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CONTENT

USING OF CARBOCALK FOR IMPROVEMENT OF SOIL FERTILITY

REVIEW ARTICLE

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ABSTRACT

Carbocalk is a byproduct of sugar factory and is suitable for liming of acid soils. In the autumn of 2000 two field experiments in four replicates were conducted with increasing rates of carbocalk application (0, 15, 30, 45 and 60 t ha^{-1}). In this study, yields of the field crops and cadmium (Cd) concentrations in the leaves and grains were shown. In both experiments maize was grown eight times (four years) and wheat four years in total. The lowest rate of applied carbocalk was adequate for both maize and wheat yield considerable increases by 16% and 28%, respectively (maize 6.92 and 8.04 t/ha; wheat 4.85 t/ha and 6.20 t ha^{-1}). Applying higher quantities of the carbocalk, the yields were less increased. Sunflower, sugar beet, barley and soybean were grown only one growing season and by liming the yields were increased for 48%, 43%, 30% and 8%, respectively. Cd concentrations in the leaves of tobacco were considerably higher (mean 2.4 mg Cd kg^{-1}) in comparison with those of maize (0.10 mg Cd kg^{-1}) and soybean (0.15 mg Cd kg^{-1}), while in grain of wheat and barley they were considerably lower (0.02 mg Cd kg^{-1}). In general, liming considerably affected decreases of Cd uptake by plants as follows: for 45% in tobacco leaves (3.6 and 2.0 mg Cd kg^{-1}, for the control and mean of the carbocalk treatments, respectively, but only in one of two tested years). The corresponding values for the leaves of maize were 0.16 and 0.08 mg Cd kg^{-1} (decrease for 52%) and for soybean 0.50 and 0.06 mg Cd kg^{-1} (decrease for 87%), respectively. In the third experiment, effects of liming with carbocalk were lower, but soil properties considerably improved (for example, base saturation) as well as P and Ca uptake by maize and wheat plants.

Keywords: carbocalk, liming, yields of the field crops, cadmium, the eastern Croatia

INTRODUCTION

Soil acidity is a global factor limiting soil fertility. There is estimation that about 40% of the cultivable land is acid - acid soil in Croatia participating with 1.6 Mill. ha[1]. In Croatia, 831704 ha or 32% of agricultural soil is acid[2]. Excessive soil acidity can be alleviated or neutralized by the addition of different lime materials containing calcium (Ca) and magnesium (Mg) ions in adequate quantities. The first written trace regarding liming in Croatia is the report of an unknown author more than 200 years ago [3, 4]. There are numerous data on more or less useful effects of liming on field crop yields in the eastern Croatia. Ameliorative ploughing up to 100 cm depth of acid soil developed on calcareous substrate about fifty years ago neutralized soil acidity of the surface layer and increased yields of maize and wheat [4, 6]. In the last decade, review [7, 8, 9] and scientific [10, 11, 12] studies on results of the stationary field experiments with liming and phosphorus (P) fertilization in Croatia were published.

Carbocalk is a by-product of sugar factory suitable for liming of soil because it is rich with Ca. At the same time the use of carbocalk resolves the problem of factory waste. For example, two sugar factories in Croatia situated in Osijek and Virovitica produce approximately 50 thousand tonnes of carbocalk every year.

The aim of this study is to review some recent results of carbocalk application effects on yields of field crops with emphasis on cadmium (Cd) concentrations in plants. Cd is well-known as a detrimental trace element, extremely toxic and Cd uptake by plants is considerably decreased with the increase of soil pH [13, 14.]

MATERIALS AND METHODS

The weather, soil management, sampling, yield determination, chemical and statistical analyses

These parts of Materials and methods were elaborated in the cited scientific studies in detail. The plant samples were digested by a wet-ashing procedure by conc. nitric and sulphuric acids and elemental composition was determined by Jobin-Yvon Ultrace 238 ICP-OES spectrometer in the RISSAC Budapest, Hungary.

Carbocalk properties

Carbocalk originating from Osijek Sugar Factory is defined as a soil inorganic conditioner suitable for liming of acid agricultural soils. Quantities of heavy metals in carbocalk are lower than maximal permissible amounts. Average samples of carbocalk were taken for chemical analyses before the distribution for the field experiment I. Based on the results, carbocalk is very rich in calcium (34.4%) and rich in Mg (1.1%), phosphorus (0.5%), aluminum (0.4%), iron and sulfur (0.3%). The heavy metal concentrations are very low (Table 1).

Table 1. Composition of carbocalk

Total concentrations in carbocalk (mg kg^{-1})											
Ca	Mg	P	Al	Fe	S	K	Na	Sr	Mn	Zn	Ba
344300	10945	4653	3620	2770	2660	1390	276	271	152	38.5	27.2
Cu	Cr	B	Ni	Pb	Co	Cd	Mo	Sn	Se	As	Hg
19.4	6.63	6.28	3.12	1.39	0.76	0.28	0.21	0.18	<0.46	<0.33	<0.24
Remaining analysis of carbocalk (%)		$CaCO_3$		CaO		Organic matter		Organic C		Total N	
		76.5		42.8		6.33		3.67		0.38	

The field experiments

Two stationary field experiments were done in the autumn of 2000. Both experiments had equal treatments (four replicates) as follows (air-dried material): 0, 15, 30, 45 and 60 t ha^{-1}. In the third experiment conducted in spring of 2003 liming was combined with NPK-fertilization.

The experiment I: The experiment was conducted close to Pleternica (Pozega-Slavonian County). Gross of the basic plot was 64.3m^2. The results originating from this experiment were published in the original studies[15, 16, 17, 18, 19, 20].

The experiment II: The experiment was conducted in Sopje (Virovitica-Podravina County). Carbocalk was distributed in four plots each 350 m^2 of area. At an early stage of crop, each plot was divided in four sub-plots representing four replicates[21, 22, 23, 24, 25, 26].

The experiment III: The field experiment of liming with carbocalk (0, 10 and 20 t ha^{-1}) combined with 0, standard and doubled NPK-fertilization was applied (three replicates, basic plot 70 m^2) in Donji Miholjac (Osijek-Baranja County) dystric luvisol[27, 28, 29, 30].

RESULTS AND DISCUSSION

Liming by carbocalk considerably increased soil pH (Tables 2 and 4). However, in the agricultural practice it is adequate to increase soil pH by liming at above 5.0 for soil improvement, while application of the higher rates of lime that increases soil pH close to neutral is unreasonable because of economic reasons. Our data also suggest the lower lime rates as adequate for yield increases (Tables 3 and 4).

Table 2. Effects of liming on soil pH (the surface 0-30 cm depth)

Soil Ph	Liming with carbocalk in 2000 (t ha^{-1})						LSD	
	0	15	30	45	60	90	5%	1%
	The experiment I [16]							
pH (H_2O) pH (1nKCl)	5.33 3.89	5.81 4.71	6.52 5.80	7.12 6.62	7.35 6.95	7.72 7.30	0.25 0.32	0.34 0.44
	The experiment II [25]							
pH (H_2O) pH (1nKCl)	4.52 3.42	6.34 5.65	7.12 6.41	7.39 6.65	7.81 6.87		0.49 0.49	0.71 0.71

In both experiments the application of the lowest rate of lime in amount of 15 t ha^{-1} resulted in a considerable increase of maize yield (4-year averages) by 16% compared to the control (the exp. I: 7.62 and 6.56 t ha^{-1}; the exp. II: 8.45 and 7.29 t ha^{-1}). With the additional rates of carbocalk, the yield was increased compared to the lowest rate only by 7% and 9%, respectively (Table 3). Considerable effects of the factor „year“ with respect of intensity of liming impacts were found. We presume that the weather characteristics are a main responsible factor for these differences. In general, the lower yields of maize are in connection with the lower precipitation and the higher air-temperatures compared to usual ones[31], especially in two summer months July and August. Our data (Table 3) are in agreement with this general opinion. For example, under drought stress in 2003 maize yield in the experiment II was 5.51 t ha^{-1} or 50% lower than under normal conditions in 2002 (10.96 t ha^{-1}).

Wheat was grown for three years in the experiment I. Depending on the year, the effects of liming on yield increases were from 30% to 44%. As in maize, the highest effects were found by application of the lowest carbocalk rate.

Soybean, barley sunflower and sugar beet were grown only one growing season in the experiments I and II. Sunflower, barley and sugar beet yields were increased by liming by 48%, 30%, and 43% respectively. Soybean response to liming was relatively low because yield was increased only by 8% (Table 3).

The response of tested field crop to liming in the experiment III was lower than in the experiments I and II. Maize yields were significantly increased by 8%, wheat 17%, while differences of soybean yields were insignificant. By liming, however, soil properties considerably improved regarding base saturation and increasing P and Ca uptake by plants (Table 4), and these phenomena could have the long-term effects on improvement of soil properties and yields of field crops.

In general, liming considerably affected decreases of Cd concentrations in leaves of tested plants. Cd in tobacco leaves is especially dangerous for human health [32]. Cd and other toxic substances (for example, pesticide residues) transform into a gaseous form during smoking under conditions of temperatures between 835 and 884 °C [33]. Therefore, tobacco smoke is harmful to both smokers and nonsmokers. An average smoker inhales 30 µg Cd daily by cigarette smoking[34]. According to the FAO/WHO criteria[35] tolerable daily intake is 1 µg Cd for every kg of body weight.

Table 3. Liming effects on yields of field crops

The field		Carbocalk (t ha^{-1})					LSD		Carbocalk (t ha^{-1})					LSD
Crop	Year	0	15	30	45	60	5%	Year	0	15	30	45	60	5%
	Grain yield (t ha^{-1}): The experiment I [15,16,17,18,20]							Grain yield (t ha^{-1}): The experiment II [25, 26]						
Maize	2001	5.04	6.19	6.39	6.72	7.56	1.05	2001	7.36	10.2	10.3	11.2	10.3	0.77
Maize	2002	5.51	6.66	6.77	6.82	7.50	0.92	2002	9.89	10.5	10.7	11.2	12.5	0.77
Sunflower	2003	2.72	3.77	4.04	3.46	3.31	0.41							
Barley	2004	5.79	7.50	7.29	6.82	7.05	0.76							
Maize	2006	7.28	7.68	7.88	8.26	8.06	0.56	2003	4.42	5.14	5.81	6.63	5.53	0.77
Wheat	2007	5.04	6.77	7.03	7.25	6.66	0.34	2007	3.84	4.62	4.99	4.52	3.80	0.51
Wheat	2009	5.04	6.96	7.09	7.14	7.07	0.66							
Maize	2010	8.40	9.94	9.69	9.73	9.58	0.89	2010	8.40	9.94	9.69	9.73	9.58	ns
Soybean	2011	2.13	2.09	2.09	2.19	2.30	0.11							
Wheat	2012	5.49	6.46	6.80	6.58	7.30	0.45							
Sugar beet (yield of root: the experiment II)								2004	30.0		43.0		34.0	6.6

Table 4. Impacts of liming (the experiment III) on yields and soil properties [27, 28, 29]

Liming with carbocalk (t ha^{-1}) in spring of 2003 impacts on yields and soil properties*									
The crop	Year	Carbocalk (t ha^{-1})					Carbocalk (t ha^{-1})		
		0	10	20			0	10	20
		Grain yield (t ha^{-1})			Soil status (3-year averages)				
Maize	2004	8.7a	8.7a	9.4a	pH $_{KCl}$		4.20a	5.26b	6.00c
Wheat	2005	4.7a	4.9ab	5.5b	Hy (cmol kg^{-1})		4.33a	2.64b	0.79c
Soybean	2006	3.4a	3.6a	3.9a	Base saturation (%)		53.5a	70.0b	90.8c
Phosphorus (P) and calcium (Ca) removal from soil by straw and grain yields (kg ha^{-1})									
Maize	P	17.5a	31.5b	32.2b	Wheat	P	9.0a	25.6b	28.6b
	Ca	22.3a	28.3b	29.8b		Ca	5.9a	19.3b	21.3b

*differences between values with the same letters are not significant at the 1% level

Table 5. Liming effects on cadmium concentrations in plants (the experiment I)

The crop	Year	Carbocalk application in 2000 (t ha^{-1})					LSD	Grain-cadmium (mg Cd kg^{-1})
		0	15	30	45	60	5%	
		Leaf-cadmium concentrations (mg Cd kg^{-1})						
Barley [36]	2004							below detection limit: < 0.02
Tobacco [19]	2005	3.6	2.2	2.1	1.9	1.9	0.4	
Tobacco [36]	2008	2.7	2.2	2.0	2.7	2.7	Ns	
Maize [36]	2006	0.15	0.07	0.06	0.05	0.05	0.01	below detection limit: < 0.02
Maize [36]	2010	0.19	0.10	0.10	0.11	0.09	0.06	below detection limit: < 0.02
Soybean [20]	2011	0.50	0.12	0.08	0.02	0.03	0.07	

In our study, liming considerably affected the decreases of Cd concentrations in tobacco leaves, but only in the growing season 2005, while leaf-Cd in maize and soybean were decreased by liming in all tested years. Leaf-Cd in tobacco leaves were considerably higher (mean 2.4 mg Cd kg^{-1}) than in maize leaves (0.10 mg Cd kg^{-1}) and soybeans (0.15 mg Cd kg^{1}). Also, grain-Cd in maize was below detection limit (Table 5). In the other study[37], considerable effects of liming on decreases of Cd in alfalfa hay by 58% were found (0.113 and 0.047 mg Cd/kg, for 0 and 20 t ha^{-1} of lime, respectively). Therefore, beside effects on yields of field crops, liming is useful soil management practice for decreasing Cd transfer into the food chain (plant-animal-human population).

CONCLUSIONS

Carbocalk has been indicated as a useful waste product of sugar industry for improvement of acid soils and yields of field crops in crop rotation. Decreases of Cd concentrations in plants have been found as additional useful effects on food quality. Application of carbocalk in amount of 15 t ha^{-1} is adequate for practical using in soil management.

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INTERACTION OF WATER/SEDMINET AS A FACTOR OF LEAD DISTRIBUTION IN THE WATERS OF NERETVA

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

During the last few decades of the 20-th century, the water from the researched area of the river Neretva, from Konjic to Mostar, had been exposed to different sorts of harmful pollutants that originated from production/industrial complexes which are located on the shores of the river Nerevta. By the intensification of these production capacities, as well as many agricultural and other activities, the degradation of the quality of water of the river Neretva may occur. A particular problem is the fact that the waters of the river Neretva have been held in the existing accumulation lakes for a long period of time (Jablaničko, Grabovica, Mostar), which makes the process of eutrophication easier and faster. The aim of this research is to establish if there is a connection between the content of the lead in the water and the content of metal in the river sediment in the form of adsorption isotherms of the Freundlich type, through the data on the distribution of lead and its sorption balance. The research covers eight sites from which the samples were collected, five of which are on the main watercourse and three from the tributaries of the river Neretva. The metal content data in water and especially the way it binds in the sediment as well as the possibility of its mobilisation, transport and accumulation is important for grading the quality of water and for understanding the chemism which takes place in the water system. According to the obtained results the following comment can be given: the results of the research show a low level of lead in the water and river sediments of the river Neretva and the measured values of metals were within the prescribed limits and meet the Directive of the EU Water Framework.

Key words: interaction, distribution of metal, water, river sediment, Neretva.

INTRODUCTION

Water is one of the most important natural resources. Daily activities of modern humans increase the risky contaminations of water, air and soil. Water and soil have always had a great impact on the development of civilization, because they are highly related to food production. The Neretva, with its tributary, represents a life source for the population of Hercegovina, hence the monitoring of its quality is necessary in order to sustain this natural resource as drinking water, for irrigation, industry, recreation and other uses. One of the measures of protection and conservation of this watercourse is permanent monitoring of all parameters in the water, especially dangerous and harmful substances. Planned researches will be performed through a series of procedures where the level of lead distribution will be determined in the water and river sediment of the river Neretva along the watercourse from Konjic, Jablanica to Mostar. The results will be used for calculations of the sorption equilibrium between metal concentrations in water and sediment, in order to determine a detailed ecochemical characterisation of this part of the Neretva watercourse.

The main aim of this research was to establish a connection between the content of lead in the water and the content of metal in the river sediment in terms of adsorption isotherms of the Freundlich type. The content of metal in water, and especially the way of its binding in the sediment and the possibility of mobilisation, transport and accumulation is crucial for understanding the chemism which takes place in the water system.

To accomplish this, following tasks were defined:

- To determine the locations of study, in the examined area of the river Neretva,
- To determine the content of lead in the water and river sediment along the watercourse of the Neretva,
- To statistically analyse the results (correlation and regression analysis)
- To establish whether there is a connection between the content of lead in the water and the content of lead in the zone of easy movable water sediments, by calculating the interaction between water/sediment.

MATERIAL AND METHODES

a) Sources of contamination in the studied region of the river Neretva

During the last few decades the watercourse of the Neretva was exposed to harmful influences of different materials, especially from factories which were part of the war industry complex „Igman“ in Konjic, and different industries in Konjic, Jablanica and the north valley of Mostar. Since these factories are of metal processing character they were the heavy metal contaminants which originate from waste waters which are made in different technological processes, especially from waste waters which are made during galvanization.

Waste waters from these industries have abundantly flown into the waters of the Neretva and they could have had direct influence on the quality of water of this river, as well as had an effect on the chemism which takes place in the water system and the pedogenetic processes.

b) Locations of study

Respecting the standards of EU for validity of experimental data, the locations of study were chosen from which the water samples were collected. On the examined territory 8 locations were chosen for this study. Looking downstream, from Spiljanski bridge near Konjic until the north entrance of the Neretva into Mostar. The order of the locations for sample collection was following:

1. Neretva (100 m above Spiljanski bridge)
2. Trešanica (estuary in the city of Konjic)
3. Neretva (near the motel Konjic)
4. Neretvica (estuary near Butrović land)
5. HE- Geabovica (300-400 m, beneath the dam)
6. Drežanka (50 m, above the bridge in D. Drežnici)
7. HE- Salakovac (200-300 m, beneath the dam)
8. HE- Mostar (300-400 m, beneath the dam)

c) Sampling water and river sediment for the determination of the content of lead

From each researched region a composite sample was collected from eight individual samples. The samples were collected from the left and right shore and the middle of the watercourse, in ratio 3:3:3, according to the scheme shown in Figure 1. Collection of individual samples of sediment was done with a nonmetal scissor catcher type Veenhapper, from the surface layer 0-15 cm, in amounts of 2 – 3 kg.

After collecting individual samples and throwing away the larger materials (stones, branches), composite samples were made and packed into plastic bags, labelled and transported to the laboratory [10].

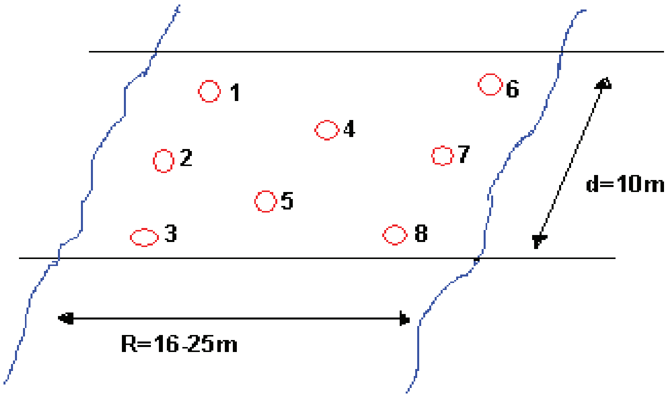

Figure 1. Schematic representation of the distribution of locations where the water samples were collected on the watercourse of the Neretva

d) Determining the content of metal in the water

The content of lead in the water was determined from acid water solutions [11] on the instrument SHIMADZU, type AA-6200, technique FAAS in the laboratories of Herkon d.o.o. – Mostar, a company for quality and quantity control. The standards used for quality control with certificate were: HC 781141 for metal (Pb). The used standard is from the manufacturer Merck.

Table 1. The condition of AAS instruments for individual metal determination

METAL	Wave length *(nm)*	Width of the slit *(nm)*	Characteristic concentration *(mg/l)*	DETECTION LIMIT *(µg/l)*
Pb	217.0	0.5	0.58	0.01-100

RESULTS

Results of the AAS analysis for all samples are shown in table 2 and represent the average values from three determinations. The precision of the results is shown through standard deviation ±. Also, there is data given in Table 3 for the amount of lead in dissolved HCl- fraction in the river sediment of Neretva, and the amount of lead in $MgCl_2$ fraction in the river sediments of Neretva is presented in Table 4.

Table 2. Amounts of lead (Pb) in the waters of Neretva

	Location of sampling							
	1	2	3	4	5	6	7	8
Content of metal (Pb) $\mu g/dm^3$	1.74 ±0.84	5.61 ±1.46	69.82 ±6.32	65.45 ±4.79	75.30 ±4.72	28.06 ±2.82	72.40 ±4.52	79.73 ±5.12

Based on the obtained values of lead concentration, it can be noticed that the highest concentrations of lead are present in the water downstream of the dam. The results of the study show a low lead concentration in the waters of the Neretva and that these measured values are within the prescribed limitations and meet the Framework Water Directives.

Table 3. Amounts of lead in dissolved HCl- fraction in the river sediment of Neretva

	Location of sampling							
	1	2	3	4	5	6	7	8
Content of metal (Pb) *mg/kg*	27.4	19.0	18.8	20.2	47.3	24.2	40.2	51.2

The „dissolved“ fraction of metal is represented in the HCl extract, which was isolated from the river sediment through 2M HCl solution treatment. By observing the content of metal in the HCl fraction of the river sediment of the Neretva, it can be concluded that the lead, as in water, is differently distributed. The maximum concentration of lead 51.2 mg/kg was registered in the lower part of the Neretva watercourse on the position 8. The minimal concentration of lead 18.8 mg/kg was registered in the upper watercourse of the Neretva.

Table 4. Amounts of lead in $MgCl_2$ fraction in the river sediments of Neretva

	Location of sampling							
	1	2	3	4	5	6	7	8
Content of metal(Pb) *mg/kg*	2.7	2.5	2.2	2.3	7.4	2.9	6.8	8.6

The “easy movable” fraction of metal, which is represented by $MgCl_2$ – extract, was isolated from the river sediment with 2M $MgCl_2$ solution. By observing the results in Table 4 it is obvious that the concentration of adsorbed metal in the river sediment is different from one location to the other. The highest concentration of adsorbed lead of 8.6 mg/kg was registered in location 8.

DISCUSSION

Looking at the distribution of dots on the correlation diagram Pb_s – Pb_v (Figure 2.2), within the limits of the existing population of samples, two different groups can be seen: one covers the samples 6, 3, 2, 8 which are represented in the form of the correlation curve equation $(Pb_s)=2.879$ ($Pb_v)=5.415$, with a correlation coefficient of 0.8245 (P>95%), and the other covers the samples 1, 4, 5, 7, which are represented in the form of the correlation curve equation $(Pb_s)=3.468$ $(Pb_v)=-1.390$ with a correlation coefficient of r=0.99301 (P>95%). A high correlation coefficient value represents a significant indication that the sorption equilibrium of lead can be expected in the examined water medium.

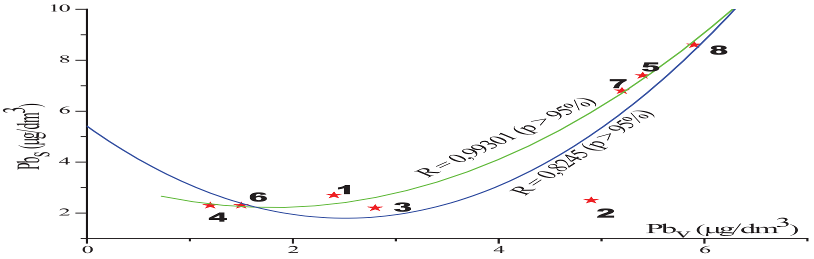

Figure 2. Correlation diagram Pb_s – Pb_v in the Neretva watercourse

The Freundlich adsorption isotherm (Figure 3), which covers the examined samples along the watercourse is determined by a regression curve equation $\log(Pb_s)=0.738\ \log(Pb_v)0.198$, where $K_F= 1.576$ *mg/kg* and (n)= 0.738. Distribution of the dots, in regards of the regression curve, show that the affinity of the sorption sediment, and thereby the intensity of lead adsorption, is significantly different in different locations. That is especially outlined when the distribution of lead is observed through the appropriate geological segments. Thus, on the diagram there is a partial adsorption isotherm that shows the relation between the changeable and water fraction of lead. The constructed adsorption isotherm is defined by the equation $\log(Pb_s)=0.883\ \log(Pb_v)0.174$, and has the value $K_F=1.493$ *mg/kg* and (n)=0.883.

As seen from the diagram, the dependence between the lead content in the fractions which are statistically significant(r=0.99301), exists only in samples 5, 7, 8 which have, as the K_F sorption affinity of sediment to lead shows a higher affinity of sediment in the dots (1, 2, 3). The samples (5, 7, 8) are located downstream from the damp on the Neretva and represent the most significant source of lead in the sediment.

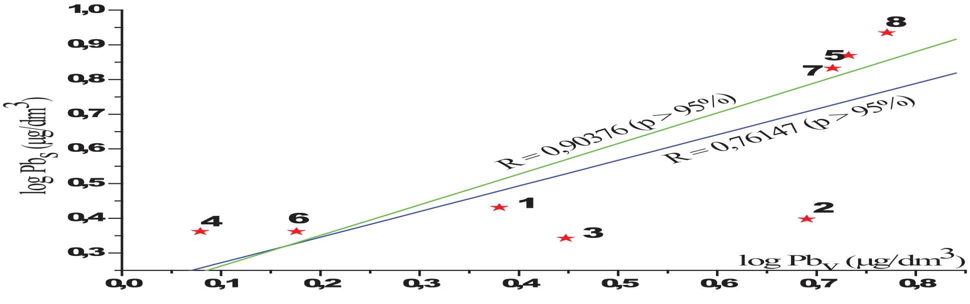

Figure 3. Adsorption isotherm of lead in the Neretva.

CONCLUSIONS

Based on the gathered analyzed data the following can be concluded:

1. The differences that appear in the lead content in the water and river sediment are specificity of different locations. Those specificities are in most part conditioned by different geological consistence of the surrounding rocks, or different consistency of river sediments in the Neretva and its tributary as well as the possible anthropogenic sources of metal (proximity of highway M-17, as well as many landfills with suspected composition)
2. The sorption-changing content of the „easy movable“ fraction of the river sediment of the Neretva and its tributary varies from one location to the other, and is within the limits: Pb (2.2-8.6 *mg/kg*).
3. Oscillations in relation between the sorptionly dependent metal and the level of metal in the HCl fraction are a consequence of different mineral composition of the surrounding rocks, the difference in chemical and granulometric sediment composition, as well as the different effect of erosion at specific locations.
4. By determining the relation between metal Pb contents in water and the content of sorptive dependent metal (Pb) by adsorption isotherms, it is concluded that for the examined metal in the Neretva there is the equilibrium of the Freundlich type.
5. The existence of the sorption equilibrium of metal (Pb) represents the proof that the sorption of metal ions on the sediment of the Neretva is the dominating

mechanism for its distribution in water medium. That also represents a high indication of undisturbed that is natural state of the waters of the Neretva.

With this study, through the interaction of water/ sediment as a factor, we determined the distribution and level of lead in the water and river sediment of the river Neretva, which could have gotten into the water as a consequence of many years of work of industries in Konjic and Jablanica. This study represents a significant contribution to the better understanding of the interaction of the water/ sediment system, which together with all the other studies should allow the prevention of heavy metal pollution and promote adequate monitoring for better perseverance of the watercourse of the Neretva.

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IDENTIFICATION OF ACACIA HONEY BY FLUORESCENCE SPECTROSCOPY

PRELIMINARY COMMUNICATION

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ABSTRACT

As several analytical methods have to be used together for a reliable authentication of the botanical origin of honey, such work is time consuming and costly. Thus, there is a real need for new methods that allow a rapid and reproducible authentication of the botanical origin of honey at low cost. Compared to spectroscopic techniques based on absorption, fluorescence spectroscopy offers a 100-1000-fold higher sensitivity and it was used to investigate authentication of the botanical origin of honey.

Excitation spectra of acacia, meadow and honeydew honeys were scanned from 220 to 400 nm with the emission measured at 420 nm.

All honey types showed significant fluorescence maxima of intensity between 220 nm and 257 nm. Samples of meadow honey had higher intensity of fluorescence when compared to acacia honey (3-fold), while honeydew honey showed different intensities of fluorescence.

Acacia honey exhibits second maximum between 325 nm and 385 nm while honeydew and meadow honeys did not show significant fluorescence intensity in this range.

Emission spectra at 420 nm with an excitation from 220 to 400 nm on Spectrofluorimeter may be used for the identification of acacia honey.

KEYWORDS: **honey**, botanical origin, authenticity, fluorescence spectroscopy, acacia honey

INTRODUCTION

As several analytical methods have to be used together for a reliable authentication of the botanical origin of honey, such work is time consuming and costly. Most of the numerous new analytical techniques proposed during the last decades for the authentication of the botanical origin of honey, have not been tested in this respect. This may also explain why none of them is being routinely used in honey analytics[1].

Thus, there is a real need for new methods that allow a rapid and reproducible authentication of the botanical origin of honey at low cost[2,3]. Compared to spectroscopic techniques based on absorption, fluorescence spectroscopy offers a 100-1000-fold higher sensitivity and it was used to investigate authentication of the botanical origin of honey[4,5].

The major challenge in the authentication of the botanical origin is to distinguish the unifloral honeys from the polyfloral ones. However, the unifloral honeys account only for a minor proportion of the honeys produced; the majority of the honeys on the market contain important proportions of nectar or honeydew from different sources and are therefore considered as polyfloral honeys[6]. In this work the potential of fluorescent spectroscopy for the identification of pure unifloral honey has been shown.

MATERIALS AND METHODS

The study included 73 different samples of natural honey: 11 acacia, 25 meadow, 9 honeydew, 7 forest-meadow, 4 lime, 2 heather, 1 sunflower and 14 mixed honeys. All samples were collected from individual producers from Bosnia and Herzegovina.

