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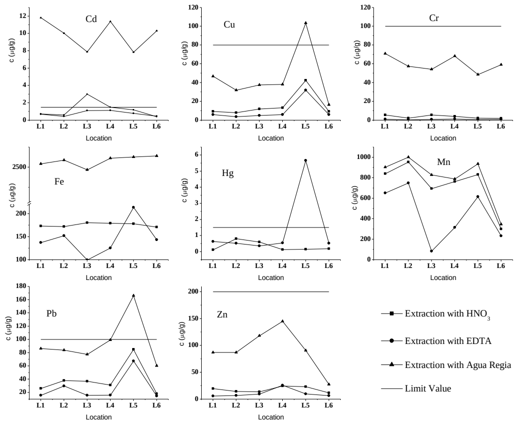

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Nasturtium-leaf	1,858
Broccoli-flower	1,809
Cauliflower-leaf	1,741
Kale-flower	1,230
Ramson-leaf	0,616
Cauliflower-flower	0,518
Cabbage-leaf	0,440
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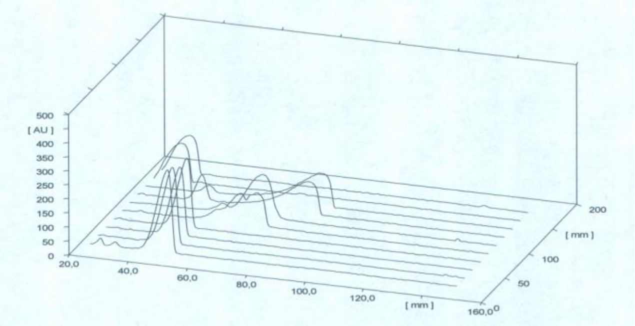

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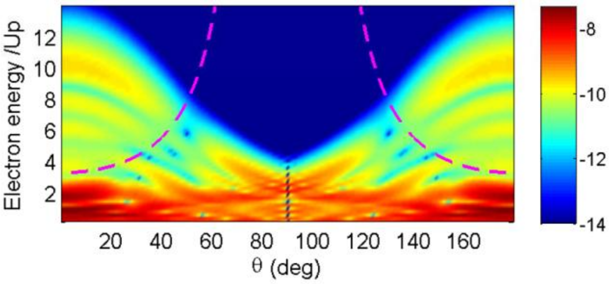

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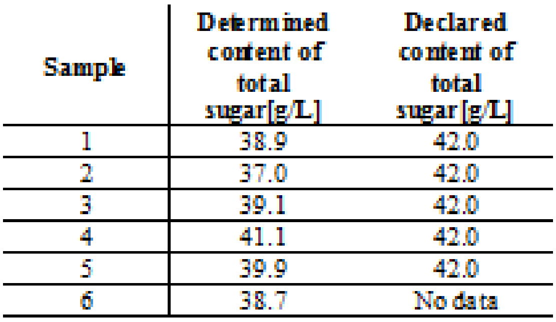
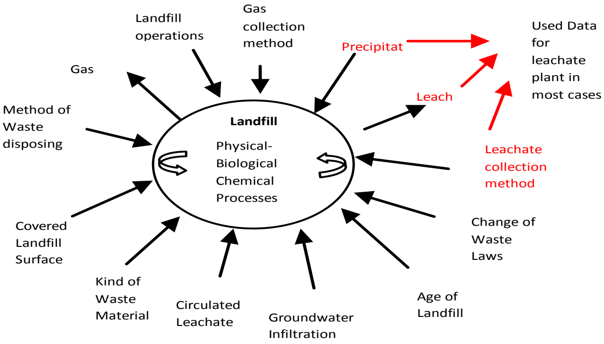
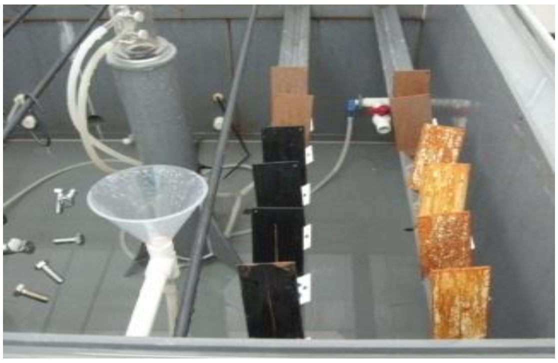


Sample	Determined content of total sugar[g/L]	Declared content of total sugar[g/L]
1	38.9	42.0
2	37.0	42.0
3	39.1	42.0
4	41.1	42.0
5	39.9	42.0
6	38.7	No data





SHORT COMMUNICATION

Element	Mean concentrations by month, μgL^{-1}			MRL, μgL^{-1}
	November	January	February	
Cr	1,19	1,57	2,42	1-6

Editorial

Air pollution has increased in recent decades in the industrialized world, resulting in a raise of allergic and other diseases that are becoming growing public health problem. Air pollution is a mixture of solid particles and gases in the air. Particle pollution, called particulate matter or PM, is a combination of fine solids and aerosols that are suspended in the air we breathe. Particles can come in almost any shape or size, and can be solid particles or liquid droplets. The particles are divided into two major groups. These groups differ in many ways. One of the differences is size, the bigger particles are called PM10 and the smaller particles PM2.5. The big particles are between 2.5 and 10 micrometers (from about 25 to 100 times thinner than a human hair). The small particles are smaller than 2.5 micrometers (100 times thinner than a human hair). Inhaling them can increase the chance to have health problems. People with heart or lung disease, older adults and children are at greater risk from air pollution. Particle size is an important determinant of the fraction of inhaled particles deposited in the various regions of the respiratory tract. Most aerosols present in natural and work environments are polydisperse. This means that the constituent particles within an aerosol have a range of sizes and are more appropriately described in terms of size distribution parameters. Size is not the only difference. Each type of particle is made of different material and comes from different places.

Atmospheric particles are emitted from a wide variety of anthropogenic and natural sources, and consequently their physical and chemical properties may vary widely. Car emissions, chemicals from factories, dust, pollen and mold spores may be suspended as particles. Susceptibility of an individual to adverse health effects of PM can vary depending on a variety of host factors such as age, physiological activity profile, genetic predisposition, or preexistent disease.

Recent epidemiological studies have provided solid evidence for the association of airborne particulate matter (PM) concentrations with adverse respiratory health effects. Acute and chronic inhalation can result in damage to the lung including cancer, and chronic inhalation affects other organ systems. However, the mechanism is unknown. Thus, determining, identifying and tracking air pollution and its adverse effect on health, requries continuous focus in attempt to address and stop this serious problem of modern world.

Editors

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1-5

Metal Levels in Surface Soils after Different Extraction Procedures

Bećiragić, S., Huremović, J.*, Muhić-Šarac, T., Memić, M., Selović, A., Žero, S.

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Abstract: Surface soil samples collected from the Kiseljak area, Bosnia and Herzegovina, were analyzed for eight different heavy metals (Cd, Cr, Cu, Fe, Hg, Mn, Pb and Zn) using atomic absorption spectrometry – flame and cold vapour techniques. Three procedures of extraction of metals from soil were used: 1) extraction with ethylenediamine tetraacetic acid (EDTA), 2) extraction with HNO_3 solution, and 3) extraction with aqua regia. Results for metals are reported for the first time in soils for this region. Pseudo total metal content in soil samples can be presented by the following descending series: Fe > Mn > Pb > Zn > Cu > Cr > Cd > Hg. The categories of increased pollution and highly polluted soils are almost equally presented in the Kiseljak area. Location Kiseljak-Centar by the content of Pb and Cu belong to the contaminated soil. In the case of Cd all analyzed soil samples belong to contaminated soils.

INTRODUCTION

Soil is a medium of interaction between the atmosphere, the biosphere and the lithosphere, and the presence of toxic elements in soil can be harmful to plants, animals and humans, under certain circumstances (Kelepertsis, Argyraki, and Alexakis, 2006). Although some metals are essential for biological functions and most occur naturally in the soil environment, the soils of urban environments commonly hold increased metal contents due to the contamination from surrounding traffic and industries (Ljung, Oomen, Duits, et al., 2007). Soils represent an excellent media to monitor heavy metal pollution because anthropogenic heavy metals are usually deposited in topsoils (Dantu, 2009). Independent of the correlations found in each of the particular cases studied, data available

in the bibliography indicates that pH, organic matter content, cation exchange capacity, specific surface area, carbonate content and iron oxide content are the principal soil characteristics that determine the capacity to retain heavy metal pollutants (Lafuente, Gonzalez, Quintana, et al., 2008). The total heavy metal content in soils provides a convenient means of expressing a measure of pollution, but numerous reports have highlighted that such measures are deficient in predicting toxicity of metal pollutants. Heavy metals may be distributed among many components of the soil or sediment and may be associated with them in different ways (Yusuf, 2007). Extraction tests are commonly used to study the mobility of metals in soils and sediments by mimicking different environmental conditions or dramatic changes on them. The results obtained by determining the extractable elements are dependent on the extraction procedure applied. In the literature, EDTA is the most frequently cited as a chelating agent for extracting potentially toxic trace metals from soils for its efficiency, availability and low cost, relative to other synthetic aminopolycarboxylic acids (Andrade, Prasher, and Hendershot, 2007). Also, most studies on helateinduced phytoextraction have focused on EDTA phytoextraction (Epelde, Hernandez-Allica, Becerril, et al., 2008). On the other hand, extraction with aqua regia is often referred to as pseudo total concentrations.

The study site has been the subject of numerous investigations, particularly those pertaining to the chemistry of mineral waters. The main goal of this study was to investigate chemical characteristics of the surface soil after different extraction procedures in the area of Kiseljak.

EXPERIMENTAL

Study area

The broader Kiseljak area is characterized by the relatively long and narrow Lepenica Valley, which extends from Han Ploča in the southeast to Kiseljak in the northwest. The Kiseljak area is in a belt with a moderate continental climate, while the southwestern sections of the terrain have high peaks and a harsh alpine climate. The climate is characterized by harsh winters, moderately warm summers, rainy autumns, relatively high precipitation irregularly distributed through the year and a lack of strong winds (Skopljak and Vlahović, 2012). What makes this place known, and by which the city was named, is certainly natural source of mineral water - kiseljak.

Soil sampling

Sampling locations in the Kiseljak area are shown in Figure 1. Soil samples were collected in December 2009, at six locations: Gromiljak (L1), Lepenica (L2), Kiseljačić (L3), Topole (L4), Kiseljak-Centar (L5), and Bilalovac (L6). Larger particles, e.g. stones and plant residues, were firstly removed. Samples were placed in polyethylene bags, and dried to constant weight at 105 ºC (for mercury determination at 35 ºC) in a drying oven. Samples were then grounded and homogenized in an agate mortar. The results were expressed on a dry weight basis.

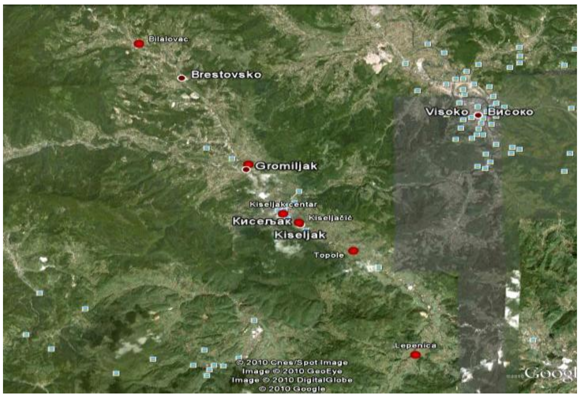

Figure 1. Satellite image of the Kiseljak area with sampling locations (lat. 43°56'24.75", lon. 18°04'43.86", elev. 490 m; background image GoogleEarth)

Soil pH determination

Soil pH is determined potentiometrically in a slurry system using pH meter (CG 841, Schott). Soil pH was determined in water (soil:water 1:2.5).

Extraction of metals from soil

In this work the three procedures of extraction of metals from soil were compared: 1) extraction with ethylenediamine tetraacetic acid (EDTA), 0.05 mol/L; 2) extraction with HNO3 solution (1:2 v/v) and 3) extraction with aqua regia which is often referred to as pseudo-total concentrations. Extraction was performed on shaker, 10 hours.

Determination of the metals

Metal (Cd, Cr, Cu, Fe, Mn, Pb and Zn) content was determined by atomic absorption spectrometry (SpectraAA-10, Varian), flame technique (FAAS) and Hg by cold vapor technique (CVAAS). To ensure the reliability of the results, appropriate quality procedures and precautions were taken. All the reagents were of analytical grade. Glassware was properly cleaned. Blank and standards were run repeatedly after set of five measurements to calibrate the instrument. Spike sample analysis (or known addition) is used to test the method at varying concentrations of analyte. Known amounts of analyte were added to a sample and the percent recovery was calculated. The acceptable percent recovery of known additions were obtained (extraction with EDTA: Fe (85-93 %); Zn (96-109 %); Pb (80-86 %); Mn (84-88 %); Cr (106-117 %); Cu (86-104 %); Cd (100-108 %); extraction with nitric acid solution: Fe (81-87 %); Zn (99-108 %); Pb (80-85 %); Mn (90-94 %); Cr (80-86 %); Cu (98-103 %); Cd (94-114 %); extraction with aqua regia: Fe (80-86 %); Zn (98-101 %); Pb (81-85 %); Mn (81-87 %); Cr (83-86 %); Cu (100-105 %); Cd (93-110 %). The sample and blank analyses were performed in triplicate.

The obtained metal contents were interpreted on the basis of the limit values specified by the Regulation of determination permitted quantities of harmful and hazardous substances in the soil and methods of their analysis (Sl. novine FBiH, 52/09) and the classification of soil contamination by Basić (1994). The classification was done by comparison of obtained values and limit values, i.e. by the degree of contamination - Cd:

% Cd = obtained pseudo total metal content in soil (mg/kg)*100/limit value

On this basis, the soils are classified into five groups:
GROUP I: clean soil - Cd up to 25%
GROUP II: increased pollution - Cd 25-50%
GROUP III: high pollution - Cd 50 - 100%
GROUP IV: polluted soil - Cd 100-200%
GROUP V: contaminated soil - Cd more than 200%

RESULTS AND DISCUSSION

Soil pH

The pH of the soil samples ranged from 6.66 to 7.15. According to data for soil pH, the follwing can be concluded:

- soil samples at locations Gromiljak, Lepenica and Bilalovac belong to slightly acid soil;
- soil samples at locations Kiseljačić, Topole and Kiseljak - Centar fall into weakly alkaline soils.

Extraction of metals

EDTA has been widely used to express metal availability to plants or other biological organisms (Dai, Nahmani, Becquer, et al., 2002; Wang, Shan, Zhang, et al., 2004; Meers, Laing, Unamuno, et al., 2007). The aqua regia of extractable content is considered as the pseudo total metal fraction.

Limit values of metal content in soil are defined by Regulation (Sl. novine FBiH, 52/09) depending on the soil texture (sandy, silty-clay and clay).The texture of the soil was not determined, so threshold for clay soil was selected with the highest metal content. Limit values (mg/kg air dried soil) are as follows: 1.5, 100, 80, 1.5, 100 and 200 for Cd, Cr, Cu, Hg, Pb and Zn, respectively. Concentration of Hg was the lowest at all locations and ranged from 0.36 to 5.67 mg/kg. Mercury content was exceeded at one location, Kiseljak-Centar. At the same location the highest concentrations of Cu (103.41 mg/kg) and Pb (166.13 mg/kg) were found and obtained concentrations exceeded the limit value. The content of cadmium in all locations was above the limit value. The lowest variability in the pseudo total Fe content was observed at all sites (2466.47 to 2640.27 mg/kg), while Mn content considerably vary (345.03-1000,82 mg/kg) with the location. Chromium content ranged from 48.52 to 70.80 mg/kg, and was below the limit values.

In most cases the lowest pseudo total contents of all analyzed metals were found at two sites, Bilalovac and Kiseljačić. Pseudo total metal content in soil samples can be presented by the following descending series: Fe > Mn > Pb > Zn > Cu > Cr > Cd > Hg. The Kiseljak area is characterized by frequented roads, gasoline stations, mineral water processing plants, textile and military industry which can be potential sources of the metals in the soil..

Results of multielemental (Cd, Cr, Cu, Fe, Hg, Mn, Pb and Zn) anaysis of the surface soil samples after different extraction procedures are given in Figure 2. The extractions were performed in triplicates and the results are presented as averages of the triplicate extracts.

In all soil samples, the metal concentrations after extractions with EDTA and HNO_3 solution were lower then the pseudo total amount. The highest amount of extracted Mn was obtained with EDTA in comparison to the pseudo total content. The content of all metals after extraction with EDTA was below the limit value.

The degree of soil contamination with heavy metals in Kiseljak area is given in table 1.

Table 1. The degree of soil contamination with heavy metals in the Kiseljak area

Sample	% C_d					
	Zn	Pb	Cr	Cu	Cd	Hg
Gromiljak	43	86	71	58	788	33
Lepenica	44	84	57	40	668	16
Kiseljačić	59	78	54	47	525	24
Topole	72	99	68	48	759	36
Kiseljak Centar	45	166	49	129	523	410
Bilalovac	14	60	59	21	687	34

The categories of increased pollution (25-50% C_d) and highly polluted (50 to 100% C_d) soils are almost equally presented in the Kiseljak area. Location Kiseljak-Centar by the content of Pb and Cu can be cosidered as contaminated soil. In the case of Cd all analyzed soil samples belongs to V group - contaminated soils.

Taking into account that the metals from natural and anthropogenic sources accumulate together, it is difficult to determine which metal fraction in the soil is natural, and which is of anthropogenic origin. Any study of the soil requires basic information about the chemical properties before any assessment of the presence of anomalous metal concentrations. A reliable method for this assessment is the normalization of geochemical data. Aluminum, iron, lithium, scandium, titanium and zirconium can be used for normalization and detection of anomalous concentrations of trace elements. In this work normalization of elements in relation to iron was used (Trefry, and Presley, 1976; Yanguo, Shijun, Xianguo, et al. 2002; Suh, and Birch 2005; Vreca, and Dolenec, 2005). Values of Pearson´s correlation coefficients are: Cd-Fe (r=0.24), Cr-Fe (r=0.03), Cu-Fe (r=0,15), Hg-Fe (r=0,38), Mn-Fe (r=0,35), Pb-Fe (r=0,30) and Zn-Fe (r=0,39). A positive correlation exists between Fe and all other elements but values are very low which can indicate that only low percentage of metals natural variability can be attributed to the textural and mineralogical variability of the soils (Sakan, Đorđević, and Trifunović, 2011).

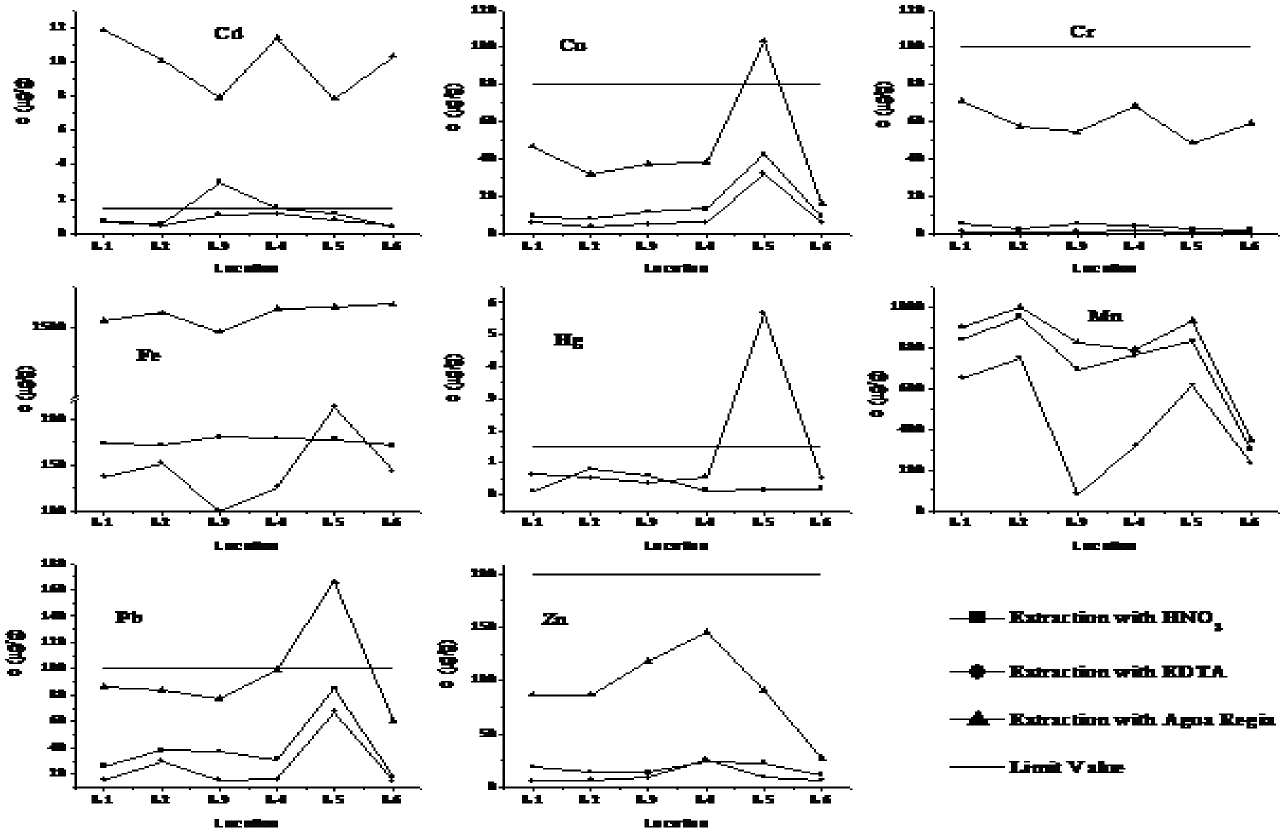

Figure 2. Metal content in soil samples from the area Kiseljak after different extraction procedures

CONCLUSIONS

Soils represent an excellent media to monitor heavy metal pollution because anthropogenic heavy metals are usually deposited in topsoils. Surface soil samples collected from the Kiseljak area, Bosnia and Herzegovina. In all soil samples, the metal concentrations after extractions with EDTA and HNO_3 solution were lower then the pseudo total amount. In most cases the lowest pseudo total contents of all analyzed metals were found at two sites, Bilalovac and Kiseljačić. The highest concentrations of Cu, Hg and Pb were found at the Kiseljak area Pseudo total metal content in soil samples can be presented by the following descending series: Fe > Mn > Pb > Zn > Cu > Cr > Cd > Hg.

