].

Zaradi mešane mikrobne združbe bi pričakovali, da so procesi, ki se odvijajo v biofilmu, naključni. Vendar je proces nastajanja biofilma natančen in gensko uravnan. V številnih genetskih in molekularnih raziskavah skušajo razvozlati mehanizem nastajanja biofilma. Z razumevanjem teh mehanizmov raziskovalci pričakujejo, da bodo lahko ciljno pristopili k reševanju problematike nastajanja biofilma. V poskusu avtorjev, kjer so uporabili *Streptococcus mutans*, so preučevali pomen posameznih genov pri nastajanju biofilma. Z inaktivacijo posameznih genov, so ugotovili njihov pomen pri nastajanju biofilma. Podobne navedbe zasledimo pri preučevanju nastajanja biofilma *E. coli* in *Actinobacillus actinomycesetemcomitans*. Preučevanje nastajanja biofilma pri *Bacillus subtilis* kaže, da je proces krmiljen s sporulacijskim transkripcijskim faktorjem, ki je bistven pri sporulacijskih procesih. Z mutacijo regulatorja opažajo poleg nesposobnosti sporulacijskih procesov tudi nesposobnost ustvarjanja biofilma, mutiranega *B. subtilis*. Podobne raziskave so bile opravljene tudi na *E. coli*, kjer so z mutacijo gena dosegli nesposobnost pritrjevanja na površino. Ustvarjanje biofilma je tudi razlog za odpornost pri zdravljenju okužb s *Candida albicans*, in zdravljenju cistične fibroze, tvorbi biofilma pri *Pseudomonas aureofaciens*, in pri *Vibrio cholerae* [3,4,5,6].

Vendar pa je začetek nastajanja biofilma odvisen od vpliva okolja, v katerem se mikroorganizmi znajdejo. Vplivi okolja v nekaterih mikroorganizmih pod nadzorom genov sprožijo nastajanje kinolonskih in homoserinskih laktonskih spojin, ki vplivajo na nastajanje biofilma. Sproščanje kinolonov in homoserinskih laktonov omogoča, da se pri sosednjih mikroorganizmih sočasno odvijajo enake aktivnosti (razmnoževanje, ustvarjanje virulence, ustvarjanje metabolitov idr.), kar je pomembno za obstoj samega biofilma [7].

VPLIVI BIOFILMOV

Veliko raziskav je usmerjenih v preučevanje pomena bakterijskih biofilmov, saj predstavljajo v industrijskih procesih resen problem. Težave se kažejo v mehanski ovirah, slabšanju prevajanja toplote, biološkem obremenjevanju kovinskih delov opreme in delov iz umetnih snovi z mikroor-

Debelitev biofilma otežuje prehajanje kisika, pri čemer se postopoma v spodnjih plasteh ustvarjajo anaerobni pogoji.

Proces nastajanja biofilma je natančen in gensko uravnan.

Začetek nastajanja biofilma je odvisen od vpliva okolja, v katerem se mikroorganizmi znajdejo.

Poleg obremenjevanja vode z mikroorganizmi, pripomore biofilm k večjim izgubam energije ob gretju vode in večjim obremenitvam strojne opreme pri uporabi filtrov v živilskopredelovalni industriji.

Rezultati raziskave francoskih avtorjev so pokazale, da prisotnost biofilma poleg bakterijskih okužb lahko povzroča tudi izločanje endotoksinov ob izvajanju dialize.

ganizmi, kar pomeni posebej v živilski industriji možnost kontaminacije živil z mikroorganizmi [7].

Zaradi svoje zgradbe se biofilm zoperstavlja delovanju zunanjih vplivov. Vzroki za njegovo trdovratnost so:

- ustvarjanje eksopolisaharidne plasti, ki varuje mikroorganizme pred zunanjimi vplivi,
- kemična reakcija in fizikalna absorpcija eksopolisaharida z biocidi povzroči inaktivnost biocidov in s tem njegovo neučinkovitost,
- prisotnosti hranilnih snovi, ki so potrebne za njegov obstoj in
- gensko uravnan proces pritrjevanja mikroorganizmov na površino.