In this article fluorescence spectra obtained for acacia honey were presented. All samples were analized in duplicate. Analysis of electric conductivity showed that all analysed samples had the values in range for acacia honey according to Bogdanov *et.al.*[9] and requirements of the EU Honey Directives (Councile Directive 2001/110/EC). Also, all acacia samples had the content of proline above the 180 mg/kg, which is the minimum value for genuine honey[10].

Fluorescence spectra of honey samples were recorded at spectrofluorimeter RF-5301 PC (Shimadzu, Japan). Fluorescence emission at 420 nm was carried out after the excitation scan between 220 nm and 400 nm. Fluorescence emission spectra from 290 nm to 650 nm were recorded after excitation of the honey samples at 220 nm. The entrance and exit slits for the excitation light-beam were both 1.5 nm.

Honey samples that were used for recording fluorescence spectra were prepared in the following manner: 20 g honey samples were incubated 8 h in a water bath at 40° C, cooled to room temperature and then used for recording fluorescence spectra in 1 cm quartz cell, without dilution and filtering.

RESULTS AND DISCUSSION

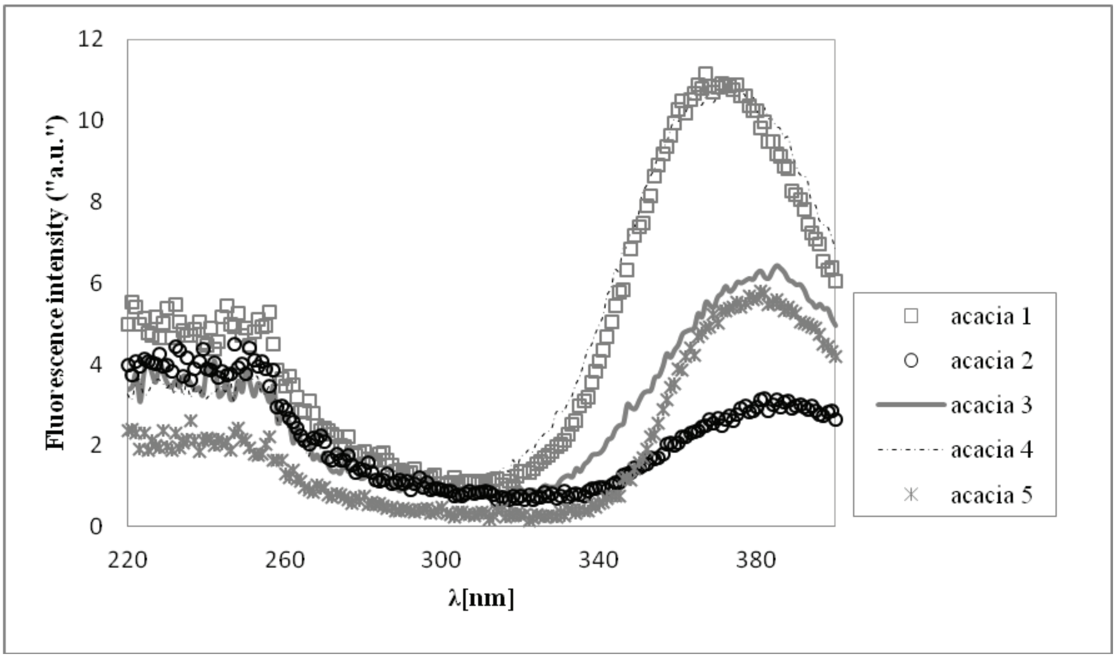

Figure 1. Fluorescence emission of five different acacia honey samples at 420 nm after excitation from 220 nm to 400 nm in 1 cm cell at 25 °C.

Fluorescence emission of five different acacia honey samples at 420 nm after excitation from 220 nm to 400 nm in 1 cm cell at 25 °C is presented at Figure 1. Acacia 2D spectra showed two maxima, between 220 nm and 257 nm and between 325 nm and 385 nm of excitation, respectively.

Fluorescence emission of acacia, meadow and honeydew honeys at 420 nm after excitation from 220 nm to 400 nm are presented at Figure 2. All honey types showed significant fluorescence maxima of intensity between 220 nm and 257 nm. In this range, samples of meadow honey had the highest intensity of fluorescence. Only acacia honey had the fluorescence maxima between 325 nm and 385 nm of excitation.

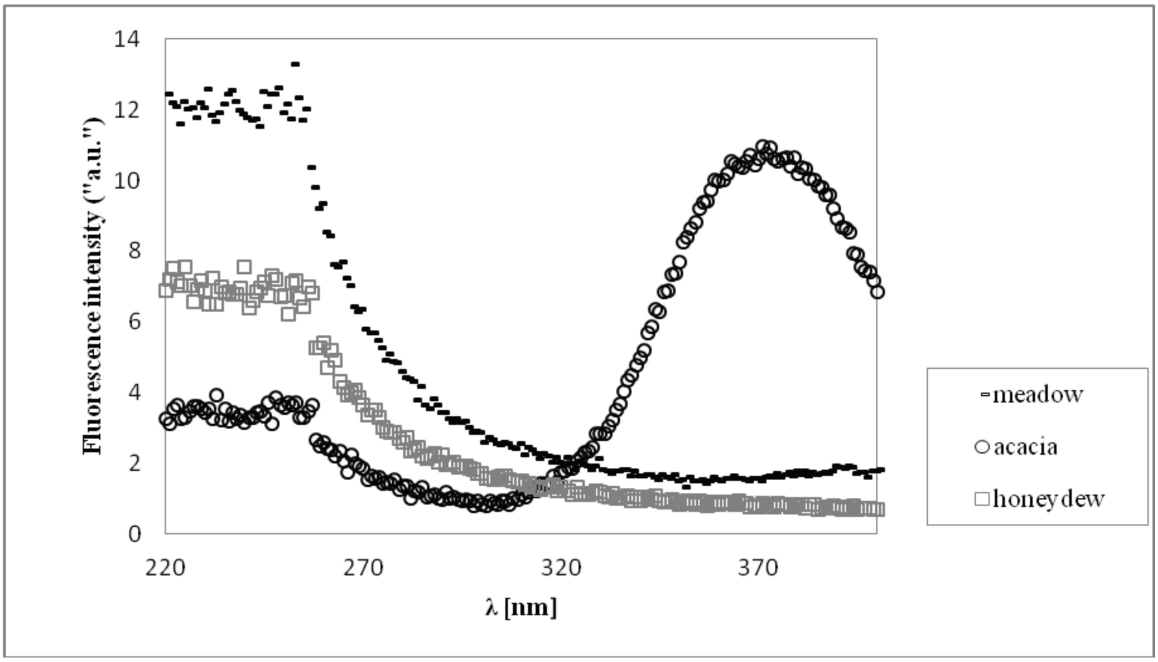

Figure 2. Fluorescence emission of acacia, medow and honeydew honeys at 420 nm after excitation from 220 nm to 400 nm in 1 cm cell at 25 °C.

This fluorescence maximum is the characteristic of acacia honey and represent the simple and fast method for a reliable identification of botanical origin of this type of honey.

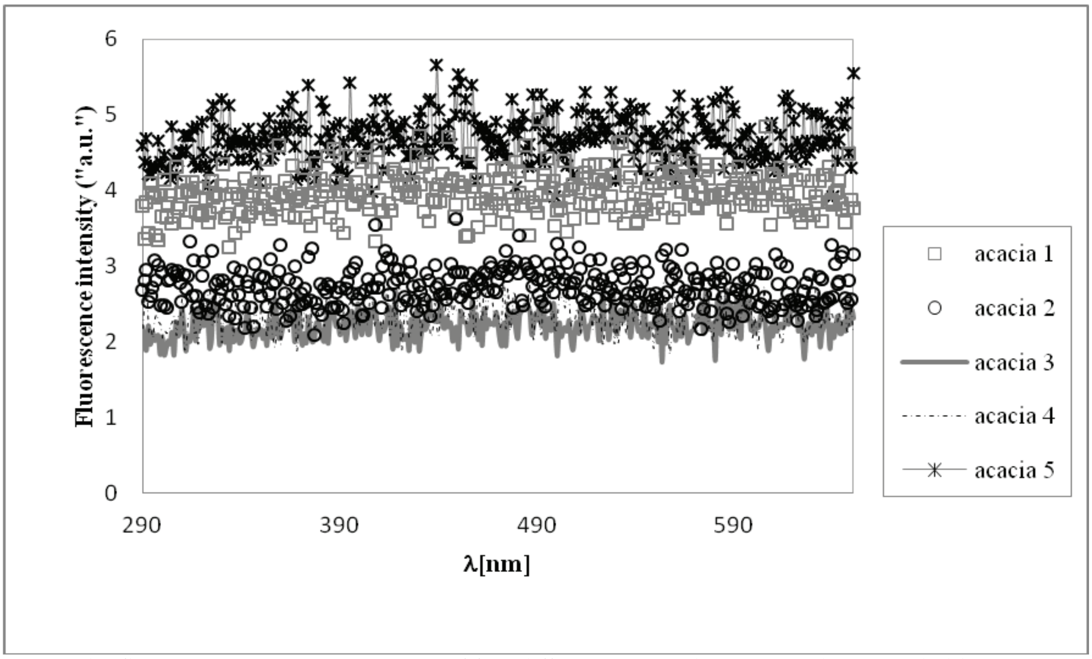

Figure 3. The fluorescence emission spectra of five different acacia honey samples recorded between 290 nm and 650 nm with excitation wavelength at 220 nm in 1 cm cell at 25°C.

Fluorescence emission spectra of five different acacia honey samples were scanned from 290 to 650 nm with the excitation wavelength at 220 nm (Figure 3).

The results of different acacia honeys emission spectra did not show any maxima, between 290 nm and 650 nm.

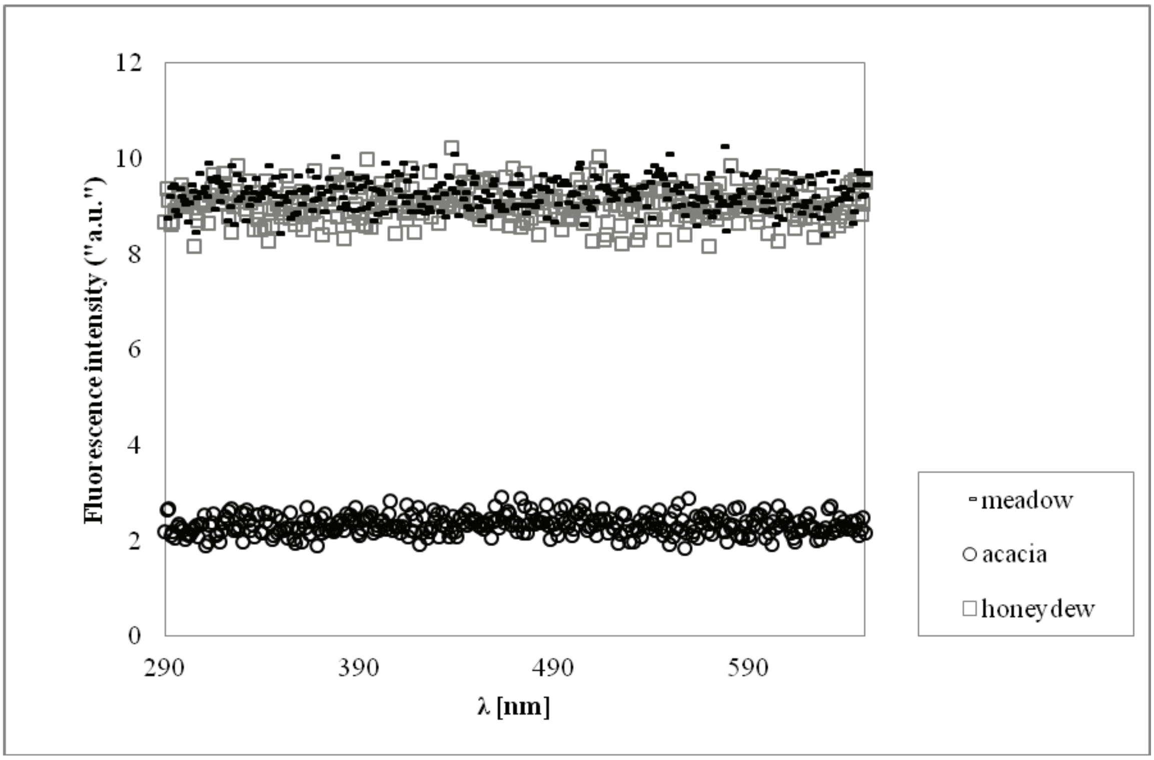

Figure 4. The fluorescence emission spectra of different honey types recorded between 290 nm and 650 nm with excitation wavelength at 220 nm in 1 cm cell at 25°C.

When excited at 220 nm, the emission spectra of acacia, honeydew and meadow honey did not show any peak of fluorescence intensity (Figure 4). The fluorescence intensity of emission spectra of acacia is significantly lower when compared to meadow and honeydew spectra (Figure 4).

Honey contains numerous components which act as fluorophores, like aminoacids, peptides, proteins, vitamines and polyphenols[7,8]. Some of them have been already suggested as a markers for some unifloral types of honey. Chemical nature of components which contribute to fluorescence of acacia honey, obtained in our results, is not identified for now. This will be the subject of our further investigation.

CONCLUSION

Our results showed that acacia honey showed characteristic fluorescence spectra in two different application modes of fluorescence spectroscopy: fluorescence emission at 420 nm after excitation from 220 nm to 400 nm and fluorescence emission spectra between 290 nm and 650 nm with excitation wavelength at 220 nm. So, this method and this particular combination of excitation and emission wavelengths may be useful technique in identification of acacia honey.

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MONITORING OF RADIOACTIVITY IN NATIONAL PARK UNA BY ANALYSIS OF SOME BIOINDICATOR SPECIES

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

Monitoring the levels of radionuclides in the environment in some areas is extremely important, not only for insight into the pollution state of the environment with radioactive substances, but also for radiation safety of the human population in a certain area.

In this paper we sought to examine contaminationof the National Park „Una“, located on the territory of the Municipality of Bihac, Una-Sana Canton and covers an area of 19 800 ha, by examinating the contamination of bioindicators.

In 15 samples of mosses, ferns and nettles, which were collected at 5 sites in the National Park „Una“, levels of activity ^{137}Cs were determined. Radiationload for the examined specieswas obtained calculating thedose rate, whichprovided insight into thecontamination of area with radioactiveisotopes.

^{137}Cs content in plant samples at all five locations, ranged from 0.37 ± 0.10 Bq/kg to 64.60 ± 3.30 Bq/kg. Considering the literature data, it can be concluded that measured values are not significant, and measured values could not affect the radiation safety of human population in this area.

Keywords: national, park, ^{137}Cs, radionuclide, bioindicators

INTRODUCTION

The main sources of artificially created radionuclide contamination are nuclear weapons tests in the atmosphere, executed in fifties and sixties,duringthe last century[1]. Use of nuclear energy for peaceful purposes, can also lead to the contamination of the environment by radioactive substances. Evidences of that are Chernobyl accident in April 1986 and Fukushima accident in March 2011. It is estimated that Chernobyl accident released around $7x10^{17}$ Bq radioactivity into the atmosphere [2].

For analysis of radioactive contamination of the environment, besides monitoring of natural radionuclide ^{40}K, monitoring of artificial radionuclides, fission products ^{90}Sr and ^{137}Cs, is very important. Due to their physico-chemical properties, they are highly toxic and contribute to the irradiation[3]. Disintegration of ^{137}Cs leads to emission of β and γ-rays, which is why it is easier to establish his presence, unlike ^{90}Sr, which is a pure β emitter. Both isotopes have a relatively long half-life period, ^{90}Sr twenty-nine and ^{137}Cs thirty years.

Nuclear accidents lead to radioactive contamination of the soil in the larger areas. Because of its chemical similarity to Ca and K, Sr and Cs can easily reach the biosphere. Radionuclidesin plants are incorporated by foliar deposition and resorption by roots. Through plants, as a part of food chains, radionuclides are being transfered in the bodies of animals and humans.In recent years, numerous studies have been conducted on the input of ^{137}Cs in plants. Also, various theories about the parameters that affect the deposition are disclosed. Intake of ^{137}Cs in plants is approximately proportional to the content of dissolved ^{137}Cs in the soil per volume unit, which is also approximately proportional to the moisture content in the soil[4]. Many researchers suggest that Cs as analog K, enters the plants[5,6]. Cs^+ and K^+ are competitively ions. It is known that the abundance of K in the soil can block entry of Cs in plants[7].

Numerous studies have shown that the mosses can accumulate high dose of radioactivity. They are one of the most important collectorsof radionuclides from the air[8]. Although they are not a significant link in the food chain, they are bioindicator of radioactiveprecipitations[9].

Mosses have the ability to retain a large part of the wet radioactive deposition.Considering they do not have roots and make the accumulation on passive way, do not have epidermis or cuticle, mosses have a great ability to absorb, and great surface/volume ratio relationship [10]. It was found that the primary binding site of ^{137}Cs, at cellular level, are moss membranes. From 26.1% to 43.1% of the initial Cs is accumulated in them[11]. Considering the prevalence and ease of sampling, mosses provide a comprehensive insight into the contamination of the tested area.

Ferns are interesting radioecological objects, since they are sensitive to the radioactive contamination[12]. There are not many studies on the transfer of Cs in ferns, although it was found that this plant accumulates radionuclides abundantly[13]. Fern accumulates Cs effectively compared to grass and mosses. It can be used to clean the soil, since the radionuclides accumulate more in stalk than in root[14].

Given the prevalence and ease of sampling,nettle is also used as a bioindicator of radioactive contamination of the environment. However, compared to the mosses and ferns, there are a smaller number of studies, on the content of ^{137}Cs in the nettle[15,16,17].

The study was conducted in the National Park "Una", located in the northwest of Bosnia and Herzegovina, in the Municipality of Bihac. Geographically, the National Park „Una“ belongs to Una-Korana plateau. National park includes parts of the valleys of the rivers Una and Unac and orographic slopesof mountains Plješevica, Grmeč and Osječenica.

The aim of this study was to provide insight into the area of National Park „Una“ state of contamination, by determination of it´s content in samples of mosses, ferns and nettles.

MATERIALS AND METHODS

The content of ^{137}Cs in fifteen samples of mosses, ferns and nettles (Fig. 1), is determined.

Fig. 1: a) moss (Polytrichum sp. L.), b) fern (Aspidium filix-masL.), c) nettle (Urtica dioica L.)

Sampling was carried out in the National Park "Una", at five different locations randomly (Fig. 2), during the September 2012. Nettles and ferns samples are cut 4-5 cm above the soil, while the mosses samples collected from the surface. The mass of each sample was 0.7 kg.

Fig. 2: Locations of sampling of biological material in the National Park „Una“

Samples were cleaned from the ground and dried at room temperature. The content of water is determined by drying at 105°C. Samples were then homogenized.

Radionuclide activity was measured at the high-resolution HPGe gamma detector, manufactured by ORTEC (diameter of 55.9 mm, length of 73.3 mm, the relative efficiency of 30%), with an integrated electronic system and a program for processing spectra, analysis and evaluation of results measurement GAMMA VISION. Time counts ranged from 21.600 seconds. Measurement uncertainty is expressed as a combined standard uncertainty by Eurachem / reader Guide 2000, with the factor 1.

All analyzes were performed at the Department of Public Health, Center for Protection from Radiation, Sarajevo.

RESULTS AND DISCUSSION

The results of measurement are given in Table 1. Results clearly show the existence of two groups of biological material, due to the significant difference in the content of ^{137}Cs.

Table 1. The results of gamma spectrometric analysis of ^{137}Cs in samples of mosses, ferns and nettles at locations in the National Park "Una"

Location	**Type of sample**	**^{137}Cs**	
		Activity (Bq/kg)	**Limit of detection (LOD) (Bq/kg)**
Ćukovi	moss	64,60±3,30	0,40
	fern	7,85±0.45	0,27
	nettle	0,53±0,16	0,37
Orašac	moss	21,98±1,15	0,38
	fern	Below LOD	0,46
	nettle	0,37±0,10	0,27
Kulen-Vakuf; Ostrovica	moss	60,16±3,07	0,38
	fern	Below LOD	0,57
	nettle	Below LOD	0,30
Kulen-Vakuf; Gečet	moss	7,85±0,48	0,42
	fern	Below LOD	0,46
	nettle	Below LOD	0,28
Martin Brod	moss	15,45±0,86	0,42
	fern	Below LOD	0,31
	nettle	Below LOD	0,32

In all tested locations, largest value of ^{137}Cs is present in samples of mosses, followed by ferns and nettles, with the smallest values of ^{137}Cs activity.

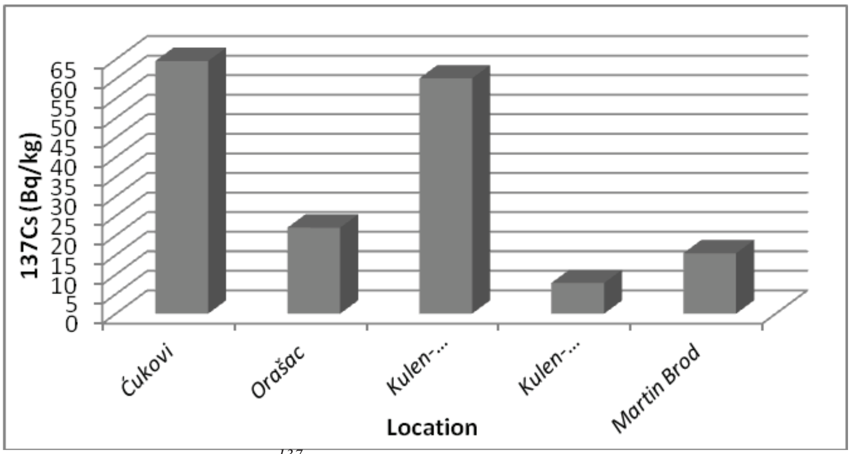

Fig. 3:^{137}Cs activity in samples of mosses

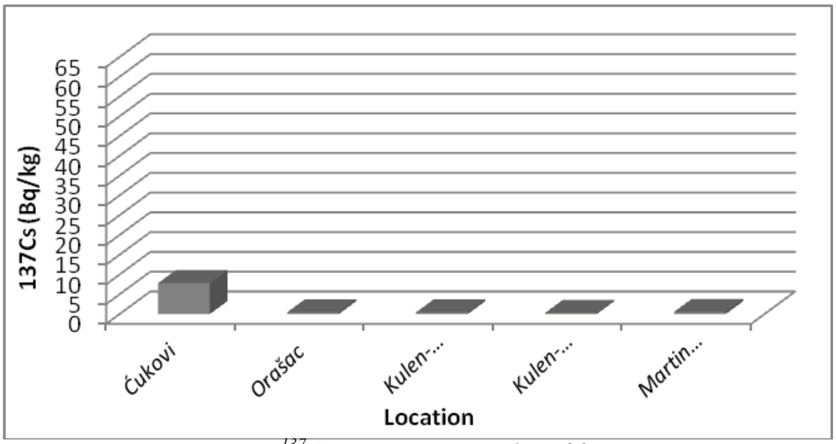

Fig. 4:^{137}Cs activity in samples of ferns

Content of ^{137}Cs in samples of mosses, is shown in Figure 3.Highest activities ^{137}Cs in samples of mosses,were measured at location Ćukovi, where ^{137}Cs content was 64.60 ± 3.30 Bq/kg, and at location Kulen-Vakuf; Ostrovica,with a value of 60.16 ± 3.07 Bq/kg. The lowest activity of radionuclides in mosses was determined at location Kulen-Vakuf; Gečet, with a value of 7.85 ± 0.48 Bq / kg. The values of ^{137}Cs in samples of mosses in eastern Salzburg (Austria), from May 2006ranged from 2863 ± 26 Bq/kg,on a dry matter basis[18].

^{137}Cs activity in samples of ferns is low,and significantly lower than values obtained for samples of mosses (Fig. 4). The activity of radionuclides occurs, as well as in samples of mosses, at location Ćukovi, with 7.85 ± 0:45 Bq/kg. At other locations ^{137}Cs activity is below the lower limit of detection.

Research in Belarus, where they analyzed samples of ferns at two locations, have shown that the activity of ^{137}Cs was 175 ± 0,13 kBq/kg, on a dry matter basis[19]. ^{137}Cs activity in samples of ferns, which are measured in Poland 1987., 1993., and 2001., showing a trend of decreasing activity of ^{137}Cs in samples.In samples from the same location, samples from the 1987. showed ^{137}Cs activity from 15900 ± 234 Bq/kg, and six years later, only 870 ± 32 Bq/kg. Decreasing trend in radionuclide activity was confirmed also in 2001., when the determined activity of ^{137}Cs was 8 ± 1 Bq/kg.

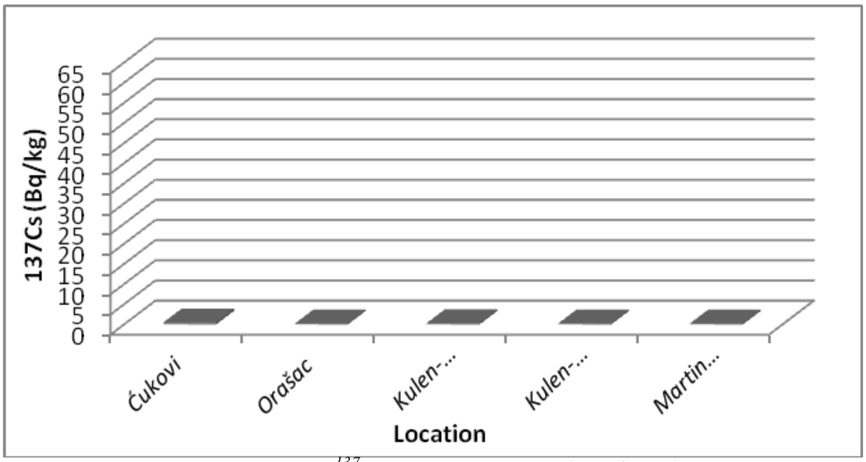

Fig. 5:^{137}Cs activity in samples of nettles

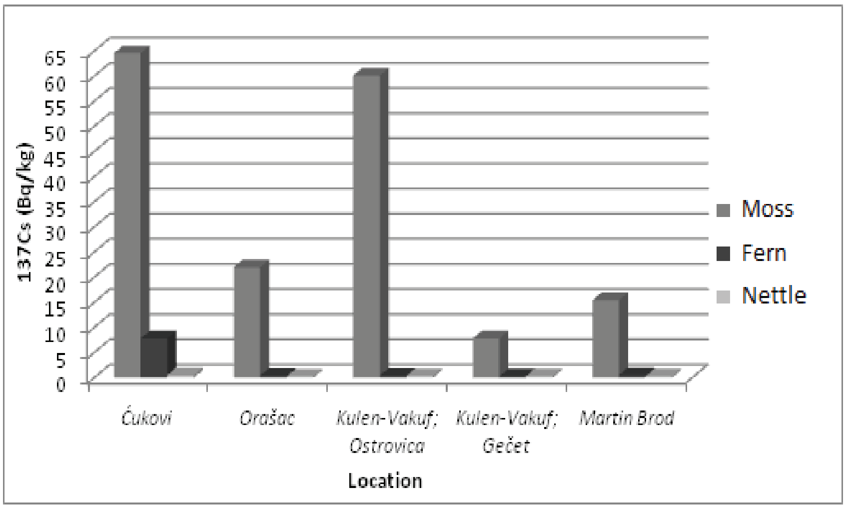

Fig. 6: Activity of ^{137}Cs in all samples

Graphical representation of ^{137}Cs (Fig. 5) shows a very low content of radionuclides in samples of nettles. The values are below the lower limit of detection. Slightly higher ^{137}Cs activity occurs only at the locations Ćukovi (0.53 ± 0.16 Bq/kg) and Orašac (0.37 ± 0.10 Bq/kg).

There are a small number of studies with nettle, as a bioindicator of radioactive contamination. Results of the research of ^{137}Cs activity in samples of nettles in the East Ural, amounted to 960 Bq/kg, in the overhead phytomasse[15]. Also, research on the transfer of radionuclides from soil to plants, in the radioactive area of the central Ural, Russia, is determined content of ^{137}Cs in surface samples of nettles. At a distance of 5 and 90 km from the source of contamination (nuclear power "Beloyarskaja") determined activity of ^{137}Cs was 960 ± 120 Bq/kg and 2.7 ± 1.4 Bq/kg[17].

CONCLUSION

All National Park „Una“ locations, where the ^{137}Cs content in samples of mosses, ferns and nettles was determined showed presence of this radionuclide. Maximum activity of ^{137}Cs was measured at location Ćukovi, in the sample of moss. Given the content of ^{137}Cs following locations are: Kulen-Vakuf; Ostrovica, Orašac, Martin Brod, Kulen-Vakuf; Gečet.As bioindicator, highest content of ^{137}Cs showed samples of mosses, and smaller samples of ferns and nettles. Possible reason for that,is the fact that for the analysis are used only above-ground parts of this plants. ^{137}Cs content in plant samples, at all five locations, ranged from 0.37 ± 0.10 Bq/kg to 64.60 ± 3.30 Bq/kg. Considering the literature data, we can conclude that a significant values are not found, values which could affect the radiation safety of human population in this area. However, these results can serve, as a basis, for monitoring and further investigations regarding the radiation safety of the population in the area of the National Park "Una".

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EFFECTS OF PROCESSING HYDROCRACKED BASE OILS BY ACTIVATED BENTONITE

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

At processes of production of base oils, different adsorbents are used in order to improve colour, stability and removal of undesirable compounds. Effects of processing by adsorbents largely depend on their characteristics, such as: specific surface area, pore volume, particle size, chemical composition, etc. These characteristics can be improved by processes of chemical activation. In local refineries, imported commercial clays are used for the processing of base oils. Natural bentonites from Šipovo area, Republic of Srpska, due to their structure have good adsorption characteristics, however, in order to be applied in treatment of base oils they should be activated by acid or alkaline process. This paper investigated the influence of bentonite activation by sulfuric acid, on adsorption characteristics of bentonite, and the effects achieved by processing of hydrocracked base oils and a commercial active clay. The results of investigation obtained by using different methods (XRD, DTA, DTG, TG, BET) show that activated bentonite has very good adsorption characteristics. Also, characterization of refined base oils activated by bentonite has shown that achieved effects are similar to the effects of processing by active alumina. However, it is important to note that usage of activated bentonite for processing of oils, leads to a greater reduction of polycyclic aromatic hydrocarbons (PCA) in oils, than in the case of commercial clays usage.

