Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

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Bulletin of the Chemists and Technologists
of Bosnia and Herzegovina

Print ISSN: 0367-4444
Online ISSN: 2232-7266

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CAPlus (Chemical Abstracts Plus):

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Glasnik hemičara i
tehnologa
Bosne i Hercegovine

*Bulletin of the Chemists and Technologists
of Bosnia and Herzegovina*

2024
Issue 63

Print ISSN: 0367-4444
Online ISSN: 2232-7266

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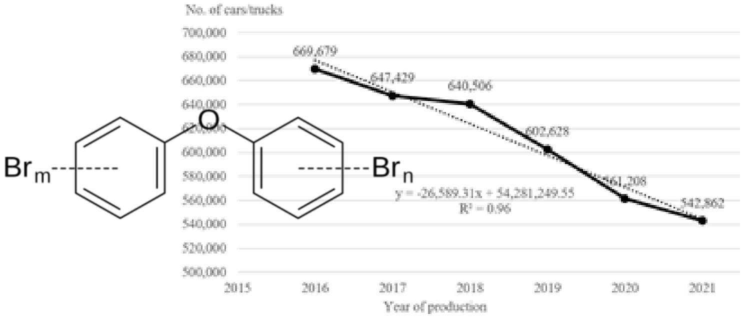
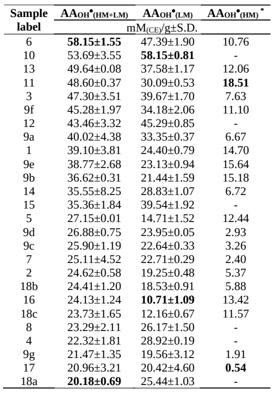
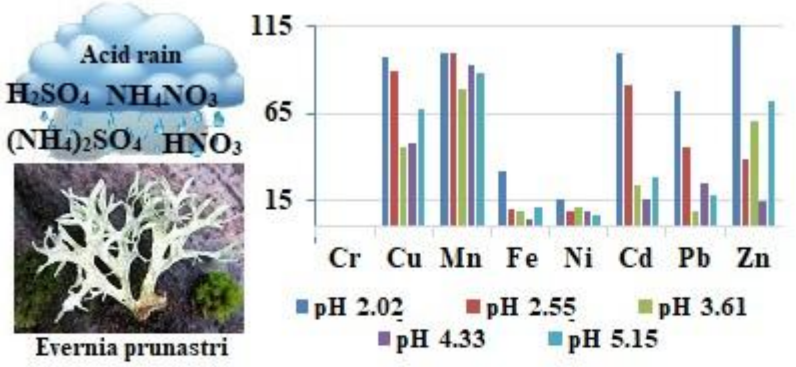




Sample label	$AA_{OH^{\bullet}(HM+LM)}$	$AA_{OH^{\bullet}(LM)}$	$AA_{OH^{\bullet}(HM)}$ *
		mM$_{(CE)}$/g±S.D.	
6	**58.15±1.55**	47.39±1.90	10.76
10	53.69±3.55	**58.15±0.81**	-
13	49.64±0.08	37.58±1.17	12.06
11	48.60±0.37	30.09±0.53	**18.51**
3	47.30±3.51	39.67±1.70	7.63
9f	45.28±1.97	34.18±2.06	11.10
12	43.46±3.32	45.29±0.85	-
9a	40.02±4.38	33.35±0.37	6.67
1	39.10±3.81	24.40±0.79	14.70
9e	38.77±2.68	23.13±0.94	15.64
9b	36.62±0.31	21.44±1.59	15.18
14	35.55±8.25	28.83±1.07	6.72
15	35.36±1.84	39.54±1.92	-
5	27.15±0.01	14.71±1.52	12.44
9d	26.88±0.75	23.95±0.05	2.93
9c	25.90±1.19	22.64±0.33	3.26
7	25.11±4.52	22.71±0.29	2.40
2	24.62±0.58	19.25±0.48	5.37
18b	24.41±1.20	18.53±0.91	5.88
16	24.13±1.24	**10.71±1.09**	13.42
18c	23.73±1.65	12.16±0.67	11.57
8	23.29±2.11	26.17±1.50	-
4	22.32±1.81	28.92±0.19	-
9g	21.47±1.35	19.56±3.12	1.91
17	20.96±3.21	20.42±4.60	**0.54**
18a	**20.18±0.69**	25.44±1.03	-



Editorial

Lithium in Shaping Modern Technology – Sustainability *versus* Controversy

Few topics in modern discourse draw as much focus as lithium. Lithium, the lightest of all metals and an alkali metal, is a silvery-white and highly reactive element renowned for its unique properties. As the strongest reducing agent, it owes its reactivity to low ionization and sublimation energies and the high hydration energy of its small Li^+ ion. Lithium reacts readily with water, nitrogen, and oxygen, even at room temperature. These distinctive properties make metallic lithium a cornerstone of modern technology and a critical element in the ongoing energy transition, enabling the shift away from fossil fuels to greener technologies. Its primary application is in lithium-ion batteries, which power a vast array of devices, from smartphones and laptops to electric vehicles. Lithium significance extends beyond batteries to pharmaceuticals in treating bipolar disorder, glass and ceramics in improving thermal shock resistance, industrial greases in increase of high-temperature stability, hydrogen storage and synthetic chemistry.

In public discourse, metallic lithium, its compounds, and lithium ores are often confused, leading to misunderstandings about the risks involved in lithium production. A common misconception equates the mining of lithium-rich minerals with the production of metallic lithium. However, mining ores like spodumene or lepidolite is no more hazardous than mining other metal ores, such as copper or iron. The actual challenges lie in the chemically intensive processes required to extract, purify, and refine lithium ores into usable compounds or metallic lithium.

Industrially, lithium is obtained either from brines or through chemical and electrochemical processing of ores. Extraction from brines involves pumping lithium-rich saline water into evaporation ponds, followed by chemical treatment of the concentrated brine to produce lithium carbonate, which can be further processed into lithium chloride. In contrast, lithium extraction from ores is more complex and chemically intensive, requiring roasting of the ore, chemical treatment to isolate lithium, purification to obtain lithium carbonate, and conversion into lithium chloride. Metallic lithium is then produced via high-temperature electrolysis of a eutectic mixture of LiCl and KCl at 750°C. These processes require large quantities of chemicals such as sulfuric acid, hydrochloric acid, sodium carbonate, and calcium hydroxide, posing significant risks of water and soil contamination if waste is not properly managed or processes are poorly executed.

While the mining of lithium ores itself shares environmental impacts common to all mining, such as deforestation, biodiversity loss, and soil degradation, the advanced stages of processing introduce these unique challenges related to water depletion, chemical waste generation, and contamination of surrounding ecosystems.

In Bosnia and Herzegovina, significant lithium reserves have been identified, particularly in Lopare and Ugljevik, with an estimated 1.5 million tons equivalent to lithium carbonate. Explorations are ongoing in areas such as Čajniče, Jezero, Šipovo, Bijeljina, Zvornik, and Brčko, encompassing a total of 20 potential sites. In Serbia, the Jadar Valley contains one of the world's largest lithium deposits, featuring the unique mineral jadarite—a lithium and boron mineral critical for battery and glass production. The Jadar project, led by Rio Tinto, is projected to meet over 10% of global lithium demand. However, the project faces substantial opposition due to concerns about potential soil and water contamination, with environmental activists and local residents rightly worried about its long-term impact on the environment.

Neither Bosnia and Herzegovina nor Serbia currently have the capacity to process lithium ores into high-value final products. Existing infrastructure supports only mining and the extraction of lithium-rich compounds like lithium carbonate or lithium chloride, which are exported to developed countries for further refinement and battery production—processes that carry significant economic value. This dependence limits the financial benefits for domestic economies and shifts most of the economic value abroad. It also raises questions about whether the potential economic gains justify the environmental and social costs of mining, as extraction and chemical processing can impose significant burdens on local ecosystems and communities.

Editors

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2024

63

1-10

Print ISSN: 0367-4444
Online ISSN: 2232-7266

Original Scientific Article
DOI: 10.35666/2232-7266.2024.63.01
UDK:543:903.2(497.6 Ilijaš)

Preliminary archaeometry investigation of artifacts from the Medieval Bosnian town of Dubrovnik (Ilijaš, BiH)

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Article info
Received: 28/02/2024
Accepted: 24/09/2024

Keywords:
Archaeometry
Medieval Bosnia
FAAS
SEM-EDS
Dubrovnik
Golden thread

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Abstract: The aim of this paper was to analyse archaeological artifacts found in the medieval town of Dubrovnik, near Sarajevo, using FAAS and SEM-EDS techniques. A total of 11 samples of different types were chosen: ceramic sherds, an iron nail, slag, and a piece of brocade thread. The results of the ceramic samples showed high levels of iron and manganese, most probably due to the geological bedding of the location. Trace amounts of chromium, copper, nickel, lead, and zinc further support this assumption, which means that the clay used to make ceramic was supplied in the vicinity of Dubrovnik. A painted sample of green ceramic had high levels of lead, suggesting that this metal was deliberately added to strengthen the properties of the vessel. SEM-EDS measurements of the slag showed the presence of As, Hg and Sn, and one possible explanation is that they were used in gold or silver refining processes, which according to historical reports were performed in Dubrovnik. A piece of golden thread has proven useful in determining the time frame of its origin. EDS measurement showed that the type of the manufacturing process used in the production of this golden thread did not exist before the 14th century.

INTRODUCTION

During the Medieval times, the territory of present-day Bosnia and Herzegovina (BiH) was at the crossroads of armies, politics, religion, and trade. Trading documents from the medieval period are probably the most important source for the researchers and historians of medieval Bosnia. From there, they can find out a lot about trade routes, types of merchandise and the places named. Most of the time, these place names have yet to be ubicated, but some are well known and located, although the sources about them are scarce and border on legends and myths. One such location is Dubrovnik, a medieval town, 30 kilometers northeast, of Sarajevo, the capital of Bosnia and Herzegovina. Its origin is shrouded in mystery and unknown facts that have yet to be discovered. But, based on the folk legends, the town was founded back in those days when the Bosnian ruler Kulin Ban gave permission to two Dubrovnik merchants to exploit mines and ores between the city of Olovo and Jagodna mountain, who then erected the city and smelted iron and silver ore, mined from the above-mentioned mountain (Filipović, 1924). The main objectives of this study were: (1) to determine the content of the selected metals: Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn in ceramic fragments, from the location of the medieval Bosnian town Dubrovnik by flame atomic absorption spectrometry technique (further FAAS); (2) to determine the composition of ceramic fragments, slag, and piece of golden thread using scanning electron microscopy and energy dispersive X-ray spectroscopy technique (SEM-EDS); (3) based on the results obtained by two techniques used to gather at least some knowledge about the medieval lifestyle of the inhabitants who lived at the location of Dubrovnik.

In addition, this paper presents the first results of the study of the ceramic potsherds, iron slag and accompanying artifacts excavated from Dubrovnik, in 2017 archaeological campaign.

Location

The old town of Dubrovnik is situated on a hill above the river Misoča, which is created by the confluence of two streams Zenik and Rijeka, at an altitude of 882 meters (coordinates: 34TBP8641274566, Figure 1.). It is oriented east-west and looks as if it had a well (cistern) in the town center (Mazalić, 1939). Many medieval necropolises are in the vicinity, the most famous being Kopošići (Filipović, 1924). Unfortunately, there are not many historical accounts of the formation and history of this town. The first information about Dubrovnik dates back to 1404. Dubrovnik is also mentioned in 1468/69., that is, only a few years after the Ottomans conquered these regions (Aličić, 2008). The next mention of Dubrovnik come from 1503 and 1519, that is, two peace treaties concluded between the Hungarian kings and the Ottoman Empire. The last mention of Dubrovnik dates from 1709, when the mosque in Dubrovnik was abandoned, and since there was no crew, and apparently no residents in the suburbs, it was not repaired (Kreševljaković 1953). By that time, the area of Dubrovnik and its surroundings had lost its military, economic, and commercial importance, so it was abandoned, and the population moved to much lower areas closer to new communications. Dubrovnik was among a few trading places (*trgovište* in Bosnian) in medieval Bosnia that existed in the second half of the 14th and the first half of the 15th century, but after the Ottoman conquest, which happened in the 1463, the number of these trading places was halved (Anđelić,1963).

MATERIAL AND METHODS

All artifacts that were part of this study were collected during the 2017 archaeological campaign. A total of 11 samples were chosen for analysis, based on their significance and importance; more valuable artifacts, like brocade thread and painted ceramic (K10) were chosen for non-destructive analysis by SEM-EDS technique. Although a small number of artifacts were included in the analysis, nevertheless they will provide valuable first data for any subsequent analysis or research in this area. Table 1. summarizes the artifacts that were part of this study, with short descriptions of each of them. Figure 2. shows the material used in this study.

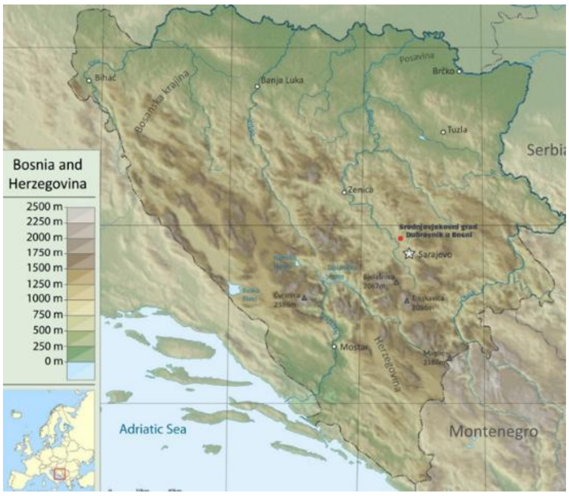

Figure 1. Location of Medieval town of Dubrovnik in Bosnia

Only incomplete, broken ceramic fragments were utilized for the analysis. Into the open PTFE vessel containing 0.2 g (± 0.2 mg) of finely powdered and homogenized sample, 25 mL of concentrated HNO_3 was added. After the evaporation of nitrogen oxides, the reaction vessel was sealed and left to react for 12 hours at 60°C. The dissolved samples were then adjusted to 50 mL with Milli-Q water. For SEM-EDS observations, a JEOL JSM-6610LV microscope was used. Before the analysis, all the samples were carefully washed with ethanol, in order to clean the surface used for analysis from soil and dirt, accumulated over time. Previously, a thin coating of Au was added to the non-conducting samples (ceramic samples) using a coating device (LEICA SCD005). As for the quantification, elements with $Z \leq 5$ could be quantified, with a detection limit of approximately 0.1 mass%, and a resolution of 126 eV.

Table 1. Artifacts used during this study

Sample	Type of sample	Short description of samples
Dub 1	Black ceramic	Fragments of coarse clayed ceramic, probably fired under reduction conditions, as can be seen by color and from roughly shaped vessels. White, different sized inclusions of rock suggest use of low-quality, unpurified clay for molding and baking of pottery vessels.
Dub 2	Black ceramic	
Dub 3	Black ceramic	
Dub 4	Black ceramic	
Dub 5	Black ceramic	Fragment of vessels edge, fine ceramic, better quality clay. Reducing conditions.
Dub 6	Grey ceramic	Fragment of grey, unglazed ceramic. Reducing conditions.
Dub 8	Red ceramic	Fragment of red, partially glazed ceramic, black colored inside. Probably fired under combination of reduction and oxidation conditions, since the red glaze color is not uniform.
RF	Slag	Greyish black piece of metallic slag, resembling iron type slag.
K10	Glazed pottery	Green glazed type of Islamic pottery, fine grained clay.

Figure 2. Samples from the Dubrovnik used for the analysis

Quality control

Recovery evaluations were conducted for all types of samples to ensure the accuracy of the research. The analyzed samples were spiked with a standard solution of each metal at three different levels of concentration to cover the measurement range. The recovery values ranged from 89% to 101% for all metals determined. The recovery values are presented in Table 2.

Table 2. Recovery values for spiked samples

Metal	Recovery (%)
Cd	98
Cr	96
Cu	90
Fe	99
Mn	97
Ni	89
Pb	98
Zn	101

Three times the standard deviation of the blank solution signal was used to calculate the detection limits (LOD). The LOD values were: Cd (0.002 mg/L), Cr (0.006 mg/L), Cu (0.003 mg/L), Fe (0.006 mg/L), Mn (0.003 mg/L), Ni (0.01 mg/L), Pb (0.01 mg/L), Zn (0.001 mg/L).

RESULTS AND DISCUSSION

The obtained results will be presented and discussed separately for FAAS and SEM-EDS measurements. The challenge of comparing the results will be complex due to the lack of previous similar analyses in B&H. Therefore, interpretation and discussion will involve reference to neighboring countries and Europe, focusing on the same period and similar ceramic and slag types.

Results obtained by FAAS technique

In the Table 3. results for the samples measured by FAAS technique are presented.

