Glasnik hemičara i tehnologa Bosne i Hercegovine

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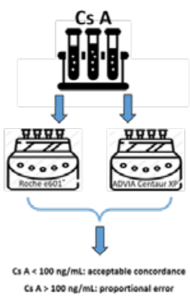

Editorial

General public views and opinions on the transition towards the clean, carbon-neutral future are often reduced to the false dilemma between hydrogen and batteries, which arises from the oversimplified and narrow view of their current and future uses for the decarbonisation of road transport. In such debates, the comparison of batteries and hydrogen is based on several physical quantities (energy density, power, rate of charge), energy conversion efficiency, and loose environmentally concerned arguments. While the first two sets of arguments may seem sufficient for comparing hydrogen and batteries directly applied in vehicles, they fall short when highlighting the importance of investing in research, development, and production of these systems.

Hydrogen plays a crucial role in various industries (refining, ammonia production, methanol, and other chemicals), and has a perspective to become a key factor in several other areas, like the steel industry and heating (by combustion of clean hydrogen or blends with natural gas). Almost all of the 8.7 Mt of hydrogen consumed by the European Union in 2020 was produced from fossil reserves, leading to significant CO_2 output and water withdrawal and consumption, which points to the conclusion that decarbonization of different sectors means decarbonization of hydrogen production. Water electrolysis, in which the energy is supplied from the surplus of the energy produced from renewable resources, is the ultimate method to produce carbon-neutral hydrogen. According to the 2022 Clean Hydrogen Monitor, the current water electrolyzer production capacity in Europe is 3.3 GW per year, out of which, 60 % is based on the alkaline and 40 % on the PEM technology. The following years will witness a significant increase in the production capacity, which will consequently increase the demand for personnel qualified in chemistry, chemical engineering, chemical technology, and materials, needed for research, development, production, operation, and maintenance of these systems. The same profiles are also essential for research and development, production, operation and maintenance of hydrogen utilization systems (fuel cells), as well as batteries and other chemical systems for energy storage and conversion. These projections emphasize the importance of investment, not only for the development of new technologies and additional production capacities but also for the investment in the education of required personnel.

Editors

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Phytochemical Analysis of Eight *Genista* L. taxa (Fabaceae) from Natural Populations in Bosnia and Herzegovina

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Abstract: Phytochemical analysis of aerial parts of eight autochthonous *Genista* L. taxa (brooms; Fabaceae; *G. germanica*, *G. januensis*, *G. pilosa*, *G. radiata*, *G. sagittalis*, *G. sericea*, *G. sylvestris* ssp. *dalmatica* and *G. tinctoria*) from natural populations in Bosnia and Herzegovina was performed in this study. Using fast phytochemical methods, for the first time, emodin was identified in the genus; coumarins, fatty acids, saponins, steroids, tannins and terpenoids in some taxa, but also the presence of phenolic compounds or the absence of anthocyanins in all studied taxa. The analysis of total phenol (TPC), flavonoid (TFC), phenolic acids (TPA) and alkaloid (TA) contents and antioxidant activity (DPPH), determined by spectrophotometry method, indicated the existence of differences between the studied taxa ($p<0.01$). The taxa differed significantly from each other in TPA and TA, and the least in terms of antioxidant activity. There is a positive correlation between TPA, TPC and TFC in one hand, and TA and antioxidative activity in other ($p<0.01$). The Euclidean dendrogram indicates two main clusters: the first cluster includes *G. januensis* and *G. pilosa*, and the second is derived from the remaining six taxa. Obtained PCA clusters were more diffused than those generated by Euclidean distance dendrogram but in a good agreement with them. The obtained data indicate the need for further phytochemical and pharmacological research of the genus *Genista*, as a very interesting source of natural active compounds, as well as population research with special emphasis on the influence of microclimate on SMs content.

INTRODUCTION

Plants produce different groups of secondary metabolites (SMs) that play important roles in stabilizing cellular structures, plant adaptation, and defence reactions to biotic and abiotic stresses. They are low molecular weight compounds that are chemically very diverse and complex, with different pathways of biosynthesis as well as biological and pharmaceutical activity. Secondary metabolites are distributed differently in the plant kingdom, with diverse and important functions (protection from herbivores, insects, pathogens, UV radiation, or stress; attract pollinators and seeds-spreading animals; mediate in plant-plant competition; and exhibit antioxidative properties). They vary in quality and quantity for certain plant species that grow in different ecological conditions (He, He, Farrar,*et al*., 2017; Santos-Sánchez, Salas-Coronado, Villanueva-Cañongo,*et al*., 2019).
The genus *Genista* L. (brooms; Fabaceae) includes about 140 taxa of shrubs and herbs which can slow down/prevent soil erosion, and with three nitrogen-fixing species (Lewis, Schrire, Mackinder, *et al*., 2005; Andrews and Andrews, 2017). The genus shows a very large variety of SMs, especially flavonoids, isoflavonoids, and alkaloids with various biological activities. Brooms have been of interest to human study since ancient times and were used in folk medicine (Kerkatou, Menad, Sarri, *et al*., 2013; Wink, 2013; Grafakou, Barda, Tomou, *et al*., 2021).

The aims of this study were to: 1) do a fast phytochemical screening for the presence of nine groups of SMs (anthocyanins, coumarins, emodins, fatty acids, phenols, saponins, steroids, tannins, and terpenoids) in aqueous extracts, 2) quantify total phenolic, flavonoid, phenolic acids and alkaloid contents in methanol extracts, and 3) evaluate antioxidant activities in methanol extracts of different aerial parts of analyzed eight autochthonous *Genista* taxa from natural populations in Bosnia and Herzegovina.

MATERIAL AND METHODS

Site characteristics, plant material and authentication

In the area of the inner and outer Dinaric Alps, there are eight localities where plant material was collected in natural populations in Bosnia and Herzegovina during 2019-2021. (Table 1). Determination of taxa was done according to Flora Europaea (Gibbs, 1992) and Index Florae Bosna et Hercegovinae (Mišić and Šoljan, 2014). Vouchers are deposited in Herbarium of Faculty of Forestry University of Sarajevo.