Keywords: process of adsorption, hydrocracked base oils, activated bentonite, polycyclic aromatic hydrocarbons

INTRODUCTION

The process of adsorption is based on surface interaction of individual solid materials and components of gases or liquids. Depending on the nature of relationships established between adsorbents and components which they adsorb, adsorption can be chemical, or physical. At chemical adsorption, the adsorbate binds to the adsorbent by chemical bonds of covalent, ionic or coordination type. Physical adsorption occurs as a result of binding the adsorbate on adsorbent through Van der Waals forces. The process of adsorption is quantitatively described by adsorption isotherms that give the relationship between the amount of adsorbed adsorbate per unit mass of adsorbent and the equilibrium concentration of adsorbate, or the equilibrium partial pressure when adsorption is performed from the gas mixture. Experimentally, it is established that there are five basic types of adsorption isotherms and their usage can provide basic information on type and structure of the adsorbent, adsorbate and concentration of the adsorbate, the heat effect of adsorption and other physico-chemical characteristics. There are numerous equations of adsorption isotherms that are used to describe adsorption, and some of them are Lagmuir, Freundlich, BET etc.

In oil industry, adsorption is widely used at many processes of separation and refining. At processes of refining of vacuum distillates of petroleum, where different types of lubricants, insulating and various special-purpose oils are obtained, processing with adsorbents is used as an additional method to achieve adequate quality. Also, processes of adsorption are applied in regeneration of used lubricating oil. Organic compounds are present in mineral base oils, their presence may be desirable or undesirable and they can be classified into four groups: n-paraffins and iso-paraffins, naphthenes, aromatics and hetero compounds. Desirable components are isoparaffins and monocyclic compounds containing naphtenic or aromatic ring. Undesirable components are n-paraffins of high molecular weight, due to poor low temperature properties, then poly-condensed aromatic hydrocarbons, due to their low volatility oxidation, colour and low viscosity index values[1]. Also, some polycondensed aromatic structures show carcinogenic properties[2]. In production of mineral base oils, processes of adsorption can be run with a contact procedure of finely ground adsorbents and with a procedure of percolation through a stagnant layer of granulated adsorbent. In these processes there is no adsorption of one component, but a group of components, and there is a monitoring of alteration of some characteristic size of the system, which is proportional to the composition and can be expressed as the amount of adsorption. At industrial adsorption processes, silica gel, activated alumina, zeolites, activated carbon, active clays and other types of synthetic and modified adsorbents are used. The effectiveness of adsorbents depends on particle size, porosity and specific surface area[3], and can be improved by different activation processes. To apply natural adsorbents (bentonite, bauxite) as adsorbents, they must be activated by acid and alkaline procedure, to improve their adsorption properties[4]. During the activation of bentonite with HCl and H_2SO_4 concentration of 10% m/m and 15% m/m respectively, it has been found that there is a higher specific surface area and pore size achieved, but this effect is more pronounced with using of H_2SO_4[5]. Although hydrocracked base oils (HC) have significantly better properties than the base oils obtained by conventional processes, it is necessary to process them with activated clay, in order to achieve better colour and oxidative stability[6].

MATERIALS AND METHODS

This paper presents the part of experiments conducted during the study of adsorption characteristics of domestic, natural adsorbents (bentonite and bauxite) and other materials (activated alumina, colloidal silica, precipitated silica and activated carbon) for the adsorption treatment of hydrocracked and solvent neutral base oils, as possible alternative to imported active clay[7,8].

Materials

- pattern of hydrocracked base oil from a domestic producer (HU),
- natural bentonite from the location of Šipovo, Bosnia and Herzegovina (B),
- commercial active clay „AMCOL“ (AG).

Methods

- activation of natural bentonite with sulfuric acid,
- processing of initial hc base oil with activated bentonite and commercial clay,
- characterization of natural and activated bentonite,
- characterization of initial and refined patterns of hc base oil.

Activation of natural bentonite with sulphuric acid

For activation of natural bentonite with sulfuric acid in laboratory conditions, the most favourable parameters were selected: sulfuric acid (20% m/m); ratio sulfuric acid: bentonite = 1:5; activation temperature 95°C ± 1°C; activation time 3 hours.

A certain amount of ground, natural bentonite was weighed, then it is poured in a glass with a previously prepared solution of 20% sulfuric acid and intensively stirred for 3 hours at the set temperature. After the activation was completed, filtration was done on Büchner's funnel and then activated bentonite was rinsed with water until the pH value changed from 1-2 to 4-5. After filtering and washing, the activated bentonite was dried for 2 hours at a temperature of 110 °C. Dried bentonite was milled in the mill and sifted through a sieve of mesh, sizes of 160 μm and 100 μm.

Processing of base oil by adsorbents

Processing of initial oil with activated bentonite (AB) and active clay (AG) was done through contact procedure and following parameters: temperature of refining 110±1°C; contact time 30 min; proportion of adsorbent: 0,5% w/w; 0,75% w/w; 1,0% w/w; 1,5% w/w; 2,0% w/w; 3,0% w/w.

A certain amount of base oil sample was poured into a 500 mL glass cup. Then, a previously weighed mass of adsorbent was added. The required amount of adsorbent was calculated on the basis of given relations between oil and adsorbent. The glass with the sample was placed in a water bath with a thermostat, with intensive mixing at a temperature of 110°C±1°C.

After completion of the refining process, the filtering of the sample of refined base oil was done on the apparatus for vacuum filtration. By this way, treatment of base oil was carried out with activated bentonite and active clay, i.e. 12 samples of refined base oils were obtained.

Characterization of natural and activated bentonite

In order to determine the effects of acid activation on the adsorption characteristics of bentonite, characterization was done before and after the activation. The characterization of adsorbents was carried out using different methods to determine:

- chemical composition,
- present phases (XRD),
- qualitative and quantitative structure (DTA, DTG, TG),
- specific surface area (BET method).

Characterization of the initial base oil and refined samples

In order to study effects of refining by activated bentonite and commercial clay, a determination of typical characteristics of initial oil and samples of refined base oil, the structural composition and polycyclic aromatic hydrocarbons (PCA) and oxidation stability were done. These tests were done in accredited laboratories, according to standardized methods.

RESULTS AND DISCUSSION

To determine the chemical composition of natural and activated bentonite, standardized methods were used: gravimetric method for determination of SiO_2, volumetric method for determination of Fe_2O_3, potentiometric method for determination of Al_2O_3, spectrophotometric method for determination of TiO_2, atomic absorption spectrophotometry for determination of calcium oxide CaO, and loss on ignition. Table 1 shows the chemical composition of

B and AB, and AG obtained from the producer. Table 2 shows their ash content and pH. It can be concluded that the activation of bentonite reduced proportions of Al_2O_3, Fe_2O_3, CaO and Na_2O, with an increase of SiO_2 and TiO_2, which is a prerequisite for improving adsorption power. The proportion of SiO_2 is significantly greater in the sample of active clay than in activated bentonite, while the proportion of Al_2O_3 is much smaller.

Table 1. Chemical composition of adsorbents

Component, % m/m	**B**	**AB**	**AG**
Al_2O_3	23.72	18.14	10.6
Fe_2O_3	6.80	4.40	3.4
SiO_2	52.88	54.88	70.8
TiO_2	0.83	0.86	0.4
CaO	1.83	0.12	0.7
Na_2O	0.02	0.00	0.4
MgO	-	-	2.6
K_2O	-	-	1.9

Table 2. Some physical characteristics of adsorbents

Characteristic	**B**	**AB**	**AG**
Moisture content, %, (2h, 110 °C)	6.85	21	6.42
pH (10%-suspension)	7	4	4

Identification of the phases of natural and activated bentonite was performed using X-ray powder diffraction (XRD) at automatic powder diffractometer PHILIPS PW-1710 CuKα anticathode under the following conditions: voltage 40 kV, current intensity 50 mA, angular range 5-60°, velocity counters 0,02°). Figure 1 and 2 show the diffractogram of natural and activated bentonite. Identification of resulting phases was performed using computer software that contains a database with ASTM cards (Culity, 1978). In the sample of natural bentonite, based on characteristic peaks, the following phases were registered: quartz (3.34; 4.26; 1.82 Å), montmorillonite (13.6; 4.47; 3.34Å), quartz mixture illite-montmorillonite (4.45; 2.55; 12.20 Å), while the peaks with d-values 2.98; 3.04; 2.89 Å may correspond to calcium silicate.

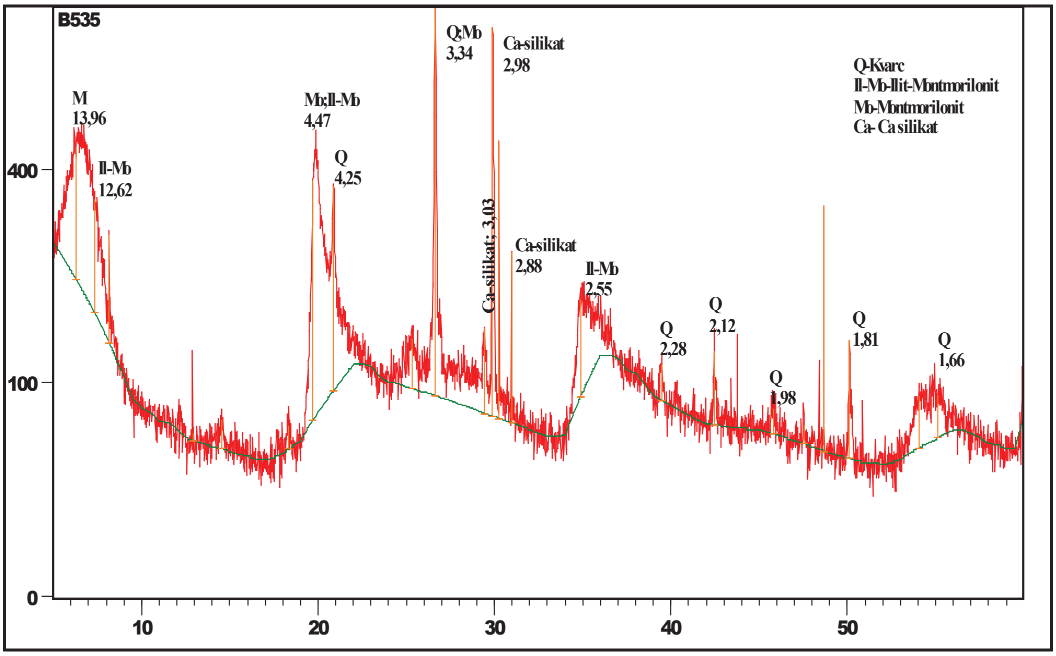

Figure 1. Diffractogram of natural bentonite (B)

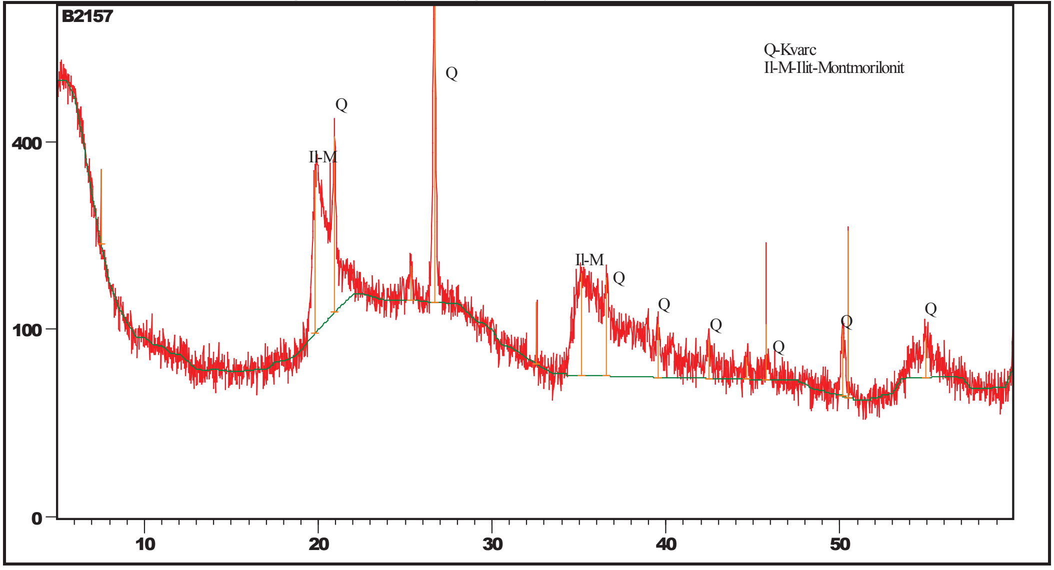

Figure 2. Diffractogram of activated bentonite (AB)

Based on diffractogram of characteristic d-values of activated bentonite, the following phases were identified: quartz (3.34;4.26;1.82Å), and mixture illite-montmorillonite (4.45;2.55;12.20 Å).

Thermal analyses were performed on the machine type „DERIVATOGRAPH“, Hungarian firm MOM Budapest. Conditions of recording samples of natural and activated bentonite were: sample weight 200 mg, heating rate 15°C/min, maximum time of 130,0 min, temperature of 1000˚C, and inert material of Al_2O_3. Qualitative characterization is reflected in determining the temperature of the phase transformations which occur during thermal activation of samples, and quantitative characterization is reflected in determination of mass loss of the sample during its thermal activation and mineralogical composition.

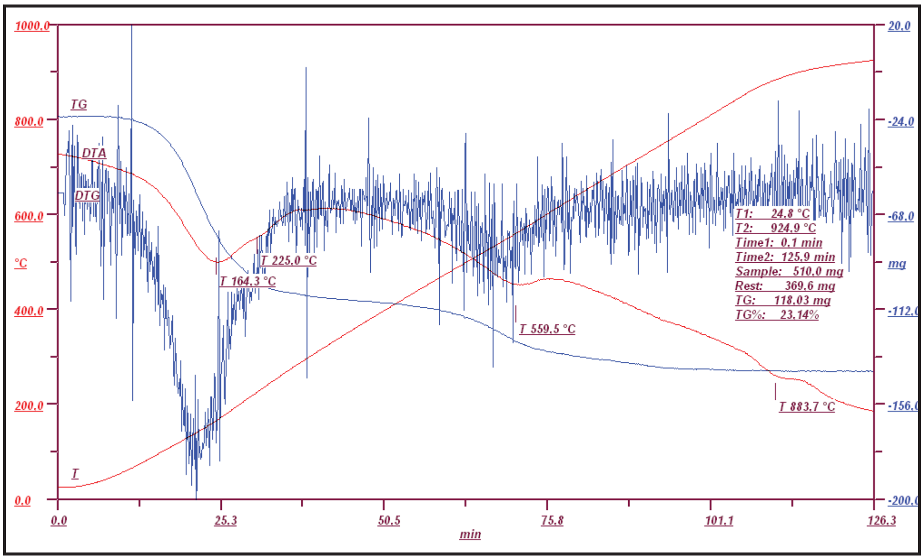

Figure 3. Thermogram of natural bentonite (B)

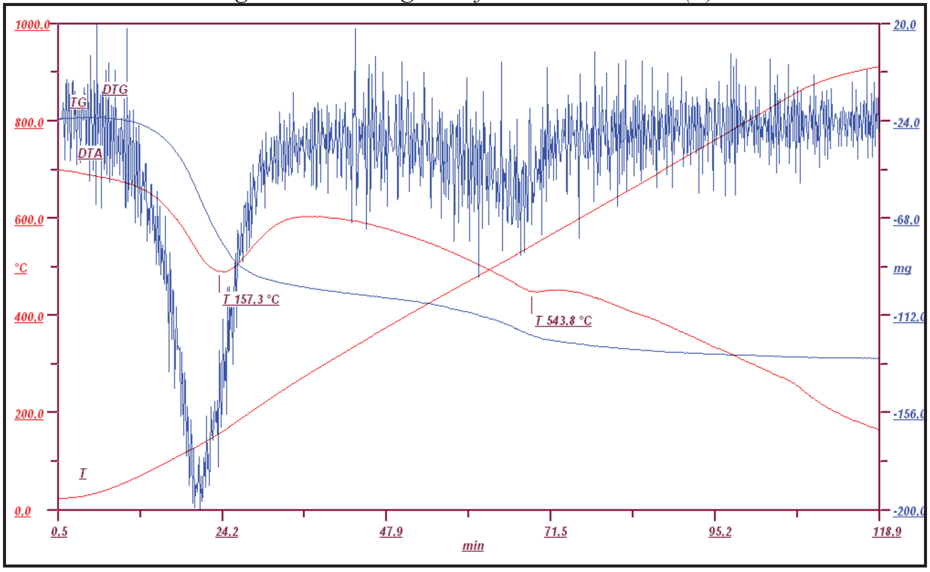

Figure 4. Thermogram of activated bentonite (AB)

The DTA curve of the natural bentonite shows two endothermic peaks with maximum temperature at 164.3 °C and 559.5 °C, which could correspond to dehydration of illite and kaolinite, on which basis we can conclude that these two phases are present in the sample. Also, we may observe the endothermic peak at temperature around 883.7°C, which comes from montmorillonite and muscovite. However, the endothermic peak at lower temperature area of 164.3°C is slightly wider and gives the impression that it was created by the overlapping of two peaks. According to the literature, the dehydration endothermic peak in this temperature range is a characteristic of montmorillonite. Mass loss by TG instrument scale is 20.39 %. The DTA curve of activated bentonite shows two endothermic peaks with temperature maximum at 157.3 °C and 543.8°C. Intervals of mass loss, registered by TG curves, also correspond to these changes. According to the type of sample, these changes could correspond to dehydration of illite, montmorillonite and kaolinite. Mass loss by TG instrument scale is 19.20 %.

The specific surface area of natural and activated bentonite was determined by low temperature adsorption of nitrogen at one point (BET) and it is 95.47 m^2/g, or 236.24

m^2/g. Increasing the specific surface area is a precondition for increasing the adsorption efficiency of bentonite after activation. Tested physical-chemical characteristics of the initial sample of base oil HU and the refined samples are given in Table 3.

Table 3. Typical characteristics of initial hydrocracked baseoil (HU)

Characteristic	**Method of examination**	**Value**
Viscosity (υ_{40}), mm^2/s	BAS ISO 3104	50.05
Viscosity (υ_{100}),mm^2/s	BAS ISO 3104	7.93
Viscosity Index (VI)	BAS ISO 2909	127
Index of Refraction (n_D)	ISO 5661	1.4680
Aniline point (AT) °C	ISO 2977	127
Density (γ_{15}), kg/m^3	ASTM D 5002	846.2
Acid number (KB), mgKOH/g	ISO 6618	0.035
Colour (B), ASTM	ASTM D 6045	D8.0

The structural composition of the initial base oil HU and the samples refined by adsorbents was determined by an IR spectrophotometer PERKIN ELMER/FTIR 1600 (method CEI IEC590), and the results are given in Tables 4 and 5.

Table 4. Structural composition of base oil HU after treatment with activated bentonite

Refined base oil samples of activated bentonite	**c_A, %w/w**	**c_P, %w/w**	**c_N, %w/w**
$HU_{0,5AB}$	5.32	72.60	20.08
$HU_{0,75AB}$	5.32	71.83	22.85
$HU_{1,0AB}$	5.32	71.60	23.07
$HU_{1,5AB}$	5.32	72.34	22.34
$HU_{2,0AB}$	5.32	72.14	22.54
$HU_{3,0AB}$	5.32	73.86	20.82
$HU_{0,5AB}$	5.41	73.26	21.33

Table 5. Structural composition of base oil HU after treatment with active clay

Refined base oil samples of activated clay	**c_A, %w/w**	**c_P, %w/w**	**c_N, %w/w**
$HU_{0,5AG}$	5.32	72.60	20.08
$HU_{0,75AG}$	5.32	72.72	22.41
$HU_{1,0AG}$	5.32	72.47	22.21
$HU_{1,5AG}$	5.32	72.60	22.08
$HU_{2,0AG}$	5.41	72.60	21.99
$HU_{3,0AG}$	5.41	69.90	24.69
$HU_{0,5AG}$	5.32	72.73	21.95

It can be concluded that the processing of initial base oil by mentioned adsorbents resulted in slight changes in structural composition, and they were approximately the same in samples treated by activated bentonite and active clay. Processing the initial base oil by adsorbents also leads to changes in some of their psysico-chemical characteristic. Tables 6-9 present the results of determination of some typical characteristics of base oil HU after treatment with activated bentonite and active clay.

Table 6. Effect of activated bentonite (AB) on some characteristics of base oil (HU)

Code of sample	Examined characteristics			
	υ_{40}, mm^2/s	υ_{100},mm^2/s	VI	n_D
$HU_{0,5AB}$	46.29	7.53	128	1.4670
$HU_{0,75AB}$	46.14	7.59	131	1.4675
$HU_{1,0AB}$	46.24	7.58	130	1.4675
$HU_{1,5AB}$	49.75	7.90	128	1.4680
$HU_{2,0AB}$	46.10	7.56	129	1.4670
$HU_{3,0AB}$	46.30	7.55	128	1.4670

Table 7. Effect of active clay (AG) on some characteristics of base oil (HU)

Code of sample	Examined characteristics			
	υ_{40}, mm^2/s	υ_{100},mm^2/s	VI	n_D
$HU_{0,5AG}$	46.54	7.61	130	1.4670
$HU_{0,75AG}$	46.37	7.60	130	1.4670
$HU_{1,0AG}$	46.64	7.60	129	1.4665
$HU_{1,5AG}$	46.45	7.62	131	1.4665
$HU_{2,0AG}$	45.90	7.53	129	1.4660
$HU_{3,0AG}$	46.10	7.55	129	1.4660

Table 8. Effect of activated bentonite (AB) on some characteristics of base oil (HU)

Code of sample	Examined characteristics			
	γ_{15}	AT	B	KB
$HU_{0,5AB}$	847.3	125	3.4	0.033
$HU_{0,75AB}$	846.0	125	2.5	0.012
$HU_{1,0AB}$	846.0	125	2.5	0.016
$HU_{1,5AB}$	848.9	125	2.0	0.037
$HU_{2,0AB}$	844.0	126	2.0	0.016
$HU_{3,0AB}$	845.0	126	2.0	0.023

Table 9. Effect of active clay on some characteristics of base oil HU

Code of sample	Examined characteristics			
	γ_{15}	AT	B	KB
$HU_{0,5AG}$	846.1	125	4.9	0.023
$HU_{0,75AG}$	845.5	125	4.0	0.017
$HU_{1,0AG}$	847.7	125	3.1	0.017
$HU_{1,5AG}$	846.2	125	2.4	0.017
$HU_{2,0AG}$	846.4	125	2.2	0.016
$HU_{3,0AG}$	844.4	125	1.9	0.017

From the ecological point of view, it is essential to reduce the concentration of polycyclic aromatic hydrocarbons (PCA) in lubricating oils because some of them are carcinogenic. The content of PCA in initial base oil HU and samples after the treatment with adsorbents was determined by method IP 346, in quantities of 0.5 % m/m and 3.0 % m/m respectively (Figure 5). A greater effect in reducing the PCA was achieved by treatment of HU with activated adsorbent, compared to the treatment with active clay.

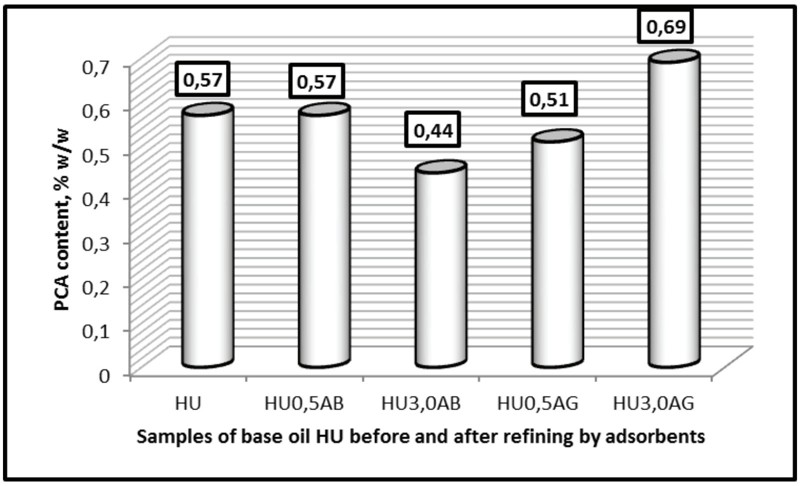

Figure 5. Effect of adsorbents on PCA content in refined samples of base oil (HU)

Oxidation stability of base oils indicates their life expectancy. HC base oil has poor oxidative stability, however, the addition of certain additives leads to significant improvements. Influence of processing the initial base oil HU with or without inhibitors, by activated bentonite and active clay is shown in Figure 6 (ASTM D 2272).

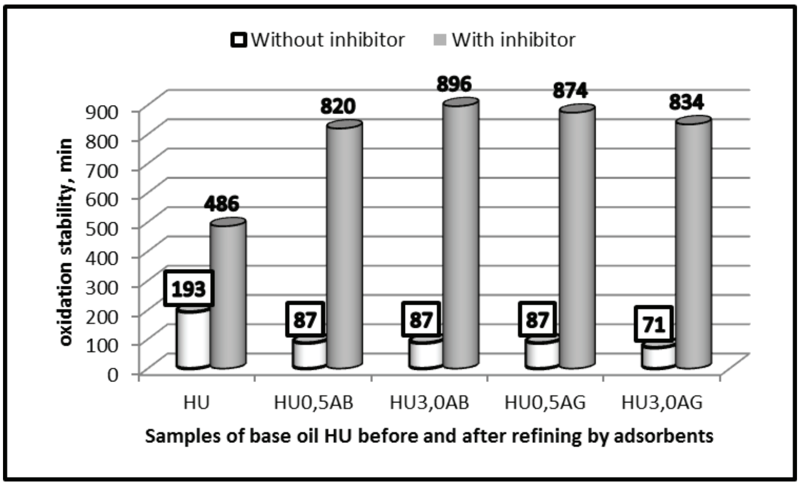

Figure 6. Effect of adsorbents on oxidative stability base oil (HU) with or without inhibitors

CONCLUSIONS

Based on research results it can be concluded:

- Activation of natural bentonite by dilute sulfuric acid in laboratory conditions leads to changes in its chemical composition, i.e. a significant decrease in content of Al_2O_3, Fe_2O_3 and CaO and an increase in specific surface area and pore size;
- Application of activated bentonite for refining hydrocracked base oils HU leads to an improvement of its characteristics (colour, viscosity index, etc);
- IR spectroscopy showed that there is a slight change in structural composition of initial oil after refining by adsorbents;
- It is very important to note that there is a reduction of polycyclic aromatic hydrocarbons (PCA), which is

significant not only for technical applications, but also for the ecology;

- Impact of oxidation inhibitors in highly refined base oils such as the tested hydrocracked base oil HU, showed maximum effects;
- Addition of oxidation inhibitors to the base oil HU, which was refined with activated bentonite and active clay, leads to a significant improvement of its oxidation stability, and even better results are achieved by using activated bentonite;
- Analysis of the effects of activation has shown that by this way activated bentonite achieved better effects than commercial active clay, during the processing of initial base oil HU, in colour improvement, PCA reduction and increased oxidative stability;
- It would be possible to determine the effects of refining, i.e. their influence on the structural composition and functional characteristics of HC base oils, by their comprehensive investigation applying some modern methods of laboratory analysis, such as MS and NMR;
- Further research on other hydrocracked base oil samples is necessary with the aim of eventual application of bentonite at semi-industrial and industrial conditions.

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RHEOLOGICAL PROPERTIES OF MOLTEN CHOCOLATE MASSES DURING STORAGE - INFLUENCE OF EMULSIFIERS

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

Production trend in the chocolate industry is to purchase finished cocoa liquor or finished chocolate mass which is then used in production of different chocolate products. If the finished chocolate mass is supplied to the facility, it is used within 30 days. The rheological properties of chocolate are highly important in quality determination and consumer preference. Since chocolate is a non-Newtonian fluid, its properties could be changed during the afore mentioned storage period. The aim of this research was to monitor the rheological properties of dark chocolate during 30-day storage at 50°C. Different emulsifiers (lecithin, PGPR, citric acid esters of and mono- and diglycerides of fatty acids (citrem) and citric acid esters of and mono- and diglycerides of fatty acids 2in1 (citrem 2in1)) were added to the chocolate in order to elucidate the influence of an emulsifier on the rheological properties during storage. The results showed that the rheological properties of all chocolate samples changed during storage. The addition of lecithin lowered plastic viscosity and yield value. At the end of the storage period, the yield value of the chocolate mass with lecithin increased, while other emulsifiers decreased its value.

Keywords: chocolate mass, storage, lecithin, PGPR, citrem, citrem 2in1

INTRODUCTION

Currently, it is common in the chocolate industry to purchase finished cocoa liquor or finished chocolate mass and use it in production of different chocolate products. If the finished chocolate mass is supplied to the factory, it is used within 30 days. Flow properties of chocolate are important quality aspects due to manufacturing requirements[1] and consumer preference[2].

From a rheological point of view, chocolate is a suspension of non-fat particles in the continuous fat phase[2]. The flow properties of chocolate are highly influenced by its composition: sugar, milk and fat particles, and emulsifiers influence these properties[3-7]. Researches have shown that the situation is even more complex when different proportions of these compounds are used[8]. Therefore, not only type, but quantity of a specific compound can have a marked impact on chocolate quality and consumer preference.

Chocolate viscosity is usually monitored through two parameters: yield stress and plastic viscosity. According to Goncalves & Lannes, "Yield stress is a material property which denotes the transition between pseudo-solid and pseudo-liquid behaviours – related to minimum shear stress at first evidence of flow – or transition from elastic to viscous deformation. Plastic viscosity determines pumping characteristics, filling of rough surfaces, coating, and sensory character of chocolate mass."[2] Both of these parameters can be influenced by addition of emulsifiers.