The results obtained by the FAAS technique, for the measured metals, most probably reflect the specific geological background of this geographical area. In the case of the ceramic samples DUB 1 to DUB 5, iron (Fe) was the most abundant element, particularly in the sample DUB 5 where the level of Fe exceeded 5000 µg/kg. Manganese was another metal that was found in significant quantities in the above-mentioned samples. The results for these two metals could potentially be explained by the fact that Dubrovnik is situated between two geological zones, Vareš and Čevljanovići, which are known for their rich deposits of iron and manganese minerals. Braunite, a manganese-silicate mineral, is particularly abundant in these zones, while hematite (Fe2O3) is a commonly associated mineral in these deposits (Vujanović, 1962). Vujanović also reports significant quantities of lead, chromium, copper, and nickel in the primary minerals of the area. Therefore, the presence of these elements in the ceramic samples can be attributed to the geological composition of the clay used in pottery production. Regarding zinc, its presence in the samples can also be explained by the geological setting of the location. Significant amounts of zinc have been found in the Borovica – Vareš-Čevljanovići geological zone at a few locations (Operta & Huseyni, 2016).

Results obtained by SEM-EDS technique

DUB 6, DUB 8 and K10 ceramic fragments; RF slag of iron type, an iron nail, and a piece of golden brocade thread were chosen for the analysis by SEM-EDS technique, and their results are presented in Table 4, 5 and 6, respectively.

Table 3. Results obtained by FAAS technique for five ceramic samples from Dubrovnik.

Metals content/ Sample	**µg/kg**							
	Mn	**Fe**	**Cr**	**Cu**	**Ni**	**Cd**	**Pb**	**Zn**
Dub 1	195.8	4856.0	49.9	14.9	41.5	0.79	14.6	44.8
Dub 2	1284.8	5046.9	10.8	10.8	22.8	3.5	227.3	693.3
Dub 3	158.3	4639.0	37.4	17.1	28.8	< LOD	32.1	65.9
Dub 4	203.4	4703.1	33.1	14.1	36.8	<LOD	11.9	39.6
Dub 5	196.6	5172.4	31.7	16.0	25.8	0.25	32.2	130.5

**<LOD* below the limit of detection

Table 4. Results for the SEM-EDS analysis of samples DUB 6, DUB 5 and K 10 (in wt.%).

Sample	**DUB 6**			**DUB 8**	**K10**	
	Site 1		**Site 2**	/	/	/
Spectrum	**1**	**2**	**1**	**1**	**Glaze**	**Body**
O	55.28	32.59	55.84	55.89	35.05	58.33
Mg	0.65	0.75	0.47	0.79	1.27	0.69
Al	13.94	18.35	8.84	8.33	3.9	7.18
Si	22.3	35.57	28	22.23	13.22	18.97
K	2.47	3.69	1.89	1.1	0.36	2.22
Ca	1.47	2.42	1.06	4.52	3.05	8.39
Ti	<LOD	0.56	<LOD	0.42	<LOD	0.48
Fe	2.73	4.71	2.9	5.98	1.3	3.42
Cu	1.16	1.37	1	0.74	4.48	<LOD
Cl	<LOD	<LOD	<LOD	<LOD	<LOD	0.31
Pb	<LOD	<LOD	<LOD	<LOD	37.37	<LOD

**<LOD* below the limit of detection

K10 sample was a typical representative of green Ottoman glazed pottery from the areas ruled by Ottoman Empire for several centuries. A fragmented, green glazed sherd, with a crack at the surface of the glaze was chosen as a suitable for the analysis (Figure 3).

The glaze itself, at the surface of the ceramic has a twofold role. The first and more important is to prevent absorption of liquid and corrosion, while the other is purely decorative (Oztoprak et al., 2016). It mostly consists of silicon dioxide, as the primary constituent that gives it a glassy appearance, and metal oxides, which give distinctive colors (Yoon et al. 2001, 253; Oztoprak et al., 2016). As for the Ottoman glazed pottery, it is known for its high lead content, with the green coloring coming from the presence of Cu^{2+} and Fe^{2+} ions in the glaze matrix (Oztoprak et al., 2016; Correia & Chaves, 2018, 1160; Kuzmanovic et al., 2021). The analysis of glazed ceramics is in most cases focused on two areas: the glaze itself and the actual body of ceramic, i.e., the area beneath glaze, since they represent two separate parts, with different ratios and content of metals. In our work, we also took the same approach.

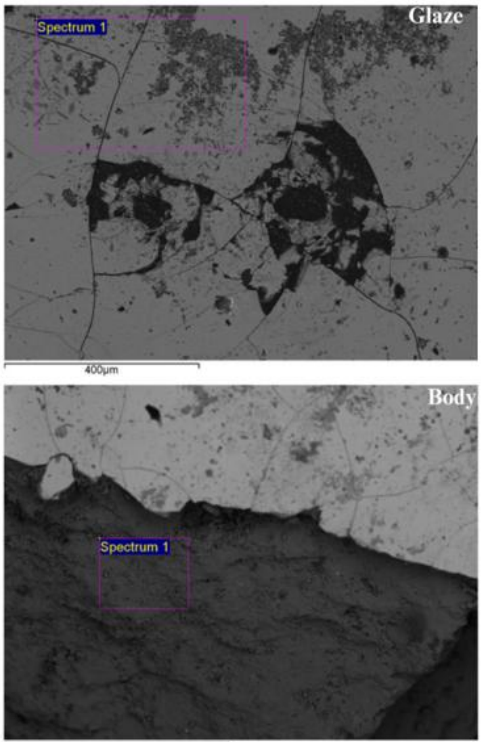

Figure 3. EDS image of the sample K 10

The glaze from Dubrovnik showed a high value for copper, resulting in a distinctive green color. Based on a large dataset of different medieval ceramics, analyzed by Tite et al. (1998), Dubrovnik ceramic can be grouped into lead – alkali type of the medieval ceramic, where the content of lead oxide is 25-35% and alkali content (soda plus potash) are 5-10%. On the other hand, the inner part showed no traces of lead or copper, so there was no transfer of Pb or Cu ions from the glaze to the inner parts of the ceramics. Additionally, the red coloring of the inner part could be due to the presence of Fe3+ ions, in the reducing atmosphere during the production process (Lyubomirova et al., 2017). Iron, although a minor constituent of ceramic, can give valuable insight into the nature of the raw materials used and firing conditions, such as the temperature during baking a clay (Wagner & Wagner, 2004). The results for this sample are in accordance with the results from Bulgaria (Yoleva et al. 2015, Lyubomirova et al., 2017), Morrocco (Correia & Chaves, 2018), Serbia (from the old city Ras, near Novi Pazar and Beograd, a fortress during the Austrian rule) (Vasilic et al., 2020; Kuzmanovic et al., 2021), Italia (Bruno et al., 1994) and Cilicia (Turkey) (Burlot & Waksman, 2022).

The DUB 6 sample was examined at two different sites, each at a different level of magnification (Figure 4.). The results obtained by the EDS mapping clearly indicate the presence of compositional heterogeneity among the grains. It is significant that the large inclusions observed at site 2 can be predominantly attributed to coarse quartz inclusions. This observation potentially suggests the use of unrefined sandy clay, meaning that it was utilized in the form in which it was originally collected, without undergoing sieving (Yoleva et al., 2015). The medium and small-sized inclusions, on the other hand, mainly consist of magnesium and potassium. Furthermore, the presence of iron and copper in this sample contributes to the red coloring observed at the fragment's edges, as clearly depicted in Figure 2 (DUB 6).

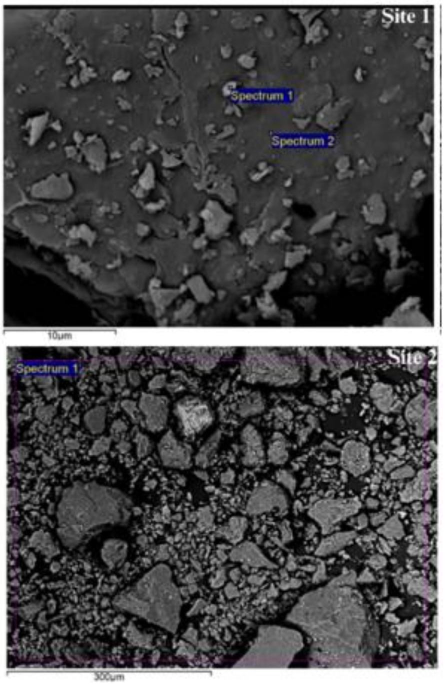

Figure 4. EDS image of the sample DUB 6

When it comes to the sample DUB 8, its most noteworthy characteristic is its coloring (Figure 1, DUB 8). The outer surface is adorned with a delicate red coating, occasionally featuring black to grey color inclusions. Conversely, the interior is coarse and black. This pattern suggests that the ceramic underwent firing under alternating reducing and oxidizing conditions, with the reducing conditions preventing the color transformation from black to red (Wagner and Wagner, 2004; Tite, 2008). Furthermore, among all the samples examined by the SEM-EDS technique, this sample exhibited the highest iron content, likely accounting for its distinctive red hue. Figure 5. reveals the EDS image of sample DUB 8, highlighting the significant heterogeneity observed in its inclusions, with quartz being the predominant component.

Figure 5. EDS image of the sample DUB 8

To wrap up the discussion of samples DUB 6, DUB 8 and K10, it is important to mention one more aspect of the ceramics, namely the CaO content. According to Tite (2008), a content below 5% suggests the use of non-calcareous clay and a content above 10% suggests the use of calcareous low-refractory clay. Based on SEM-EDS results, ceramic from Dubrovnik, showed that sample DUB 6 is representative of non-calcareous clay, while the K10 and DUB8 samples are calcareous clay (Tite, 2008; Vasilic et al., 2020).

A significant quantity of iron nails was discovered during the excavations (Bujak, 2018). Among these nails, one with a white spot at its head was selected for analysis. The examination revealed that this area is predominantly composed of CaO, indicating it is likely lime. Furthermore, the substantial carbon content (15.37%) suggests that the nail may have been in contact with an organic matter, which was probably used to join the wooden beams and construction plaster.

Table 4. Results of iron nail analysis by means of SEM-EDS technique (in wt.%).

Elements	Percentage (%)
C	15.37
O	48.19
Al	0.48
Si	0.69
Cl	0.08
Ca	34.57
Fe	0.63

Two of the most interesting findings from Dubrovnik were a piece of metallic slag and brocade threads, and the latter was found inside an intact ceramic pot, in situ (Bujak, 2018).

The visual appearance of the metallic slag (sample RF) suggested that it most likely contains high amounts of Fe, in the magnetite form. This was confirmed by basic testing of its properties on different surfaces, where it showed magnetic traits, across different parts, meaning that its magnetism was unevenly spread across the sample. This assumption was confirmed by SEM-EDS measurements on different parts of the sample (total of four distinctive spots and five spectra). The results are summarized in Table 5, and Figure 6. shows EDS image of the sample RF.

Table 5. Results of RF sample analysis by means of SEM-EDS technique (in wt.%).

Site	**A**	**B**		**C**	**D**
Spectrum	**1**	**1**	**2**	**1**	**1**
O	33.07	8.12	48.12	36.11	12.02
Al	0.68	<LOD	<LOD	0.29	<LOD
Mg	<LOD	0.18	<LOD	<LOD	<LOD
Si	2.05	0.22	0.49	0.36	<LOD.
S	0.44	<LOD	0.28	5.83	<LOD
Cl	0.35	3.34	0.43	6.12	<LOD
K	0.49	<LOD	<LOD	<LOD	<LOD
Ca	2.37	2.06	1.38	8.05	<LOD
Fe	58.01	50.8	24.67	9.4	3.14
As	2.54	0.19	1.45	<LOD	<LOD
Cu	<LOD	1.18	0.56	0.55	<LOD
Ag	<LOD	33.91	<LOD	33.29	46.02
Sn	<LOD	<LOD	22.61	<LOD	<LOD
Hg	<LOD	<LOD	<LOD	<LOD	38.82

**<LOD* below the limit of detection

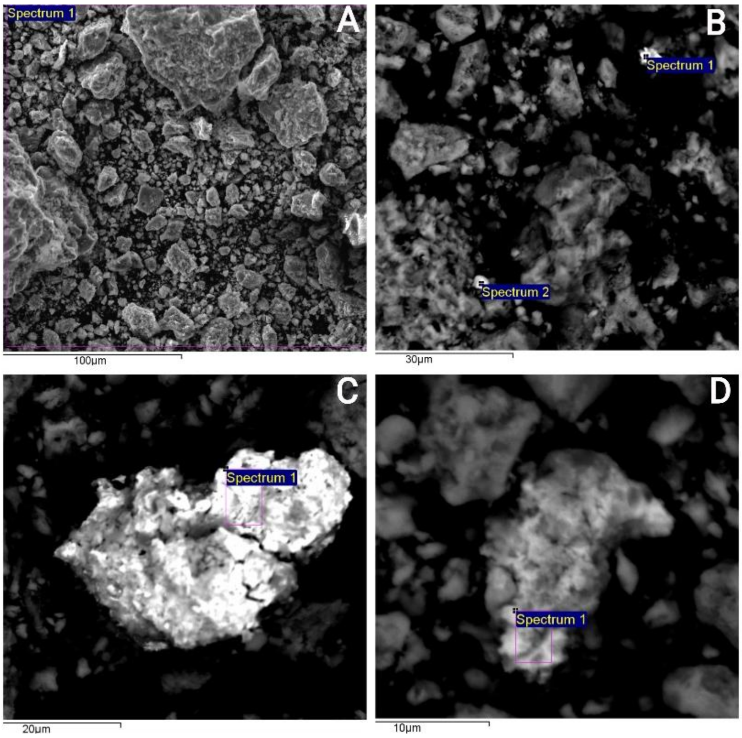

Figure 6. EDS image of the sample RF.

As can be seen from the EDS image, it is easy to distinguish heavy (brighter) and light (darker) element-containing particles, with grains of different dimensions and relatively heterogenous chemical composition. Some parts of the RF sample have high quantities of iron, which corresponds to its content in the magnetite ore (Fe_3O_4), and Figure 6a clearly demonstrates that is almost completely composed of Fe and O. Additionally, during the SEM analysis of the sites A and B, we encountered strong magnetic interference and we were unable to take better images with the instrument.

The most important aspect of this sample was the relatively high quantities of As, Ag, Sn and Hg in some of its parts. This can be easily seen from the sites C and D of Figure 6., where we have large grain inclusions of Ag, Hg and Sn. A possible explanation for the appearance of these metals in the sample could be twofold. First, during medieval times, Hg was used for the so-called mercury silvering, using the amalgamation technique (La Niece 1993; Giumlia-Mair, 2020). Second, arsenic, apart from its primary ores realgar and orpiment which in the BiH are found together with the mercury ores, can also be found in the ores that were used as a source of silver during medieval times. As already mentioned, Dubrovnik lies between two mineralogical zones, Vareš and Čevljanovići, where there are deposits of tetrahedrite (Cu, Fe, Ag, Hg)$_{12}$(Sb, As, Bi)$_4$S$_{13}$, proustite (Ag_3AsS_3) and cinnabar (HgS) were recorded (Operta, 2009). So, there is a possibility that As found its way to the sample simply because it was a part of the primary ore, which was used in the metallurgical process, and therefore represented the geological bedding of the location.

The presence of As, Ag, Sn and Hg in the RF sample can significantly contribute to our grasp of historical industrial processes in the region. These elements may provide valuable insights into the historical mining, metallurgical, and manufacturing activities that took place in the area. Understanding the occurrence and distribution of these elements in a sample could shed light on the utilization of specific ore deposits, mining techniques, and the production of various materials during different historical periods. Analysis of additional samples can help in reconstructing the intricate relationship between the local geological resources and the industrial processes that shaped the region's historical development. Further investigation and analysis of additional artifacts from Dubrovnik, as well as a thorough examination of the geology of the surrounding area, are necessary to determine the origin and purpose of the samples, especially the RF one. Without these crucial pieces of information, it is impossible to conclusively assert whether the sample was involved in deliberate smelting activities in Dubrovnik or if it merely represents the natural geological composition of the region. As for the other elements present, their low concentration suggests that the primary ore used in the process also had these metals present only as impurities.

The most interesting artifact that was analyzed was a part of the brocade thread, found inside an intact ceramic pot. Figure 7. shows the analyzed piece of the brocade thread, under the x12 and x80 magnification and the distance between the coils. Analysis revealed that the thread was made of a combination of Au/Ag, with uniform distance between the coils of the thread of 257.803 µm. The Au content was 63.94% and Ag 36.06%.