Table 1: Origin of analyzed *Genista* taxa with data about site geological substrate, rhizosphere soil types and their physicochemical characteristics (Soil Map of B&H, 2013).

Taxon	Latitude	Longitude	Altitude (m)	Exposure	Substrate	Soil type
G. germanica	43°55'52.97" N	17°46'21.35" E	1774	SE	Shale	Dystric Leptosol, Dystric Cambisol
G. januensis	43°15'30.25" N	18°20'59.21" E	937	S-SE	Limestone	Lithic Leptosol
G. pilosa	44°08'47.26" N	17°34'51.24" E	876	S	Shale	Eutric Leptosol
G. radiata	44°21'11.29" N	17°30'46.31" E	1132	SW	Conglomerate	Rendzic Leptosol
G. sagittalis	43°32'07.05" N	18°35'14.53" E	650		Limestone	Lithic Leptosol, Rendzic Leptosol
G. sericea	42°48'19.05" N	18°24'31.32" E	571	E	Limestone	Rendzic Leptosol
G. sylvestris* ssp. *dalmatica	43°50'10.56" N	17°00'10.19" E	935	S	Limestone	Rendzic Leptosol, Cambisol
G. tinctoria	43°47'54.33" N	18°05'22.58" E	661	SW	Philite	Dystric Leptosol, Dystric Cambisol

The stems and inflorescences were immediately separated and stored in paper bags in the field. Collected materials were then rinsed with running tap water and dried at room temperature (20°C), in dark and well-ventilated area, for seven days. The dried samples were then grinded into powder, and stored in glass vials, at room temperature, until extraction.

Soil samples

Soil samples (individual plant rhizosphere) were cleaned, air-dried and sieved through a 1mm sieve. The texture, gravel content (%), drainage and water holding capacity (WHC) were determined during field studies. The soil organic carbon (SOC; ISO14235), carbonate content ($CaCO_3$; ISO10693), and pH values in water and 1M KCl reagent solute (ISO10390) were determined during laboratory studies.

Chemicals and reagents

Atropine, caffeic acid, gallic acid, rutin, and DPPH (1,1-diphenyl-2-picryl-hydrazyl) are of HPLC purity. All other used chemicals and reagents were of analytical grade (Sigma-Aldrich, Deinheim, Germany).

Fast phytochemical screening

After homogenization of the crushed plant material with 50 mL of sterile distilled water, the solutions were transferred to a water bath (temperature 50° C, incubation 30 min). The contents were then filtered through Whatman No.1 filter papers, and the filtrates were centrifuged for 15 min at 2,500 rpm. The isolated supernatants were immediately used for phytochemical screening for the presence of SMs.

Qualitative phytochemical analysis of aqueous extracts of aerial parts of studied *Genista* taxa were performed to determine the presence of:

Anthocyanins: In 2 mL of aqueous extract 2 mL of 2N hydrochloric acid and 2 mL of ammonia was added. The presence of anthocyanins is proven by discoloration of red-pink colour in the blue-violet (Paris and Moyse, 1969).

Coumarins: The volume of 3 mL of 10% sodium hydroxide was add to 2 mL of the aqueous extract. The presence of coumarins is proven by the appearance of a yellow color (Rizk, 1982).

Emodin: Mix 2 mL of ammonium hydroxide and 3 mL of benzene was mixed with 2 mL of the aqueous extract. The appearance of red color indicates the presence of emodin (Rizk, 1982).

Fatty acids: The volume of 5 mL of ether was added to 0.5 mL of the aqueous extract. The solution was poured on filter paper and dried. The presence of fatty acids is indicated by the transparency of the filter paper (Savithramma, Linga Rao, and Ankanna, 2012).

Phenolics: The volume of 2 mL of 2% iron (III) chloride solution was mixed with2 mL of aqueous extract. The presence of phenols is proven by the appearance of a blue-green or black color (modified Gibbs, 1974).

Saponins: The volume of 5 mL of the aqueous extract was diluted with 20 mL of distilled water. Resulting solution was mixed for 15 minutes. Formation of foam indicates the presence of saponins (Kumar, Ilavarsn, Jayachandran, *et al.*, 2009).

Steroids: The volume of 1 mL of the aqueous extract was added to 10 mL of chloroform, and then 10 mL of concentrated sulphuric acid to the edges of the test tube was added. If the upper layer of the solution (chloroform) turns red, and the lower layer (sulphuric acid) turns yellow with green fluorescence, then the presence of steroids is indicated (Gibbs, 1974). *Tannins*: A few drops of 1% lead acetate were added to 2 mL of the aqueous extract. The presence of tannins is indicated by the appearance of a yellowish precipitate (Trease and Evans, 1983). *Terpenoids*: The volume of 2 mL of chloroform was added to 0.5 ml of aqueous extract. The volume of 3 mL of concentrated H_2SO_4 was added to form a layer. The presence of terpenoids is indicated by the appearance of a reddish-brown color (Ayoola, Coker, Adesegun, *et al*., 2008).

Quantitative determination of phenolic compounds and alkaloids

Per 0.5 g of ground plant material was extracted with 25 mL of 80% methanol in two replicates using an ultrasonic bath (Elma sonic S 60 H) for 30 min, and then were centrifuged at 1,800 rpm for 10 min. The supernatants were stored at +4°C for further analysis.

For determination of polyphenolic and alkaloid contents, and antioxidant activity of all methanol extracts Lambda 25 UV/VIS W/WINLAB V4 Perkin Elmer spectrophotometer was used. All values are presented as means of triplicates.

Total phenolic content (TPC) was determined by the Folin-Ciocalteu method (Luthria, Mukhopadhyay and Krizek, 2006). Absorbance was measured spectrophotometrically at 765 nm against the blank. TPC was expressed as the gallic acid equivalent per gram of dry plant material (mg GAE/g DW).