Biofilm nastaja tudi v vodovodnem sistemu, posebej na mestih, kjer je pretok vode upočasnen oziroma je občasno prekinjen. Rezultati študij kažejo, da niti visoke količine preostalega klora v vodi ne pripomorejo k preprečitvi nastajanja biofilma. V nadaljevanju ugotavljajo, da poleg obremenjevanja vode z mikroorganizmi, pripomore biofilm k večjim izgubam energije ob gretju vode in večjim obremenitvam strojne opreme pri uporabi filtrov v živilskopredelovalni industriji [8].

Biofilmi povzročajo resne zaplete tudi pri presajanju ortopedskih inplantatov. Tako je poleg priprave imunskega sistema organizma bila potrebna kompleksna antibiotična terapija z namenom preprečiti postoperativne komplikacije. Zato so v inplantate iz polimetilmetakrilata dodajali antibiotike, ki so se sproščali v nizkih odmerkih. Ugotovili so, da je sproščanje nizkih koncentracij antibiotikov pri bolnikih z inplantati vzrok za rezistenco na številne antibiotike [9,10].

Vzroke trdovratnih vnetij srednjega ušesa pri otrocih pripisujejo tudi biofilmu, ki ga ustvari *Hemophylus influenzae*. V poskusu so uporabili činčile, ki so jih okužili s kulturo mikroorganizmov izoliranih iz ušesa otrok. Ko so na činčilah ugotovili vnetje sluhovoda, so na njegovi površini dokazali prisotnost biofilma, ki ga je povzročil *Hemophylus influenzae*. Ameriški avtorji poročajo o pomenu biofilma *Mycobacterium chelonae* in *Methylobacterium mesophilicum*, ki je prisoten na površini endoskopov. V večjih zdravstvenih ustanovah potekajo postopki čiščenja in razkuževanja endoskopov v samodejnih čistilnih sistemih. Ugotavljajo, da samodejni higienizacijski sistemi raznašajo *M. chelonae* in *M. mesophilicum* na površino endoskopov. Zato za zaključek sanitacije priporočajo uporabo perocetne kisline kot sredstva za razkužbo endoskopov. Prisotnost biofilma lahko povzroči resne zaplete pri cevni sistemih dializnih aparatov. Rezultati raziskave francoskih avtorjev so pokazale, da prisotnost biofilma poleg bakterijskih okužb lahko povzroča tudi izločanje endotoksinov ob izvajanju dialize [11,12].

Prisotnost biofilmov igra pomembno vlogo pri koroziji materialov. Navedbe madžarskih avtorjev govore o pomenu sulfreduktornih mikroorganizmov pri koroziji materialov. Ob preučevanju vpliva tekoče in stoječe pitne vode na cevi iz nerjavnega materiala so ugotavljali prisotnost kovinskih ionov, predvsem železa, molibdena, niklja in kroma. Omenjeni ioni so bili prisotni v biofilmih, ki so se ustvarili na površini nerjavnega

materiala v primeru stoječe in tekoče vode, le da je bila koncentracija ionov višja pri stoječi vodi [13,14].

KAKO UGOTOVITI PRISOTNOSTI BIOFILMA

Pri ugotavljanju prisotnosti biofilma za raziskovalne kot praktične namene uporabljamo glede na njegovo obstojnost na površini, več metod. Za preučevanje biofilmov v raziskavah uporabljamo invazivne in neinvazivne metode. Z invazivnimi metodami grobo posežemo v strukturo biofilma. Namen teh metod je določiti prisotnost mikroorganizmov (kvantitativno) oziroma ugotoviti vrsto mikroorganizmov (kvalitativno). Bistvo neinvazivnih metod je preučevanje biofilma in pri tem ne poškodovati njegove strukture. Tovrstne metode omogočajo raziskovalcem preučevanje procesov, ki se odvijajo v biofilmu. Pri tem najpogosteje uporabljamo konfokalno lasersko mikroskopijo, magnetno resonanco, elektronsko mikroskopijo in druge [15].