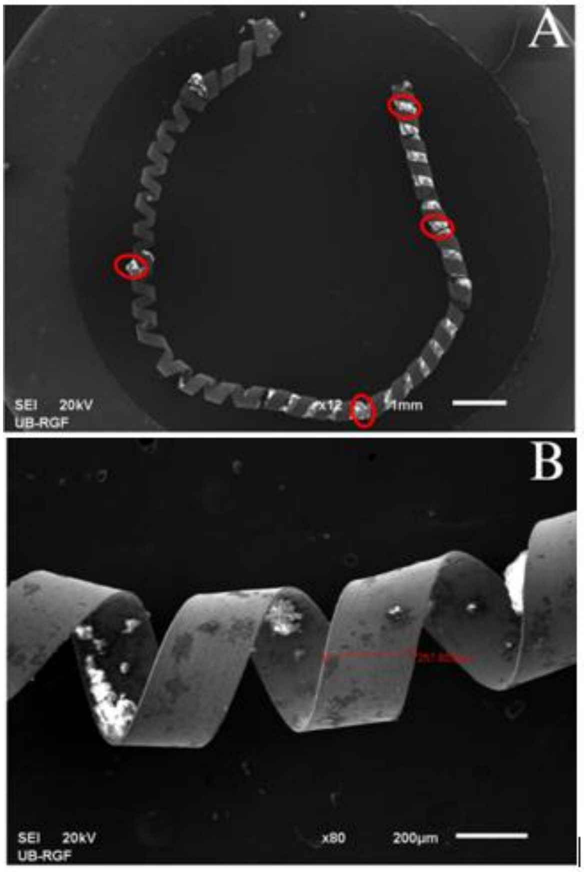

Figure 7. EDS image of the sample of golden brocade, under different magnifications

A substantial body of the literature addresses various aspects of manufacturing techniques, non-destructive analysis, and interpretation of threads from Medieval Europe (Járó, 1990; Caratzani & Rehren, 2006; Costa, de Reyer & Betbeder, 2012; Balta, Demetrescu & Lupu, 2020; Karatzani, 2021; Güzel, 2023). Here, we aim to contextualize our sample within a broader European framework, focusing on its type and dating. In terms of type, the primary classification revolves around the twist of the strip around the organic core, namely S or Z twist. In our case, the organic material likely served as a belt, as the brocade thread was discovered alongside a belt buckle in the same ceramic vessel (Bujak, 2018). Although no visible traces of the belt remain due to the probable textile decay over time, SEM images revealed distinct remnants of organic matter (Figure 7A, marked by red circles).

Further chemical analysis is required to determine its type and composition. Based on the twist type, our sample falls into the S-type category, which is more prevalent. In addition, our sample can be identified as a strip featuring gilding of gold onto a silver wire base on all sides, a technique developed in the 14th century (Karatzani and Rehren, 2006). Technologically, two manufacturing methods exist, aiding in approximate dating. The earlier method involves "beaten and cut" strips from the 13th century, while the later method employs "cast, drawn and rolled strips" from the 14th century onwards (Karatzani and Rehren, 2006; Balta, Demetrescu & Lupu, 2020). The latter displays a uniform width throughout its length and the characteristic parallel lines due to wire drawing, both of which are evident in our sample, suggesting a 14th-century timeframe. Chemical analysis by means of EDS measurements revealed that the brocade wire consists solely of gold and silver, without any copper or zinc impurities, which helps to distinguish between European and Oriental wire types, the later one using very pure precious metals, with only small quantities of impurities (Hoke and Petrascheck-Heim, 1977; Járó, 1990; Balta, Demetrescu & Lupu, 2020). Considering these data, our brocade wire most likely originates from the Orient and was produced no earlier than the 14th century.

CONCLUSIONS

Although a relatively small number of samples was analyzed in this paper, the results obtained can nevertheless provide a valuable first insight into the life and practice of the Dubrovnik inhabitants, but at the same time pave a road to further analysis and investigations. Based on the results, we can mark some of the samples as particularly interesting. DUB 2 sample had almost tenfold values of Mn, Zn and Pb compared to other samples measured by FAAS technique. A possible explanation would be that the clay used for ceramic was brought from another location, but without additional analyses of the raw material, this cannot be reliable concluded. Analysis by SEM-EDS technique showed that, unlike DUB 8 and K10 (body), a relatively high aluminum, non-calcareous clay with high potassium content was used for the sample DUB 6. Sample K10 was representative of a typical Ottoman type, green glazed pottery, found throughout Balkan Peninsula. The most interesting sample was without a doubt the brocade thread, found inside an intact ceramic pot, buried inside soil. Based on the data provided by SEM and EDS for this sample, it can be concluded that its time frame can be attested to the 14th century.

In order to draw any definitive conclusions regarding this location, additional analysis needs to be made. This would include XRD measurements of the ceramic findings and raw clay material, to determine the mineral phases and changes between source material (clay) and the finished product (ceramic). In addition, the analysis of the organic matter present in the brocade, although in minute quantities, could lead to conclusions about the type of the material that surrounded it and possibly light up its presence inside the pot, buried deep in the ground.

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Summary/Sažetak

Cilj ovog rada bio je analizirati arheološke artefakte pronađene u srednjovjekovnom gradu Dubrovniku, u blizini Sarajeva, korištenjem FAAS i SEM-EDS tehnika. Odabrano je ukupno 11 uzoraka različitih tipova: keramičke ulomke, željezni ekser, šljaka i komad brokatnog konca. Rezultati keramičkih uzoraka pokazali su visoke nivoe željeza i mangana, najvjerovatnije zbog geološke slojevitosti lokacije. Količine hroma, bakra, nikla, olova i cinka u tragovima potvrđuju ovu pretpostavku, što znači da je glina koja se koristila za izradu keramike dopremljena u okolici Dubrovnika. Oslikani uzorak zelene keramike imao je visok nivo olova, što sugerira da je ovaj metal namjerno dodan kako bi se ojačala svojstva posude. SEM-EDS mjerenja troske pokazala su prisutnost As, Hg i Sn, a jedno od mogućih objašnjenja je da su korišteni u procesima rafinacije zlata ili srebra, koji su se prema istorijskim izvještajima obavljali u Dubrovniku. Komad zlatne niti se pokazao korisnim u određivanju vremenskog okvira njegovog nastanka. EDS mjerenje je pokazalo da tip proizvodnog procesa koji se koristio u proizvodnji ovog zlatnog konca nije postojao prije 14. stoljeća.

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

Print ISSN: 0367-4444
Online ISSN: 2232-7266

Original Scientific Article
DOI: 10.35666/2232-7266.2024.63.02
UDK:543PBDE:629.014.7(497.6)"2020"

2024
63
11-17

Inventory of Polybrominated Diphenyl Ethers (PBDEs) in the Transport Sector in Bosnia and Herzegovina

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Article info
Received: 18/04/2023
Accepted: 30/09/2024

Keywords:
Polybrominated diphenyl ethers (PBDEs)
Flame retardants
Stockholm Convention
Electric/electronic equipment (EEE)
Transport vehicles and related wastes
decaBDE

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Abstract: Polybrominated diphenyl ethers (PBDEs) are flame retardants whose use is restricted to industrial production in line with the Stockholm Convention due to their persistence and toxicity. Some congeners were restricted in 2009, while the decaBDE restriction entered into force in 2017. Selected plastic products containing PBDEs are still widely used and may potentially impact human health and the environment. This paper investigates the presence of the PBDEs in transport vehicles and related wastes in BiH during 2020. The applied estimation method was proposed by the Secretariat of the Stockholm Convention, including a calculation based on the statistical data at the national level and literature data on PBDE concentration in the different vehicle types. It has been estimated that the most PBDEs come from the old transport vehicles that are still in use (4,582.72 kg), while the amount of PBDEs in imported vehicles and end-of-life vehicles is at a low level, 6.10 kg and 213.30 kg, respectively. Inadequate disposal of old vehicles has been identified as a significant environmental threat in Bosnia and Herzegovina related to PBDE contamination.

INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) are chemicals widely used since 1970s as flame-retardant additives in industrial applications because they inhibit or suppress combustion in organic materials. These substances are not single chemical compounds, but rather mixtures of several brominated substances. The entire family of PBDEs consists of 209 possible substances that are referred to as congeners (ATSDR and Hana, 2017). The consumption of PBDEs was very intensive in the period 1970 – 2005 because they were used for the production of a wide range of mainly consumer products in different manufacturing sectors: electrical and electronic industry, transport industry, furniture industry, textile and carpet industry, construction industry recycling industry. PBDEs are declared as POP (persistent organic pollutant) substances, with confirmed bioaccumulation and toxic properties, and the ability to resist degradation (Jinhui, Yuan, Wenjing, 2015). POPs undergo long–range transport (LRT), which means that they are transported to areas that are remote if compared to the source regions (Teran, Lamon, Marcomini, 2012) PBDEs are environmental endocrine disruptors, with biological toxicity (Parsons, Lange, Hutchinson, 2019), and their toxic effects on nerves are primarily reflected in changes in body movement, behaviour, and cognitive ability (Branchi, Capone, Alleva, 2003).

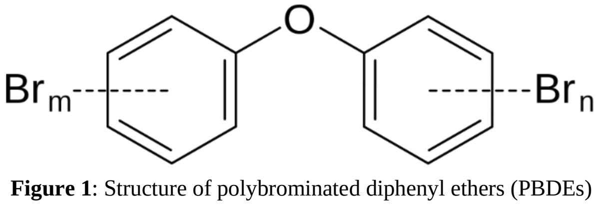

Figure 1: Structure of polybrominated diphenyl ethers (PBDEs)

Humans can be exposed to PBDEs in a wide variety of ways, including eating contaminated foods, breathing in contaminated air, or having skin contact with contaminated soil/ dust/commercial products. Soils and sediments are the major sinks of PBDEs. A variety of food items, including fish, meat, and dairy products, have been shown to contain low concentrations of PBDEs (ATSDR and Hana, 2017).

The Stockholm Convention (SC) on Persistent Organic Pollutants is a global treaty to protect human health and the environment from persistent organic pollutants (Magulova and Priceputu, 2016). The Global Monitoring Plan for SC was adopted in 2001 and entered into force in 2004, after being ratified by 50 countries. By 2023, 152 countries have ratified the convention and committed to take measures to eliminate or reduce the release of POPs into the environment (Fiedler, Kallenborn, De Boer, 2019).

The first PBDE substances were added to the list for elimination (Annex A) of the Stockholm Convention in May 2009. These were the mixture of hexaBDE and heptaBDE (comercial octabromodiphenyl ether; c-octaBDE) and the mixture of tetraBDE and pentaBDE (commercial pentabromo-diphenyl ether C-pentaBDE). Some exemptions in use were still allowed for the recycling of articles that contain or may contain these substances. However, these specific exemptions will expire no later than 2030 (part Annex A, Parts IV and Part B, of the Stockholm Convention). DecaBDE (commercial mixture, c-decaBDE) was in free use until 2017, when the Conference of Parties adopted amendments to the Annex A to include c-decaBDE to the list, with various specific exemptions. Due to their POP characteristics, products and environment contaminated with PBDEs are a constant threat to human health. Therefore, the SC requires its parties to identify all stockpiles, products, emission points, contaminated sites, or any source of contamination with POPs, and shift their policy respond to elimination and constant monitoring.

Bosnia and Herzegovina (BiH) ratified the Stockholm Convention on May 30, 2010 and committed to meeting the requirements of the Convention. The first steps were undertaken in 2012, starting with the activities of the preliminary inventory of POPs, and the analysis of the national institutional and policy framework to develop an implementation plan that will bring BiH to meet the SC requirements. The National Implementation Plan for the Stockholm Convention in Bosnia and Herzegovina was adopted in 2016 (Tabučič, 2017). Further activities were undertaken in the period 2020–2022 when a detailed inventory of POPs was carried out as a part of the project Environmentally Sound Management of Persistent Organic Pollutants (POPs) in Industrial and Hazardous Waste Sectors in Bosnia and Herzegovina, which is implemented by the United Nations Development Programme.

The transport sector (cars, buses, trucks, trains, planes, and ships) is one of the large material flows of goods and ultimately a major stream of waste and recycling. The end-of-life management in the transport sector is a highly relevant material flow for the recovery of materials and for managing pollutants. A large proportion of c-PentaBDE use has been within the transport sector; the major use was for treatment of flexible PUR foams (automotive seating, head rests, car ceilings, acoustic management systems, etc.) and a minor use was for the back-coating of textiles used for car seats. C-OctaBDE has also been used to some extent in plastic vehicle parts (steering wheels, dashboards, door panels, etc.) (Jinhui et al., 2015). Polyurethane (PU) foot pads and PU seat covers are significant potential sources of PBDEs in indoor air and dust of the automobile microenvironment (Jin, Zhang, He, 2021). Automotive shredding and metal recycling facilities are the sources of PBDE contamination, since concentrations of PBDE have been determined in air samples near a large outdoor automotive shredding and metal recycling facility and the surrounding local area (Hearn, Hawker, Mueller, 2012).

This paper summarizes the results of the inventory of PBDEs in the transport sector in BiH, carried out in period 2020–2022.

METHODOLOGY

PBDE inventory in BIH was based on the methodology provided in the Guideline proposed by the Secretariat of the Stockholm Convention (Jinhui et al., 2015). The Guideline recognizes four different sources of PBDE as follows:

- POP-PBDEs in electrical and electronic equipment (EEE) and related waste (WEEE)
- POP-PBDEs in the transport sector
- POP-PBDEs in other use
- POP-PBDEs-contaminated sites

This paper is focused on inventory of PBDE in the transport sector only.

The following formula is used to calculate the POP-PBDEs content in vehicles for the different categories (cars, trucks or buses) at different life cycle stages.

Quantity of POP-PBDEs Vehicle category = Number of vehicles category x POP-PBDEs category x F-regional

Where:

- Number of vehicles category is the number of vehicles (manufactured in 1975-2004) present in the category (car, bus or truck) calculated for the different life cycle stages
- POP-PBDEs category is the quantity of POP-PBDEs in an individual car, truck or bus treated with POP-PBDEs (160 g of c-PentaBDE per car/truck, 1000 g of c-PentaBDE per bus)
- F-regional is the regional factor for vehicles. The Guideline suggests a factor of 0.05 as a regional adjustment factor for Europe (5 % of cars produced in the region between 1975 and 2004 are estimated to be impacted by POP-PBDEs). This factor was derived from measurements of European automotive shredder residues that contained an average of approximately 7 g of c-PentaBDE per car in around 2000, which corresponds to 4.4% of the impacted cars when considering 160 g of c-PentaBDE for an impacted car (Morf, Taverna, Daxbeck, 2003).

Finally, the listed hexaBDE and heptaBDE (from c-OctaBDE) need to be calculated from the c-OctaBDE total amount, taking in consideration that the average c-OctaBDE consists of the 43 % of heptaBDE homologue and 11 % the hexaBDE homologue.

The assessment of POP-PBDEs in the transport sector includes the inventory of vehicles in the following life cycle stages:

- POP-PBDEs of vehicles in current use/sale
- POP-PBDEs in imported/exported vehicles
- POP-PBDEs in ELVs for the respective inventory year

In general, cars and other vehicles (trucks and buses) are the major portion of the transport sector containing the largest volume of POP-PBDEs. Therefore, the inventory is focused on these vehicles. The used Guideline does not include ships and airplanes in the described calculation methodology to simplify the details, but also this transport sector is not relevant for BiH. Since POP-PBDEs were produced and used in the period from 1975 to 2004, only vehicles produced during that period were inventoried for POP-PBDEs.

RESULTS AND DISUSSION

The number of registered vehicles in BiH is available on the official web page of the Agency for Identification Documents (www.iddeea.gov.ba) distributed in two groups of data relevant for this assessment: first time registered vehicles and all registered vehicles. The number of the first-time registered vehicles was used as a source of data for “POP-PBDEs in imported/exported vehicles”, while all registered vehicles group of data were used to express “POP-PBDEs of vehicles in current use/sale”.

The vehicle classification used in the IDDEEA BiH database follows the rules set by the Inland Transport Committee of the United Nations Economic Commission for Europe (www.unece.org):

“Category M1”: Vehicles used for the carriage of passengers which, in addition to the driver's seat, have a maximum of eight seats
"Category M2": Vehicles used for the carriage of passengers, which, in addition to the driver's seat, have more than eight seats and whose maximum permissible mass does not exceed 5 tons.
"Category M3": Vehicles used for the carriage of passengers, which have more than eight seats in addition to the driver's seat, and whose maximum permissible mass exceeds 5 tons.
“Category N - Power-driven vehicles having at least four wheels and used for the carriage of goods
"Category N1": Vehicles used for the carriage of goods and having a maximum mass not exceeding 3.5 tons.
"Category N2": Vehicles used for the carriage of goods and having a maximum mass exceeding 3.5 tons but not exceeding 12 tons.
"Category N3": Vehicles used for the carriage of goods and having a maximum mass exceeding 12 tonnes.

Moreover, the IDDEEA BiH database enables filtering of data per different categories such as the year of production and the type of the vehicle that was used for this study. The first filtering data step was to extract all vehicles produced between 2015–2004, while second filtering data step was to select vehicles listed under category M1 (as cars), M2/M3 (as buses) and N1/N2/N3 (as trucks)

The number of the first-time registered vehicles (imported), and all registered vehicles in the year of 2020,that were produced in the period 1975 – 2004 are presented in table 1.