To assess the presence of anomalous metal concentrations normalization of elements in relation to iron was used. A positive correlation exists between Fe and all other elements ,but values are very low which can indicate that only low percentage of metals natural variability can be attributed to the textural and mineralogical variability of the soils.

The categories of increased pollution (25-50% C_d) and highly polluted (50 to 100% C_d) soils are almost equally presented in the Kiseljak area. Location Kiseljak-Centar by the content of Pb and Cu can be cosidered as contaminated soil. In the case of Cd, all analyzed soil samples belongs to V group - contaminated soils.

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Službene novine F BiH br. 52/09. Pravilnik o utvrđivanju dozvoljenih količina štetnih i opasnih tvari u zemljištu i metode njihovog ispitivanja.

Summary/Sažetak

Analizirano je osam teških metala (Cd, Cr, Cu, Fe, Hg, Mn, Pb i Zn) u uzorcima površinskih tala sakupljenih na području Kiseljaka, Bosna i Hercegovina. Korištena je metoda atomske apsorpcione spektrometrije, plamena i tehnika hladnih para. Za ekstrakciju metala iz tla korišteni su sljedeći postupci: 1) ekstrakcija sa etilendiaminotetraacetatnom kiselinom (EDTA), 2) ekstrakcija sa HNO_3, i 3) ekstrakcija sa zlatotopkom. Pseudo ukupni sadržaj metala u uzorcima tla predstavljen je sljedećim opadajućim nizom: Fe > Mn > Pb > Zn > Cu > Cr > Cd > Hg. Kategorije povećanog onečišćenja (25-50% So) i visokog onečišćenja tala (50 to 100% So) su gotovo podjednako zastupljene na području Kiseljaka. Lokacija Kiseljak-Centar po sadržaju Pb i Cu pripada zagađenim tlima. U slučaju Cd svi analizirani uzorci tla spadaju u kontaminirana tla.

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Original scientific article

GC/MS Analysis of the Essential Oil of *Thymus Longicaulis* Presl. from Kosovo

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Phone: 00-377-44-145-049

Abstract: Three samples of essential oil of *Thymus longicaulis* Presl. were analyzed for their chemical composition. The plants were collected in their natural habitat on three different locations in Kosovo. Aerial parts of the plant were submitted to hydrodistillation using Clevenger apparatus. Gas chromatography – mass spectrometry analysis was performed for each extracted essential oil sample. Essential oil yield resulted with 0.68-1.54% on dried weight. Analyzed essential oil constituents resulted with variation in their quality and quantity. One sample was characterised as thymol/carvacrol chemotype, resulting with 15.26% of thymol and 12.65% of carvacrol as its main constituents. Two other analyzed samples had very small amounts of thymol and carvacrol and resulted with α-terpineol (14.23%), α-terpinyl acetate (11.81%), linalool (9.63%), and myrcene (6.59%) as the main constituents for the first, and α-phellandrene (19.07%), linalool (14.22%), *β*-caryophyllene (9.27%), and limonene (7.83%) for the second sample, respectively.

INTRODUCTION

The genus *Thymus* (*Lamiaceae*) is noteworthy for the numerous wild-growing plants biosynthesizing a remarkable amount of volatile compounds. *Thymus* species are perennial, aromatic herbs and sub shrubs native to Europe, North Africa and Asia (Morales, 1997). *Thymus* species, a taxonomically complex group of plants, are well known as medicinal plants containing tannins, bitter substances, and flavonoids, and are aromatic due to their essential oil content. The essential oils themselves are products in demand not only as a ingredients in the production of perfumes and cosmetics, but also for medicinal purposes due to their antiseptic, antispasmodic and antitussive properties (Stahl-Biskup, 2002). The existence of genetically based polymorphisms for secondary compound production has been reported in a range of *Thymus* spp. (Stahl-Biskup, 2002; Thompson, 2002). This variability is caused both by environmental factors and genetic variation (Thompson, Manicacci, and Tarayre, 1998). Numerous chemotypes of wild *Thymus* species have been defined, such as carvacrol and thymol, α-terpineol, thujone, geraniol, linalool, and others (Thompson, Chalchat, Michet *et al*., 2003). Thymol and carvacrol low quantity is a characteristic feature of the wild *Thymus* species growing in the Northern European countries (Loziene, Vaiciuniene, Venskutonis, 1998; Raal, Paaver, Arak, *et al*., 2004; Paaver, Orav, Arak, *et al*., 2008). Moreover, (*E*)-nerolidol, caryophyllene oxide, myrcene, β-caryophyllene and germacrene D, linalool, linalyl acetate and geranyl acetate were reported as main constituents of wild thyme (Raal, Paaver, Arak, *et al*., 2004).

In Kosovo, *Thymus* spp. is categorized as a harvestable commercial wild herb found in large quantities, with a potential production of tens of thousands of kg annually (Blakley, 2003). It is used traditionally as folk medicine in Kosovo, as infusion and decoction, mainly for the treatment of respiratory tract inflammation. (Mustafa, Hajdari, Krasniqi, *et al*., 2012).

The aim of the study was essential oil analysis of three population samples of *Thymus longicaulis* Presl. collected in south-western parts of Kosovo, Sharr mountain area and Rugova mountain area.

EXPERIMENTAL

Plant material and reagents

The plant material was collected from its natural habitat from three different locations in Kosovo at two different areas: Sharr mountain area (south Kosovo) and Rugovë mountain area (west Kosovo). Plants were harvested in full flowering state in June 2012. A voucher specimen of each sample was deposited in Department of Pharmacy, Medicine Faculty, University of Tirana. The geographic area and altitude were recorded for each collection point (Table 1). All reagents used in this study were of the highest purity available and obtained from the Sigma Chemical Company.

Sample preparation

The plants were dried at room temperature. In order to isolate essential oil, hydrodistillation was carried out for 2 hours using 30 g of dried plant material. The oil yield was recorded for each sample and calculated on dried plant weight (Table 1). The essential oil samples were dried over anhydrous sodium sulphate and were kept at -4°C in dark, until analysis.

Table 1: Harvest location, harvest date and essential oil yield of *Thymus longicaulis* Presl.

Sample	Location	Altitude (m)	Yield (%)
L1	Brod (Dragash)	1380 m	1.54
L2	LiqenatiKuqishtës	1500 m	0.68
L3	Bjeshkë e Kuqishtës	1310 m	0.71

Gas chromatography-mass spectrometry

The composition of volatile content was determined by gas chromatography-mass spectrometry technique. GC-MS was carried out on a Shimadzu GCMS-QP2010S gas chromatograph fitted with a fused silica ZB-5 (5% phenyl methyl siloxane) capillary column (30 m x 0.25 mm, 0.25 μm film thickness), and with an autosampler AOC-20. Column temperature was programmed from 60°C to 240°C at 3°C/min, and helium was used as carrier gas (1.1 mL/min). Other operating conditions were as follows: inlet pressure 9.43 psi, injector temperature 250°C, detector temperature 280°C, split ratio 1:25, injection volume 1 μL. Ionization of the sample components was performed in the EI mode, (70 eV), with scan range 40-450 amu.

Qualitative and quantitative analysis

The linear retention indices, RI, for all compounds were determined (Van Den Dool, and Kratz, 1963). The identification of the volatile constituents was accomplished by the visual interpretation, comparing their retention indices and mass spectra with literature data (Adams, 2007), by computer library search (Wiley 8.0, NIST/EPA/NIH Mass Spectral Library 2.0, and Mass Finder 4.2 Computer Software and Terpenoids Library), and in the laboratory own database. Compounds concentrations (as % content) were calculated by integrating their corresponding chromatographic peak areas (TIC mode).

RESULTS AND DISCUSSION

Samples of *Thymus longicaulis* Presl. essential oils yielded from 0.68 to 1.54% of dried weight. Identified compounds in *Thymus* species studied with their relative percentages and retention indices are given in Table 2.

Table 2: Constituents of the three samples of *Thymus longicaulis* Presl. essential oil.

RI	Compound	L1 RA %	L2 RA %	L3 RA %
806	(2E)-Hexenal	-	1.8	0.45
885	α-Thujene	-	0.2	0.29
896	α-Pinene	0.83	-	-
899	Tricyclene	-	0.46	0.27
911	Camphene	0.92	2.44	7.26
935	Sabinene	1.56	1.89	0.35
939	1-Octen-3-ol	0.98	-	-
946	3-Octanone	0.73	1.76	-
950	Myrcene	2.94	6.59	6.32
975	α-Phellandrene	1.2	5.34	19.07
991	*p*-Cymene	9.45	2.87	1.91
993	Limonene	-	1.49	7.83
996	Sylvestrene	2.31	-	-
998	1,8-Cineole	-	3.97	2.36
1006	Δ-3-Carene	0.2	-	tr
1015	(*Z*)-β-Ocimene	0.08	-	-
1017	(*Z*)-β-Ocimene	-	-	0.24
1026	β-Phellandrene	9.67	-	-
1035	*cis*-Sabinene hydrate	1.83	-	-
1076	Linalool	0.46	9.63	14.22
1091	1-Octen-3-yl acetate	1.34	-	-
1105	Octanol acetate	0.67	1.04	-
1120	Camphor	0.23	2.04	4.69
1142	Borneol	-	0.43	0.41
1153	Terpinen-4-ol	2.62	0.96	-
1166	α-Terpineol	6.42	14.23	0.66
1206	Nerol	-	-	0.66
1211	Thymol methyl ether	0.29	-	0.16
1220	Carvacrol methyl ether	0.93	0.14	0.14
1243	Geraniol	-	0.32	0.28
1263	Bornyl acetate	-	1.3	3.09

1274	Thymol	15.26	-	-
1310	Carvacrol	12.65	0.17	-
1348	*α*-Terpinyl acetate	11.74	11.81	0.84
1357	Neryl acetate	0.78	0.91	0.36
1379	Geranyl acetate	-	1.9	0.6
1402	*β*-Caryophyllene	3.88	1.03	9.27
1423	*β*-Longipinene	0.11	-	-
1433	*α*-Humulene	0.38	0.26	-
1442	(*E*)-*β*-Farnesene	-	-	3.29
1460	*γ*-Amorphene	0.15	-	-
1461	Germacrene D	0.67	0.86	0.31
1469	(*Z*)-*β*-Farnesene	-	0.28	-
1469	6,9-Guaiadiene	tr	-	tr
1484	*α*-Amorphene	0.11	-	0.23
1496	*β*-Bisabolene	-	-	0.33
1509	*δ*-Amorphene	0.63	0.1	0.96
1536	Elemol	-	1.04	0.21
1643	*α*-Cadinol	-	-	0.91
1643	Khusimone	-	tr	-
1671	*epi*-*α*-Bisabolol	0.32	-	-
1703	(2*E*, 6*Z*)-Farnesal	1.88	-	-
	Aliphatic compounds	3.72	4.60	0.45
	Aromatic compounds	38.58	3.18	2.21
	Monoterpene hydrocarbons	19.71	18.41	41.70
	Oxygenated monoterpenes	24.08	47.50	28.17
	Sesquiterpene hydrocarbons	5.95	3.60	14.64
	Oxygenated sesquiterpenes	2.20	-	0.91
	Total identified	94.24	77.29	88.08

RI= Retention indices calculated on ZB-5 column; RA=Relative area; tr=traces (<0.1%); L1, L2 and L3=samples of *Thymus longicaulis* Presl.hydrodistilled oil.

Fifty-two constituents were identified representing 77.29-94.24% in total. Present compounds were oxygenated monoterpenes (24.08-47.5%), monoterpene hydrocarbons (18.41-41.70%), aromatic compounds (2.21-38.58%), sesquiterpenes (3.60-14.64%), oxygenated sesquiterpenes (0.91-2.20%), and aliphatic compounds (0.45-4.60%). In sample L1 thirty-five constituents were identified. Main compounds were aromatic compounds with 38.58%, oxygenated monoterpenes with 24.08%, and monoterpene hydrocarbons with 19.71%. Main constituents were thymol (15.26%), carvacrol (12.65%), *α*-terpinyl acetate (11.74%), and *β*-phellandrene (9.67%). In sample L2 77.29% of compounds were identified, with thirty-one constituents resulted in total. Main compounds were oxygenated monoterpenes (47.50%) and monoterpene hydrocarbons (18.41%). Main constituents were *α*-terpineol(14.23%), *α*-terpinyl acetate (11.81%), linalool (9.63%), and myrcene (6.59%). In L3 sample 88.08% of the compounds were identified with thirty-three constituents resulted in total. Main compounds were monoterpene hydrocarbons (41.70%), oxygenated monoterpenes (28.17%), and sesquiterpene hydrocarbons (14.64%). Main constituents were *α*-phellandrene (19.07%), linalool (14.22%), *β*-caryophyllene (9.27%), limonene (7.83%), and camphene (7.26%).

In these samples of *Thymus longicaulis* Presl. essential oil, monoterpenoids in general resulted with most abundant levels. Also in the literature monoterpenoids have been reported as the main compounds of the *Thymus longicaulis* essential oil (De Martino, Bruno, Formisano, *et al.*, 2009).

According to the literature, different chemotypes of *Thymus longicaulis* Presl. were reported, providing evidence of intraspecific chemical variability of this plant species.

In a study conducted in Greece, three chemotypes of Thymus longicaulis C. Presl subsp. chaoubardii (Reichenb. fil.) Jalas were detected: limonene (18.7%) and thymol (19.4%); geraniol (56.8%) and geranyl acetate (7.6%); linalool (63.1%) and α-terpinyl acetate (20.4%). (Tzakou O., Verykokidou E., Roussis V. *et al.*, 1998).

Chemical profile of thymus longicaulis from Serbia consisted of α-terpineol acetate (67.52%), thymol (4.47%), limonene (3.58%), α-terpineol (3.43%) and γ-terpinene (2.07%) as main constituents, with a content of monoterpene hydrocarbons (11.20%), oxygenated monoterpenes (80.07%), sesquiterpenes (2.94%), oxygenated sesquiterpenes (0.79%), and the rest 4.86%. The oil was poor in sesquiterpene hydrocarbons (3.66%), of which only small amounts of β-caryophyllene (1.54%), β-bisabolene (0.70%), and germacrene D (0.50%) were present. (Grujić Jovanović S., Marin P.D., Dzamić A. *et al.*, 2009).

Thymol, *α*-terpinyl acetate, geraniol were the predominant components in each of the three different chemotypes detected in Turkey (Baser, Ozek, Kirimer, *et al.*, 1993).

Thymol and carvacrol were the main components in the oils of *Thymus longicaulis* according to another study report from Turkey, with other major components identified as *p*-cymene, *γ*-terpinene and borneol (Azaz, Irtem, Kurkcuoglu, *et al.*, 2004). The same plant species from southern Italy had cymene, *γ*-terpinene, myrcene and limonene as the main constituents (De Martino, Bruno, Formisano, *et al.*, 2009). In a study conducted in Croatia, thymol (46.3%), *γ*-terpinene (16.2%), thymol methyl ether (11.4%) and *p*-cymene (9.4%) were identified as the most abundant compounds followed by borneol, *β*-caryophyllene, *δ*-3-carene and linalool ranged from 1.4% to 2.2%. It is clear that *Thymus longicaulis* originating from Croatia belongs to a thymol chemotype (Kuštrak, 2005; Vladimir-Knežević, Kosalec, Babac, *et al.*, 2012). The oxygenated monoterpene *α*-terpinyl acetate present as the main constituent in essential oil samples L1 and L2, was reported in literature as α-terpinyl acetate chemotype of *Thymus* spp. (Ćavar, Maksimović, and Vidic, 2009; Mockute, and Bernotiene, 2011).

CONCLUSIONS

Essential oil of *Thymus longicaulis* from Kosovo resulted with a quantitative and qualitative variation of constituents. Phenolic and non-phenolic chemotypes were detected within the analyzed essential oil samples, thus being in accordance with the literature data.

The observed differences in essential oil profile of investigated species confirm the influence of origin of the plant material, as well as environmental conditions on the nature of plant chemical composition.

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Summary/Sažetak

Ispitivan je hemijski sastav tri uzorka esencijalnog ulja *Thymus longicaulis* Presl. Biljni material je prikupljen sa prirodnog staništa na tri različite lokacije na Kosovu. Nadzemni dijelovi biljaka su podrvgnuti hidrodesitlaciji uz korištenje Clevenger aparature, te analirizirani kombinovanom tehnikom gasna hromatografija-masena sprektrometrija. Prinosi esencijalnog ulja su varirali od 0.68 do 1.54%. Jedan od uzoraka se pokazao kao predstavnik timol/karvakrol hemotipa, sa 15.26% timola i 12.65% karvakrola. Preostala dva uzorka su pokazala veoma nizak sadržaj timola i karvakrola. Prvi uzorak sadržavao je 14.23%, α-terpineola 11.81% α-terpinil acetate, 9.63% linalola i 6.59% mircena, a drugi 19.07% α-felandrena, 14.22% linalola, 9.27% β-kariofilena i 7.83% limonena.

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Determination of Total Sulphur Content in Biological Samples by Using High Performance Ion Chromatography and Elemental Analysis

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Abstract: The aim of this work was the assessment of total sulphur (S) content in the plants with the highest S metabolic needs and different natural and artificial fertilizers by using a High Performance Ion Chromatographic (HPIC) method and Elemental analysis (EA). 7 plants and 11 diferent fertilizers were prepared by digestion and oxidation with a mixture of perchloric and nitric acid. The HPIC method was performed with a Shimadzu Ion Chromatograph equipped with conductivity detector CDD-10A. For an EA, samples of plants and fertilizers were analyzed in an elemental analyzer LECO SC-132. The total S content in analysed plants varies from 1,85 mg S/g to 0,30 mg S/g; and in fertilizers varies from 15,82 mg S/g to 0,71 mg S/g. The used HPIC method and EA are simple, sensitive, and accurate methods that can be applied as a reliable analytical tool for the determination of total S content in the fertilizers and plant material. Results of this analysis suggest consumption of brocoli as a significant natural source of organo-sulphur compounds and could recommend soil fertilization with sheep and rabbit manure for the cultivation of the plants with a highest S metabolic needs.

INTRODUCTION

Sulphur (S) is assimilated from the soil as a sulphate ion and is further being reduced to the organosulphide species. The application of a farmyard manure is a core principle in organic farming systems and enables management of soil organic matter. Soil organic matter is crucial to maintain and improve physical, chemical and biological properties of agricultural soils and is essential for the storage of energy and nutrients as a structural stabilizer and as a chemical buffer for introduced xenobiotics. This work has been aimed to assess the total S content in different fertilizers (cow, chicken, sheep, horse, goat, rabbit and lumbrihumus) and plants like *Brassica oleracea* Botrytis Cultivar Group (cauliflower), *Brassica oleracea* Italica Group (broccoli), *Nasturtium officinale* (nasturtium), *Brasscica oleracea* Acephala Group (kale) Brassica oleracea Capitata Group (cabbage), *Allium ampeloprasum* (leek) and *Allium ursinum* (ramson) with a great gastronomic, nutritional and global healthcare importance by using a high Performance Ion Chromatographic (HPIC) method and Elemental analysis (EA).

EXPERIMENTAL

Biological material

Plant samples (leafs and flowers)- Cauliflower, Broccoli, Nasturtium, Kale, Cabbage, Leek and Ramson were prepared using digestion and oxidation with a mixture of perchloric and nitric acid.

Fertilizer samples (artificial and natural)–NPK, cow, chicken, sheep, horse, goat, rabbit and lumbrihumus were prepared by digestion and oxidation with a mixture of perchloric and nitric acid.

HPIC method

HPIC method was performed with a Shimadzu Ion Chromatograph equipped with conductivity detector CDD-10A, pump LC-10AD, autosampler SIL-10Ai, deggaser DGU-14A, owen CTO-10A. Deggased and diluted samples were analysed on Shodex IC SI-90G strong exchange column, using a mobile phase: carbonate buffer $NaHCO_3$ (0,0017 M) and Na_2CO_3 (0,0018 M), during the 20 minutes at 40°C and 1.0 mL min^{-1} flow rate. Magnesium sulphate was used for the preparation of calibration curve.

Elemental analysis

Fertilizer and plant samples were dried at 105°C (sterilizer INKO ST 200) and ground to a size of 0.25 mm (mill IKA Werke M20). Samples of fertilizers were dried on 40°C (sterilizer INKO ST 200) for 24 hours, crushed and sieved to 0.25 mm. 0.2 g sample was burned in a stream of pure oxygen, flow rate 300 mL/min at 1350°C in an elemental analyzer LECO SC-132 (V_2O_5 is used as a catalyst). The resulting SO_2 was measured in the infrared cell (ISO 15178: 2000).

RESULTS AND DISCUSSION

The total S content determined by using an HPIC method in different plants is shown in Table 1.

Table 1. The total S content in different plant material detrmined by HPIC

Plant material	Total S content (mg/g)
Nasturtium-leaf	1,858
Broccoli-flower	1,809
Cauliflower-leaf	1,741
Kale-flower	1,230
Ramson-leaf	0,616
Cauliflower-flower	0,518
Cabbage-leaf	0,440
Leek-leaf	0,300

The highest concentration of S was measured in Nasturtium and Broccoli. The total S content determined by using an HPIC method in different fertilizers is shown in Table 2.

