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***Glasnik hemičara i tehnologa  
Bosne i Hercegovine  
Bulletin of the Chemists and Technologists of  
Bosnia and Herzegovina***

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**60**

**June, 2023.**

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**Univerzitet u Sarajevu - Prirodno-matematički fakultet, Sarajevo  
University of Sarajevo - Faculty of Science, Sarajevo**



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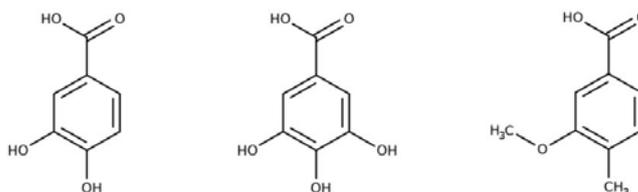
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| Cow's milk 2 (short-lived, sterilized) | 2.8          | 459.8±100.4 |
| Cow's milk 3 (pasteurized)             | 2.8          | 731.9±134.5 |
| Cow's milk 4 (permanent, pasteurized)  | 3.2          | 637.6±56.70 |
| Cow's milk 5 (permanent, pasteurized)  | 3.8          | 663.8±155.4 |

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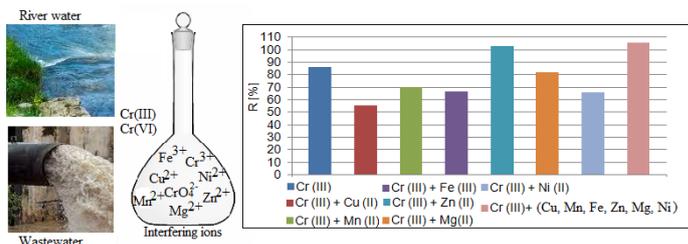
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## **Editorial**

In January 2022, the University of Sarajevo's Department of Physics started to implement a comprehensive project that aimed at all-encompassing modernization of the Department. Recently, the project has been successfully completed, resulting with a completely modernized research and teaching infrastructure.

The following capital research equipment was purchased: X-ray diffractometer, scanning electronic microscope, atomic force microscope, differential scanning calorimeter and UV-Vis spectrometer. This equipment is supposed to be used for conducting fundamental and applied research in condensed matter physics, and other natural sciences.

In addition, all the Department's rooms were completely refurbished, including the computer lab and introductory physics labs. Thus the quality of the learning and study environment has been significantly improved. Especially the conditions for the development of computer and laboratory skills are now much better.

The net value of the project was around 1.5 million euros and was fully financed by the "Three Physicists" Foundation, established by physicists Edmond, Maja and Jan Offermann.

**Editors**

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## Content of Metals in Cow, Sheep and Goat Milk Samples

Korać, (R) S., Huremović, J.\*, Žero, S., Ljubijankić, N.

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### Article info

Received: 26/07/2022  
Accepted: 14/03/2023

### Keywords:

Milk  
Metals  
FAAS

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**Abstract:** Milk is an excellent source of many essential nutrients, including Ca, proteins and vitamin D. Nine metals (Ca, Cd, Cu, Fe, Mg, Mn, Zn, Ni and Pb) in raw and pasteurized milk of cow, sheep and goat were determined by atomic absorption spectrometry, flame technique. The levels of Cd, Fe, Mn, Ni and Pb were below the detection limit of the used method. The concentration ranges for Ca, Mg, Zn and Cu, in all milk samples, were as follows: 459.8-992.8, 59.5-206.8, <LOD-22.31 and <LOD-3.20 mg/L, respectively. The order of the metal levels in regards to concentration was Ca>Mg>Zn>Cu. The highest content of Ca and Mg was found in goat milk. In the case of cow's milk, the content of Ca, Mg and Zn increased with increasing amount of milk fat in processed milk. Matrix correlation analysis showed that there is a significant correlation for the following pairs: Ca-Mg ( $r = 0.830$ ), Zn-Cu ( $r = 0.799$ ) and Ca-Zn ( $r = 0.624$ ). Also, a strong correlation was found between milk fat and Ca, Mg and Zn (Pearson factor,  $r > 0.600$ ). Based on daily consumption of 200 mL of milk, the milk consumption does not meet the daily requirements for determined elements. Most significant intake was in case of Ca.

## INTRODUCTION

Milk and milk products are basic dietary products and constitute an important source of nutrients in the humans' daily diet required for normal human development and metabolic processes. As far as the basic composition of milk is known, elemental composition is generally unknown (Dobrzański, Koacz, Górecka, et al., 2005; Khan, Choi, Nho, et al., 2014). Various minerals are essential for the health, especially during the growth and in old age. The efficacy and/or excess of minerals can result in pathological changes and metabolic irregularities (Garcia, Lorenzo, Cabrera, et al., 1999). Consumption of milk is recommended by many nutritionists to meet daily needs for calcium, vitamins B12 and D, and animal proteins. Heavy metals and metallic compounds, as a result of water, air, and soil pollution, pass to animals and people through the food chain and can have a negative impact on health (Karasakal, 2020).

The milk analysis is important because milk is a significant diet source that can contain toxic metal ingested by humans. Milk is also a source of essential nutrients and an indicator of environmental contamination. Animals reduce human trace metals exposure; for example, the levels present in different environmental matrices are higher than those found in food (Pérez-Carrera, Arellano and Fernández-Cirelli,

2016). Trace elements in cow milk, in recent years, have been considered to be good bio-indicators of pollution in the agriculture. The concentration of minerals in raw cow milk vary according to different factors, such as animal species and health status, the season, contamination of environment, climate, lactation period and the dietary composition of animal feed (Vahčić, Hruškar, Marković, et al., 2010).

According to research by Vahčić et al. (2010), the children of 8 to 9 years of age consume 0.46 L of milk per day and average daily milk consumption of an adolescent (15-18 years old) is similar with value of 0.47 L/d when compared to children consumption, but not sufficient. In the United States, the national guidelines for dietary recommendations note that adults should drink three cups or 732 mL/d of milk (Dietary Guidelines for Americans, 2010). However, such level of consumption is rarely observed. According to the Canadian dairy information center (Dairy Facts and Figures) from 2016 year, the mean per capita milk consumption in the United States was 196 mL/d in 2014 and in Europe 171 mL/d, with great heterogeneity in consumption depending on the country (Mullie, Pizot and Autier, 2016). The contents of nine trace elements of toxicological or nutritional importance (Ca, Mg, Mn, Fe, Cu, Zn, Cd, Ni and Pb) in samples of raw and pasteurized cow, sheep and goat milk were measured by atomic absorption spectrometry, flame

technique (FAAS). There are not large numbers of published papers on the elementary analysis of goat's milk compared to cow's milk. In addition, matrix correlations between elements were done and estimated daily intakes of metals from milk samples were calculated.

## EXPERIMENTAL

### Sample collection, preparation and heavy metal determination

Milk samples were collected from local farms in Sarajevo, Bosnia and Herzegovina and Sarajevo markets, during the summer of 2020. Milk samples were placed into plastic bottles that were rinsed with 10 % HNO<sub>3</sub> in Milli-Q water prior to collection.

One mL of the sample was annealed in a porcelain crucible at 550 °C for 1 h. The resulting white ash was then moistened with Milli-Q water (10 drops), dissolved in 10 mL of 3 mol/L HCl, filtered through Whatman No. 1 paper into a 100 mL volumetric flask, and diluted to original volume with 3 mol/L HCl.

All reagents used were of analytical reagent grade (Merck, Darmstadt, Germany). Milli-Q water was used during the complete analysis process. The content of metals in milk samples was determined by FAAS (Atomic absorption spectrometer, model Varian AA240FS, Mulgrave, Australia).

The lipid content in raw milk from farms was not determined, while the data for milk fat (table 1) in the milk samples from the markets were taken from the milk packaging.

### Analytical quality control

All milk samples were analyzed in triplicate, and standard deviation was calculated. Reagent blanks were also analyzed after each batch of 10 samples. The mean concentration for each milk sample was reported. The limit of detection (LOD, three times the standard deviation of the blank absorbance signal, n = 10) for each metal was calculated. LODs were: 0.035 µg/mL for Ca;

0.021 µg/mL for Cd; 0.071 µg/mL for Cu; 0.941 µg/mL for Fe; 0.014 µg/mL for Mg; 0.210 µg/mL for Mn; 0.163 µg/mL for Ni; 1.091 µg/mL for Pb; 0.660 µg/mL for Zn. The assessment of accuracy was performed by spiking four already analyzed milk samples with varying analyzed elements concentrations. Samples were spiked at three different known concentrations of standards, high, medium and low, covering the working range. Satisfactory recovery factor values, from 84 % to 109 %, were obtained for all metals.

### Estimated daily intake (EDI) of metals through the milk consumption

EDI (mg/kg/day) from milk ingestion was calculated by combining the data on the consumption of milk with the determined levels (c, mg/L) of Ca, Cu, Mg and Zn.

EDI was calculated based on the assumptions that:

- 1) body weight is 60 kg, and
- 2) daily intake of milk is 200 mL.

EDI (mg/kg/day) = metal concentrations in milk × 200/1000/60 (Vahčić *et al.*, 2010; Bašić, Beganović, Huremović, *et al.*, 2020)

### Statistical analysis

For the data analysis for metals concentrations in milk samples, descriptive statistical parameters such as mean values, standard deviation and Pearson's matrix correlations were used.

## RESULTS AND DISCUSSION

The concentrations of Ca, Cd, Cu, Fe, Mg, Mn, Zn, Ni and Pb in raw and pasteurized cow, sheep and goat milks were investigated. The content of Cd, Fe, Mn, Ni and Pb was below the detection limit of the method used. The results of the elemental milk analysis are given in Table 1.

**Table 1:** Content of Ca, Mg, Zn and Cu (mg/L) ± standard deviation in different milk samples, n = 3.

| Sample   | Milk fat (%) | Ca (mg/L)   | Mg (mg/L)  | Zn (mg/L)   | Cu (mg/L)   |
|--|--------------|-------------|------------|-------------|-------------|
| Cow's milk 1 (short-lived, sterilized)         | 0.9          | 507.5±99.12 | 68.3±7.92  | *<LOD       | *<LOD       |
| Cow's milk 2 (short-lived, sterilized)         | 2.8          | 459.8±100.4 | 59.5±14.3  | *<LOD       | *<LOD       |
| Cow's milk 3 (pasteurized)                     | 2.8          | 731.9±134.5 | 181.5±22.1 | 7.00±0.000  | * <LOD      |
| Cow's milk 4 (permanent, pasteurized)          | 3.2          | 637.6±56.70 | 186.5±8.76 | 10.11±0.61  | *<LOD       |
| Cow's milk 5 (permanent, pasteurized)          | 3.8          | 663.8±155.4 | 172.4±5.54 | 13.00±2.09  | *<LOD       |
| Lactose-free cow's milk                        | 1.5          | 700.2±146.4 | 175.4±33.3 | 22.31±0.01  | *<LOD       |
| Chocolate cow's milk                           | 2.3          | 469.2±64.08 | 85.8±14.2  | *<LOD       | 0.50±0.11   |
| Cow's milk (with addition of Ca and D vitamin) | 3.2          | 757.7±111.9 | 160.6±19.8 | 1.40±0.7    | *<LOD       |
| Raw cow's milk 1                               | -            | 740.4±98.11 | 183.3±7.00 | 8.41±0.61   | 3.20±0.9    |
| Raw cow's milk 2                               | -            | 725.7±121.8 | 182.8±23.1 | 4.40±0.08   | 0.51±0.00   |
| Raw sheep milk                                 | -            | 992.8±223.2 | 186.7±54.0 | 5.31±0.90   | 0.30±0.14   |
| Raw goat milk 1                                | -            | 988.7±242.1 | 206.8±30.1 | 4.40±0.76   | 0.90±0.52   |
| Raw goat milk 2                                | -            | 931.3±199.9 | 204.0±9.98 | 2.80±1.15   | 0.21±0.13   |
| Goat milk (pasteurized)                        | 2.8          | 732.1±198.7 | 180.4±25.7 | 1.21±0.09   | *<LOD       |
| Range  | -            | 459.8-992.8 | 59.5-206.8 | *<LOD-22.31 | *<LOD -3.20 |

\*<LOD - below the detection limit of the method used

Concentrations of Cd, Fe, Mn, Ni and Pb in all milk samples were below the detection limit of the method used. The highest content of Ca and Mg was found in raw goat milk and sheep milk. The content of Ca, Mg and Zn mainly increased with the increase of milk fat in processed milk. The concentration of Ca in cow milk with 3.8 % fat was very similar to the concentration of Ca in cow milk with the addition of Ca. The highest Ca, Mg, Zn and Cu content in cow milk samples was found in raw, unprocessed milk. By concentrations, the metals were arranged as the following diminishing series Ca>Mg>Zn>Cu.

Ca was the major metal of interest in milk. It is the most common mineral and needed for the bone formation and bodily functions. It also plays a vital role in activation of different enzymes (Karasakal, 2020). Mg is a cofactor of many important enzymes, including peptidases, arginase, cholinesterase, pyruvate carboxylase, phosphoglucomutase, mitochondrial superoxide dismutase and several glycosyltransferases and phosphates (Vahčić et al., 2010). The Table 2. provides an overview of previous research on the metal content in milk samples in the world.

**Table 2:** Results of mineral content in different milk samples in the world.

| Metal Sample (Origin)   | Concentration of metals in milk (mg/L) |                 |              |             | Reference  |
|---|--|-----------------|--------------|-------------|--|
|   | Ca                                     | Mg              | Zn           | Cu          |  |
| <b>Cow's milk</b> -processed and raw milk samples (Sarajevo, Bosnia and Herzegovina)    | 459.8-757.7                            | 59.5-186.5      | <LOD-22.31   | <LOD-3.20   | Present study                                      |
| <b>Goat's milk</b> -pasteurized and raw milk samples (Sarajevo, Bosnia and Herzegovina) | 732.1-988.7                            | 180.4-206.8     | 1.21-4.40    | 0.2 -0.90   | Present study                                      |
| <b>Raw sheep's milk</b> (Sarajevo, Bosnia and Herzegovina)                              | 992.8±223.2                            | 186.7±54.0      | 5.31±0.90    | 0.30±0.14   | Present study                                      |
| <b>Cow's pasteurized milk</b> (Zagreb, Croatia)   | 901.2±80.2                             | 110.8±17.4      | 3.22±0.23    | 0.19±0.10   | Vahčić, <i>et al.</i> , 2010                       |
| <b>Raw cow's milk</b> (Virginia, USA)   | 1164±56                                | 101.4±7.7       | 3.522±0.526  | 0.020±0.008 | Lopez, Collins, and Williams, 1985                 |
| <b>Cow's pasteurized milk</b> (Virginia, USA)   | 1169±63                                | 99.82 ±8.08     | 3.488± 0.427 | 0.043±0.019 | Lopez <i>et al.</i> , 1985                         |
| <b>Raw goat's milk</b> (Virginia, USA)  | 988.9±50.4                             | 120.7±10.0      | 3.426±0.507  | 0.084±0.050 | Lopez, <i>et al.</i> , 1985                        |
| <b>Pasteurized goat's milk</b> (Virginia, USA)  | 992.1± 56.4                            | 120.2 ±12.9     | 3.286±0.570  | 0.138±0.085 | Lopez, <i>et al.</i> , 1985                        |
| <b>Milk powder</b> (Brazil)   | 731±14                                 | 155±5           | 2.93±0.12    | -           | Hamid, Abd El-Samad, Soliman, <i>et al.</i> , 2017 |
| <b>Plain cow's milk</b> (Seoul, Korea)  | 1085±0.29                              | 104.8±0.03      | -            | -           | Khan <i>et al.</i> , 2014                          |
| <b>Skimmed cow's milk</b> (Seoul, Korea)  | 1184±0.26                              | 115.2±0.04      | -            | -           | Khan <i>et al.</i> , 2014                          |
| <b>Raw cow's milk</b> (Wroclaw, Poland)   | -                                      | -               | 3.124±0.650  | 0.077±0.105 | Dobrzański, Koacz, Górecka, <i>et al.</i> , 2005   |
| <b>Plain cow's milk</b> (California, USA)   | -                                      | -               | 3.700±0.023  | -           | Voth, 1963   |
| <b>Domestic cow milk</b> (Dhaka, Bangladesh)  | -                                      | -               | -            | 0.127±0.029 | Muhib, Chowdhury, Easha, <i>et al.</i> , 2016      |
| <b>Fresh cow's milk</b> (Constanta, Romania)  | -                                      | 214.00          | 0.98         | 0.17        | Birghila, Dobrinas, Stanciu, <i>et al.</i> , 2008  |
| <b>Pasteurized cow's milk</b> (Constanta, Romania)                                      | -                                      | 212.82          | 0.85         | 0.11        | Birghila, <i>et al.</i> , 2008                     |
| <b>Powder milk</b> (Constanta, Romania)   | -                                      | 919.80          | 3.24         | 0.54        | Birghila, <i>et al.</i> , 2008                     |
| <b>Cow's milk</b> (Butajira and Meskan districts, south central Ethiopia)               | 380.1-532.4                            | 159.6-397.9     | 0.0-8.2      | 0.03-1.1    | Teklu, <i>et al.</i> , 2022                        |
| <b>Cow's milk</b> (Erode District, Tamil Nadu, India)                                   | -                                      | -               | 1.22-20.94   | BDL*-0.35   | Yasothea, <i>et al.</i> , 2021                     |
| <b>Cow's milk</b> (Nuer Zone and Gambella town, Ethiopia)                               | 755.15-812.75                          | 1675.87-2050.65 | -            | -           | Damtew and Gebre, 2020                             |

\* indicates below detection limit

From the data given in Table 2 it can be seen that the metal concentrations obtained through this research were similar to the research conducted in other countries. In this study, slightly lower values for Ca content were obtained compared to previously published papers. Lower values for Mg in milk samples from this study compared to samples from Ethiopia (Damtew and Gebre, 2020) have been obtained.