Table 1: Number of vehicles registered in 2020 and produced in period 1974–2004

Month/2020	First registration		All registered	
	cars/trucks	buses	cars/trucks	buses
January	105	9	34,753	148
February	89		37,575	179
March	55		42,128	134
April	29		47,062	69
May	46		51,468	119
June	55		53,416	144
July	86		58,066	115
August	45	1	49,854	173
September	44		47,601	318
October	58	1	50,441	168
November	37		44,459	159
December	48		45,720	129
Total	697	11	562,543	1,855

The amount of c-PentaBDEs calculated in vehicles (cars, buses, trucks) that were used and imported into BiH in 2020, with the production date between 1975 and 2004 is presented in Table 2 and Table 3.

Table 2: Amount of POP-PBDEs in PUR foam in vehicles in use in 2020 and produced in period 1974–2004

Number of vehicles in use (age1975 - 2004)		Average quantity of c-pentaBDEs per vehicles(kg)		Regional factor		Quantity of POP-PBDEs$_{\text{Vehicle in use}}$ (kg)
CARS/TRUCKS						
561,246	x	160 g per car (0.16 kg)	x	0.05	=	4,489.96
BUSES						
1,855	x	1000 g per bus (1 kg)	x	0.05	=	92.75
TOTAL QUANTITY OF c-pentaBDEs - vehicles in use						**4,582.72**

Table 3: Amount of POP-PBDEs in PUR foam in vehicles imported in 2020 and produced in period 1974–2004.

Number of imported vehicles (age1975 - 2004)		Average quantity of c-pentaBDEs per vehicles(kg)		Regional factor		Quantity of POP-PBDEs$_{\text{Imported vehicles}}$ (kg)
CARS/TRUCKS						
697	x	160 g per car (0.16 kg)	x	0.05	=	5.6
BUSES						
11	x	1000 g per bus (1 kg)	x	0.05	=	0.6
		TOTAL QUANTITY OF c-pentaBDEs - imported vehicles				**6.1**

There are no statistical data on end-of-life (ELV) vehicles in BiH. According to the available data on the total number of registered motor vehicles, it can be concluded that the average age of motor vehicles in BiH is 16.5 years (www. bihamk.ba).

Car scraping dealers are widely present in BiH. The project team visited three companies that receive rejected old cars, extract and trade with the valuable parts. Based on the conducted interviews it was concluded that after removal of valuable parts of the cars, metal parts of cars are commonly used for recycling and sold as secondary raw materials to metal processing companies and ironworks.

Plastic and foam parts of the wasted vehicles (steering wheels, dashboards, door panels, etc.) represent a major problem for the car scrapping dealers since there is no market for them. Secondary raw materials operators do not accept this type of plastic, because there are no clients abroad who are interested in trade. Therefore, plastic/foam parts of the vehicles are usually illegally burned or disposed on non-sanitary waste disposal sites. This represents a significant burden on the environment.

Initially, the number of end-of-life vehicles was estimated from the above-mentioned database of registered cars, by comparing the number of registered vehicles in 2019 and 2020 produced in 1975-2004 (see table 4). It is assumed that non - registered cars are no longer in use and will be disposed of as car scrap. As 2020 was the year of COVID 19 global pandemic outbreak, with transportation restriction in force, this result was not fully reliable. Therefore, ELV estimations through the analyses of the registered vehicles produced in period 1974 – 2004 was extended for the 5 year period, 2016 – 2021, and the average data was taken as more reliable.

The number of registered vehicles, produced in the period 1974–2004 has a decreasing trend, following a linear regression with a reliable R-squared value: for the cars/trucks 0.9641, and for buses 0.8522 (See Figure 2. And Figure 3). Following 5-year data, the average number of non-registered vehicles between two consecutive years is 25,363 in total for personal cars and trucks, and 207 for buses or similar vehicles aimed for the transport of more than 8 passengers, assumed to be vehicles that are no longer in use and disposed of as car scrap i.e ELV.

In addition, following the rules of the regression, it is predicted that in the next 5 years (up to 2025) more than 400.000 cars/tracks per year and more than 200 buses per year, produced between 1975 and 2004, will still be a part of the transport sector in BiH. It is expected that ELV disposal will be significantly increased, following more strict rules of the vehicle registration, especially for the old vehicles that hardly pass technical evaluation procedure including vehicle emission standards.

Table 4: Number of registered vehicles in BiH in the period 2016–2021, produced in period 1974–2004.

Year	2021		2020		2019		2018		2017		2016	
	cars/ trucks	bus	cars/ trucks	bus	cars/ trucks	bus	cars/ trucks	bus	cars/ trucks	bus	cars/ trucks	bus
January	32,194	155	34,753	148	38,029	180	40,455	220	38,451	200	40,479	197
February	35,718	165	37,575	179	39,152	189	41,187	214	43,681	210	44,872	235
March	45,022	132	42,124	134	48,220	245	53,739	243	56,185	237	56,516	265
April	46,919	66	47,062	69	56,510	249	58,499	285	56,413	283	59,811	273
May	48,284	117	51,468	119	56,504	274	57,795	281	58,643	310	57,273	304
June	52,317	160	53,409	144	51,448	148	57,979	173	59,508	145	64,096	162
July	54,827	118	58,055	114	63,183	149	63,783	133	62,780	147	63,112	165
August	50,545	229	49,847	173	55,386	230	59,876	292	62,899	271	64,012	265
September	46,247	300	47,597	317	47,483	283	50,998	298	52,053	309	55,533	314
October	45,491	148	50,441	168	52,369	201	58,120	234	58,814	211	57,682	228
November	42,497	122	43,163	159	45,465	195	49,903	226	48,303	232	52,659	211
December	42,801	108	45,714	129	48,879	160	48,172	166	49,699	186	53,634	235
Total	542,862	1,820	561,208	1,853	602,628	2,503	640,506	2,765	647,429	2,741	669,679	2,854
	2020 - 2021		2019-2020		2018-2019		2017-2018		2016-2017			
ELV per year	18,346	33	41,420	650	37,878	262	6,923	-24	22,250	113	-	-
ELV 5-year average	Cars/trucks: 25,363						Buses: 208					

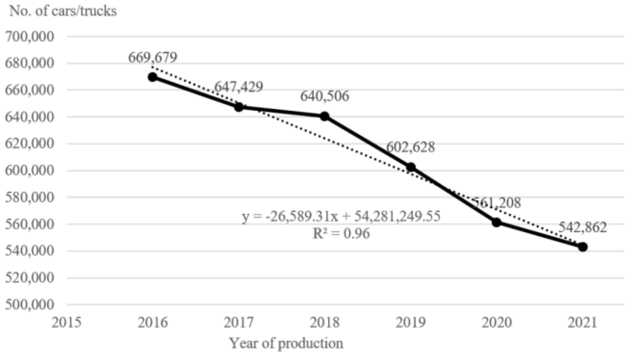

Figure 2: Number of registered personal cars/trucks in BiH in the period 2016–2021, produced in the period 1974–2004.

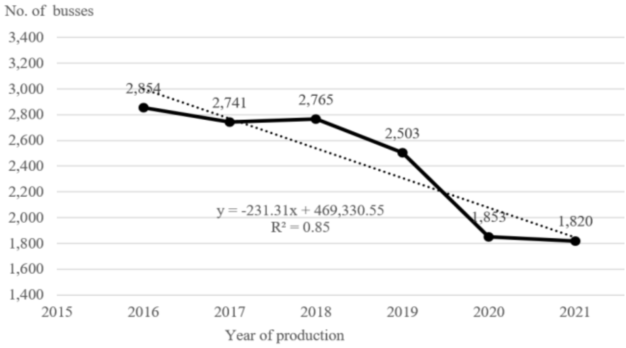

Figure 3: Number of registered buses in BiH in the period 2016 – 2021, produced in the period 1974–2004.

The total amount of c-PentaBDEs in estimated ELV vehicles (cars, buses, trucks) is presented in Table 5.

Table 5: Amount of POP-PBDEs in PUR foam of end-of-life vehicles estimated for year 2020.

Number of ELV vehicles (age 1975 - 2005)		Average quantity of c-pentaBDEs per vehicles(kg)		Regional factor*		Quantity of POP-PBDEs$_{\text{Vehicle in use}}$ (kg)
		CARS/TRUCKS				
25,363	x	160 g per car (0.16 kg)	x	0.05	=	202.90
		BUSES				
208	x	1000 g per bus (1 kg)	x	0.05	=	10.40
TOTAL QUANTITY OF c-pentaBDEs - ELV vehicles						213.30

According to the Stockholm Convention, the amounts of c-pentaBDE or c-OctaBDE in the flow of materials are not reported; only the relevant PBDEs homologues: tetraBDE, pentaBDE, hexaBDE and heptaBDE which are on the list of chemicals of the Stockholm Convention are reported. Differentiation between different homologues is done due to the fact that not all PBDEs homologues were on the list of chemicals of the Stockholm Convention, such as octa, nona and decaBDE (decaBDE was added in 2017), and that not all homologues are present in the same quantities in products covered by this group of substances. The quantities of BDE in the transport sector are shown in Table 6.

Table 6: Recalculation of POP-PBDEs present in the transport sector to the listed POP-PBDEs homologues (tetraBDE, pentaBDE, hexaBDE and heptaBDE) for the relevant life cycle stages

	Distribution homologues c-PentaBDE	POP-PBDEs in vehicles currently in use (kg)	POP-PBDEs Imported in vehicles 2020 (kg)	POP-PBDEs in end-of-life vehicles (kg)
Inventoried PBDE*		4,582.72	6.1	213.30
tetraBDE	33%	1,512.30	2.01	70.39
pentaBDE	58%	2657.98	3.54	123.71
hexaBDE	8%	366.62	0.49	17.06
heptaBDE	1%	45.83	0.06	2.13

The total amount of BDE in the transport sector identified during 2020 in Bosnia and Herzegovina was 4,802.12 kg, which is equivalent to approximately 1.4 tons per million inhabitants. This calculation considers the B&H Census results from 2013, which reported a population of 3.5 million inhabitants (Agency for Statistics of Bosnia and Herzegovina, 2016). The methodology employed in the assessment, derived from the Stockholm Convention PBDE inventory guidance, was implemented in Nigeria, resulting in the identification of 270 t of POP-PBDEs in the transport sector (Babayemi, Osibanjo, Sindiku, 2018). Given the estimated national population of Nigeria in 2018 at 196 million (United Nation Children's Fund, 2019), the BDE amount per million inhabitants is 1.4, equivalent to that found in Bosnia and Herzegovina.

The quantity of c-PentaBDE present in vehicles currently in use or available for sale in Sri Lanka is estimated to be 5,445.6 kg (www.env.gov.lk), which equates to 0.2 tons per million inhabitants, based on population of 21.8 million (Central Bank of Sri Lanka, 2018). Both countries have identified the final disposal of end-of-life vehicles as their most significant issue and challenge. The primary concern is the disposal method of waste transport devices containing PBDEs, which pose a risk to environmental pollution and human health.

CONCLUSION

Although restricted by the Stockholm Convention, PBDE substances are highly present in different products used in every day human routine. The transport sector (cars, buses, trucks, trains, planes, and ships) is one of the large material flows of goods and ultimately becomes a large waste and recycling flow. The end-of-life management of the transport sector is a highly relevant material flow for the recovery of materials and for managing pollutants. It has been estimated that the most of the PBDEs in BIH come from the old transport vehicles still in use 4,582.72 kg, while PBDE in imported vehicles and end-of-life vehicles is at a low level, 6.10kg and 213.30kg, respectively. It is predicted that by 2025 more than 400.000 cars/tracks per year and more than 200 buses per year, produced between 1975 and 2004, will still be a part of the transport sector in BiH. Car scraping dealers are widely present in BiH, and motor vehicles in BiH are quite old (16.5 years based on the data from 2019). Investigation done through this survey showed that after the removal of valuable parts of the cars, the metal parts are commonly used for recycling and are sold as secondary raw materials to metal processing companies and ironworks such as Arcelor Mittal d.o.o. Plastic and foam parts of the ELV vehicles (steering wheels, dashboards, door panels, etc.) represent a major problem for the car scrapping dealers since there is no market for them. Therefore, plastic/foam parts of the vehicles are usually illegally burned or disposed on non-sanitary waste disposal sites. There is still no practical solution for this type of waste and it represents a significant burden on the environment. Finding a solution for the final disposal of this type of waste is recognised as a priority for the future actions regarding PBDE waste removal. Future studies should focus on the presence of PBDE in the environment (soil, groundwater, river sediments) surrounding car scraping dealers' facilities.

ACKNOWLEDGEMENT

This research was conducted within the framework of the project entitled Environmentally Sound Management of Persistent Organic Pollutants (POPs) in Industrial and Hazardous Waste Sectors in Bosnia and Herzegovina, which is implemented by UNDP and financed by the Government of Sweden.

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Summary/Sažetak

Polibromirani difenil eteri (PBDE) su usporivači gorenja čija je upotreba ograničena na industrijsku proizvodnju u skladu sa Štokholmskom konvencijom zbog njihove postojanosti i toksičnosti. Neki su kongeneri ograničeni 2009., dok je ograničenje dekaBDE-a stupilo na snagu 2017. Odabrani plastični proizvodi koji sadrže PBDE još uvijek se široko koriste i mogu potencijalno utjecati na ljudsko zdravlje i okoliš. Ovaj rad analizira prisutnost PBDE-a u transportnim vozilima i povezanom otpadu u BiH tokom 2020. godine. Primijenjenu metodu procjene predložio je Sekretarijat Štokholmske konvencije uključujući izračun na temelju statističkih podataka na nacionalnom nivou i literaturnih podataka o koncentraciji PBDE-a u različitim vrstama vozila. Procjenjuje se da većina PBDE dolazi od starih transportnih vozila koja su još u upotrebi (4.582,72 kg), dok je PBDE u uvezenim vozilima i otpadnim vozilima manje zastupljen i iznosi 6,10 kg odnosno 213,30 kg. Neadekvatno zbrinjavanje starih vozila identificirano je kao značajna prijetnja okolišu u BiH u vezi s kontaminacijom PBDE-om.

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2024

63

19-24

Print ISSN: 0367-4444
Online ISSN: 2232-7266

Original Scientific Article
DOI: 10.35666/2232-7266.2024.63.03
UDK:638.162:577.1(497.6)

Correlations of total phenolics and antioxidant activity in royal jelly from Bosnia and Herzegovina

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Article info
Received: 07/11/2023
Accepted: 06/05/2024

Keywords:
Phenolics
Antioxidant activity
Correlations
Royal jelly
Spectrophotometric method

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Abstract: In this study, the total phenolics content (TPC), and antioxidant activity against hydroxyl ($AA_{OH}^{\bullet}$) and peroxyl ($AA_{ROO}^{\bullet}$) free radicals in centrifuged (low molecular weight fractions, LM) and uncentrifuged (the bulk of low molecular weight and high molecular weight, LM+HM fractions) samples of fresh royal jelly (RJ) from Bosnia and Herzegovina (B&H), as well as correlations between these parameters were determined. For determination of the TPC, spectrophotometric method with gallic acid (GA) as the standard was used. For the determination of AA, the oxygen radical absorbance capacity (ORAC) assay with catechin as the standard was used. The highest TPC expressed in equivalent mass of GA per mass of RJ (LM+HM fractions) was found in the sample from Kalinovik, 5.54±0.76 mg GAE/g, and in the sample from Bosanska Krupa, 4.07±0.04 mg GAE/g for LM phenolics of RJ. The highest $AA_{OH}^{\bullet}$ expressed as mmol catechin equivalents (CE) per mass of LM+HM fraction of RJ was found in the sample from Cazin 58.15±1.55 mM CE/g, and in the sample from Ključ, 58.15±0.81 mM CE/g for LM fraction. The highest $AA_{ROO}^{\bullet}$ per mass of LM+HM fraction of RJ was found in the sample from Bosanska Krupa, 8.04±0.04 mM CE/g, in which also found the highest $AA_{ROO}^{\bullet}$ for LM fractions, 7.58±0.39 mM CE/g. There were very high positive correlations between $AA_{OH}^{\bullet}{}_{(LM+HM)}$, as well as $AA_{OH}^{\bullet}{}_{(LM)}$) and $TPC_{(LM+HM)}$, as well as $TPC_{(LM)}$ (Pearson correlations (P.c.)). It was moderate positive correlation $AA_{OH}^{\bullet}{}_{(HM)}$ to $TPC_{(HM)}$. Also, there were weak positive correlations between $AA_{(ROO^{\bullet})(LM+HM)}$, as well as $AA_{(ROO^{\bullet})LM}$ and $TPC_{(LM+HM)}$, as well as $TPC_{(LM)}$, and weak negative correlation $AA_{(ROO^{\bullet})(HM)}$ to $TPC_{(HM)}$. Based on a very high TPC and significant AA, it can be concluded that RJ from B&H is a good source of natural antioxidants and is an important dietary supplement.