Table 2. The total S content in different fertilizers determined by HPIC

Fertilizer	Total S content (mg/g)
NPK fertilizer	15,825
Commercially available Horse + cow manure	8,514
Commercially available Chicken manure	8,494
Biological-organic fertilizer Stallgrena	5,067
Rabbit manure	2,521
Sheep manure	1,512
Lumbrihumus	1,290
Chicken manure	1,142
Goat manure	0,918
Cow manure	0,759
Horse manure	0,710

The highest concentration of S was measured in NPK (artificial fertilizer) and rabbit manure (natural fertilizer). The total S content in different plants determined by EA is showed in the Table 3.

Table 3. The total S content in different plants determined by EA.

Plant material	Total S content (mg/g)
Broccoli -flower	19,6
Cauliflower -flower	13,30
Kale-flower	11,45
Cauliflower-leaves	10,95
Cabbage-leaves	10,65
Ramson-leaves	8,05
Nasturtium-leaves	7,75
Leek-leaves	4,45

The highest concentration of S was measured in Broccoli and Cauliflower.

The total S content in fertilizers determined by EA is showed in the Table 4.

Table 4. The total S content in different fertilizers determined by EA

Fertilizer	Total sulphur content (mg S/g)
NPK fertilizer	11,5
Commercially available Chicken manure	8,70
Biological-organic fertilizer Stallgrena	6,10
Sheep manure	4,90
Horse manure	4,50
Goat manure	4,20
Cow manure	4,06
Lumbrihumus manure	3,40
Commercially available Horse + cow manure	3,00
Chicken manure	2.95
Rabbit manure	2,65

The highest concentration of S was measured in NPK (artificial fertilizer) and sheep manure (natural fertilizer). A number of methods, such as induced coupled plasma (ICP), HPIC, turbidimetric analysis, elemental analysis, etc. (Benton, 2001; Kalra, 1998; Tubino and Queiroz, 2009; Soon et al., 1996), are used to determine the total S content in plant material and soil. The reason for such a large number of methods is that no single method fulfills all the conditions, such as precision, accuracy, selectivity, speed, etc. In all methods the sample must be degraded, by wet or dry digestion. In order to quantify the use of any of the above mentioned methods, sulfur must be translated into the appropriate form (to be reduced to sulfide or that is oxidized to sulfate). In some cases, the translation of sulfur into the correct form and its quantification is performed in one instrument (LECO elemental analyzer). In this paper of the HPIC and EA methods were used to determine amount of total S content in the fertilizers and plant material. Because fertilizers are complicated biological matrix, and similar to plant material, the HPIC method requires the preparation of the sample, where the sample has to be decomposed and S has to be converted to the SO_4^{2-} ion. Some other methods such as turbidimetry and inductively coupled plasma require this kind of preparation (Soon et al., 1996; Parkinson, 1987). Translation of sulfur in sulfate ion and decomposition of the sample is carried out with a mixture of concentrated acids $HClO_4/HNO_3$ (Kalra, 1998; Zafar, 1992). Problem with digestion is that, when sample is heated in such acidic conditions, S may evaporate in the form of its volatile compounds, so it is very important to control heat to avoid evaporation of S (Benton, 2001; Kalra, 1998; Sagger, 2012). Certain ions, such as chromium ions, can be oxidized in such acidic conditions to form precipitate with Ba^{2+} ions and interfere with the determination of SO_4^{2-} by turbidimetric method. Also, acidity must be taken into consideration when S is determined by turbidimetric method, because the acidity of the solution affects on the structure and precipitation of $BaSO_4$ (Tubino and Queiroz, 2009). Before quantification, the HPIC method requires the separation of ions and these ions do not have affect on obtained result. According to some authors (Tubino and Queiroz, 2009), the results obtained by ICP and HPIC methods show no significant difference in the results, especially if the samples are prepared in the same manner. ICP method is 10 times faster than the HPIC method, but there is the possibility of spectral interferences for ICP method, especially when analyzed samples have a complex matrix (Soon et al., 1996). The HPIC method contrary to conventional methods, offers the following advantages: speed, sensitivity, selectivity, simultaneous determination, stability of separation column, and determination of sulphate ion by ion chromatography is already routine chromatographic method (ISO 15178:2000), for analysis of soil samples (Weiss, 2004). According to some authors (Soon et al., 1996) the method of elemental analysis proved to be a reliable method for the determination of total S content in plant material. Combustion of the sample, translation S to the form of SO_2 ion and its detection in the infrared cell is performed using only one instrument (Soon et al., 1996; Pöykiö, 2002). Sonn et al. in their paper (1996) showed that this method is more precise than the other methods. Also, results for total S content in plant material and fertilizer by this method are provided very fast (2 min), but the high price of this instrument is the biggest drawback. The biggest problem for all methods for the determination of total S in plant material and fertilizers is a decomposition of the sample, translation of S into the proper form while losses of S are none or negligible small, and lack of reference materials for the plant material, and especially for fertilizers is great disadvantage (Soon et al., 1996; Sager, 2012).

CONCLUSIONS

The used HPIC and EA methods are simple, sensitive and accurate, and can be applied for the determination of total S content in the different fertilizers and plants. Results of this analysis suggest consumption of brocoli as a significant natural source of organo-sulphur compounds. According to the results of this analysis the soil fertilization with commercially available sheep and rabbit manure for the cultivation of the plants with a highest S metabolic needs is recommended .

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Summary/Sažetak

Cilj rada je bio odrediti ukupni sadržaj sumpora (S) u biljkama sa povećanom metaboličkom potrebom za S i različitim prirodnim i umjetnim fertilizerima upotrebom jonske kromatografije visokih performansi (HPIC) i elementarnom analizom (EA). 7 biljaka i 11 različitih fertilizera je pripremljeno digestijom i oksidacijom smjesom koncentrovane perkloratne i nitratne kiseline. HPIC metoda izvedena je s Shimadzu jonskim kromatografom opremljenim konduktometrijskim detektorom CDD-10A. Za EA uzorci biljaka i fertilizera analizirani su u elementarnom analizatoru LECO SC-132. Ukupni sadržaj S u analiziranim biljkama kretao se u opsegu od 1,85 mg S/g do 0,30 mg S/g; i u fertilizerima kretali su se u opsegu od 15,82mg S/g do 0,71 mg S/g. Rezultati ovog ispitivanja sugeriraju konzumaciju brokolija, kao značajnog prirodnog izvora organo-sumpornih komponenata i mogli bi preporučiti fertilizaciju tla ovčijim i zečijim gnojivom, za kultiviranje biljaka sa povećanom metaboličkom potrebom za S.

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Original scientific Article

Evaluation of Purity of some Coumarin Derivatives by Melting Point Measurments, TLC and Scanning Densitometry

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Abstract: Impurity remains after syntheses greatly influence properties and effectiveness of synthesized compounds. Therefore it is very important to determine its presence and remove them from the synthesized product. In order to obtain crystals of high purity, synthesized bis-4-hydroxycoumarins and benzopyranocoumarins were purified by recrystallization and column chromatography. The success of the purification of the synthesized compounds was examined by the melting point measurements, thin layer chromatography and scanning densitometry. Only one of nine tested mobile phase systems proved successful for separation of the analyzed compounds. Detection of the compounds on TLC plates was carried out under UV light at wavelength of 254 nm, as well as quantitative analysis of the present impurities. The results of scanning densitometry showed that the corresponding compounds obtained by purification had a satisfactory degree of purity (less then 2% unknown impurities). Percentage of impurities can also be calculated from data attained by spectrometric methods and elemental analysis.

INTRODUCTION

It is very important to identify and quantify impurities in products. This represents a crucial step in quality control of new drugs, as much as, active compounds. Different mechanisms of degradation of drugs can lead to a very wide spectra of possible effects such as: loss of pharmaceutical quality (loss or increase of an active compound's concentration, change in uniformity of content, endangering of microbiological status, forming toxic degradation products), changes in bioavailability and also a modification of any other factor that can represent a threat to the validity of drug. (Jiben, 2002; Vijayalakshmi, Kumaravel and Anbazhagan, 2012).

Impurities in an active compound can occur during the synthesis, purification and during the validity time. They can be classified as: inorganic impurities (reagents, catalysts, ligands, inorganic salts, heavy metals or other residual metals), organic impurities (starting materials, process-related impurities, intermediates, and degradation products) and residual solvents (organic and inorganic liquids). (ICH. Q3A (R2), 2006; Lunn and Faustino, 2007).

Impurities that remain after the synthesis greatly affect the properties and efficiency of mentioned synthesized compound. In order to get crystals with a high degree of purity, synthesized compounds must undergo purification with different procedures such as recrystallization and chromatography methods (column chromatography, HPLC, TLC). (Görög, 2000 a).

There are two major approaches to determine the purity of a chemical compound. (Görög, 2000b; Beckett and Stenlake, 1988). The first is the absolute approach, based on thermodynamic methods; the total amount of impurities can be determined without detailed knowledge of these impurities. The second is the chromatographic approach (TLC, HPLC, GC), which gives information about the detection and determination of impurities as far as their nature and chemical behavior are known. (Ferenczi-Fodor, Vegh and Renger, 2006; Nyiredy, 2001; Nageswara Rao and Nagaraju, 2003). Chromatographic methods can be applied for the determination of purity due to their many advantages such as high efficiency, speed and the possibility of their utilization in automatized systems. Investigation and quantification of impurities presented in synthesized coumarin derivatives were achieved by measuring melting points, and using thin layer chromatography and scanning densitometry. (Gumieniczek, Inglot and Kończak, 2011).

EXPERIMENTAL

General procedures for the preparation of compounds

Benzylidene-bis-(4-hydroxycoumarin) derivatives (**1**-**2**) and fused benzopyranocoumarin derivatives (**3**-**4**) were prepared in moderately good yields by Claisen condensation of 4-hydroxycoumarin (4-HC in further text) (Sigma Aldrich, 98%) and appropriate substituted aromatic aldehydes, all being commercially available [4-bromobenzaldehyde (Merck, 98%), 4-(trifluoromethyl) benzaldehyde (Sigma Aldrich, 98%), 2-fluoro benzaldehyde (Sigma Aldrich, 97%) and 5-bromo-2-hydroxybenzaldehyde (Merck, 97%)].

According to the previously published procedure (Završnik, Muratović, Makuc, *et al*., 2011) 4-HC (0.324 g, 2 mmol) was dissolved in hot ethanol (6 mL) (Sigma Aldrich, puriss. p.a., absolute, ≥99.8%), the appropriate aldehyde (1 mmol) was added, and the reaction mixture was refluxed for 24 h. After cooling to room temperature, the solid was filtered out and purified in parallel by recrystallization and column chromatography to give products benzylidene-bis-(4-hydroxycoumarin) derivatives **1** and **2**, and 3-(6-oxo-(1*H*)-benzopyrano[4,3-b]benzopyran-7-yl)-4-hydroxycoumarin derivatives **3** and **4** (Figure 1).

General procedures for analytical and experimental section

Melting points (uncorrected) were determined with Büchi melting point B-545. Precoated silica gel 60F-254 plates were used for thin-layer chromatography (TLC); samples were applied to plate by CAMAG Linomat 5 and the spots were detected and scanned by CAMAG TLC Scanner 3, under UV light of wavelength 254 nm.

Column chromatography was performed using silica gel 60 (0.063-0.2 mm); glass column was slurry-packed under gravity. Solvent system used for TLC and column chromatography was dichloromethane (Sigma Aldrich, puriss. p.a., 99.6%) / ethyl acetate (Sigma Aldrich, puriss. p.a., 99.6%) = 2:1. Solvent used for recrystallization was chloroform (Sigma Aldrich, puriss. p.a., 99.0-99.4%).

Mass spectra were recorded with an Autospec: ESI/Q-TOF Premier instrument. ^{1}H-NMR spectra were recorded at 600 MHz, in deuterated DMSO-*d*6 (Sigma Aldrich, 99.8%), on Bruker (UXNMR/XwinNMR) spectrometer, using tetramethylsilane (TMS) as internal reference. 1D and 2D NMR spectra were recorded at 25 °C on Varian Unity Inova 300 spectrometers. ^{1}H chemical shifts were referred to residual signal of $CDCl_3$, CD_2Cl_2 and DMSO-d6 (δTMS 0.0 ppm). Elemental analyses were performed in the Central Analytic Service, Ruđer Bošković Institute Zagreb, Croatia, using a Perkin Elmer 2400 Elemental Analyser. The infrared spectra were obtained from potassium bromide triturate containing 0.5% of the product on a Perkin-Elmer 1000 FT-IR spectrophotometer. All data were recorded at 25 °C unless specified otherwise.

Determination of purity with TLC

Thin-Layer Chromatography (Nyiredy, 2001) is a simple and inexpensive technique that is often used to judge the purity of a synthesized compound or to indicate the extent of progress of a chemical reaction. The analysis was performed on precoated 20x20 cm (0.25 mm thick) 60F-254 silica gel TLC plate. Nine different mobile phases (Table 1.) were selected according to their polarity to establish the R_f value for investigated compounds.

Table 1. Mobile phase systems studied

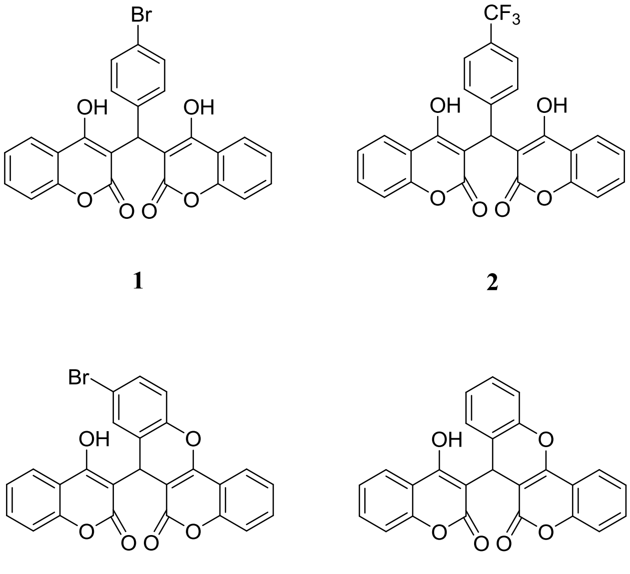
	Solvent system	v/v
1	toluene : chloroform : acetone	8 : 8 : 1
2	hexane : acetone : glacial acetic acid	5 : 5: 2
3	cyclohexane : chloroform : glacial acet. acid	5 : 5 : 2
4	cyclohexane : chloroform : glacial acet. acid	15 : 15 : 4
5	n-hexane : acetone	2 : 1
6	dichloromethane : ethyl acetate	2 : 1
7	n-hexane : ethyl acetate	11 : 3
8	dichloromethane : methanol	9 : 1
9	toluene : chloroform : acetone	8 : 8 : 1

On TLC plate 10 μL of probe solutions were applied as spots 6 mm length and 0,45 mm width band. The plates were than dried at room temperature and afterwards were developed in a vertical chamber previously saturated with mobile phase vapors for 60 minutes, at room temperature. The development distance was 4/5 of a plate. After development, the plates were dried on hot plate. The spots were detected and scanned under UV light (wavelength 254 nm) automatically by CAMAG TLC Scanner 3. The R$_f$ value is the identification characteristic in TLC and depends on the used combination of solvents and the chemical nature of substance.

RESULTS AND DISCUSSION

Chemistry

Synthesis and characterization of four biscoumarin compounds were described in previously published paper (Završnik, Muratović, Makuc, *et al.*, 2011). Chemical structure of bis-4-hydroxycoumarins and benzopyrano- coumarins are presented on Figure 1.



1 **2**

3 **4**

Figure 1. Benzylidene-bis-(4-hydroxycoumarin) derivatives (**1** and **2**) and 3-(6-oxo-(1*H*)-benzopyrano[4,3-b]benzopyran-7-yl)-4-hydroxy-coumarin derivatives (**3** and **4**)

3,3'-(4-Bromobenzylidene)-bis-(4-hydroxycoumarin) (1) (Završnik, Muratović, Makuc, *et al.*, 2011).

Yield= 74%. mp 267.2 °C. Anal. calcd for $C_{25}H_{15}O_6Br$: C 61.12, H 3.08. Found: C 61.07, H 3.13. All values are given in percentages. IR (KBr) n 3276 2730 1772 1668 1604 1564 1488 1454 646 cm^{-1}. ^{1}H-NMR (600 MHz, DMSO-*d*6): d 6.02 (s, 1H, H*), 7.10 (m, 2H, H-2' and H6'), 7.38 (m, 2H, H-6 and H-6"), 7.41 (m, 2H, H-8 and H8"), 7.44 (m, 2H, H-3' and H-5'), 7.64 (m, 2H, H-7 and H-7"), 7.99 (d, *J* 7.7, 1H, H-5 and H-5"), 8.07 (d, *J* 7.3, 1H, H-5 and H-5"), 11.32 (s, 1H, OH), 11.54 (s, 1H, OH). MS *m/z*: 491 (M^{+}H, 100).

3,3’-(4-Trifluoromethylbenzylidene)-bis-(4-hydroxy-coumarin) (2) (Završnik, Muratović, Makuc, *et al.*, 2011).

Yield 72%. Mp 271.3 °C. Anal. Calcd for $C_{26}H_{15}O_6F_3$: C 65.01, H 3.15, F 11.86. Found: C 65.11, H 3.25, F 11.97. All values are given in percentages. IR (KBr) n 2918 1778 1652 1470 1198 1028 cm^{-1}. ^{1}H-NMR (600 MHz, DMSO-*d*6): d 6.34 (d, *J* 0.9, 1H, H*), 7.27 (m, 2H, H-6 and H-6”), 7.31 (m, 2H, H-8 and H-8”), 7.34 (d, *J* 8.4, 2H, H-2’ and H-6’), 7.55 (m, 2H, H-7 and H-7”), 7.55 (d, *J* 8.4, 2H, H-3’ and H-5’), 7.85 (dd, *J* 7.8, 1.5, 2H, H-5 and H-5”). ^{19}F-NMR (DMSO-*d*6): d –60.03 (s, CF_3). MS *m/z*: 481 (M^{+}H, 100).

3-(6-Oxo-(1*H*)-18-bromobenzopyrano[4,3-b]benzo-pyran-7-yl)-4-hydroxycoumarin (3) (Završnik, Muratović, Makuc, *et al.*, 2011).

Yield 72%. Mp 296 °C. Anal. Calcd for $C_{25}H_{13}O_6Br$: C 61.9, H 2.68. Found: C 61.7, H 2.68. All values are given in percentages. IR (KBr) n 3274 2886 1772 1610 1570 1558 1496 1220 586 cm^{-1}. ^{1}H-NMR (600 MHz, DMSO-*d*6): d 5.71 (s, 1H, H*), 7.31 (d, *J* 1.8, 1H, H-6’), 7.32 (m, 1H, H-8), 7.34 (d, *J* 8.7, 1H, H-3’), 7.36 (m, 1H, H-6), 7.45 (m, 1H, H-8”), 7.49 (m, 1H, H-6”), 7.52 (m, 1H, H-4’), 7.61 (m, 1H, H-7), 7.71 (m, 1H, H-7”), 8.03 (b, 1H, H-5), 8.11 (dd, *J* 7.9, 1.4, 1H, H-5”), 12.19 (b, 1H, OH). MS *m/z*: 488 (M^{+}H, 100).

3-(6-Oxo-(1*H*)-benzopyrano[4,3-b]-(1)10-hydroxybenzo-pyran-7-yl)-4-hydroxycoumarin (4) (Završnik, Muratović, Makuc, *et al.*, 2011).

Yield 82%. Mp 241.7 °C. Anal. Calcd for $C_{25}H_{14}O_6$: C 73.17, H 3.43. Found: C 72.7, H 2.99. All values are given in percentages. IR (KBr) n 2958 1616 1570 1488 1456 1230 cm^{-1}. ^{1}H-NMR (600 MHz, DMSO-*d*6): d 5.74 (s, 1H, H*), 7.14 (m, 1H, H-5’), 7.20 (m, 1H, H-6’,), 7.32 (m, 1H, H-8), 7.34 (m, 1H, H-4’), 7.35 (m, 1H, H-3’), 7.36 (m, 1H, H-6), 7.46 (m, 1H, H-8”), 7.49 (m, 1H, H-6”), 7.60 (m, 1H, H-7), 7.71 (dd, *J* 7.5, 1.7, 1H, H-7”), 8.03 (m, 1H, H-5), 8.11 (dd, *J* 7.9, 1.4, 1H, H-5”,), 12.21 (b, 1H, OH). MS *m/z*: 411 (M^{+}H, 100).

TLC and scanning densitometry

Analyzed compounds were chromatographed with each of nine mobile phases that were chosen according to their polarity. Only a mobile phase **6** of those tested (Table 1.), yieldedsuccessful separation of synthesized compounds, whilst other mobile phases were less efficient. Results are presented for the mobile phase **6** (dichloromethane/ethyl acetate = 2:1) and are shown on Figure 2. Plate was investigated under the UV light at wavelength of 254 nm.

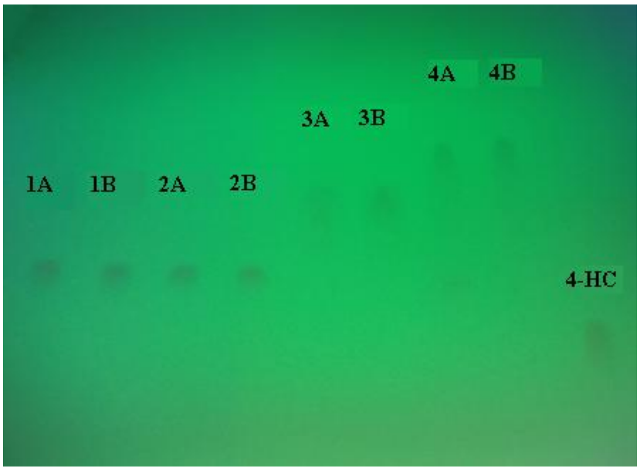


Figure 2. TLC plate developed with mobile phase **6**, under the UV light on 254 nm for the compounds **1** – **4** (raw substances (**A**) and after purification process (**B**); 4-HC at the end)

Thin layer chromatography can be applied for the quantitative analysis of samples and possible impurities. TLC plates were analyzed with scanning densitometer, in order to get quantitative data on percentage of present impurities. Resulting densitogram is presented on Figure 3.