The total flavonoid content (TFC) was performed by the method of Quettier-Deleu, Gressier, Vasseur, *et al.* (2000). The absorbance of coloured samples was taken at 415 nm against the blank. TFCwas expressed as the rutin equivalent per gram of dry plant material (mg RE/g DW).

Total phenolic acids content (TPA) was determined by Arnow method (Szaufer-Haydrich and Goślińska, 2004). The absorbance at 490 nm against the blank was measured. TPAwas expressed as the caffeic acid equivalent per gram of dry plant material (mg CAE/g DW).

Total alkaloid content (TA) was determined by method of Patel, Patel and Trivedi (2015). Absorbance was measured at 415 nm against blank immediately after collection of the chlorophorm layer. TA content was expressed as the atropine equivalent per gram of dry plant material (mg AE/g DW).

Antioxidant activity

The DPPH free-radical scavenging activity (methanolic solutions of 100, 80, 60, 40, and 20 μL) was estimated by method of Thaipong, Boonprakob, Crosby, *et al.* (2006). The results were expressed as percent inhibition (IC_{50}) calculated from the control, where lower IC_{50} values indicate higher antioxidant activity.

Statistical analysis

The results were expressed as the mean of three replicates ± standard deviation. The data was analyzed using a one-way ANOVA, followed by Duncan's multiple range test, Pearson's correlation coefficient and Euclidean distance (r; IBM SPSS Statistics version 20, IBM Corp., Armonk, NY, USA), considering $p<0.01$ as very significant. The Principal Component Analysis (PCA) was performed in RStudio Team (2020).

RESULTS

Soil

General observations corresponding to all sites are soil shallowness, coarse texture, high percentage of gravel content, high water conductivity, and low water holding capacity in each site (Table 2). Most soil samples had neutral to alkali reaction, except soil sample number 1 which was extremely acidic. **Fast screening for secondary metabolites**

The presence of phenolics, tannins and terpenoids in the aboveground parts of all analyzed taxa was determined by fast screening methods (Table 3). Coumarins were present in aboveground parts of *G. pilosa* and *G. sagittalis*, in the stem of *G. sericea*, and in the inflorescences of *G. radiata*. Emodin was present only in the aboveground parts of *G. pilosa*. Only *G. sericea* had fatty acids in stem and inflorescences, and saponins in the stem. All analyzed taxa, except *G. sericea*, had steroids in aerial parts. On the other hand, presence of anthocyanins was not proven for any of the analyzed *Genista* taxa

Table 2: Soil traits at sampling site. Water Holding Capacity (WHC); Soil Organic Carbon (SOC); carbonate content ($CaCO_3$); pH value in water (pH_{H2O}); pH value in 1M KCl reagent solute (pH_{KCl}).

Taxon	Texture	Gravel (%)	Drainage	WHC	SOC (%)	$CaCO_3$ (g kg^{-1})	pH_{H2O}	pH_{KCl}
G. germanica	Sandy loam	0	Excessive	Weak	9.41	0.00	4.29	3.69
G. januensis	Loamy sand	10-20	Excessive	Weak	2.00	10-20	6.5	7.11
G. pilosa	Sandy loam	50-55	Excessive	Weak	1.09	20-80	7.01	5.92
G. radiata	Sand	0	Strong	Weak	2.43	80-160	7.26	7.00
G. sagittalis	Sandy loam	0	Strong	Weak	3.25	160-200	7.11	6.81
G. sericea	Sandy loam	5-10	Excessive	Weak	2.55	20-40	6.30	6.44
G. sylvestris* ssp. *dalmatica	Loam	70-80	Excessive	Weak	6.64	10-20	7.25	6.45
G. tinctoria	Loamy sand	50-60	Excessive	Weak	1.95	0.00	6.80	6.40

Table 3: Results of phytochemical analysis of secondary metabolites presence by rapid screening methods for analyzed *Genista* taxa.

Taxon	Plant part	Secondary metabolites															
		Coumarins		Emodin		Fatty acids		Phenols		Saponins		Steroids		Tannins		Terpenoids	
		OD	LD	OD	LD	OD	LD	OD	LD	OD	LD	OD	LD	OD	LD	OD	LD
G. germanica	S	–	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–
	I	–	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–
G. januensis	S	–	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–
	I	–	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–
G. pilosa	S	+	–	+	–	–	–	+	+	–	–	+	–	+	–	+	–
	I	+	–	+	–	–	–	+	+	–	–	+	–	+	–	+	–
G. radiata	S	–	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–
	I	+	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–
G. sagittalis	S	+	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–
	I	+	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–
G. sericea	S	+	–	–	–	+	–	+	–	+	–	–	–	+	–	+	–
	I	–	–	–	–	+	–	+	–	–	–	–	–	+	–	+	–
G. sylvestris ssp. *dalmatica*	S	–	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–
	I	–	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–
G. tinctoria	S	–	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–
	I	–	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–

Note: S – stem and leaves; I – inflorescence; (–) – negative result; (+) – positive result; OD – our data; LD – literature data.

Table 4: Total phenolic, flavonoid, phenolic acids and alkaloid contents and DPPH scavenging activity in stem and leaves (S) and inflorescences (I) of eight analyzed *Genista* taxa.