INTRODUCTION

Royal jelly (RJ) is a secretion product of the cephalic glands of nurse bees that has been used for centuries for its extraordinary properties and health effects (Pavel *et al*., 2011). It is produced under partial digestion of pollen and nectar (Stocker, 2003). Due to its complex composition1(water, proteins, lipids, carbohydrates, amino acids, mineral salts, vitamins, enzymes, hormones, oligo-elements, natural antibiotics), RJ has a multitude of biological activities: antioxidant (Nagai and Inoue, 2004; Nagai *et al*., 2006), hypoglycemic (Pourmoradian *et al*., 2014; Maleki *et al*., 2019), hypocholesterolemic (Chiu *et al*., 2017; Pan *et al*., 2018; Petelin *et al*., 2019) and hepatoprotective (Almeer *et al*., 2018; Bilgic *et al*., 2018), hypotensive and blood pressure regulatory (Liang *et al*., 2018; Pan *et al*., 2019), antitumor (Albalawi *et al*., 2022), antibacterial (Alreshoodi and Sultanbawa, 2015; Gevorgyan *et al*., 2021; Guo *et al*., 2021), anti-inflammatory (Aslan and Aksoy, 2015) immunomodulatory and anti-allergic (Guendouz *et al*., 2017), general tonic, healthy aging and longevity (Kunugi and Ali, 2019) etc.

The subject of this paper was to investigate the correlations between total phenolics content (TPC) and antioxidant activity against hydroxyl and peroxyl free radicals of fresh royal jelly from Bosnia and Herzegovina. There is no data on these correlations in the available literature. Therefore, the scientific significance of this work could be in the direction of determining TPC in correlation with the antioxidant activity of Bosnian royal jelly.

MATERIAL AND METHODS

Chemicals - Fluorescein, Standard Fluka for fluorescence – free acid was obtained from Fluka Chemie GmbH, Steinheim, Germany. Catechin hydrate and 2,2'-azobis (2 amidino-propane) dihydrochloride (AAPH) were obtained from Sigma, cupric sulfate pentahydrate and hydrogen peroxide from Kemika, Zagreb, Croatia, and Folin-Ciocalteu reagent, sodium carbonate, sodium phosphate dibasic, potassium phosphate monobasic were purchased from Semikem, Sarajevo, Bosnia and Herzegovina. All chemicals were analytical grade.

Samples - The samples of royal jelly originating from the area of three cantons from the Federation of Bosnia and Herzegovina and two cities from Eastern Bosnia are listed in Table 1.

All samples were stored at -32 °C until analysis.

Instrumentations - All analyses were performed on UV/Vis spectrometer Lambda 25, and fluorescence spectrometer LS 55 (all by Perkin-Elmer). The samples were centrifuged on a Hettich Mikro 22R centrifuge. For weighing the samples, the balance Mettler Toledo AB 104 was used. Thermostate KP 20-Lauda was used for temperature regulation.

Sample preparation - Approximately 0.05 g of RJ sample dissolved in 1 ml of distilled water (concentrated RJ solution obtained: 50 mg/ml). This RJ solution was diluted[1] and they were analysed before and after centrifugation at 15000 rpm, 15 minutes, at 15 °C (after centrifugation, the supernatants were used for the analyses).

Determination of total phenolic content (TPC)

The total phenolics content (TPC) was determined by spectrophotometry, using gallic acid (GA) as a standard, according to the method described by Keskin-Šašić *et al.*, (2012). All measurements were done in triplicate at 743 nm.

Determination of antioxidant activity – Oxygen Radical Absorbance Capacity (ORAC)

For the analyses of antioxidant activity (AA) of RJ the oxygen radical absorbance capacity (ORAC) assay was used (Cao and Prior, 1999) with catechin (C) as standard. For the analyses of AA of RJ, all samples were measured before [AA of the low-molecular (LM) + high-molecular (HM) antioxidants, $AA_{(LM+HM)}$] and after centrifugation [AA of the low-molecular antioxidants, $AA_{(LM)}$] (Tahirović *et al.*, 2017).

Table 1. Royal jelly samples.

Sample label	**Sample location**	**Sample label**
1	Bjelašnica (Rakitnica)	1
2	Kalinovik (Jezero)	2
3	Bosanska Krupa 1 (Vranjska)	3
4	Bosanska Krupa 2 (Krčevine)	4
5	Milići (Johovača)	5
6	Cazin 1 (Stijena)	6
7	Bosanska Krupa 3 (Ljusina)	7
8	Bosanska Krupa 4 (Ljusina)	8
9a	Konjic 1 (Seonica)	9a
9b	Konjic 2 (Čuhovići)	9b
9c	Konjic 3 (Čuhovići)	9c
9d	Konjic 4 (Čuhovići)	9d
9e	Konjic 5 (Seonica)	9e
9f	Konjic 6 (Seonica)	9f
9g	Konjic 7 (Čuhovići)	9g
7	Bosanska Krupa 3 (Ljusina)	7
8	Bosanska Krupa 4 (Ljusina)	8
9a	Konjic 1 (Seonica)	9a
9b	Konjic 2 (Čuhovići)	9b
9c	Konjic 3 (Čuhovići)	9c
9d	Konjic 4 (Čuhovići)	9d
9e	Konjic 5 (Seonica)	9e
9f	Konjic 6 (Seonica)	9f
9g	Konjic 7 (Čuhovići)	9g
10	Ključ (Hanlovići)	10
11	Sanski Most 1 (Skucani Vakuf)	11
12	Sanski Most 2 (Čaplje)	12
13	Sanski Most 3 (Čaplje)	13
14	Bosanska Krupa 5 (Velika Jasenica)	14
15	Cazin 2 (Klisa)	15
16	Bratunac 1 (Suha)	16
17	Bratunac 2 (Suha)	17
18a	Modriča 1 (Svilaj)	18a
18b	Modriča 2 (Svilaj)	18b
18c	Modriča 3 (Svilaj)	18c

Statistical analysis - For the statistical analysis of the obtained results Kolmogorov-Smirnov and Shapiro-Wilk by SPSS 17 were used as normality tests. According to these tests, data were in normal distribution, thus average values with standard deviations, and the Student's t-test with p=0.05 as the statistical level of significance, were used.

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[1] The dilution factor was taken into account in the calculation

RESULTS AND DISCUSSION

For analysis of TPC, all measurements were performed at 743 nm. The equation of the calibrated curve was: $y = 0.0927x + 0.0129$; $R^2 = 0.9999$. Obtained values of TPC for 26 samples of royal jelly (RJ), expressed as mass (in mg) of gallic acid equivalents per mass of RJ (mg GAE/g) are shown in the Table 2. Normality tests showed that values were in a normal distribution, and in that case, they are listed as mean from three determinations with standard deviations (±S.D.).

Table 2. Total phenolics in 26 analyzed samples of royal jelly.

Sample label	TPC^{I} (mg GAE/g±S.D.)		
	TPC^{II}	**TPC^{III}**	**TPC^{IV}**
2	**5.54±0.76**	3.80±0.05	1.75±0.78
8	5.47±0.24	**4.07±0.04**	1.40±0.20
3	5.30±0.01	3.55±0.02	1.76±0.02
4	5.27±0.28	3.50±0.01	1.65±0.27
9e	5.25±0.06	3.62±0.09	1.63±0.01
9a	5.14±0.42	2.97±0.05	**2.17±0.37**
9f	4.94±0.05	3.21±0.06	1.76±0.07
6	4.89±0.28	3.40±0.04	1.48±0.30
9b	4.77±0.11	3.15±0.14	1.62±0.03
9d	4.52±0.07	2.68±0.04	1.84±0.04
16	4.49±0.09	3.27±0.20	1.22±0.18
9c	4.46±0.04	2.84±0.25	1.62±0.23
9g	4.40±0.06	3.31±0.02	1.09±0.06
1	4.33±0.52	3.22±0.34	1.11±0.75
5	4.12±0.10	3.30±0.21	0.94±0.12
18c	3.95±0.03	2.78±0.01	1.16±0.03
7	3.89±0.12	2.87±0.06	1.03±0.09
11	3.70±0.14	2.99±0.07	0.71±0.19
10	3.65±0.04	2.88±0.07	0.76±0.09
18a	3.62±0.01	3.01±0.04	0.60±0.03
13	3.60±0.04	3.01±0.08	0.59±0.11
17	3.57±0.10	2.89±0.23	0.68±0.16
15	3.55±0.13	2.48±0.02	1.07±0.11
14	3.45±0.13	2.65±0.10	0.80±0.07
12	3.36±0.03	2.99±0.21	**0.37±0.19**
18b	**3.28±0.09**	**2.35±0.05**	0.99±0.12

$^{\mathbf{I}}$Mean from three determinations±standard deviations
$^{\mathbf{II}}$Total phenolics in the bulk (uncentrifuged samples)
$^{\mathbf{III}}$Low-molecular (LM) total phenolics (centrifuged samples)
$^{\mathbf{IV}}$High-molecular (HM) total phenolics (IV=II-III)

Obtained TPC values were slightly lower than those obtained by Nabas *et al.*, (2014).

The TPC values of uncentrifuged samples of RJ, which are a mixture of low molecular weight (LM) and high molecular weight (HM) phenolics (TPC$_{(LM+HM)}$) were statistically significantly higher than the TPC values of centrifuged samples of RJ (TPC$_{(LM)}$) ($p^{***}<0.001$; Student's t-test) (mean values 4.42±0.72 GAE/g, and 3.16±0.38 mg GAE/g, respectively). The values of high molecular weight fractions of phenolics (TPC$_{(HM)}$) (mean value 1.26±0.48 mg GAE/g) were statistically significantly lower than TPC$_{(LM)}$ ($p^{***}<0.001$; Student's t-test).

There was no statistically significant difference between both: the TPC$_{(LM+HM)}$ values for the samples of RJ from Una-Sana Canton to the TPC$_{(LM+HM)}$ values for the samples of Herzegovina-Neretva Canton (mean values 4.37±0.84 mg GAE/g, and 4.88±0.41 mg GAE/g, respectively), and between TPC$_{(LM)}$ values for the samples of RJ from these locations ($p>0.05$; Student's t-test) (mean values 3.13±0.46 mg GAE/g, and 3.24±0.33 mg GAE/g, respectively). The results showed that the TPC$_{(HM)}$ values for the samples of RJ from Herzegovina-Neretva Canton were statistically significantly higher than the TPC$_{(HM)}$ values for the samples of RJ from Una-Sana Canton ($p^{**}<0.01$; Student's t-test), (mean values 1.69±0.30 mg GAE/g, and 1.06±0.46 mg GAE/g, respectively). There were no statistically significant differences between the TPC values for RJ from Federation of Bosnia and Herzegovina to the TPC values of RJ in another entity of Bosnia and Herzegovina ($p>0.05$; Student's t-test, in all analyzed fractions).

Analysis of antioxidant activity

The antioxidant activity of royal jelly was examined using several methods, but data obtained using the ORAC method are not available (Liu *et al.*, 2008; Park *et al.*, 2020).

Antioxidant activity against hydroxyl free radicals

The equation of the calibrated curve for antioxidant activity (AA) of royal jelly (RJ) against hydroxyl free radicals ($OH^\bullet$) was: $y = 346.78x + 486.68$, and the obtained results, expressed as mmol catechin equivalents per mass of RJ (mM CE/g) are shown in Table 3.

Normality tests show that values for AA$_{OH^\bullet(LM+HM)}$, AA$_{OH^\bullet(LM)}$, and AA$_{OH^\bullet(HM)}$ were in a normal distribution, and in that case they are listed as mean from three determinations with ±S.D. The AA values for 26 uncentrifuged samples of RJ, which are a mixture of low molecular weight (LM) and high molecular weight (HM) antioxidants (AA$_{OH^\bullet(LM+HM)}$) were statistically significantly higher than the AA values for 26 centrifuged samples - low molecular weight (LM) fractions of antioxidants (AA$_{OH^\bullet(LM)}$) ($p^{**}<0.01$; Student's t-test) (mean values 33.91±11.51 mM CE/g, and 28.01±11.32 mM CE/g, respectively). The values of both: AA$_{OH^\bullet(HM+LM)}$ and AA$_{OH^\bullet(LM)}$ were statistically significantly higher than the AA$_{OH^\bullet(HM)}$ values ($p^{***}<0.001$; Student's t-test).

The results showed that there were no statistically significant differences between AA$_{OH^\bullet}$ values for the samples of RJ from Una-Sana Canton to the AA$_{OH^\bullet}$ values of RJ from Herzegovina-Neretva Canton in all analyzed fractions ($p>0.05$; Student's t-test).

Also, the values for AA$_{OH^\bullet(LM)}$ for the samples of RJ from the area of Bosanska Krupa, where natural meadow food for bees prevailed, were statistically significantly higher than the AA$_{OH^\bullet(LM)}$ values for the samples of RJ from the area of Konjic, where the same diet for bees prevailed

($p^{*}<0.05$; Student's t-test) (mean values 30.90±5.99 mM CE/g, and 21.90±1.87 mM CE/g, respectively).

Table 3. Antioxidant activity of RJ against hydroxyl free radicals.

Sample label	$AA_{OH^{\bullet}(HM+LM)}$	$AA_{OH^{\bullet}(LM)}$	$AA_{OH^{\bullet}(HM)}$ *
	mM$_{(CE)}$/g±S.D.		
6	**58.15±1.55**	47.39±1.90	10.76
10	53.69±3.55	**58.15±0.81**	-
13	49.64±0.08	37.58±1.17	12.06
11	48.60±0.37	30.09±0.53	**18.51**
3	47.30±3.51	39.67±1.70	7.63
9f	45.28±1.97	34.18±2.06	11.10
12	43.46±3.32	45.29±0.85	-
9a	40.02±4.38	33.35±0.37	6.67
1	39.10±3.81	24.40±0.79	14.70
9e	38.77±2.68	23.13±0.94	15.64
9b	36.62±0.31	21.44±1.59	15.18
14	35.55±8.25	28.83±1.07	6.72
15	35.36±1.84	39.54±1.92	-
5	27.15±0.01	14.71±1.52	12.44
9d	26.88±0.75	23.95±0.05	2.93
9c	25.90±1.19	22.64±0.33	3.26
7	25.11±4.52	22.71±0.29	2.40
2	24.62±0.58	19.25±0.48	5.37
18b	24.41±1.20	18.53±0.91	5.88
16	24.13±1.24	**10.71±1.09**	13.42
18c	23.73±1.65	12.16±0.67	11.57
8	23.29±2.11	26.17±1.50	-
4	22.32±1.81	28.92±0.19	-
9g	21.47±1.35	19.56±3.12	1.91
17	20.96±3.21	20.42±4.60	**0.54**
18a	**20.18±0.69**	25.44±1.03	-

*Values obtained from the difference $AA_{OH^{\bullet}(HM+LM)}$ - $AA_{OH^{\bullet}(LM)}$.

The $AA_{OH^{\bullet}(LM+HM)}$ and $AA_{OH^{\bullet}(LM)}$ values of RJ samples from Federation of Bosnia and Herzegovina were statistically significantly higher than the $AA_{OH^{\bullet}(LM+HM)}$ and $AA_{OH^{\bullet}(LM)}$ values of RJ samples from another entity of Bosnia and Herzegovina ($p^{**}<0.01$; Student's t-test) (mean values 37.71±11.21 mM CE/g, and 23.60±2.35 mM CE/g for $AA_{OH^{\bullet}(LM+HM)}$, 34.93±9.90 mM CE/g, and 20.36±4.73 mM CE/g for $AA_{OH^{\bullet}(LM)}$, respectively). It was no statistically significant differences between $AA_{OH^{\bullet}(HM)}$ fractions in these two entities.

Antioxidant activity against peroxyl free radicals

The equation of the calibrated curve for AA of RJ against $ROO^{\bullet}$ was: $y = 691.38x + 212.9$, and the obtained results, expressed as mmol catechin equivalents per mass of RJ (mM CE/g) are shown in Table 4.

Normality tests show that values for $AA_{ROO^{\bullet}(LM+HM)}$, $AA_{ROO^{\bullet}(LM)}$, and $AA_{ROO^{\bullet}(HM)}$ were in a normal distribution, and in that case, they are listed as mean from three determinations with ±S.D.

The $AA_{ROO^{\bullet}(LM+HM)}$ values for 26 samples of RJ were statistically significantly higher than the values of both: $AA_{ROO^{\bullet}(LM)}$ and $AA_{ROO^{\bullet}(HM)}$ ($p^{***}<0.001$ in both cases; Student's t-test). Also, the values of $AA_{ROO^{\bullet}(LM)}$ were statistically significantly higher than the values of $AA_{ROO^{\bullet}(HM)}$ ($p^{***}<0.001$; Student's t-test).

There were no statistically significant differences between the $AA_{ROO^{\bullet}}$ values of RJ from Una-Sana Canton and from Herzegovina-Neretva Canton in all analyzed fractions ($p>0.05$; Student's t-test).

Also, the $AA_{ROO^{\bullet}(LM)}$ values for the samples of RJ from the area of Bosanska Krupa, where natural meadow food for bees prevailed, were statistically significantly higher than the $AA_{ROO^{\bullet}(LM)}$ values for the samples of RJ from the area of Konjic, where the same diet for bees prevailed ($p^{**}<0.01$; Student's t-test), (mean values 5.82±1.91 mM CE/g, and 2.31±1.06 mM CE/g, respectively), while the $AA_{ROO^{\bullet}(HM)}$ values from the area of Konjic were statistically significantly higher than the $AA_{ROO^{\bullet}(HM)}$ values from the area of Bosanska Krupa (mean values 1.94±1.17 mM CE/g, and 0.34±0.20 mM CE/g, respectively) ($p^{**}<0.01$; Student's t-test).