Use of this technique allows identification of analyzed compounds not only by usual comparison of R_f values with standard compounds but also by comparison of their absorption or fluorescence spectra. Quantification is based on comparison of height or area under chromatographic peak (curve) of analyzed sample with height or area under the peak of standard on the same plate. Quantitative analyses of each compound from recorded densitogram are presented on Table 2.

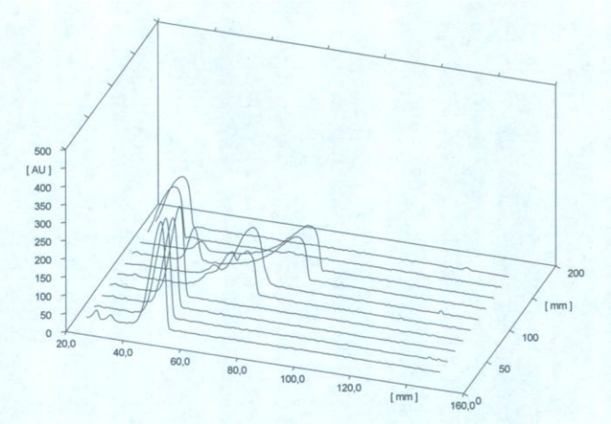


Figure 3. Densitogram recorded after TLC plate scanning at 254 nm in the absorbance mode

Table 2. Quantitative analysis after densitometry scanning for synthesized compounds and 4-HC

Peak	Start mm	Start Height	Max mm	Max Height	Max %	End mm	End Height	Area	**Area %**	Assigned substance
RAW COMPOUND **1** (**1A**)										
1	28.6	0.3	31.6	16.5	4.9	34.0	0.7	379.5	**2.1**	4-HC
2	39.3	3.7	49.3	294.5	88.1	52.6	1.9	16966.1	**95.2**	**1**
PURIFIED COMPOUND **1** (**1B**)										
1	24.2	1.9	24.9	3.9	1.4	26.2	1.0	54.1	**0.4**	4-HC
2	37.9	3.5	48.3	273.7	96.8	52.2	2.1	15406.7	**99.2**	**1**
RAW COMPOUND **2** (**2A**)										
1	23.5	0.7	25.2	5.6	2.2	28.1	0.2	125.9	**1.0**	4-HC
2	38.1	1.1	47.9	248.1	97.8	51.7	1.4	12942.2	**99.0**	**2**
PURIFIED COMPOUND **2** (**2B**)										
1	24.9	1.0	27.0	4.0	1.6	28.4	0.3	74.3	**0.6**	4-HC
2	38.9	3.4	48.2	245.2	98.4	52.4	1.7	12986.4	**99.4**	**2**
RAW COMPOUND **3** (**3A**)										
1	24.2	1.3	29.6	8.2	3.3	32.2	1.8	270.0	**1.6**	4-HC
2	49.3	9.0	63.6	111.4	45.4	65.2	89.8	9283.1	**54.4**	unknown
3	65.3	90.0	68.9	120.8	49.3	73.7	4.5	7350.3	**43.1**	**3**
PURIFIED COMPOUND **3** (**3B**)										
1	26.0	0.9	30.0	4.6	2.2	31.0	2.7	147.7	**0.8**	4-HC
2	48.8	7.5	54.6	35.2	16.9	57.0	25.0	1873.6	**10.3**	unknown
3	57.1	25.1	68.2	155.1	74.3	76.3	3.7	15921.2	**87.6**	**3**
RAW COMPOUND **4** (**4A**)										
1	23.3	0.4	24.7	5.5	2.4	26.0	0.1	246.4	**1.2**	4-HC
2	37.6	2.3	44.8	95.8	42.0	48.1	10.3	4197.5	**20.5**	unknown
3	61.0	18.1	80.9	115.9	50.9	85.9	2.0	15905.2	**77.8**	**4**
PURIFIED COMPOUND **4** (**4B**)										
1	38.2	1.2	44.7	29.3	19.6	48.1	6.6	1354.6	**7.6**	unknown
2	64.5	23.9	82.2	119.9	80.4	87.8	0.3	16440.2	**92.4**	**4**
4-HC										
1	23.3	1.4	32.4	136.5	92.4	36.5	0.2	11309.0	**97.3**	4-HC
2	70.1	0.3	72.8	6.4	4.3	75.5	1.5	196.0	**1.7**	unknown

As seen from Table 2. purification process by column chromatography was successful for these raw synthesized compounds. Analysis unambiguously showed increase in main compound percentage after purification process and decrease in percentage of 4-HC and other unknown impurities (present in some of the samples), indicating higher purity (bold column "**Area %**" in Table 2.). Impurities likely come from 4-HC itself as a reagent (having in mind its labeled purity percentage and results from Table 2.) or represent byproducts of syntheses.

CONCLUSIONS

Investigated compounds were analyzed on TLC plates with nine different mobile phases. Only mobile phase dichloromethane/ethyl acetate = 2:1 showed good separation of analyzed compounds. Plates were detected under the UV light at wavelength of 254 nm. Scanning densitometry analysis of present impurities was performed by scanning of TLC plate at 254 nm as well. Results of scanning densitometry showed that purification processes yielded compounds of higher purity.

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Summary/Sažetak

Onečišćenja koja zaostaju nakon sinteza u velikoj mjeri utiču na svojstva i djelotvornost sintetiziranog spoja, te je od velikog značaja utvrđivanje njihovog prisustva i uklanjanje iz sintetiziranog produkta. U cilju dobivanja kristala visokog stepena čistoće, sintetizirani spojevi bis-4-hidroksikumarina i benzopiranokumarina prečišćeni su postupcima prekristalizacije i kolonske hromatografije. Uspješnost prečišćavanja sintetiziranih spojeva ispitana je određivanjem tački topljenja, te hromatografijom na tankom sloju i skenirajućom denzitometrijom. Samo jedna od devet ispitivanih kombinacija mobilnih faza se pokazala dovoljno uspješnom za razdvajanje ispitivanih spojeva. Detekcija ploča je provedena pod UV svjetlom na talasnoj dužini 254 nm, kao i denzitometrijska kvantitativna analiza prisutnih onečišćenja. Rezultati skenirajuće denzitometrije su pokazali da su odgovarajućim prečišćavanjem dobiveni spojevi zadovoljavajućeg stepena čistoće (manje od 2% nepoznatih onečišćenja). Procenat čistoće se također može izračunati i iz podataka dobivenih spektroskopskim metodama i elementarnom analizom.

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Original Scientific Article

Laser-induced Processes of Diatomic Molecules: Homonuclear vs. Heteronuclear Species

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Abstract: We investigate high-order above-threshold ionization (HATI) and high-order harmonic generation (HHG) of homonuclear and heteronuclear diatomic molecules using modified molecular strong-field approximation (MSFA) and Stark-shift-corrected MSFA respectively. We present our results for N_2 and CO molecules. Their initial highest occupied molecular orbitals (HOMO) are characterized by $3\sigma_g$ and 5σ symmetry respectively, as for heteronuclear species parity property *gerade* or *ungerade* is no longer relevant. N_2 and CO HATI and HHG spectra for different molecular orientation with respect to laser polarization axis, and for different values of the electron or photon energies and emission angle, clearly reflect influence of the electron-density distribution symmetry of the HOMO. Prominent difference between these spectra is the absence of the left-right symmetry in HATI spectra for paralel orientation and selection rules violation in HHG spectra for CO. Spectra for CO could be reconstruct with contributions that accounts for ionization from the C atom only.

INTRODUCTION

High-order above-threshold ionization (HATI) and high-order harmonic generation (HHG) are two important nonperturbative phenomena, which occur when an atom or a molecule is exposed to an intense laser field. Especially, molecular HATI and HHG processes have received much attention in the last few years (Lin *et al.*, 2010; Lein and Chirilă, 2008).

In the first step of these processes, the considered molecular system absorbs more photons from the laser field than is necessary for ionization. The electron, liberated in such a way, can go directly to the detector. We call this process the direct above-threshold ionization (ATI) (Milošević, 2006). Due to the influence of the laser field, the ionized electron may also return to the parent molecular ion (second step) and elastically scatters off it (third step), before reaching the detector. In this process, the electron can absorb many more photons from the laser field than in the direct ATI. This process was named high-order ATI (HATI) (Busuladžić, Gazibegović-Busuladžić, Milošević, *et al.*, 2008). For the HHG process, in the third step, the electron recombines with the parent ion and one high-energy photon is emitted (Chirilă and Lein, 2006; Odžak and Milošević, 2009). The energy spectra of molecular HATI and molecular HHG processes are characterized by a plateau which manifests itself as a broad energy interval of the spectrum in which the photoelectron (HATI) or photon (HHG) yield is practically constant. These intervals are followed by abrupt cutoffs. By analyzing these spectra one can extract information about moleculare structure.

In fact, even direct ionization (direct ATI) without rescattering reveals the initial symmetry of the molecular system (Grasbon, F., Paulus, G. G., Chin, *et al*., 2001). For example, the O_2 molecule shows a suppression in the low-energy electron spectra due to its π_g symmetry, while the N_2 molecule, having σ_g symmetry, does not show such a suppression (for a more recent experimental and theoretical study see Okunishi, Shimada, Prümper, *et al*., 2007; Busuladžić and Milošević, 2010; Durá, Grün, Bates, *et al*., 2012). Including rescattering process there are four rescattering contributions to the ionization rate, which are responsible for the high-energy plateau in the electron (photon) spectra and which interfere in a complicated manner. The spectra are even more complicated due to the different symmetry properties of the atomic orbitals of which a particular molecular orbital consists.

By analyzing these spectra for different values of the angle between the molecular orientation and the laser polarization axis, and for different values of the electron emission angle with respect to the laser polarization axis, one can obtain information about molecular symmetry. As the molecules are multicenter systems, the ionization, as well as the rescattering and recombination, can happen at different centers, producing interference structures in the electron or photon spectrum (Hetzheim, Figueira de Morisson Faria, and Becker, 2007; Lein and Chirilă, 2008). This novel two-source two-rescattering-centers interference survives the focal averaging and even has been observed in the HATI experiment with unaligned molecules (Okunishi, Itaya, Shimada, *et al.*, 2009).

The double-slit-type or two-point emitter interference in HHG also manifests itself as minima and maxima in the harmonic yield for particular values of the angle between the internuclear axis and the laser polarization axis which are accompanied by a jump of the harmonic phase by π (Odžak and Milošević, 2009). It has been recently observed in HHG from the H_2 molecule that the interference occurs at lower harmonic order than would be the case if the nuclei were static (Baker, Robinson, Lein, *et al.*, 2008).

All above cited papers have been mostly devoted to the nonlinear processes with homonuclear diatomic molecules. More recently, behavior of polar molecules in strong laser field was attracted more attention (Holmegaard, Hansen, Kalhjøj, *et al.*, 2010; Dimitrovski, Martiny, and Madsen, 2010; Etches, Gaarde, and Madsen, 2011). Regarding considered phenomena in polar molecules, Stark shifts of the ionizing molecular orbital can play a signifcant role. Very recently, Stark-corrected molecular strong-field approximation (SC-MSFA) was introduced in order to appropriately describe the ATI process in polar molecules (see for example Dimitrovski *et al.*, 2010).

One of the most useful theoretical approaches based on three-step model is the modified molecular strong-field approximation (see for example Busuladžić, *et al.*, 2008). Our aim was to generalize our theoretical approach in order do describe above-mentioned processses with polar molecules. First of all, we consider the influence of the Stark effect on (H)ATI spectra of heteronuclear diatomic molecules (Hasović, Busuladžić, Becker, *et al.*, 2011). Analogous to the previous case, some features of partial contributions and total spectrum can be related to the shape of the molecular orbital (Busuladžić, Hasović, Becker, *et al.*, 2012). A similar theory of high-order harmonic generation by heteronuclear diatomic molecules exposed to an arbitrary polarized laser field was presented by Odžak and Milošević (2012). Again, the laser-field-caused Stark shift of the effective ionization potential of the highest occupied molecular orbital changes the structure of the harmonic spectra.

An interference minima condition that is valid for arbitrary heteronuclear diatomic molecules are derived for both HATI and HHG spectra (Hasović, *et al*.,2011; Odžak and Milošević, 2012). We also compare the key similarities and differences between heteronuclear and homonuclear diatomic molecules. In this paper, our results are illustrated using examples of CO and N_2 molecules.

This paper is organized as follows. A brief review of our SC-MSFA theory, which includes the most important results obtained for linear polarization case, is presented in Section 2. Our numerical results are presented in Section 3, while conclusions are given in Section 4. We use atomic system of units.

THEORY

We have developed a theory describing ionization of diatomic molecules by a strong linearly polarized laser field (Milošević, 2006). This was done within the modified molecular strong-field approximation (MSFA) approach. In the next step, we have generalized this theory so that it describes high-order processes which are the subject of our investigation (Busuladžić, *et al.*, 2008; Odžak and Milošević, 2009). Using the MSFA, we successfully simulated the earliest HATI experiments (Okunishi, Itaya, Shimada *et al.*, 2008; Okunishi, *et al.*, 2009). More recently, we have extended our theory of HATI and HHG by homonuclear diatomic molecules (Busuladžić, *et al.*, 2008; Odžak and Milošević, 2009) to heteronuclear molecules (Hasović, *et al.*,2011; Busuladžić, *et al.*,2012; Odžak and Milošević, 2012).

We briefly present here our results for HATI and HHG of heteronuclear molecules and emphasize differences between heteronuclear and homonuclear diatomic molecules. We assume that the ionization of a molecule occurs from the highest occupied molecular orbital (HOMO), which is presented as a linear superposition of atomic orbitals (LCAO) (Atkins and Friedman, 2001; Levine, 2000)

$$\phi_{ei} = \sum_{sa} c_{sa} \psi_a e^{iI_p t} \ , \quad (1)$$

where the coefficients c_{+1a} and c_{-1a} are related to two different centers. For heteronuclear molecules they are not equal up to a sign, as was the case for homonuclear molecules (Hasović, *et al.*,2011). The laser dressing of the initial state of the homonuclear diatomic molecules was performed at the level of atomic orbitals (Milošević, 2006), while in the heteronuclear case the dressing of the whole molecular orbital was done (Hasović, *et al.*, 2011). This corresponds to the replacement in the exponent of the term with the ionization energy $I_p t$ by $(I_p - \Delta_s)\cdot t + \mu_S(t) + \alpha_S(t)$. A more deatiled explanations about these procedures can be found in our previously published papers (Hasović, *et al.*,2011; Busuladžić, *et al.*,2012). The first of the three additional terms, $-\Delta_S \cdot t$, contains the polarizability-induced Stark shift, which increases the effective ionization potential of the molecule and, therefore, leads to a decrease of the ionization rate (Hasović, *et al.*,2011). The remaining two time-dependent terms, $\mu_S(t)$ and $\alpha_S(t)$, are related to the dipole moment and the polarizability, respectively. Both of them enrich the oscillatory structure of the HATI and HHG spectra (Hasović, *et al.*,2011; Odžak and Milošević, 2012).

Now, we are going to present the expression of the total rate which accounts for the coherent sum of the direct term $T_{fi}^{(0)}(n)$ and the rescattering term $T_{fi}^{(1)}(n)$ (Hasović *et al.*, 2011)

$$w = 2\pi p_f \left|T_{fi}(n)\right|^2, \quad (2)$$

where the *T*-matrix elements $T_{fi}^{(0)}(n)$ and $T_{fi}^{(1)}(n)$ are the Fourier transforms of the expressions given by Eq. (18) and Eq. (19) in the paper by Hasović, *et al.* (2011). These matrix elements were obtained in the length gauge. First of them accounts for contribution of direct electrons, while second one decribes high-energy part of the HATI spectra. The direct matrix element $T_{fi}^{(0)}(n)$ can be decomposed into two terms, while the matrix element $T_{fi}^{(1)}(n)$, related to the rescattering process, can be presented as a sum of four different terms (Hasović, et al., 2011; Busuladžić, et al., 2012). Therefore, as the molecules are multicenter systems, the ionization and the rescattering can happen at different centers. As a consequence, we have six different terms. Four of them are responsible for the high-energy part of the HATI spectrum and may cause interference structure.

It can be shown that our rescattering matrix element is proportional to $\cos[(\vec{p}_f - \vec{k}_{st})\cdot\vec{R}_0/2]$, regardless of the symmetry of the atomic orbital of which the considered molecular orbital consists (Busuladžić, *et al.*, 2008). In the last equation, $\vec{p}_f$ is the final momentum of the detected electron, $\vec{k}_{st}$ is the stationary intermediate electron momentum between the ionization and the rescattering (it is in the direction of the laser electric field vector), and $\vec{R}_0$ is the internuclear axis. Therefore, if we present the rescattering ionization rate in the (E_{p_f}, θ) plane, we will have local minima if the condition

$$R_0\left|p_f\cos\theta - k_{st}\cos\theta_L\right| = (2m+1)\cdot\pi, \quad (3)$$

with m integer, is fulfilled. Here, θ is the angle between the final momentum of detected electron and the internuclear axis, while θ_L is the angle between the internuclear axis and the laser polarization axis.

Now, we will consider HHG process on molecular targets. For a periodic laser field, having the period $T = 2\pi/\omega$, the rate of emission of a harmonic photon of the frequency $\omega_{\vec{K}} = n\omega$, wave vector $\vec{K} = \omega_{\vec{K}}\hat{\vec{K}}/c$, and the unit complex polarization vector $\hat{\vec{e}}_{\vec{K}}$, into a solid angle $d\Omega_{\hat{\vec{K}}}$, is given by (Odžak and Milošević, 2012)

$$w_{\vec{K}} = \frac{1}{2\pi}\left(\frac{\omega_{\vec{K}}}{c}\right)^3\left|T_{\vec{K}}\right|^2. \quad (4)$$

The T-matrix element, within the MSFA and for four possible combinations [the field-free, $q = u$ (undressed), or laser-dressed, $q = d$ (dressed)] of the initial and final molecular bound states, can be presented in the form of a five-dimensional integral which is given by Eq. (15) in our previously published paper (Odžak and Milošević, 2012).

We calculate the components of the complex vector $\vec{T}_{\vec{K}} = \vec{T}_{\vec{K}}\hat{\vec{e}}_{\vec{K}} = T_{\vec{K}}^{\hat{\vec{\varepsilon}}}\hat{\vec{\varepsilon}} + T_{\vec{K}}^{\hat{\vec{k}}\times\hat{\vec{\varepsilon}}}\hat{\vec{k}}\times\hat{\vec{\varepsilon}}$ along the major ($T_{\vec{K}}^{\hat{\vec{\varepsilon}}}$) and the minor ($T_{\vec{K}}^{\hat{\vec{k}}\times\hat{\vec{\varepsilon}}}$) semiaxis of the laser-field polarization ellipse. Knowing $T_{\vec{K}}^{\hat{\vec{\varepsilon}}}$ and $T_{\vec{K}}^{\hat{\vec{k}}\times\hat{\vec{\varepsilon}}}$ we can calculate the harmonic emission rate using $\left|T_{\vec{K}}\right|^2 = \left|T_{\vec{K}}^{\hat{\vec{\varepsilon}}}\right|^2 + \left|T_{\vec{K}}^{\hat{\vec{k}}\times\hat{\vec{\varepsilon}}}\right|^2$. For aligned molecules this rate depends on the angle θ_L between the major semiaxis of the polarization ellipse and the molecular axis.

As in the case of HATI, one can observe interference minima in the molecular HHG spectra. The local minima appear if the condition

$$\sum_{s=\pm1}\sum_a c_{sa}e^{-is[\vec{k}_{st}+\vec{A}(t)]\cdot\vec{R}_0/2}\vec{e}_{\vec{K}}^{\,*}\cdot\vec{\mathrm{m}}_a(t) = 0, \quad (5)$$

is fulfilled. In the last equation, the term $\vec{\mathrm{m}}_a(t)$ is the recombination matrix element of the particular atomic orbital a. Since the recombination step is responsible for the interference, the positions of interference minima can be obtained by solving the transcendental equation (5). This is a general expression that can be applied to arbitrary diatomic molecules. It is important to stress that all other paramethers appearing in the Eq. (5) are defined in the same manner as in the case of HATI process.

It should be mentioned that various versions of the interference minima condition exist in the literature.

Their explicit form depends on the model used for calculation of the harmonic
emission rate.

RESULTS AND DISCUSSION

We will consider CO molecule, as an example of heteronuclear molecular species. Furthermore, spectra of the CO molecule will be compared with the homonuclear molecule N_2. For the N_2 molecule, the initial HOMO is $3\sigma_g$. Twelve atomic orbitals will be taken into account in the corresponding LCAO: a=1s, 1s', 2s, 2s', 3s, 2p, 2p', 2p'', 3d, 3d', 3d'', and 4f. Because of the lack of a center of inversion for heteronuclear molecules, the parity property gerade or ungerade is no longer relevant. As a consequence, the HOMO of CO is 5σ and is modeled by a linear combination of the 1s, 1s', 2s, 3s, 2p, 2p', 2p'', and 3d atomic orbitals. Atomic orbitals which enter LCAO are the Slater-type orbitals obtained using the Hartree-Fock-Roothaan method (Atkins and Friedman, 2001; Levine, 2000). The coefficients c_{sa} and the STO ψ_a that appear in Eq. (1) are tabulated in literature (Cade and Wahl, 1974; Cade and Huo, 1975). In our calculation, we use the equilibrium internuclear distance for both molecular targets: $R_0 = 2.068\,\text{a.u.}$ for N_2 and $R_0 = 2.132\,\text{a.u.}$ for CO. Also, the next value of the ionization potential is taken into account: $I_p = 15.58\,\text{eV}$ (for N_2) and $I_p = 13.99\,\text{eV}$ (CO). The dipole and polarizability of the HOMO of CO are found by calculating the ionization potential for a range of values of the static electric field strength and fitting the resulting curve to a second-order polynomial (Hasović, *et al.*, 2011). This yields a dipole of $\mu = 1.546\,\text{a.u.}$ pointing from C to O and a polarizability with $\alpha_\parallel = 5.3673\,\text{a.u.}$ and $\alpha_\perp = 5.49\,\text{a.u.}$ The mass asymmetry parameter for CO molecule is

$$\lambda = \frac{M_A - M_B}{M_A + M_B} = 0.142701. \quad (6)$$

In the last equation, M_B and M_B are the masses of the particles A and B. For homonuclear molecules we have $\lambda = 0$.