Taxa	Sample	Phenols (mg GAE/g DW)	Flavonoids (mg RE/g DW)	Phenolic acids (mg CAE/g DW)	Alkaloids (mg AE/g DW)	IC_{50} (mg/mL)
G. germanica	S	26.128 ± 0.16^{e}	36.265 ± 0.02^{f}	7.220 ± 0.06^{c}	0.419 ± 0.03^{c}	0.223 ± 0.04^{a}
	I	$16.621 \pm 0.12^{a,b}$	13.118 ± 0.07^{b}	4.421 ± 0.08^{a}	0.902 ± 0.02^{f}	0.259 ± 0.04^{a}
G. januensis	S	43.411 ± 0.86^{g}	75.566 ± 2.89^{h}	32.291 ± 0.31^{i}	1.258 ± 0.01^{g}	0.187 ± 0.05^{a}
	I	43.859 ± 1.22^{g}	64.972 ± 2.20^{i}	29.633 ± 0.85^{h}	$0.598 \pm 0.03^{d,e}$	0.198 ± 0.04^{a}
G. pilosa	S	58.027 ± 1.12^{h}	32.498 ± 1.20^{f}	51.031 ± 0.55^{j}	0.195 ± 0.01^{a}	0.174 ± 0.05^{a}
	I	55.030 ± 8.03^{h}	36.547 ± 0.67^{e}	55.388 ± 0.89^{k}	0.208 ± 0.01^{a}	0.164 ± 0.10^{a}
G. radiata	S	$16.938 \pm 0.48^{a,b}$	9.068 ± 0.40^{c}	4.732 ± 0.08^{a}	6.508 ± 0.04^{k}	1.783 ± 0.31^{e}
	I	$26.703 \pm 0.99^{e,f}$	16.111 ± 0.83^{a}	$6.525 \pm 0.12^{b,c}$	1.337 ± 0.04^{h}	1.348 ± 0.08^{d}
G. sagittalis	S	$17.410 \pm 1.05^{a,b}$	19.818 ± 0.20^{d}	7.554 ± 0.11^{c}	0.653 ± 0.01^{e}	0.976 ± 0.32^{c}
	I	$18.468 \pm 0.31^{a,b,c}$	20.879 ± 0.49^{d}	9.648 ± 0.33^{d}	0.228 ± 0.01^{a}	0.597 ± 0.12^{b}
G. sericea	S	$18.409 \pm 0.07^{a,b,c}$	35.989 ± 0.15^{e}	4.184 ± 0.03^{a}	2.345 ± 0.07^{i}	$1.073 \pm 0.01^{c,d}$
	I	14.115 ± 0.18^{a}	32.058 ± 0.19^{f}	5.923 ± 0.12^{b}	2.494 ± 0.03^{j}	0.625 ± 0.01^{b}
G. sylvestris ssp. *dalmatica*	S	$19.637 \pm 0.81^{b,c,d}$	20.086 ± 0.60^{g}	13.155 ± 0.39^{e}	0.559 ± 0.01^{d}	$0.477 \pm 0.15^{a,b}$
	I	31.168 ± 1.02^{f}	41.198 ± 1.36^{d}	16.498 ± 0.24^{f}	0.328 ± 0.01^{b}	$0.347 \pm 0.06^{a,b}$
G. tinctoria	S	$24.240 \pm 0.16^{d,e}$	41.668 ± 2.09^{d}	17.795 ± 1.16^{g}	0.415 ± 0.01^{c}	$0.371 \pm 0.07^{a,b}$
	I	$23.192 \pm 1.12^{c,d,e}$	21.685 ± 0.77^{g}	10.282 ± 0.19^{d}	0.324 ± 0.01^{b}	0.605 ± 0.13^{a}

Note: GAE– Expressed as mg of gallic acid per g of dry plant material; RE– Expressed as mg rutin per g of dry plant material; CAE– Expressed as mg of caffeic acid per g of dry plant material; AE– Expressed as mg of atropine per mL of dry plant material; Values are expressed as means ± standard deviations (n = 3); Means in the same column with different letters in superscript are significantly different at $p<0.01$.

Active compounds: total phenols, flavonoids, phenolic acids, and alkaloids

The TPC, TFC and TA values of analyzed *Genista* taxa are shown in Table 4. Analyzed taxa had relatively high and fairly uniform amounts of TPC, where *G. pilosa* and *G. januensis* had the highest values and *G. radiata* (stem) and *G. sericea* (inflorescences) the lowest. On the contrary, TFC values are very variable and in a wide range. The amount of TPA is relatively low and uniform in most of the analysed taxa, except in *G. pilosa* and *G. januensis*. Based on available literature data, TA spectrophotometric determination was done for the first time in this study. The stem of *G.radiata* had a very high content of TA, followed by *G. sericea* and *G. januensis*. Among the inflorescence extracts, the highest content of TA was recorded in *G. sericea* and *G. radiata*, while *G. pilosa* had the lowest amount of alkaloids in both stem and inflorescence.

DPPH free radical scavenging

Genista pilosa and *G. januensis* showed the highest antioxidant activity (Table 4), while *G. radiata*, *G. sericea* and *G. sagittalis* had the lowest antioxidant activity.

Multivariate analysis of phytochemical characteristics of *Genista* taxa

ANOVA showed that all the studied characters indicate the presence of mutual differences ($p<0.01$; Table 4). The results of the Duncan's test confirmed statistically significant differences in individual comparisons of the studied taxa. The largest number of significant interspecies differences is related to testing of TPA and TA, while the smallest number of interspecies differences was found in the antioxidant activity. Also, significant differences were observed in the analysed samples of stem/leaves and inflorescences in the individuals of the same taxon. Further data analysis showed a positive relationship between TPA, TPC and TFC, and TA and antioxidative activity at $p<0.01$ level. The Euclidean distance dendrogram showed two main clusters, where the first cluster includes *G. januensis* and *G. pilosa*, and the second is formed from the remaining six taxa (Figure 1). Taxa within the second cluster are relatively close although they are grouped into three subclusters.

According to principal component analysis (PCA), the first two principal components accounted for 82.90% of total variability between taxa (Figure2). The PC1 correlated with TPC, TPA and TFC, while PC2 correlated with TA and antioxidant activity. Obtained PCA clusters were more diffused than those generated by Euclidean distance dendrogram but in a good agreement with them.