### Matrix correlation analysis

The strength of the correlation between analyzed elements in raw and pasteurized cow, sheep and goat milks is expressed using the guide where Fantu Garcia (1996) described the absolute value of Pearson correlation factor,  $r$ : 0.00–0.19, very weak; 0.20–0.39, weak; 0.40–0.59, moderate; 0.60–0.79, strong and 0.80–1.0, very strong.

Results of matrix correlation analysis are shown in Table 3.

Matrix correlation analysis showed that there is a very strong correlation for Ca-Mg and strong correlation for Cu-Zn and Ca-Zn pairs. The Cu content was only significantly correlated with Zn, and similar results, for these two metals, were obtained by Garcia *et al.* (1999). Positive association between Ca and Mg was also found in study by Steck, Omofuma, Su, *et al.* (2018). Fantuz, Maglieri, Lebboroni, *et al.*, (2009) investigated ass's milk and they found positive and significant correlation between Ca and Mg ( $r=0.63$ ), Zn and Cu ( $r=0.50$ ) and Zn and Mg ( $r=0.45$ ). Also, in this study, a strong correlation was found between milk fat and Ca, Mg and Zn.

**Table 3:** Correlation matrix between concentrations of trace metals and milk fat in cow, sheep and goat milk samples.

|          | Milk fat | Ca           | Mg           | Zn           | Cu           |
|----------|----------|--------------|--------------|--------------|--------------|
| Milk fat | 1        | <b>0.640</b> | <b>0.706</b> | <b>0.784</b> | 0.000        |
| Ca       |          | 1            | <b>0.830</b> | <b>0.624</b> | 0.158        |
| Mg       |          |              | 1            | 0.571        | 0.192        |
| Zn       |          |              |              | 1            | <b>0.799</b> |
| Cu       |          |              |              |              | 1            |

For easier visual analysis, correlation values  $> 0.6$  are bold highlighted

### EDI values for metals from milk

People need a certain daily intake of minerals. EDI of metals (Ca, Mg, Zn and Cu) depended on the metal concentration level and the amount of milk consumption.

Calculated EDI values for metals from raw and pasteurized cow, sheep and goat milk samples are presented in Table 4.

**Table 4:** EDI of metals from milk samples.

| Sample /metals                            | EDI     |       |         |       |         |      |         |       |
|---|---------|-------|---------|-------|---------|------|---------|-------|
|   | mg/kg/d | mg/d  | mg/kg/d | mg/d  | mg/kg/d | mg/d | mg/kg/d | mg/d  |
|   | Ca      |       | Mg      |       | Zn      |      | Cu      |       |
| Cow's milk 1 (short-lived, sterilized)    | 1.691   | 101.5 | 0.228   | 13.66 | -       | -    | -       | -     |
| Cow's milk 2 (short-lived, sterilized)    | 1.53    | 146.4 | 0.198   | 11.90 | -       | -    | -       | -     |
| Cow's milk 3 (pasteurized)                | 2.44    | 146.4 | 0.605   | 36.3  | 0.023   | 1.38 | -       | -     |
| Cow's milk 4 (permanent, pasteurized)     | 2.13    | 127.5 | 0.622   | 37.3  | 0.034   | 2.04 | -       | -     |
| Cow's milk 5 (permanent, pasteurized)     | 2.21    | 132.8 | 0.575   | 34.48 | 0.043   | 2.60 | -       | -     |
| Lactose-free cow's milk                   | 2.33    | 140.0 | 0.585   | 35.08 | 0.074   | 4.46 | -       | -     |
| Chocolate cow's milk                      | 1.56    | 93.8  | 0.286   | 17.16 | -       | -    | 0.002   | 0.10  |
| Cow's milk (addition of Ca and D vitamin) | 2.52    | 151.5 | 0.535   | 32.12 | 0.005   | 0.28 | -       | -     |
| Raw cow's milk 1                          | 2.47    | 148.1 | 0.611   | 36.67 | 0.028   | 1.68 | 0.011   | 0.64  |
| Raw cow's milk 2                          | 2.42    | 145.1 | 0.609   | 36.56 | 0.015   | 0.88 | 0.002   | 0.10  |
| Raw sheep milk                            | 3.31    | 198.6 | 0.622   | 37.34 | 0.018   | 1.06 | 0.001   | 0.06  |
| Raw goat milk 1                           | 3.29    | 197.7 | 0.689   | 41.36 | 0.015   | 0.88 | 0.003   | 0.18  |
| Raw goat milk 2                           | 3.10    | 186.3 | 0.680   | 40.80 | 0.009   | 0.56 | 0.001   | 0.042 |
| Goat milk (pasteurized)                   | 2.44    | 146.4 | 0.601   | 36.08 | 0.004   | 0.24 | -       | -     |

Most significant intake was in case of Ca. The Food and nutrition board has defined the recommended daily intake (RDI) values for a normal adult population, and for Ca,

Mg, Zn and Cu is 1000–1200 ppm, 320–420 ppm, 4–25 ppm, 1.0–1.6 ppm, respectively. The results showed that daily milk consumption of 200 mL does not meet the daily

requirements for the determined metals. In accordance with the obtained EDI values, it would be necessary for adults to consume about 2 L of milk to meet the needs for Ca, Mg, Zn and Cu, while neglecting the intake of these minerals through other food products. The content of these metals is not necessarily the amount that human body will absorb.

## CONCLUSION

Minerals are found in all foods, including milk and milk products. Although the topic is very important, there have not been many published papers dealing with elements in cow, sheep and goat milk. The highest concentration of Ca and Mg were found in raw goat and sheep milk. The content of Ca, Mg and Zn mainly increased with the increase of milk fat. Based on daily consumption of 200 mL of milk, it was shown that the milk consumption does not meet the daily Ca, Mg, Cu and Zn requirements. Most significant intake was in case of Ca. Matrix correlation analysis showed that there is a very strong correlation between Ca and Mg and strong correlation for pairs: Cu-Zn and Ca-Zn. Strong correlation was also found between milk fat and Ca, Mg and Zn.

The content of Ca, Mg, Zn and Cu is the measure of these elements in different milks but not necessarily the amount that human body will absorb. Therefore, the bioavailability of certain minerals should be investigated in future research.

## Conflict of interest

The authors declare no conflict of interest.

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### Summary/Sažetak

Mlijeko je odličan izvor mnogih esencijalnih nutrijenata, uključujući Ca, proteine i vitamin D. Devet metala (Ca, Cd, Cu, Fe, Mg, Mn, Zn, Ni i Pb) u sirovom i pasteuriziranom kravljem, ovčijem i kozjem mlijeku je određeno atomskom apsorpcionom spektrometrijom, plamenom tehnikom. Sadržaj Cd, Fe, Mn, Ni i Pb bio je ispod granice detekcije korištene metode. Rasponi koncentracija za Ca, Mg, Zn i Cu, u svim uzorcima mlijeka, bili su sljedeći: 459.8-98.83, 59.5-206.8, <LOD-8.41 i <LOD-3.20 mg/L, respektivno. Redoslijed metala je bio Ca>Mg>Zn>Cu. Najveći sadržaj Ca i Mg nađen je u kozjem mlijeku. U slučaju kravljeg mlijeka sadržaj Ca, Mg i Zn se povećavao sa povećanjem mliječne masti. Korelaciona analiza je pokazala da postoji značajna korelacija za sljedeće parove: Ca-Mg ( $r = 0.830$ ), Zn-Cu ( $r = 0.799$ ) i Ca-Zn ( $r = 0.624$ ). Takođe, nađena je jaka korelacija između mliječne masti i Ca, Mg i Zn (Pearsonov faktor,  $r > 0.600$ ). Konzumacijom 200 mL mlijeka dnevno, potrošnja mlijeka ne zadovoljava dnevne potrebe za utvrđenim elementima. Najznačajniji unos bio je u slučaju Ca.

## Investigation of Antioxidant Activity of Gallic, Protocatechuic and Vanillic Acids using the Briggs-Rauscher Reaction as Tool

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### Article info

Received: 17/03/2022  
Accepted: 18/04/2023

### Keywords:

Briggs-Rauscher Reaction  
Hydroxybenzoic Acids  
Antioxidant Activity

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**Abstract:** Hydroxybenzoic acids are an important class of polyphenols because their strong antioxidative activity. In this study, the antioxidant activity of three hydroxybenzoic acids was investigated using the Briggs-Rauscher reaction. Besides individual gallic, protocatechuic, and vanillic acids at concentrations of 250, 500, and 1000  $\mu\text{M}$  respectively, the various mixtures of two and three hydroxybenzoic acids were also tested. The highest antioxidant activity showed protocatechuic acid at a concentration of 1000  $\mu\text{M}$  while the lowest antioxidant activity was observed for vanillic acid at a concentration of 250  $\mu\text{M}$ . The most of investigated hydroxybenzoic acids mixtures showed some degree of antagonistic effect. The highest antagonistic effect was found for the equimolar mixture of protocatechuic and vanillic acid at concentration of 250  $\mu\text{M}$ . On the contrary, the equimolar mixture of gallic acid and vanillic acid at the same concentration indicated a high synergistic effect.

## INTRODUCTION

Phenolic acids are an important group of secondary metabolites with a basic chemical structure of  $\text{C}_6\text{-C}_1$  (hydroxybenzoic acids) or  $\text{C}_6\text{-C}_3$  (hydroxycinnamic acids), consisting of a phenolic ring and a carboxyl substituent (Saxena, Saxena and Pradhan, 2012; Kahkeshani, Farzaei, Fotouhi et al., 2019). Gallic acid, protocatechuic acid, and vanillic acid are the most common members of hydroxybenzoic acids.

Gallic acid (GA) exists in different forms in many plants (oak, carob, sumac), vegetables (onions, potatoes), fruits (strawberries, grapes, pineapple, banana, lemon, mango), nuts (walnut, hazelnut), olive oil, coffee, tea, and wine. It has antioxidant, anti-inflammatory, antibacterial, antiviral, antifungal, antimelanogenic, antimutagenic, anticancer, cardioprotective, gastroprotective, and neuroprotective activities among others (Brglez Mojzer, Knez Hrnčić, Škerget et al., 2016; Nayeem, Asdaq, Salem et al., 2016; Fernandes and Saldago, 2016; Kahkeshani et al., 2019). Because of its many biological and pharmacological activities, gallic acid and its derivatives are commonly used in the food and pharmaceutical industries (Mota, Queimada, Pinho et al., 2008; Kahkeshani et al., 2019). It

is soluble in water unlike other phenolic acids (Brglez Mojzer et al., 2016).

Protocatechuic acid (PCA) is widely distributed in many fruits (plums, grapes, gooseberries), medicinal plants (rosemary, hibiscus, melissa), spices (anis), nuts (almond), rice, olive oil, and white wine (Kakkar and Bais, 2014). It has various biological activities like antioxidant, antibacterial, anticancer, antidiabetic, antiviral, anti-inflammatory, analgesic, etc. (Kakkar and Bais, 2014).

Vanillic acid (VA) is an oxidized form of vanillin, and is widely used as flavoring, additive, and preservative in the food industry. It is found in some fruits (mango, strawberry), cereals (wheat, rice), herbs, spices, green tea, juices, beer, and wine (Zuo, Wang and Zhan, 2002; Kumar, Prahalathan and Raja, 2011; Palafox-Carlos, Gil-Chávez, Sotelo-Mundo et al., 2012; Almeida, Cavalcante, Vicentini et al., 2016). It has antibacterial, antimicrobial, antifilarial, antioxidant, anti-inflammatory properties, free-radical scavenging ability, cardioprotective, chemopreventive, and hepatoprotective effects (Itoh, Isoda, Kondoh et al., 2009; Itoh, Isoda, Kondoh et al., 2010; Raja and Mol, 2010; Kim, Kim, Um et al., 2010; Kumar et al., 2011; Almeida et al., 2016).

When more than one antioxidant is present in a sample, synergistic effect of those compounds towards increasing antioxidant activity can be noticed, yet the mechanism behind those interactions is still not thoroughly explained. Synergistic antioxidant activity depends on the type of compounds and their concentration in the mixture. A particular combination may show synergism at one concentration ratio while antagonism at the other (Sonam and Guleria, 2017).

Interactions between phenolic acids can lead to the changes in overall antioxidant activity. Because phenolic acids are usually present in the form of mixtures in nature, it is impossible to explore their interactions on the total antioxidant activity of samples (Skroza, Generalić Mekinić, Svilović *et al.*, 2015).

In this study, the antioxidant activity of individual hydroxybenzoic acids (gallic acid, protocatechuic acid, and vanillic acid) and different mixtures of two or three hydroxybenzoic acids were investigated using the Briggs-Rauscher reaction method. This method is based on the inhibitory effects of antioxidants on the oscillations of the Briggs-Rauscher reaction mixture (Cervellati, Höner, Furrow *et al.*, 2001).

## EXPERIMENTAL

### Reagents

All used reagents were of analytical grade. Potassium iodate, sulfuric acid, hydrogen peroxide, and ethanol were obtained from Semikem (Sarajevo, BiH), malonic acid, manganese(II) sulfate monohydrate, and starch were obtained from Merck (Darmstadt, Germany), gallic acid, protocatechuic acid, and vanillic acid were obtained from Sigma (St. Louis, USA).

### Preparation of the solutions of hydroxybenzoic acids

A stock solution of gallic, protocatechuic, and vanillic acids, 2000  $\mu\text{M}$ , were prepared by dissolving pure hydroxybenzoic acid in 2 mL of ethanol and diluting with distilled water to 100 mL. Other solutions of hydroxybenzoic acids in the concentrations of 250, 500, and 1000  $\mu\text{M}$  were obtained by diluting the stock solution with distilled water. Thus solutions were used for the preparation of the mixtures of two and three hydroxybenzoic acids.