In Figure 1, we present $3\sigma_g$ and 5σ HOMO wave functions of N_2 (upper panel) and CO (bottom panel), respectively. Both wave functions are presented in the XZ plane (coordinate space).

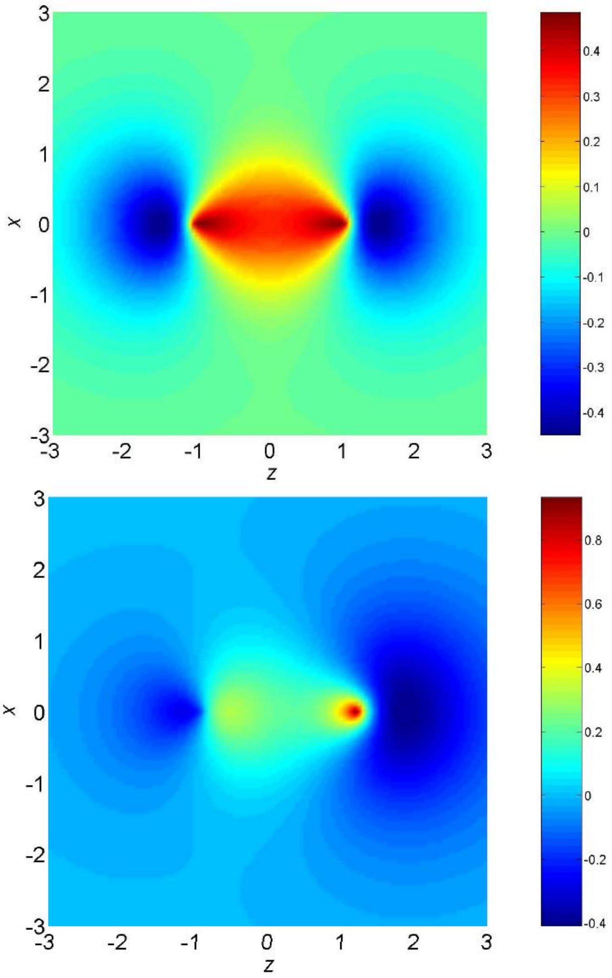

Figure 1: Coordinate space wave function for the $3\sigma_g$ HOMO of N_2 (upper panel) and for 5σ HOMO of CO, presented in the XZ plane (lower panel). In the lower panel, the *z* axis is oriented from the O center (left) to the C center (right).

In Figure 2, we present angle-resolved electron energy spectra for the N_2 molecule for the fixed molecular orientation with respect to the laser polarization axis: $\theta_L = 0^o$. Laser field is linearly polarized having intensity $I = 1.2 \cdot 10^{14}\,\text{W/cm}^2$ and wavelength $\lambda = 800\,\text{nm}$. The total spectrum which include all six contributions is presented in the uppermost panel of the figure. First of all, we can observe pronounced minima in the HATI spectra, particularly in the cutoff region, in accordance with Eq. (3). For chosen laser and molecular parameters, we have minima for value $m = 0$ in Eq. (3). The dashed curves presented in the panel are based on Eq. (3). This equation provides a good fit of the positions of the interference minima in the (E_{p_f}, θ) plane, especially for larger values of θ_L, and becomes exact fit for θ_L. In the remaining three lower panels we have shown the spectra corresponding to the partial *T*-matrix contributions, as denoted in the caption of Figure 2. It is clear that neither of these spectra exhibit the mentioned interference minima. This confirms our previous finding that the interference minima are due to the destructive interference of all partial *T*-matrix contributions. Now, we remember that our *T*-matrix element related to the rescattering process (high-energy part of the spectrum) can be decomposed into four terms:

$$T^{(1)} = T^{++} + T^{+-} + T^{-+} + T^{--}. \quad (7)$$

The electron can tunnel out of either atom. These possibilities are denoted by superscript "+" or "−". With $T^{++}(T^{--})$ we denote the *T*-matrix contribution of the electron which, after excursion in the continuum, rescatters at the same center "+" ("−"). The terms T^{+-} and T^{-+}, correspond to the cases where the electron is ionized and rescatters off different centers.

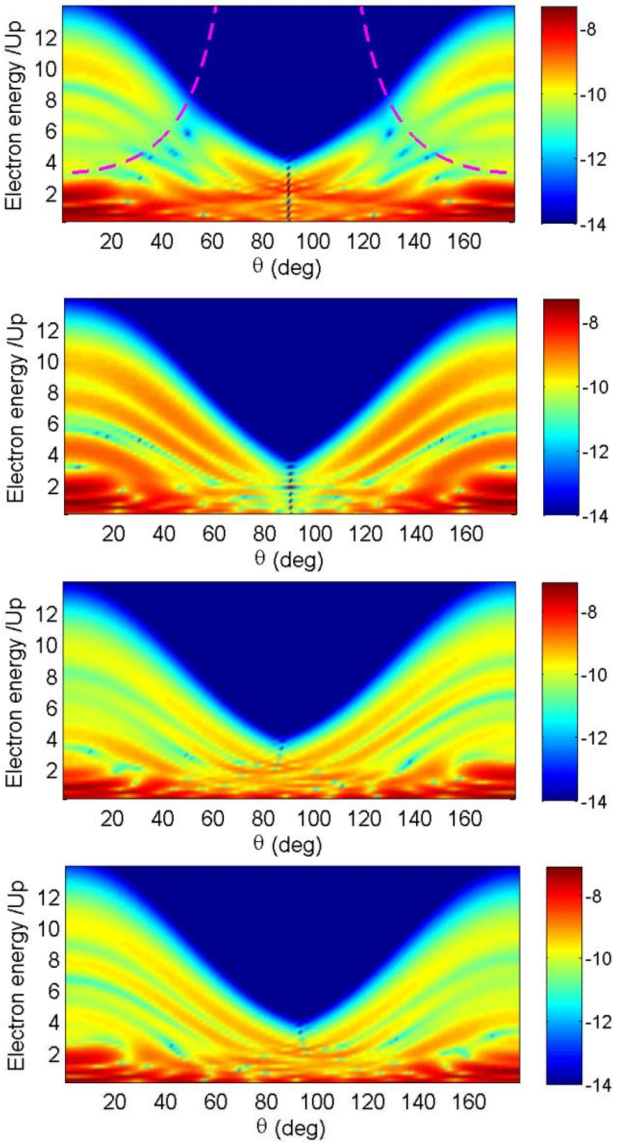

Figure 2: Logarithm of the differential ionization rate of N_2, coded in false colors in the (E_{p_f}, θ) plane, for $\theta_L = 0^o$ is presented in the uppermost panel. In the remaining panels of the figure, presented spectra are obtained taking into account only the corresponding partial contributions: T^{++--}, T^{+-}, and T^{-+}. In our notation is $T^{++--} = T^{++} + T^{--}$. Intensity of the linearly polarized laser field is $I = 1.2 \cdot 10^{14}$ W/cm^2 and the wavelength is 800 nm. The electron kinetic energy E_{p_f} is presented in units of U_P, where U_P is defined as the ponderomotive energy of the electron in the laser field. The dashed pink curves are based on Eq. (3) (see the text). All HATI spectra for the N_2 molecule are obtained using the dressed length-gauge modified MSFA (Busuladžić, *et al.*, 2008).

More precisely, interference of four possible contributions to the high-energy part of the spectrum is responsible for observed minima in the HATI spectra of the N_2 molecule. This is an example of the HATI process with homonuclear molecules.

The angle- and energy-resolved HATI spectra of the CO molecule are shown in Fig. 3 for $\theta_L = 0^o$. Laser parameters are the same as in Fig. 2. Total rate or cohrerent sum of all six contributions is presented in the uppermost panel. As in the case of a homonuclear molecule, we observe pronounced minima of the ionization rate presented in the (E_{p_f}, θ) plane (uppermost panel). The results presented in the remaining two lower panels of Figure 3 are obtained taking into account only the electrons ionized from the C center (T^{C}, T^{CC}, and T^{CO}) and O center (T^{O}, T^{OO}, and T^{OC}), respectively. By comparing the spectra of partial contributions (two lower panels of Figure 3) with the total rate spectrum (uppermost panel of Figure 3), we conclude that scenarios in which the electron is born at the carbon core are dominant. This is valid for almost all molecular orientations and directions of the emitted electrons. The only exception is for parallel orientation and $\theta > 120^o$ (Hasović, *et al.*, 2011). Let us explain these results. If we look at the valence electron distribution of the CO molecule in coordinate space (Figure 1), we notice the absence of the inversion symmetry. More precisely, most of the electron-density probability is located on the side of the C atom. It is also important to stress that the atomic centers are not equidistant from the coordinate origin. The distance from the origin to the carbon atom is 1.218 a.u., while the distance to the oxygen atom is 0.914 a.u. As a consequence, the shape of the ground-state wave function of the CO molecule suggests that the ionisation probability of the CO molecule from the C center located on the right-hand side is much higher than the ionisation probability of the same molecule from the O center (on the left-hand side). Therefore, the partial contributions T^{C}, T^{CC}, and T^{CO} are dominant in the total HATI spectrum.

Also, it is important to stress that in the heteronuclear diatomic case, the symmetry property $\theta \leftrightarrow \pi - \theta$ for $\theta_L = 0^o$ which is satisfied for homonuclear molecular species, is violated (compare the uppermost panels of Fig. 2 and Fig. 3). It is obvious that this twofold symmetry is violated because the heteronuclear molecules have two different centers (Hasović, *et al.*, 2011; Busuladžić, *et al.*,2012).

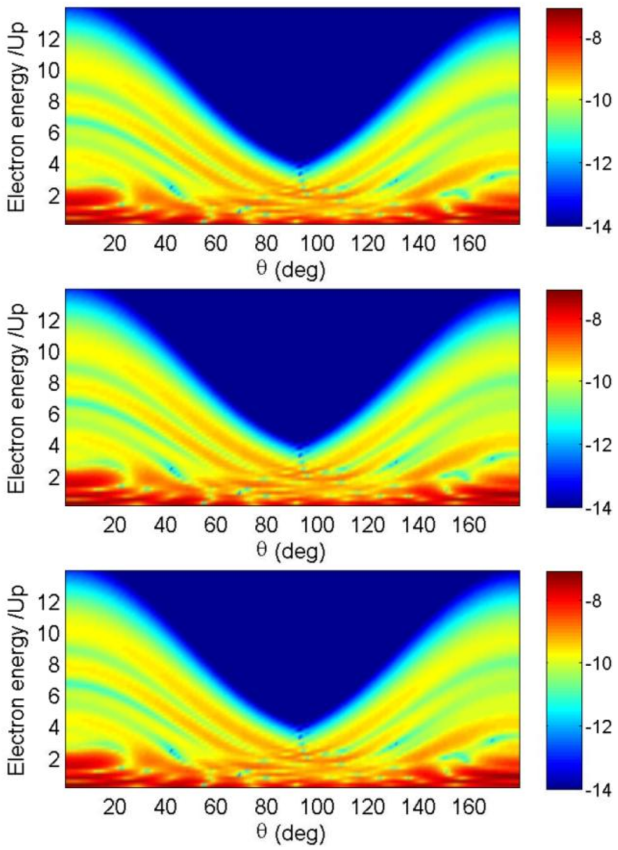

Figure 3: Logarithm of the differential ionization rate of CO, coded in false colors in the (E_{p_f},θ) plane, for $\theta_L = 0^o$ is presented in the uppermost panel. In the remaining panels of the figure, presented spectra are obtained taking into account only the electrons ionized from the C center (T^C, T^{CC}, and T^{CO}) and O center (T^O, T^{OO}, and T^{OC}), respectively. Laser parameters are the same as in Fig. 2. The dashed pink curves are based on Eq. (3) (see the text). All HATI spectra for CO molecule are obtained using the Stark-corrected undressed length-gauge version of our modifed MSFA (Hasović, *et al.*, 2011; Busuladžić, *et al.*, 2012).

Now, we turn our attention to the observed minima in the ionization rate, presented in the energy-angle plane. In all panels of Figure 3 one can notice pronounced minima in the high-energy region of the spectra at specific values of the angle θ. Therefore, minima appearing in total spectra have almost the same position as those appearing in spectra obtained by taking into account only the electrons ionized from the C center. It can be shown that even if we take into account only the two partial rescattering amplitudes in which the electron was ionized at the C core we can obtain the condition for the location of these minima which is given by Eq. (3).

More recently, we have found that the imprint of the molecular multicenter structure can be observed in the above-threshold ionization spectra obtained by a circularly polarized laser field. It manifests itself as minima in the rate of the ionized electrons as a function of their energy. The locations of these minima are strongly influenced by the symmetry of the corresponding highest occupied molecular orbital as well as the internuclear separation. Analyzing the interference structures of the electron spectra one can obtain information about the molecular symmetry (Busuladžić, Gazibegović-Busuladžić, Becker, *et al.*, 2013).

In Figure 4 we present the harmonic spectra of the N_2 molecule. Laser field is linearly polarized having intensity $I = 2.0\cdot 10^{14}$ W/cm^2 and wavelength $\lambda = 800$ nm. The harmonic emission rate is shown in false color as a function of the angle between the major semiaxis of the polarization ellipse and the molecular axis (plotted along the horizontal axis), and the harmonic order (vertical axis).

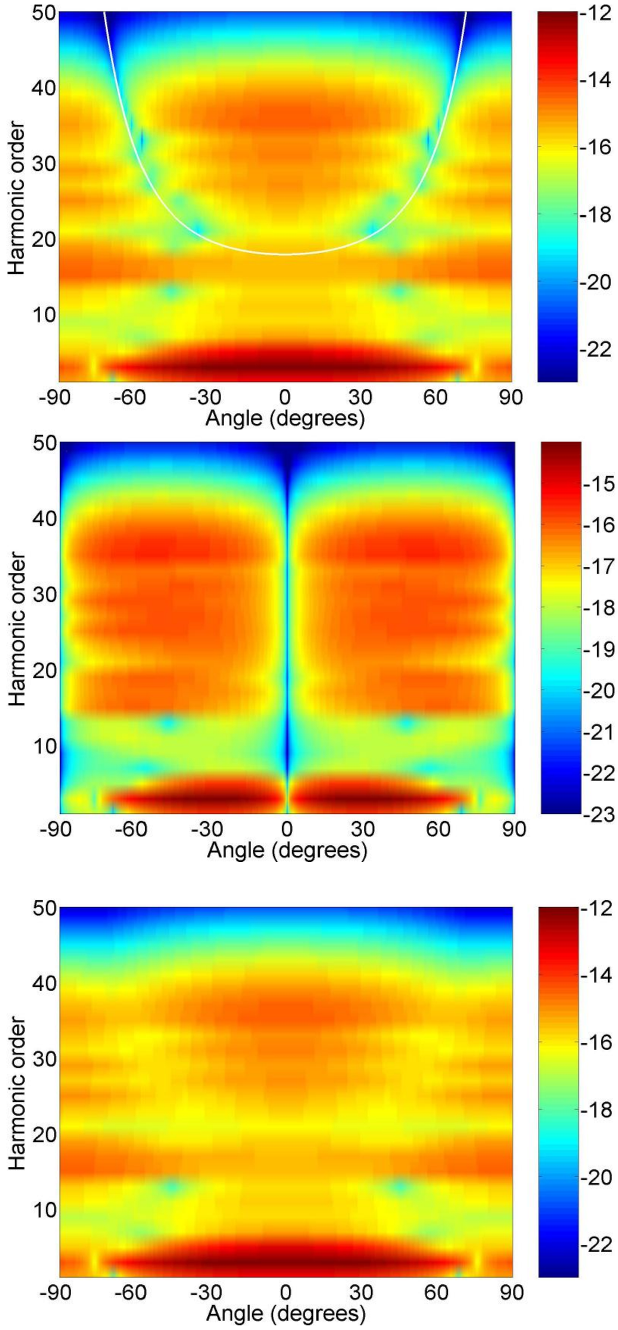

Figure 4: High-order harmonic spectra of the N_2 molecule, obtained using a linearly polarized laser field having the intensity $I = 2.0\cdot 10^{14}$ W/cm^2 and wavelength $\lambda = 800$ nm. The harmonic emission rate is shown in false color as a function of the angle between the major semiaxis of the polarization ellipse and the molecular axis (plotted along the horizontal axis), and the harmonic order (vertical axis). The results shown in the uppermost (middle) panel are obtained using only the component $T_{\vec{K}}^{\hat{\varepsilon}}$ ($T_{\vec{K}}^{\hat{k}\times\hat{\varepsilon}}$) along the major (minor) semiaxis of the laser-field polarization

ellipse. The results in the lowest panel represent the sum of both contributions: $|T_{\vec{K}}|^2 = |T_{\vec{K}}^{\hat{\varepsilon}}|^2 + |T_{\vec{K}}^{\hat{k}\times\hat{\varepsilon}}|^2$.

The results shown in the uppermost panel are obtained using only the component of the vector $\vec{T}_{\vec{K}}$ along the major semiaxis of the laser-field polarization ellipse. These spectra show clear two-center interference minima. The solutions $n_{\min} = n_{\min}(\theta_L)$ of the transcendental equation (5) fit well the interference minima obtained numerically. The results shown in the middle panel are obtained using only the component $T_{\vec{K}}^{\hat{k}\times\hat{\varepsilon}}$ along the minor semiaxis of the polarization ellipse. In this case, minima for parallel ($\theta_L = 0^\circ$) and perpendicular orientation ($\theta_L = \pm 90^\circ$) are clearly visible (Odžak and Milošević, 2010). The reason for existence minima for $\theta_L = 0^\circ$ is lack contribution of *s* atomic orbitals, while for *p, d* and *f* atomic orbitals $T_{\vec{K}}^{\hat{k}\times\hat{\varepsilon}} = 0$. For $\theta_L = \pm 90^\circ$ only the *p* and *f* orbitals can contribute to $T_{\vec{K}}^{\hat{k}\times\hat{\varepsilon}}$. In this case, interference factor is proportional to $\sin(a \cdot \cos\theta_L)$ $\left(a = \frac{1}{2} p R_0\right)$ leading again to $T_{\vec{K}}^{\hat{k}\times\hat{\varepsilon}} = 0$.

As a consequence complex mixing contributions of *s, p* and *f* atomic orbitals in the total harmonic spectrum determined by $|T_{\vec{K}}|^2 = |T_{\vec{K}}^{\hat{\varepsilon}}|^2 + |T_{\vec{K}}^{\hat{k}\times\hat{\varepsilon}}|^2$ the interference minima are not clearly visible. In the total spectra, shown in the third column of Fig. 2, the interference minima are not clearly visible. The reason is that the interference minima that originate from the $T_{\vec{K}}^{\hat{k}\times\hat{\varepsilon}}$ component are covered by the contribution of the dominant $T_{\vec{K}}^{\hat{\varepsilon}}$ component.

In all presented panels of Figure 4, the existence of only odd harmonics is clearly visible. This is related to the fact that the inversion symmetry is fulfilled for homonuclear diatomic molecules, i.e. valid $\vec{E}(t + T/2) = -\vec{E}(t)$.

In Figure 5 we present the harmonic spectra of the CO molecule. Laser parameters are same as in the case of molecule N_2.

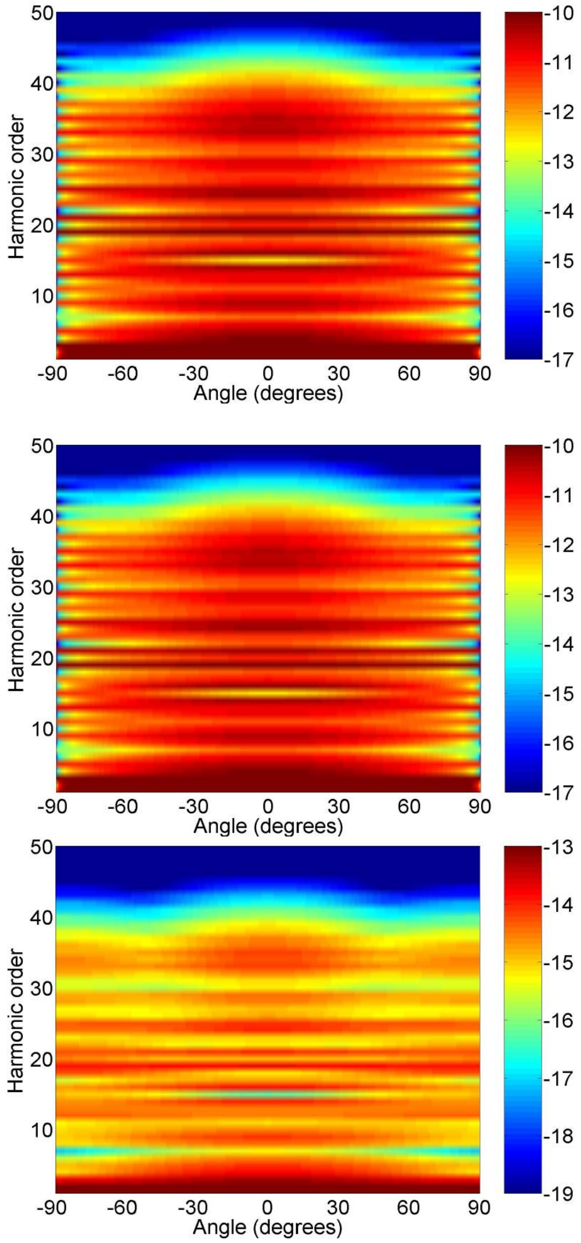

Figure 5: Same as for the N_2 molecule, but for CO molecule.