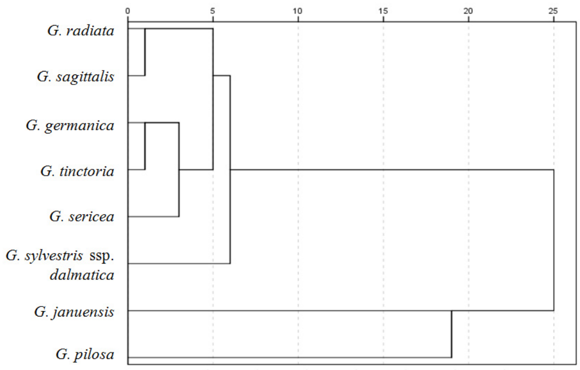

Figure 1. Dendrogram resulting from a cluster analysis selecting the Euclidean distance as similarity measurement of means of total phenol, flavonoid, phenolic acids and alkaloid contents, and antioxidant activity of studied *Genista* taxa.

DISCUSSION

Fabaceae species possess a distinctive and specific chemical composition characterized by variable bioactive phenolic compounds, especially flavonoids. Namely, certain genera or species are rich in different types of SMs whose concentrations vary significantly in different plant parts, seasonally or in relation to environmental conditions, especially from sun's radiation and temperature (Wink, 2013; Tsypysheva, Petrova, Baykova, *et al.*, 2014).

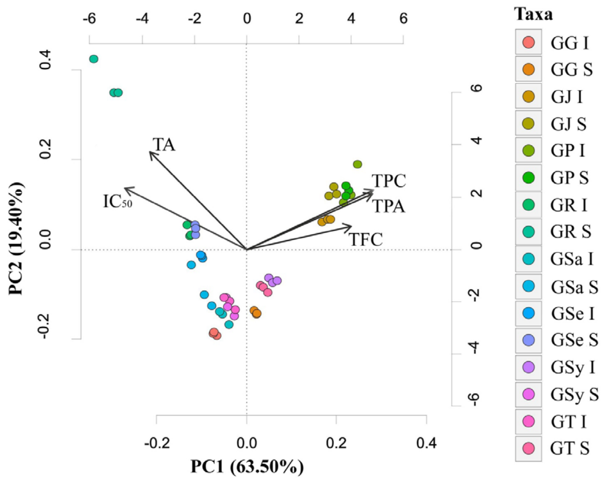

Figure 2. Principal component analysis (PCA) of the different phytochemicals of *Genista* taxa methanolic extracts. Principal component 1 (PC1) = 63.50% and component 2 (PC2) = 19.40%. I – inflorescences; S – stem and leaves; GG – *G. germanica*; GJ – *G. januensis*; GP – *G. pilosa*; GR – *G. radiata*; GSa – *G. sagittalis*; GSe – *G. sericea*; GSy – *G. sylvestris* ssp. *dalmatica*; GT – *G. tinctoria*.

Preliminary qualitative tests for SMs in eight *Genista* taxa showed the presence of steroids in all taxa (except for *G. sericea*), tannins and terpenoids for the first time, while the occurrence of phenolic compounds was consistent with published data. For some of the analysed taxa, the presence of coumarins, emodin, fatty acids and saponins was recorded for the first time. These results indicate the potential use of the investigated *Genista* taxa as a source of bioactive compounds that have not yet been sufficiently investigated. Despite its significant roles in protecting plants from many biotic and abiotic stressors, preliminary qualitative tests have shown that there are no anthocyanins in the aboveground parts of all analysed taxa. According to the literature, only Lrhorfi, Dahmani, Elyahyoui, *et al.* (2016) reported that *G. quadriflora* possess the anthocyanins and leukoanthocyanidins.

Coumarins and flavonoids are known to have a number of beneficial effects on human health, which are related to antioxidant activity as previously demonstrated (Lrhorfi, Dahmani, Elyahyoui, *et al.*, 2016). In this work, coumarins were detected in the stem of *G. sericea*, inflorescences of *G. radiata* while *G. pilosa* and *G. sagittalis* had coumarins in all aboveground parts. Emodin was found only in *G. pilosa* while fatty acids in *G. sericea*. Previous results showed the presence of fatty acids in essential oils of *G. numidica* and *G. saharae* (Lograda, Chaker, Chalard, *et al.*, 2009).

Phenols were present in the aboveground parts of all analyzed taxa with a content that was relatively high and fairly uniform. In the genus *Genista* the presence of phenols has already been proven (Boukaabache, Boumaza, Mekkiou, *et al.*, 2015; Guetaff, Abidli, Kariche, *et al.*, 2016; Hanganu, Olah, Benedec, *et al.*, 2016; Lrhorfi, Dahmani, Elyahyoui, *et al.*, 2016; Ati, Salima and Warda, 2017; Barek, Rahmoun, Aissaoui, *et al.*, 2020) and their content varies depending, among other things, on solvents used, which may indicate the polyphenolic composition of the extracts (Zhang, Cai and Cheng, ~~*et al.*,~~ 2022). Saponins and steroids were detected only in *G. sericea* but they were also detected in aboveground parts of some other *Genista* taxa (Boutaghane, Voutquenne-Nazabadioko, Harakat, *et al*., 2013; Boukaabache, Boumaza, Mekkiou, *et al*., 2015; Guetaff, Abidli, Kariche, *et al.*, 2016). In the aboveground parts of all investigated taxa, except in *G. sericea*, the presence of steroids was confirmed for the first time in this study. Tannins, which provide protection of plants from insects, pests and herbivores, were previously detected in several *Genista* species. Tannins and some phenolic compounds are usually associated with antioxidant activity (Guetaff, Abidli, Kariche, *et al.*, 2016; Lrhorfi, Dahmani, Elyahyoui, *et al.*, 2016; Ati, Salima and Warda, 2017). In this study, for the first time, the presence of tannins in the aboveground parts of all analyzed *Genista* taxa was confirmed. Terpenoids play very important roles in all life processes (Adamski, Blythe, Milella, *et al.*, 2020), and have previously been identified only in other four *Genista* taxa (Lograda, Chaker, Chalard, *et al*., 2009; Boukaabache, Boumaza, Mekkiou, *et al.*, 2015; Guetaff, Abidli, Kariche, *et al.*, 2016; Lrhorfi, Dahmani, Elyahyoui, *et al.*, 2016). Terpenoids were noticed in the aboveground parts in all investigated *Genista* taxa for the first time in this study.