Za ugotavljanje prisotnosti biofilma v praksi, uporabljamo brise za določanje prisotnosti mikroorganizmov na površinah, UV svetilke za ugotavljanje fluorescence beljakovinskih snovi in določanje vrednosti ATP z metodo bioluminiscence. Slednja dobiva vse večji praktični pomen predvsem v živilsko predelovalni industriji za takojšnje ugotavljanje higienskega stanja površin pred začetkom proizvodnega procesa [16].

METODE ODSTRANJEVANJA BIOFILMA

V mnogih raziskavah so preučevali možnosti odstranjevanja biofilma. V grobem jih lahko razdelimo na fizikalne, kemične in biološke.

Fizikalne metode odstranjevanja biofilma

Pri odstranjevanju biofilmov so raziskovalci uporabljali številne fizikalne metode, kot so super visoki magnetni valovi, ultrazvok, visoke električne pulzacije, vendar brez večjega uspeha. Fizikalne načine odstranjevanja biofilma so dopolnjevali z uporabo antibiotikov in s tem dosegli boljše učinkovitost pri odstranjevanju biofilma.

Ponovno lahko opazimo dela avtorjev, kjer so preučevali učinek visokotlačnega čiščenja. Na površini z biofilmom *Pseudomonas aeruginosa* in *Staphylococcus aureus* so uporabljali različne tlake. Ugotovili so, da pri čiščenju z delovnimi tlaki 34,5 bar, 51,7 bar in 68,9 bar ni značilnih razlik v primerjavi z delovnim tlakom 17,2 bar. Preučili so tudi vpliv oddaljenosti šobe od površine za doseganje največjega čistilnega učinka. Razdalja naj bi bila med 125 mm in 250 mm od površine. Zanimal jih je tudi vpliv časa pri postopku čiščenja. V tem so uporabljali delovni tlak 17,2 bar in ugotavljali učinek časa na biofilm. Ugotovitve kažejo, da ni značilnih razlik, če se s tlačno šobo zadržujejo na mestu čiščenja 1 ali 10 sekund. Tudi če so za čiščenje uporabljali alkalne, kisle ali nevtralne detergente niso ugotovili boljšega učinka pri odstranjevanju biofilma s površine.

V raziskavah so raziskovalci preučevali vpliv hrapavosti površin in nastajanje biofilma v živilskopredelovalni industriji, ki jih predpisuje evrop-

Za preučevanje biofilmov v raziskavah uporabljamo invazivne in neinvazivne metode.

Tudi če so za čiščenje uporabljali alkalne, kisle ali nevtralne detergente niso ugotovili boljšega učinka pri odstranjevanju biofilma s površine.

ska direktiva EEC 93/43. S prisotnostjo testnih mikroorganizmov so ugotavljali razlike pri čiščenju površin različne kakovosti, ki jih priporoča omenjena direktiva. Ugotovili so, da pri čiščenju površin, ki jih predpis priporoča za stene in tla, ni značilnih odstopanj. V nadaljnjih raziskavah poročajo, da je vrsta nečistote glavni dejavnik, ki vpliva na uspešnost čiščenja [13].

Kemijske metode odstranjevanja biofilma

V veliki meri je učinek razkužil odvisen od števila mikroorganizmov. Čim več jih je, tem slabši bo učinek dezinficijensov. Poleg števila mikroorganizmov ima pomembno vlogo tudi debelina nastalega biofilma. Raziskovalci tudi ugotavljajo, da se z naraščanjem debeline biofilma znižuje učinek razkužil. Raziskovalci prav tako ugotavljajo, da čiščenje in razkuževanje nista zadostna pri preprečevanju in odstranjevanju biofilma, ki nastaja na površinah.