Inspection of Figure 5 it should be noted two main differences. Firstly, in the case of CO molecule there exist pronounced two-center interference minima only for $T_{\vec{K}}^{\hat{k}\times\hat{\varepsilon}}$ component of vector $\vec{T}_{\vec{K}}$. By analyzing partial contributions of atomic orbitals to the harmonic spectrum, we concluded that in this case, the interference minima are caused by the contribution of the *p* atomic orbitals to the HOMO. Also, the solutions of the transcendental equation (5) fit well the interference minima obtained numerically. Fitting curve is presented by white curve in the middle panel of Figure 5. Secondly, in all presented panels for CO molecule, the existence of odd and even harmonics is clearly visible. The existence of even harmonics is related to the violation of the inversion symmetry for heteronuclear molecules.

CONCLUSIONS

In this paper we have presented various numerical results for HATI and HHG of homonuclear and heteronuclear diatomic molecules. The results are obtained using our improved MSFA approach for homonuclear and Stark-shift-corrected MSFA theory for heteronuclear diatomic species. As the corresponding examples we have taken N_2 (characterized by $3\sigma_g$ symmetry of its HOMO) and the CO (5σ) molecules.

In order to reproduce total HATI spectra for the CO molecule, one can take into account only the contributions from the ionization of the C atom. In the case of the N_2 molecule, we have to calculate the coherent sum of all contributions from both atomic centers. That behavior can be related to the shape of their HOMO-electron-density distribution. For both molecules, destructive interference minima are observed. Analytical formula for the minima positions can be found in both cases.

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Summary/Sažetak

U ovome radu istražujemo procese jonizacije iznad praga višeg reda (eng. HATI) i generacije viših harmonika (eng. HHG) na dvoatomskim homonuklearnim i heteronuklearnim molekulama u jakom laserskom polju. Pri analizi je korištena modifikovana molekularna aproksimacija jakog polja (eng. MSFA) koja u slučaju heteronuklearnih sistema uključuje i Starkov efekat. U radu smo poredili rezultate za molekule N_2 i CO. Njihove najviše zaposjednute molekularne orbitale (eng. HOMO) su karakterisane $3\sigma_g$ i 5σ simetrijom, respektivno. Kako heteornuklearni sistemi posjeduju dva različita atomska centra, to je svojstvo simetrije označeno sa *gerade* i *ungerade* u slučaju homonuklearnih molekula odsutno kod heteronuklearnih sistema. Analizirajući dobivene HATI i HHG spektre za različite laserske i molekularne parametre, moguće je izvesti određene zaključke o samoj strukturi posmatranih molekula. Značajna razlika između dobivenih HATI spektara za ove dvije molekule je gubitak odgovarajuće prostorne simetrije za paralelnu orijentaciju u slučaju CO molekule. Također, u slučaju HHG spektara CO molekule narušena su pravila prijelaza koja vrijede u slučaju N_2 molekule. Dobivene spektre CO molekule možemo reproducirati uzimajući samo one doprinose koji dolaze sa C atomskog centra, dok u slučaju molekule N_2 doprinosi sa oba centra igraju značajnu ulogu u dobivenim spektrima.

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Technical Article

Determination of Sugar Content in Flavored Waters

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Abstract: Flavored waters mostly consists of water, natural or artificial flavorings, and sugar or artificial sweeteners. This work aimed to assess total sugar content in 25 different flavored water samples commercially available from the Slovenian local market by using an high pressure liquid chromatography (HPLC) method with refraction index detection (RID). HPLC (Agilent 1200 Series) was performed with an isocratic elution of mobile phase 15 mM K_2HPO_4 water solution. Degassed and diluted samples were analyzed on Supelcogel K column (300 x 7.8 mm, with 9 μm particles size), at 80 °C and 0.5 mLmin^{-1} flow rate. The total sugar content in flavored waters varies according to the types and brands, from 11.0 g/L to 44.1 g/L. Concentration values of sucrose, glucose and fructose were found to be in the ranges (g/L): 10.0 – 32.8; 1.3 - 21.7 and 3.0 -37.2, respectively. The results suggest caution in the use of high amount of flavored waters especially by diabetic patients.

INTRODUCTION

Over 70% of all food consumed by humans contain some quantities of sugar. In the same time, consuming of different non alcoholic drinks increased about five times from 1950s till now. Meta analysis showes that consumption of sweetened drinks is related to different disseases such as diabetes, metabolic syndrome and cardiovascular disease development, in the adults as well as in children (Bray and Popkin, 2013).

Phrase „flavored water“ could seem like water with healty fruit flavorings, but ingredients varies from brand to brand. It is well known that flavored waters contain plane water, any variety of added fruit ingredients and other flavorings, and sugar. In the comparison with recommendation from medical experts that eight glasses of plane water is required for daily usage it is very sad truth that small bottles of flavored waters contain sugar content which is equal to the amount of sugar in some desserts. Although, water consumption is quality and tastefull hidratation, drinking of 500 ml flavored water means that adult person could take 1/3 daily recommended sugar quantities. That fact was a reason that some world companies had to avoid advertising for vitaminin supllemented water products and flavored waters. It was not possible to convince their consumers that these products are healthy and harmless even if 420 ml water contain about 42 g of sugar content.

That is why, especialy children should not drink any other water than plane water. It is necessary to insist that children do not drink this type of water, cause during the groving period, sugar is the main reason for teeth decay. Adults who still like to drink aromatized waters should be aware that these waters are full of sugar and some negative efects of it could be avoided only with extreme physical activities (Bray and Popkin, 2013).

MATERIAL AND METHOD

Material: 25 different flavored water samples commercially available from the Slovenian local market: 1.DANA with flavor of lemon & melissa (Dana d.o.o. Mirna Slovenia), 2.DANA with flavor of forest strawberries & aloe vera (Dana d.o.o. Mirna Slovenia), 3.DANA with flavor of orange & Echinacea purpurea (Dana d.o.o. Mirna Slovenia), 4.DANA with flavor of pomegranate, blueberry aroma & ginseng (Dana d.o.o. Mirna Slovenia), 5.DANA with flavor of plum & green tea (Dana d.o.o. Mirna Slovenia), 6.DANA sport with taste of pink grapefruit (Dana d.o.o. Mirna Slovenia), 7.COSTELLA with flavor of limes & Kumquat (Uskok d.o.o. Kostel Slovenia), 8.COSTELLA with flavor of peach (Uskok d.o.o. Kostel Slovenia), 9.COSTELLA with flavor of blackberries, blueberries & forest strawberries (Uskok d.o.o. Kostel Slovenia), 10.COSTELLA with flavor of grapefruit & aloe vera (Uskok d.o.o. Kostel Slovenia), 11.OAZA with flavor of tangerine (Radenska d.d. Radenci Slovenia), 12.OAZA with flavor of apricot & white tea (Radenska d.d. Radenci Slovenia), 13.OAZA with flavor of cherry & white tea (Radenska d.d. Radenci Slovenia), 14.ZA LIFE with flavor of apple (Pivovarna Union d.d. Ljubljana Slovenia), 15.ZA HARMONY with flavor of white peach (Pivovarna Union d.d. Ljubljana Slovenia), 16.ZA SYMPHONY with flavor of pears (Pivovarna Union d.d. Ljubljana Slovenia), 17.ZA SPORT ISOTONIC (Pivovarna Union d.d. Ljubljana Slovenia), 18.WATER WITH TASTE - lemon (for Mercator produces Radenska d.d. Radenci Slovenia), 19.WATER WITH TASTE – apple & cranberry (for Mercator produces Radenska d.d. Radenci Slovenia), 20.JANA with flavor of strawberry & Guana (Jamnica d.d. Zagreb Croatia), 21.JANA with flavor of lemon & limes (Jamnica d.d. Zagreb Croatia), 22.JANA with flavor of blueberry & cranberry (Jamnica d.d. Zagreb Croatia), 23.NATIVA WATER with flavor of green tea & peach (Rauh Fruchsäfte Rankweil Austria), 24.NATIVA WATER with flavor of jasmine tea & passion fruit (Rauh Fruchsäfte Rankweil Austria), 25.VIVIA LIFE with flavor of lemon & limes (Vitinka a.d. Kozluk Bosnia and Herzegovina).

Method: 25 different flavored water samples commercially available from the Slovenian local market were analyzed by using high pressure liquid chromatography (HPLC) method with refraction index detection (RID). HPLC (Agilent 1200 Series) was performed with an isocratic elution of mobile phase 15 mM K_2HPO_4 water solution. Degassed and diluted samples were analyzed on Supelcogel K column (300 x 7.8 mm, with 9 µm particles size), at 80 °C and 0.5 mLmin^{-1} flow rate.

RESULTS

Table 1. Content of sucrose, glucose and fructose (g/L) in flavored waters

Sample	Sucrose content [g/L]	Glucose content [g/L]	Fructose content [g/L]
1	28.2	4.7	6.0
2	21.8	7.0	8.1
3	10.4	15.4	13.3
4	28.9	5.7	6.5
5	15.4	13.6	10.9
6	0.0	20.8	17.9
7	0.0	20.7	15.7
8	0.0	21.2	16.2
9	0.0	21.7	17.0
10	0.0	21.0	17.3
11	13.7	6.1	19.1
12	0.0	4.7	30.2
13	0.0	4.9	31.4
14	32.8	4.2	5.8
15	32.2	5.2	5.9
16	28.9	6.9	8.3
17	0.0	0.0	37.2
18	23.8	1.3	2.5
19	25.8	1.5	3.0
20	18.4	5.0	5.6
21	10.0	4.4	5.0
22	19.4	5.0	5.3
23	0.0	0.0	11.2
24	0.0	0.0	11.0
25	22.4	7.2	9.3

Results showed the highest sucrose content in sample of ZA LIFE with apple (32.8 g/L). The highest content of glucose was determined in sample of COSTELLA blackberries, blueberries & forest strawberries (21.7g/L). The highest content of fructoze was in sample of ZA SPORT ISOTONIC (37.2 g/L).

Table 2. Determined and declared content of total sugar in flavored waters.

Sample	Determined content of total sugar[g/L]	Declared content of total sugar[g/L]
1	38.9	42.0
2	37.0	42.0
3	39.1	42.0
4	41.1	42.0
5	39.9	42.0
6	38.7	No data
7	36.4	40.0
8	37.4	40.0
9	38.7	40.0
10	38.4	40.0
11	38.9	40.0
12	34.9	38.4

13	36.2	38.0
14	42.8	43.0
15	43.3	42.0
16	44.1	43.0
17	37.2	42.0
18	27.6	31.0
19	30.3	31.0
20	28.9	29.0
21	19.5	20.0
22	29.7	29.0
23	11.2	16.0
24	11.0	16.0
25	38.9	No data

Results showed the highest total sugar content in sample of ZA SYMPHONY with pears (44.1 g/L). The highest contribution in the total sugar content in this flavored water is from sucrose.

DISCUSION

In the last decades, consumption of sugar-sweetened beverages (SSB) generally increased between world populations. These beverages included different types of non alcoholic drinks and different types of flavored, enhanced or vitamin waters. Only few of the sugars occurring in nature are used extensively as sweeteners in these beverages. Besides sucrose, other important sugars like glucose maltose, lactose and fructose are imported to SSB. These saccharides are physiologically interesting. Whether compounds will be successful as a sweetener depends on nutritional, physiological and processing properties, cariogenicity as compared to sucrose, economic impact, and the quality and intensity of the sweet taste. New and functional sugar isomaltulose is compound used in the production of food and beverages with low glicemic index. Commercial isomaltulose is produced from sucrose by enzymatic rearrangement. From intravenous studies it can be assumed that any systemic isomaltulose would be hydrolyzed as well, or excreted in urine. In several subchronic toxicity studies, the administration of large doses (up to 7.0 and 8.1 g/kg body weight/day in male and female rats, respectively) of isomaltulose, did not result in adverse effects (Lina, Jonker and Kozianowski 2002). Unfortunately, physiological effects of isomaltulose on humans are not completely described (Duffey and Popkin, 2008; Holub et al., 2010).

SSB are the main source of added sugars in the food. High consumption of SSB has been linked to unhealthy weight gain and nutrition related chronic disease. (Malik et al., 2006; 2010). Growing evidence supports a mechanistic link between the high sugar, and specifically high fructose, content of SSBs,and the risk of chronic disease and excess weight gain. The unique metabolic and hormonal effects of fructose are linked to a number of adverse short-term effects including enhanced de novo lipogenesis and triglyceride production resulting in dyslipidemia, increased systolic blood pressure, reduced insulin and leptin sensitivity, impaired appetite control and visceral adiposity (Basciano, Federico and Adeli, 2005; Jalal et al., 2010; Stanhope et al., 2009; Teff et al., 2004).

These conditions may contribute to an increased risk of longer term chronic health conditions such as type 2 diabetes, cardiovascular disease, cardio-renal disease, and obesity (Johnson et al., 2007). As common caloric sweeteners such as sucrose, high fructose corn syrup and fruit juice concentrate have relatively high concentrations of fructose they have similar metabolic effects (Malik et al., 2010).

CONCLUSIONS

The results suggest caution in the use of high amount of flavored waters especially for diabetic patients. Used HPLC-RID method could be performed for fast determination of total sugar content and sugar composition in flavored waters.

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Summary/Sažetak

Aromatizirane vode se sastoje uglavnom od vode, prirodnih ili vještačkih aromatizera i šećera ili vještačkih zaslađivača. Cilj ovoga rada je određivanje ukupnog sadržaja šećera u 25 različitih aromatiziranih voda, koje se mogu nabaviti na slovenačkom lokalnom tržištu, korištenjem metode visokotlačne tekućinske hromatografije (HPLC) sa detekcijom refrakcijskog indeksa (RID). HPLC (Agilent 1200 Series) izvedena je izokratskim eluiranjem mobilne faze 15 mM K_2HPO_4 vodene otopine. Degasirani i razrjeđeni uzorci analizirani su na Supelcogel K koloni (300 x 7.8 mm, sa veličinom zrnaca 9 μm), na 80 °C i protokom od 0.5 $mLmin^{-1}$. Ukupni sadržaj šećera u aromatiziranim vodama varirao je od brenda do brenda , od 11.0 g/L do 44.1 g/L. Vrijednosti koncentracije saharoze, glukoze i fruktoze kretao se u rasponima (g/L) : 10.0 – 32.8; 1.3 - 21.7 and 3.0 -37.2, respektivno. Rezultati sugeriraju oprez prilikom upotrebe velikih količina aromatiziranih voda posebno za dijabetičare.

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Technical Article

Interdependencies Between Landfill Operation Procedures and Leachate Treatment

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landfill body

Abstract: Leachate of landfills with organic waste consist a complex composition of different ingredients. Therefore it is not enough to design a leachate treatment plant only on the base of leachate analysis from a selected period. As a consequence, an examination of the expected development of landfill body over next 20 to 30 years must be an important part of the design in order to get an optimum solution of leachate treatment.

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INTRODUCTION

Leachate treatment is one of the most complex tasks in the field of wastewater treatment, and is next to the treatment of landfill gas an important parameter for economical landfill operation. Wastewater of landfills have an extremely variation of components. This has to be kept in mind while designing a new treatment plant or refitting an old one.

Different leachate treatment systems are available and cannot be used universally in the same way in every case. Depending on the contaminant loads and outlet limits, applied technologies have to be varied and adjust. Designer of leachate treatment plants need all information about landfill operation conditions as well as analysis data of leachate to select the best treatment process. In sum, the landfill body and the belonging management is a complex system with interdependencies of type of waste storage, types of waste, local precipitation, the arising leachate, type of leachate catchment, type of landfill gas catchment/treatment etc. Biological leachate treatment systems are one of the most used solutions for landfills in high activity phase with lots of biodegradable organics. Depending on experiences with SBR treatments plants for leachate, this article shows some essential notes that have to be regarded using biological treatment systems. Recently we see more and more large equalization tanks being built at leachate treatment plant inlets in order to reduce the treatment capacity to an economically justifiable extension.

Looking at the aforementioned explanation about the complexity of landfill body following aspects are mainly responsible for the content of leachate:

Precipitation or surface water

- Type of deposited waste and kind of deposition method
- Age of landfill body
- Efficiency of landfill degassing system
- Size of uncovered surface or surface covering solutions
- Recirculated leachate
- Changes in waste composition

As an example, we take a look at the landfill age. As shown in figure 2 the components of a landfill change significantly during a long-term period. Leachate components like COD and especially BOD decrease significantly, nitrogen increases. This has dramatic consequences on biological treatment processes.

Another example is the kind of landfill degassing. Landfill gases consist almost to 100 % of CH_4 and CO_2 (Ehrig, H. J, 1989). If the aerosis is more effective in the landfill, the rate of carbon atoms in the landfill gas decrease and do not get to the leachate.

If a biological treatment is designed and realized regarding common design criteria (Bishof, W. 1998) at a period with fully operation of a landfill big problems will be expected maybe 10 or 15 years later when the landfill is full and must be covered. The biological treatment needs external COD like e.g. acetic acid to reduce nitrogen.

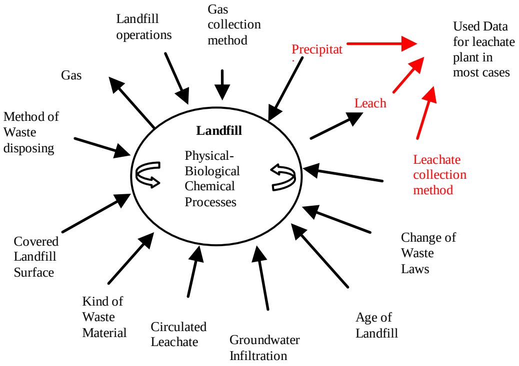

Figure 1: Complex interdependencies in a landfill (Mueller-Czygan G. 2010)

Design guidelines for efficient and sustainable leachate treatment

As shown above, it is not sufficient to use only a spot of leachate composition as sole design criteria. Waste water experts should always coordinate the selection of the appropriate treatment method and the according facility design together with landfill experts and operators. Table 1 is an excerpt of recommended design guidelines for municipal landfills and can be used as a checklist. Mainly it is used to analyze the interdependencies of a landfill and to ask important questions to the landfill operators during design. The additional costs at design doing an evaluation along the shown guidelines are only a small fraction of complete costs that can be saved if an optimum leachate treatment solution will be chosen.

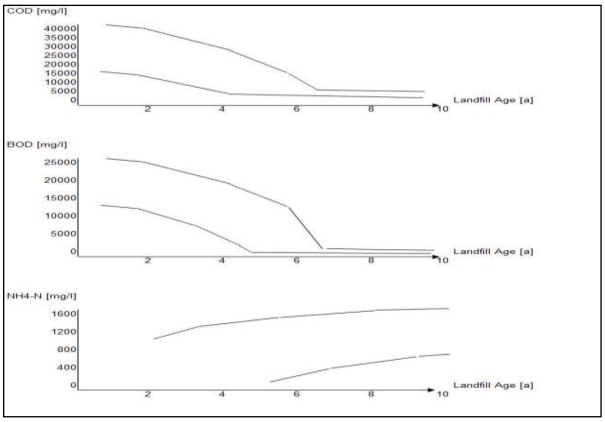

Figure 2: Leachate composition depending on landfill age (Ehrig, H. J.1989)

Available leachate treatment technology

The overview of chosen technologies for leachate treatment is shown. Table 2 is a summary of lecture material from Technical University of Dresden that is available as a download. As each landfill has its own characteristic, universal methods for leachate treatment do not exists. To get a well done solution for each single leachate, it is recommended to spend needed effort for analyzing the influence parameters from landfill body and waste management at landfill even on creating a likely scenario how the landfill body will develop in the future incl. expected influences to leachate characteristics. In lots of cases, solutions with modular systematics are preferred in order to have the possibility adapting the treatment process onto expected changes of conditions in the landfill body. Furthermore, the selection of a suitable control and automation technology like Scada V 10 or the little sister Scada.web supports a maximum flexibility in operation control.

Example SBR-plant of landfill Jakuševac (Croatia)

To improve the existing leachate treatment plant of landfill of Jakuševac (Zagreb, Croatia) the plant was ordered to change the automation system and to renew the dosage system. Several years before, the owner of the landfill built a SBR treatment plant with standard control solution based on common design criteria (see e.g. Chang, L., 1993). The plant does not hold the outlet limits about COD < 700 mg/l. The main problem was that during start of design and end of commissioning of new SBR plant, the NH_4 load inside of landfill body increased significant and COD decreased. As the original control system was not flexible to adjust the SBR cycles in a way adapting the operation steps of nitrification and denitrification onto the change of inflow conditions the performance of SBR was unsatisfying. Furthermore the possibility to add acetic acid was not foreseen that is needed if NH_4 is rising while parallel COD concentration is reducing. After refitting the old SBR plant with control and automation system Scada V10 and new dosage equipment for adding acetic acid within 6 weeks the outlet limits could be hold.