Alkaloids have different biological activities, but in plants they act as allelopathic compounds, and have a defensive role against herbivores and pathogens (Wink, 2013). In Fabaceae family, quinolizidine alkaloids and some piperidine alkaloids are principal SMs for almost all taxa of the Genistoid clade (Wink, 2008; Küçükboyaci, Özkan and Tosun, 2012; Wink, 2013). The presence of alkaloids, mainly of quinolizidine alkaloids, was detected in 23 *Genista* taxa, of which only two of the eight taxa were analyzed in this study (*G. tinctoria* and *G. sagittalis*) (Kirch, Veit, Waetzig, *et al.*, 1995; Christov and Evstatieva, 2000; Tero-Vescan, Vari and Vlase, 2014; Küçükboyaci, Özkan and Tosun, 2020). Tsypysheva, Petrova, Baykova, *et al.* (2014, and references therein) found that the alkaloids concentration in *G. tinctoria* was highest in twigs during the flowering period, while Tero-Vescan, Vari and Vlase (2014) found that extracts of *G. tinctoria* and *G. sagittalis* have very low content of alkaloids. Some studies have shown that alkaloid compositions in *Genista* were similar although very different alkaloid values were determined (Küçükboyaci, Özkan and Tosun, 2012; Tero-Vescan, Vari and Vlase, 2014). The TA content varied both among the taxa and between the analyzed plant parts in this study. Most of the analyzed taxa had more-or-less approximate TA concentrations in stem/leaves and inflorescences, except *G. radiata*, *G. sericea* and *G. januensis*. Different studies showed that *Genista* taxa are rich in alkaloids, but data about TA contents we could not find (Wink, 2013; Ati, Salima and Warda, 2017).

Natural antioxidant compounds have a key defensive role against the free radicals in prevention of the antioxidation processes. Due to the complex chemical composition of plant extracts, it is difficult to estimate correlations between their active compounds and antioxidant activities. Many authors are agreed that antioxidant activity depends on the plant species, plant part, concentration, structure, and synergism or antagonism of present antioxidants in the extracts (Zheng and Wang, 2001; Barek, Rahmoun, Aissaoui, *et al.*, 2020). However, most of them agree that different groups of phenolic compounds, especially phenols and flavonoids (Zheng and Wang, 2001; Aoruhaon, Fazouane, Benayache, *et al*., 2019), or their structures (Kaur and Mondal, 2014) are most responsible for the antioxidant activity. Using DPPH free radical scavenging method, we found a fairly high to moderate antioxidant activity in almost all analyzed methanol extracts. Many studies show that the main contributors to antioxidant activity of numerous *Genista* taxa were phenols and/or flavonoids in the extracts (Rauter, Martins, Lopes, *et al.*, 2009; Serrilli, Graziosi, Ballero, *et al*., 2010; Orhan, Tosun, Tamer, *et al.*, 2011; Kerkatou, Menad, Sarri, *et al.*, 2013; Meriane, Genta-Jouve, Kaabeche, *et al*., 2014; Guettaf, Abidli, Kariche, *et al.*, 2016; Hanganu, Olha, Benedec, *et al.*, 2016; Ati, Salima and Warda, 2017; Aourahoun, Fazouane, Benayache, *et al.*, 2019; Barek, Rahmoun, Aissaoui, *et al.*, 2020; Simões, Pinto, Neves, *et al.*, 2020; Wafaand Sofiane, 2021). In contrast, Chebbah, Marchioni, Sarri, *et al.* (2016) found that antioxidant activity is probably due to the presence of phenolic acids, and Bouchouka, Djilani and Bekkouche (2012) reported no correlations between antioxidant activity and TPC and flavonoid contents in extracts of *G. saharae*. Among the investigated taxa, only for *G. sagittalis* and *G. tinctoria* there are data on the antioxidant activity of aboveground parts (Hanganu, Olah, Benedec, *et al.*, 2016), and their values were significantly lower than those recorded in this study. High antioxidant activity of *G. pilosa* and *G. januensis* in this study can be associated with the presence of high concentrations of TPC, TFC and TPA. All these results indicate that antioxidant compounds in higher concentrations contribute and act through multiple mechanisms, directly or indirectly, which requires further chemical and pharmacological investigations.

Based on cluster analysis, it is possible to describe the structure of data and determine natural groups within the data set. Thus, based on the similarity of analyzed total phenolic compounds, total alkaloids and antioxidant activity, the studied *Genista* taxa can be divided into two major groups. The PCA clusters, in comparison with Euclidean distance dendrogram, were more diffused but still in a good agreement with them. According to those analyses, *G. januensis* and *G. pilosa* separated from others based on TPA, TPC and TFC, while other *Genista* taxa are grouped in three subclusters without the possibility of clear mutual discrimination.

CONCLUSIONS

The use of qualitative fast phytochemical screenings of eight *Genista* taxa has proven the presence of new groups of SMs both in the genus and in certain taxa, which represent a potential source of bioactive compounds. Also, the presence of significant differences in the analyzed samples of the studied *Genista* taxa was found for TPA and TA, and the lowest for antioxidant activity. The results obtained in this study are promising, and it would be desirable and very interesting to continue in two directions: 1) phytochemical and pharmacological research of the potential alternative sources of bioactive natural compounds; and 2) population analyzes with special emphasis on correlations of microclimatic conditions and SMs content.