Učinek razkužil je odvisen tudi od adhezije mikroorganizmov na površino, kar je potrebno upoštevati pri testiranju ustreznosti in učinkovitosti dezinfekcijskih sredstev. Učinek razkužil je odvisen tudi od adhezije mikroorganizmov na površino, kar je potrebno upoštevati pri testiranju ustreznosti in učinkovitosti dezinfekcijskih sredstev. Če mikroorganizmi niso pritrjeni na površino, je učinek dezinficijensa neprimerno boljši. Zato je potrebno mehanično ali kemično zlomiti polisaharidno zaščito biofilma, ki ščiti mikroorganizme pred zunanjimi vplivi. Avtorji navajajo detergente, ki učinkujejo na polisaharidno zaščito in vsebujejo kelatna sredstva, kot so EDTA in etilen glikol-bis (β -aminoetil) eter. Sposobnost odstranjevanja biofilma so ugotovili tudi pri nekaterih razkužilih kot npr. perocetna kislina, klorne spojine, jodove spojine in vodikov peroksid. Pri odstranjevanju biofilma igrajo pomembno vlogo tudi pH razkužila. Značilne učinke na odstranjevanje biofilma *L. monocytogenes* so ugotovili pri vrednosti pH razkužil med 11,6 in 12,5, kar avtorji navajajo kot pomemben dejavnik sanitacije v živilsko predelovalni industriji.

V zadnjem času se vse bolj uveljavlja elektrookisgenirana voda. Zaradi svojega edinstvenega delovanja, enostavnosti uporabe in nizke toksičnosti za okolje, jo vse bolj uporabljamo tudi za odstranjevanje biofilmov. Svojo uporabnost kaže na vseh področjih živilorejske proizvodnje, posebej pri odstranjevanju trdovratnih oblik biofilmov iz cevni in napajalnih sistemov (npr. *Legionella spp.*) [15].

Biološke metode odstranjevanja biofilma

Na upočasnitev rasti biofilmov in njihovo odstranjevanje vplivajo tudi naravni furani, ki jih izloča alga *Delisea pulchra*. Med biološke načine delovanja na biofilm prištevamo tudi selektiven učinek zaščitnih starterških kultur pridobljenih iz mleka, ki zavirajo rast nekaterih patogenih mikroorganizmov v perutninskih rejah. Zasedimo lahko tudi objave raziskovalcev, ki mikroorganizme uporabljajo ob ekoloških katastrofah izlitja nafte oziroma pri obdelavi odpadnih produktov iz naftne industrije z namenom znižati obremenjevanje okolja.

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Odstranjevanje biofilma z encimi

Med biološke načine odstranjevanja biofilmov uvrščamo tudi uporabo encimov. Encimi so naravni katalizatorji, zgrajeni iz beljakovinskih molekul, in jih najdemo v vseh živih bitjih od mikroorganizmov, rastlin, živali in ljudi. Uporabo encimov najpogosteje zasledimo v živilskopredelovalni industriji, pri proizvodnji papirja in tekstilni industriji.

Vedno pogosteje so sestavni del čistilnih sredstev. Encimi so zanimivi zaradi učinkovitega delovanja pri nizkih temperaturah, delujejo v nizkih koncentracijah, so razgradljivi in razpadejo v naravi prijazne razgradne snovi. Po čiščenju z encimskimi sredstvi je površine potrebno temeljito sprati z vodo, s čimer preprečimo obremenjevanje živil z encimi (npr. proteolitičnimi), kar bi vplivalo na organoleptične in kvalitativne lastnosti živila. Kljub temu pa je uporaba encimov še nekoliko omejena predvsem zaradi njihove visoke cene in specifičnega delovanja. Zaradi heterogene sestave nečistot in biofilmov je potrebno uporabiti encimske mešanice, ki bodo delovale na vse sestavine nečistot.