Many researchers have investigated the influence of emulsifiers on chocolate viscosity[8-10]. However, to authors' knowledge, no research has been conducted to elucidate this influence during chocolate storage. Therefore, the aim of this research was to examine the influence of lecithin, PGPR and citrem addition on the rheological properties of chocolate during storage.

MATERIALS AND METHODS

Lecithin (liquid) was supplied from ADM, Netherlands, citric acid esters of mono- and diglycerides of fatty acids (citrem), citric acid esters of mono- and diglycerides of fatty acids 2in1 (citrem 2in1) and polyglycerolpolyricnoloeate (PGPR) were supplied by Danisco, Denmark.

Chocolate masses were produced by the classic process in Zvečevo dd, Croatia on the Carle & Montanari production line. Conching time was 24 h. The emulsifiers were added in laboratory conditions as follows. The chocolate masses were kept at 50°C and emulsifiers were added in 0.4% with shearing at 1200 rpm during 10 min. After emulsifier addition, the chocolate masses were stored in laboratory thermostat ElektromehanikaLabo, Zlatar Bistrica, Croatia, at 50°C during 30 days.

Every 10 days, the rheological properties of chocolates were monitored. A DIN Viscometer VT 550 Digital Rheometer, Thermo Haake (Germany), with SV DIN rotor was used for this purpose. The Viscometer is computer controlled via RheoWin software, which is used for data analysis as well. Prior to viscosity measurement, the samples were stirred at room temperature to decrease sample temperature to 40°C, and at this temperature, the viscosity was measured with the increase of shear rate from 0 to 100 rpm. Measurements were done in duplicate.

The NCA/CMA Casson model was used to calculate flow parameters as follows:

$$(1 + a)\sqrt{\tau} = 2\sqrt{\tau_o} + (1 + a)\sqrt{\mu D}$$

Where:

a = ratio of outer radius of measuring rotor to inner radius of measuring cup
τ = shear stress (Pa)
τ_0 = Cross-border flow (yield stress) (Pa)
μ = plastic viscosity (Pas)
D = shear rate (s^{-1})

RESULTS AND DISCUSSION

Plastic viscosity and yield stress of the analysed chocolate samples are shown in Table 1. Immediately after addition, the most pronounced effect on yield stress PGPR had, followed by citrem, lecithin and citrem 2in1. Plastic viscosity was most influenced by addition of lecithin, followed by citrem, PGPR and citrem 2in1 (Table 1). This observation is in accordance with work of Shantz, Linke & Rohm (2003), who reported a more pronounced reduction of apparent viscosity when lecithin was used than with PGPR[9]. In addition, lecithin has been reported to significantly reduce both yield stress and plastic viscosity when used in less than 0.5% due to the action of phosphatidylcholine[8]. PGPR, on the other hand, is reported not to significantly influence plastic viscosity, but reduces yield stress by 50% when used in 0.2% and removes it when used in 0.8%[8,10].

Table 1 Casson plastic viscosity (μ_{CA}) and Casson yield stress (τ_{CA}) of molten dark chocolate during 30-day storage

Emulsifier	**Storage days**			
	0	**10**	**20**	**30**
	τ_{CA}[Pa]			
NO EMULSIFIER	45.15 ± 0.19			
Lecithin	15.16 ± 0.18	34.82 ± 0.26	33.72 ± 0.31	18.78 ± 0.47
PGPR	8.55 ± 0.41	0.30 ± 0.02	12.64 ± 0.25	0.09 ± 0.05
Citrem	23.61 ± 0.28	18.14 ± 0.25	16.08 ± 0.25	16.13 ± 0.38
citrem (2in1)	13.24 ± 0.68	9.26 ± 0.25	9.00 ± 0.25	8.29 ± 0.29
	μ_{CA} [Pa·s]			
NO EMULSIFIER	38.42 ± 0.53			
Lecithin	2.10 ± 0.03	1.71 ± 0.18	2.04 ± 0.18	2.51 ± 0.11
PGPR	3.36 ± 0.34	3.80 ± 0.11	3.06 ± 0.10	3.53 ± 0.43
Citrem	2.46 ± 0.44	1.93 ± 0.03	1.93 ± 0.06	2.32 ± 0.00
citrem (2in1)	3.39 ± 0.03	3.59 ± 0.28	2.88 ± 0.22	3.63 ± 0.18

Chocolate mass composition: sugar, cocoa liquor, cocoa butter, CBE, emulsifier (cocoa parts 38%). PGPR, polyglycerolpolyricinoleate; citrem, citric acid esters of and mono- and diglycerides of fatty acids.

After 10 days of storage the yield stress of chocolate mass with lecithin increased significantly, whereas other masses showed a decrease of yield stress, with the most pronounced change when PGPR was used. This phenomenon could be explained by research of Knoth et al. (2005), who reported that lecithin-stabilised water-in-oil emulsions formed aggregates[11]. Similar aggregates could be formed in chocolate with lecithin during storage, making it harder to start to flow. In PGPR-stabilised emulsions water droplets were singly distributed[11], which is indicative of better distribution of droplets in chocolate as well.

Plastic viscosity of samples was not significantly influenced by storage, regardless of the emulsifier used. Sugar particles are better dispersed in the oil phase when the emulsifier is used[12], which could be the reason for the enhanced stability of chocolate viscosity. Namely, without the emulsifier, sugar particles tend to settle in cocoa butter, forming compact sediment, but when lecithin, for example, is added sedimentation is limited[12]. In addition, aggregates which form in lecithin-stabilised water-in-oil emulsion are disintegrated during shearing[11].

Shear stress – the shear rate ratio of dark chocolate with lecithin addition is shown in Figure 1. From the results shown it can be seen that shear stress decreases continuously during storage (for same shear rate) with the most remarkable change after first ten days of storage. Note that no significant difference was observed between 10 and 20 days of storage.

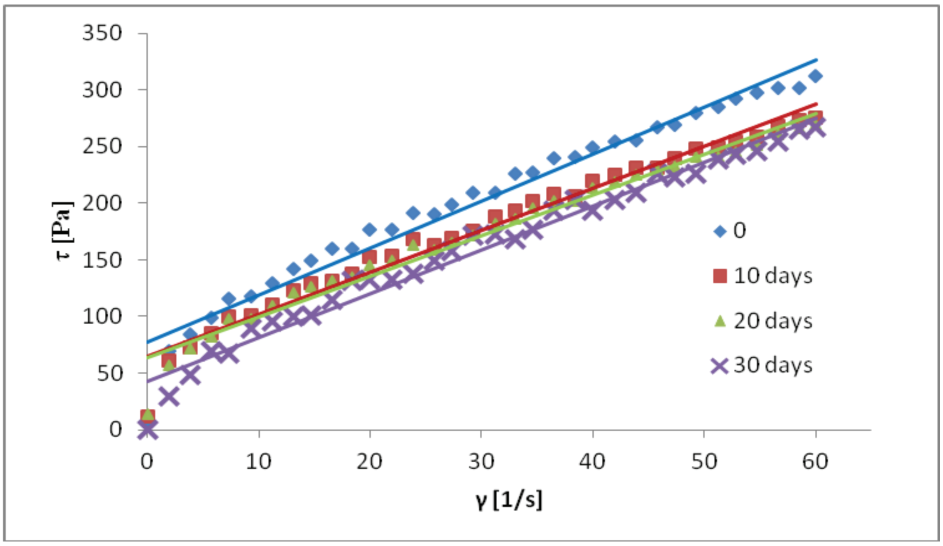

Figure 1. Shear stress (τ) – shear rate (γ) ratio of molten dark chocolate with lecithin addition during 30-day storage at 50°C

When PGPR was used instead of lecithin (Figure 2), a different trend was observed. The shear stress increased after 10 days (for same shear rate), than decreased after 20 days to values lower then at the beginning of storage, to increase after 30 days to values similar to those of 10-day-storage.

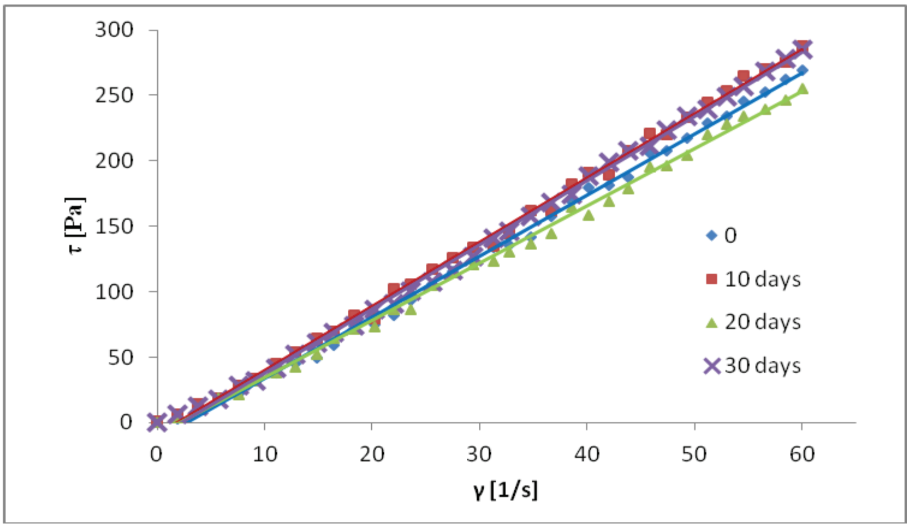

Figure 2 Shear stress (τ) – shear rate (γ) ratio of molten dark chocolate with PGPR addition during 30-day storage at 50°C

Citric acid esters of mono- and diglycerides of fatty acids (citrem) (Figure 3) and citrem 2in1 (Figure 4) had a similar effect: shear stress decreased after 10 and further after 20 days of storage. After 30 days, its value increased close to the initial values when citrem was used and close to the 10-day-values when citrem 2in1 was used.

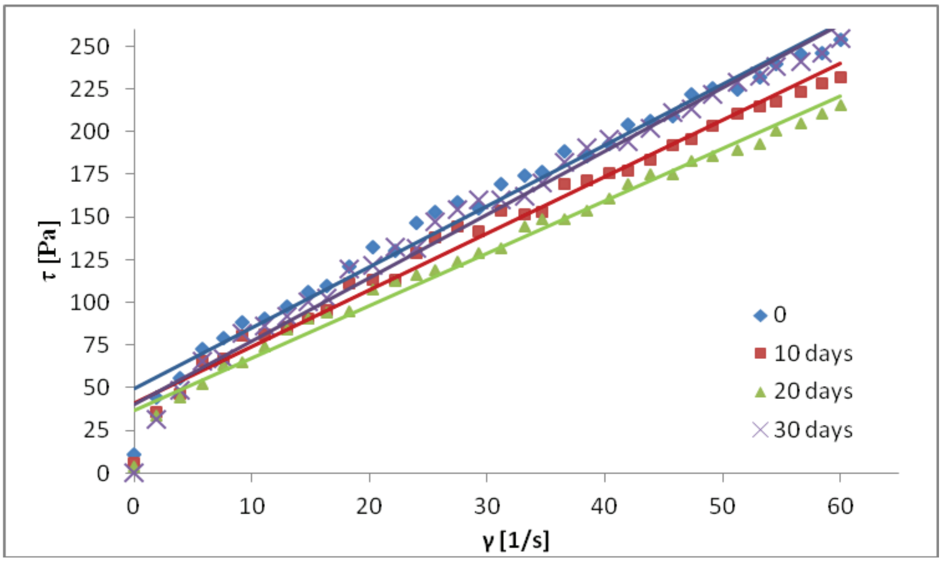

Figure 3 Shear stress (τ) – shear rate (γ) ratio of molten dark chocolate with added citric acid esters of and mono- and diglycerides of fatty acids during 30-day storage at 50°C

Slope approximated by the linear regression of measured values for shear stress – shear rate correlation forms an angle which is indicative of absolute chocolate viscosity. From Figures 1 – 4 it can be seen that within first 20 days of storage viscosity (expressed through slope angle) decreased, whereas after 30 days it approximates to initial values, which is most pronounced when PGPR and citrem were used.

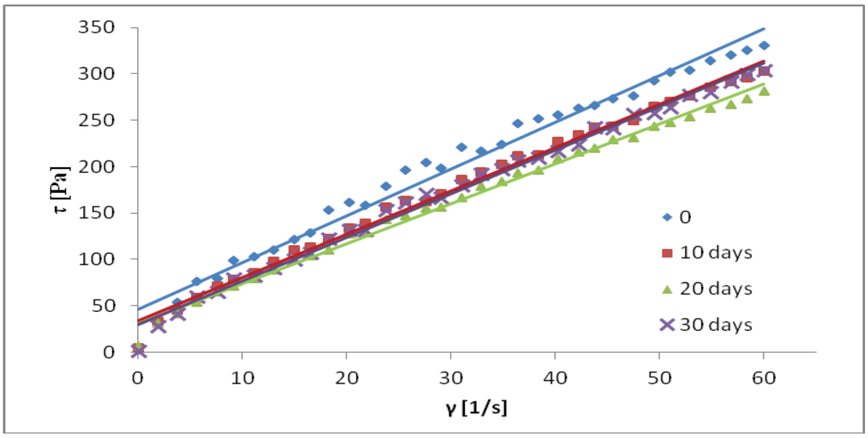

Figure 4 Shear stress (τ) – shear rate (γ) ratio of molten dark chocolate with added citric acid esters of and mono- and diglycerides of fatty acids 2in1 during 30-day storage at 50°C

CONCLUSION

Chocolate rheology is influenced by many factors. In addition to composition – the influence of sugar, milk particles, fat and emulsifier, this research has shown that storage time also has an impact on chocolate flow properties. The storage influence can be varied by time and/or selection of an emulsifier and this is something chocolate producers have to consider when using chocolate mass in production.

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WATER BORNE POLYMER/GRAPHENE COMPOSITES: ANALYSIS OF THE THERMAL DEGRADATION PROCESS

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

A study of a thermal degradation of polymer/graphene composites is presented. The composites were prepared by the emulsion mixing technique, where the water borne polymer poly(methyl methacrylate/butyl acrylate) p(MMA/BA) (monomers ratio 50/50 wt% and 40 % solid content) was mixed by ultrasonication with graphene nanoplatelets (GNPs) aqueous dispersion in the presence of poly(vinyl pyrrolidone) (PVP), allowing formation of stable hybrid latexes. The composite films containing GNPs of 0.5 wt%, 1 wt%; 2 wt%; and 3 wt% were prepared from these latexes by water evaporation under standard ambient conditions and characterized by means of SEM imaging and Raman spectroscopy. The thermal properties of the composites were examined by Thermo Gravimetric Analysis (TGA).

The composites have shown the improved thermal properties by introducing higher amount of GNPs, which resulted in decreased rate of degradation and increased amount of carbonaceous/GNPs chars. The calculated activation energies have confirmed augmentation of thermal stability, since the addition of 3% of GNPs in relation to polymer increased the activation energy for about 260%. The excellent thermal conductivity of the GNPs was found as a main cause for it, since the GNPs in the polymer matrix allowed a better distribution of thermal energy and homogeneous heating of the composite materials.

Keywords: graphene nanoplatelets, poly(methyl methacrylate/butyl acrylate), poly(vinyl pyrrolidone), emulsion mixing technique, Thermogravimetric analysis

INTRODUCTION

Graphene is a material with remarkable properties, such as excellent electronic transport properties, high thermal conductivity, superior mechanical properties etc. [1-4]. These intrinsic properties of graphene have generated enormous interest for its possible implementation in a myriad of devices [5].

Different approaches for synthesis of GNPs/polymer have been studied [1-8]. As a method of choice, an emulsion mixing process (blending of polymer latex with GNPs) [3,4] offers a multitude of advantages: it is simple and versatile and it offers a control of polymer properties during synthesis, without the presence of GNPs. As well, it offers a better distribution of GNPs in the polymer matrix because the GNPs during preparation are dispersed in a limited space between polymer particles, which partially prevent the aggregation. Finally, it is an environmentally friendly method, since both components (polymer and GNPs) are dispersed in water and the composite films are prepared by simple water evaporation.

GNPs have been used as a filler in different polymeric materials in order to introduce electrical conductivity [3,4], improve mechanical properties [9-12] or optical properties [13], introduce sensing and biosensing properties [14,15] or improve thermal properties [16-19] of the composites. GNPs have been considered a very good candidate for heat control in electronic devices, because of a very high thermal conductivity of GNP, around 5000 $Wm^{-1}K^{-1}$ [20]. Almost in all of published works an improvement of the thermal properties has been demonstrated by means of increased degradation temperature and glass transitions temperatures. By integrating graphene platelets into the polymer matrix, the highly improved thermal properties of the composites were achieved. Yu et al. [18] examined the thermal stability of graphene oxide (GO), GNPs and funcionalized GNPs (fGNPs) within the thiolene matrix. They confirmed that fGNPs and GNPs composites are thermally more stable comparing to GO composites, but all of them are thermally more stable by increasing filler content. Das and Prusty [19] explored the thermal conductivity, by utilization of graphene-based materials in the fabrication of nanocomposites within different polymer matrixes. They achieved four times higher thermal conductivity with 5wt.% GO-filled epoxy resin comparing with neat epoxy resin.

As presented, the thermal properties were examined by a lot of authors but to the best of author's knowledge, there is no data on mechanism and kinetics of the thermal degradation process presented, yet. In this paper, beside synthesis and characterization of water-borne GNPs/polymer composites, we present the investigation of kinetics of the thermal degradation process. On the one hand, the deeper knowledge will allow an improvement of the properties of the composites, and on the other a base for possible industrial processing or thermal recycling of composites can be set up.

Non isothermal or dynamic thermogravimetry (TGA) has been used for kinetic study of the polymer/graphene composites, heating by two different rates up to around 723 K. The analytical model of kinetic curve was used, by means of Gropjanov`s method [21], in order to determinate the kinetic of thermal degradation of the composites.

EXPERIMENTAL PART

Materials

Methyl methacrylate (MMA, Quimidroga) and butyl acrylate (BA, Quimidroga) were used as received. Graphene powder with thickness of 8nm was purchased from a Graphene supermarket. Sodium dodecyl sulfate (SDS, Aldrich) and Poly(vinyl pyrrolidone) (PVP, Aldrich) were used as stabilizers. Initiator 4,4 –Azobis(4-cyanovaleric acid) (V-501, Aldrich) which was dispersed in a 1N solution of sodium hydroxide (NaOH Aldrich) was used to initiate the free radical polymerization.

Synthesis of p (MMA/BA)

p(MMA/BA) with monomer ratio of 50/50, was prepared by semicontinuous seeded emulsion polymerization using 4,4`– Azobis(4-cyanovaleric acid) (V-501) as a free radical initiator. The seed was prepared with a solid content of 10% by a batch reaction in a 1L jacketed glass reactor at 75°C under a nitrogen atmosphere for 180 minutes. Agitation was provided by a stainless steel impeller at 250 rpm. An initial charge containing the seed latex, the total amount of initiator and part of the water was added to the reactor and let to reach 70°C. The pre-emulsion containing the rest of water and surfactant was feed until a 40% solid content (SC) was reached, during 240 minutes, after which it was left to react additional 60 minutes. Complete conversion was achieved. Particle size was 199.9 nm. The formulation used is shown in Table 1.

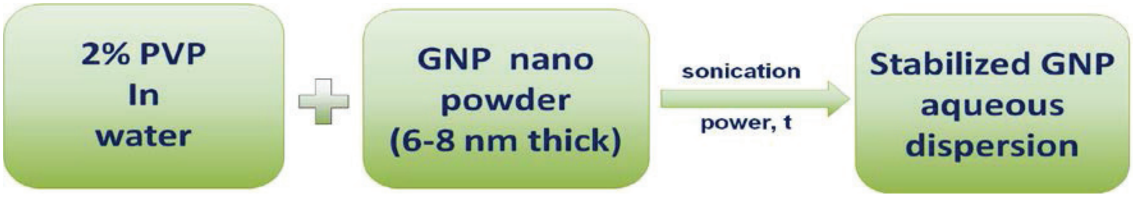
Table 1. Formulation for 40% solid content latex

Compound	Batch reaction	Semicontinuous reaction	
	10 wt% s.c. seed[1] Charge (%)	Initial charge (%)	40 wt% s.c. latex Stream (%)
MMA	5	-	18.8
BA	5	-	18.8
SDS	0.1	-	0.4
V-501	0.1	0.37	-
H_2O	89.1	12.4	23.1
Seed latex	-	19.8	-

[1] *Seed emulsion polymerization is a process of particle growth in the system*

Preparation of graphene dispersion

0.42g of Poly(vinyl pyrrolidone) (PVP) were added in 20ml distilled water and mixed well, after which 0.8g of graphene was added. The dispersion was sonicated (UP200Ht, ultrasonic processor, with amplitude 80% and 50 % puls), for 1h in the presence of ice. The procedure was repeated four times. The preparation of graphene dispersion is presented in Scheme 1.



Scheme 1. Preparation of graphene dispersion

Preparation of composites

Emulsion mixing technique was used for preparation of polymer/graphene composites, changing the amount of added graphene to achieve the final graphene content of: 0.5%, 1%, 2% and 3%wt. The composite films were prepared by water evaporation under ambient temperature conditions. The latex was casted into a 40 mm diameter silicon moulds and allowed to form the film for 3 days. The whole procedure is presented in Fig.1.

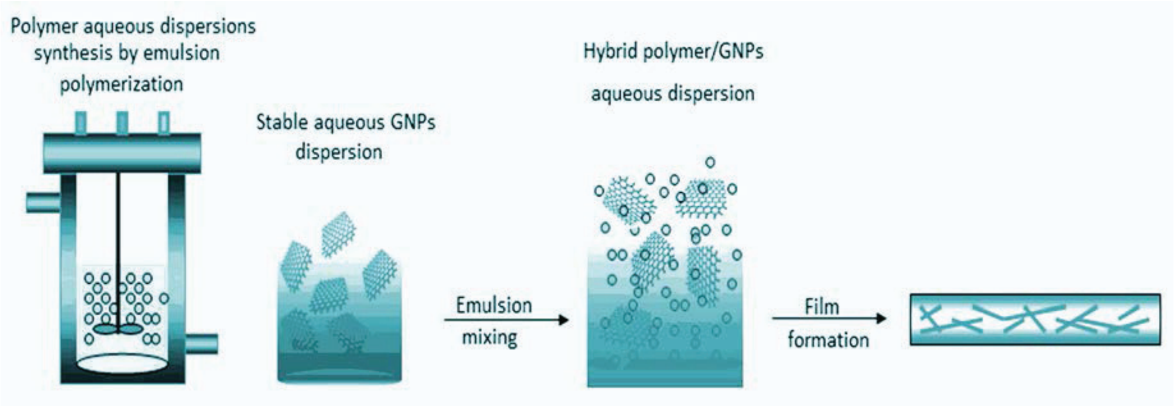


Fig.1 Preparation of polymer/graphene composites

Characterization of the composites

The morphology of the composite films was studied by scanning electron microscopy (SEM) (Hitachi S-4800, Tescan Indusem) at an accelerating voltage of 5 kV. The samples were scanned as-prepared without sputtering metal on their surface. Cross-sectional images were taken after breaking the samples under liquid nitrogen.

Raman spectroscopy was used to determine the extent of exfoliation of GNPs in the frame of the composites. The spectra were recorded on a Renishaw (a Ramascope model 1000) Raman microscope with a CCD detector using the exciting beam of an Ar-ion laser (514.4 nm) 50µW.

The samples were thermogravimetricaly analyzed (TGA, Cahn D-200 Digital Recording Balance) by heating the sample up to around 723 K at the rate: dT/dτ = 4 and dT/dτ = 10^{o}/min. The changes in the samples weight was determined by using Cahn D-200 recording microbalances in a stream of argon (flow rate 100 ml/min).

Calculation of activation energy

The calculation of the activation energies of the thermal decompositions was conducted by using Friedman analysis [21], based on the Arrhenius equation, which applies the logarithm of the conversion rate, dx/dt as a function of the reciprocal temperature at different degrees of conversion (Eq. (1)):

$$\ln\left(\frac{dx}{dt}\right) = lnA - \frac{E(x)}{RT} + \ln(f(x))$$

(1)

where dx/dt is the rate of reaction and the f(x) is the function dependent on the decomposition mechanism, E(x) the activation energy, A is the frequency factor, R the gas constant and T the thermodynamic (Kelvin) temperature. The thermal decomposition is complex processes. For this reason, and because of limited amount of samples available, the simplified form of the analysis, Gropjanov's method [22] was applied, where thermogravimetric decay curves were measured only at two values of the heating rate, namely, at 4 and 10 °C/min. The activation energy E(x) is then calculated according to Eq. (2):

$$E(x) = \frac{R[\ln\left(\frac{dx}{dt}\right)2 - \left(\frac{dx}{dt}\right)1}{[\left(\frac{1}{T1}\right) - \left(\frac{1}{T2}\right)]}$$

(2)

where $(dx/dt)_1$ and $(dx/dt)_2$ are the conversion rates taken for the equal value of the conversion x from the both curves measured at the abovementioned different heating rates. In this simplified form of analysis, the E(x) is obtained with curves of only two values of the heating rate.

RESULTS AND DISCUSSION

In Fig. 2 the fracture surface of the composite film containing 1 wt.% of GNPs is presented, showing that the graphene platelets are well distributed. The layered structuring of the composite film is obvious, resulting from the way of formation of the films: the graphene platelets are placed in the limited space between the polymer nanoparticles (see Fig. 1) in aqueous dispersion, thus during water evaporation the high aspect ratio graphene filler will induce rearrangements in layers. In the inset of Fig.2, one platelet under higher magnification is shown, indicating that the platelets are well exfoliated in few layers.

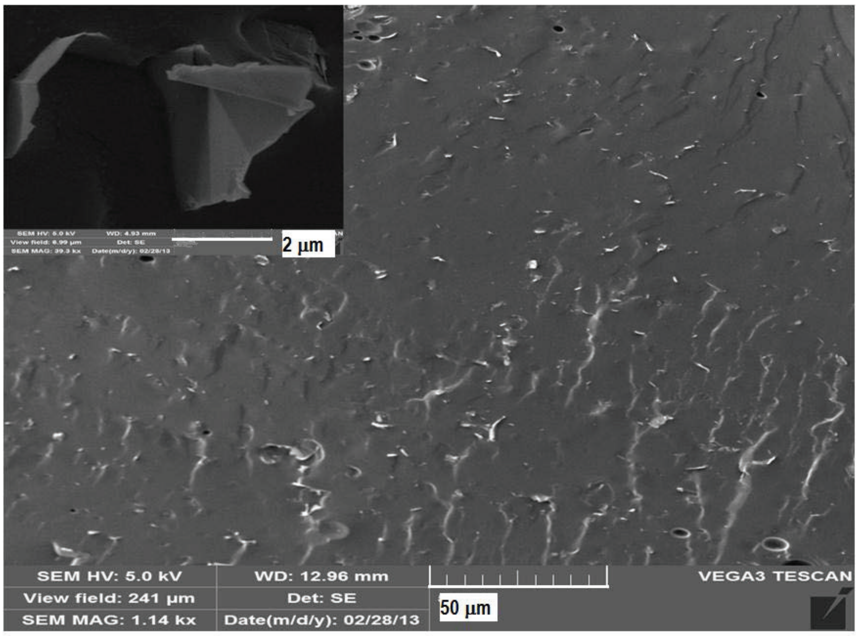


Figure 2. SEM image of fracture surface of polymer/graphene composite film

The exfoliation of graphene platelets is confirmed by Raman spectra measurements. In Fig. 3 Raman spectrum of the composite containing 1% of graphene are presented. In Fig.3a the spectra was obtained by focusing the laser beam at polymeric part, thus the characteristic vibration of the polymer are dominating, although the graphene characteristic peaks, G and the 2D peaks, are also presented. The Raman spectum presented in Fig. 3b was obtained by focusing the laser beam at graphene platelets, and the shape of these two graphene bands (wide and almost symmetric) and their ratio (the 2D peak is more than half of the intensity of the G peak) it could be concluded that the GNPs in the composites are stacks of few layers, however not more than 2-3 [23].

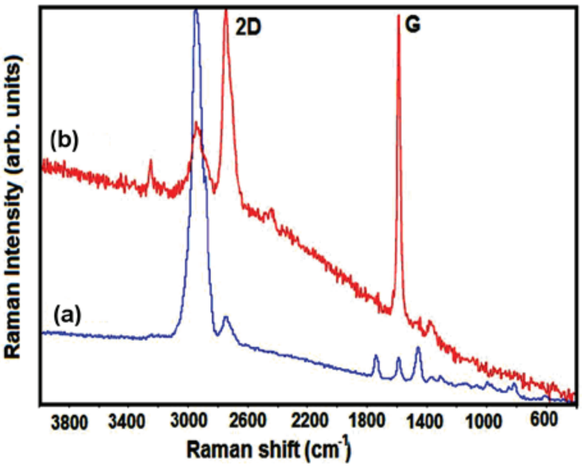


Figure 3. Raman spectra of polymer/graphene composite containing 1% graphene

Polymer/graphene composites were thermally treated in inert atmosphere, up to 723K, using a heating rate of 4 and 10^{o}C/min. The dependence of weight loss on temperature for the heating rate of 4^{o}C/min and 10^{o}C/min is shown in Figs. 4a and 4b, respectively.