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Summary/Sažetak

U ovom radu urađena je fitohemijska analiza nadzemnih dijelova osam autohtonih svojti roda *Genista* L. (žutilovke; Fabaceae; *G. germanica*, *G. januensis*, *G. pilosa*, *G. radiata*, *G. sagittalis*, *G. sericea*, *G. sylvestris* ssp. *dalmatica* i *G. tinctoria*) iz prirodnih populacija u Bosni i Hercegovini. Kvalitativne fitohemijske metode pokazale su se korisnim jer su neki od spojeva po prvi put identificirani u rodu (emodin) i u svojtama (kumarini, masne kiseline, saponini, steroidi, tanini i terpenoidi), kao i potvrđena prisutnost fenolnih spojeva ili odsutnost antocijanina u svim proučavanim svojtama. Analiza sadržaja ukupnih fenola (TPC), flavonoida (TFC), fenolnih kiselina (TPA) i alkaloida (TA) te antioksidativnog djelovanja (DPPH), određena spektrofotometrijskim očitanjem metanolnih ekstrakata, pokazala je da postoje razlike između proučavanih svojti ($p<0,01$). Svojte su se međusobno značajno razlikovale u TPA i TA, a najmanje po antioksidativnom djelovanju. Korelacijska analiza provedena u ovoj studiji pokazala je pozitivan odnos između TPA, TPC i TFC s jedne strane te TA i antioksidativne aktivnosti s druge strane ($p<0,01$). Dendrogram Euklidske udaljenosti, na temelju sličnosti hemijskog sastava i antioksidativnog djelovanja analiziranih svojti *Genista*, ukazuje na dva glavna klastera: prvi klaster uključuje *G. januensis* i *G. pilosa*, a drugi je izveden od preostalih šest svojti. Dobiveni klasteri PCA bili su raspršeniji od onih generiranih dendrogramom Euklidske udaljenosti, ali su se dobro podudarali s njima. Dobiveni podaci ukazuju na potrebu daljnjih fitohemijskih i farmakoloških istraživanja roda *Genista*, vrlo zanimljivog izvora prirodnih aktivnih spojeva, kao i populacijskih istraživanja s posebnim naglaskom na uticaj mikroklime na sadržaj sekundarnih metabolita.

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GHG Emissions in the Current and Future MSW Management System in Zvornik, Bosnia and Herzegovina

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Abstract: Each and every step in the process of municipal solid waste (MSW) management generates the greenhouse gases (GHG). Therefore, it is imperative to focus on MSW from the source to the final waste disposal in order to decrease the negative impact on the environment. This study aims to calculate the GHG emissions at the present moment (Status Quo) for waste management as well as on the improved MSW management that should be implemented in this local community by 2027 (Scenario 2027). To visualize waste streams in these two scenarios, the STAN 2.5 software was used, and for the calculation of GHG emissions in the City of Zvornik, the IWM-2 software was used. The MSW management Status Quo is basically characterized by the collection communal of waste and its deposition on the landfill without a degasification system and landfill gas treatment. The guidelines and recommendations for MSW management improvement, Scenario 2027 propose the establishment of separate collections of secondary raw materials and biodegradable waste, and improved collection and treatment of landfill gas at the landfill site. The implementation of these measures would result in a reduction of approximately 40% in GHG emissions compared to the Status Quo. The most significant impact would be realized in the environment due to the collection and treatment of landfill.

INTRODUCTION

Traditionally, the term "waste" has a negative connotation and comprehends something undesirable, discarded by people due to inadequate or inaccurate thinking about the waste (Seadon, 2010).

Waste is any substance or object that the holder discards, intends to discard, or is required to discard; waste management means the collection, transport, recovery, and disposal of waste, including the supervision of such operations and the subsequent care of disposal sites, including actions taken as a dealer or broker (Directive 2008/98/EC). Communal waste is household waste, as well as other waste that is, by nature or due to its content, similar to household waste (Official Gazette of the Republic of Srpska no. 111/13, 106/15, 16/18, 63/21, 65/21). The impact of waste on the environment is mainly achieved by the pollution emitted throughout the entire life cycle of the waste, from the point of waste creation, when a product has no utility, through waste collection, waste treatment processes (recycling, composting, combusting, depositing on the landfill, etc.) (Vergara and Tchobanoglous, 2012).

In 2020, Bosnia and Herzegovina (B&H) generated 1200000t of municipal waste, and the population of B&H produced an average of 354 kg of municipal waste (Agency of Statistics of B&H, 2022).

Waste depositing is still the major method of waste treatment in B&H.

There are several active regional sanitary landfills in B&H: Sarajevo, Banja Luka, Bijeljina, Zenica, Mostar, Zvornik, Živinice (still under construction), and Prijedor (still under construction). However, the number of illegal dumpsites is still high. Also, the country lacks facilities for disposal of special categories of waste, which usually ends up at municipal landfills, threatening human health and the environment. Current recycling rates are far lower than those achieved in other European countries (Ionkova, Doychinov, Silajdzic, *et al*., 2019). A number of illegal dumpsites, low recycling rates, and the number of non-compliant municipal landfills threaten the environment and climate change through pollution and human health in general (Bjelić, Čarapina, Markić, *et al*., 2015).

Human activities and natural systems are the two main sources of greenhouse gases (GHG) (Xi-Liu and Qing-Xian, 2018). The three most important greenhouse gases in terms of abundance and contribution to the greenhouse effect are water vapor (H_2O), carbon dioxide (CO_2), and methane (CH_4) (Berman, Fladeland, Liem, *et al*., 2012). Human activities are the major cause of the increased CO_2 concentration in the atmosphere. In recent decades, two-thirds of the greenhouse effect has been caused by human activities (Songolzadeh, Soleimani, Takht Ravanchi, *et al*., 2014). The total GHG emissions generated by waste management contribute up to 5% of the total GHG emissions into the atmosphere. (Gautam and Agrawal, 2021). The GHG compounds relevant to climate change caused by solid waste management activities include CH_4, CO_2, and N_2O (King and Gutberlet, 2013).

There are currently several dedicated research studies on the GHG emissions caused by MSW management in B&H.

In Daul's (2014) research, three different waste management scenarios in the Federation of B&H were compared using GHG emission calculation. This research concludes that the primary cause of the substantial amount of GHG emissions is directly attributed to waste management. Therefore, it is necessary to supplement existing waste management practices with waste recycling in order to decrease GHG emissions.