Table 1: Examples for basic conditions that should be considered during design/ redesign/ optimizing of leachate treatment plants

Topic	What questions must be answered?	Possible measures
Precipitation - surface water	Is the flow measured automatically?	It can be derived from this value if the leachate consists mainly of rain water or if other water sources like i.e. groundwater intrusion exist.
	Do prognoses exist for future rain events?	The size of a mixing and buffer tank is directly related to the amount of rain expected.
	Is information how surface water is collected and removed available?	If surface water removal is insufficient, leaks in the surface seal are likely the cause. An immediate repair of such leakages reduces the leachate flow.
	Do alternative storage options to a mixing and buffer tank for extreme rain events exist?	If a strong rain event happens only a few times a year, it might be more economical to reduce the size of the mixing and buffer tank and to truck the peak flows to the municipal WWTP. The use of prognostic models like the Virtual Rain Gauge allows planning the logistics for such measures ahead of time. Alternatively it should be checked if sufficient storage volume might be available inside the landfill. This could provide several days of storage. Further alternatives like add. retention basins, unused but already sealed areas are to be checked. Leachate recirculation might be another useful alternative.
Type of waste and method of installation	Is the composition of the waste going to remain similar for a longtime?	Leachate data at the beginning of the design phase is data of the past. The chosen process technology based on this data is usually suitable only for limited contaminant compositions. Changes in composition often mean process adjustments with significant costs.
	If not, when and how does the composition change?	If it is known from the beginning that significant types of waste are left out in a few years, this can be considered in phased or modular concept. Is for example the entire green waste within 2-3 years excluded, can the biological process split in multiple units that can be used as buffer tanks after the exclusion. Respective flexibility in piping should be added in design.
	Are open installation areas minimized during operation?	Mainly good communications between treatment plant and landfill operator is important here. When the treatment plant runs at or over capacity, should the operator ensure that as little area as possible is uncovered (maybe interim storage of waste necessary)
Age of landfill	In which process phase is the landfill?	At the beginning of a landfill the BOD is relatively high. Later the amount of hard to treat carbons (COD) increases. It can be wise to install a first phase biological plant and to remove the rest COD with mobile activated carbon units. Is the easy to treat carbon content to low should a chemical-oxidative unit (ozone, H_2O or UV) added. The construction of a chemical-oxidative unit at the beginning is usually uneconomical. A high degree of flexibility can be achieved through respective piping design, if all tanks are connected in a way that their function can be modified. It can be economical for smaller landfills to choose some technical components as mobile container solutions. That way new or alternative process phases can be realized by simply exchanging the container unit.
Landfill degassing	How efficient is the current landfill degassing system?	Efficient landfill degassing reduces the biologically easy to treat carbons. This can cause problems when choosing the bio-reactor, if not considered. With sufficient amounts of CH_4 energetic use is desired which can cover part of the energy demand of the leachate plant.
	Is landfill gas routed through leachate pipes?	It has to be checked, if and in what amount landfill gas leaks through the leachate piping system. Respective measures should be taken if this is the case.

Table 2: Overview of the common methods for leachate treatment

Process	Comments	Limits
Physical Methods		
Sedimentation	low cost	suitable only for un-dissolved components
Concentration	concentration of ingredients	costly, corrosion, only partial solution (COD is removed but not destroyed).
Physical-chemical Methods		
Adsorption on activated carbon	suitable for hydrophobic waste water contents	only partial solution, thermal regeneration of carbon necessary
Adsorption on adsorption resins	for chloride hydro-carbons, hydro-carbons, condiments usable	only partial solution, costly
Membrane technology/ reversed osmosis	reversed osmosis, good retention	concentrate might have to be disposed of, coagulation of colloids on the membrane („Fouling“) possible.
Ion exchange	suitable only for special problems (Ions)	Organic matter and colloids interfere
Flocculation and coagulation (with $Ca(OH)_2$ + Fe-salts or $Al_2(SO_4)_3$)	is used often, partial COD elimination, is considered as outdated	disposal of sludge and salts necessary (35 kg/m³ leachate!)
Chemical Methods		
Wet oxidation w/ H_2O_2	No accumulation, elimination of rest COD and AOX contents	not always suitable for direct dischargers (too much salt), high energy requirements
Wet oxidation w/ Ozone/ UV radiation	No accumulation, elimination of rest COD and AOX contents	not always suitable for direct dischargers (too much salt), high energy requirements
Wet oxidation w/ Ozone /packed bed catalyst	high reaction speed	high energy requirement
Bio-chemical Methods		
Anaerobic treatment	no energy required for aeration, no excess sludge	retention of biomass, sensitive, no extensive treatment
Anoxic treatment	Denitrification	prior Nitrification necessary
Aerobic treatment (COD/BOD5-Elimination)	the most common, cheapest method	not efficient for biological hard to treat matter, therefore local effluent requirements not always reachable (more suitable for indirect dischargers)
Nitrification	Oxidation of ammoniac nitrogen via nitrite to nitrate	sensitive method (Inhibition)

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Summary/Sažetak

Procjedne vode deponija sa organskim otpadom sadrže različite komponente složenog sastava. Kako je tijelo deponije biološki reaktor, to se sastav procjednih voda kao i sastav razvijenog plina mijenja sa promjenom pokrova deponije. Stoga nije dovoljno osmisliti postrojenje za tretman otpadnih voda samo na osnovu analize procjednih voda za odabrani period. Pa izrada studije vjerovatnog razvoja deponije za period od 20 do 30 godina treba biti važan dio dizajna za izbor optimalnog rješenja tretmana procjednih voda.

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Anticorrosion Performance of Pco-friendly Paint Coatings

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Abstract: Principal solvent and diluent used in water-soluble paints is water, although they can contain minimum quantities of potentially hazardous organic solvents. Corrosion protection performance of this type of paints is still low compared to the organic solvent-based paints. In this paper, monitoring of dry coating thickness and adhesion, as well as salt chamber and field testing were performed in order to test coating quality. Organic solvent-based paints show superior performance compared to the water soluble paint coatings.

INTRODUCTION

Implementation of environmental legislatives and laws that regulate allowed quantities of organic solvents in waste effluents led to comprehensive modifications of composition of protective coatings. In order to produce safe products, manufacturers were forced to develop new formulations with reduced content of organic solvent, eventually minimizing it to trace levels (Stojanović, Alar, Juraga, 2012). Introduction of so-called eco-friendly coatings have two main goals: to reduce fraction of organic components that can be released in environment and to avoid protection pigments that contain heavy metals. Water-soluble coatings, for which water is the main solvent and diluent, contain minimum quantities of environmentally hazardous organic solvents and heavy metals. Application of these coatings can be beneficial because it provides reduced emissions of hazardous organic substances in the atmosphere, as well as release of heavy metals, especially lead and chromium, into the environment (Rašković, Samardžić, Milošević, 2013).

Beside its potential benefits, improvement of protection performance of water-soluble coatings is subject open for further investigations. Although most coatings of this type provide rather good protection performance, even in very aggressive environments, it is still considerably below organic solvent-based coatings. Also, technology, which is new in industrial applications, should be adapted for ordinary user.

In order to ensure good corrosion protection of steel, one must be familiar with the technology of paint application. Even the most expensive and high quality coatings could be damaged and their performance impaired by incompetent handling before, during and after application.

Application of coating includes following procedures:

1. Preparation of substrate – cleaning and conditioning of substrate surface;
2. Application of coating;
3. Finishing (drying and hardening of coating).

One of the potential consequences of improperly performed process can be reduced protection performance, even if the coating system is properly chosen. Pretreatment of substrate ensures good adhesion of primary layer on the metal, which is important in order to prevent detachment of coating during mechanical stress of the interface. Poor protection performance is often consequence of poor substrate pretreatment.

EXPERIMENTAL PART

Protective paints were coated on steel substrates prepared and provided by company Pretis d.d. One group consisted of phosphatized steel samples while the other one was used without phosphatizing pretreatment. Three coating systems were used (one organic and two water-based, from different manufacturers) in the following ways:

- Volatile organic solvent based coating, applied in two layers – as primary (base) and finishing layer – on both phosphatized and unphosphatized samples;
- Water based coating, also applied in two layers on both types of samples;
- Water based coating, applied in one layer („2 in 1“ system) on both types of samples.

Measurements of film thickness and adhesion, as well as salt chamber and field tests were performed after coating process.

Materials

Substrates used in this work were made from rolled, soft annealed and 100% cleaned steel, according to EN 10204 standard. Steel was coded as Č.1737 VP.

Three coating systems (paints) were used: one based on organic solvent and two based on water. Paints were obtained from two different manufacturers.

First coating system consisted of: 1. quick-drying protection base, formulated from modified alkyd resin and protection pigments, diluted by synthetic solvent; 2. quick-drying protection lacquer with pigments, based on modified alkyd resins, diluted by nitro solvent.

Second coating system was acrylic dispersion base paint with pigments, diluted by water. This paint is designed for protection of iron and steel surfaces in both open and closed spaces and it can be considered as eco-friendly.

Third coating system was paint based on aqueous dispersion of acrylic binders. This protection paint has metallic color and is also used for decorative purposes.

Coating test methods

Measurement of dry coating thickness

Measurement of dry coating thickness was performed by magnetic method with DIGI-DERM 2100 tester, according to EN ISO 2360 standard. Five measurements were performed on every sample.

Dry coating adhesion test

Coating adhesion was tested by cross-hatch test method, according to EN ISO 2409 (ISO 2409:2007). In this test, squared hatch is incised in coated surface using the special knifes, while experimental setup is adjusted according to dry film thickness:

- below 60 μm – knife separation 1 mm
- 60 – 120 μm – knife separation 2 mm

After incision and brushing, transparent duct tape was pressed over the hatch. After removing the tape, fraction of free surface was determined. Depth of incision should be adjusted so that knifes are able to penetrate the film all to the substrate surface.

Salt chamber test

Salt chamber testing is performed in order to simulate the exposure of samples to marine atmosphere. Tests were performed in salt chamber JW-150-NS, in company SurTec Eurosjaj d.o.o. Konjic, according to EN ISO 9227 standard (ISO 9227:2006). Salt chamber used in this investigation as well as experimental setup are shown in Figure 1.

Figure 1. Salt chamber front panel and position of tested samples during the test

Parameters for salt chamber testing are given in table 1.

Table 1. Test parameters

Test parameters	Salt spray test according to EN ISO 9227
Temperature	(35±2) °C
Average collected quantity on horizontal collector of 80 m^2 area	(1,5±0.5) mL/h
NaCl concentration (collected solution)	(50±5) g/L
pH value of collected solution	6,5 do 7,2
Conductivity of distilled water	Max. 20 μS/cm (25±2)°C

Field testing

Field corrosion tests were performed in atmospheric conditions, in order to determine the effects of environment on coating durability and protection performance. Samples have been mounted on inclined (45° from the surface) plate and placed near the main road in Semizovac, from 01.07.2012. to 01.10.2012.

Sample preparation

Total number of thirty 100×50 mm steel samples were prepared for experimental tests. Samples were split in two lines (phosphatized and nonphosphatized) and coated with described paints.

Figure 2. Steel substrate

Figure 3. Substrate surface surface after phosphatizing coating

Air spray coating has been carried out in company Pretis d.d. (Fig. 4. and 5.). Pretreated samples and paints were keept at room temperature prior to the coating process.

Figure 4. Paintshop in company Pretis d.d. (samples ready for painting)

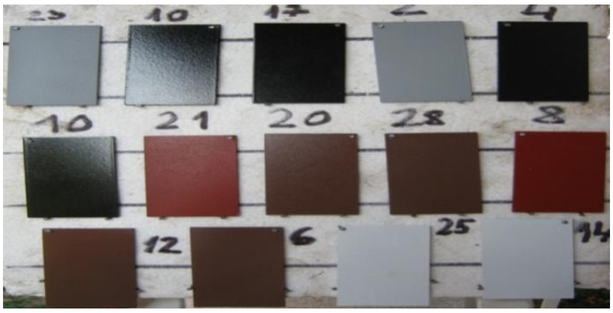


Figure 5. Appearance of samples after painting

RESULTS AND DISCUSSION

Dry coating thickness

Five measurements were performed on every sample. Measured values obtained for organic solvent based coatings are shown in Table 2., while those for water based coatings are presented in Tables 3. and 4.

It is obvious, from the results presented above, that thicker coatings were obtained from organic solvent based paints, compared to those obtained from water based paints.

Table 2. Film thicknesses for organic solvent based coatings

Film thickness / µm							
Phosphatized samples				Nonphosphatized samples			
Sample	Min. thickness	Max. thickness	Average	Sample	Min. thickness	Max. thickness	Average
1(FO)T	45,10	59,00	53,13	1(BFO)T	47,60	52,40	50,23
2(FO)T	48,50	54,10	52,17	2(BFO)T	58,70	75,80	65,20
3(FO)TP	107,40	112,40	110,10	3(BFO)TP	110,00	123,50	117,30
4(FO)TP	110,20	122,00	116,53	4(BFO)TP	115,00	134,00	125,20
5(FO)P	65,40	70,00	67,83	5(BFO)P	40,40	52,00	46,33
6(FO)P	73,50	80,40	76,43	6(BFO)P	48,30	56,90	52,13

Table 3. Film thicknesses for water based coatings

Film thickness / µm							
Phosphatized samples				Nonphosphatized samples			
Sample	Min. thickness	Max. thickness	Average	Sample	Min. thickness	Max. thickness	Average
1(FV1)T	27,00	34,20	30,33	1(BFV1)T	24,90	35,70	29,40
2(FV1)T	30,50	34,70	32,77	2(BFV1)T	25,60	32,30	28,70
3(FV1)TP	54,30	69,00	60,73	3(BFV1)TP	45,80	63,20	56,03
4(FV1)TP	52,80	60,80	56,03	4(BFV1)TP	49,40	65,00	57,96
5(FV1)P	40,50	43,30	42,00	5(BFV1)P	28,90	36,50	32,96
6(FV1)P	41,00	46,30	31,33	6(BFV1)P	35,90	41,40	38,50

Table 4. Film thicknesses for water based coatings, system 2 in 1

Film thickness / µm							
Phosphatized samples				Nonphosphatized samples			
Sample	Min. thickness	Max. thickness	Average	Sample	Min. thickness	Max. thickness	Average
1(FV2)TP	32,30	42,30	36,80	1(BFV2)TP	28,40	35,00	31,30
2(FV2)TP	35,00	40,10	37,70	2(BFV2)TP	25,00	35,80	31,26

Dry coating adhesion

Coating adhesion was tested by cross-hatch test method, according to EN ISO 2409 (ISO 2409:2007). Samples tested by this method were the same as the samples used for field testing. One group of samples was tested immediately after painting and used as a reference, while the other group consisted of samples that were exposed in atmosphere between 14.06.2012. and 24.10.2012.

Samples that were tested immediately after painting are:

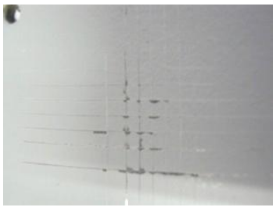
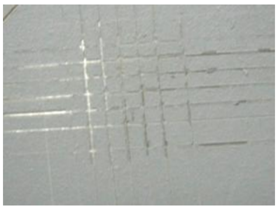
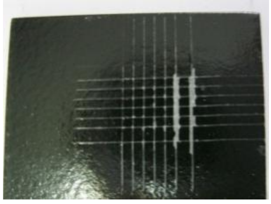
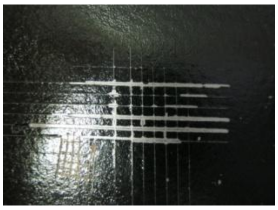
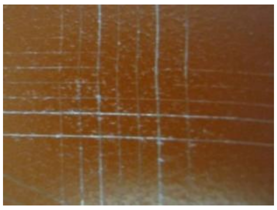
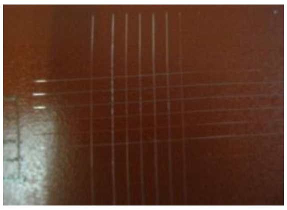
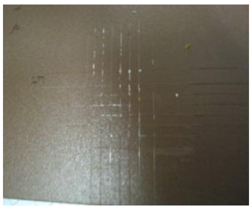
- 19(FO)T; 1(BFO)T
- 30(FO) TP; 3BFO)TP
- 22(FV1)T; 7(BFV1)T
- 18(FV1)TP; 5(BFV1)TP
- 29(FV2)TP; 15(BFV2)TP

Samples that were tested after field testing are:

- 23(FO)T; 2(BFO)T
- 16(FO)TP; 4(BFO)TP
- 21(FV1)T; 8(BFV1)T
- 20(FV1)TP; 6(BFV1)TP
- 25(FV2)TP; 14(BFV2)TP

Experimental results and adhesion ratings are presented in Tables 5. and 6.

Table 5. Adhesion ratings according to EN ISO 2409 standard (samples after painting)

Adhesion testing after coating			
Sample	Sample appearance	Coating type	Adhesion rating
19		- phosphate layer - base layer	2
1		- base layer	2
30		- phosphate layer - base layer - finishing layer	3
2		- base layer - finishing layer	3
22		- phosphate layer - base layer	1
7		- base layer	0
18		- phosphate layer - base layer - finishing layer	0
5		- base layer - finishing layer	1

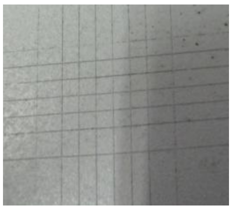
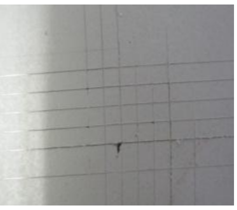
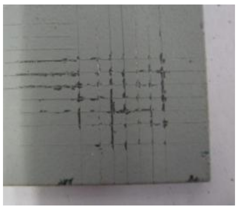
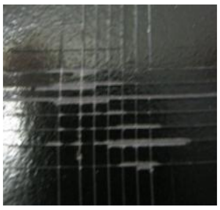
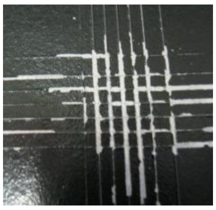
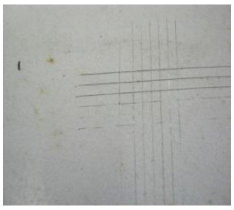
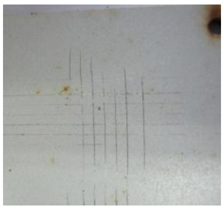
29		- phosphate layer - base layer - finishing layer	0
15		- base layer - finishing layer	1

Table 6. Adhesion ratings according to EN ISO 2409 standard (samples after field testing)

Adhesion testing after field tests			
Sample	Sample appearance	Coating type	Adhesion rating
23		- phosphate layer - base layer	2
2		- base layer	2
16		- phosphate layer - base layer - finishing layer	3
4		- base layer - finishing layer	3
21		- phosphate layer - base layer	1
8		- base layer	1

20		- phosphate layer - base layer - finishing layer	1
6		- base layer - finishing layer	1
25		- phosphate layer - base layer - finishing layer	1
14		- base layer - finishing layer	1

On the basis of obtained results, it can be noticed that water based coatings have, in general, better adhesion than organic solvent based coatings for both, phosphatized and nonphosphatized samples.

Salt chamber

Testing was performed on 12 samples, six of which were phosphatized prior the coating. After coating, incisions were made on six samples in order to increase corrosion rate. Before insertion, thickness of dry coating was determined for all samples. Results of thickness measurement are presented in Tables 7., 8. and 9.

Table 7. Thickness of organic solvent based coating

Film thickness / µm							
Phosphatized samples				Nonphosphatized samples			
Sample	Min. thickness	Max. thickness	Average	Sample	Min. thickness	Max. thickness	Average
1(FO w/o incision)TP	112,60	121,00	116,20	5(BFO w/o incision) TP	115,40	127,20	119,40
8(FO w incision) TP	139,20	152,40	145,73	11(BFO w incision) TP	120,60	138,60	127,87

Table 8. Thickness of water based coating

Film thickness / µm							
Phosphatized samples				Nonphosphatized samples			
Sample	Min. thickness	Max. thickness	Average	Sample	Min. thickness	Max. thickness	Average
22(FV1 w/o incision)TP	46,60	61,20	55,13	12(BFV1 w/o incision)TP	48,90	61,40	56,33
14(FV1 w incision)TP	45,60	67,10	55,53	15(BFV1 w incision)TP	49,60	66,30	56,90

Table 9. Thickness of water based coating system 2 in 1

Film thickness / µm							
Phosphatized samples				Nonphosphatized samples			
Sample	Min. thickness	Max. thickness	Average	Sample	Min. thickness	Max. thickness	Average
26(FV2 w/o incision)TP	23,4	25,8	24,63	20(BFV2 w/o incision)TP	19,1	23,8	21,56
23(FV2 w incision)TP	21,2	24,2	23,06	29(BFV2 w incision)TP	17,2	25,2	22,06

Results of salt chamber testing are shown in Table 10. Duration of testing was 168 hours (from 02.04.2013. to 09.04.2013.), and it was conducted on following samples:

- 1(FO w/o incision); 8(FO w incision)
- 5(BFO w/o incision); 11(BFO w incision)
- 22(FV1 w/o incision); 14(FV1 w incision)
- 12(BFV1 w/o incision); 15(BFV1 w incision)
- 26(FV2 w/o incision); 23(FV2 w incision)
- 29(BFV2 w/o incision); 20(BFV2 w incision)

Samples chosen for testing were put in salt chamber on 02.04.2013. at 13:00.

Table 10. Salt chamber testing results

Salt chamber test			
Sample No.	Sample appearance 03.04.2013. (13:30)	Sample appearance 06.09.2013. (14:00)	Sample appearance 09.04.2013. (14:00)
1			
8			
5			
11			
22			
14			
12			

15			
26			
23			
29			
20			

Organic solvent based coatings possesses the best protection performance, as can be seen from the experimental observations. No corrosion manifestations at all could be seen at samples with organic solvent based coatings, in contrast to samples with water based ones. Also, emergence of bubbles was symptomatic for water based coatings, especially for 2 in 1 systems, where the emergence of bubbles was noticed after 24 hours.

Field testing

Field corrosion tests were performed in atmospheric conditions. Samples have mounted near the main road in Semizovac, from 01.07.2012. to 01.10.2012. Samples selected for field testing were:

- 23(FO)T; 2(BFO) T
- 16 (FO)TP; 4 (BFO)TP
- 21 (FV1)T; 8 (BFV1)T
- 20 (FV1)TP: 6 (BFV1)TP
- 25 (FV2) TP; 14 (BFV2)TP

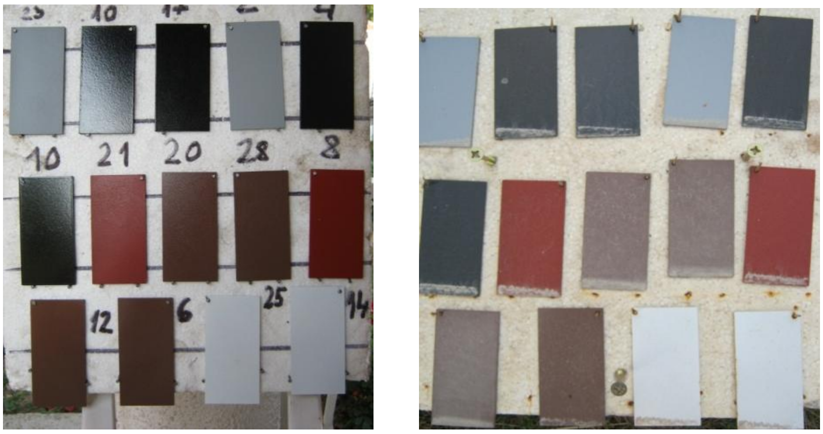


Figure 11. Tested samples at the begining (01.07.2012.) and at the end of testing (01.10.2012.)