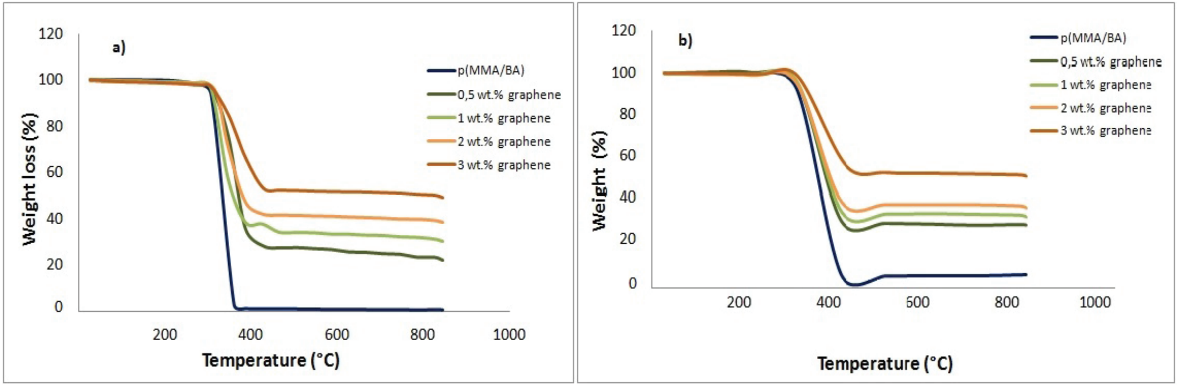


Figure 4. Dependence of weight loss of graphene polymer composites with different graphene content on temperature for heating rate of (a) 4^{o}C/min and (b) 10^{o}C/min

Fig. 4a shows that the polymer degraded in one step and completely until 400 ºC by process of main-chain pyrolysis, which is in accordance with literature data in the same copolymer system [24]. The presence of GNPs improves the thermal stability of the composite materials, since the rate of degradation slightly decreased with increasing the graphene content, which could be the indication of the existence of small interaction between the polymer and the filler. As a result of these interactions, the polymer chains next to the graphene platelets will have decreased mobility and induce slower degradation rates. However, as the decrease in weight by increasing in temperature is one step process, one can conclude that only the polymeric part has been decomposed. Additionally, the polymer decomposition is not complete because there is high amount of created carbonaceous chars. Fig 4b presents similar trends, however the increase rate of heating slows down the rate of polymer degradation, whereas almost did not influence the composite degradation rates. It is worth mentioning that by increasing the GNPs content the solid residual in form of carbonaceous char increased significantly, thus for 3 % graphene content is almost 60% of the composite remain in the residual. One possible reason for this decreased rate of degradation is formation of char earlier for the higher heating rate heat [25]. The second possible reason could be when heating starts, it induces additional bonding between the GNPs and polymer, creating incredibly thermally stable materials.

By means of Gropjanov's non-isothermal method for investigation of the formal kinetic of polymer and polymer/graphene composites [22] and using the TGA results presented in Figs. 4a and 4b the activation energies were calculated according to the equation 2 (Table 2). The values presented in Table 2 confirmed the enhanced thermal stability of the composites. The activation energies increased significantly. The presence of 1wt% of graphene in the composites increased the needed energy for starting the degradation to double of the value of pure polymer, while 3% of graphene presented in the composites increased it up to around 260%. As well known, the GNPs are one of the best known thermally conductive materials. During heating of the composites the presence of GNPs will allow better distribution of thermal energy and homogenous heating of the samples, avoiding local high heating. As a result, initially the higher energy will be necessary to start the degradation process.

Table 2. Activation energies of polymer/graphene composites

Name of example	**wt% of graphene in terms of polymer**	**Activation energy (Ea) (kJ/mol)**
Polymer	0	90,24
GPVP18	0,5	139,26
GPVP19	1	172,68
GPVP20	2	213,135
GPVP21	3	241,86

CONCLUSION

From the results it can be concluded that with introducing a small amount of graphene nanoplatelets into the polymer matrix by the emulsion mixing technique, nice and homogeneous composites were synthesized by the emulsion mixing technique. The thermal stability of the composites was improved by increasing the graphene content and the rate of degradation decreased. The calculated energies of activation confirmed the increased thermal stability; therefore higher energy is necessary to start the thermal degradation process, as a result of the high thermal conductivity of the GNPs presented in the composites, which allowed better distribution of thermal energy within the composite matrix.

These results confirmed the excellent improvement of the thermal properties of GNPs/polymer composites that were prepared only by simple emulsion mixing without inducing specific interactions between the polymer and the filler. The synthesized materials could find application as heat control devices in various applications.

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DISTRIBUTION OF SPECIES *ICHTHYOSAURA ALPESTRIS* (LAURENTI, 1768) IN THE HERZEGOVINA AREA

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

We surveyed Hercegovina area, recorded presence of the Alpine newt (*Ichthyosaura alpestris* Laurenti, 1768), and compared its present distribution with previously reported data. Its presence was confirmed at 12 localities: Crvenjak Lake on mountain Čvrsnica, Orlovačko Lake on mountain Čvrsnica, Džamija Plateau on mountain Visočica, Teleća lastva on mountain Velež (Podveležje), Presjeka village (Nevesinje Field), Kruševljani village (Nevesinje Field), Kruševac on mountain Prenj, sites Ošljak, Prekača and Žeravac on mountain Prenj, Zijemlje village on the southern slopes of mountain Prenj (2 sites). All of the sites studied belong to the high mountain regions of Herzegovina, considering that none of the specimens was sampled at elevations below 800 meters above sea level. According to the vertical gradient, these sites ranged from 869 m to 1974 m above sea level, Zijemlje and Visočica respectively. Apart from the mentioned sites, the research was also conducted at other areas where individuals *Ichthyosaura alpestris* were not found. These sites located in Nevesinje Field were: Alagovac Lake, the Zalomka River catchment area (locality Ponori), Sopilja village, water meadows in Sopilja, Gornja Bijenja village (pond at Ilino hill), Plužine village (flood meadows) and Veleško Lake. At the site of Veleško Lake, the individuals of Smooth newt *Lissotriton vulgaris* Linnaeus, 1758 were found, which is the only existing record of this species within the researched area.

Key words: *biological resources, Ichthyosaura alpestris (Laurenti, 1768), distribution, region of Herzegovina, biodiversity.*

INTRODUCTION

Biodiversity of Bosnia and Herzegovina is one of the most diverse in all of Europe, and a high level of endemic and relict fauna of this region gives importance to the level of global biodiversity. The high-mountainous and mountainous area of Bosnia and Herzegovina as a part of the Balkan Peninsula is one of six centres of European biodiversity. On a small geographical area, as rarely in Europe, exists more endemic developmental centres, which are still occurring processes of the formation of new species (Redžić et al., 2008). The reasons for the very high level of biodiversity in this region are numerous. They involve very different geological base dominated by carbonate rocks, a significant area of karst and different types of soil. Extraordinary heterogeneity of these factors has created very favourable conditions for the development of a rich mosaic of biodiversity in these areas (Redžić et al., 2008). During the Ice Age the territory of Bosnia and Herzegovina was a refugium (spatial units have a lower impact of climate change) for a large number of plant and animal species. Owing to its territory, Bosnia and Herzegovina inhabits many relict and endemorelict species. In the process of a reduction of global biodiversity, declining amphibian populations are most affected. Globally and locally, amphibian populations have greater public importance because they rapidly respond to changes in the outside environment and can be used as bio-indicators of the general state of the natural environment. Amphibian fauna of Bosnia and Herzegovina has attracted the attention of many zoologists, starting from the end of the 19th Ages until today. The first data are related to the research Werner (1893, 1897). The initial list of fauna of Bosnia and Herzegovina, whose author is Austrian zoologist Möllendroff, includes data on six species of amphibians (Möllendroff, 1873). Later, continuous Herpetological Research of Bosnia and Herzegovina was conducted by Werner (Werner, 1897, 1898, 1907), then by Bolkay (Bolkay, 1919, 1922, 1924, 1929). It is necessary to mention that Radovanović (1932-1958) made an exceptional contribution to the study of amphibians in this region by publishing a monograph "Amphibians and reptiles of our country" (1951), which gives a detailed overview of batrahofaune in Bosnia and Herzegovina (15 amphibians, Radovanović, 1851). In recent years, a significant contribution to the study of amphibians in Bosnia and Herzegovina has been given by Škrijelj and Korjenić, 2000; Lelo and associates, 2003; Adrović and Mujić, 2006; Šunje and Lelo, 2008; Lelo, 2010.

MATERIALS AND METHODS

Field research in the area of Herzegovina was conducted at a number of localities in the period from March 2011 to April 2012. The presence of the investigated species was confirmed in 12 localities: Crvenjak Lake on mountain Čvrsnica, Orlovačko Lake on mountain Čvrsnica, Džamija Plateau on mountain Visočica, Teleća Lastva on mountain Velež (Podveležje), Presjeka village (Nevesinje Field), Kruševljani village (Nevesinje Field), Kruševac on mountain Prenj, sites Ošljak, Prekača and Žeravac on mountain Prenj, Zijemlje village on the southern slopes of mountain Prenj (2 sites). The collected data were entered into a database and the map of distribution in the study area was made with the help of GIS technology. Specimens were taken for analysis after which they were returned to their original habitat. However, some of them have been preserved in 76% ethyl alcohol. At each of the above mentioned sites, the presence of species *Ichthyosaura alpestris* was determined by the altitude, latitude and longitude (GPS device Garmin - Colorado 400t GPS receiver). Specimens were determined using identification keys (Lelo 2007, Arnold et al., 1992). The current nomenclature and taxonomic review are aligned with the data on the portal: research.org / vz / Herpetology / index.php amphibia (Frost et al., 2006).

RESULTS AND DISCUSSION

The exploration of spatial distribution of *Ichthyosaura alpestris* lasted from March 2011 until April 2012. The presence of the investigated species was found in twelve locations (map 1). All the investigated locations spatially belong to the high-mountain regions of Herzegovina, since the species were not sampled at altitudes below 800 meters above sea level. According to the vertical gradient, the sites ranged from 869 meters to 1974 meters above see level, Zijemlje and Visočica respectively. In relation to the type of aquatic ecosystems, the species of mountain Tritons is most common in permanent and periodic ponds; less frequent in smaller lakes, and quite rare in larger ones, especially in those that are populated by salmon fish. The presupposition that species *Ichthyosaura alpestris* inhabits habitats belonging to the high-mountain regions was confirmed by the field research carried out in this study. It is necessary to mention that, despite the fact that the six investigated localities where the presence of the analyzed species was confirmed belong to the mountain region Prenj, does not prove that the species *Ichthyosaura alpestris* prevails in the region. A significant number of mountain Alpine newt populations in this area can be explained by a time interval of the research, which was longer than in the other study areas. During this study the Alpine newt was ascertained on the following sites:

Location 1; spring Ošljak on Prenj, near a mountain lodge. Ošljak is located at an altitude of 979 m, the geographical coordinates are 43°28'00.97"S 17°58'0510"I. This site was first visited in April 2011, which was analyzed several times during the study, primarily because the life cycle of mountain Alpine newt was observed. Therefore, it is necessary to emphasize that a stable population of Alpine newt exists at this site indicated by the significantly higher number of individuals in a period of the aquatic life cycle, compared to other analyzed sites.

Location 2; Kruševac (coordinates: 43°28'31.07"S 17°57'11.61"I, 1023 m asl.

Location 3; Prekača located at a lower altitude of 902 m (coordinates are 43°27'12.48"S 18°00'04.04"I). The species *Ichthyosaura alpestris* were found in April 2011. It is an oval-shaped swamp with a diameter of 6 meters whose depth does not exceed 1 metre, and which is filled with groundwater coming from surrounding wells. During summer, however, it dries up due to the water level decreases in those wells.

Location 4; Zijemlje is located on the southern slopes of the mountain, an altitude is 907 m (coordinates: 43°25'24.69"S 18°00'26.53" I). The species was found in a water tank near a house in this village in June 2011. Only four adult males were found, while females and larvae were not identified.

Location 5; Zijemlje (coordinates: 43°25'28.14"S 18°00'12.62"I, altitude: 869 m). In this village, by finding another mountain Alpine newt, it was ascertained that a microhabitat is identical to the previous location in the same villlage; and a small number of this kind was found in a pool of water (two males). This location is also the site with the lowest altitude at which alpine newt was found in the researched area (in Montenegro, Alpine newt was found at the sea level).

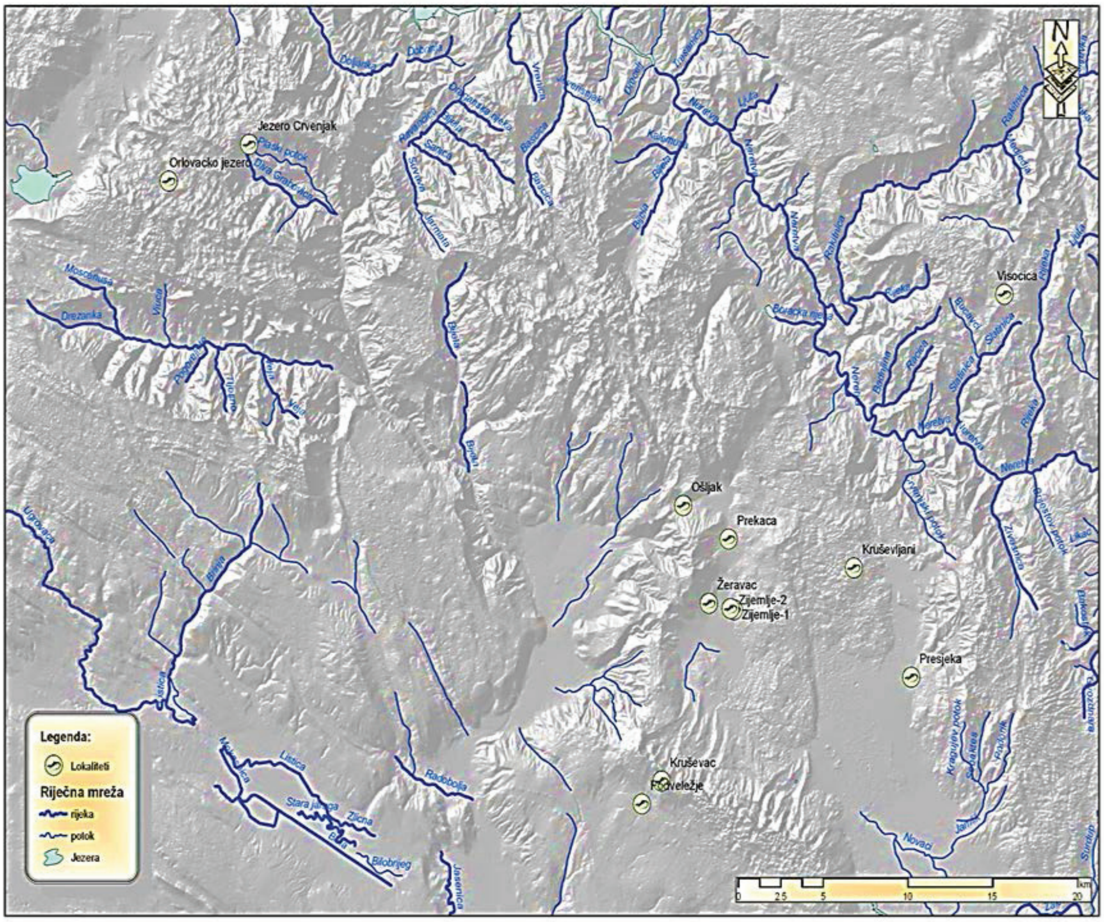
Location 6; Žeravac (coordinates: 43°25'41.53"S 17°59'17.37"I, altitude: 998 m) are flooded meadows that do not dry up during summer, because they are formed by several smaller sources. The site was visited in May 2011 when seven males and five females were taken for analysis.

Location 7; Presjeka (coordinates: 43°23'45.55"S 18°08'01.36"I, altitude: 985 m) is a village in the Nevesinje Field, the species was found in a pool near the village in May 2011. A mountaineer B. Jusic also confirmed this finding by discovering some individuals later in May of that year.

Location 8; Kruševljani (coordinates: 43°26'35.62"S 18°05'50.30"I, altitude: 1134 m). The investigated site is located at the foot of mountain Crvanj. Specimens were sampled at the source Kruševac in early June 2011. In addition to adults, a larval stage was also found indicating the stability of the population in this locality.

Location 9; Podveležje (coordinates: 43° 20'11.69"S 17°56'24.84"I, altitude: 1343 m). It is a plateau in the central area at the foot of mountain Velež. *Ichthyosaura alpestris* was found on the slopes of Mount Velež, particularly in the area of Podveležje Plateau, a hill called Teleća Lastva.

Location 10; Visočica (coordinates: 43°33'59.61"S 18°12'1.13" and altitude: 1591 m). Individuals were sampled in a pool next to the hiking trail that leads to the highest peak of Visočica - „Džamija“ (1974 m above see level). This site represents the extreme eastern border inhabited by the mountain Alpine newt in the study of the Herzegovina area.



Map 1. Distribution of species Ichthyosaura alpestris in Herzegovina

Location 11; Crvenjak Lake on Mountain Čvrsnica (coordinates: 43°37'54.34"S 17°39'01.97"I, 1970 m). It is an oval-shaped lake which is 70 meters long, 50 meters wide with an approximate depth of 9 meters. The area around the lake is surrounded by impassable forest vegetation.

Location 12; Orlovačko Lake on Mountain Čvrsnica (coordinates: 43°38'55.77"S 17° 5'49.57"I, altitude: 1,831 m) is located at the foot of the northern slopes of the peak Mali Vilinac in the area of Donjih Mejdana. It is a glacial lake formed by a nearby source. The lake is modest in size which is accessible from Risovac, a distinct highland terrain. Sampling was carried out in May 2011.

During field studies, which lasted from April 2011 to April 2012, excepting the mentioned localities, the research was also carried out in other areas where the species *Ichthyosaura alpestris* were not identified. The above research included the entire area of Nevesinjsko Field and on that occasion the following locations were visited: Lake Alagovac, Zalomka River catchment area (locality sink), Sopilja village (pool), flooded meadows in Sopilja village, Gornja Bijenja (pond on Ilin hill), Plužine village (flooded meadows) and Velež Lake. At the site of Velež Lake, the species *Lissotriton vulgaris* - Smooth newt (Linnaeus, 1758) – were found, the only record of this species in the locality. During this field study, Alpine newt individuals were found at elevations below 869 metres above see level (Zijemlje site), which confirms the assumption that this species inhabit only high mountain ecosystems in Herzegovina.

The species were not found at lower altitudes in the areas of Svitava and Stolac during April 2012. It is very similar to the spatial distribution in Montenegro, where the Alpine newt inahbits eco-geographical regions in the upper zone of Montenegrin karst, and is limited to altitudes above 870 meters above sea level (Ćirović et al., 2008). In terms of conservation, there are no clear guidelines designed to protect Alpine newt in Herzegovina. In the present study, a significant degree of vulnerability of these animals was noted as a result of natural and anthropogenic impacts such as construction of inappropriate cottage settlements. The most pronounced changes in natural ecosystems have been observed in hydrographic objects. The biodiversity of aquatic ecosystems have performed for decades powerful ichthyologic pressure which resulted in the eradication of indigenous species *Ichtyosaura*.

CONCLUSIONS

An image of amphibian fauna is significantly changed in Bosnia and Herzegovina. This study showed that individuals *Ichtyosaura alpestris* inhabit only high-mountain regions or regions above 800 meters above sea level in Herzegovina. The lowest altitude at which Alpine newt was found is 869 m (Zijemlje site).

Analyzing specimens on Prenj, confirmed the presence of individuals whose life cycle is characterized by facultative paedomorphosis.

Because of the special role that amphibians occupy in many ecosystems, it is necessary to prevent their extinction. These factors can, as it is usually the case, act synergistically - two or more factors acting simultaneously and the impact of certain factors reinforce each other. Research increasingly shows that there is no single most prominent cause of global amphibian extinction, but that all the above reasons threaten their populations. A particular site depends which one (or more factors at once) will predominated. This clearly points to the dangers which threaten amphibians and the need to protect this group of organisms as soon as possible.

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CLASSIFICATION OF WINES BY CHEMOMETRIC ANALYSIS

PROFESSIONAL PAPER

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ABSTRACT

A combination of FTIR and chemometrics was applied to classify Slovenian white wines. The identification of the geographical origin of wines is of great interest for wine consumers and producers since it may provide determinant criteria for guarantee of quality. Many researches described the possibilities of prediction of wine origin using efficient methods of wine components analysis connected with multivariate data analysis. The aim of our work is focused on white wine classification. Wine samples were scanned in transmission on a WineScan FT 120 from wave numbers 926 to 5012 cm^{-1}. All samples were sourced from 2004 to 2006 vintage. The present study deals with description and classification of the wine samples originated from several regions of south-west Slovenia by means of the following variables: relative density, content of total, non-volatile and volatile acids, ash, reducing sugar, free sugar, SO_2, ethanol and pH. An important additionally available variable was sensorial quality of wine. This sensory variable positively correlates with content of ethanol and content of volatile acids. The effect of the variety of wine and geographical origin was examined and many significant differences were found in the mentioned descriptors.

Key words: chemometrics, wine, WineScan FT120, classification.

INTRODUCTION

The identification of the geographical origin of wines is of great interest for wine consumers and producers since it may provide determinant criteria for guarantee of quality. Wine belongs to the commodities, which are very frequent objects of falsification.[1,3] Wine is considered falsified when it has not been made in accordance with a specified method of production and is presented as a valuable product under an official trademark or when the declared location of production is not true. Wine classification according to its producer or locality as well as year of production is also frequently demanded. The question of geographic identification of wines becomes even more interesting when it relates to small production areas.

Methods of multivariate data analysis (MVA) are frequently used for investigation of relations and interactions inside a large data table. For wine classification the MVA methods are especially useful.[1,3,5] Performed research focused on the classification of Slovenian white varietal wines, based on the results of chemical analysis. Eleven selected chemical descriptors (variables), the most frequently used for wine characterization, were obtained by FTIR (Fourier transformed infrared spectroscopy). This study concerns an optimal choice of variables with regard to their effective use for wine classification according to the target factors – variety, and geographical origin. For this purpose, principal component analysis (PCA), cluster analysis (CLU) and linear discriminant analysis (LDA) were used as the most important chemometrical tools.[2,6]

MATERIALS AND METHODS

Wine samples

Altogether 177 samples of white varietal wines, namely Chardonnay (36 samples), Rebula (20), Sauvignon (41), Malvasia (25), Pinot Gris (33) and Yellow Muscatel (22) were analysed successively. The wines originated from four regions of Primorska area, south-west Slovenia (Gorica, Koper, Kras and Vipava area) and three vintages (2004 – 2006). Sampling was made by the Chamber of Agriculture and Forestry of Slovenia (KGZS) in Nova Gorica. The following enological descriptors were determined: relative density in g/mL at 20 °C (*dens*), contents of total extract in g/L (*extract*), total acidity in g/L (*TotAc*), non-volatile acidity in g/L *(NVolAc*), volatile acidity in g/L (*VolAc*), ash in g/L (*Ash*), free SO_2 in mg/L (*SO2*), reducing sugars in g/L (*RedSug*), sugar-free extract in g/L (*SugFree*), ethanol in % (*alc*), and pH. In addition, the sensorial variable *mark* describing the sensory quality of the wine samples was obtained by a group of experts evaluating the wine properties (colour, barrique aroma and taste) using a twenty point scale in total.

Analytical Methods

All analytical methods were accomplished according to Official Gazette Republic of Slovenia No. 43/01 [6], and sensorial analysis was performed according to Official Gazette RS No. 32/00. The WineScan FT 120 instrument utilizing FTIR was employed for simultaneous determination of mentioned wine descriptors. The temperature of samples was set to 40 °C. Samples of wine were filtered through filter paper to expel CO_2, and catch any sediment. The instrument was zeroed before any set of analyses with the zeroing solution. Samples were scanned from 926 to 5012 cm^{-1}.

For the calibration of WineScan FT 120 the standard analytical methods were used for the real samples. The iodometric titration methods were applied for determinations of free SO_2 and reducing sugars contents. Potentiometric methods using glass and saturated calomel electrodes were employed for determination of total acidity, volatile acidity, non-volatile acidity and pH. A calibrated pycnometer was used for measuring density. The difference of the total extract, finally obtained also by pycnometry and the determined content of reducing sugars were used for calculating the value of the sugar-free extract. The ash content was determined by ignition of the wine extract at 550 °C followed by a gravimetric endpoint.[7,8]

Statistical Analysis

Statistical treatment of the obtained data was performed using program packages SPSS IBM Statistics 19, STATISTICA 7 and Microsoft EXCEL. SPPS was used for calculations of linear discriminant analysis. Calculations of principal component analysis and cluster analysis were performed by STATISTICA 7. MS EXCEL was used for the data preparation and summarization of the results.

RESULTS AND DISCUSSION

Correlation analysis

For the investigation of interrelations among the analysed variables the Pearson correlation analysis was performed. Statistically significant correlations were found in case of numerous variables (Table 1). The highest correlation coefficients were observed between variables *extract* and *dens* (0.99), *extract* and *RedSug*(0.99) and *NVolAc* and *TotAc*(0.98). In case of four strongly correlated variables *dens*, *extract*, *RedSug* and *SugFree* was *Ash* in significant positive correlation. The sensorial variable *mark* was positively correlated to *alc* (0.27) and *ash* (0.22). The variable *alc* was in negative correlation to *dens* (-0.63), *extract* (-0.51), *RedSug* (-0.50) and *SugFree* (-0.40). *VolAc* was negatively correlated to *NVolAc* (-0.39) and positively to *pH* (0.23). *pH* was negatively correlated to *NVolAc* (-0.44) and *TotAc*(-0.42) as expected; the positive correlation between *pH* and *Ash* (0.66) is worth to be noted.

Table1. Reduced correlation matrix with Pearson correlation coefficients. The statistically significant correlations are denoted by ***violet*** *($p<0.05$),* ***blue*** *($p<10^{-4}$) and* ***red*** *colour ($p<10^{-12}$).*

	dens	***extract***	***RedSug***	***SugFree***	***pH***	***TotAc***	***NVolAc***	***VolAc***	***Ash***	***SO2***	***alc***
extract	**0.99**										
RedSug	**0.98**	**0.99**									
SugFree	**0.72**	**0.72**	**0.60**								
pH	0.17	0.19	0.14	**0.33**							
TotAc	0.03	0.00	-0.06	**0.23**	**-0.42**						
NVolAc	0.03	-0.01	-0.06	**0.24**	**-0.44**	**0.98**					
VolAc	0.01	0.03	0.06	-0.10	**0.23**	-0.21	**-0.39**				
Ash	**0.35**	**0.37**	**0.30**	**0.57**	**0.66**	-0.14	-0.16	0.14			
SO2	0.12	0.10	0.10	0.09	0.06	-0.07	-0.06	0.00	-0.04		
alc	**-0.63**	**-0.51**	**-0.50**	**-0.40**	-0.01	**-0.23**	**-0.24**	0.12	-0.09	-0.15	
mark	-0.04	0.00	0.02	-0.07	0.13	-0.17	-0.16	0.02	**0.22**	0.05	**0.27**

Principal Component Analysis

Principal component analysis (PCA) is a basic way used for characterizing multidimensional data, providing a satisfactory representation of the studied objects by projecting the original data set from the high dimensional space onto the lower dimension space. [4,10]

In our case, the first two PC's calculated from all variables, account for 62.3% of the total data variability. The mutual position of the descriptors was in accordance with the correlation analysis. The variety Yellow Muscatel was separated from the other varieties and the samples were situated at negative *PC 1* and positive *PC 2* values as follows from the score plot in Figure 1. This is connected to its sweet taste and high level of reducing sugars compared to the other varieties.

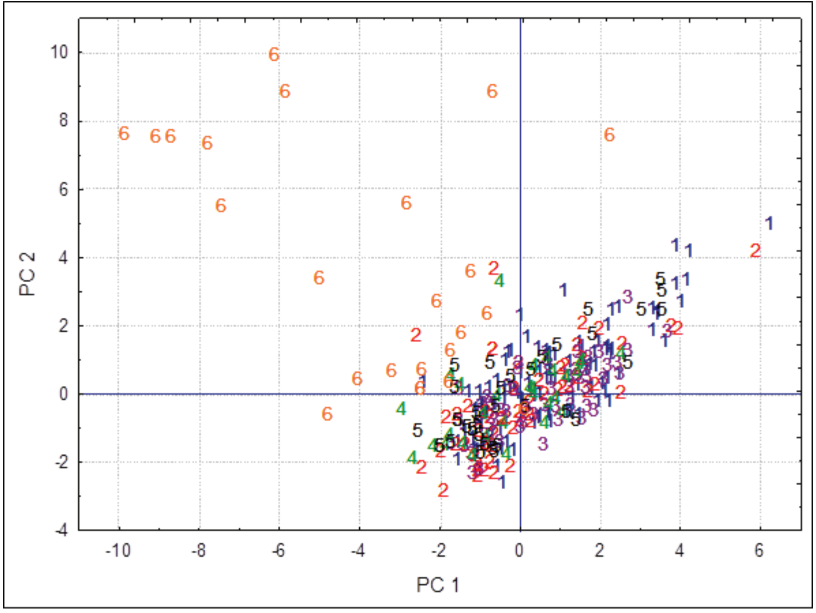

Fig. 1. PCA score plot in the plane PC 2 vs. PC 1. The objects are labelled by the wine variety. The Yellow Muscatel (6) is clearly separated from the others varieties. Software STATISTICA 7.

Cluster Analysis

The aim of cluster analysis (CLU) is the recognition of groups of objects or variables based on their similarity, which is given, using appropriate metrics, by the distances between the objects in the multidimensional space of the chosen variables.[4,9] The most effective agglomerative clustering algorithm is the Ward´s method, which we have applied using the squared Euclidean distance as the similarity measure. The descriptor *mark* representing sensorial quality of the wine samples was most closely related to *SugFree* and to *alc*, which reflects the importance of the mentioned descriptors for the quality of white wines. Further observed clusters are expected: (1) *TotAcid* and *NVolAc*, (2) *Dens* and *VolAc*, (3) *Ash* and *pH*. In general, the mentioned results of cluster analysis were in a good agreement with the PCA results.

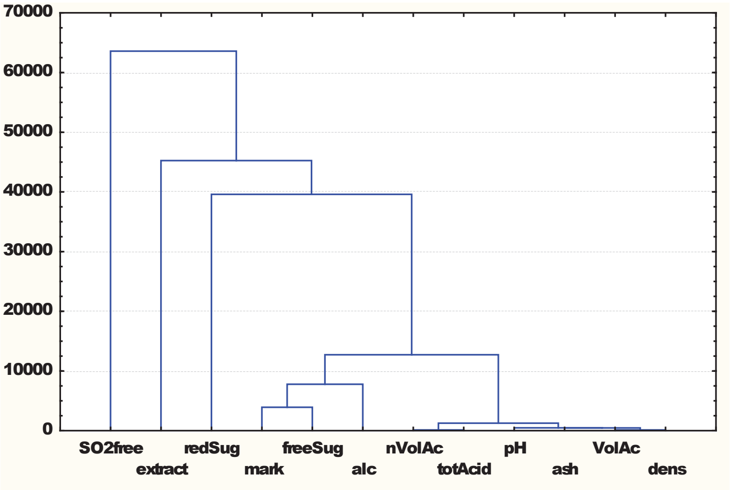

Fig. 2. Dendrogram – graphical output of the cluster analysis using Ward´s method for 6 varieties of wine in year of production from 2004 to 2006.