### Procedure for the determination of antioxidant activity

The antioxidant activity of individual hydroxybenzoic acids and their mixtures was determined by the Briggs-Rauscher reaction. The original procedure (Cervellati *et al.*, 2001) was slightly modified. The Briggs-Rauscher reaction mixture was prepared according to the procedure described in our previous study (Gojak-Salimović and Ramić, 2020). The oscillations of the Briggs-Rauscher reaction mixture were followed potentiometrically at temperature  $25 \pm 0.5^\circ\text{C}$ . A Pt electrode was used as a working electrode, while Ag/AgCl was used as the reference electrode. After the third oscillation, 1 mL solution of tested hydroxybenzoic acid, at corresponding concentration (or their mixtures) was added to 30 mL of an active, well stirred Briggs-Rauscher reaction mixture.

The total antioxidant activity of individual hydroxybenzoic acids and their mixture was expressed as the inhibition time ( $t_{\text{inhib}}$ ). The inhibition time is defined as the time elapsed between the end of the addition of tested hydroxybenzoic acids (or their mixtures) and the first regenerated oscillation (Cervellati *et al.*, 2001).

## RESULTS AND DISCUSSION

### Antioxidant activity of selected hydroxybenzoic acids

In this study, the antioxidant activities of gallic, protocatechuic, and vanillic acids were evaluated at different concentrations (250, 500, and 1000  $\mu\text{M}$ ) using the Briggs-Rauscher reaction. The obtained results are reported in Table 1.

**Table 1:** Inhibition times of individual hydroxybenzoic acids

| Hydroxybenzoic acid | $t_{\text{inhib}}$ (s) |                       |                        |
|---------------------|------------------------|-----------------------|------------------------|
|                     | 250 ( $\mu\text{M}$ )  | 500 ( $\mu\text{M}$ ) | 1000 ( $\mu\text{M}$ ) |
| GA                  | 48                     | 117                   | 165                    |
| PCA                 | 547                    | 1173                  | 1874                   |
| VA                  | 17                     | 394                   | 1351                   |

The inhibition time increased with increased concentration, and linearity was found in the tested concentration range of hydroxybenzoic acid added. The parameters of the straight-lines are reported in Table 2.

**Table 2:** Parameters of straight-lines equations

| Hydroxybenzoic acid | $m$ ( $\mu\text{M}^{-1}\text{s}$ ) | $q$ (s) | $R^2$ |
|---------------------|------------------------------------|---------|-------|
| GA                  | 0.147                              | 24.0    | 0.916 |
| PCA                 | 1.717                              | 196     | 0.975 |
| VA                  | 1.798                              | -461    | 0.996 |

The obtained results confirmed that the antioxidant activity of the investigated hydroxybenzoic acids varies depending on their structure and concentration. The lowest inhibition time was detected when using vanillic acid at a concentration of 250  $\mu\text{M}$  (17 s), and the longest with protocatechuic acid at a concentration of 1000  $\mu\text{M}$  (1874 s). The inhibition time for certain acids changed significantly with increasing concentration from 250 to 1000  $\mu\text{M}$ . At a concentration of 250  $\mu\text{M}$  antioxidant activity increased in the following order: vanillic acid, gallic acid, protocatechuic acid, while at concentrations of 500 and 1000  $\mu\text{M}$  the order was different: gallic acid, vanillic acid, protocatechuic acid.

In the literature, the ranking order of the antioxidant activity of the investigated phenolic acids differed from method to method. Rice-Evans, Miller and Paganga (1996) reported the total antioxidant activity expressed as TEAC values in the following order: gallic acid > *p*-coumaric acid > ferulic acid > vanillic acid > syringic acid > caffeic acid > *m*-coumaric acid > protocatechuic acid > gentisic acid > *o*-coumaric acid > salicylic acid > *p*-hydroxybenzoic acid. Schlesier, Harwat, Böhm *et al.* (2002) determined the antioxidant activity of four antioxidants and some beverages using six different *in vitro* methods (TEAC, TRAP, DPPH, DMPD, PCL and FRAP). They found that gallic acid was the strongest antioxidant in all methods except the DMPD method.

Karamać, Kosińska and Pegg (2005) evaluated the radical-scavenging activity of selected phenolic acids using DPPH method. They found that tested phenolic acids exhibited radical-scavenging in the following order: gallic acid > gentisic acid > syringic acid > caffeic acid > protocatechuic acid > sinapic acid > ferulic acid > isoferulic acid > vanillic acid > *p*-coumaric acid > *o*-coumaric acid > *m*-coumaric acid > salicylic acid » *p*-hydroxybenzoic acid. Skroza *et al.* (2015) found the lowest antioxidant activity was observed for gallic acid by the Briggs-Raucher reaction method. Skroza, Šimat, Vrdoljak *et al.* (2022) examined the antioxidant activity of gallic, gentisic, protocatechuic, syringic, and vanillic acids at concentrations of 2.5  $\mu\text{M}$  and 5  $\mu\text{M}$  using the ORAC method. The ORAC value for certain acids did not change significantly with increasing concentration from 2.5 to 5  $\mu\text{M}$ . At a concentration of 2.5  $\mu\text{M}$  antioxidant activity increased in the following order: gallic acid, vanillic acid, syringic acid, gentisic acid, protocatechuic acid, while at a concentration of 5  $\mu\text{M}$  the order was different: gallic acid, syringic acid, gentisic acid, vanillic acid, protocatechuic acid.

#### Antioxidant activity of hydroxybenzoic acids mixtures

The obtained results for the inhibition time of the mixture of two and three combination hydroxybenzoic acids are reported in Table 3. The results for the inhibition time ranged from 16 s for the mixture of protocatechuic acid and vanillic acid at a concentration of 250  $\mu\text{M}$  (1:1) to 1602 s for the same mixture at a concentration of 1000  $\mu\text{M}$  (1:1). In order to evaluate the impact of interactions between constituents on their antioxidant activity, the inhibition time obtained experimentally for the different mixtures of the investigated hydroxybenzoic acids were compared with theoretical values calculated by adding up the effects of two or three individual hydroxybenzoic acids analyzed separately.

**Table 3:** Inhibition times for mixtures of hydroxybenzoic acids

| Mixtures          | $t_{\text{inhib}}$ (s) |                       |                        |
|-------------------|------------------------|-----------------------|------------------------|
|                   | 250 ( $\mu\text{M}$ )  | 500 ( $\mu\text{M}$ ) | 1000 ( $\mu\text{M}$ ) |
| GA:VA (1:1)       | 46 (32)                | 81 (255)              | 323(758)               |
| GA:PCA (1:1)      | 73 (297)               | 277 (645)             | 865(1019)              |
| PCA:VA (1:1)      | 16 (282)               | 458 (783)             | 1602 (1612)            |
| GA:VA:PCA (1:1:1) | 53 (204)               | 256 (561)             | <b>1256 (1129)</b>     |
| GA:VA (3:1)       |                        | 122 (186)             |                        |
| GA:PCA (3:1)      |                        | 234 (322)             |                        |
| PCA:VA (3:1)      |                        | 786 (978)             |                        |
| GA:VA (1:3)       |                        | 303 (325)             |                        |
| GA:PCA (1:3)      |                        | 839 (909)             |                        |
| PCA:VA (1:3)      |                        | 504 (589)             |                        |
| GA:VA:PCA (2:2:1) |                        | 84 (439)              |                        |
| GA:VA:PCA (2:1:2) |                        | 104 (595)             |                        |
| GA:VA:PCA (1:2:2) |                        | 257 (650)             |                        |

\*The values in parentheses are the sum of antioxidant activities of individual hydroxybenzoic acids at corresponding concentrations.

Our results indicate that most of the investigated mixtures to some extent showed a difference in antioxidant activity when compared to their theoretical values. A slight synergistic effect was observed between gallic acid and vanillic acid at a concentration of 250  $\mu\text{M}$  (1:1).

The other investigated of two-component mixtures showed a high antagonistic effect. The mixtures of three hydroxybenzoic acids showed an antagonistic effect except their equimolar combination at concentration of 1000  $\mu\text{M}$  which showed a slight synergistic effect.

Palafox-Carlos *et al.* (2012) investigated individual antioxidant activity and the interactions of four major phenolic compounds (chlorogenic acid, gallic acid, protocatechuic acid, and vanillic acid) found in 'Ataulfo' mango pulp using the DPPH method. They found that the antioxidant activity decreased in the following order: gallic acid > protocatechuic acid > chlorogenic acid > vanillic acid. The majority of all combinations showed significant synergism while the combination between protocatechuic acid-chlorogenic acid-vanillic acid and gallic acid-vanillic acid had a small antagonism. López-Martínez, Santacruz-Ortega, Navarro *et al.* (2015) analyzed the interactions and mechanisms of major phenolic acids found in mango (gallic, protocatechuic, chlorogenic, and vanillic acids) and papaya (caffeic, ferulic, and *p*-coumaric acids) using  $^1\text{H}$  NMR and evaluated the effect of the antioxidant mixtures using the DPPH method. They found that the ability of the phenolic acid to neutralize the DPPH radical decreased in the following order in mango: gallic acid > chlorogenic acid > protocatechuic acid > vanillic acid. The majority of all combinations showed significant synergism while the combination between gallic acid and vanillic acid had a small antagonism.

Hajimehdipoor, Shahrestani and Shekarchi (2014) investigated effects of mixtures between caffeic acid, gallic acid, rosmarinic acid, chlorogenic acid, rutin, and quercetin using FRAP method. They found that combination of rutin, rosmarinic acid, and gallic acid showed an antagonistic effect (-18.5%), while combination of quercetin, gallic acid, caffeic acid (59.4%), quercetin, gallic acid, rutin (55.2%) showed the most synergistic effects. Skroza *et al.* (2015) investigated the interaction between resveratrol and gallic acid, caffeic acid, catechin, quercetin in equimolar binary mixtures using FRAP, DPPH and the Briggs-Rauscher reaction methods. The high synergism (45.4%) in the mixture of gallic acid and resveratrol were detected using the Briggs-Rauscher reaction method.

Skroza *et al.* (2022) investigated antioxidant activity of gallic, vanillic, protocatechuic, syringic and gentisic acids using the FRAP method. Besides individual phenolic acids, the equimolar mixtures of two, three, four, and all five acids were also tested at different concentrations (100, 500, 1000  $\mu\text{M}$ ). The antioxidant activity decreased in the following order: gallic acid > gentisic acid > syringic acid > protocatechuic acid > vanillic acid. Paper reports the highest synergism for the mixture of protocatechuic, syringic, and gentisic acids at a concentration of 100  $\mu\text{M}$  while the highest antagonism for the mixture of vanillic and protocatechuic acids at same concentration. Also, Skroza *et al.* (2022) investigated the antioxidant activity of equimolar mixtures of gallic, gentisic, protocatechuic, syringic, and vanillic acids at a concentration of 5  $\mu\text{M}$  using the ORAC method. Only two mixtures of phenolic acids showed an antagonistic effect (gallic acid+syringic acid and gallic acid+vanillic acid), while all the others showed a synergistic effect.

Our previous study (Aljović and Gojak-Salimović, 2017) investigated the antioxidant synergistic and antagonistic effects between ferulic acid, homovanillic acid, and vanillic acid using the Briggs-Rauscher reaction method. The most of the investigated phenolic acids mixtures showed some degree of synergistic effect. Also, the results obtained for the most of investigated two-component and three-component mixtures of ferulic acid, caffeic acid, and rosmarinic acid showed some degree of synergistic effect (Gojak-Salimović and Ramić, 2020).

## CONCLUSIONS

Our results suggest differences in the antioxidant activity of gallic acid, protocatechuic acid, and vanillic acid individually as well as in their mixtures. Most of the hydroxybenzoic acid mixtures showed some degree of antagonistic effect. The antioxidant interaction of the investigated hydroxybenzoic acids varies depending on their structure and concentration. It can be concluded that the antioxidant activity of the hydroxybenzoic acids mixtures cannot be predicted from the individual values of their constituents. One method is not enough to fully explain antioxidant activity of the mixtures. For better understanding the antioxidant interactions between the selected hydroxybenzoic acids when present together more studies are needed. Those studies should include various acid combinations, and most preferably additional experimental methods included.

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**Summary/Sažetak**

Antioksidacijska aktivnost galne, protokatehinske i vanilinske kiseline ispitivana je primjenom Briggs-Rauscher oscilirajuće reakcije. Osim pojedinačnih hidroksibenzojevih kiselina pri koncentracijama 250, 500 i 1000  $\mu\text{M}$ , testirane su i različite kombinacije smjesa dviju i sve tri kiseline. Najbolju sposobnost inhibicije oscilacija Briggs-Rauscher reakcije smjese pokazala je protokatehinska kiselina pri koncentraciji od 1000  $\mu\text{M}$ , dok je najmanja antioksidacijska aktivnost opažena za vanilinsku kiselinu pri koncentraciji od 250  $\mu\text{M}$ . Većina ispitivanih smjesa hidroksibenzojevih kiselina pokazala je određeni stepen antagonističkog efekta. Najveći antagonistički efekat nađen je za ekvimolarnu smjesu protokatehinske i vanilinske kiseline pri koncentraciji od 250  $\mu\text{M}$ . Nasuprot tome, ekvimolarna smjesa galne kiseline i vanilinske kiseline pri istoj koncentraciji pokazala je značajan sinergistički efekat.



## Addressing Linguistic and Cultural Heterogeneity in Chemistry Classrooms: Findings from the In-Service Teacher Training Program

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### Article info

Received: 27/01/2023

Accepted: 29/03/2023

### Keywords:

Linguistic heterogeneity

Cultural heterogeneity

ESTA project

Laboratory work

ICT in education

Chemistry education

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**Abstract:** One of the challenges in the teaching science in Bosnia and Herzegovina is its plurality of languages and cultural diversity. In the previous initial teacher education, there was a lack of quality preparation for these modern challenges; opportunities to teach cultural and linguistic diversity were not recognized. Therefore, the challenge is to encourage experienced chemistry teachers to develop competencies for an intercultural teaching environment within the Educating Science Teachers for All (ESTA) project. The participants of the in-service professional development program were chemistry teachers from three cantons in Federation of Bosnia and Herzegovina. The aim of the program was to develop and improve professional competencies through workshops and work in groups, with discussions on selected topics and laboratory exercises with the use of modern laboratory equipment and ICT. Teaching opportunities were offered in linguistically diverse classes, as well as in classes with other forms of student diversity. The results showed that teachers are aware of the need to be sensitized for student differences. They expressed positive impressions and willingness to adjust the teaching material according to the needs of their students, but also the need for further support through collaboration with the university staff.

## INTRODUCTION

Bosnia and Herzegovina (BiH) is a country with a long and rich history, which is reflected in its cities, monuments, symbols, artifacts, and rare books. BiH is home to three constituent nations and 17 national minorities, which reflect cultural diversity and an invaluable contribution to BiH society. The development of national identity is based on the understanding of nationality in BiH, most often caused by the narratives of certain ethnic groups.

There are several educational systems in BiH that exist side by side. At the level of primary and secondary education, students are often separated according to the religious and cultural group they belong to.

According to the recent population census (2013), more than 96% of residents belong to one of three main ethnic groups: Bosniaks (predominantly Muslims), Serbs (predominantly Orthodox Christians), and Croats (predominantly Catholic Christians).

They all live in the same country, but learn different interpretations of historical events, have different religious education, and are taught in different languages, which they speak now.

However, these main ethnic groups in BiH share a significant part of the ancient gene pool that is unique to the Balkan region (Marjanovic, Fornarino, Montagna, et al., 2005).

### Linguistic heterogeneity

The disintegration of the common Serbo-Croatian or Croato-Serbian language (official language in SFR Yugoslavia) happened in the 1990s, along with the disintegration of the state. For linguists, this was not unexpected because the preconditions existed long before. The Serbo-Croatian language has always had two variants (Eastern/Belgrade, and Western/Zagreb), as well as two expressions (Bosnian and Montenegrin), which had their own special linguistic upgrades according to different linguistic heritage, cultural and traditional peculiarities.

The disintegration of the common state only accelerated the process of creating independent national languages.

Today, there are three standard languages in official use in BiH: Bosnian, Croatian, and Serbian (all three in Ijekavian pronunciation), and two alphabets (Latin and Cyrillic). Teaching in BiH is conducted in the official languages.