Table 4. Antioxidant activity of RJ against peroxyl free radicals.

Sample label	$AA_{ROO^{\bullet}(LM+HM)}$	$AA_{ROO^{\bullet}(LM)}$	$AA_{ROO^{\bullet}(HM)}$*
	mM$_{(CE)}$/g±S.D.		
8	**8.04±0.04**	**7.58±0.39**	0.46
9e	7.83±0.25	7.13±0.10	0.70
3	7.07±0.30	6.96±0.38	**0.11**
1	6.76±0.13	6.58±0.47	0.18
11	6.49±0.11	2.68±0.32	**3.81**
9b	6.18±0.17	3.04±0.17	3.14
15	5.70±1.36	3.22±0.53	2.48
6	5.64±0.38	5.32±0.24	0.32
7	5.09±0.02	4.06±0.02	1.03
9d	4.90±0.71	3.39±0.31	1.51
5	4.72±0.70	4.03±0.67	0.69
13	4.69±0.24	3.53±0.03	1.16
9c	4.02±0.33	1.44±0.02	2.58
4	3.96±0.20	5.43±0.76	-
14	3.75±0.01	3.29±0.30	0.46
2	3.71±0.01	4.42±0.32	-
10	3.62±0.19	1.44±0.09	2.18
12	3.48±0.18	3.05±0.12	0.43
9a	3.05±0.17	2.40±0.34	0.65
9f	2.59±0.23	1.75±0.08	0.84
18b	2.37±0.15	1.55±0.03	0.82
16	2.20±0.11	1.62±0.02	0.58
18a	2.13±0.07	1.49±0.15	0.64
17	1.94±0.29	**1.22±0.06**	0.72
9g	1.87±0.05	1.36±0.12	0.51
18c	**1.66±0.14**	1.41±0.10	0.25

*Values obtained from the difference $AA_{ROO^{\bullet}(HM+LM)}$ - $AA_{ROO^{\bullet}(LM)}$.

The $AA_{ROO^{\bullet}(LM+HM)}$ and $AA_{ROO^{\bullet}(LM)}$ values of RJ samples from the Federation of Bosnia and Herzegovina were statistically significantly higher than the $AA_{ROO^{\bullet}(LM+HM)}$ and $AA_{ROO^{\bullet}(LM)}$ values of RJ samples from another entity of Bosnia and Herzegovina ($p^{**}<0.01$, and $p^{*}< 0.05$, respectively, by Student's t-test) (mean values 4.99±1.77 mM CE/g, and 2.68±1.11 mM CE/g for $AA_{ROO^{\bullet}(LM+HM)}$, 3.88±1.98 mM CE/g, and 2.25±1.26 mM CE/g for $AA_{ROO^{\bullet}(LM)}$, respectively). It was no statistically significant differences between $AA_{ROO^{\bullet}(HM)}$ fractions in these two entities.

Correlations between total phenolics and antioxidant activity

There were very high positive correlations between $AA_{OH^{\bullet}(LM+HM)}$, as well as $AA_{OH^{\bullet}(LM)}$) and $TPC_{(LM+HM)}$, as well as $TPC_{(LM)}$ (Pearson correlations (P.c.): 0.880, and 0.879 respectively, $p^{**}<0.01$ in both cases). It was moderate positive correlation $AA_{OH^{\bullet}(HM)}$ to $TPC_{(HM)}$ (P.c. 0.684, $p^{**}<0.01$). Also, there were weak positive correlations between $AA_{(ROO^{\bullet})(LM+HM)}$, as well as $AA_{(ROO^{\bullet})LM}$ and $TPC_{(LM+HM)}$, as well as $TPC_{(LM)}$ (P.c. 0.013, and 0.116 respectively, $p>0.05$ in both cases), and weak negative correlation $AA_{(ROO^{\bullet})(HM)}$ to $TPC_{(HM)}$ (P.c. -0.058).

Considering that most of the samples were from the area of two Bosnian-Herzegovinian cantons (Una-Sana Canton and Herzegovina-Neretva Canton), which are spatially separated from each other, a statistical analysis of these two groups of data was also performed. The results of this analysis showed that there were very high positive correlations between $AA_{OH^{\bullet}(LM+HM)}$ - $TPC_{(OH^{\bullet})(LM+HM),}$ $AA_{OH^{\bullet}(LM)}$ - $TPC_{(OH^{\bullet})(LM),}$ (P.c. 0.903, and 0.919 respectively, $p^{**}<0.01$ in both cases), and moderate positive correlation $AA_{OH^{\bullet}(HM)}$ - $TPC_{(OH^{\bullet})(HM)}$ (P.c. 0.770, $p^{**}<0.01$) for Una-Sana Canton, but there was very high correlation only between $AA_{OH^{\bullet}(LM)}$ - $TPC_{(OH^{\bullet})(LM)}$ for Herzegovina-Neretva Canton (P.c. 0.935; $p^{**}<0.01$). Also, the results showed that there was a moderate positive correlation between $AA_{(ROO^{\bullet})(LM)}$ and $TPC_{(LM)}$ of RJ samples from Una-Sana Canton (P.c. 0.390; $p^{**}<0.01$), and a very weak negative correlation between $AA_{(ROO^{\bullet})(LM)}$ and $TPC_{(LM)}$ of RJs from Herzegovina-Neretva Canton (P.c. -0.068; $p>0.05$).

CONCLUSIONS

The obtained results showed that royal jelly from Bosnia and Herzegovina is rich in phenolics. Also, royal jelly from Bosnia and Herzegovina has very high antioxidant activity against both: hydroxyl and peroxyl free radicals. Because the results for the antioxidant activity of royal jelly against these free radicals showed significant statistical differences, it is necessary to investigate the effects of these parameters on a larger group of samples. There were observed very strong positive correlations between total phenolics and antioxidant activity against hydroxyl free radicals, and a moderate positive correlation between total phenolics and antioxidant activity against peroxyl free radicals. The obtained results support the recognition of the quality of royal jelly from Bosnia and Herzegovina.

ACKNOWLEDGEMENT

This work was supported by the Federal Ministry of Education and Science, Bosnia and Herzegovina (Grant No 05-39-2603-1/18).

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Summary/Sažetak

U ovom istraživanju, određen je sadržaj ukupnih fenola (SUF) i antioksidativna aktivnost protiv hidroksilnih ($AA_{OH}^{\bullet}$) i peroksilnih ($AA_{ROO}^{\bullet}$) slobodnih radikala, kao i korelacije između ovih parametara u uzorcima svježe matične mliječi (MM) porijeklom iz Bosne i Hercegovine. Za određivanje SUF korištena je spektrofotometrijska metoda sa Folin-Ciocalteu reagensom i galnom kiselinom (GA) kao standardom. Za određivanje AA korištena je metoda apsorbirajućeg kapaciteta kisikovih radikala (ORAC) sa katehinom kao standardom i fluoresceinom kao fluorescentnom metom. Najviši SUF od ukupno 26 uzoraka MM izražen u ekvivalentnoj masi GA po masi svježe necentrifugirane MM nađen je u uzorku iz Kalinovika, 5,54±0,76 mg GAE/g, dok je među centrifugiranim uzorcima MM najviši SUF nađen u uzorku iz Bosanske Krupe, 4,07±0,04 mg GAE/g. Najviša $AA_{OH}^{\bullet}$ izražena u mmol katehin ekvivalenata (KE) po masi svježe necentrifugirane MM (frakcija nisko- i visokomolekularnih antioksidanasa u otopini, $AA_{OH}^{\bullet}{}_{(n.m.+v.m.)}$) nađena je u uzorku iz Cazina, 58,15±1,55 mmol KE/g, dok je najviša $AA_{OH}^{\bullet}$ od centrifugiranih uzoraka MM (niskomolekularna frakcija antioksidanasa, $AA_{OH}^{\bullet}{}_{(n.m.)}$) nađena u uzorku iz Ključa 58,15±0,81 mmol KE/g. Najviša $AA_{ROO}^{\bullet}{}_{(n.m.+v.m.)}$ nađena je u uzorku MM iz Bosanske Krupe 8,04±0,04 mM KE/g, u kojem je nađena i najviša $AA_{ROO}^{\bullet}{}_{(n.m.)}$, 7,58±0,39 mmol KE/g.Uočena je vrlo visoka pozitivna korelacija između $AA_{OH}^{\bullet}{}_{(n.m.+v.m.)}$ prema $SUF_{(n.m.+v.m.)}$, (Pearson-ova korelacija (P.k.)), kao i između $AA_{OH}^{\bullet}{}_{(n.m..)}$ prema $SUF_{(n.m.)}$. Između $AA_{OH}^{\bullet}{}_{(v.m..)}$ i $SUF_{(v.m.)}$ postojala je umjerena P.k.. Također, postojala je slaba pozitivna P.k. između $AA_{ROO}^{\bullet}{}_{(n.m.+v.m.)}$ prema $SUF_{(n.m.+v.m.)}$, kao i između $AA_{ROO}^{\bullet}{}_{(n.m..)}$ i $SUF_{(n.m.)}$. Između $AA_{ROO}^{\bullet}{}_{(v.m..)}$ i $SUF_{(v.m.)}$ postojala je slaba negativna P.k. Na osnovu vrlo visokog SUF i značajne AA može se zaključiti da je MM sa prostora Bosne i Hercegovine dobar izvor prirodnih antioksidanasa i značajan dodatak prehrani.

Glasnik hemičara i
tehnologa
Bosne i Hercegovine

Bulletin of the Chemists and Technologists
of Bosnia and Herzegovina

2024

63

25-30

Print ISSN: 0367-4444
Online ISSN: 2232-7266

Original Scientific Article
DOI: 10.35666/2232-7266.2024.63.04
UDK:543[582.29:551.577.13]

Metal Desorption from the Lichen *Evernia prunastri* (L.) Ach. Using Simulated Acid Rain pH Solutions

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Article info
Received: 26/09/2024
Accepted: 31/10/2024

Keywords:
Evernia prunastri
Washed and Unwashed Lichen
Acid Rain
Desorption
Heavy Metals

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Abstract: In this study, the content of Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn in washed and unwashed lichen *Evernia prunastri* (L.) Ach. was determined. Solutions similar to acid rain (H_2SO_4–HNO_3–$(NH_4)_2SO_4$–NH_4NO_3) with pH 2.02. 2.55, 3.61, 4.33, and 5.15 were used to investigate the desorption of metals from washed lichen *Evernia prunastri*. The metal analysis was conducted by flame atomic absorption spectrometry (FAAS). Content of Fe and Pb was higher in unwashed lichen, Mn and Ni were similar in washed and unwashed lichen, and Cr, Cu, Cd, and Zn were higher in the washed lichen sample. Using solution with pH 2.02, the percentage of desorbed Cu, Mn, Cd, and Zn was higher than 95%, while for Pb it was ~ 80%. Mn was desorbed in a mass percentage higher than 79% in all simulated acid rain solutions, while the percentage of Cr desorption was less than 1%. Solutions with pH 3.61, 4.33, and 5.15 desorbed all metals except Mn in a percentage lower than 68%. The results indicate that the lichen *Everina prunastri* can become a secondary environment pollutant and that the mass percentage of desorbed metals depends on the strength of the bond between the metal and the components of the lichen tissue or cell wall, as well as on the pH value of the precipitation with which the lichen comes into contact.

INTRODUCTION

Lichen are symbiotic organisms of fungi and algae. They lack roots and a protective cuticula layer and stomata, so they accumulate substances (mineral elements, including heavy metals) from the atmosphere using their complete tallus surface at levels that exceed their metabolic requirements. Concentrations of elements found in lichen thalli can be directly related to those present in the environment, so that is one of the reasons that lichens are used as bioindicators of air pollution. The accumulation of metals in lichens is affected by a variety of factors, including metal availability, lichen properties, and climate conditions. The life of lichens depends on the mineral nutrients obtained by wet atmospheric deposition (precipitation, fog, dew) and dry atmospheric deposition (sedimentation, gaseous absorption). The lichen thallus stores metals in its intracellular spaces by particle capture, ion exchange, adsorption, and active uptake (Bačkor and Loppi, 2009). Lichens can survive high levels of heavy metals by storing them as oxalate crystals or lichen acid complexes. They are slow-growing and long living organisms that can provide long-term data on the amounts of heavy metals and other pollutants in the atmosphere. Lichens use the cation exchange mechanism to rapidly absorb soluble substances over their entire surface. Soil particles and aerosols deposited on the surface also contribute to the thalli overall element concentrations (Tošić et al. 2022). Depending on the pH of the environment and competition between metal ions, the lichen can accumulate and desorb metals. Although the accumulation of metals in different lichen species is extensively reported (Conti et al., 2004 Lopi and Paoli 2015; Ramić et al., 2019; Adžemović et al., 2023; Aničić Urošević et al. 2024; Vannini et al., 2024;), information on metal desorption is limited (Piervittori et al. 1997; Čučulovic et al., 2014; Vannini et al., 2021a; Vannini et al., 2021b. Due to the lack of information on metal desorption from the lichen, the aims of this study were: a) to determine the content of eight heavy metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) in washed and unwashed lichen *Evernia prunastri* (L.) Ach.; b) to estimate the desorption of Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn from the thallus of the washed *Evernia prunastri* using five simulated acid rain pH solutions (2.02, 2.51, 3.61, 4.33, and 5.12); c) to determine whether the lichen species *Evernia prunastri* could become a secondary environmental pollution source.

EXPERIMENTAL

Sampling and sample preparation

Lichen sampling was carried out under stable weather conditions. Sampling took place at Vuksovići village, about 40 km from Sarajevo the capital of Bosnia and Herzegovina,. The sample location (Figure 1) is 7.1 km from the major road and at an altitude of about 1100 m a.s.l; there are no production facilities nearby. To avoid contamination of the samples, the lichen was removed from the tree's bark using a plastic knife. Samples were gathered from branches that were 150 cm above ground. Rubber gloves were utilized for sampling. The samples were maintained in paper bags labelled with the sampling date, location, and name of the individual who collected the samples. The samples were kept at room temperature in a dark environment, and after being cleared of physical contaminants, one part of the sample was air dried for seven days, and the other part was washed with demineralized water and air-dried for seven days. The samples were washed in demineralized water since one of the research objectives was to determine whether the metals accumulated inside the lichen are desorbed.

Figure 1 - Vuksovići village, location of sampling lichen *Evernia prunastri*

Metal analysis of the washed and unwashed lichen

Lichen samples from the location of the village of Vukasovići were analyzed for metal content by washing one part of the sample in distilled water, and the other part of the lichen sample did not undergo any cleaning treatment except for the removal of traces of tree bark. The aim of the analysis of the metal content in washed and unwashed lichen was to check how washing the lichen in distilled water affects the metal content in the lichen *Evernia prunastri*. It was ensured that the analysis of washed and unwashed lichen was evenly distributed in the radius of the sampling location. Metal analysis of the washed and unwashed lichen samples was performed as follows: A total of about 1 g (± 0.1 mg) of dry lichens (washed/unwashed) was weighted directly in polytetrafluoroethylene (PTFE) digestion vessels. Both washed and unwashed lichen samples were analyzed in triplicate. After adding 25 mL of concentrated HNO_3 and after the evaporation of nitrogen oxides, the vessel was sealed and left to react at 60°C for 12 h. Then, 5 mL of H_2O_2 was added and the solutions were heated for 10 min, transferred to 50 mL volumetric flasks and filled to the mark with double-distilled water. The metal analysis was performed using a flame atomic absorption spectrometer, model AA240FS, Varian.

Solutions used to simulate acid rain were prepared as follows:

- Five (A) solutions were prepared: pH 2.02 (A1), 2.55 (A2), 3.61 (A3), 4.33 (A4), and 5.12 (A5) by adding concentrated H_2SO_4 to 500 mL of demineralized water until the desired pH value was reached. Then, 2.5 g of $(NH_4)_2SO_4$ and 2.5 g of NH_4NO_3 were added to each of these solutions.
- Solutions (B1, B2, B3, B4, B5) were made in the same way as solutions A, only nitric acid was used instead of sulfuric acid.
- Solutions A1 and B1, A2 and B2, A3 and B3, A4 and

B4, and A5 and B5 were mixed in a ratio of 1:1, and the following solutions were obtained 2.02 (C1), 2.55. (C2), 3.61 (C3), 4.33 (C4) and 5.12 (C5). The pH value of each of the five final solutions (C1-C5) was adjusted, if necessary, by adding concentrated H_2SO_4. Solutions C1-C5 were used as acid rain solutions to perform metal desorption experiments from the lichen.

Buffer solutions with pH 4.00 and 7.00 (Reagecon) were used to calibrate the pH meter (MP200, METTLER TOLEDO, Swiss).