In case of clustering of the objects no well separated clusters were observed regardless the target factors *variety* and *production area*.

Linear Discriminant Analysis

Linear discriminant analysis (LDA) is a supervised learning method in which a classification model is constructed using the data of the objects pre-categorized into known categories (the training data set). The developed model is then employed for classification of the samples, which were not used in the model calculation (the validation data set). The validation of the classification model is necessary in order to verify its prediction ability. An important task in the optimisation of the discrimination model is an appropriate selection of the input variables. The backward selection method was used for optimizing the set of input variables and the validation of the LDA model was accomplished by leave-one-out method.

Table 2. Results of classification of wine samples according to the production area using LDA for all selected wines

variety	*production area*	*results of classification*		*selected input variables*
		training set	**validation set**	
Sauvignon	Gorica (19) Vipava (22)	90.2%	90.2%	*dens, pH, Ash*
Malvasia	Koper (18) Vipava (7)	96.0%	88.0%	*pH, SO2, alc, TotAc, SugFree*
Pinot Gris	Gorica (27) Vipava (6)	87.9%	81.8%	*Ash, SugFree, dens, TotAc, alc*
Yellow Muscatel	Gorica (5) Koper (7) Vipava (10)	77.3%	68.2%	*TotAc, dens, alc*
Rebula	Gorica (6) Koper (17)	69.6 %	69.6 %	*Extract, Ash, RedSug, dens, SO2*
Chardonnay	Koper (9) Kras (6) Vipava (21)	100%	89.2%	*TotAc, alc, pH, NVolAc, RedSug*

Not satisfactory results in classification of white wine samples originated from all vintages by *variety* were obtained when using all six categories of *variety*. Despite of very good classification rate for Chardonnay (100 % in training and 89.2 % in validation) and Yellow Muscatel (77.3 % and 68.2 %, resp.), the remaining categories were not well distinguished. An independent LDA calculation with three categories of *variety* – Chardonnay, Pinot Gris and Rebula for samples originated from vintage 2005 resulted in improving of the classification rate – 89.2 % for the training data and 81.1 % for leave-one-out validation using variables *VolAc, dens, alc, SugFree, pH, mark* and *extract*.

The classification of the wine samples into the four categories of *production area* using all varieties was not satisfactory. Therefore, an additional approach was attempted – to classify the samples by *production area* separately for particular varieties. The corresponding LDA results were very good excepting the classification of Rebula wines (Table 2).

CONCLUSIONS

The sensory quality descriptor is significantly positively correlated to the ethanol and ash content which reflects the importance of the mentioned descriptors for the quality of white wines. Further considerable interrelations were observed by the correlation analysis; noteworthy among them is a positive significant correlation of the ash content and wine pH as well as a negative high correlation between the ethanol content and relative density. The most important two principal components calculated from all descriptors, account for 62.3 % of the total data variability. The samples of the variety Yellow Muscatel differ clearly from others and their distinctness was revealed also in the wine classification by variety. The PCA loading plots are concordant with the outputs of correlation analysis and in several aspects similar to the results of cluster analysis.

The classification of white wines according to the wine variety was not satisfying. Despite of very good classification rates for the varieties Yellow Muscatel and Chardonnay, the discrimination of other white wine varieties was not sufficient when used wine samples from all vintages. If the chosen simple enological descriptors are used it is possible to fully differentiate only several of the studied white wine varieties.

The studied enological variables have good discrimination power for classification of wine samples according to the area of production when the samples of a single variety were used.

Acknowledgement

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THE EFFECTS OF SURFACTANT STRUCTURE IN MIXED SURFACTANT SOLUTION AT THE PRECIPITATION OF CA(II) IONS

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

The mixed surfactant solution has a wide range of applications in modern society and an important place in many areas of chemistry, industry and everyday life. Therefore, surfactants are significant pollutants in wastewater. The interaction between these surfactants and existing ions in water and soil can result in forming hardly soluble anionic surfactants salts and soluble nonionic surfactants complexes. The adsorption of present cations to surface of mixed surfactant micelle is also possible. The concentration of the products depends on concentration and structure of surfactants, and it results in influence on the distribution of metal cations in soil. Similar reactions can be carried out in the analytical systems, thereby affecting the interference in determining of cations. Knowledge of cation-surfactant and cation-mixed micelle interactions is particularly interesting in analytical procedures involving separation by precipitation or complexing.

Today, it is particularly interesting to study the influence of nonionic polyether-type of surfactants at the precipitation of hardly soluble calcium salts, in the presence of anionic surfactants and coexistence of carbonate. In this paper, it was proven that the tolerance to the precipitation of Ca(II) ions increases with synergism, e.g. achieving maximum concentration of hydrophilic components on the micelle surface through bonding at the micelle/solution interface.

This paper also investigates and analyzes the effect of the concentration and the structure of anionic and nonionic polyether-type of surfactants on complexometric determination of Ca(II) ions, as well as the possibility of separation of Ca(II) ions as carbonates. It was found that addition of EDTA, during the volumetric determination of Ca(II) leads to formation of more stable Ca(II)-EDTA complex in micellar solutions compared to solutions without surfactants.

In this study were used:

- *Anionic surfactant*– Sodium dodecylbenzene sulfonate (SDBS)
- *Nonionic surfactant* - Polyethylenglycol p-(1,1,3,3-tetramethylbutyl) phenyl ether (Triton X-100) and nonaethylenglycolmonadodecyl ether ($C_{12}E_9$)

Methods used in this study: tensiometry, volumetry, conductometry and spectrophotometry.

Kaywords: anionic surfactant SDBS, nonionic surfactant $C_{12}E_9$, nonionic surfactant Triton X-100, Ca(II) ions, precipitation of $CaCO_3$

INTRODUCTION

The mixed surfactant solutions have a wide range of applications in different areas of everyday life, including various industrial fields.[1] Hence, the presence of these compounds in waste water increases. The interactions of anionic surfactants with polyvalent cations often result with formation of hardly soluble salts, especially in high salinity conditions.[2] This can be overcome by adding an amount of nonionic surfactants which can increase the solubility of formed hardly soluble mixed salts. This can be explained with formation of mixed anionic-nonionic micelles. Nishikido et *all.*[3] concluded that Craft's point of bivalent metal-dodecyl sulphates decreases in the presence of nonionic surfactants, thus contributing to higher tolerance to precipitation.

Recent studies have a tendency to examine the possibility of practical implementation of the above mentioned interactions for precipitation control of $CaCO_3$, not only from an aspect of quantitative yield of the process but also of crystal morphology. A polar head group of anionic surfactants (e.g.

sulphonates) represents the active centre for $CaCO_3$ bonding due to the electrostatic interactions between positive charged Ca(II) ions and negative heads of anionic surfactants. On the other hand, it can be expected that the structure of nonionic surfactant yields even more complex species interacting with cations, compared to anionic surfactants.

Calcium carbonate crystallizes in different crystall shapes: calcite, aragonite, vaterite, calcium carbonate monohydrate and calcium carbonate hexahydrate.[4] Precipitation from micellar solutions can favor only one crystall shape of $CaCO_3$.

Hao Wei and co-workers[5] investigated the influence of concentration of anionic surfactant-polimeric complexes on $CaCO_3$ crystallization. They proved that sodium dodecyl sulphonate (SDDS) as well as the complex poly(N-vinyl-1-pyrrolidine) (PVP)/SDDS does not influence significantly on polymorfism and the shape of calcium carbonate crystalls, while sodium dodecylbenzene sulphonate (SDBS) and also its PVP complex significantly change crystal shapes and morphology of formed crystals.

Jun-Hwan Bang and co-workers[6] examined the influence of critical micellar concentration (CMC) of sodium dodecyl sulphate (SDS) dissolved in carbonate and calcium solution during the precipitation of $CaCO_3$ on the crystall characteristics. SDS in carbonate solution causes polymorfism of $CaCO_3$. The solution which contains lower SDS concentration than the CMC is favourable for crystallization of vaterite configuration. Increasing the concentration of SDS above the CMC leads to changes in crystall shape, e.g. calcite is dominant in that case.

In this paper, influences of concentration and the structure of nonionic and anionic polyether surfactant were analyzed based on an investigation of interferencies during complexometric determination of Ca(II) as well as the possibility of Ca(II) separation in its carbonate form. Investigations were based on a theoretical study of surfactant characteristics, especially on: surface tension, critical micellar concentration, possibility of cation complexation as well as other interactions that lead to the precipitation of bivalent cations.

MATERIALS AND METHODS

In experimental research the following materials were used:

- Anionic surfactant: Sodium dodecylbenzene sulphonate (SDBS) $CH_3(CH_2)_{11}C_6H_4SO_3Na$ (80%, Fluka). The solutions were prepared in the concentration range of $1\cdot10^{-4}$ to $1\cdot10^{-2}$ mol/l.
- Nonionic surfactants:
  - Polyethylene glycol p-(1,1,3,3-tetramethylbutyl) phenyl ether (Triton X-100) $C_{14}H_{22}O(C_2H_4O)nOH$ (n=9-10) (99%, Sigma). The solutions of this surfactant were prepared in the concentration range of $1\cdot10^{-5}$ to $1\cdot10^{-3}$ mol/l.
  - Nonaethylene glycol monododecyl ether ($C_{12}E_9$) $C_{30}H_{62}O_{10}$ (98%, Sigma). The solutions of this surfactant were prepared in the concentration range of $1\cdot10^{-5}$ to $1\cdot10^{-3}$ mol/l.
- Basic solution of Ca(II) ions with the concentration of 0.1 mol/l, prepared by dissolving $Ca(NO_3)_2$ x $4H_2O$ in double destilled water, (98,5 %, Alkaloid Skopje).
- Na_2CO_3, concentration of 0.1 mol/l. (p.a. 99,5% Kemika Zagreb)
- Disodiumsalt of ethylene-diamine tetraacetic acid (EDTA) at concentration 0.01 mol/l. EDTA is a powdery white substance, the primary standard.
- 1 mol/l NaOH for regulation of pH value.
- Metal indicator murexide.

Instrumentation was used as followed:

- Tensiometer "Krüss" K10ST, with a thermostat and measurement precisions of ± 0.1 mN/m. The surface tension measurements were carried out by using the platinum-iridium ring at constant temperature of 25±1°C.

- UV/VIS a spectrophotometer "CECIL CE 2021", with a quartz cuvette (b = 1.00 cm).
- Conductometer GLP 31 Crison Instruments. Electrical conductivity was measured in a glass cell, with two platinum electrodes. Cell constant is 1.000 cm^{-1}.
- pH-meter "CRISON" with a combined electrode "GLP" 21
- .

RESULTS AND DISCUSSION

1. Determination of the critical micelle concentration (CMC)

In order to determine the optimal conditions for the research in micellar solutions (since the formation of micellar aggregates is required for the experiment) surface tension measurements were carried out as a function of surfactant concentration to determine the CMC. The results are shown in Table 1.

*Table 1. CMC values of used surfactants (*experimental results)*

Surfactant	**CMC* (mol/l)**	**CMC[6] (literature)[7]**	**σ_{min}* (mN/m)**
$CH_3(CH_2)_{11}C_6H_5SO_3Na$ (SDBS)	$1.6 \cdot 10^{-3}$	$1.2 \cdot 10^{-3}$	33
$C_{14}H_{22}O(C_2H_4O)_nOH$ (Triton X-100)	$4 \cdot 10^{-4}$	$3.6 \cdot 10^{-3}$	31
$CH_3(CH_2)_{10}CH_2(OCH_2CH_2)_9OH$ ($C_{12}E_9$)	$1.1 \cdot 10^{-4}$	$1 \cdot 10^{-4}$	28

1. Micelle formation in mixed systems

The mixed solutions of SDBS-Triton X-100 and SDBS-$C_{12}E_9$ were prepared, with different molar fractions of anionic surfactants (SDBS) (Table 2). CMC values of each system were determined. Table 2 shows that the CMCs of mixed systems decreases from the CMC value of anionic surfactant toward the CMC values of pure nonionic surfactants, with increasing mole fractions of nonionic surfactant. The experimental CMCs for mixtures are lower than those obtained by ideal mixing.

Table 2.The effect of structure and the concentration of the surfactant on the CMC

	α_{SDBS}	**1**	**0,9**	**0,75**	**0,5**	**0,25**	**0**
Triton X-	**α_{TX-100}**	0	0.1	0.25	0.5	0.75	1.0
	CMC_{ideal}(mol/l)	$1.6 \cdot 10^{-3}$	$1.2 \cdot 10^{-3}$	$9 \cdot 10^{-4}$	$6.4 \cdot 10^{-4}$	$4.9 \cdot 10^{-4}$	$4 \cdot 10^{-4}$
	$CMC_{exp.}$ (mol/l)	$1.6 \cdot 10^{-3}$	$4 \cdot 10^{-4}$	$4 \cdot 10^{-4}$	$3 \cdot 10^{-4}$	$3 \cdot 10^{-5}$	$4 \cdot 10^{-4}$
	$pCMC_{exp.}$	2.8	3.4	3.4	3.5	3.5	3.4
$C_{12}E_9$	**α_{C12E9}**	**0**	**0.1**	**0.25**	**0.5**	**0.75**	**1**
	CMC_{ideal}(mol/l)	$1.6 \cdot 10^{-3}$	$6.8 \cdot 10^{-4}$	$3.6 \cdot 10^{-4}$	$2 \cdot 10^{-4}$	$1.4 \cdot 10^{-4}$	$1.1 \cdot 10^{-4}$
	$CMC_{exp.}$ (mol/l)	$1.6 \cdot 10^{-3}$	$3 \cdot 10^{-4}$	$3 \cdot 10^{-4}$	$3 \cdot 10^{-4}$	$2 \cdot 10^{-4}$	$1.1 \cdot 10^{-4}$
	$pCMC_{exp}$	2.8	3.5	3.5	3.5	3.7	3.95

Theory of mixed micelles[8]

In an ideal mixed system, the ideal CMC of mixed surfactant solutions can be predicted with the following term (Rosen, 2004):

$$\frac{1}{CMC_{ideal}} = \frac{\alpha}{CMC_{TX-100}} + \frac{1-\alpha}{CMC_{SDS}} \qquad (1.)$$

where, α – the mole fraction of TX-100 in the mixed solutions, and (1-α) – the mole fraction of SDS. According to Rubingh'snonideal solution theory (1979), the deviation of CMC_{exp} from CMC_{ideal} can be represented by the parameter β which also represents the interaction between Triton X-100 and SDS in mixed solutions. The more negative β-value indicates the stronger attraction and synergism between the Triton X-100 and SDS. The positive value indicates antagonism and if β=0 then the mixed micelle is ideal. β can be calculated as follows:

$$\beta = \frac{ln\left(\frac{\alpha CMC_{exp}}{X_{TX}^{M} CMC_{TX}}\right)}{(1-X_{TX}^{M})^2} \qquad (2)$$

whereX_{TX}^{M} is the mole fraction of TX-100 in the mixed micelles and can be determined from the following relationship which was derived by Rubingh (1979):

$$1 = \frac{(X_{TX}^{M})^2 ln\left(\frac{\alpha CMC_{exp}}{X_{TX}^{M} CMC_{TX}}\right)}{(1-X_{TX}^{M})^2 ln\left(\frac{(1-\alpha) CMC_{exp}}{(1-X_{TX}^{M}) CMC_{SDS}}\right)} \quad (3)$$

According Rubingh's regular solution theory for mixed micelles, the mixed CMC (CMC*) for TX-100+SDS systems can be estimated from the following equations:

$$\frac{1}{CMC*} = \frac{\alpha}{f_{TX} CMC_{TX}} + \frac{1-\alpha}{f_{SDS} CMC_{SDS}} \quad (4)$$

$$f_{TX} = \exp\left[\beta\left(1-X_{TX}^{M}\right)^2\right] \text{ and } f_{SDS} = \exp\left[\beta\left(1-X_{SDS}^{M}\right)^2\right] \qquad (5)$$

wheref_{TX} and f_{SDS} is the activity coefficients of surfactants in mixed micelle. In case of ideal behavior$f_{TX} = f_{SDS}$.Using the terms from 1 to 5 the synergistic parameters for mixed system SDBS-Triton X-100 and SDBS-$C_{12}E_9$ were calculated. The results are shown in Tables 3 and 4.

Table 3. Synergism data for mixed system SDBS-Triton X-100

α(TX-100)	**α(SDBS)**	**X^M_{TX}**	**X^M_{SDBS}**	**β**	**f_{TX}**	**f_{SDBS}**	**CMC***
0.1	0.9	0.43973	0.56027	-4.71806	0.2274	0.4016	$3.9 \cdot 10^{-4}$
0.25	0.75	0.526965	0.473035	-3.33243	0.474416	0.396387	$4 \cdot 10^{-4}$
0.5	0.5	0.623286	0.376714	-3.58019	0.60164	0.248879	$3 \cdot 10^{-4}$
0.75	0.25	0.725174	0.274826	-3.36323	0.775676	0.170576	$3 \cdot 10^{-4}$

Table 4.Synergism data for mixed system SDBS-$C_{12}E_9$

α($C_{12}E_9$)	**α(SDBS)**	**X^M_{TX}**	**X^M_{SDBS}**	**β**	**f_{TX}**	**f_{SDBS}**	**CMC***
0.1	0.9	0.5449	0.4551	-3.3415	0.5005	0.3708	$2.9 \cdot 10^{-4}$
0.25	0.75	0.7377	0.2623	-1.1453	0.9242	0.5361	$2.9 \cdot 10^{-4}$
0.5	0.5	1	0	1.9226	1	6.808	$3 \cdot 10^{-4}$
0.75	0.25	-	-	+	-	-	-

Negative *β*-values in Table 3 indicate the interaction (synergism) between SDBS and Triton X-100. Therefore, a bit stronger synergism for the mixed system SDBS-Triton X-100 is found for the lower mole fraction of Triton X-100. The synergism also can be found in the mixed system SDBS-$C_{12}E_9$ for the lower mole fractions of nonionic surfactants, while the higher concentration of $C_{12}E_9$ has no significant impact in mixed systems. For these reasons (maximum synergism) the values of mole fraction of Triton X-100 from 0.1 to 0.5 in the mixed system SDBS - Triton X-100 were chosen to investigate of the Ca(II) precipitation.

3. Precipitation of Ca(II) ions with anionic surfactant in the absence and in the presence of nonionic surfactant

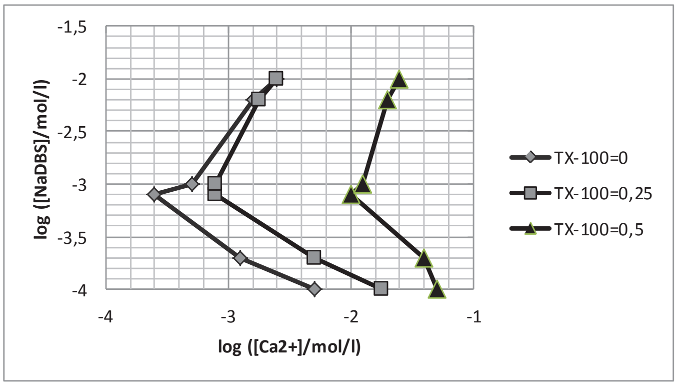

***Figure 1**. Precipitation boundaries for Ca(II) ions with anionic surfactant in the absence and in the presence of different mole fractions of nonionic surfactant at 25°C.*

Figure 1 shows that in all cases the addition of the nonionic surface active agent causes a shift of the precipitation boundary toward higher concentrations of calcium ions, which corresponds to an increase in tolerance toward salinity and hardness.

4. Precipitation of calcium carbonates ($CaCO_3$)

$CaCO_3$ was precipitated from the 100 ml 0.1 mol/l solution of $CaCl_2$ by adding an *equivalent quantity of Na_2CO_3 1 mol/l solution, in the absence and in the presence at different mole fraction of anionic (SDBS) and nonionic (Triton X-100 and $C_{12}E_9$) surfactants. The pH value of the solution is maintained at pH=11. Crystallization system is constantly mixed with the magnetic stirrer 1h (200 rpm). The specific molar conductivity was measured and the* results are shown in Figure 2.

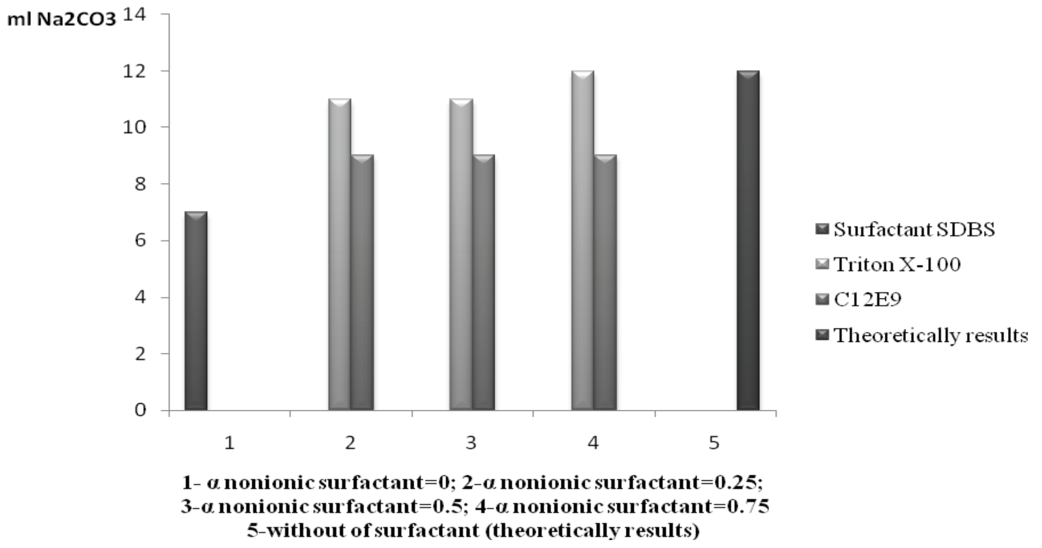

***Figure 2**. Comparison of precipitation efficiency for Ca(II) ions in the form of $CaCO_3$, in the presence of varying amounts of anionic and nonionic surfactants*

The results in Figure 2 show that theoretically the volume of Na_2CO_3 solution (taking into account the pH of the solution andK_{sp} for $CaCO_3$) required for the precipitation of Ca(II) ions presented in the solution is significantly different from the volume for the precipitation from micellar solutions. The most important decrease can be noted if the precipitation of Ca(II) ions occurs from solution which contains only anionic surfactant (in concentration above the CMC). This experimental result is probably a consequence of formation of hardly soluble salt $Ca(DBS)_2$ which *Ksp* is about 10^{-10}mol/l and it is lower than*Ksp* ($CaCO_3$)= $3.9 \cdot 10^{-10}$mol/l.

5. Spectrophotometric determination of Ca(II) ions with complexometric titrations in micellar solutions

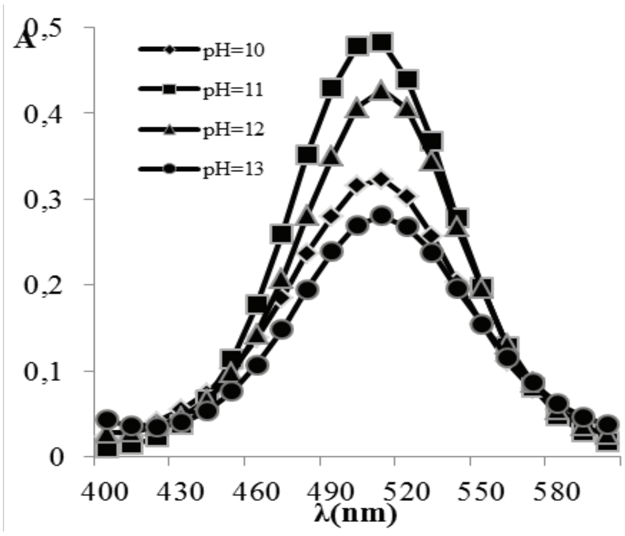

***Figure 3.** Spectrophotometric determination of the maximum wavelength (λmax) at the different pH values for colour 1 (Ca-murexide complex)*

The measurement was performed in the range of wavelengths 400-600 nm. The solutions were prepared by mixing 0.1 ml of 0.1 mol/l solution of $Ca(NO_3)_2 \cdot 4H_2O$ with indicator murexide. pH values of the solution were 10, 11, 12 and 13. The results in Figure 3 show optimal conditions for the spectrophotometry determination of Ca(II) ions with indicator murexide, λmax is 510 nm at pH=11.

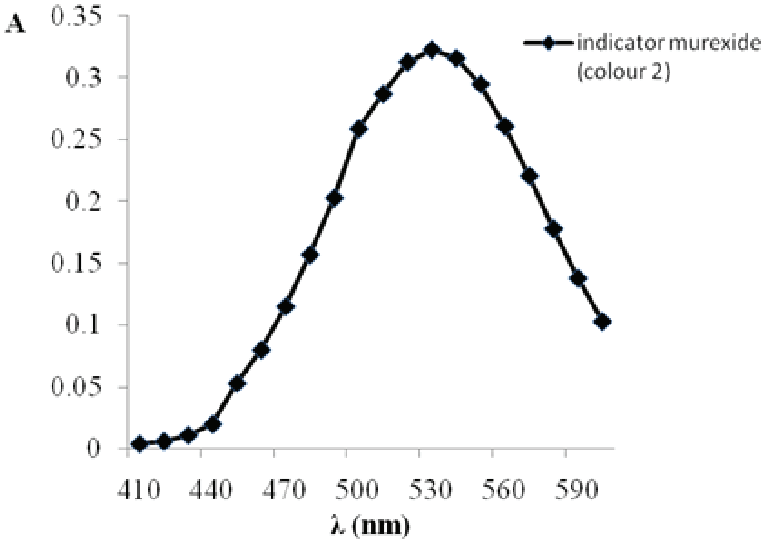

Figure 4.*Spectrophotometic determination of the maximum wavelength (λmax) at the pH=11 for colour 2 (free indicator murexide)*

Results in Figure 4 show that λmax for colour 2 (free indicator murexide) is 530 nm.

Table 5*. Comparison of results for spectrophotometric determination of Ca(II) using complexometric titration*

Present surfactant	**Volume of EDTA in equivalent point (ml)**
Without surfactant	1
SDBS	1
SDBS + Triton X-100	1.05
SDBS + $C_{12}E_9$	1.05

The solutions contained 0.1 ml 0.1 mol/l of $Ca(NO_3)_2 \cdot 4H_2O$ mixed with indicator murexide and various surfactants (at concentrations above the CMC). The pH value of solution was 11. Absorption was measured at two wavelengths: 510 nm (λmax for color 1) and 530 nm (λmax of colour 2) depending on the volume of the EDTA solution (0.01 mol/l). The results are shown in Table 4.

CONCLUSIONS

- Tolerance to the Ca(II) precipitation increases with synergism, e.g. achieving maximum concentration of hydrophilic components on the micelle surface through bonding at the micelle/solution interface.
- Addition of the anions (carbonates or sulphates) leads to formation of hardly soluble salts with Ca(II) and depends on the speed of formation and quantitative amount of formed centres of crystallization in micellar solutions.

- Addition of EDTA, during the volumetric determination of Ca(II) leads to formation of more stable Ca(II)-EDTA complex in micellar solutions compared to solutions without surfactants.
- Adsorption/desorption equilibrium which occurs at the micelle/solution interface actually define the extent of performance simultaneous processes: decomposition of Ca-metalochromic indicator and formation of Ca-EDTA complex.

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METAL POLLUTION ASSESSMENT IN SEDIMENTS OF THE SPREČA RIVER

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

Concentration of metallic elements (Al, Co, Cr, Cu, Fe, K, Mn, Ni and Zn) was determined in sediment samples collected along the Spreča River stream to assess the environmental pollution status of the river. Samples were taken at 12 positions from springhead to confluence into the Bosna River. Determination of the concentration of metal elements was performed by X-ray fluorescence (WDXRF). The pH value, electrical conductivity and content of organic matter were also measured. The sediments were found to be contaminated with Cr, Cu and Ni which has been attributed mainly to dispersion from major urban and industrial areas. The relatively high content of organic matter is mainly related to the high organic matter flux to sediments from direct discharge of domestic and industrial wastewaters. Electrical conductivity showed higher values in all samples, which indicate the high content of soluble salts in sediment. A high concentration of chromium and nickel were found in the sediment samples downstream from Lukavac town.

Key words: sediment, metal elements, pollution, heavy metals wastewater, X-ray fluorescent spectroscopy

INTRODUCTION

Sediments are defined as the material deposited at the bottom of rivers, which are silt and deposits[1]. The sediments contain organic and inorganic materials as well as solid particles derived from the weathering processes of sand, pebbles, silt, mud and fine-grained soil upstream and is susceptible to transport downstream by the water. Sediments in rivers have high ecological value because they are an integral and dynamic part of the water ecosystem forming diverse habitat and the environment. Sediments in aquatic environments have an important role in the nutrient cycle and are also responsible for the transport of essential nutrients and pollutants. Contaminated sediment can cause lethal and sub-lethal effects especially in benthic and other sediment associated organisms[2]. By studying sediments we can get information about the pollution of an investigated area at a given moment or in any historical period. Chemical analysis of sediments can serve as an efficient tool for the determination of chemical equilibrium and indirectly monitor the quality of aquatic ecosystems.