POVZETEK

S prisotnostjo biofilmov se pogosto srečujemo v veterini, medicini, številnih industrijskih panogah in vsakdanjem življenju. Pri njihovem odstranjevanju je pomembno, v kakšnem okolju nastanejo. Tako ni vseeno, ali se srečujemo z biofilmom v veterinarski medicini, papirni ali mlečno-predelovalni industriji. Pri kontroli nastajanja biofilma je namreč pomembno kakšni mikroorganizmi in mehanizmi pripomorejo k nastanku biofilmov. Glede na to se odločamo o izbiri sredstev za odstranjevanje biofilma s površin. V postopkih čiščenja vedno pogosteje srečujemo uporabo encimskih čistilnih sredstev. Z encimskimi čistilnimi sredstvi bolj ciljno delujemo na posamezno vrsto nečistot (mlečne beljakovine, celulozo, maščobe, ...) in pri nižjih temperaturah čiščenja. Uporabljamo jih v nižjih koncentracijah in so zaradi hitrega razpada encima ekološko prijaznejša. Seveda pri čiščenju uporaba encimskih sredstev ne nadomešča mehaniskega učinka in časa, ki sta pomembna dejavnika uspešnega čiščenja.

Pri odstranjevanju biofilmov lahko uporabimo sredstva iz skupine biocidov, ki sproščajo kisik. Svojo učinkovitost pa je pokazal tudi biocid, ki ga pridobimo iz elektrooksigenirane vode. S svojim širokim spektrom delovanja, enostavno uporabnostjo, brez posebne osebne zaščite, ekološke čistosti, in tudi možnosti izvajanja razkužbe ob prisotnosti živali. Perspektivni potencial se kaže v uporabi ob razkužbah cevni sistemov (katetrov, sond, vodovodnih sistemov), razkuževanju površin, saj jih ni potrebno spirati s površin, lahko jih uporabimo tudi za hladno zamegljevanje prostorov in operacijskih dvoran. Ker ni škodljiva za celice višjerazvitih organizmov pa se varnost uporabe pri nadzoru prisotnosti biofilmov še toliko bolj poveča.

Med biološke načine delovanja na biofilm prištevamo tudi selektiven učinek zaščitnih starterskih kultur pridobljenih iz mleka, ki zavirajo rast nekaterih patogenih mikroorganizmov v perutninskih rejah.

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2010 EFFoST Annual Meeting – What are the current trends?

Andrej **OVCA**

The EFFoST annual meeting was held in Dublin, Ireland from 10th to 12th of November 2010. Twenty-three plenary lectures were given and 272 posters presented. The scientific programme was divided in the four main sections: *Nutrition and Health*; *Functional Foods*; *Processing for Healthy Foods* and *Food Safety and Health*. The Journal does not permit a detailed description of all sub-topics; therefore, only the contributions representing the most significant breakthroughs are presented and further discussed.

As shown in the first section, **Nutrition and Health**, research trends as well as applications are strongly moving towards personalised nutrition, because (as is well known) not all individuals have the same response to the same diet. The concept of personalised nutrition is, as presented at the meeting, related to details of an individual's genetic code. This code may allow researchers to understand diet and gene interactions in the metabolism. However, because of very large number of genetic codes, definitive dietary advice based on a single code is not possible, but there is the possibility that dietary advice could be tailored to clusters of individuals who share a common metabolic profile or so-called *metabotype*. Of course, one needs to be aware that while personalised nutrition has major potential in medical treatment, no one will produce personalised cornflakes. Nevertheless, there are some issues regarding the approach of personalised nutrition for common use. Recently there has been growing interest in pursuing personalised nutrition, not based on genetic data but based on phenotypic and dietary data. Many services in the public and private sectors offer the opportunity to submit details of one's habitual diet and receive back details of one's pattern of nutrient intake together with advice on where the balance of nutrients needs to be improved. Such databases store quite personal information about individuals; some questions regarding the security of this data needs to be solved.