Table 12. Results of field testing

Adhesion tests after field testing				
Sample	Coating type	Sample appearance 01.07.2012.	Sample appearance 01.10.2012.	Description of change
23	- phosphate layer - base layer			Emergence of surface corrosion – small spots
2	- base layer			Emergence of surface corrosion – small spots
16	- phosphate layer - base layer - finishing layer			No traces of corrosion
4	- base layer - finishing layer			No traces of corrosion

21	- phosphate layer - base layer			Emergence of surface corrosion – small spots
8	- base layer			Emergence of surface corrosion – small spots
20	- phosphate layer - base layer - finishing layer			No traces of corrosion
6	- base layer - finishing layer			No traces of corrosion
25	- phosphate layer - base layer - finishing layer			Emergence of corrosion on surface and edges – small spots
14	- base layer - finishing layer			Emergence of corrosion on surface and edges – small spots

Samples have been exposed to atmosphere during the period of three months. Corrosion was observed on samples with only a base layer, as well as on samples with 2 in 1 coating. Corrosive changes have not observed on samples with organic solvent based coatings as well as on water based coatings with two layers of paint.

CONCLUSIONS

Comparison and determination of protection performance for two different systems – water based and organic solvent based – was conducted.

On the basis of dry coating thickness determination, adhesion tests, salt chamber and field tests, following conclusions can be drawn:

- Thicker films are obtained for coatings with base and finishing layers;
- Water based coatings show better adhesion, compared to organic solvent based ones;
- Organic solvent based coatings show better performance in salty fog;
- Coatings based on organic solvent also show better performance in field testings.

Although water based coatings show satisfactory protection performance, it is still far bellow the performance of coatings based on organic solvent.

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Summary/Sažetak

Vodorazrjedivi premazi koriste vodu kao otapalo i razređivač, a sadrže minimalan udio organskih otapala koji zagađuju okoliš. Zaštita od korozije koju imaju ove boje još uvijek nije na nivou koji pružaju premazi na bazi otapala. Kvalitet prevlake je praćen mjerenjem debljine suhog premaza, ispitivanjem prionjivosti suhog sloja, ispitivanjem u slanoj komori i kroz terensko korozijsko ispitivanje. Pokazalo se da su debljine premaza na bazi otapala u slučaju kada je nanesen temeljni i završni sloj veće, bolja prionjivost kod vodorazrjedivih premaza, da u uslovima slane magle bolju korozijsku postojanost pokazuju premazi na bazi otapala, da su prilikom terenskog ispitivanja najbolju korozijsku postojanost pokazali premazi na bazi otapala.

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Short Communication

Preliminary Analysis of Heavy Metals Content in the River Bosna Upstream and Downstream from the Industrial Plants in Zenica and Pollution Assessment

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Abstract: This paper presents the results of an analysis of monthly average contents of ten heavy metals in the flow of the river Bosna.. The amount of toxic heavy metals, Pb,As,Cd,Hg and in addition, potentially harmful metals such as Cr, Ni, Zn, Cu, Mn, Fe was determined in order to assess the influence of industry in Zenica on the quality of the river Bosna. Samples were collected during the period of three months: November of 2010, January and February of 2011, during the normal water level of river Bosna. The sampling sites were chosen upstream and downstream from the industrial plants in Zenica with the aim of determining the influence of industrial complexes, mainly ArcelorMittal Zenica, on the quality of the water in river Bosna. The rating of the level of pollution in the river Bosna is based on the comparison of concentrations of heavy metals from the results of the analysis with the highest allowable concentrations of heavy metals by individual classes of water, MDK. The results showed that the industrial waste in Zenica has no significant influence on increase of heavy metals' concentrations in river Bosna above the maximum allowable concentration for waters of I and II classes. The results showed increased mercury content in the river Bosna, not only in the downstream flow from the industrial complex in Zenica but also from the above of mentioned plants.

INTRODUCTION

Serious pollutants of natural waters include heavy metals. Special attention for the protection of the environment should be given to heavy metals which are toxic to living organisms. The following toxic heavy metals are present in natural waters: cadmium, lead, arsenic and mercury. Arsenic is a metalloid that is classified as a toxic heavy metal due to its extreme toxicity. However, the content of heavy metals in natural waters is generally low except iron, due to their low representation in the lithosphere and pedosphere, thus, they are called micronutrients. Water pollution by heavy metals occurs due to intensive industrial development, ecosystems because of their high toxicity and tendency to accumulate. If heavy metals get into the water, accumulate in aquatic organisms, bacteria, hydrophytes, invertebrates and fish, through the food chains, they can get to land plants, animals and humans. The content of heavy metals in river bottom sediments is often used as an indicator of their anthropogenic pollution (Frankowski et al. 2008). If contaminated water is used for irrigation of agricultural land, heavy metals can also accumulate in soil and crop plants through which they can be further involved in a cycle, or in to the food chain, and cause different environmental consequences.

The use of cultivated plants from such soil for human and animal nutrition can threaten the health of people and animals. Some kinds of toxic sediments kill benthic organisms, reducing the food available to larger animals such as fish (Begum1 et al. 2009). The presence of heavy metals in contaminated water bodies may pose a threat to the existing ecosystem of an area as a whole (Nongbri et al. 2012).

Due to the increasing pollution of heavy metals and their toxic effects, many countries in the world have adopted strict regulations regarding allowable values for concentrations of heavy metals in the water used for drinking, fish cultivation and irrigation of agricultural land in order to protect human health. One of the biggest anthropogenic sources of environmental pollution with heavy metals is the metal industry. We should not forget mining, transport, urban waste, etc. (Bikić and Omerović, 2012). Inadequately treated industrial waste water discharged into rivers is a potential danger of pollution of rivers with heavy metals. The atmosphere is an important mean of transporting heavy metals. Soil is often polluted with heavy metals emissions whose origin could be hundreds of miles away. Metals are usually present in the atmosphere in the form of aerosol particles. Aerosols remain in the atmosphere usually for 10 to 30 days from where they deposit into water and soil. They are rapidly diluted and precipitated as insoluble carbonates, sulfates and sulfides at the bottom of the water system. When the adsorption capacity of sediments is exhausted, the concentration of metal ions in water increases (Goletić, 2005). Metals may exist in water as reversibly bound to inorganic anions or to organic compounds as metal complexes, or they may be present as organometallic compounds containing carbon-to-metal bonds (Stanley, 2003).

EXPERIMENTAL

The main objective of the research was to analyze the content of toxic heavy metals, Pb, As, Cd, Hg, and harmful and potentially harmful heavy metals Cr, Ni, Zn, Cu, Mn, Fe in the river Bosna.

Water samples were collected with appropriate access and possible places for sampling water from the river Bosna from following locations:

A. Kamberovića Polje, under the bridge that connects Hotel Dubrovnik, fifty meters above the main wastewater discharges from an industrial zone where ArcelorMittal Zenica is located,
B. Fifty meters under the main wastewater discharges from an industrial zone where ArcelorMittal Zenica is located,
C. Banlozi, under the railway bridge, about 2.5 km from the location of the last discharge of the industrial zone of ArcelorMittal Zenica.

Samples from these locations were collected two meters from the shore and in the depth of half a meter. Water sampling was carried out at 4 locations, during the three months, four times a month. Samples were collected in November of 2010, January and February of 2011, during the periods with normal water levels of the river Bosna. After sampling, the samples were properly stored in the chemical laboratory of the Faculty of Metallurgy and Materials. Water samples were filtered through filter paper "black tape". Then super pure HNO_3 was added to the samples in order to achieve a concentration of 0.2% HNO_3 (Somenath, 2003; PerkinElmer, 1996). For the preparation of calibration curves, standard metals from PerkinElmer producer were used.

Analysis of the samples of examined heavy metals was carried out in the chemical laboratory of the Faculty of Metallurgy and Materials on the device: "Atomic Absorption Spectrophotometer, Perkin Elmer, AAnalyst 800". Analysis of the majority of metals, Pb, As, Cd, Cr, Ni, Cu, Mn, Zn and Fe in part was done by graphite furnace technique. Zinc and iron were analyzed with graphite furnace technique and flame techniques. Mercury was analyzed by Flow Injection Mercury/Hydride technique.

RESULTS AND DISCUSSION

Results of the analysis of heavy metals on defined locations given in Table 1 are calculated as the mean of the concentrations of heavy metals measured in different months.

Relative standard deviation (RSD) was calculated for all data, amounted to a maximum value of 3%. Rating of the level of pollution in the river Bosna is based on the comparison of concentrations of heavy metals from the results of the analysis with the highest allowable concentrations of heavy metals by individual water classes MDK (Uredba o opasnim i štetnim materijama u vodama, 2007; Uredba o klasifikaciji voda i voda obalnog mora Jugoslavije u granicama SR BiH 1990).

Table 1. Metal concentrations in Bosna river (μgL^{-1})

Location	Element	Mean concentrations by month, μgL^{-1}			MRL, μgL^{-1}
		Nov.	Jan.	Feb.	
A	Cr	1,19	1,57	2,42	1-6
B		1,06	1,88	1,92	
C		5,03	3,42	3,07	
D		1,20	2,27	1,88	
A	Ni	1,87	1,04	0,85	15-30
B		1,36	0,90	1,20	
C		0,69	0,30	0,60	
D		0,91	0,88	1,16	
A	Mn	**51,73**	43,18	44,63	50
B		38,63	**53,93**	**62,65**	
C		1,38	1,46	3,38	
D		30,87	40,61	**61,90**	
A	As	2,50	1,02	0,95	50
B		1,72	1,22	1,07	
C		0,18	0,20	0,00	
D		1,51	1,19	1,47	

A	Cd	0,04	**0,65**	0,01	0,5
B		0,10	**0,54**	0,02	
C		0,02	0,02	0,01	
D		0,11	0,04	0,01	
A	Pb	0,74	1,55	0,00	2
B		0,89	1,50	0,00	
C		0,20	0,20	0,00	
D		0,10	0,65	0,00	
A	Hg	**0,48**	**0,67**	**0,57**	0,02
B		**0,19**	**0,35**	**1,40**	
C		0,00	**0,51**	**0,51**	
D		**0,16**	**0,75**	**1,04**	
A	Fe	**159,00**	**133,00**	78,12	100
B		**153,00**	**124,5**	83,61	
C		**144,50**	91,50	98,10	
D		**170,00**	107,00	72,50	
A	Cu	1,33	**10,87**	1,29	2-10
B		0,85	1,08	1,65	
C		0,88	0,29	1,09	
D		1,33	0,96	0,46	
A	Zn	7,50	6,91	2,08	50-80
B		27,00	41,93	34,13	
C		13,5	11,11	0,61	
D		15,00	9,75	1,12	

Comparison of the heavy metal concentrations obtained from the performed analysis with MDK results show that the concentrations of certain heavy metals in certain locations and in certain months were higher than MDK of water I and II class. Specified overruns of concentrations are shown in Table 1 in the bold.

As Table 1 shows, the maximum allowable concentration for water I and II class exceeded in the case of following elements: Mn, Cd, Hg, Cu and Fe. With the exception of mercury, listed overruns of other elements are sporadic and are not as dangerous as it is the case with iron.

Mercury concentrations in all tested locations of the river Bosna exceeded the maximum permissible concentration for water I and II class. The results showed that downstream of the industrial complex in Zenica, specifically downstream of the plant ArcelorMittal Zenica, concentrations of heavy metals were not significantly altered in comparison to concentrations of these heavy metals in upstream from the industrial zone. As conclusion, the most of the measured values are within an allowable limits for water of I and II class.

CONCLUSION

Based on the results, we may draw two general conclusions:

1. Industry in Zenica has no significant influence on increasing concentrations of heavy metals in the Bosna river above the maximum allowable concentration for water of I and II class,
2. Mercury concentrations in all tested locations of the river Bosna exceeded the maximum permissible concentration for water I and II class.

Confirmation of the first conclusion comes out of comparison of the results of analysis of heavy metals in locations A, B, C and D.

Mercury concentrations were increased not only in the downstream flow of the industrial complex in Zenica but also at the upstream location of mentioned plants. Increased levels of mercury are caused by industrial plants upstream from Zenica. As the mercury and its compounds are very dangerous to a human health, the issue of mercury in the river Bosnia should be given a special attention.

Acknowledgement

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Summary/Sažetak

U ovom radu su dati rezultati analiza sadržaja deset teških metala u dijelu toka rijeke Bosne. Analiziran je sadržaj toksičnih teških metala, Pb, As, Cd i Hg, zatim štetnih i potencijalno štetnih teških metala Cr, Ni, Zn, Cu, Mn, Fe u cilju utvrđivanja uticaja industrije u Zenici na kvalitet rijeke Bosne. Uzorci su prikupljani tokom tri mjeseca, u novembru 2010. godine, te u januaru i februaru 2011. godine, pri normalnom vodostaju rijeke Bosne za navedeno doba godine. Za uzorkovanje su izabrane lokacije ispod i iznad industrijskog kompleksa grada Zenice s ciljem utvrđivanja uticaja industrijskog kompleksa, prvenstveno kompanije ArcelorMittal Zenica na kvalitet rijeke Bosne. Ocjena stepena zagađenja rijeke Bosne ispitivanim teškim metalima je vršena na osnovu poređenja koncentracija navedenih teških metala iz rezultata dobivenih analiza s najvećim dozvoljenim koncentracijama teških metala po pojedinim klasama voda, MDK. Rezultati pokazuju da industrija u Zenici ne utiče značajno na povećanje koncentracije ispitivanih teških metala u rijeci Bosni iznad maksimalno dopuštenih koncentracija za vode I i II klase. Rezultati pokazuju povećan sadržaj žive u rijeci Bosni i to ne samo na toku nizvodno od industrijskog kompleksa u Zenici nego i iznad navedenih postrojenja.

Doc. dr. sc. Marija Janković

(1936 – 2013)

S velikom tugom smo primili vijest o smrti naše drage kolegice i prijateljice doc. dr. sc. Marije Janković koja nas je napustila 9. aprila 2013., u 77. godini života. Doc. dr. sc. Marija Janković je na Prirodno-matematičkom fakultetu provela najveći dio svog radnog vijeka. Svojim nastavnim, naučnim i stručnim radom obilježila je Katedru za organsku hemiju i biohemiju, Odsjeka za hemiju, na kojem je radila od 1965. do penzionisanja 2005. Želeći izraziti našu ljubav i poštovanje prema kolegici Mariji Janković, ovim se kratkim tekstom s nekoliko uobičajenih šturih podataka iz njene biografije i po kojim ličnim mišljenjem i osjećajem, opraštamo od nje.

Doc. dr. sc. Marija Janković rođena je u Sarajevu 26. septembra 1936. gdje je završila osnovnu školu i gimnaziju, te upisala Filozofski fakultet, Odsjek za hemiju, na kojem je diplomirala 1960. Nakon završetka studija, zaposlila se kao diplomirani hemičar – analitičar u Analitičkom laboratoriju za kontrolu lijekova Tvornice Bosnalijek u Sarajevu gdje radila dvije godine do prelaska za gimnazijskog profesora na Sokocu. Godine 1965. izabrana je za asistenticu na Katedri za organsku hemiju i biohemiju, Odsjeka za hemiju, Prirodno-matematičkog fakulteta Univerziteta u Sarajevu, gdje je u zvanju docentice i završila svoju nastavnu i naučnu karijeru. Njen pegagoški angažman protezao se i izvan matične kuće, pa je tako pamte i brojne generacije studenata Farmaceutskog fakulteta u Sarajevu kojima je pomagala u usvajanju znanja iz njima važnog predmeta organske hemije. U naučno-nastavno zvanje docentice izabrana je 1986., za naučnu oblast Organska hemija, na predmetu Mehanizmi hemijskih reakcija.

Završila je postdiplomski studij iz *Mehanizama i kinetike hemijskih reakcija*, na Odsjeku za hemiju, te magistrirala 1974. Doktorirala je u oblasti organske hemije 1981. Doc. dr. sc. Marija Janković bila je uključena u nastavnu djelatnost Odsjeka za hemiju i kroz predmet *Istorija hemije* za koji je objavila udžbenik *Historija hemije - Povijest kemije* (1999) i bila mentor pri izradi diplomskih radova budućih srednjoškolskih profesora hemije. Nastavni, naučni i stručni interes doc. dr. sc. Marije Janković kretao se u području organske hemije, odnosno hemije prirodnih proizvoda, što je rezultiralo objavom niza naučnih i stručnih radova u međunarodnim i domaćim časopisima među kojima je bio i ovaj u kojem upravo čitate nekrolog ovoj izuzetnoj ženi.

Nije hemija bila jedino polje njenog interesa. Voljela je književnost, puno čitala i imala je jedinstven dar za pisanje. Bila je dobitnica nagrade za nabolju kratku priču - pripovjetku književno-kulturne manifestacije *Susreti Zija Dizdarević* (2003). Riječima je vladala u svakom smislu, te ne čudi da se ogledala u pjesništvu u kojem je našla utočište i spas od životne nepodnošljive lakoće postojanja. Kao i kod velikih pjesnika, bio je to izraz velike ljubavi, bola i tuge i pobune protiv jedne životne tragedije, nenadoknadivog gubitka kćeri Sandre, studentice medicine. Rado su čitane i citirane njene zbirke poezije *Kao najljepša istina* (1991), *Vječno zeleno* (2003), *Izlet u visine* (2004), kojima je u mnogim pjesmama pružila vidik na punoću ovog svijeta. Sjetu i tugu su zamijenile pjesme radovanja životu koje je potaknulo rođenje njene unučice Natalie na koju je bila tako ponosna i kojoj je posvetila svoje zbirke dječije poezije.

Pored hemije, egzaktne nauke, istraživala je tajne skrivene u rukopisima velikih i običnih ljudi. Kroz učešća u međunarodnim asocijacijama i kao kolumnistica u nekoliko američkih časopisa i domaćem tjedniku, te kao sudski vještak, grafologija joj je bila više od hobija.

Hemija je bila njeno profesionalno opredjeljenje i ljubav. Duboko je cijenila prvog među svojim profesorima hemije koji su je uveli u naučna istraživanja, uvaženog akademika, profesora Mladena Deželića, kojem je posvetila svoju knjigu *Mladen Deželić-Život i djelo* (2004) i nije nevažna činjenica da je prvenstveno njenom inicijativom i zalaganjem Hemijski amfiteatar na Prirodno-matematičkom fakultetu ponio ime ovog „oca hemije u Bosni i Hercegovini“, u novembru prošle godine, a što je bila njena posljednja aktivnost u Odsjeku za hemiju.

Sve nas, koji smo Mariju poznavali i s njom sarađivali, iskreno je rastužila vijest o njezinoj smrti. Nama na Odsjeku za hemiju naša Marija bila je pojam skromnosti, poštenja i pravednosti. Pamtit ćemo je po njenom radu, plemenitosti, dobroti prema studentima, kolegama i saradnicima. Pamtit ćemo je i po razgovorima o kulturnim događanjima u našem gradu, pozorišnim predstavama, izložbama, o književnosti, o modi, o sportu, o *politici. Ostat će u sjećanju kao jednostavna i pristupačna osoba, strpljiva i susretljiva.*

Uz osjećaje dubokog poštovanja, nama bliskim saradnicima ostat će uzor kojeg treba slijediti: kako se sve surovosti života mogu zajedno svladati i živjeti ljepšu stranu. Iza sebe ostavila nam je primjer kako se mogu i trebaju graditi međusobni odnosi, odnosi saradnje, zajedništva, razumijevanja, uvažavanja mišljenja i sugestija drugih, odnosi bez sukoba, rivalstva, egoizma i sve to prenositi na mlade naraštaje vlastitim primjerom.

Bila nam je čast biti njenim studentima, a kasnije i saradnicima. Upoznala sam je davnih sedamdesetih godina kada je započela naša kolegijalna saradnja i prijateljstvo, koje je neprekidno trajalo više od 35 godina. Za nas koji smo je dobro poznavali i koji smo imali čast i sreću da nas je smatrala svojim prijateljima, Marija Janković živi i živjet će u našim sjećanjima.

Draga Marija, hvala Ti što si bila dio našeg života.

Prof. dr. sc. Milka Maksimović

INSTRUCTIONS FOR AUTHORS

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   ^{13}C NMR (125 MHz, $CDCl_3$) d 12.0, 14.4, 23.7, 26.0, 30.2, 32.5, 40.6 (C-3), 47.4 (C-2'), 79.9, 82.1, 120.0 (C-7), 123.7 (C-5), 126.2 (C-4).

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   IR (KBr) n 3236, 2957, 2924, 1666, 1528, 1348, 1097, 743 cm^{-1}.

   Abbreviation: n, wavenumber of maximum absorption peaks in reciprocal centimetres.
5. ***Mass Spectrometry***:

   MS *m/z* (relative intensity): 305 (M^+H, 100), 128 (25).

   HRMS–FAB (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{38}N_4O_6$, 442.2791; found, 442.2782.

   Abbreviations: *m/z*, mass-to-charge ratio; M, molecular weight of the molecule itself; M^+, molecular ion; HRMS, high-resolution mass spectrometry; FAB, fast atom bombardment.
6. ***UV–Visible Spectroscopy***:

   UV (CH_3OH) l_{max} (log e) 220 (3.10), 425 nm (3.26).

   Abbreviations: l_{max}, wavelength of maximum absorption in nanometres; e, extinction coefficient.
7. ***Quantitative analysis***:

   Anal. calcd for $C_{17}H_{24}N_2O_3$: C 67.08, H 7.95, N 9.20. Found: C 66.82, H 7.83, N 9.16. All values are given in percentages.
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