The surface water of an aquatic system is often the most polluted phase of the environment. This is because pollutants in the air and soil ultimately end up in the aquatic systems via local precipitation leaching from rocks, solid wastes and water surface run-off[3]. In polluted rivers, sediment serves as a pool that can retain metals or release metals to the water column by various processes of remobilization[4,5,6]. Numerous studies have demonstrated that the concentrations of metals in sediments can be sensitive indicators of contaminants in aquatic systems[7,8,9]. Discharges of inorganic and organic pollutants and radioactivity from various industrial, agricultural and domestic sources have resulted in permanently contaminated water, polluted sediments and the accumulation of chemicals in the aquatic food-chain[10]. An important part in determining capacity to carry away sediments is the speed at which water flows. Higher water velocity results in higher amounts of sediment. On the other hand, slower velocity has a lower rate of sediment movement. Metals are known to constitute highly persistent environmental pollutants, thus their tendency to remain as an environmental contaminant for a long period of time and to be magnified through the food chains is high[11]. Some of the metals such as calcium, magnesium, potassium and sodium are essential to sustaining life and must be present for normal body functions. While others such as cobalt, copper, iron, manganese, molybdenum and zinc are needed at low levels as catalysts for enzyme activities[12]. High contamination of aquatic systems with toxic heavy metals is a major concern as these elements are not biodegradable and may be immobilized within the stream sediment by main processes such us adsorption, flocculation and co-precipitation. In view of the persistence of many toxic organic and inorganic pollutants and their potential for bioaccumulation, sediments are regarded as an important source that seriously threatens natural ecosystems[13]. In Bosnia and Herzegovina, most domestic and industrial wastewaters from urban, industrial and rural areas are released into the environment without prior treatment. For more than four decades, the Spreča River receives all domestic and industrial wastewater directly from urban and industrial areas without prior treatment. Further, wastewaters from rural agglomerations and runoff from agricultural fields enter into this river. The agricultural lands are often flooded and the river water is used for irrigation crops, concerns have risen about contamination by toxic metals in this area and the potential impact on food systems and human health. The Spreča River is of particular importance for this area of Bosnia and Herzegovina, its valley contains the largest agricultural area. The current state of the Spreča basin watercourses is characterized by pollution from domestic, industrial, agricultural and landfill leachate wastewaters. Due to poorly developed infrastructure for flood protection, flooding of agricultural land in the coastal areas is frequent. Significant amounts of sediment remain on the fields after flooding. Since the sediments originate from different sources they could contain highly toxic metal elements that can have a long-term negative impact or completely destroy the wildlife in the Spreča River Valley. Previous research on the quality of the Spreča River[14] shows a strong evidence of pollution of this ecosystem, but no investigation has been conducted to characterize the status of metal pollution in sediment. The aim of this study was measuring the pH value, conductivity, total organic matter, metallic and non-metallic elements to determine the composition of the sediment, which would clearly establish the status of water ecosystems. Protection of sediment quality is an important part of monitoring and restoring the biological integrity of the Spreča River as well as protecting aquatic and human health.

MATERIALS AND METHODS

Study area: the Spreča River is one of the longest rivers in Bosnia and Herzegovina flowing 115,7 km through 11 communities, inhabited by about 483,425 residents, it covers about 10% of the territory and 25% of the population[15]. It flows into the Bosna River near Doboj town. According to the research[14], it is the most polluted river in the drainage basin of Bosnia and Herzegovina. The watercourse in the plain part, especially in the lower part of the river meanders. This process is supported by a small slope, narrowing the flow in some areas due to the disposal of different types of construction and demolition waste as well as large amounts of sediment taken in the alluvial plain of its tributaries. The most important tributaries of the Spreča River on its right side are: Bukovica, Bjelova, Krivača and Jala, and on its left side are: Mramorak, Sajtovica, Brod, Oskova with Gostelja, Sušica and Gribaja. The river downstream from Modrac Lake is a less developed river network, due to less water in its tributaries. Tributaries on the right in this section are: Lukavačka rijeka, Rašljevska rijeka, Sokoluša and Stanićki potok. The most important tributaries of the left side are: Prenja, Velika rijeka, Mala rijeka, Ponikva, Jadrina i Kamenička rijeka. The Spreča River has a catchment area of about 1945 km2. In the hydrographic system, the river network density is 550 m/km2 which means that rainwater passes an average path of 550 m before it flows into one of the streams in the basin[16].

Sediment sampling and analysis

Sediment sampling was conducted during the period (July, 2013) when water was in its base-flow regime. A total of 12 sediments samples (0-5 cm) were collected from main sampling points covering the full length of the Spreča River and the areas expected to be polluted: springhead (marked with S1), Kalesija town (S2), between Kalesija town and Živnice town (S3), Živinice town (S4), Spreca΄s bridge (S5), Modrac village (S6), Lukavac town (S7), Puračić village (S8), Miričina village (S9), Pribava village (S10), Klokotnica village (S11) and Doboj town (S12), collection locations marked as shown in Figure 1.

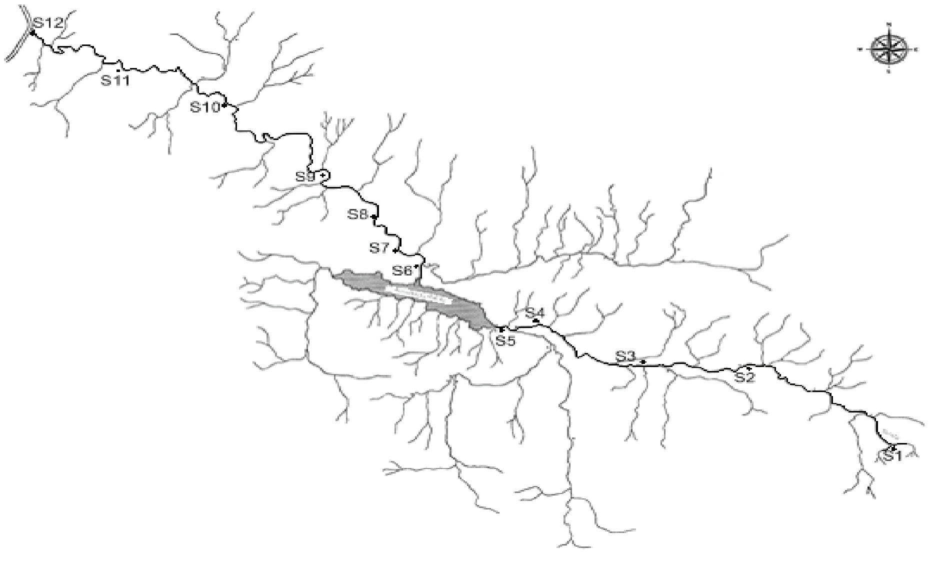

Figure 1. Sampling locations of sediments in the Spreča River

The sampling positions were recorded using GPS localization. The sediments were chosen as this layer controls the exchange of metals between sediments and water[17]. The samples for analysis were collected, prepared and stored, according to the procedures[18,19]. Each sampling area was approximately 50–100 m on both river banks. The surface sediment (0–5 cm depth) was collected with a flat hand shovel as sub-samples from several points with low current velocities, in order to obtain approximately 2 kg the finest-grained sediment. Taken samples were dried in the open air, crushed by hand in a porcelain mortar and sieved through a 2 mm screen. Air-dried <2 mm samples were stored in plastic bags for subsequent analysis. pH of sediments and conductivity was measured *in situ* in the wet sediments. Organic matter was determined by ignition at 550°C for 4 h in a muffle furnace according to standard methodology[20]. The percentage of loss on ignition was considered as Total Organic Matter (TOM). The analyses were made by spectrometry of wavelenght dispersive X-ray fluorescence (WDXRF) because this method is not destructive and the same samples can be analyzed by others methods[21,22]. The advantages of this method in term of sediment analysis is that samples are not destroyed or changed by exposure to X-rays and they can thus be saved for future reference or used for othertypes of testing that may be destructive. Also, samples can be examined with little pre-treatment, including almost all samples in solid state. Destructive methods, on the other hand, require dissolution procedures that are both time-consuming and costly in terms of the acids or other reagents required. The elemental content of samples was determined using S8 TIGER 4K spectrometer having an X-ray tube with Rh anode, two collimators (0.23° and 0.46°) and five analyzer crystals. SPECTRA^{plus} software packages for qualitative, standardless and quantitative analysis was used to interpret the XRF spectra. The accuracy and precision of results were evaluated by the standardless calibration of SPECTRA^{plus}. The sensitivity of method is 1xppm.

RESULTS AND DISCUSSION

Physicochemical analysis

Results of the physicochemical analyses are reported in Table 1 and Fig. 1. pH of the sediments is a measure of their acidity or alkalinity and is one of the stable measurements and also has the primary importance in deciding the quality of sediments. Sediment of the Spreča River exhibits a small fluctuation in pH values that range from 6,84 to 8,89 with an average of 8. The neutral to slightly alkaline pH, probably related to the carbonate nature of the sediment and the organic matter accumulation in sediment, seems to be favorable for the immobilization of heavy metals[23]. It is well known that electrical conductivity (EC) is a good measure of dissolved solids. High level of electro conductivity can affect both the adoption and the release of primarily heavy metals from sediments. Electrical conductivity shows higher values in all samples (483,5-1583,7 µS/Cm) compared to the previous research[24], these values indicate the amount of minerals existing in the water column as well as in sediment. This increase in conductivity is attributed to the high content of soluble salts in sediment[25]. Changes values of EC from S1 to S12 are related with changing in chemical composition of sediment as well as river water. High level of EC is associated with deposition of high dissolved salts in sediments it due to effluents discharged from various industries in catchment area of Spreča River. At sites S7, S8 and S9 are found the largest EC levels. Site S7 is approximately 3500 m away from confluence of Jala River into Spreče River. This right tributary is recipient of untreated industrial wastewater from salt factory Solana Tuzla, mentioned factory emitted large quantity of wastewater which consist: NaCl, Na_2SO_4, Na_2CO_3, $CaCO_3$, NaOH and $Mg(OH)_2$. Between sites S6 and S7 on the right side if Spreča River is a deposition pond for waste mud from Sisecam Soda Lukavac pond used as sedimentator, overflow water discharge in Spreča River without treatment. Waste mud consists: $CaCl_2$, NaCl and $CaSO_4$. Large quantities of wastewater from these two factories lead to high level of ions such as chloride, sulphate, calcium and magnesium in sediments from site S6 to

S12. Chloride ion (Cl$^-$) is completely soluble and very mobile, Cl$^-$ is toxic to aquatic life and there is no natural process by which chlorides are broken down. With another cations such as potassium or magnesium, Cl$^-$ toxicity increase. The presence of soluble salts also release metals from sediment into water which can lead to inhibit nutrients and dissolved oxygen within aquatic species rely on. It should be noted that between sites S6 and S10 during hot weather in summer no sign of aquatic life. Lower EC at S1 is not from anthropogenic sours it is indicates of highly silicates natural material. TOM is measured as 2,35- 58,90% with an average of 18, 1%. The relatively high content of TOM is mainly related to the high organic matter flux to sediments due to direct discharge of domestic and industrial wastewaters. High level of organic matter creates reducing conditions which leads fermentations with the release of chemical species of unpleasant smell. Extremly high content of TOM at site 5 is related to discharge of fine coal particles from coal mines.

Table 1. Physicochemical parameters of sediments from different stations

Stations	TOM (%)	pH	EC (µS/cm)
S1	2,35	7,16	483,5
S2	3,53	6,83	720,2
S3	10,2	7,58	814,7
S4	19,32	7,65	877,9
S5	58,90	8,20	984,6
S6	7,54	7,32	802,1
S7	10,64	8,52	1162,6
S8	13,81	7,95	1583,7
S9	18,42	8,34	1056,3
S10	37,31	7,90	990,4
S11	11,59	7,87	955,8
S12	23,37	7,91	920,0
Min.	2,35	6,83	483,5
Max.	58,90	8,34	1583,7
Mean	18,08	7,77	946,0

Concentrations of elements (Al, Co, Cr, Cu, Fe, K, Mn, Ni and Zn) in the Spreča River sediment are presented in Table 2. The criterion for the selection of the monitored elements was their possibility negative effect and in the case of K as a indicator of its fertilize origin. Heavy metal concentrations are compared with values reported from other rivers sediments in order to further evaluate the likely contamination in the Spreča River (Table 3). Of the various sampling sites, site S1 was found to be the least polluted in relation to the metals analysed. This was attributed to the fact that there were no anthropogenic activities at this point. For site S2, the increase in concentrations of the metals except Cu and Co was caused by the discharge of domestic and landfill leachate wastewater from Kalesija town and landfill Vis located close to it.

Table 2. Metal concentrations (mg/kg) in sediments

Stations	Al	Co	Cr	Cu	Fe	K	Mn	Ni	Zn
S1	3360,4	0,0	0,0	0,0	2875,2	1096,6	586,2	0,0	0,0
S2	39774,7	0,0	383,1	0,0	34814,0	11505,2	414,3	232,6	6,3
S3	53274,6	17,3	252,4	51,9	36964,7	13454,3	1201,1	144,6	94,7
S4	30249,1	20,4	448,1	57,4	18016,5	6527,1	612,6	161,9	100,3
S5	62630,8	44,7	583,5	84,6	47157,7	13291,6	1248,3	586,2	158,2
S6	30000,3	7,4	688,9	75,0	55284,1	5693,7	569,2	249,9	166,2
S7	47051,2	12,7	780,6	78,3	26798,9	12080,4	1307,2	1224,5	175,9
S8	39414,8	28,3	1806,0	75,8	30933,1	10982,2	774,4	334,0	148,3
S9	38626,3	32,5	1191,7	92,5	37262,6	7949,9	731,8	578,3	128,5
S10	38742,7	27,2	1016,6	109,4	38104,7	8450,4	941,7	631,8	141,3
S11	58593,0	30,6	952,5	121,6	63879,7	14027,9	3953,3	472,1	114,8
S12	54735,2	25,9	1202,0	91,1	45875,7	13075,7	1022,2	510,0	222,5
Min.	3360,4	0,0	0,0	0,0	2875,2	1096,6	414,3	0,0	0,0
Max.	62630,8	44,7	1806,0	121,6	63879,7	14027,9	3953,3	1224,5	222,5
Mean	41371,1	20,6	775,4	69,8	36497,3	9844,6	1113,5	427,2	121,4

Site S3, which is located about 8000 m away from site S1, had higher concentration of Mn, Zn, Cu, Co, K and Fe metals when compared to sites S1 and S2. Site S4 had higher concentrations of Ni, Zn, Cr, Cu and Co than sites S1, S2 and S3. High Ni concentration at S2 is probably associated with leachate from landfill Vis which located approximately 200 m away upstream from S2. Leachate from this landfill is discharged into the Spreča River without any treatment. Approximately 100 m away upstream from S2 is confluence of Dubnica creek, this stream is recipient of municipal wastewater from Kalesija town, also without any treatment. Distance between S2 and S3 is about 5000 m, this part of watercourse characterized small number of confluences with very low flow of water. Around this 5000 m of watercourse there are not anthropogenic activities which can be responsible for pollution of Spreča River, also agricultural activities are not so expressed near watercourse because these areas is often flooded. This is reason for decrease of Cr and Ni concentration from S2 to S3. Site 3 is located near Živinice town where the discharge of sewage sludge from residential areas and farms takes place. Site 5 is located downstream from Živinice before the Spreča River flows into Modrac Lake. Concentration increase of metal levels in sediment is associated to several anthropogenic activities and discharge of wastewater from residential, urban and industrial areas. The quality of sediment at S5 site is affected by domestic wastewater from Živinice urban area and the tributary river Gostelja, which is the main recipient of domestic wastewater from Banovici area as well as wastewater from the coal mines Banovići and Đurđevik. At this site, an extremely high concentration of organic matter was recorded. Measuring site S6 is located at a distance of about 400 m from the dam of Modrac Lake. At this site, concentrations of Mn, Cu, Co and Ni were lower when compared to site S5. Also, the concentration of organic matter at this site was the lowest. It can be concluded that a significant amount of sediment and organic matter and metals as its constituents remain in Modrac Lake. Sediment within Modrac Lake is not analyzed. Measuring site S7 is located at the distance of about 200 m from the point where the Jala River flows into the Spreča River. Site S7 had higher concentrations of Mn, Ni, Zn, Zr and Cr than sites S1 to S6. Concentrations of Cu and Cu were higher than at site S6. Contributing to high concentrations of these metals, specifically Mn, Ni and Zn, might be associated with the highly contaminated Jala River which is a reciver of domestic wastewater from Tuzla, the largest town in the Spreča catchment area and industrial wastewater. Relatively higher metal values were also observed at sites S8, S9, S10, S11 and S12 that are close to rural, urban and industrial areas. Activities in these areas contribute to sediment contamination of the Spreča River. The highest concentration of certain metal elements such as Mn, Zr, Cr, Cu and Co was recorded at those sites. Upstream of measurement site S8 are large industrial complexes. Based on the results obtained from this study, the concentrations of heavy metals in sediment sample for site S3 was slightly polluted, while the levels for other sites were moderate to heavily polluted. Heavy metal concentrations are compared with values reported from sediments of other rivers in order to further evaluate the likely contamination in the Spreča River (Table 3).

Table 3. Comparison concentration of heavy metals (mg/kg) obtained in this research with the sediment from different regions

Rivers	*Cr*	*Ni*	*Cu*	*Fe*	*Zn*	*Reference*
This study	*0-1806*	*0-1224,5*	*0-121,6*	*36497,3-63879,7*	*0-222,5*	
Danube River	*17,8-45,3*	*23,7-116,4*	*17,8-45,3*	-	*49,4-389,5*	*Milenković* et al.*(2005)*
Lianshan River	-	-	*29-106*	-	*114-797*	*Zheng* et al.*(2008)*
Hindon River	*42,9-250,4*	-	*9,4-195,1*	*221,2-237,0*	*3,9-85*	*Suthar* et al.*(2009)*
Han River	*2,5-13*	-	*7,6-14*	*14100-51200*	*34,3-55*	*Kim* et al.*(2011)*
Day River	*11,6-52,3*	-	*32,6-40,7*	*15670-36010*	*49,8-149,1*	*Barakat* et al. *(2012)*
Sava River	*45,3-186,0*	*102,0-141,9*	*32,0-47,1*	-	*93,1-127,2*	*ISRBC*[29]
Bosna River	*135,4-193,4*	*187,7-236,9*	*46,8-55,9*	-	*44,1-182,4*	
Vrbas River	*42,0-61,3*	*74,1-120,1*	*30,8-53,7*	-	*87,1-118,4*	
Una River	*51,9-70,5*	*74,1-110,3*	*30,0-73,6*	-	*88,2-123,6*	
Drina River	*51,1-128,3*	*86,7-130,1*	*34,5-59,4*	-	*73,4-357,8*	

The highest Cr concentration of 1806 mg/kg was observed in site S8, while Cr was not found at site S1. Also there was a significant increase in concentrations of chromium at sites S4 to S8. Chromium and its compounds are known to cause cancer of the lung, nasal cavity and paranasal sinus and suspected to cause cancer of the stomach and larynx[26]. Probable effect level is established at concentration of 197 mg/kg[27]. From the results of these analyses, the concentrations of chromium exceeded EPA maximum permissible limit[28] of 76,00 mg/kg from S2 to S12. The high Cr values detected at sites S2 and S12 suggests that the major source of Cr appears to be anthropogenic. Chromium at S2 probably comes from municipal wastewater of Kalesija town and leachate of landfill Vis. Down stream from S3 to S4 Spreča River is recipient of wastewater from open pit coal mine Dubrave and farm Spreča. Untreted municipal wastewater comes from many villages between Kalesija and Živinice town. Left tributaries Oskova and Gostelja are recipient of wastewater from coal mine Đurđevik and Banovići. These sours of pollution affect to increase of Co, Cr, Cu, Ni and Zn. Zinc concentrations ranged from 0,0 to 222,5 mg/kg with an average of 121,4 mg/kg. Compared to regional studies, the Zn ranges were lower than those of the Danube**[28]** and the Drina River[29]. Zinc is used in a number of alloys including brass and bronze, batteries, fungicides and pigments. Zinc is an essential growth element for plants and animals but at elevated levels it is toxic to some species of aquatic life[30]. The levels of Zn in the sediment samples exceeded the WHO guideline value[30] of 123 mg/kg at sites S5, S6, S7, S8, S9, S10 and S12, but did not exceed probable effect level[27] of 271 mg/kg. Iron is a major element in all sediment samples, the maximum concentration of 63879,7 mg/kg for Fe was observed at site S11, while the minimum level of 2875,2 mg/kg was detected at site S1. Elevated Fe concentrations can be associated with anthropogenic origin. The Fe content in the Spreča River is higher than the values reported in EPA guideline[31] value (30 mg/kg of Fe in sediment is acceptable[32]), while over 30 mg/kg may cause a condition known as haemochromatosis. The level (1224,5 mg/kg) of Ni in the sediment samples was highest at site S7, while at site S1 it was not present. Nickel is used mainly in the production of nickel-cadmium batteries, stainless steels, non-ferrous alloys and super alloys and electronic products. It is estimated that 8% of nickel is used for household appliances[30]. The WHO guideline value[30] of 20 mg/kg and toxic effect threshold[27] of 6 mg/kg showed that the concentrations of Ni in sediment samples of the Spreča River is very high and indicate possible high toxic pollution. The maximum concentration for manganese (1307.2 mg/kg) was observed at site S7, while the minimum level (414.3 mg/kg) was detected at site S2. Manganese is an essential element for plants and animals, in common aqueous species is predominantly Mn^{2+} and Mn^{4+}. Manganese can be adsorbed onto soil, the extent of adsorption depends on the organic content and cation exchange capacity of the soil[33]. The levels of manganese in the sediment samples exceeded the EPA limit[31] of 30 mg/kg. The source of manganese in the sediment of the Spreča River is from agricultural areas where Mn is used as fertilizer, varnish and fungicides. Another source is domestic wastewater from households where potassium permanganate is used as an oxidant for cleaning, bleaching and disinfection purposes. The maximum concentration of (121.6 mg/kg) for Cu was observed at site S11, while at site S1 was not presented. Copper is widely used in electrical wiring, roofing, various alloys, pigments, cooking utensils, piping, and in the chemical industry. Copper compounds are used as or in ammunitions, alloys, fungicides, algaecides, insecticides, wood preservatives, in electrical wiring, and electroplating. Copper compounds are added to fertilizers and animal feeds as a nutrient to support plant and animal growth. The levels of copper in the sediment samples were above the WHO guideline standard values[30] of 25 mg/kg for the survival of aquatic organism. The highest Cu content was detected in the downstream samples (S10 and S11) close to a main industrial discharge point suggesting an anthropogenic contribution to total Cu concentrations in the Spreča River sediment. The concentrations of Cu, Cr, Ni, Zn, Fe and Mn were compared with sediment quality guidelines to assess an environment condition and impact of industrial and economic activities in this area. Table 4 shows several guidelines which were used in this research such as: sediment criteria proposed by EPA[31], CBSOG Consensus –Based Sediment Quality Guidelines[34], New York Sediment Criteria for metals[35]and Provincial Sediment Quality Guidelines for metals[36,37,38]. Aluminium is the most abundant metal and the third most abundant element in the earth's crust, comprising about 8.8% by weight (88 g/kg)[39]. Aluminium can be highly toxic to aquatic biota, its toxicity is strongly depend on pH values, at pH below 6,5 Al is more toxic because low pH favours the formation and solubilisation of cationic aluminium (Al^{+}), highest toxicity occurring around pH 5,5 value[40]. Concentrations of Al in all sites are lower than[39] and pH values is between 6,83-8,34 on these facts we can concluded that Al has no negative impact on sediment quality. There is currently no quality guideline for aluminium. Potassium is a widely distributed non-toxic element which is an essential nutrient for plant and animal growth[41]. In the study, the potassium concentration of sediments ranged between (1096,6 to 14027,9 mg/kg) it is in the range of (1957,0 to 17700,0 mg/kg) [42] for major human activities prevalent in the area of agriculture. The high values of K is due to the leaching of K through rain water from the surrounded fields, which contain potash in the form of fertilizer. Under high K concentration the growth rate and photosynthesis of algae becomes strong and respiration decrease in river ecosystem.

Table 4. Comparison of average concentration of heavy metals (mg/kg) obtained in this research with sediment quality guidelines

Rivers		*Cu*	*Cr*	*Ni*	*Zn*	*Fe*	*Mn*
This study		*0-121,6 (69,8)*	*0-1806 (775,4)*	*0-1224,5 (427,2)*	*0-222,5 (121,4)*	*2875,2-63879,7 (36497,3)*	*414,3-3953,3 (1113,5)*
EPA (1999)- Sediment quality proposed	*Not-polluted*	*< 25*	*< 25*	*<20*	*<90*	*-*	*-*
	Slightly polluted	*25-50*	*25-75*	*20-50*	*90-200*	*-*	*-*
	Severely polluted	*>50*	*>76*	*>50*	*>200*	*-*	*-*
CBSOG SQG (2003)-Consensus –Based Sediment Quality Guidelines	*Threshold effect n concentration (TEC)*	*< 32*	*< 43*	*< 23*	*< 120*	*<20000*	*460*
	Probable effect on concentration (PEC)	*>150*	*>110*	*>49*	*>460*	*>40000*	*1100*
	Midpoint effect concentration (MEC)	*91*	*76,5*	*36*	*290*	*30000*	*780*
New York sediment Criteria	*Lowest effect level*	*16*	*26*	*16*	*120*	*20000*	*460*
	Severe effect level	*110*	*110*	*50*	*270*	*40000*	*1100*
ISQG (1992)-Interim Sediment quality criteria guideline	*Lowest effects range (ISQG-low)*	*65*	*80*	*21*	*200*	*-*	*-*
	High effects range - (ISQG-high)	*270*	*370*	*52*	*410*	*-*	*-*
DENSQ (2003) - Development and Evaluation of Numerical Sediment Quality	*Probable effect level level (PEL)*	*197*	*90*	*36*	*315*	*-*	*-*
	Toxic effect threshold threshold (TET)	*86*	*100*	*61*	*540*	*-*	*-*

CONCLUSION

Sediment of the Spreča River was analyzed along its course to investigate and analyze the properties and characteristics of the metal concentration distribution. The results of this study proved that the activities within the urban, rural and industrial areas are responsible for the elevated levels of the metals and organic content in the sediment samples. Therefore, it is clear that the concentrations of heavy metals showed pronounced levels of pollution. Although, part of the metals was probably a contribution from the geochemical weathering of the parent rocks around and their subsequent juvenile inputs into the river. The levels of Cu, Cr and Ni in the sediment samples were higher than the sediment guideline limits. The concentrations of Cu, Cr and Ni in sediment samples were much higher than levels reported in sediments from other rivers. It can be concluded that sediments serve as reservoirs for metals and their concentrations are controlled by a variety of physical and chemical factors. Because the heavy metals (Cu, Cr, Ni and Zn) exceeded concentrations given in different sediment quality guidelines, it is important to prevent further contamination by identifying sources of pollution and managing their input into the Spreča River. The present data on pollution in sediments in the Spreča River also points out to the need to establish a regular monitoring of sediment and agricultural soil in inundated areas as well as resources, and to further improve the domestic and industrial wastewater treatment methods. If the existing situation of contamination is allowed to continue unabated, it is most likely that the food web in the Spreča valley might be at the highest risk of induced heavy metal contamination.

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THE INFLUENCE OF DIFFERENT COMPOSITION AND THICKNESS ON PHYSICO-MECHANICAL, STRUCTURAL AND BARRIER PROPERTIES OF STARCH BASED EDIBLE PACKAGING FILMS

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT

Interest in maintaining food quality while reducing packaging waste has encouraged the exploration of the edible films. The present study investigates different composition and thickness effect of edible films of corn starch-based edible films on structural, barrier and mechanical properties. Films were obtained from solutions containing gelatinized starch, polyol, guar-xantan gum modified mixture and water by casting it on a Petri dish and evaporating water at room temperature for 72h. Sorbitol was used in three different concentrations (counted on dry starch basis) as polyol because it is compatible with starch improving film flexibility, facilitating its handling and preventing cracks. Two sample groups were obtained: 100 μm and 200 μm thick films. Within every group there were three additional subgroups regarding three different sorbitol concentrations used, which means that six film samples were obtained. Physical, mechanical, structural and barrier properties are determined: thickness, tensile strength and elongation at break, film structure (FT-IR) and water vapour and gas permeability. Films proved to be strong, moderately flexible with good barrier properties to oxygen and water vapour. Film thickness proved to be directly related to all tested film properties. The incorporation of plasticizers resulted in more flexible and manageable films. Mechanical strength of films depends on plasticizer addition resulting in increased tensile strength and elongation at break. Gas permeability decreases while water vapour permeability increases as plasticizer content of the film increases.

Key words: packaging, edible films, starch, composition, characteristics

INTRODUCTION

Edible films are thin layers of biopolymers that are used for the food packing. Edible films may be used to separate layers having different water activities retarding water transfer from one layer to the other[1], may be a barrier for O_2 decreasing oil or fat oxidation, could be used as a barrier for oil uptake in deep fat frying[2], used as a carrying agent for antimicrobial or functional substances[3]. Edible film coating with encapsulated antimicrobial substances can retard growth of microorganisms[4]. Predominant type of molecules in the film structure of biopolymers determines the basic physico-mechanical and barrier properties so there are edible films based on polysaccharides, lipids or proteins. Regarding polysaccharide group there are many sources used in the edible film production such as alginate, pectin, carrageenan, starch and cellulose derivatives. Starch is one of the most frequently used biopolymer for edible films production. There are many reasons why starch has being used for film making: the fact that could be obtained from large number of raw materials, its production costs are cheap, it is renewable and biodegradable biopolymer that has the ability to form films. Starch based edible films are tasteless, odorless and transparent, thus prevent a change of taste, flavor and appearance of food products[5]. Main advantages of starch films are excellent barrier properties to gasses (O_2 and CO_2)[6]. Moreover, starch films are better barriers to O_2 than CO_2 and they may act as selective barriers[7]. On the other hand there are some facts about starch based edible films that limit their usage.