The washed lichen *Evernia prunastri* was used to analyze the desorption of metals by simulated acid rain solutions. About 2.5 g (± 0.1 mg) of the washed lichen sample was weighed, and the analysis was done in triplicate. The washed lichen samples were transferred into fifteen 250 mL laboratory beakers and 50 mL of the corresponding solution C1, C2, C3, C4 and C5. The extraction was carried out at room temperature for 24 h with occasional mixing of the solution. After extraction, the lichen solution was filtered, and the lichen was dried to a constant mass at room temperature. Then 50 mL of a new portion of the solution (C1-C5) with the same pH was used for successive desorption of the metal from the same replicate of the lichen sample, and the procedure of filtering and drying was repeated. The desorption process was repeated three times on the same replicate of the lichen with a solution of the same pH (C1-C5) (three replicates of lichen were treated three times with the same solution e.g. C1). The contents of Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn were determined in the solutions after each repeated extraction by FAAS.

Analytical quality control

All substances used were of analytical grade and supplied from Merck (Darmstadt, Germany). To ensure accurate heavy metal assessment in lichens, a certified reference material (CRM-IAEA-336-ichen) was analyzed. All samples, blanks, and CRMs were prepared in triplicate. The recovery was calculated and ranged from 95% to 102% for all metals. The value of the detection limit (LOD) (calculated as three times the standard deviation of the blank signal) were: Cd (0.002 mg/L), Cr (0.007 mg/L), Cu (0.06 mg/L), Fe (6.22 mg/L), Mn (0.20 mg/L), Ni (0.01 mg/L), Pb (1.00 mg/L), and Zn (0.75 mg/L).

RESULTS AND DISCUSSION

One of the aims of this study was to determine the content of eight metals: Cr, Cu, Fe, Mn, Ni, Cd, Pb, and Zn in washed and unwashed lichen samples. Table 1 presents the results of determined metals in washed and unwashed lichen samples and the results for the percent relative standard deviation (%RSD). %RSD was used to evaluate homogeneity in the samples between the unwashed and washed procedures. Table 2 presents the results of the mass percentage of desorbed metals after each extraction and the mass percentage of total metals extracted by solutions simulating acid rain.

Table 1 - Average metal content (mg/kg ± SD) and %RSD in washed and unwashed lichen *Evernia prunsatri*

Metals	**Mean ± SD (mg/kg)**		**The difference between unwashed and washed samples**		**%RSD**		
	Unwashed	**Washed**	**Amount (mg/kg)**	**Percent (%)**	**Unwashed**	**Washed**	**Unwashed/ washed ratio**
Cr	5.29 ± 0.896	5.81 ± 0.36	- 0.52	↑ 10	16.94	6.1	2.8
Cu	3.94 ± 0.283	5.78 ± 0.07	- 1.84	↑ 47	7.18	1.19	6
Mn	11.52 ± 0.114	11.49 ± 0.004	0.03	↓ 0.3	0.99	0.035	28.3
Fe	401 ± 21.37	284 ± 10.20	117	↓ 29	5.33	3.59	1.5
Ni	10.61 ± 0.35	10.71 ± 0.22	- 0.1	↑ 1	3.30	2.1	1.6
Cd	0.132 ± 0.008	0.222 ± 0.003	- 0.09	↑ 68	6.06	1.35	4.5
Pb	6.53 ± 0.018	6.21 ± 0.06	0,32	↓ 5	0.28	1	0.28
Zn	29.94 ± 0,66	36.45 ± 2.20	- 6.51	↑ 22	2.20	6.04	0.36

The results presented in Table 1 showed that the metal content in unwashed and washed lichen was arranged as the following diminishing series: Fe > Zn > Mn > Ni > Pb > Cr > Cu > Cd. The highest %RSD in washed and unwashed lichen was for Mn (28.3), Cu (6), Cd (4.5) and Cr (2.8) while for the other analyzed metals, the %RSD was below 2%. Paoli et al. (2012) point out that high amounts of lithogenic elements in lichen, including Fe, Cd, Cr, and Ni, originate from airborne soil dust. The content of Fe and Pb was higher in the unwashed lichen sample. One of the reasons for this could be that lichen had particles on the surface that were rich in these two metals. Celik et al. (2005) mentioned that Pb pollution on a local scale is caused by industrial emissions, and on a larger scale it is caused by emissions from motor vehicles using leaded gasoline. Leaded gasoline has stopped being used in the European Union since 2000, and in Bosnia and Herzegovina since 2010. It is possible that lead has accumulated in lichen from the years when leaded gasoline was still used, or it originates from some other anthropogenic atmospheric sources. Parzych et al. (2016) reported that 73% to 95% of the Pb content in plants is of atmospheric origin (atmospheric particles, combustion of solid fuels used for heating households and residential buildings). The content of Mn and Ni was similar in washed and unwashed lichen, while the content of Cr, Cu, Cd, and Zn was higher in the washed samples. Some authors (Boonpeng et al. 2020; Garty and Garty-Spitz

2015; Kularatne and de Freitas 2013) mentioned that these variations in results may mainly depend upon the contamination level of the sampling location, the chemical composition of the pollution accumulated in the lichen thallus, as well as the accumulation sites of elements (for this study, samples of *Evernia prunastri* were taken in the radius of 200 m). Garty (2001) and St. Clair et al. (2002), in their studies, mentioned that areas with humidity and clean air accumulate metals in minimal amounts and that most metals are intracellular. One of the reasons why the content of some metals is higher in the washed lichen could be that elements accumulated intracellularly are not or are less affected by distilled water washing. Soil particles in clean-air areas with a dry climate can have a significant impact on the amounts of elements in lichen thalli. As a result, big soil particles can collect on thallus surfaces or outside the cell. The elements that accumulate in these places are washed away by water with less difficulty compared to those in the intracellular site, particularly water-soluble elements (Boonpeng et al. 2020; Tošić et al. 2022). The results for metal content in this study were compared with other studies in Europe and the world that used *Evernia prunastri* as a lichen sample, and the results were in the same range as in the studies of: Paoli et al., 2012; Loppi and Paoli, 2015; Vannini et al., 2017. Tošić et al., 2022; Studzińska-Sroka et al., 2023;

Table 2 - Mass percentage of desorbed metals from lichen after successive extractions (I, II, III) with solutions simulating acid rain (C1-C5) and percentage of total extracted metals from lichen

Mass percentage of desorbed metals after extractions with solutions simulating acid rain

Metals/ No of extraction n_x		**pH**	**Cr**	**Cu**	**Mn**	**Fe**	**Ni**	**Cd**	**Pb**	**Zn**
I	C1	2.02	*ND	60.07	72.60	13.01	8.93	84.19	38.26	65.15
I	C2	2.55	*ND	26.77	74.32	7.82	6.32	72.99	25.41	18.34
I	C3	3.61	*ND	21.28	60.71	6.51	7.20	24.25	9.57	23.18
I	C4	4.33	0.86	14.59	74.37	2.71	5.48	15.27	12.61	7.28
I	C5	5.12	*ND	15.45	72.78	6.65	3.58	24.30	*ND	20.59
II	C1	2.02	*ND	22.71	19.42	8.71	6.73	15.53	26.50	33.68
II	C2	2.55	*ND	58.84	17.56	1.35	2.94	8.04	20.40	9.81
II	C3	3.61	*ND	19.90	12.74	1.11	4.04	*ND	*ND	24.69
II	C4	4.33	0.18	29.68	12.04	0.70	3.92	0.44	13.15	7.79
II	C5	5.12	*ND	49.10	10.44	3.32	3.46	3.82	17.83	39.31
III	C1	2.02	*ND	14.88	7.61	9.89	*ND	*ND	13.13	16.73
III	C2	2.55	0.94	3.67	7.65	1.57	*ND	*ND	0.51	11.24
III	C3	3.61	*ND	4.73	5.65	1.53	*ND	*ND	*ND	12.52
III	C4	4.33	*ND	3.95	6.32	1.35	*ND	*ND	*ND	*ND
III	C5	5.12	*ND	3.57	4.74	1.84	*ND	*ND	*ND	12.19

The mass percentage of total extracted metals

	Cr	Cu	Mn	Fe	Ni	Cd	Pb	Zn
2.02	ND*	97.7	99.6	31.6	15.7	99.7	77.9	115.6
2.55	0.94	89.3	99.5	10.7	9.3	81.0	46.3	39.4
3.61	ND*	45.9	79.1	9.2	11.2	24.3	9.6	60.4
4.33	1.04	48.2	92.7	4.8	9.4	15.7	25.8	15.1
5.12	ND*	68.1	88.0	11.8	7.0	28.1	17.8	72.1

*ND- not desorbed

The results presented in Table 2 show that using un acid solution with a pH 2.02, the percentage of desorbed Cu, Mn, Cd, Zn was higher than 95%, while the percentage of desorbed Pb was around 80%. This could indicate that these metals, while accumulating in lichen tissue, build highly insoluble compounds or complexes with higher stability constants. If the pH of the acid solution was 2.55, 81% of Cd and around 90% of Cu were desorbed from *Evernia prunastri*. These results showed that H^+ ions have an effect on the desorption of metals from the lichen.

Using solutions with pH 3.61, 4.33, and 5.15, all analyzed metals except Mn were desorbed in percentages less than 68%. There are no literature data for metal desorption from *Evernia prunastri* with acid solutions ranging from 2.02 to 5.15. Čučulović et al. (2007); Čučulović et al. (2008); Čučulović et al. (2014), investigated the desorption of various elements (K, Al, Ca, Mg, Fe, Cu, Ba, Zn, Mn, Sr, and ^{137}Cs) from the lichen *Cetraria islandica* (L.) Ach with solutions pH 2.00 to 5.75; these solutions were prepared with a variety of components (water, HNO_3; H_2SO_4; H_2SO_4–HNO_3–$(NH_4)_2SO_4$; H_2SO_4–HNO_3–$(NH_4)_2SO_4$–NH_4NO_3). The results of this study compared to the results presented in the study of Čučulović et al. (2008) and Čučulović et al. (2014) showed that the mass percentage of desorbed metals (Cu, Mn, Fe, and Zn) from *Evernia prunastri* after three extractions was higher than that presented in *Cetraria islandica* after five extractions. What is similar in this study and other studies investigating metal desorption from the lichen *Cetraria islandica* is that the use of lower pH solutions increased metal desorption. Also, it is very important to know that the metal accumulation in lichens depends on their age, reproduction, location, temperature, moisture, and substrate properties, including chemical composition and acidity.

CONCLUSION

This study showed that the lichen washing procedure did not affect the content of Mn and Ni, while it can increase the content of Cr, Cu, Cd, and Zn in *Evernia prunastri*. In this work, the desorption of metals Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn was analyzed using a simulated acid rain solution. Solutions with pH values between 2.02 and 5.15 were used, which corresponds to the composition of acid rain (H_2SO_4–HNO_3–$(NH_4)_2SO_4$–NH_4NO_3). There is no previously published data for desorption of metals from *Evernia prunastri* with solutions whose pH values are from 2.02 to 5.15. Based on the presented results, it can be concluded that certain concentrations of the analyzed metals will be desorbed by acid rain; it depends on the pH of acid rain, the stability of chemical bonds and complexes formed between metals and components from lichen tissue or cells, and it also depends on the lichen species. A low mass percentage of metal desorption could indicate that the metal is tightly bound within the lichen cell itself; either they build highly insoluble compounds or complex compounds with lower or higher stability constants. A high mass percentage of metal desorption can indicate that the metals are linked with an ionic bond to the corresponding chemical groups in the lichens. Decreasing the pH value of acid rain will increase the desorbed concentration of metals, except for Cr.

The results suggest that acid rain with pH values below 2.6 may enhance the desorption of metals from *Evernia prunastri*, indicating that this lichen could serve as a secondary source of environmental pollution with the analyzed metals.

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Summary/Sažetak

U ovom istraživanju određen je sadržaj Cd, Cr, Cu, Fe, Mn, Ni, Pb i Zn u opranom i neopranom lišaju *Evernia prunastri*. Rastvori koji simuliraju kiselu kišu (H_2SO_4–HNO_3–$(NH_4)_2SO_4$–NH_4NO_3) sa pH 2,02. 2.55, 3.61, 4.33 i 5.15 su korišteni za ispitivanje desorpcije metala iz opranog lišaja *Evernia prunastri*. Analiza metala je provedena plamenom atomsko apsorpcionom spektrometrijom (FAAS). Sadržaj Fe i Pb je bio veći u neopranom lišaju, Mn i Ni je sličan u opranom i neopranom lišaju, a sadržaj Cr, Cu, Cd i Zn je veći u opranom uzorku lišaja. Kod rastvora sa pH 2,02 procenat desorbovanog Cu, Mn, Cd i Zn bio je veći od 95%, dok je za Pb bio oko 80%. Mn se desorbirao u masenom procentu većem od 79% u svim rastvorima koji simuliraju kiselu kišu, dok je procenat desorpcije Cr bio manji od 1%. Rastvori sa pH 3,61, 4,33 i 5,15 desorbirali su sve metale osim Mn u procentu nižem od 68%. Rezultati pokazuju da lišaj *Evernia prunastri* može postati sekundarni zagađivač okoliša i da maseni procenat desorbiranih metala ovisi o jačini veze između metala i komponenti unutar tkiva ili ćelijskog zida lišaja, kao i od pH vrijednosti padavina sa kojima lišaj dolazi u kontakt.

Glasnik hemičara i tehnologa Bosne i Hercegovine
Bulletin of the Chemists and Technologists of Bosnia and Herzegovina
2024
63
31-37
Print ISSN: 0367-4444
Online ISSN: 2232-7266
Original Scientific Article
DOI: 10.35666/2232-7266.2024.63.05
UDK:620.196[669.268:661.875'027.1]

Modification of the Surface of Objects for the Aviation Industry by the Procedure of Hard Chrome Plating

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Article info
Received: 21/11/2023
Accepted: 17/10/2024

Keywords:
Hard Chrome Plating
Shroud-Fuel Nozzle
Reparation
Coating Quality

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Abstract: In the paper, the quality of the hard chrome plating on standard steel plates and shroud-fuel nozzle used in the aviation industry was examined, where the thickness of the coating, hardness, microscopic structure and resistance to bending were determined. The entire galvanization process involved chemical, electrochemical and mechanical preparation of the examined parts prior to chromating, while the chromating itself took place in a solution of chromic acid. The aim of the work is to obtain high-quality hard chrome coatings through the process of reparation of worn shroud-fuel nozzle, which will be able to replace commercial new parts in terms of quality and characteristics. Also, taking into account that some parts are no longer even commercially available, the reparation procedure should enable extended use of the same parts.

INTRODUCTION

Researchers and engineers are concentrating on increasing the operating temperatures of gas turbines and enhancing plant efficiency while minimizing breakdowns. This advancement is largely made feasible through the development of new materials that resist heat, corrosion, and erosion, utilizing various surface engineering techniques. (Prashar and Vasudev, 2024).

The corrosion process can lead to increased maintenance and repair costs for materials. Additionally, failures caused by corrosion can present safety hazards and lead to accidents, which in turn exacerbate the economic impacts (Tamalmani and Husin, 2020).

The process of covering the surface of one material with a thin layer of metal is referred to as metal coating (Artkin, 2023, Ivanov, Alsaraeva, Gromov et al., 2015; Pachurin, Mukhina, Kuzmin, et al al., (2020). Various metal coating techniques are used for different applications, depending on the material being coated and the desired properties of the coating (Artkin, 2023).

The technology of material protection using coatings is very demanding and complex, and it is necessary to know all the steps required for the coating to be applied well, from surface preparation, and application of the coating to drying (Juraga, Alar, et al., 2012; Stupnišek-Lisac, 2007; Esih, 2007). To prevent corrosion and rusting of materials, protective coatings are applied to vehicle bodies, which increases durability and preserves their visual appeal. (Motlalte, Ray, Ojijo et al., 2022). Steel surfaces can be coated with metal using one of four popular techniques. These include sherardizing, electroplating, thermal spraying, and hot-dip galvanizing. The level of corrosion protection provided by metallic coatings is mostly determined by the kind and thickness of the coated metal, with the application technique having a minimal effect (Demirel, Biskiner, Sahin 2023). In addition, corrosion protection coatings play a vital role in the aerospace industry, as aircraft face a range of environmental challenges, such as moisture, high-altitude conditions, and chemical contaminants. (Pecho, Vel'ky, Bugaj et al., 2023).

Galvanization is the most widely used procedure of applying metal coatings to metal and non-metal objects by electrolysis of ionic solutions, i.e. electrolyte (Ozturk, Evis, Kilic, 2017). It is based on the cathodic reduction of metal ions from the electrolyte using an electric current (Munger, Vincent, 1999).

Chromizing or chrome plating is a surface engineering method widely used in industries to obtain a hard metal surface. This method is preferred because it is easy to apply and is more economical (Hasanah, Priadi, Dhaneswara, 2023). The chromium plating process is one of the most effective methods for safeguarding the base material from extreme environments or enhancing its surface properties (Islam, Hasan, Poroma et al., 2024).

Chromium plating, particularly when applied at thicknesses exceeding 2 μm, holds significant industrial importance." (Adachi, Kitaba, Fukami et all., 2020).