However, teachers encounter difficulties when they have to plan and realize the teaching process in practice for each student in his/her native language, which is guaranteed by the Constitution of Bosnia and Herzegovina (1995). It was quite simple in “mono-national” environments where classes are conducted in the language of the majority of students, but problems have arisen in multinational environments and have not been fully resolved. Most of the solutions so far have not been recognizing and nurturing linguistic heterogeneity. For example, in FBiH there are “two schools under one roof” – a model where the teaching process is performed in one building but in two different languages. Students are segregated based on their ethnicity and therefore seriously discriminated (Ibrahimović, 2015). Last, but not least, there are schools whose students speak languages different from the official languages of Bosnia and Herzegovina (English, French, Turkish, etc.).

### **The importance of language for learning chemistry**

Language plays various and difficult roles in the teaching and learning chemistry, especially given the changing character and diversity of the student population in terms of aptitude, language, and culture. The relevance of communication as the ability is demonstrated by numerous activities that take place in the classroom, such as reading, writing, listening, and speaking. All these activities require different aspects of language use (Markic, Childs, 2016).

It is worth mentioning that for teaching the language of chemistry, teachers use the language spoken in the country where they work. The scientific language of chemistry (chemish) is essential for communication and understanding of chemistry, but it is also one of the major difficulties in teaching and learning chemistry in a school context (Mönch and Markic, 2022). Regardless of the students' native language, the principles of chemistry language (nomenclature, formulae etc.) are common to all students (Markic, Childs, 2016) and follow the universal guidelines prescribed by IUPAC (International Union of Pure and Applied Chemistry).

**Group work in context of linguistic and cultural diversity**  
Group work is a superior technique for conceptual learning, creative problem solving, and academic linguistic skills development, but it also improves intergroup relations by increasing trust and friendliness. It increases and deepens opportunities to learn content and develop language and thus has the potential to build equitable classrooms (Cohen and Lotan, 2014).

Teacher-centered approaches result in considerably less active practice than a group activity, where students talk and share knowledge and ideas. Language acquisition experts agree that children learn language in a more casual, meaningful setting (Cohen and Lotan, 2014).

### **Multimedia learning**

Extensive research has shown many positive effects of multimedia-based learning. It combines interactive media with text, static and dynamic images or videos in a teaching subject matter (Nazir, Rizvi, Pujeri, 2012). It enables teachers to deliver more innovative materials and motivate students to learn, as they have proven to be more effective than the teacher's lecture material itself (Nazir *et al.*, 2012, Osamah, Fong, Ziad, 2010).

Since the necessary measurement devices for chemistry and science education are often too expensive, and sometimes difficult to use and outdated, quantitative measurements has long been a challenge in chemistry teaching (Wejner and Wilke, 2022). Instruments such as conductometers, pH-meters etc. are usually intended for professional use, which has specific requirements regarding reliability, accuracy, and compatibility with some other equipment or software. Their distribution is performed through limited and specialized channels. It is not easy for an interested teacher to purchase them (Koželj and Istenič, 2013).

However, there are various possibilities for implementing group work and integrating ICT in chemistry classrooms. In order to increase the effectiveness and meaningful adoption of ICT in schools, it is important to look at the perspectives and practices of instructors. International suppliers (e.g. Phywe, Vernier, Pasco) offer devices at acceptable prices, optimized for educational purposes. The device requires a mobile phone or tablet with a free measuring application installed. Moreover, collections of experiments are freely available on the Internet as a valuable resource for teachers.

There is evidence from certain national and international research that using tablets in chemistry lectures improves students' motivation, attention, and independence (Wu, Lee, Chang, *et al.*, 2013). For instance, Augmented Reality (AR) technology enables the viewer to simultaneously examine real and virtual content, using appropriate applications. This technology is increasingly interesting for education purposes due to its free accessibility and widespread availability on mobile devices. It provides the opportunity to design personalized, interactive learning environments (Huwer and Seibert, 2018). Moreover, some studies (e.g. Gallardo-Williams and Dunnagan, 2021) showed that creating learning material without students' input minimizes teachers' bias regarding students' races, gender identities, and ethnicities, as well as their talent. Some students might find it difficult to work with an instructor in a personal setting, so this approach can offer them the opportunity for personalized learning environment.

The ability to visualize events that cannot be observed, such as the submicroscopic level of representation, makes it particularly useful for chemistry instruction. The learning process can be supported by the simultaneous presence of the real environment (chemical experiment) and the virtual content (visualized description of chemical processes (Thyssen, 2017, Aufenanger, 2017).

### Intercultural education

One of the major challenges for science education in Bosnia and Herzegovina is the pluralism of languages and cultures in the country. Therefore, teachers should understand and apply the main parameters of intercultural education.

Intercultural education promotes values, empathy, openness, coexistence, assertiveness, curiosity, readiness for change, and respect for and preservation of cultural heritage (Bartulović and Kušević, 2016; Piršl, 2016). By promoting these values, the education process enables learners to accept and respect different cultural identities and their coexistence in a particular community. The main task of intercultural education is to develop respect for diversity and human rights. Intercultural education supports diversity in all forms of human life, promotes equality and human rights. At the same time, it opposes discrimination, segregation, and disrespect.

The education system is responsible for shaping and developing cultural identity, just as it is responsible for the conceptualization of educational indicators of cultural identity.

It is important to encourage intercultural dialogue between the nations and national minorities of BiH and to develop sensitivity towards diversity. The main aim is to build peace and coexistence, and to raise awareness of the multicultural wealth of BiH society.

### Educating Science Teachers for All

Cultural and linguistic diversity in the education system of BiH encouraged cooperation with international partners within the Erasmus+ CBHE project Educating Science Teachers for All (ESTA). One of the project goals is to share knowledge and skills with in-service and pre-service science teachers and thereby contribute to more inclusive and higher-quality science teaching. The innovative aspect of these courses is their interdisciplinarity: educators from science disciplines collaborate with educators from languages, general pedagogy, and intercultural education to create new instructional materials, with the intention of improving learning outcomes.

## EXPERIMENTAL

As part of the project, a training program was organized for chemistry teachers from primary and secondary schools, with the aim to achieve the goal of sharing knowledge and skills with in-service science teachers. Activities within the courses were planned according to the aims of the ESTA project. The idea of CLIL (Content and Language Integrated Learning) was followed, which focuses not only on students' linguistic skills but also on their intercultural competencies. There is evidence that this approach has benefits in the teaching and learning of chemistry (e.g. Nurdillayeva and Zhuman, 2021, Nurdillayeva, Baisalova, Zhuman, 2020, Nikula, 2015).

### Research questions

According to the aims of the ESTA project, the following research questions were formulated:

RQ1: What are in-service teachers' expectations from professional development (PD) program regarding cultural and linguistic diversity?

RQ2: What are in-service teachers' experiences regarding cultural and linguistic diversity in their classroom?

RQ3: What are the benefits of PD program implemented within the project and how it can be upgraded?

### Instruments

The prerequisite for enrolling in the program was to fulfil an initial questionnaire (QI) that contained 36 questions about participants background (age, education level, mother tongue of them and their students, language of the literature they use to prepare lessons etc.). The QI was administered online, along with an invitation to the course sent to the corresponding ministries of education in Republic of Srpska (RS) and three cantons in Federation of Bosnia and Herzegovina (FBiH): Sarajevo Canton, Zenica-Doboj Canton and Central Bosnia Canton.

After attending and completing all requirements within the course, participants were asked to complete a paper-and-pencil questionnaire (QC), which contained 19 items about their impressions of the PD program and their perceptions of linguistically and culturally diverse material and its applicability in their teaching practice.

### Participants

A total of 58 primary and secondary school chemistry teachers completed the QI, while 14 primary and 24 secondary school teachers attended and completed a two-day course held in November and December 2021 for primary school teachers and in May and June 2022 for secondary school teachers. There were still certain restrictions and precautions due to the COVID-19 pandemic, which explains the lower number of participants of the courses comparing to the number of teachers who filled the QI. The participants were divided into groups based on the data from QI, so that the groups were heterogeneous with respect to mother tongue. Each group consisted of at least one person proficient in English and IT in chemistry teaching, so that communication and cooperation that arose from the need to solve a common problem was at a high level.

### About the courses

Participants were offered carefully planned activities (lectures, discussions, and workshops) aiming to develop knowledge, skills, and attitudes about (1) key concepts of intercultural education, (2) values of the intercultural dimensions and their integration into the educational process, (3) awareness of science and cultural capital and their role in the classroom, (4) the philosophy of nonviolence and nonviolent communication (an example of a conversation between a giraffe and a snake (Rosenberg, 2006)). Communication between participants was encouraged through group work.

During the course, the teachers were introduced to the basic linguistic characteristics of all three official languages in BiH, their similarities and differences at the grammatical and lexical linguistic level. The emphasis was set on the terms related to science/chemistry, which can affect the process of acquiring chemistry knowledge, and thus the achievements of students. Participants were encouraged to describe how they felt while reading a text in a language and script they rarely use. Those who have already had the opportunity to teach in linguistically diverse environments shared their experiences and pointed out the challenges they encountered.

Laboratory exercises were designed for the target group of participants (applicable at the primary and/or secondary level of chemistry education, depending on the group). The concept was based on the fact that each group receives a new exercise in a different language/alphabet or as a visual material. Additionally, the laboratory part of the course included the application of modern, digital laboratory and IT equipment (laboratory sensors for pH, temperature and conductivity, tablets and the corresponding applications for data collection and analysis).

Two experiments were selected from the teaching material *Lilu's House: Language Skills through Experiments* (2018): *Lilu, Alina and the Scientific Experiment* and *Lilu, Alina and Scientific Models*, based on their connections with current chemistry curriculum for primary schools. They were adapted with respect to the linguistic diversity of the participants (primary school chemistry teachers). Two experiments were prepared with the aim to use tablets and laboratory sensors: *Energy from food* and *Investigating Physical and Chemical Changes of Matter*. They were also adapted to another group of participants (secondary school chemistry teachers).

As laboratory part of the course required written material for the participants, it was delivered in different languages and/or alphabets (Bosnian, Croatian, Serbian, English, pictures; Latin or Cyrillic), in order to sensitize the participants to how the learning process can be hindered if the material was delivered to the students in a language or alphabet they are not familiar with.

Images support the development of students' language skills. Analyzing pictures offers an opportunity for meaningful student-to-student engagement because our perception of what we see is subjective. Genuine communication occurs during this type of oral exchange (Baker, 2015).

Before performing the laboratory exercises, the participants should (1) recognize the language standard used, (2) notice the linguistic features of the text, (3) find linguistic forms that might cause difficulties for students in understanding the content, and (4) offer synonyms for the forms found in the text. The goal was to familiarize the participants with the features of language standards in BiH, but also to develop sensitivity for students stemming from different linguistic backgrounds. This can result in planning an adequate teaching process for students in heterogeneous environments.

## RESULTS AND DISCUSSION

The initial questionnaire (QI) that was applied with the invitation for the PD program, showed that a large number of teachers filled out the questionnaire using language forms characteristic only for certain language systems (e.g. *hemija* for Bosnian and Serbian; *kemija* for Croatian). Only a few teachers entered both language forms (e.g. *hemija/kemija*), thus respecting the culture of other language standard in Bosnia and Herzegovina. This indicated that some teachers are familiar with teaching in a linguistically heterogeneous environment, and that to a certain extent they have a developed sensitivity towards students who come from different speaking backgrounds. However, as pointed by Duverger (2007), teachers of "non-linguistic subjects" (such as chemistry) are not language teachers and therefore require specific training to apply CLIL (Jameau and Le Henaff, 2018).

There were 32.7% of participants with teaching experience up to 5 years. The second group consists of teachers with 20 and more years of experience (25.9%), while the other three groups (5-10, 10-15 and 15-20 years) were represented by 12-15%. More than 36% of teachers stated that they often teach students who speak a different mother tongue, while 17.2% stated they never had this opportunity.

The language of the literature that these teachers use to prepare for classes is represented in Table 1 (Likert-type questions, 5 - everyday, 1 - never).

**Table 1:** Literature used for preparing the class

| Language             | M*   | SD** |
|----------------------|------|------|
| Bosnian              | 4,26 | 1,09 |
| Croatian             | 4,14 | 1,02 |
| Serbian              | 3,22 | 1,43 |
| English <sup>1</sup> | 2,84 | 1,41 |

\*M – mean, \*\*SD – standard deviation

Teachers also stated that their knowledge of the Cyrillic alphabet is at a high level ( $M = 4.36$ ,  $SD = 0.97$ ). This was relevant because in the cantons to which the invitations were sent, Latin alphabet is dominant in the education system. Likert-type questions were also used to explore what types of teaching resources teachers use.

**Table 2:** The frequency of using different teaching materials and aids

| Type of teaching material/aid      | M    | SD   |
|------------------------------------|------|------|
| Visual (schemes, pictures, photos) | 4,57 | 0,53 |
| Videos (e.g. YouTube)              | 3,90 | 0,85 |
| Mobile/tablet apps                 | 3,55 | 1,13 |
| Other types of IT                  | 4,00 | 0,99 |

Static visual teaching aids are most frequently used, while teachers rarely encouraged the use of mobile or tablet applications.

At the beginning of the course, the participants were asked about their expectations from the program they are attending, the lecturers, and themselves. The results are presented in Table 3.

<sup>1</sup> English and other foreign languages

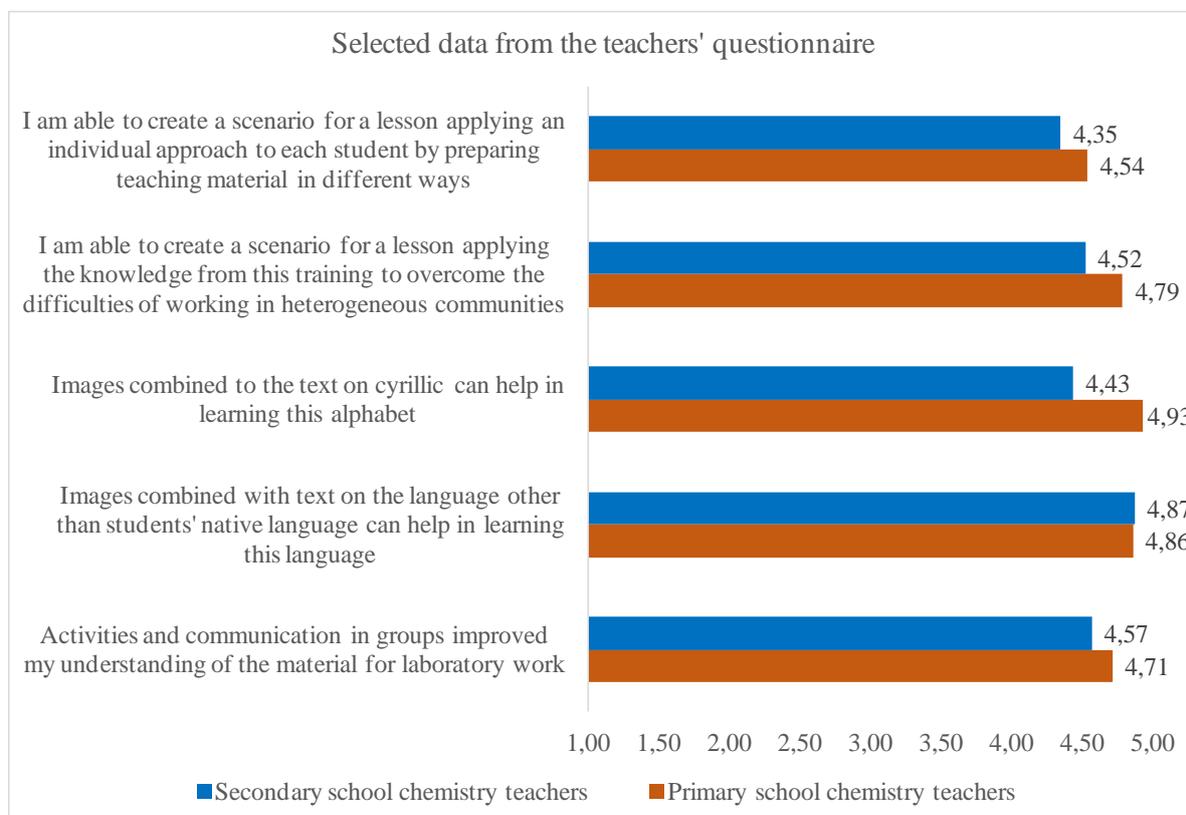
**Table 3:** Expectations of course participants

| Type                               | Expectation   |
|------------------------------------|---|
| From the training program          | To learn new knowledge and skills                                   |
|                                    | To improve acquired knowledge                                       |
|                                    | To experience new methods and forms of teaching                     |
|                                    | To improve pedagogical and didactic skills                          |
|                                    | To familiarize with the basic principles of intercultural education |
|                                    | To learn from innovative and inspiring teaching materials           |
|                                    | To make a new friendship and exchange experiences with colleagues   |
| From the ESTA project team members | Competence  |
|                                    | Interesting and creative contents                                   |
|                                    | Examples of good teaching practice                                  |
|                                    | Support with the challenges of teaching in heterogeneous groups     |
| From the participants themselves   | Respect for the needs and interests of the participants             |
|                                    | Active participation during the training program                    |
|                                    | Application of the new knowledge in teaching                        |
|                                    | Commitment and willingness to cooperate                             |
|                                    | Success during the training program                                 |
|                                    | Enjoy activities and socializing with colleagues                    |

These findings are consistent with the relevant scientific literature that explores teachers' expectations of in-service professional training programs, mostly highlighting relevant themes (Avidov-Ungar, 2020), as well as interactive learning and classroom applications

possibilities (Armour and Makopoulou, 2012; Bellibas and Gumus, 2016).