Starch based edible films have weaker barrier properties to the water due to the high hydrophilicity. Also, films prepared from plain biopolymers are often too fragile to stand handling (bending, stretching…). To solve this shortcoming, starch has been blend with different biopolymers[8] or hydrophobic materials such as oils or waxes[7] which decrease interactions between the biopolymer chains (amylose and amylopectin) thus preventing their close packing which results in lower degree of crystallinity in the film[7]. By adding plasticizer, mechanical characteristics have been meliorated which results in elastic and flexible films, but this addition doesn't improve water vapour permeability (WVP). Thickness of the film has been reported to be inversely proportional to the WVP[9-11]. This phenomenon is explained to be originated from increased resistance of the film to mass transfer because of increased water vapor pressure on the film surface[9]. WVP decreases with increasing crystalline zones because permeation occurs through amorphous zones in the film. Plasticizer addition has been shown to retard starch crystallization which was the reason why WVP of the films with plasticizer do not change during storage[12]. The most common process to produce films on a laboratory scale is casting. Solvent is evaporated from the solution in order to form the film[13-15]. Environmental properties, such as temperature and air relative humidity, during the evaporation stage could be used to control some of the film properties[16, 17].

MATERIALS AND METHODS

Film preparation

Starch films were prepared by casting aqueous starch solution with different sorbitol concentrations. Aqueous solution of 5% (w/w) maize starch was prepared and heated at 90 ^{0}C for 60 minutes in a water bath. A weight of sorbitol equal to 70, 85 and 100% of the original starch was added and the solution was kept hot with mechanical stirring for 10 more minutes. Finally, guar-xantan modified mixture was added in a portion of 0.5% to initial starch weight. Guar-xantan modified mixture had role to enable better film folding and handling. The film-forming solution was degassed under vacuum to remove dissolved air and then cast into Petri dishes. The Petri dish was coated with film forming solution on a leveled surface and left to dry at room temperature for 3 days. Two sample groups were obtained: 100 µm and 200 µm thick films. Within every group there were three additional subgroups regarding three different sorbitol concentrations used, which means that six film samples were obtained:

S100/70: 0.100mm film with 70% sorbitol
sorbitol
S100/85: 0.100mm film with 85% sorbitol
sorbitol
S100/100: 0.100mm film with 100% sorbitol
sorbitol

S200/70: 0.200mm film with 70%
S200/85: 0.200mm film with 85%
S200/100:0.200mm film with 100%

Mechanical properties

Film thickness was measured using a micrometer with sensitivity of 0.001 mm. Ten-thickness measurements were carried out on each film, from which an average was obtained. Tensile strength (TS) and elongation at break (EB) of films were measured on an Instron Universal Testing Instrument (Model No 4301, Instron Engineering Corp.), according to ASTM D882-01[18]. TS (MPa) was calculated by dividing the given peak load by the cross-sectional area of the film. EB was calculated as the percent of change by dividing film elongation at the moment of rupture by initial gage length of the specimen (50 mm) and multiplying by 100. TS and EB measurements were repeated 10 times, from which an average was obtained.

Water vapor barrier properties

Water vapor barrier properties of films were determined gravimetrically according to the ASTM E 96-95 desiccant method[19].

Oxygen permeability

Oxygen permeability was measured using the Lyssy method, according to DIN 53 380[20] on a oxygen permeability tester (Lyssy GPM-200) with an appropriate gas chromatograph (GC-320, Gasukuro Kogyo) and an integrator (HP 3396, Hewlett-Packard).

Fourier transform spectroscopy

FTIR analysis of the film samples was carried out in the wave number range 4000 to 400 cm^{-1}, at a resolution of 4 cm^{-1}, using the IR spectrophotometer (Nicolet IS10, Thermo Scienticif) and attenuation total reflection (ATR) extension. IR spectrophotometer is controlled via a computer equipped with software Omnic.

Statistical analysis

Descriptive statistical analyses for calculating the means and the standard error were performed using MicroSoft Excel software (MicroSoft Office 2007). All obtained results were expressed as the mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Regarding film thickness, two groups of samples were obtained: 100μ (group 1) and 200μ (group 2) thick films and that is how samples were marked. Table 1 gives more detailed results.

Table 1. Film thickness (mm)

Sorbitol concentration	Group 1	Group 2
70	0.108±0.011	0.202±0.016
85	0.110±0.010	0.214±0.305
100	0.106±0.150	0.206±0.257

Successful repeatability of film making process has led to uniform film thickness values. Results indicate film thickness uniformity no matter the amount of sorbitol added. Film thickness is important because it influences mechanical and barrier characteristics of every packaging material.

Usually, in the mechanical testing of the film a stress-strain experiment is carried out where a film sample is stretched at a constant rate until it breaks. Thus, the films have to be plasticized to obtain more flexible and elastic films. Results related to mechanical properties are shown in Fig. 1a and Fig. 1b.

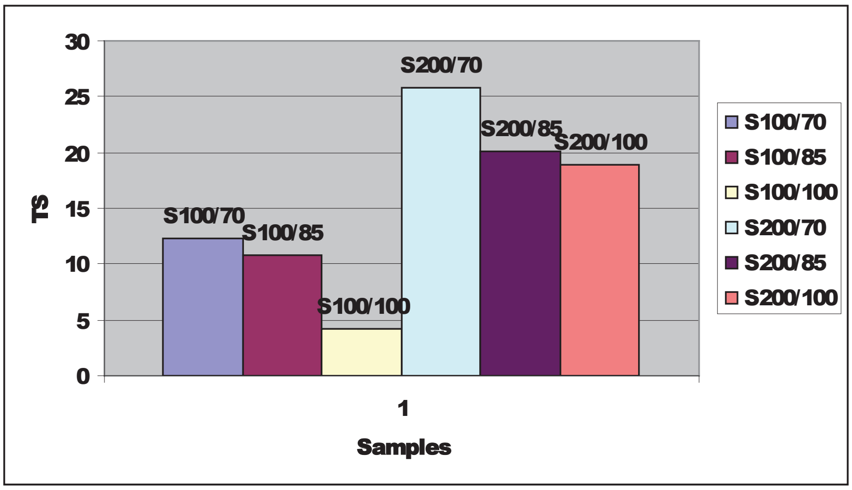

Figure 1a. Tensile strength of films with different composition and thickness (MPa) (Mean ± SD from 10 measurements): S100/70: 0.100mm film with 70% sorbitol; S200/70: 0.200mm film with 70% sorbitol; S100/85: 0.100mm film with 85% sorbitol; S200/85: 0.200mm film with 85% sorbitol; S100/100: 0.100mm film with 100% sorbitol and S200/100:0.200mm film with 100% sorbitol

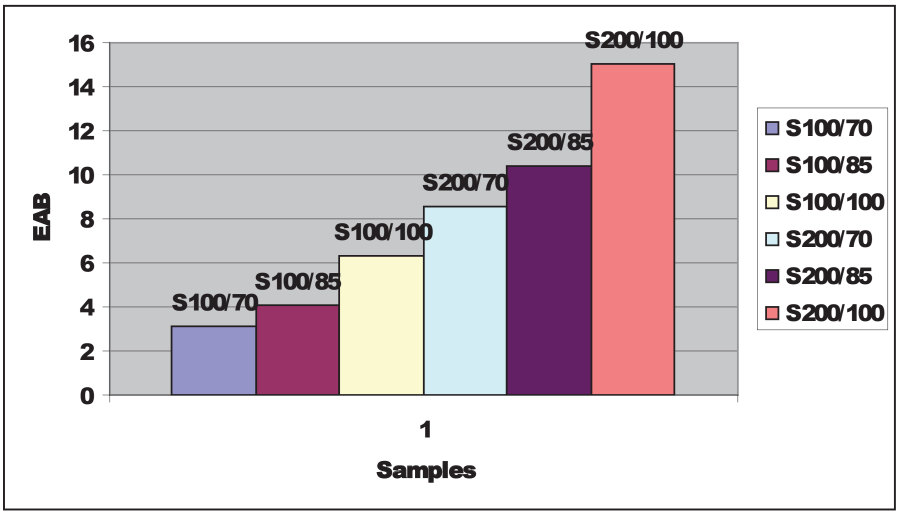

Figure 1b. Elongation at break of films with different composition and thickness (%) (Mean ± SD from 10 measurements): S100/70: 0.100mm film with 70% sorbitol; S200/70: 0.200mm film with 70% sorbitol; S100/85: 0.100mm film with 85% sorbitol; S200/85: 0.200mm film with 85% sorbitol; S100/100: 0.100mm film with 100% sorbitol and S200/100:0.200mm film with 100% sorbitol

Tensile strength value of tested films decreased due to plasticizer addition while elongation at break increased. Results are in agreement of other author findings[12, 21, 22]. Plasticizer addition resulted in more elastic and flexible films. Due to plasticization better handling properties may be obtained. Different film thickness also influenced mechanical behaviour. As the thickness of the films was higher there was the need for greater tensile strength and therefore elongation at break was greater compared to the thinner film samples.

Regarding to barrier characteristics results are presented in Table 2. Obtained data are similar for all films with same thickness. There are differences regarding film thickness where it could be observed that thicker films are better barrier to gasses. Generally, all films are better barriers to O_2 than CO_2 which is in agreement with findings of García et al.[7].

Table 2. Gas (ml/m^{2}24h) and water vapour permeability (g/m^{2}24h) of films with different composition and thickness

Samples	CO_2	O_2	N_2	air	WVP
S100/70	121.13	50.08	41.84	44.10	80.71
S100/85	114.22	47.83	40.05	39.98	86.14
S100/100	110.95	46.02	37.37	37.13	95.75
S200/70	105.78	45.16	35.92	38.47	64.36
S200/85	101.14	40.08	33.16	32.57	75.30
S200/100	95.46	37.19	21.74	28.55	89.65

Water vapour permeability of starch films has moderate values compared to gas permeability. Results are also presented in Table 2. Value of water vapour permeability increases as sorbitol has been added which is in accordance with findings of other authors[22, 23]. Film thickness is inversely proportional to the WVP which is also in agreement with other findings[9-11]. This phenomenon is explained to be originated from increased resistance of the film to mass transfer because of increased water vapour pressure on the film surface[9].

Infrared spectra of the films are shown in Fig. 2a and 2b. All spectra are similar (have the same shape) regarding film composition and film thickness.

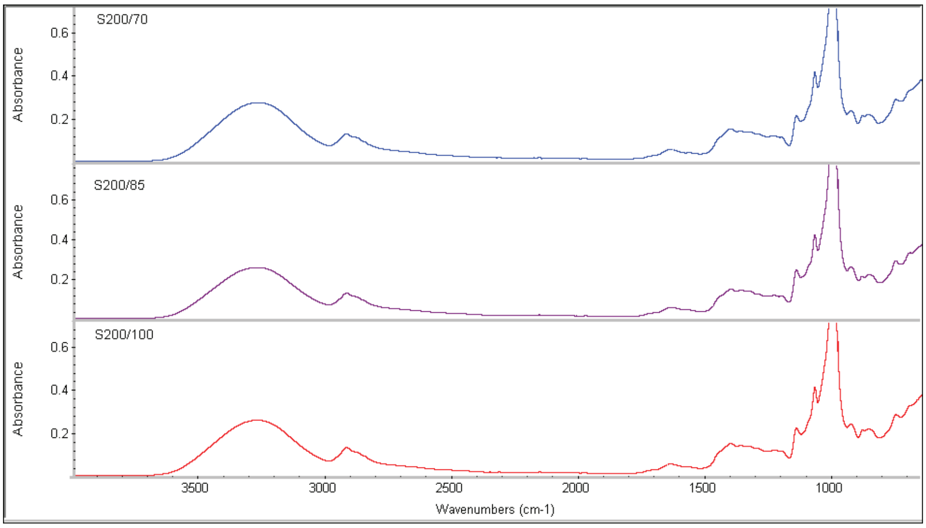

Figure 2a. IR spectra of 0.100mm thick films with different composition

It was expected that thickness would not affect spectra but slight differences of absorbance at about 3300 cm^{-1} were observed for all tested samples. These peaks correspond to monosubstituted alkynes. Very small differences in spectra are the result of different sorbitol addition. No matter differences, similar molecular structure was achieved.

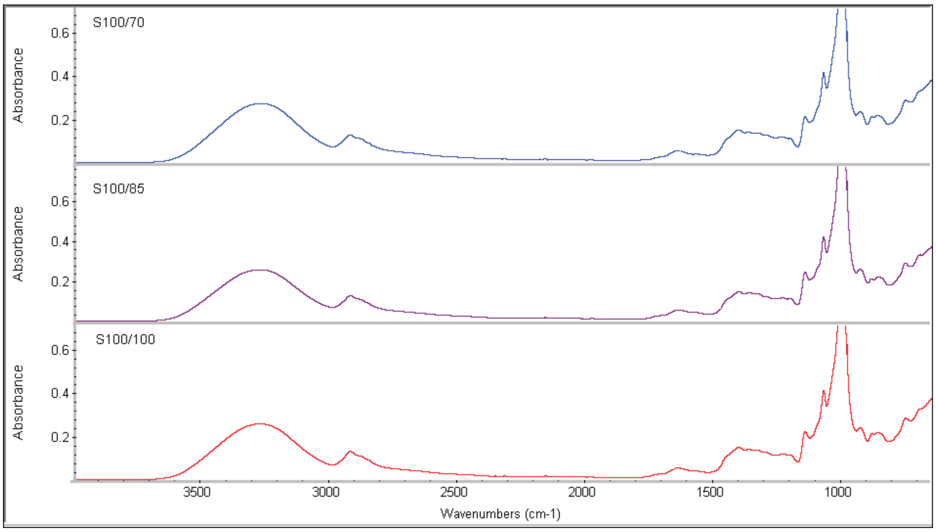

Figure 2b. IR spectra of 0.200mm thick films with different composition

CONCLUSIONS

Starch is one of the most frequently used biopolymer for edible films production. This work proved starch to be a good matrix-forming base. Some disadvantages of plane starch films could be overcome by adding plasticizers during film making. Sorbitol is a plasticizer compatible with starch and its addition meliorates mechanical and barrier properties. It was proved that sorbitol is needed to obtain cohesive films and that plasticizer content defines film properties. Regarding mechanical characteristics, sorbitol addition to films decreased tensile strength values and at the same time elongation at break values increased which resulted in more elastic and flexible films, easy to handle. As the thickness of the films was higher there was the need for greater tensile strength and therefore elongation at break was greater compared to the thinner film samples. Sorbitol addition also improved gas permeability by reducing permeability values for all tested gasses. Generally, all starch films are better barriers to O_2 than CO_2. Thicker films were better barrier to gasses than thinner. The biggest drawback of starch films is high water permeability due to high starch hydrophilicity so water vapour permeability of films has moderate values compared to gas permeability. Water vapour permeability value increases as sorbitol has been added. Thinner films proved to be worse barrier than films with higher thickness values. Structural characteristics proved to be similar for all tested samples (films) no matter composition or thickness change.

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PHYSICO-CHEMICAL AND MICROBIOLOGICAL CONTROL OF SOURCE WATER IN THE MUNICIPALITY OF BUŽIM

PROFESSIONAL PAPER

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ABSTRACT

In this work, researches on three sources were carried out in the Municipalitiy of Bužim, Una-Sana Canton. We analyzed samples of drinking water from three springs, of which one source was of untreated water, and the other two sources of samples were taken after treatment. Analyses were done during the first four months of 2012. In addition to this, every month a sample of tap water was collected from different structures of the Municipality of Bužim.

Physico-chemical and bacteriological analyses of these samples were carried out. The analyses were done at the Institute of Public Health of the Una-Sana Canton in compliance with the Safe Drinking Water Regulation (Official Gazette of Bosnia and Herzegovina number 40/10),Water Act (Official Gazette of the Federation of BiH number 70/06) and Food Act (Official Gazette of BiH number 50/04).

The analyses showed that this is water of good quality that meets these Regulations, except in February when a bacteriological analysis of water resources "Musići" was not in compliance with the Regulations.

In this sample of water, UFK-5 was identified and *E. coli* was isolated. Physical and chemical analyses of all samples had values that satisfy the above mentioned Regulations.

Key words: *springs, physico-chemical analysis, bacteriological analysis, UFK-5, E. Coli.*

INTRODUCTION

Supplying the population with drinking water is one of the basic prerequisites of good health and the right to drinking water is a basic human right. Drinking water, as well as a special section of natural waters, must possess certain characteristics, and most importantly, it must not be harmful to human health. This involves chemical and microbiological water quality and hygiene of water for human consumption as drinking water and for food preparation, but also for all other human activities that require high quality water. From the health aspect, physico-chemical and microbiological properties of water have a special place as they can have many negative effects. Medical control of water in Bužim for the Ministry of Health was carried out by the Institute of Public Health. The testing program complies with the Regulations on hygienic quality of drinking water, which defines the scope of testing, the frequency and number of samples of water supply systems, as well as the maximum allowable concentration of the studied parameters (MAC). In nature there is no chemically pure water, but any surface or ground water contains more or less dissolved salts (mostly calcium and magnesium salts), mineral and organic acids, insoluble salts, organic matter, dissolved gases, and mechanical impurities. The quality of natural waters is sensitive and changing and is becoming one of the factors limiting their use. Drinking water can be a carrier of toxic substances, pathogens and can cause poisoning or epidemic. Coliform bacteria are generally not pathogenic however, they are determined in water since they are constantly accompanied by pathogenic forms (Duraković, 2008). It is important to analyze the presence of nitrates in groundwater because it has been shown that they have adverse health effects on children, while some studies have indicated their carcinogenicity (Zebec and Senta, 2001). Another important source of pollution is agricultural land: plant protection products and pesticides whose chemical properties can be very different. The safety of drinking water is controlled according to the Safe Drinking Water Regulation in certified laboratories. The greatest number of samples of drinking water come from local water and individual water-supply systems (wells, tanks, cisterns, not captured sources), in which water is irregular or is not controlled. The individual water supply facilities often do not receive water chlorination. Common causes of chemical defects are elevated concentrations of heavy metals, ammonia, nitrate, and microbial elevated number of total bacteria.

MATERIALS AND METHODS

Material

In the study of water quality at the source in the municipality of Bužim, the samples were collected from three sites during the first four months of 2012. In addition, each month tap water samples were collected from different places in the Municipality of Bužim. The samples were presented in Table 1 and Table 2.

Table 1. Localities of source water samples

Number of samples	Location
1.	Reservoir "Eminići" (purified water)
2.	Pump station "Mulalići" (purified water)
3.	Pump station "Musići" (untreated water)

The analyses were performed in January, February, March and April 2012.

Table 2. Localities of tap water samples

Number of samples	Location	Month
1.	SZD „ Jabuka“ (a fountain)	January
2.	Izeta Nanića Street (a sample of water from a household)	February
3.	MSŠ “Hasan Musić“ (a tap in the kitchen)	March
4.	MSŠ “Hasan Musić“ (a tap in the bathroom)	April

Each month a sample of water from different locations was randomly collected.

Methods

The samples of water were collected under the appropriate field conditions in accordance with the regulations on water sampling. All tests were done in a laboratory of the Institute of Public Health of the Una-Sana Canton. Determination of physico-chemical parameters of the quality of water samples was carried out according to the standard methods (APHA, 1989 and Water Act, Official Gazette of FBiH 70/06). Table 3 shows the methods by which the analyses were carried out.

Table 3. Methods of testing water

Parameter	Unit of measure	Method
Temperature	^{o}C	Electrochemical
pH		Electrochemical
Electrical conductivity	µS/cm	Electrochemical

Fuzziness	NTU	Turbidimetry
Consumption of KMnO4	mg/lO_2	Vol. Kubel-Tiemann
Residual chlorine	mg/l	Spectrophotometric
Ammonia	mg/l	Spectrophotometric
Nitrate, NO_3	mg/l	Spectrophotometric
Nitrite, NO_2	mg/l	Spectrophotometric
Chlorides, Cl	µg/l	Spectrophotometric

The microbiological tests of water were conducted in compliance with the Safe Drinking Water Regulation (Official Gazette no. 40/10). The analysis of microbial quality of water consisted of determining the number of viable bacteria in water, and determining the presence of coliform bacteria and *E. coli*.

Method: Membrane filtration

RESULTS AND DISCUSSION

The physico-chemical analysis

The physico-chemical parameters that determine the quality of source water in Bužim Municipality in compliance with the Safe Drinking Water Regulation depending on the type of examination are presented in Tables. Tables 4 to 7 show the results for the four months in 2012.

Table 4. Results of physico-chemical parameters of water sources for January 2012

Parameter	Unit of measure	Reservoir Eminići	Pump station Mulalići	Pump station Musići	MDK drinking water
Temperature	°C	11,3	11,2	11,9	-
Ph		7,48	7,58	7,49	6,5 – 9,5
Electrical conductivity	µS/cm	357	381	377	2500
Fuzziness	NTU	0,39	0,50	0,61	1,0 NTU
Consumption of KMnO4	mg/lO_2	0,767	0,639	0,757	5,0
Residual chlorine	mg/l	0,177	0,233	--	0,5
Ammonia	mg/l	0,081	0,032	0,065	0,5
Nitrate, NO_3	mg/l	2,322	3,151	4,687	50
Nitrite, NO_2	mg/l	0,0002	0,0003	0,0005	0,1
Chlorides, Cl	mg/l	14,49	14,99	14,99	250
Iron	µg/l	13,37	15,0	6,68	200

Table 5. Results of physico-chemical parameters of water sources for February 2012

Parameter	Unit of measure	Reservoir Eminići	Pump station Mulalići	Pump station Musići	MDK drinking water
Temperature	^oC	9,0	8,2	8,9	-
pH		7,67	7,43	7,58	6,5 – 9,5
Electrical conductivity	µS/cm	391	347	398	2500
Fuzziness	NTU	0,32	0,45	0,40	max1,0NTU
Consumption of KMnO4	mg/lO2	0,512	0,639	0,639	5,0
Residual chlorine	mg/l	0,222	0,157	--	0,5
Ammonia	mg/l	0,122	0,125	0,118	0,5
Nitrate, NO_3	mg/l	3,084	4,443	3,696	50
Nitrite, NO_2	mg/l	0,000	0,0001	0,0002	0,1
Chlorides, Cl	mg/l	14,99	15,49	14,99	250
Iron	µg/l	22,83	23,97	14,18	200

Table 6. Results of physico-chemical parameters of water sources for March 2012

Parameter	Unit of measure	Reservoir Eminići	Pump station Mulalići	Pump station Musići	MDK drinking water
Temperature	^oC	12,4	9,6	11,3	-
pH		7,09	7,15	7,12	6,5 – 9,5
Electrical conductivity	µS/cm	389	371	383	2500
Fuzziness	NTU	0,78	0,98	0,81	1,0 NTU
Consumption of KMnO4	mg/lO2	0,639	0,895	0,512	5,0
Residual chlorine	mg/l	0,244	0,298	0,341	0,5
Ammonia	mg/l	0,119	0,171	0,086	0,5
Nitrate, NO_3	mg/l	2,337	3,874	3,909	50
Nitrite, NO_2	mg/l	0,0001	0,0006	0,0008	0,1
Chlorides, Cl	mg/l	14,99	14,99	15,49	250
Iron	µg/l	17,12	19,24	19,73	200

Table 7. Results of physico-chemical parameters of water sources for April 2012

Parameter	Unit of measure	Reservoir Eminići	Pump station Mulalići	Pump station Musići	MDK drinking water
Temperature	^oC	16	16,2	15,6	-
pH		7,54	7,59	7,48	6,5 – 9,5
Electrical conductivity	µS/cm	350	391	388	2500
Fuzziness	NTU	0,30	0,33	0,35	max1,0NTU

Consumption of KMnO4	mg/lO2	0,512	0,384	0,512	5,0
Residual chlorine	mg/l	0,167	0,165	0,179	0,5
Ammonia	mg/l	0,113	0,120	0,032	0,5
Nitrate, NO_3	mg/l	3,648	3,681	3,664	50
Nitrite, NO_2	mg/l	0,000	0,000	0,000	0,1
Chlorides, Cl	mg/l	13,99	13,99	14,49	250
Iron	µg/l	1,29	3,41	4,56	200

Table 8. Measurement results of physico-chemical parameters of tap water

Parameter	Unit of measure	SZD Jabuka (fountain) **January**	Ul. Izeta Nanića (household) **February**	MSŠ Hasana Musića (kitchen) **March**	MSŠ Hasana Musića (bathroom) **April**	MDK drinking water
Temperature	°C	12,2	9,6	--	--	-
pH		7,54	7,55	7,10	7,40	6,5 – 9,5
Electrical conductivity	µS/cm	382	379	384	380	2500
Fuzziness	NTU	0,42	0,89	0,28	0,48	1,0 NTU
Consumption of KMnO4	mg/lO2	0,515	1,023	0,512	0,512	5,0
Residual chlorine	mg/l	0,167	0,235	0,205	0,289	0,5
Ammonia	mg/l	0,114	0,085	0,129	0,056	0,5
Nitrate, NO_3	mg/l	3,957	2,305	4,621	2,946	50
Nitrite, NO_2	mg/l	0,000	0,000	0,003	0,000	0,1
Chlorides, Cl	mg/l	14,99	14,99	16,96	13,99	250
Iron	µg/l	10,27	8,47	4,56	4,23	200

The chemical composition of water from some sites suggests that the region has high-quality drinking water. The water temperature was in the range of 8 °C to 16 °C in February and April respectively, which was conditioned by the increase in the air temperature during the period in which the research was conducted. The analyzed water parameters confirm the exceptional purity of water. The physical parameters of water, especially conductivity, indicate the purity of water, and no pollution at all sites. Conductivity values are within tolerable limits 350-390 S/cm, and pH from 7.09 to 7.67 indicates that the water is neutral.

The values of residual chlorine in all samples have values allowed by the Regulation. The values of nitrate, nitrite, ammonia and other dissolved salts, which can be harmful or toxic to humans, are within the prescribed standards for the value of water. The amount of iron that was identified in the samples ranged from 1.29 to 23.97 mg/l, which is also in the allowable amount.

Microbiological analyses

Microbiological analyses done in samples are shown in Table 9 and Table 10.

Table 9. Microbiological analyses of the sources

		Number of colonies at 22 ^{0}C	Number of colonies at 37 ^{0}C	Coliform bacteria in 100 ml of water	*E. coli* in 100 ml of water
I	Reservoir Eminići	0	0	0	neg.
	Pump station Musići	0	0	0	neg.
	Pump station Mulalići	0	0	0	neg.
II	Reservoir Eminići	0	0	0	neg.
	Pump station Musići	6	41	5	poz.
	Pump station Mulalići	0	0	0	neg.
III	Reservoir Eminići	0	0	0	neg.
	Pump station Musići	0	0	0	neg.
	Pump station Mulalići	0	0	0	neg.
IV	Reservoir Eminići	0	0	0	neg.
	Pump station Musići	0	0	0	neg.
	Pump station Mulalići	0	0	0	neg.

The microbiological analysis showed that it is hygienically proper water except in February when from the bacteriological standpoint, the sample from the gas station "Musići" did not correspond to the Regulations for total fecal coliform bacteria that were isolated (chart 1).

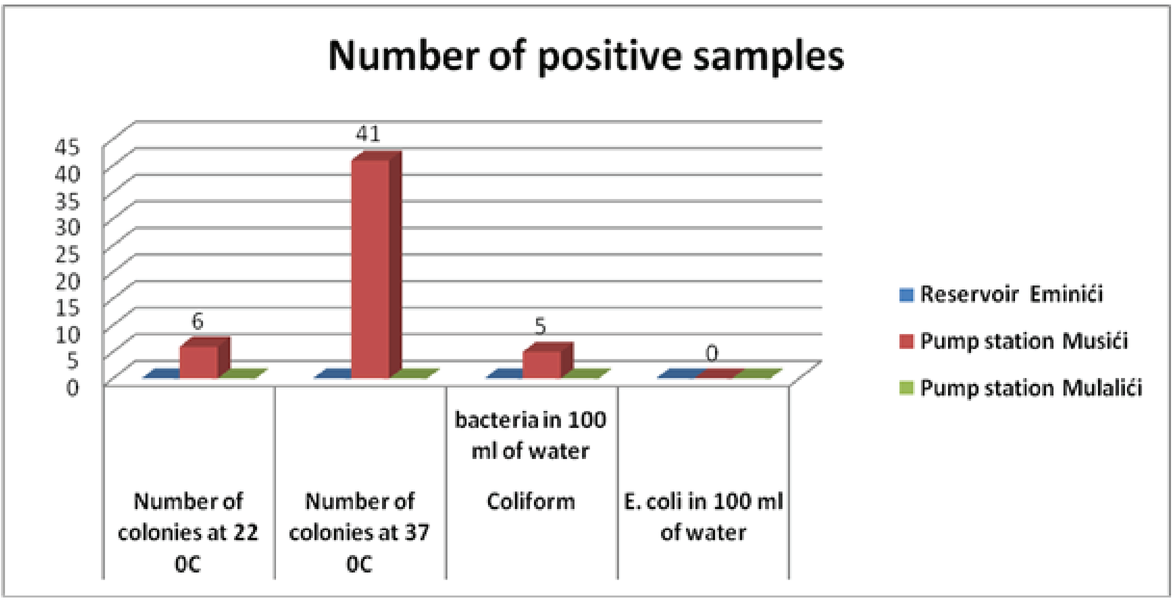

Chart 1. Isolated total fecal coliforms

Table 10. Microbiological analyses of tap water

		Number of colonies at 22^0C	Number of colonies at 37^0C	Coliform bacteria in 100 ml of water	*E. coli* in 100 ml of water
I	SZD Jabuka (fountain)	0	0	0	Neg
II	Izeta Nanića Street (household)	0	0	0	Neg
III	MSŠ Hasan Musić (kitchen)	0	0	0	Neg
IV	MSŠ Hasan Musić (bathroom)	0	0	0	Neg

The Results of the analysis of tap water collected from different sources proved to be hygienic.

CONCLUSION

Firstly, the measured parameters show that there are quality water sources in the municipality Bužim, which were mostly within the limits prescribed by the Water Act BiH except one source.

Secondly, for the purpose of a comprehensive review and final judgment on the quality of water sources tested in the Una Sana Canton, it would be necessary to collect samples more frequently and systematically explore the surrounding terrain in order to create a representative picture of quality, identify possible ways of contamination and predicted its possible changes.

Thirdly, given the state of surface and groundwater and the state of water pollution in general, it is important that the general and professional public realize the seriousness of the problem and the need for total protection of the environment, especially water as the most important factor in sustainable development. Bosnia and Herzegovina is one of the few countries in Europe and in the world, which has significant reserves of clean drinking water. In order to protect the health of current and future generations, it is necessary to prevent pollution of water resources, which requires the responsibility of each country and its population, as well as international cooperation.

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