Encapsulation of important/**functional food** ingredients was another field of research and application presented and discussed and in which

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there is exponential progress. Encapsulation is a technology of coating an active ingredient within a shell material or entrapping it in a matrix, which may be used to prolong the shelf life of the active ingredient and achieve its controlled release or targeted delivery. The main benefits of this technology are the avoidance of severe environments during digestion and avoidance of biochemical reactions with other food components. This technology should preserve the chosen ingredient up to the point of utilisation in the body. There are significant difficulties in transferring knowledge gained in laboratory experiments into industrial production; mostly, this step is not done. However, the matrix material and encapsulation process chosen should ensure that the active ingredients do not lose their functions during the encapsulation process, that the matrix material should be edible, and that the formed capsules have desirable properties including size, morphology, structure, permeability and mechanical strength. This process should also be easy to scale up and (of course) not be too expensive. It is to be aware that this technology falls within the field of nano-materials, which opens some discussion regarding health impacts.

The presentation in the field of **food allergens** was very surprising. In spite of known health problems caused by food allergens, there is still a lack of good quality data about how many people suffer from food allergies, which kind of food react to and how much of a certain food can cause a problem. Therefore, the EuroPrevall project (<http://www.euro-prevall.org/>) is very welcomed, as it brings together a multidisciplinary partnership to address these issues. Cohorts spanning the main climatic regions of Europe have been developed, including infants, school-age children and adults. Confirmatory double-blind placebo-controlled food diagnosis has been undertaken, using foods as they are eaten with titrated doses to allow no effect and the lowest observable effect levels for allergenic foods to be determined. The cohorts are also facilitating validation of novel in vitro diagnostics through the development of the EuroPrevall serum bank. New instruments to assess the socioeconomic impact of food allergies have been developed and applied, allowing an assessment to be made of the burden this disease places on allergy sufferers and their communities for the first time. The new information coming from this activity will fill many of the gaps mentioned before. In addition, international collaborations, spanning Europe, North America, Africa, India and the Far East, are giving new insight into how environmental and lifestyle factors may affect patterns of allergies. This is especially relevant in the present global market place.

In the field of **food safety**, a very interesting presentation opening new perspectives regarding foodborne disease was given. The data presented showed that, at least in Western societies, viruses are the main pathogens involved in foodborne infections, although *Salmonella spp.* and *Campylobacter spp.* are still the most frequent one in the official reports. The reason for this is that they are the most frequently isolated. To date, several food items/products have been ranked by WHO/FAO as high risk food for foodborne infections and outbreaks. The assessment of the relevance of foodborne viruses is currently being formalised by

the preparation of guidelines for viral food safety in the Codex Alimentarius. Recent insights suggest several virus families causing a range of clinical symptoms that can be foodborne. Dealing with viruses in the food chain is not an easy task, as explained by the presenter, because they are quite resistant to common food production processes. Since not only levels of shedding may be very high, infectious doses are generally quite low. As explained by the presenter, the most common route of foodborne virus transmission is human to human, followed by sea shells and water. While foodborne viruses are quite resistant to heat treatment and cleaning procedures, personal hygiene is still the most effective measure to prevent their spreading. The need for the revision of guidelines for good hygiene practices (GHP) should also be considered in this context.

Nutrition and health claims legislation in the EU were presented and discussed from a legislation point of view. Emphasis was put on the differences between the nutrition claims and health claims, where it is a crucial and primary role of food legislation that consumers are not misled. The presentation was orientated mostly on The Nutrition and Health Claims regulation (1924/2006/EC), which came into force in January 2007; this was a good demonstration of the first harmonised EU legislation to establish specific rules governing the use of nutrition and health claims made on food.

In general, all the presenters explained the need for more effective and faster transfer of knowledge gained in the laboratories into real life and industrial production, but not on the account of lower levels of scientific research. Our contribution to this meeting was given in the form of the preliminary results of two studies on the field of food safety, entitled “Insulated bags: How effective are they?” and “Hygienic status and storage conditions of domestic refrigerators”.

INSTRUCTIONS FOR AUTHORS

Scope

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

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

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

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