Participants filled out the QC after finishing the course's prerequisites. Selected results are represented in Figure 1.

**Figure 1:** Selected findings from teacher questionnaire Qc

There were no significant differences in responses between primary and secondary school chemistry teachers. Their positive impressions allow us to further develop teaching materials, as well as collaboration with teachers, both in- and pre-service. Teachers largely agree that the combination of images with Cyrillic alphabet or with a foreign language can improve a student's learning of the alphabet/language.

They also indicated that group activities and communication among group members helped in understanding the material for laboratory exercises. Perhaps the most important findings point to the abilities they developed during the courses to create adequate scenarios for lessons, with implementation in heterogeneous classrooms.

It is worth mentioning that all participants have positively evaluated the training program: the relevance of content, working conditions, quality of activities and engagement received  $M \geq 4.87$  (on a 1-5 Likert-type scale). For the most part, they had no suggestions for upgrading the courses; some of them highlighted that they are pleased with sufficient amount of laboratory exercises applicable in the classroom, since they are not often implemented in PD courses.

Some examples of the ignorance of spelling rules common for all three official language standards in BiH were registered on the written material they received from the participants. This points to the issues in language culture in general, which can further complicate the situation in heterogeneous classrooms. There is certainly a need for continuous professional development, both in the professional and in the linguistic domains.

One of the training activities was the participants' perceptions of themselves. Participants were asked to complete five sentences beginning with "I am". It is interesting that 90% wrote "I am a teacher" in the second or third sentence. This is significant information because it indicates that the participants generally accept and understand their role and reputation in society.

## CONCLUSION

Intercultural education is a vital factor in understanding different cultures and establishing positive relations between people. Its primary aim is to ensure sustainable social and cultural development, especially for vulnerable individuals or groups. Teachers need to acknowledge and accept other cultures, as well as respect their own culture in order to apply these values and attitudes in education process. Competent teachers can create a positive environment for intercultural communication and develop intercultural sensitivity in their students (Mrnjauš *et al.*, 2013, Sablić, 2013, Piršl, 2016).

During the training, the participants were exposed to unexpected situations, such as reading material in a language they (often) do not speak. These and similar teaching situations aimed for the participants to develop intercultural sensitization to the differences they could encounter in their classrooms.

RQ1: In-service teachers' expectations from PD program regarding cultural and linguistic diversity do not differ from those found in the literature; the most important

outcome from PD program is the applicability in everyday teaching practice.

RQ2: During the discussion, some teachers shared their experiences of cultural and linguistic diversity in their classrooms. They mostly encountered at least one example during their teaching practice. However, they did not have clear instructions or knowledge on how to manage them in their classrooms prior to this PD program.

RQ3: The benefits of the PD program realized within the project are numerous. Participants clearly missed such programs during the COVID-19 pandemic. They appreciated the laboratory exercises applicable in their classroom, the offer of visits to their schools to disseminate tools, methods and devices, and the combination of various teaching methods during the program. Some of them stated that they would like more practical activities.

The participants had no major difficulties in understanding content written in Latin or Cyrillic, nor did they experience any other difficulties related to linguistic issues. The reason for this is that the educational system of the former Yugoslavia respected diversity in terms of language, so students who predominantly received their education in this system are familiar with linguistic diversity. However, they experienced certain difficulties and showed resistance to material written in English. At the same time, younger participants stated that they know less about the "other" alphabet (in this case, mostly Cyrillic). They also show less confidence when working on texts written in Cyrillic, but they were more comfortable with material in English. It is reasonable to expect that teachers who experience some degree of insecurity will consider this in their classrooms.

Since themes regarding cultural and linguistic diversity with an emphasis on science education are new in teacher education in our country, there is certainly a need to develop courses which address these issues, both for in- and pre-service teachers. This confirms that the goals of the ESTA project were relevant for contemporary science education.

## ACKNOWLEDGEMENTS

*This research was part of the project "ESTA—Educating Science Teachers for All" that is co-funded by the Erasmus+ Programme of the European Union, under the grant number 609719-EPP-1-2019-1-DE-EPPKA2-CBHE-JP.*

*The European Commission's support for the production of this publication does not constitute an endorsement of the content, which reflect only the views of the authors, and the Commission cannot be held responsible for any use which may be made of the information contained therein.*

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## **Summary/Sažetak**

Jedan od izazova u nastavi prirodnih nauka u Bosni i Hercegovini jeste pluralizam jezika i kulturološka raznolikost. U dosadašnjem inicijalnom obrazovanju nastavnika nedostajalo je kvalitetne pripreme za ove suvremene izazove; prilike za poučavanje kulturne i jezične raznolikosti nisu bile prepoznate. Stoga je izazov potaknuti iskusne nastavnike kemije da razviju kompetencije za interkulturalno nastavno okruženje, a što je jedan od ciljeva u okviru projekta Educating Science Teachers for All (ESTA). Polaznici programa stručnog usavršavanja bili su nastavnici kemije iz tri kantona u FBiH. Program je imao za cilj razvijanje i unaprjeđenje stručnih kompetencija kroz radionice i grupni rad, uz raspravu o odabranim temama i laboratorijske vježbe uz korištenje suvremene laboratorijske opreme i informacijsko-komunikacijske tehnologije. Ponuđene su mogućnosti poučavanja u jezički raznolikim razredima, kao i u razredima s drugim oblicima različitosti učenika. Rezultati su pokazali da su nastavnici svjesni potrebe senzibiliziranja za različitosti učenika. Izrazili su pozitivne dojmove i spremnost da nastavne materijale prilagode potrebama svojih učenika, ali i potrebu za daljnjom podrškom kroz suradnju sa univerzitetskim nastavnim osobljem.

## The Influence of Foreign Ions on Determination and Speciation of Cr(VI) and Cr(III) from Water Samples

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### Article info

Received: 06/03/2023  
Accepted: 25/06/2023

### Keywords:

Chromium(III)  
Chromium(VI)  
Speciation  
Foreign Ions  
Wastewater  
River Water

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**Abstract:** This study reports: a) the content of Ca, Cd, Co, Cr(III), Cr(VI), Cu, Fe, K, Mg, Mn, Na, Ni, Pb, and Zn in wastewater from metal industries and rivers that flow near the metal processing industries; b) the possibility of quantitative determination of Cr(III) and Cr(VI) in the presence of the following cations: Fe<sup>3+</sup>, Cu<sup>2+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Ni<sup>2+</sup>, Mn<sup>2+</sup>; and Zn<sup>2+</sup> and anions Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, and SO<sub>4</sub><sup>2-</sup>; c) speciation of Cr(III) and Cr(VI) on silica gel modified with Nb<sub>2</sub>O<sub>5</sub>. Metals were determined by flame atomic absorption spectrometry, except for Na and K, which were determined by atomic emission spectrometry, and Cr(VI) by ultraviolet-visible spectrophotometry. When the concentration of cations is higher than Mg<sup>2+</sup> (>25 mg/L), Ni<sup>2+</sup> (>100 mg/L), Fe<sup>3+</sup> (>250 mg/L), Zn<sup>2+</sup> (>500 mg/L), and Mn<sup>2+</sup> (>1000 mg/L), an error greater than 10 % is obtained in the measurement of Cr(III). Silica gel modified with Nb<sub>2</sub>O<sub>5</sub> can be considered as an effective solid phase extraction adsorbent for speciation of Cr species and simultaneous concentration of Cr(III). The proposed method was successfully applied to the speciation and determination of Cr(III) and Cr(VI) in the analyzed samples. The content of almost all analyzed metals has been below the permitted values for wastewaters.

## INTRODUCTION

Despite different efforts to decrease it, the continuous pollution of surface water is a global issue that is not going away. The prosperous age of the industrial revolution and its modernization, along with reckless industrial operations, has sadly resulted in the degradation of the ecosystem, putting the environment in perilous conditions. As a result, harmful contaminants from diverse industrial processes have contaminated an increasing amount of surface water. Many ecosystems have disappeared as a result of this tragic issue, and many people have been left in excruciating health conditions that require constant medical monitoring (Rajput et al., 2017; Nkutha et al., 2021). The importance of chromium (Cr) speciation originates from the widespread participation of this element in various environmental and biological samples. The major sources of Cr in the environment are steel, electroplating, tanning, and chemical industries, oxidative dyeing, cooling water towers, corrosion inhibitors used in water pipes and containers, and sanitary landfill leaching (Kotas and Stasicka 2000; Dong, Chen, and Chen, 2013). The acidity

and a significant concentration of Cr compounds in the wastewater make the Cr industry a high environmental concern. Industrial wastewater is the major source of pollutants that pollute the environment. Large amounts of industrial waste have been released into rivers, lakes, and coastal areas during the past century. This has resulted in a significant water contamination problem, as well as detrimental consequences for the ecosystem and human life. Increased metal concentrations in wastewater from the metal industry are hazardous to the aquatic ecology and can be lethal (Nkutha, Naidoo, and Shooto, 2021). It has been widely recognized that the impact of detrimental heavy metals on the ecological system, biological organisms, as well as human health does not only depend on the total amount of the element but also significantly depends on its chemical forms (Kot and Namiesnik, 2000). Cr(III) and Cr(VI), the two most stable oxidation states of Cr, have different biological activities and toxic effects on living organisms (Narin, Soylak, and Kayakirilmaz et al., 2002). Chromium (III) is less toxic and less soluble than Cr(VI) and is an essential micronutrient for most biota, including humans.

Chromium(VI) is generally soluble and toxic to many plants, animals, and microorganisms in the aquatic environment, and Cr(VI) is considered mutagenic and carcinogenic (Losi, Amrhein, Frankenberger, 1994). The Cr content in surface waters should be very low, usually between 0.3 and 6 µg/L (Rakhunde, Deshpande, Juneja, 2012). Due to all of the above mentioned, and mainly because of the toxicity of certain forms of Cr, it is necessary to perform Cr speciation. Chemical precipitation, ion exchange, and solid-phase extraction are methods used for Cr preconcentration and speciation (Sarzanini, Abolino, and Mentastrì, 2001; Aydin and Soylak, 2007; Tuzen and Soylak 2007; Sulejmanović et al., 2015; Tiwari, Sharma, and Saxena, 2016; Pyrzynska, 2020). Adsorption has emerged as one of these techniques that is both effective and affordable. In trace element analysis, the two most common adsorbents are activated carbon and silica gel. Today, solid-phase extraction is a very useful technique, widely spread as part of sample preparation procedures for the determination of various inorganic and organic analytes (Chahal et al., 2012). A measurement procedure that provides quantitative and qualitative information on the chemical forms of an element in various samples is known as speciation analysis. Separation and determination are the two most common phases in speciation analysis (Kumral, 2007). The objectives of this study were: (1) to determine the content of the following metals: Ca, Cd, Co, Cr(III), Cr(VI), Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Zn in river waters that flow in the vicinity of metal processing industry and in wastewater of the metal industries (2); quantitative determination of Cr(III) and Cr(VI) in the presence of the following cations: Fe<sup>3+</sup>, Cu<sup>2+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Ni<sup>2+</sup>, Mn<sup>2+</sup> and Zn<sup>2+</sup> which are added to model solutions as chloride or nitrate salts at the concentration range of 10 - 2000 mg/L; (3) the influence of Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup> on the quantitative determination of Cr(III) and Cr(VI) which are added to model solutions in the form of sodium, potassium and ammonium salts and SO<sub>4</sub><sup>2-</sup> in the form of potassium salt so that their concentration range is 10 - 2000 mg/L; (4) speciation of Cr(III) and Cr(VI) on silica gel modified with Nb<sub>2</sub>O<sub>5</sub>.

## EXPERIMENTAL

### Instruments

A Varian model Fast Sequential Atomic Absorption Spectrometer, AA240FS was used for the determination of Ca, Cd, Co, Cr(III), Cu, Fe, K, Mg, Mn, Na, Ni, Pb, and Zn. Spectrophotometer type Cary 50 (Varian) was used for spectrophotometric determination of Cr(VI) at λ = 545 nm using 1 cm glass cells.

### Chemicals and reagents

All chemicals used were of analytical grade purchased from Merck (Darmstadt, Germany). Doubly distilled water was used throughout the experiment.

Stock solutions (1.000 mg/L) of Cr(VI) were prepared by dissolving 0.7856 g of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, previously dried for 2 hours at 175 °C, and diluted in doubly distilled water to 250 mL. A working solution with a concentration of Cr (VI) of 50 mg/L was prepared by diluting the stock solution. Stock solutions of cations (5.000 g/L): Fe<sup>3+</sup>,

Cu<sup>2+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Ni<sup>2+</sup>, Mn<sup>2+</sup>, Zn<sup>2+</sup> were prepared by dissolving appropriate amounts of the chloride and nitrate salts.

Stock solutions of anions (5.000 g/L): Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, were prepared by dissolving appropriate amounts of the following salts: NaCl, NaNO<sub>3</sub>, NH<sub>4</sub>Cl, NH<sub>4</sub>NO<sub>3</sub>, KNO<sub>3</sub>, KCl, K<sub>2</sub>SO<sub>4</sub>. A 0.5 % solution of 1,5-diphenylcarbazide was prepared by dissolving appropriate amounts of this reagent in acetone. A fresh solution was prepared every day.

### General procedure

Silica gel was modified with Nb<sub>2</sub>O<sub>5</sub> according to the procedure by Musić (2010), since this study described the used sorbent as an effective sorbent for the speciation of Cr because it acts as a cationic ion exchanger. of silica gel (250 mg) modified with Nb<sub>2</sub>O<sub>5</sub> (sorbent) was weighed on an analytical balance (Mettler Toledo New Classic MF, model: ML204/01, Switzerland), which was then quantitatively transferred to the columns. Solutions containing a mixture of Cr(III) and Cr(VI), then a solution of Cr(III) or Cr(VI), and solutions containing Cr(III) and the metal being tested as an interference were passed through the columns. According to Musić (2010) the optimal pH value for Cr speciation analysis is pH 9. In the process of speciation of Cr(III) and Cr(VI) ions, a self-made glass tube column filled with 250 mg of silica gel modified with Nb<sub>2</sub>O<sub>5</sub> was used. The solutions that passed through the columns contained metals at a concentration of 0.1 mg/L in a 100 mL volumetric flask. According to Musić (2010), in order to achieve efficient retention of the metal of interest on the given sorbent and to adjust the analyte flow through the column, the specified buffer (pH 9) was first passed through the column. The buffer solution was prepared by dissolving an appropriate amount of disodium tetraborate decahydrate (Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O) in distilled water, and the pH was adjusted using 0.1 mol/L HCl. After that, standard metal solutions were passed through the columns at a flow rate of 2 mL/min. The adsorbed metals were eluted with 8 mL HNO<sub>3</sub> (1 mol/L), in a 10 mL measuring vessel. During the speciation analysis, Cr(III) is retained on the sorbent, and Cr(VI) passes through the column unhindered because it is present in aqueous solutions as CrO<sub>4</sub><sup>2-</sup>, Cr<sub>2</sub>O<sub>7</sub><sup>2-</sup> and HCrO<sub>4</sub><sup>-</sup> (Martendal et al., 2009).