Because of its exceptional abrasive resistance, thermal stability, and chemical durability, electroplated chromium coatings are commonly used in high-temperature environments with moving parts, including hydraulic components, turbine blades, gun barrels, and other mechanical equipment and weapons, to reduce failure rates and extend service life. (Podgornik, Massler, Kafexniu, 2018).

Hard chrome coatings are becoming more prevalent in the contemporary protective industry, as they enhance resistance to wear, corrosion, and abrasion. Additionally, these coatings alter the physical and mechanical properties of material surfaces, improving the quality of the coated objects. (Kvashrin, Oganov, Samtsevich et al., 2017; Liang, Li, Ni et all., 2017). In addition to protection against corrosion, galvanic chrome coating is applied for reparation of damaged and robust parts worn in places of friction, such as turbine rotors, machine shafts, etc. (Astanin, Pukhov, Stekolnikov, et al., 2018). Chrome coatings are resistant to atmospheric corrosion at normal and elevated temperatures, are very hard and wear-resistant, have a low coefficient of friction, and can be obtained shiny without subsequent processing (Fedrizzi, Rossi, Bellei, et all, 2002). Chromium has several valence states, divalent chromium salts are not stable in air and, like electrolytes, are not suitable for chroming. Cr (III) ions in aqueous solutions are also not favorable for chroming, as they highly hydrate and are difficult to reduce. Therefore, Cr (VI) in the form of chromate and Cr (VII) in the form of dichromate are suitable because they can be reduced at the cathode when the electrolytes contain certain anions that act as a catalyst (e.g. sulfate or fluoride ions (Abys, 2011). Preparation before the galvanization process itself can be mechanical, chemical, or electrochemical (Đorđević, Maksimović, Pavlović, et all., 1998). By preparing the substrate, it is necessary to ensure good adhesion of the coating and its specific properties, and this is achieved by cleaning the surface from greasy substances, corrosion products, dust, etc., and achieving the optimal level and form of roughness (Fedrisi, Rossi, Bellei, 2002). The process of modifying and coating the surface with hard chrome plating is the process of returning used parts for exploitation/reuse (De Mello, Goncalves, Costa, 2013). The most commonly coated materials are stainless steel and its alloys. Stainless steels are iron-based alloys that contain at least 12% Cr to form a passive film, the presence of which ensures their corrosion resistance (Petrović, Stojanović, 2007). Additional surface protection of stainless steel provides increased corrosion resistance, and depending on the deposited coating, some other properties are also improved (abrasion resistance, hardness). Compact, fine-grained electrochemical chrome coatings are a good choice for solving functional requirements (Petrović, 2000).

Aircraft encounter a range of environmental influences, such as chemical contaminants, moisture, and special well as high-altitude conditions (CAP1570, 2019). Coatings on aircraft exteriors safeguard against corrosion, preserve aerodynamic efficiency, and enhance overall safety (Pecho, Veľký, Bugaj, 2023). The work aims to carry out the process of applying hard chrome to the shroud-fuel nozzle used in the aviation industry to repair worn parts and to achieve a certain thickness of the coating that will meet the required characteristics of hardness and specific resistance. At the same time, the process of hard chrome plating on standard steel plates was also carried out to compare the results and simplify the characterization. Moreover, a suitable alternative to hard chrome has not yet been found for all applications and is unlikely to be indicating that hard chrome coatings will continue to be essential (Podgornik, *Massler*, Kafexhiu, et al., 2018).

EXPERIMENTAL

In the experimental part of the work, the aim was:

- determine the chemical composition of the basic material of the shroud-fuel nozzle;
- prepare the material;
- perform the technological inspection of materials and defects;
- perform the inspection for cracks;
- measure the existing coating to accurately determine the application of chrome coating in the plating process;
- carry out the tempering process at the required temperature to relieve stress in the base material.";
- perform the hard chrome plating process;
- measure the thickness of the new forms of coating (hardness of the coating on the standard steel plate, bending test of the standard steel plate, microscopic observation of the coating);
- visual comparison of the parts before and after the repair procedure.

MATERIAL AND METHODS

- To determine the surface chemical composition of the material, the XRF method was used on the “X-Met 8000 Expert” device;
- A “Carl Zeiss” microscope, type “Neophot 21” was used for metallographic examinations;
- The “Karl Frank” device, type “38536” was used to test the microhardness of the coating;
- A microscope and a coating thickness measuring device “QuaNix 1500” were used to determine the coating thickness.
- Test for bending using a bending tool and coating adhesion using a comb.

Before the hard chrome plating process, the material was prepared. For testing the application of chrome coating, and in accordance with the tested parameters, the standard plate made of alloy steel and shroud-fuel nozzle, which was used in the aviation industry, was selected as the base material (Figure 1). The standard plates on which the hard chrome plating process is to be performed are first electrochemically degreased in an alkaline solution to remove all impurities (Li, Jia, Chen, et all., 2023), followed by a grinding process using abrasives. In this case, there is no specified requirement for the fineness or roughness of the surface when grinding reference tiles. The surface must be uniform, smooth, and clean. However, with a shroud-fuel nozzle, it is necessary to sand to remove the old coating, and the sanding is done to a certain extent. For the proper and even application of the chrome coating, the surfaces to be chromed must be placed on appropriate tools of special construction and then degreased. Mask surfaces that are not subject to chrome plating using PVC foil, lead tape, or special tools. Degrease surfaces electrochemically in a solution for electrochemical degreasing. An alkaline solution of the appropriate composition was used for this. After degreasing, the surface should be thoroughly rinsed in clean, cold water. Before applying the hard chrome coating, the parts were thermally treated to reduce the risk of hydrogen brittleness at temperatures from 130º to 150 ºC for 5 hours. Etching of the surface was carried out anodically in a chromate acid solution for 20 to 30 seconds at a voltage of 6 V.

Figure 1: View of the standard plate and shroud-fuel nozzle prepared for the hard chrome plating process

Electrolyte composition and chroming:
CrO_3 - 289 g/dm^3
Cr_2O_3 - 1.28 g/dm^3
H_2SO_4 - 2.86 g/ dm^3
bathtub volume 1200 dm^3

The anode is made of lead, which also contains antimony, silver, and other elements.

The prepared surfaces are chrome-plated in a chromate acid solution at a temperature range of 50-55 ºC and a current density of 45 - 55 A/dm^2. The surfaces to be chrome plated are placed as cathodes, in relation to anodes in a ratio of 2:1. The chrome plating time depends on the application, taking into account that an average thickness of chrome of 0.0254 mm is deposited in 1 hour at the specified working conditions. When placing the parts in the tub for chrome plating, it is ensured that the parts are 10-15 cm away from the walls of the tub, 10 cm from the upper level of the liquid, and at least 15 cm from the bottom of the tub. The parts are placed vertically in the tub and are equidistant on both sides from the anodes. Chrome plating is done in a layer that is sufficient to compensate for machining. In the case of a shroud-fuel nozzle that is mounted on tools made for this type of part, the protected parts that are not chrome-plated in this case are the nuts. The shroud-fuel nozzle is exposed to chrome plating. After the chrome plating, the plates are rinsed with water in a tub for “saving” rinsing and then in a tub with clean and cold running water. The tiles are then rinsed in a tub of hot water. After rinsing and drying, the parts are removed from the tool and the protective masks are removed. The chrome-plated surfaces were then subjected to the dehydrogenation process by heating the object at the highest possible temperature for about 2 hours. After heating, a visual control of the chrome-plated surface is performed. After inspection, the surface is sanded to the prescribed dimension, and after sanding, a visual control is performed. The coating must not be in the form of pitting, spots, and cracks. The obtained results were not compared with the experiments and results of other studies due to the lack of similar research.

The appearance of the reference plate and the injector jacket after the chrome plating process is shown in Figure 2.

Figure 2: The appearance of the standard plate and shroud-fuel nozzle after the hard chrome plating process

RESULTS AND DISCUSSION

Table 1 presents the results of testing the chemical composition of standard plates following surface preparation for the hard chrome plating process, along with the chemical composition of the shroud-fuel nozzle. X-ray fluorescence spectrometry is a non-destructive analytical method employed to identify and quantify the mass concentration of chemical elements in liquid, solid, and powder samples. The XRF spectrometer analyzes the specific wavelength components of the fluorescence emissions from a sample exposed to X-rays (Ivanek, 2016; Morgui, Queralt, de Almeida, 2022).

Table 1: Display of the results of the chemical composition of the material

Element	Chemical composition % (m/m)	
	Standard plates	Shroud-fuel nozzle
Si	0.33	0.35
Cr	11.81	12.27
P	0.01	0.03
Mn	0.74	0.24
Ni	2.59	0.73
V	0.33	0.02
Mo	1.64	0.07
Fe	the remain	the remain

The composition of the tested standard plates consists of steel alloyed with 12% of chromium and 2% of nickel. Based on the obtained chemical analysis for the shroud-fuel nozzle, it can be concluded that it is made of a very similar material as the tested standard plate. In addition to the visual inspection of the applied coating, the thickness of the hard chrome on the standard plates was also measured. The measurement of the thickness of the hard chrome coating was carried out on two standard plates at several measuring points, and the obtained results are shown in Table 2. The thickness of the coating is an important parameter for almost all types of coatings (Tadić, 2013; Bammer, Huemer, 2019). Effective control of coating thickness can lead to outcomes such as producing a product that meets customer specifications, achieving cost savings by preventing material waste, and increasing yield through the use of in-line control (Jones, Uggalla, Li et all., 2021). Methods for testing coating thickness are divided into two primary categories: destructive and non-destructive measurements (Petrili, 2021).

Table 2: Results of coating thickness measurements on standard plates

Sample	Coating thickness (µm)										The mean value of the coating thickness (µm)
	Number of measurements										
	1	2	3	4	5	6	7	8	9	10	
1	32	37	34	39	36	33	31	44	39	34	35.9
2	28	30	32	31	33	29	25	33	27	27	29.5

The obtained results show differences in the thickness of the layer at different measuring points. Namely, the different thickness of the coating is the result of the electrochemical deposition of the coating on the standard plates, which are flat and have sharp edges compared to the roundness of the surface of the shroud-fuel nozzle. The thickness of the coating was measured using the destructive microscopy method. Table 3 presents the results of coating thickness measurements from one section.

Table 3: Display of obtained coating thickness values for a standard plate by measuring with a microscope

Sample	Coating thickness (µm)										The mean value of the coating thickness (µm)
	Number of measurements										
	1	2	3	4	5	6	7	8	9	10	
1	31.25	31.2	31.0	30.31	31.1	31.56	31.2	31.0	31.1	31.1	31.18

Deviations in this measurement compared to the measurement using the QuaNix contact device are acceptable because the thickness of the section coating is measured on a much smaller surface (about 20 mm), while the measurements using the device were made at different measuring points, measuring 50x70 cm. Figure 3 displays the appearance of the plate section magnified at 400x, used for measuring the coating thickness.

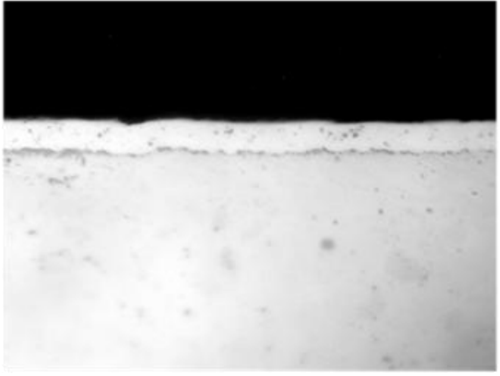

Figure 3: Microscopic observation of coating thickness at 400x magnification

Hardness tests fall into the category of destructive methods. The fundamental principle involves applying a load to the material by pressing a test specimen with a specific geometric shape, using a defined force for a set duration (Svoboda, Lysonkova, 2020).

Hardness is a key factor influencing tribological properties and serves as an indicator of a material's resistance to plastic deformation. Hardness most effectively indicates a material's resistance to wear. The hardness of the hard chrome coating was assessed using the Vickers method. Microhardness measurements were conducted on two standard plates, with the results presented in Table 4.

Table 4: Display of the measuring results of the hard chrome coating microhardness

Sample	Microhardness HV 0.2				
	Number of measurements				
	1	2	3	4	5
1	883	927	927	905	883
2	883	862	862	905	862

Based on the obtained results, it can be concluded that the microhardness of the coating on the tested standard plates met the requirements of the SNO 2328/91 standard. The standard plates were also tested for bending, where the bending was performed at an angle of 90º (Figure 4). The hard chrome coatings passed this test, meaning that there was no tearing or peeling of the coatings and that the part on the bends was still coated with hard chrome. If there is separation, peeling or cracking, the coating is not acceptable.

Figure 4: The appearance of the plate after bending at an angle of 90º

The adhesion of coatings is crucial in the coating industry, and a deeper understanding of adhesion behavior is necessary (Sweden, 2019).

Adhesion refers to the energy needed to detach the coating from the substrate (Figure 5). "Perfect" adhesion would be in case the bond between the atoms of the coating and the substrate is stronger than the bond between the atoms in the coating and the atoms in the substrate (Bajat, Mišković–Stanković, Dražić, 2007). Coating adhesion was measured by the standard notch method.

Figure 5: The appearance of the plate examined by the notch method

The results showed that the coating has good adhesion because there was no separation or peeling in any place.

Metallographic tests were performed to assess the microstructure. Three samples were taken from the plate on which the hard chrome coating was applied, and based on them, it can be said that the first two positions have an even coating that is slightly wavy, while damage to the coating can be observed in the third position, and it can be seen that it is wavy and uneven (Figure 6).

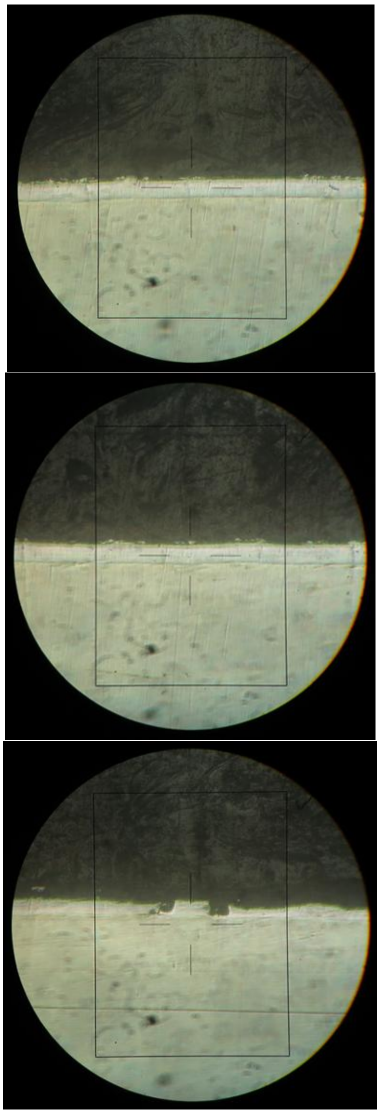

Figure 6: The appearance of three sections under a microscope

CONCLUSIONS

1. By modifying the surface of the material with the process of hard chrome coating, the quality of the processed object surface being coated increases, as well as the lifetime of the material.
2. One of the most important procedures before the hard chrome coating process is surface preparation. Preparation of the surface results in a firmer adhesion of the coating to the base material, i.e. good adhesion.
3. The results of measuring the coating thickness satisfied the thickness of about 30 microns for one hour of deposition which was predicted based on the given parameters of current density and the deposition time The measured coating thickness was approximately 30 microns after one hour of deposition, as anticipated based on the specified parameters of current density and deposition time during the hard chrome coating process. Thus, the coating thickness met the requirements for the shroud-fuel nozzle diameter.
4. The microhardness of the coating met certain requirements and increased the hardness of the part itself as an essential characteristic to obtain a fully functional part for reuse.
5. The coating also passed the bending test as well as the adhesion test in terms that there is no peeling, nor the separation of the coating from the base material. The bond between the base material and the coating is satisfactory.
6. The microstructure of the coating is good and unique, without major deviations, with one very small break in the form of an inclusion present in only one place out of three tested samples.

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Summary/Sažetak

U radu je ispitan kvalitet prevlake tvdog hroma na etalonskim čeličnim pločicama i plaštu brizgača goriva koji je korišten u avioindustriji, pri čemu je određena debljina prevlake, tvrdoća, mikroskopska struktura i otpornost na savijanje. Ukupan proces prevlačenja podrazumjevao je proces mehaničke, hemijske i elektrohemijske pripreme ispitivanih dijelova prije procesa hromiranja, a proces hromiranja proveden je u rastvoru hromne kiseline. Cilj rada je dobiti kvalitetne prevlake tvrdog hroma postupkom reparacije istrošenog plašta brizgača goriva koji će kvalitetom i karakteristikama moći zamjeniti komercijalne nove dijelove. Također, uzimajući u obzir da pojedini dijelovi nisu više ni komercijalno dostupni uvođenje postupka reparacije treba omogućiti produženo korištenje istih dijelova.

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Online ISSN: 2232-7266

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Print ISSN: 0367-4444
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