A series of model solutions containing Cr(III) and Cr(VI) and metals in varied concentrations (10 mg/L–2000 mg/L) were created and treated according to the devised method (Musić, 2010) for the determination and speciation analysis of Cr species.

### Wastewater and river water analysis

The ISO standard method was used for sampling (ISO, 2014). Water samples were collected in polyethylene bottles. River samples were taken during stable weather conditions at four locations in Bosnia and Herzegovina (BiH): Ilijaš, Misoća, Vogošća and Vareš. The sampling sites were in rivers flowing near industries. Wastewater samples, from the electroplating industries, were collected before discharge into the recipient (river water). The concentrations of Cr(III), Cu, Mn, Fe, Co, Ni, Cd, Pb, Zn, Ca, and Mg were determined using flame atomic absorption spectrometry (FAAS), and the concentrations of Na and K by flame atomic emission spectrometry

(FAES). Cr(VI) was determined from the original samples by ultraviolet and visible (UV/Vis) spectrophotometry.

#### Quality control

Recovery evaluations were conducted to ensure the accuracy of the research. Real samples of river water and wastewater were spiked with the standard solution of each metal at three different levels of concentration to cover the measurement range. The recovery values for all metals determined ranged from 86% to 104%. Recovery values are presented in table 1. For model solutions, the results are presented as recovery values in Tables 2 and 3, and on Fig. 2 – Fig. 7.

**Table 1.** Recovery values of spiked real river water and wastewater samples

| Metals  | Recovery (%) |            |
|---------|--------------|------------|
|         | River water  | Wastewater |
| Cr(III) | 97           | 98         |
| Cr(VI)  | 90           | 91         |
| Cu      | 95           | 96         |
| Mn      | 98           | 99         |
| Fe      | 95           | 95         |
| Co      | 86           | 86         |
| Ni      | 88           | 88         |
| Cd      | 93           | 93         |
| Pb      | 98           | 97         |
| Zn      | 103          | 103        |
| Ca      | 101          | 104        |
| Mg      | 99           | 100        |
| Na      | 93           | 93         |
| K       | 96           | 97         |

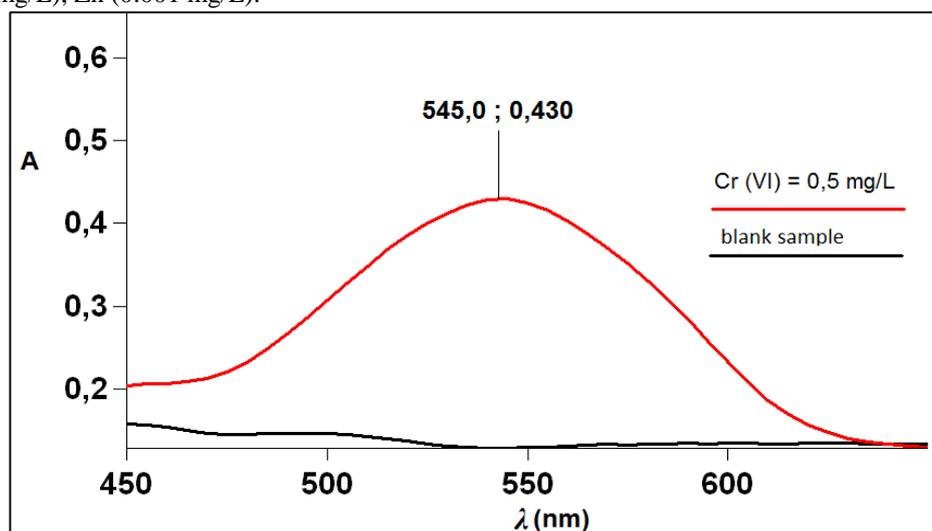
The detection limits (LOD) were calculated as three times standard deviation of the blank solution signal. The LOD values were: Ca (0.001 mg/L), Cd (0.002 mg/L), Co (0.005), Cr(III) (0.006 mg/L), Cr(VI) (0.02), Cu (0.003 mg/L), Fe (0.006 mg/L), K (0.003 mg/L), Mg (0.0003 mg/L), Mn (0.002 mg/L), Na (0.0002 mg/L), Ni (0.01 mg/L), Pb (0.01 mg/L), Zn (0.001 mg/L).

#### Influence of cations and anions on determination of Cr(III)

First, 25  $\mu$ L of the sample solution containing 1000 mg/L Cr(III) and 1000 mg/L Cr(VI) was placed in a 25 mL volumetric flask, then the tested cations ( $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ ) were added separately to the volumetric flasks so that their concentrations were 10 mg/L, 25 mg/L, 50 mg/L, 100 mg/L, 250 mg/L, 500 mg/L, 1000 mg/L, 1500 mg/L and 2000 mg/L. The same procedure described above was performed with anions ( $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ). For each model solution, a blank solution containing the interfering metal or anion as well as doubly distilled water was prepared. The concentration of Cr(III) in the prepared solutions was determined by FAAS.

#### Influence of cations and anions on determination of Cr(VI)

For the spectrophotometric determination of Cr(VI), an absorption spectrum was first recorded with the objective to determine the wavelength ( $\lambda$ ) of the maximum absorption of the colored complex Cr(VI) and diphenylcarbazide (DPC) in a range of (450–650) nm (Fig. 1). Measurements were performed using a standard solution containing 0.5 mg/L of Cr(VI), 1 mL of (1:1)  $\text{H}_2\text{SO}_4$  (v/v), and 1 mL of 0.5% DPC. The reaction is selective for Cr(VI) and very sensitive. The maximum absorption for Cr(VI)-DPC colored complex was found to be at 545 nm. The influence of cations and anions on the direct spectrophotometric determination of Cr(VI) was also investigated for determination of Cr(III). First, 250  $\mu$ L of the working solution (50mg/L Cr(VI)) was placed in a 25 mL volumetric flask, the tested cations and anions were added separately to the volumetric flasks in the same concentration range as for the determination of Cr(III), and Cr(VI) was complexed with 1 mL of 0.5% DPC in the presence of 1 mL of (1:1)  $\text{H}_2\text{SO}_4$  (v/v). A blank solution was prepared for each model solution (interfering metal or anion, 1 mL (1:1)  $\text{H}_2\text{SO}_4$  (v/v), 1 mL 0.5% DPC, and doubly distilled water).

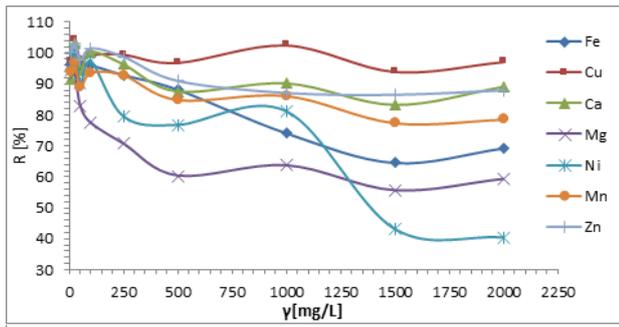


**Fig. 1 -** Spectral responses of Cr(VI) solution in the range from 450 to 650 nm.

## RESULTS AND DISCUSSION

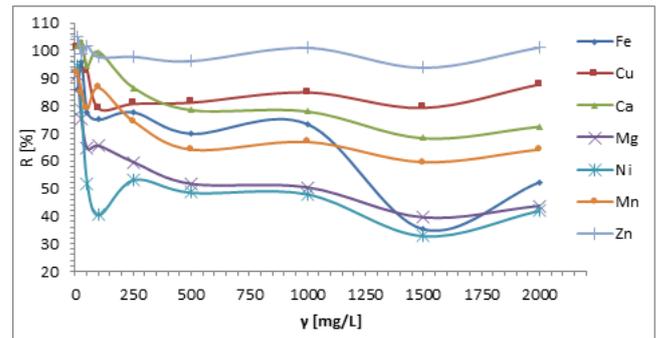
### Influence of coexisting cations on determination of Cr(III)

Matrix effects are a very problematic point in the instrumental detection of trace metal species (Unsal, Tuzen, and Soylak, 2014). The influence of cationic chloride and nitrate salts on the recovery values of Cr(III) was investigated separately. The results obtained are given in Figs. 2 and 3.



**Fig. 2** - Recovery values for Cr(III) in presence of cation chloride salts.

Figure 2 shows that  $\text{Ca}^{2+}$  and  $\text{Cu}^{2+}$  in all concentration ranges (10-2000 mg/L) do not affect the measurement of Cr(III), since the error in the determination of Cr(III) was lower than 10%. The error in the measurement of the Cr(III) is higher than 10% in the presence of these cations:  $\text{Mg}^{2+}$  (> 25 mg/L),  $\text{Ni}^{2+}$  (> 100 mg/L),  $\text{Fe}^{3+}$  (> 250 mg/L),  $\text{Zn}^{2+}$  (> 500 mg/L) and  $\text{Mn}^{2+}$  (> 1000 mg/L).



**Fig. 3**- Recovery values for Cr(III) in presence of cation nitrate salts.

The obtained results for cations in nitrate salts (Fig. 3) show that only in the presence of  $\text{Zn}^{2+}$ , the recovery values for Cr(III) are at the maximum recovery value (~100%). The error of Cr(III) measurement is higher than 10% when the concentration of metal ions is higher than  $\text{Mg}^{2+}$ ,  $\text{Ni}^{2+}$ , and  $\text{Fe}^{3+}$  (> 25 mg/L),  $\text{Cu}^{2+}$  and  $\text{Mn}^{2+}$  (> 50 mg/L), and  $\text{Ca}^{2+}$  (> 100 mg/L). The lowest recovery values for Cr(III) were achieved in the presence of nickel as  $\text{Ni}(\text{NO}_3)_2$ . If the  $\text{Ni}^{2+}$  concentration is 25 mg/L, the recovery value for Cr(III) is 78.55%, and if the  $\text{Ni}^{2+}$  concentration is 1500 mg/L, the recovery value is only 32.95%.

### Influence of coexisting anions on determination of Cr(III)

Recovery values for Cr(III) in the presence of  $\text{Cl}^-$ ,  $\text{NO}_3^-$ , and  $\text{SO}_4^{2-}$  ions are presented in Table 2.

It is clear from the results that chloride ions do not interfere with the determination of Cr(III) because the recovery values ranged from 95% to 106% whether chloride ions were added as potassium, sodium, or ammonium salt in all ranges of concentration (10 mg/L to 2000 mg/L).

**Table 2** - Recovery values for Cr(III) in presence of anions

| Concentration of anion          | 10 mg/L          | 25 mg/L | 50 mg/L | 100 mg/L | 250 mg/L | 500 mg/L | 1000 mg/L | 1500 mg/L | 2000 mg/L |
|---------------------------------|------------------|---------|---------|----------|----------|----------|-----------|-----------|-----------|
| Salts                           | R for Cr(III)(%) |         |         |          |          |          |           |           |           |
| KCl                             | 102.25           | 98.90   | 96.55   | 94.35    | 98.95    | 101.35   | 103.80    | 102.15    | 106.45    |
| NH <sub>4</sub> Cl              | 98.45            | 98.10   | 99.25   | 99.10    | 96.45    | 97.55    | 97.85     | 94.60     | 96.85     |
| NaCl                            | 96.35            | 97.30   | 99.90   | 100.60   | 102.50   | 101.50   | 104.01    | 103.50    | 102.40    |
| KNO <sub>3</sub>                | 98.85            | 98.45   | 100.55  | 100.25   | 101.05   | 100.45   | 101.55    | 102.45    | 105.80    |
| NH <sub>4</sub> NO <sub>3</sub> | 87.35            | 87.55   | 84.55   | 81.05    | 87.15    | 86.45    | 93.40     | 89.10     | 94.95     |
| NaNO <sub>3</sub>               | 111.90           | 110.65  | 113.95  | 118.25   | 113.10   | 112.20   | 110.35    | 112.10    | 109.50    |
| K <sub>2</sub> SO <sub>4</sub>  | 96.35            | 114.90  | 116.30  | 115.15   | 115.30   | 114.70   | 118.75    | 117.15    | 113.30    |

The lowest recovery values for Cr(III) were in the presence of  $\text{NH}_4\text{NO}_3$ . The recovery values in this case ranged from 87.35% for 10 mg/L of  $\text{NO}_3^-$  anion to 94.95% for 2000 mg/L of  $\text{NO}_3^-$  anion. If the nitrate anion was added to the solution as potassium salt, the recovery values for Cr(III) were 98.85% when the concentration of  $\text{NO}_3^-$  was 10 mg/L and increased with increasing nitrate concentrations.

Recovery values for Cr(III) are higher than 110% if the nitrate anion is present in the solution in the form of a sodium salt. Table 2 shows that  $\text{SO}_4^{2-}$  anion has an influence on the recovery values for Cr(III) if it is present in a concentration higher than 10 mg/L.

### Influence of coexisting cations on determination of Cr(VI)

After it was established that Cr(VI) ions have no affinity to be retained on silica gel modified with  $\text{Nb}_2\text{O}_5$ , the influence of the cations and anions on the quantitative determination of Cr(VI) was investigated using model solutions. The results are presented in Fig.4 (chloride salts), and Fig.5 (nitrate salts).

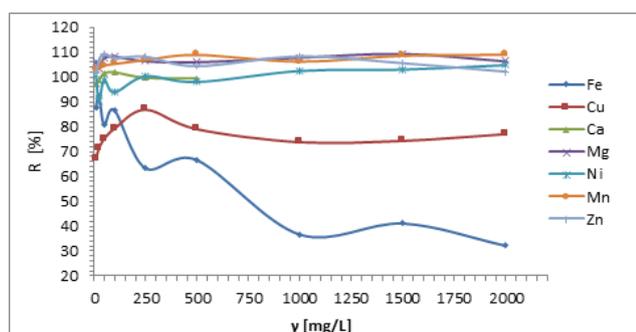


Fig. 4 - Recovery values for Cr(VI) in presence of cation chloride salts.

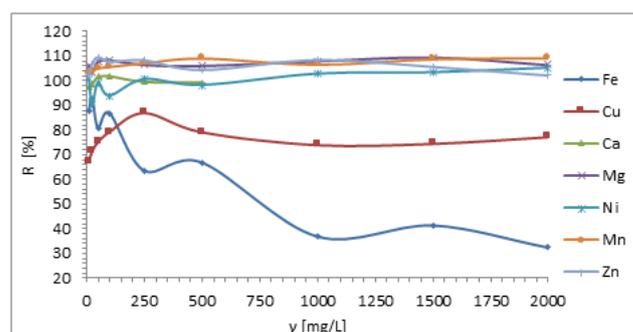


Fig. 5 - Recovery values for Cr(VI) in presence of cation nitrate salts.

As a result,  $\text{Fe}^{3+}$  ions clearly interfere with the determination of Cr(VI). Since the recovery values are around 80%, the quantitative determination of Cr(VI) is not possible if the concentration of  $\text{Fe}^{3+}$  is greater than 250 mg/L (chloride salts) or 100 mg/L (nitrate salts). Also, the color of the solutions changed from purple to red as the concentration of  $\text{Fe}^{3+}$  ions increased.

When copper ions were added to the solution, the recovery values for Cr(VI) were approximately 80% when the copper concentration was less than 500 mg/L; when the concentration of Cu(II) increased to 2000 mg/L, the recovery value for Cr(VI) was 52.16% (this occurs in the presence of chloride salt). When the  $\text{Ca}^{2+}$  ion concentration exceeds 500 mg/L, a white precipitate is formed, which makes quantitative determination of Cr(VI) impossible;  $\text{Ca}^{2+}$  concentrations less than 500 mg/L have no effect on the determination of Cr(VI). The error in the measurement of Cr(VI) is not higher than 10% in the presence of these ions  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Zn}^{2+}$ . Recovery values for Cr(VI) in the presence of  $\text{Cl}^-$ ,  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  ions are presented in Table 3.

Table 3 - Recovery values for Cr(VI) in presence of anions

| Concentration of anion   | 10 mg/L          | 25 mg/L | 50 mg/L | 100 mg/L | 250 mg/L | 500 mg/L | 1000 mg/L | 1500 mg/L | 2000 mg/L |
|--------------------------|------------------|---------|---------|----------|----------|----------|-----------|-----------|-----------|
| Salts                    | R for Cr(VI) (%) |         |         |          |          |          |           |           |           |
| KCl                      | 96.22            | 101.30  | 97.66   | 101.12   | 103.20   | 101.88   | 103.90    | 103.28    | 105.78    |
| $\text{NH}_4\text{Cl}$   | 100.96           | 99.28   | 94.62   | 94.14    | 95.34    | 97.62    | 96.96     | 98.82     | 96.23     |
| NaCl                     | 102.50           | 97.50   | 100.80  | 98.90    | 102.60   | 101.10   | 100.70    | 99.20     | 102.00    |
| $\text{KNO}_3$           | 103.64           | 100.48  | 96.90   | 95.84    | 99.64    | 95.76    | 100.78    | 97.42     | 100.28    |
| $\text{NH}_4\text{NO}_3$ | 100.44           | 98.52   | 97.66   | 97.72    | 96.32    | 97.30    | 99.56     | 95.90     | 98.80     |
| $\text{NaNO}_3$          | 91.48            | 92.36   | 94.00   | 99.78    | 92.88    | 92.70    | 98.26     | 97.42     | 99.88     |
| $\text{K}_2\text{SO}_4$  | 101.58           | 97.80   | 100.18  | 99.54    | 101.74   | 98.66    | 104.32    | 102.14    | 103.76    |

The recovery values for Cr(VI) were around 96% when the  $\text{Cl}^-$  ion was added to the solution as KCl (the concentration of  $\text{Cl}^-$  ion was 10 mg/L), and recovery values increased with increasing chloride concentrations up to 105.78% for 2000 mg/L of  $\text{Cl}^-$  ions. The concentration of  $\text{Cl}^-$  ions had no effect on the recovery values of Cr(VI) when chloride ions were added in the form of potassium and ammonium salt. The presence of

$\text{NO}_3^-$  ion in the form of sodium salt results in the lowest recovery values. The recovery values are in the range of 91.48% - 99.88%, and the recovery values increase with the increase in nitrate concentrations. From the results shown in Table 3,  $\text{SO}_4^{2-}$  anion does not affect the recovery values for Cr(VI).

### Effect of interfering ions on speciation of Cr(III) and Cr(VI)

The effect of common coexisting ions in river water and wastewater that could interfere with the speciation process (Cr(III) and Cr(VI)) and Cr(III) and adsorption on  $\text{SiO}_2\text{-Nb}_2\text{O}_5$  were studied. Solutions of 0.1 mg/L of Cr(III) in the presence of interfering ions ( $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Ni}^{2+}$  individually and in a mixture were analyzed according to the recommended procedure (Musić, 2010). If the recovery value of Cr species in the presence of a standard solution of metal ions is  $\pm 10\%$ , then the metal or a mixture of metal solutions is considered an interfering species. The recovery value for Cr(VI) was determined based on its concentration in the eluate from the sample that passed through a column filled with  $\text{SiO}_2\text{-Nb}_2\text{O}_5$ . It was found that interfering metals do not interfere with the determination of Cr(VI) because Cr(VI) does not adsorb on the  $\text{SiO}_2\text{-Nb}_2\text{O}_5$  (Fig. 6).

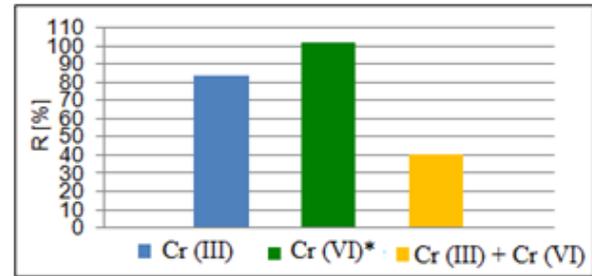


Fig. 6 - Results of preconcentration of Cr(III), Cr(VI) and mixture solution of Cr(III) and Cr(VI) on silica gel modified with  $\text{Nb}_2\text{O}_5$ .

The result of preconcentration of Cr(III) (0.1 mg/L) in the presence of metals (0.1 mg/L) individually and in a mixture is presented in Fig. 7.

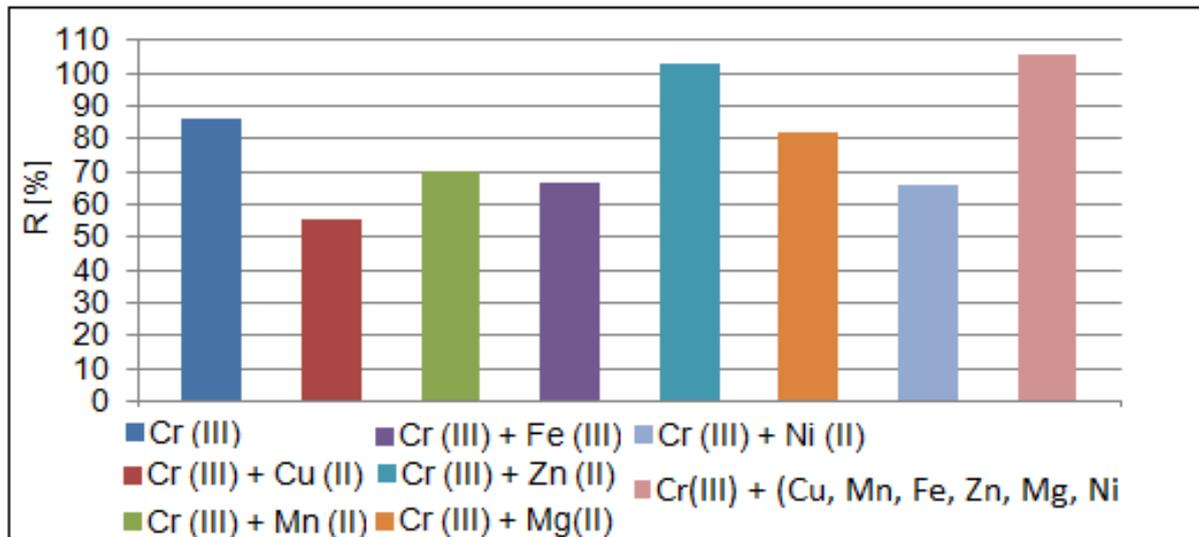


Fig. 7 - Recovery values of Cr(III) preconcentration and in a mixture with metals

The results showed that the lowest recovery values for Cr(III) were in the presence of Cu 55%. For Fe, Mn, and Ni, the recovery was below 70% and for Mg below 80%. Ions that are already known to interfere with the determination of Cr(III) are  $\text{Cu}^{2+}$  and  $\text{Fe}^{3+}$  (Wiryawan et al. 2018; Handbook, 2007). Based on the results of this study, Ni, Mn, and Mg also interfere. The best recovery values for Cr(III) are achieved in the presence of either zinc (102.6%) or all metals (105.9%). One of the reasons for this could be the formation of metal hydroxide on the surface of the sorbent, considering that in these two samples a thin yellow-colored layer was observed on the surface of the sorbent, which is lost during the elution of the columns. The results from this study were compared with the results of other studies that also analyzed the concentration of common coexisting ions that could affect the determination of Cr(III) and Cr(VI):  $\text{NO}_3^-$  and  $\text{Cl}^-$  were tolerated above 5000 mg/L,  $\text{SO}_4^{2-}$  and Ca mg/L were tolerated between 1000 and 100 mg/L, while those tolerated below 100 mg/L were  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{Cd}^{2+}$  (Tiwari et al. 2016). Zhang et al. (2008) in their study found that:  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  were tolerated for Cr(III) up to 10,000 mg/L and for Cr(VI) up to 2000 mg/L.  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Zn}^{2+}$  were tolerated for Cr(III) and Cr(VI) up to

1000 mg/L, and Fe up to 100 mg/L,  $\text{NO}_3^-$  can be tolerated up to 2000 mg/L for Cr(III) and for Cr(VI) up to 1000 mg/L.  $\text{SO}_4^{2-}$  is tolerated up to 3000 mg/L for Cr(III) and for Cr(VI) up to 1000 mg/L.  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Na}^+$  do not interfere with the determination of Cr(III) if their concentrations are less than 50 mg/L (Sulejmanović et al., 2015).

The results of this study were in good agreement with other previous published studies related to this investigated topic and also indicated that the use of silica gel or other sorbents for Cr speciation can also have an effect on the reduced interfering influence of foreign ions present in the sample. Therefore, the presence of major coexisting ions had no obvious influence on the determination of Cr(III) and Cr(VI).

### River water and wastewater analysis

The methods for Cr(III) and Cr(VI) determinations were applied to the river water and wastewater samples. Two wastewater samples were analyzed. The samples were filtered through a 0.45  $\mu\text{m}$  membrane filter (Whatman), and concentrated  $\text{HNO}_3$  was added to prevent metal ion losses and the formation of microorganisms. The concentrations of the following interfering metals (Ca,

Cd, Co, Cr(III), Cu, Fe, Mg, Mn, Ni, Pb, and Zn by FAAS, and Na and K by AES, while Cr(VI) by UV/Vis

spectrophotometry) were determined from real samples. The results are shown in Table 4.

**Table 4** -Average concentration of metals in wastewater and river water

| Samples | Wastewater I       | Wastewater II | River water Ilijaš | River water Vogošća | River water Misoča | River water Vareš |
|---------|--------------------|---------------|--------------------|---------------------|--------------------|-------------------|
| Metals  | Concentration mg/L |               |                    |                     |                    |                   |
| Cr(III) | 0.031              | 0.106         | < LOD              | < LOD               | < LOD              | < LOD             |
| Cr(VI)  | 0.162              | 0.204         | 0.038              | 0.035               | 0.04               | 0.04              |
| Cu      | 0.064              | 0.419         | < LOD              | < LOD               | < LOD              | < LOD             |
| Mn      | 21.52              | 40.7          | 0.011              | 0.009               | 0.018              | 0.064             |
| Fe      | 449.75             | 1810          | 0.156              | 0.29                | 0.177              | 0.086             |
| Co      | 0.165              | 0.301         | < LOD              | < LOD               | < LOD              | 0.012             |
| Ni      | 1.258              | 2.437         | 0.017              | < LOD               | < LOD              | < LOD             |
| Cd      | 0.011              | 0.013         | 0.002              | 0.002               | 0.003              | 0.004             |
| Pb      | < LOD              | 5.212         | 0.053              | 0.056               | 0.015              | 0.043             |
| Zn      | 2675               | 5250          | 0.636              | 0.299               | 1.763              | 1.908             |
| Ca      | 2775               | 4625          | 63.05              | 66.01               | 73.5               | 79.5              |
| Mg      | 80.00              | 120.00        | 7.5                | 9.5                 | 7.00               | 22.5              |
| Na      | 9.00               | 14.00         | 3.4                | 3.62                | 3.45               | 4.52              |
| K       | 2.512              | 5.025         | 0.937              | 1.087               | 1.255              | 1.575             |

The concentrations of Ca and Fe in the wastewater samples were higher than the concentration that affects Cr(VI) determination. The obtained concentrations for Ca, Mn, Mg, Fe, and Zn in the tested samples were higher than the concentration that has an influence on Cr(III) determination. Ca concentrations in wastewater can be high as a result of the use of CaO or Ca(OH)<sub>2</sub> as flocculants in the wastewater treatment process. The concentrations of all other investigated cations do not affect the Cr(III) and Cr(VI) determinations in wastewater samples. The results for the metal content in the river water samples showed that the concentration of all determined metals was lower than the concentrations that could affect the accuracy of the determination of Cr(III) and Cr(VI).

The content of Zn, Mn, Ni, Fe, and Cr(VI) in both wastewater samples was above the values allowed according to the Regulation on the limit values of hazardous and harmful substances for technological wastewater before their discharge into the public sewage system or into another receiver according to the Law on Water in the the Federation of Bosnia and Herzegovina ("Official Gazette of the Federation of Bosnia and Herzegovina "number 18/98; a and b).The level of Cu and Pb in Wastewater II were higher than the allowed values in the abovementioned Regulation. The metal concentration in all river water samples was below the values specified in the Regulation on limit values of hazardous and harmful substances in the waters that are discharged from the public sewage into a natural recipient after purification , except for Zn in river water collected at Misoča and Vareš sites. The obtained values are almost two times higher than the limit value for Zn (1 mg/L) ("Official Gazette of the Federation of Bosnia and Herzegovina" number 18/98; a and b).

## CONCLUSIONS

The effect of some cations and anions on the determination of Cr(III) and Cr(VI) was investigated in this study. Chloride salts of calcium and copper, as well as nitrate salts of zinc do not interfere with the determination of Cr(III). The concentration of Cr(III) in the presence of cation nitrate salts (except calcium) was lower compared to the presence of their chloride salts. Nitrate salts showed a lower recovery value for Cr(III) compared to chloride salts: nickel nitrate salts showed a 30% lower recovery value for Cr(III), iron and magnesium 17%, 16% manganese, 11% copper, and zinc with 7%. In the case of anions, chloride ions had no effect on the determination of Cr(III), and the nitrate ion had the same effect, but only if it was added as a potassium salt. If the nitrate anion is added as the sodium salt, the recovery values for Cr(III) are 10 % lower, but in the case of ammonium salt, they are 10% - 20% lower. Investigated anions in the concentration range of 10 mg/L up to 2000 mg/L do not affect the determination of Cr(III). The majority of the investigated cations have no effect on Cr(VI) determination, but recovery values are 10% lower if the cation concentration is higher than: Fe<sup>3+</sup> (> 10 mg/L), Cu<sup>2+</sup> (> 25 mg/L), and Ca<sup>2+</sup> (500 mg/L). Investigated anions in concentration range of 10 mg/L up to 2000 mg/L do not affect the determination of Cr(VI). In subsequent research, the concentration range and number of metals as potential interferers in chromium speciation could be expanded. Also, the potential use of sorbent (silica gel with Nb<sub>2</sub>O<sub>5</sub>) for the speciation of other metals that occur in two or more oxidation states could be investigated.

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### Summary/Sažetak

U ovoj studiji je predstavljen: a) sadržaj Ca, Cd, Co, Cr(III), Cr(VI), Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Zn u otpadnim vodama metalske industrije i rijeka koje teku u blizini metaloprerađivačke industrije; b) mogućnost kvantitativnog određivanja Cr(III) i Cr(VI) u prisutnosti sljedećih kationa: Fe<sup>3+</sup>, Cu<sup>2+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Ni<sup>2+</sup>, Mn<sup>2+</sup>; i Zn<sup>2+</sup> i anioni Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup> i SO<sub>4</sub><sup>2-</sup>; c) specijacija Cr(III) i Cr(VI) na silikagelu modificiranim sa Nb<sub>2</sub>O<sub>5</sub>. Specijacijska analiza Cr(III) i Cr(VI) provedena je na silikagelu modificiranim sa Nb<sub>2</sub>O<sub>5</sub>. Metali su određeni atomskom apsorpcionom spektrometrijom - plamena tehnika, osim Na i K koji su određeni atomskom emisijskom spektrometrijom, i Cr(VI) ultraljubičastom-vidljivom spektrofotometrijom. Kada je koncentracija kationa veća od: Mg<sup>2+</sup> (>25 mg/L), Ni<sup>2+</sup> (>100 mg/L), Fe<sup>3+</sup> (>250 mg/L), Zn<sup>2+</sup> (>500 mg/L) i Mn<sup>2+</sup> (>1000 mg/L), dobiva se pogreška veća od 10% u određivanju koncentraciji Cr(III), Cr(VI). Silikagel modificiran s Nb<sub>2</sub>O<sub>5</sub> može se smatrati efikasnim adsorbensom za ekstrakciju specija hroma na čvrstoj fazi i istovremeno koncentriranje Cr(III). Predložena metoda uspješno je primijenjena na specijaciju i određivanje Cr(III) i Cr(VI) u analiziranim uzorcima. Sadržaj skoro svih određivanih metala je bio ispod dozvoljenih vrijednosti za otpadne vode.



Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 2. 2. 2023. godine, donijela je

## ODLUKU O IMENOVANJU ČLANOVA

### Nadzornog Odbora

### Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

#### Član 1.

U Nadzorni odbor udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“ imenuju se:

1. Nevzeta Ljubijankić, predsjednik
2. Amira Čopra-Janićijević, član
3. Lejla Klepo, član

#### Član 2.

Izabrani članovi Nadzornog odbora Udruženja su postali kandidati sa najvećim brojem glasova. Mandat članova Nadzornog odbora traje 2 godine.

#### Član 3.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-02-03/23  
2. 2. 2023. godine  
Sarajevo

Predsjednik Skupštine

Dragan Krešić

Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 2. 2. 2023. godine, donijela je

## ODLUKU O IMENOVANJU ČLANOVA

### Skupštine Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

#### Član 1.

U Skupštinu udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“ imenuju se :

1. Dragan Krešić, predsjednik
2. Šaćira Mandal, potpredsjednik
3. Fehim Korać, glavni urednik društvenih glasila

#### Član 2.

Izabrani članovi Skupštine Udruženja su postali kandidati sa najvećim brojem glasova. Mandat članova Skupštine traje 2 godine.

#### Član 3.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-02-01/23  
2. 2. 2023. godine  
Sarajevo

Predsjednik Skupštine

Dragan Krešić

Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 2. 2. 2023. godine, donijela je

## ODLUKU O IMENOVANJU ČLANOVA

### Suda časti

### Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

#### Član 1.

U Sud časti udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“ imenuju se:

1. Danijela Vidic, član
2. Ismet Tahirović, član
3. Sabina Gojak-Salimović, član

#### Član 2.

Izabrani članovi Suda časti su postali kandidati sa najvećim brojem glasova. Mandat članova Suda časti traje 2 godine.

#### Član 3.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-02-04/23  
2. 2. 2023. godine  
Sarajevo

Predsjednik Skupštine

Dragan Krešić

Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 2. 2. 2023. godine, donijela je

**ODLUKU O IMENOVANJU PREDSJEDNIKA I ČLANOVA**  
**Upravnog Odbora**  
**Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo**

**Član 1.**

U Upravni odbor udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“ imenuju se :

1. Jelena Ostojić, predsjednik
2. Selma Burović, potpredsjednik
3. Selma Korać, sekretar
4. Sabina Begić, blagajnik
5. Safija Herenda, urednik glasila
6. Dinaida Tahirović, član
7. Anes Krečo, član

**Član 2.**

Izabrani članovi Upravnog odbora Udruženja su postali kandidati sa najvećim brojem glasova. Mandat članova Upravnog odbora traje 2 godine.

**Član 3.**

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-02-02/23  
2. 2. 2023. godine  
Sarajevo

Predsjednik Skupštine

Dragan Krešić

Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 2. 2. 2023. godine, donijela je

## ODLUKU O RAZRJEŠENJU I IMENOVANJU PREDSJEDNIKA I POTPREDSJEDNIKA

Skupštine Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

### Član 1.

Razrješavaju se dužnosti predsjednik i potpredsjednik Skupštine udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“:

1. Šaćira Mandal, predsjednik
2. Edhem Mulaosmanović, potpredsjednik

### Član 2.

Imenuju se predsjednik i potpredsjednik Skupštine udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“:

1. Dragan Krešić, predsjednik
2. Šaćira Mandal, potpredsjednik

### Član 3.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-02-06/23  
2. 2. 2023. godine  
Sarajevo

Predsjednik Skupštine

Dragan Krešić

Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 2. 2. 2023. godine, donijela je

## ODLUKU O RAZRJEŠENJU I IMENOVANJU PREDSJEDNIKA Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

### Član 1.

Razrješava se dužnosti predsjednik udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“:

Lejla Klepo, predsjednik Društva

### Član 2.

Imenuje se predsjednik udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“:

Jelena Ostojić, predsjednik Društva

### Član 3.

Izabrani predsjednik Udruženja je izabran sa najvećim brojem glasova. Mandat predsjednika udruženja traje 2 godine.

Broj: P-02-05/23  
2. 2. 2023. godine  
Sarajevo

Predsjednik Skupštine

Dragan Krešić

Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 2. 2. 2023. godine, donijela je

## ODLUKU O RAZRJEŠENJU ČLANOVA

### Nadzornog Odbora

### Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

#### Član 1.

Razrješavaju se dužnosti članovi Nadzornog odbora udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“ :

1. Nevzeta Ljubijankić, predsjednik
2. Mustafa Memić, član
3. Nermina Korać, član

#### Član 2.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-01-3/23

2. 2. 2023. godine

Sarajevo

Predsjednik Skupštine

Dragan Krešić

Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 2. 2. 2023. godine, donijela je

## ODLUKU O RAZRJEŠENJU PREDSJEDNIKA, POTPREDSJEDNIKA I GLAVNOG UREDNIKA DRUŠTVENIH GLASILA

Skupštine Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

### Član 1.

Razrješavaju se dužnosti predsjednik, potpredsjednik i glavni urednik društvenih glasila Skupštine udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“:

1. Šaćira Mandal, predsjednik
2. Edhem Mulaosmanović, potpredsjednik
3. Fehim Korać, glavni urednik društvenih glasila

### Član 2.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-01-1/23

2. 2. 2023. godine

Sarajevo

Predsjednik Skupštine

Dragan Krešić

Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 2. 2. 2023. godine, donijela je

## ODLUKU O RAZRJEŠENJU ČLANOVA

Suda časti

Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

### Član 1.

Razrješavaju se dužnosti članovi Suda časti udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“:

1. Nurudin Avdić, član
2. Milka Maksimović, član
3. Meliha Zejnilagić-Hajrić, član

### Član 2.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-01-4/23

2. 2. 2023. godine

Sarajevo

Predsjednik Skupštine

Dragan Krešić

Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 2. 2. 2023. godine, donijela je

**ODLUKU O RAZRJEŠENJU PREDSJEDNIKA I ČLANOVA**  
**Upravnog Odbora**  
**Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo**

Član 1.

Razrješavaju se dužnosti članovi Upravnog odbora udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“:

1. Lejla Klepo, predsjednik
2. Dragan Krešić, potpredsjednik
3. Almir Olovčić, sekretar
4. Sabina Begić, blagajnik
5. Safija Herenda, urednik glasila
6. Mirel Subašić, član
7. Anes Krečo, član

Član 2.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-01-2/23  
2. 2. 2023. godine  
Sarajevo

Predsjednik Skupštine

Dragan Krešić

Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 2. 2. 2023. godine, donijela je:

## ODLUKU

o razrješenju i imenovanju lica za zastupanje i predstavljanje

### Član 1.

Razrješavaju se dužnosti lica za zastupanje i predstavljanje udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“:

1. Lejla Klepo, predsjednik Društva
2. Almir Olovčić, član Upravnog odbora
3. Sabina Begić, član Upravnog odbora

### Član 2.

Imenuju se lica za zastupanje i predstavljanje udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“:

1. Jelena Ostojić, predsjednik Društva
2. Selma Korać, član Upravnog odbora
3. Sabina Begić, član Upravnog odbora

### Član 3.

Izabrana lica za zastupanje i predstavljanje su po članu 7. Statuta Udruženja DKTKS predsjednik Društva i dva odabrana člana Upravnog odbora. Mandat lica za zastupanje i predstavljanje traje 2 godine.

### Član 4.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-03/23

2. 2. 2023. godine

Sarajevo

Predsjednik Skupštine

Dragan Krešić



## INSTRUCTIONS FOR AUTHORS

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1. on a topic outside the scope of the Journal;
  2. lacking technical merit;
  3. of insufficient novelty for a wide international readership;
  4. fragmentary and providing marginally incremental results; or
  5. is poorly written.
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6. the names, full affiliation (department, institution, city and country), and
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Tables and figures and/or schemes should not be embedded in the manuscript but their position in the text indicated. In electronic version (Word.doc document) tables and figures and/or schemes should follow the text, each on a separate page. Please number all pages of the manuscript including separate lists of references, tables and figures with their captions.

IUPAC and International Union of Biochemistry and Molecular Biology recommendations for the naming of compounds should be followed.

SI units, or other permissible units, should be employed. The designation of physical quantities should be in Times New Roman font. In text, graphs, and tables, brackets should be used to separate the designation of a physical quantity from the unit. Please do not use the axes of graphs for additional explanations; these should be mentioned in the figure captions and/or the manuscript (example: "pressure at the inlet of the system, kPa" should be avoided).

*Percents* and *per mills*, although not being units in the same sense as the units of dimensioned quantities, can be treated as such. Unit symbols should never be modified (for instance: w/w %, vol.%, mol.% ) but the quantity measured has to be named, *e.g.* mass fraction,  $w=95\%$ ; amount (mole) fraction,  $x=20\%$ .

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The manuscript must contain, each on a separate page, the title page, abstract in English, (abstract in Bosnian/Croatian/Serbian), graphical abstract (optional), main text, list of references, tables (each table separately), illustrations (each separately), and legends to illustrations (all on the same page).

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Warren, J. J., Tronic, T. A., Mayer, J. M. (2010). Thermochemistry of proton-coupled electron transfer reagents and its implications. *Chemical Reviews*, 110 (12), 6961-7001.

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Corey, E. J., Kurti, L. (2010). *Enantioselective chemical synthesis*. (1<sup>st</sup> Ed.) Direct Book Publishing, LLC.

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Moody, J. R., Beck II, C. M. (1997). Sample preparation in analytical chemistry. In Settle, F. A. (Ed.), *Handbook of instrumental techniques for analytical chemistry*. (p.p. 55-72). Prentice Hall.

d) Reference to a proceeding:

Seliskar, C. J., Heineman, W.R., Shi, Y., Slaterbeck, A.F., Aryal, S., Ridgway, T.H., Nevin, J.H. (1997). *New spectroelectrochemical sensor*, in Proceedings of 37<sup>th</sup> Conference of Analytical Chemistry in Energy and Technology, Gatlinburg, Tennessee, USA, p.p. 8-11.

e) Patents:

Healey, P.J., Wright, S.M., Viltro, L.J., (2004). *Method and apparatus for the selection of oral care chemistry*, The Procter & Gamble Company Intellectual Property Division, (No.US 2004/0018475 A1).

f) Chemical Abstracts:

Habeger, C. F., Linhart, R. V., Adair, J. H. (1995). Adhesion to model surfaces in a flow through system. *Chemical Abstracts*, CA 124:25135.

g) Standards:

ISO 4790:1992. (2008). *Glass-to-glass sealings - Determination of stresses*.

h) Websites:

Chemical Abstract Service, [www.cas.org](http://www.cas.org), (18/12/2010).

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### Reporting analytical and spectral data

The following is the recommended style for analytical and spectral data presentation:

1. **Melting and boiling points:**

mp 163–165°C (lit. 166°C)

mp 180°C dec.

bp 98°C

Abbreviations: mp, melting point; bp, boiling point; lit., literature value; dec, decomposition.

2. **Specific Rotation:**

$[\alpha]_{23}^D -222$  (*c* 0.35, MeOH).

Abbreviations:  $\alpha$ , specific rotation; D, the sodium D line or wavelength of light used for determination; the superscript number, temperature (°C) at which the determination was made; In parentheses: *c* stands for concentration; the number following *c* is the concentration in grams per 100 mL; followed by the solvent name or formula.

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**3. NMR Spectroscopy:**

<sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 0.85 (s, 3H, CH<sub>3</sub>), 1.28–1.65 (m, 8H, 4'CH<sub>2</sub>), 4.36–4.55 (m, 2H, H-1 and H-2), 7.41 (d, *J* 8.2 Hz, 1H, ArH), 7.76 (dd, *J* 6.0, 8.2 Hz, 1H, H-1'), 8.09 (br s, 1H, NH).

<sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 12.0, 14.4, 23.7, 26.0, 30.2, 32.5, 40.6 (C-3), 47.4 (C-2'), 79.9, 82.1, 120.0 (C-7), 123.7 (C-5), 126.2 (C-4).

Abbreviations: δ, chemical shift in parts per million (ppm) downfield from the standard; *J*, coupling constant in hertz; multiplicities s, singlet; d, doublet; t, triplet; q, quartet; and br, broadened. Detailed peak assignments should not be made unless these are supported by definitive experiments such as isotopic labelling, DEPT, or two-dimensional NMR experiments.

**4. IR Spectroscopy:**

IR (KBr) ν 3236, 2957, 2924, 1666, 1528, 1348, 1097, 743 cm<sup>-1</sup>.

Abbreviation: ν, wavenumber of maximum absorption peaks in reciprocal centimetres.

**5. Mass Spectrometry:**

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

HRMS–FAB (*m/z*): [M+H]<sup>+</sup>calcd for C<sub>21</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub>, 442.2791; found, 442.2782.

Abbreviations: *m/z*, mass-to-charge ratio; M, molecular weight of the molecule itself; M<sup>+</sup>, molecular ion; HRMS, high-resolution mass spectrometry; FAB, fast atom bombardment.

**6. UV-Visible Spectroscopy:**

UV (CH<sub>3</sub>OH) *I*<sub>max</sub> (log *e*) 220 (3.10), 425 nm (3.26).

Abbreviations: *I*<sub>max</sub>, wavelength of maximum absorption in nanometres; *e*, extinction coefficient.

**7. Quantitative analysis:**

Anal.calcd for C<sub>17</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub>: C 67.08, H 7.95, N 9.20. Found: C 66.82, H 7.83, N 9.16. All values are given in percentages.

**8. Enzymes and catalytic proteins relevant data:**

Papers reporting enzymes and catalytic proteins relevant data should include the identity of the enzymes/proteins, preparation and criteria of purity, assay conditions, methodology, activity, and any other information relevant to judging the reproducibility of the results<sup>1</sup>. For more details check Beilstein Institut/STREND A (standards for reporting enzymology data) commission Web site (<http://www.strenda.org/documents.html>).

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- All references mentioned in the Reference list are cited in the text, and *vice versa*.

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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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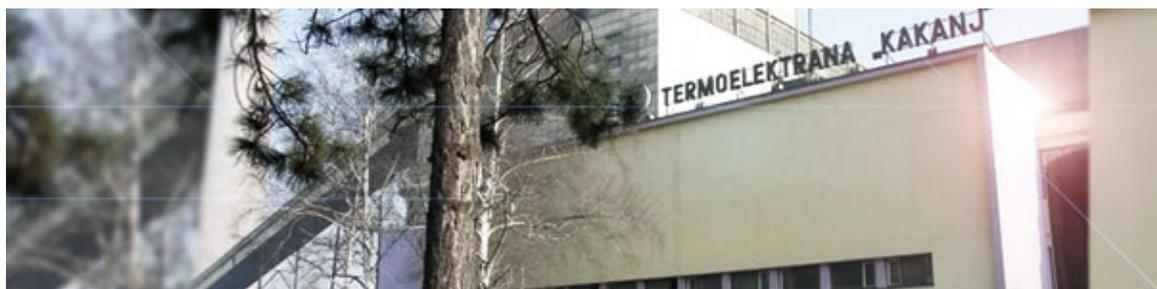


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