Therefore, it is necessary to supplement existing waste management practices with waste recycling in order to reduce greenhouse gas emissions. Bjelic, Markic, Pesic, *et al.* (2017) conducted a study analyzing three scenarios for municipal solid waste (MSW) management in the area of the city of Banja Luka (B&H). They applied the Life Cycle Assessment (LCA) to quantify emissions into the environment. One of the environmental impact categories considered in this study is the Global Warming Potential (GWP). The study revealed that the highest value of GWP is associated with waste disposal on dumpsites or illegal dumping sites, as well as on landfills without the proper collection and treatment of the landfill gas.

A significantly larger number of studies on the GHG emission from waste have been conducted in neighboring countries. For example, in the Republic of Serbia, studies by Mihajlović, Pešić, Jovanović, 2019; Stanisavljević, Ubavin, Batinić, *et al.,* 2012; and Djekic, Miloradovic, Djekic, *et al.,* 2019, were conducted. Similarly, in the Republic of Croatia studies by Anić-Vučinić, Hublin, Ružinski, 2010; Schneider, Kirac, Hublin, 2012; 2013; were carried out.

The research objective of this paper is to calculate the GHG emissions in the current waste management system for the City of Zvornik (B&H), and to predict the GHG emission by proposing improvements to the existing waste management system. This research can serve as a recommendation for improving the management of mixed communal waste. It can serve as an example of other local communities facing similar waste management challenges, aiming to minimize the negative environmental impacts and their consequences on human health and well-being.

MATERIALS AND METHODS

The City of Zvornik covers a territory of 371.95 km^2 and is located in the northeastern part of the Republic of Srpska (RS), B&H. It is considered one of the more developed self-governing local communities within the RS.

According to the last population census conducted 2013, the City of Zvornik had population of 54,407 inhabitants (Republika Srpska Institute of Statistics, 2017). Out of the total population, approximately 11,082 inhabitants, or 20.36% live within the downtown area.

The dominant fraction of municipal waste in Zvornik is organic waste (31.45%), while recyclable raw materials make up about 41.49% of MSW (11.28% paper and cardboard, 11.05% foil, 7.53% glass, 6.93% plastic, and 4.70% metal).

All quantitative indicators, including waste generation, collection, transport, and treatment, rely on a good understanding of waste flows through the entire waste management system. The MSW waste flow monitoring methodology, therefore, includes a detailed analysis of the amount of waste generated as well as further waste processing and treatment flows using the Sankey's material flow diagram, in this case specifically adapted for waste. In this study, waste streams were constructed using STAN2.5 software (Cencic and Rechberger, 2008).

For the GHG calculation, the IWM-2 software was used. As outlined by McDougall, White, Franke, et al. (2008), the IWM-2 software is a life cycle inventory (LCI) tool specifically engineered to aid in the development of sustainable solid waste management systems. IWM-2 is a widely available analytical tool based on life cycle assessment (LCA) intended for waste managers and policy makers in waste management. Within the context of Zvornik, Bosnia and Herzegovina, we applied the IWM-2 software to perform an LCA for both the current and anticipated future waste management systems.

Advanced waste management systems have the potential to substantially reduce gas emissions that contribute to GWP. The environmental impact indicator used in this research measures the effect of waste management in the City of Zvornik on global warming. The scenarios "Status Quo" and "Scenario 2027" were modeled using data from the Local Waste Management Plan for the City of Zvornik for the period 2022–2027 (UNDP, Environmental Protection and Energy Efficiency Fund of the Republic of Srpska and the City of Zvornik, 2022).

The current MSW management in Zvornik – Status Quo

Waste management in the City of Zvornik primarily involves the collection of mixed communal waste into a single container. About 60% of the inhabitants is currently covered by the waste collection service. The collected waste is subsequently transported to the regional landfill "Crni vrh" which was established in 2017. This landfill accepts waste from several municipalities, including Kalesija, Sapna, Šekovići, Osmaci, and Bratunac. The landfill span 69 hectares and has an annual capacity of 40,000 t of MSW, with an expected operational lifespan of 20 years. In 2020, about 8,220 t of MSW was collected from the area of the City of Zvornik. The waste management in Zvornik in 2020 was based on: (1) waste collection; (2) transporting the waste to the landfill; and (3) depositing the waste in the landfill (Figure 1).

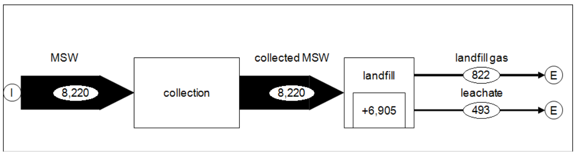

Figure 1. MSW management in Zvornik – Status quo

The waste collection is the collection of waste, including the previous separation and temporary storage of waste, for transport to the waste treatment facility (Official Gazette of the Republic of Srpska no. 111/13, 106/15, 16/18, 63/21, 65/21). The waste collection in the City of Zvornik is done by nine vehicles, seven of which are constantly used for waste collection and transport, and the remaining two are in reserve (in case of malfunction or need to engage due to extraordinary circumstances). The average age of the vehicle fleet for waste collection is 20 years. Among the vehicles used for waste collection and transport, one is equipped with a Euro diesel II engine, another with a Euro diesel V engine, while the remaining five vehicles operate on Euro diesel III engines.

In 2020, approximately 37,300 L of diesel was used for communal waste collection in Zvornik. Given the total amount of waste collected, which stands at 8,220 t, it can be concluded that around 4.54 L of diesel is required to collect 1 t of waste. Of the total diesel consumed, vehicles with Euro II engines used 7,000 L (19%), vehicles with Euro III engines used 20,900 L (56%), and vehicles with Euro V engines used 7,000 L (25%).

Taking into account the capacity of each vehicle involved in the waste collection process in 2020, as well as the number of trips made daily, weekly, and annually, we calculated the maximum possible amount of waste that each vehicle could collect. According to our findings, the maximum waste collection potential was 25,898 t. Considering that approximately 8,220 t of waste was collected in 2020 it can be concluded that the vehicles of this communal company were operating at an average capacity of 32%.

Literature data on diesel consumption for waste collection varies significantly depending on local conditions, ranging from 1.6 to 10.1 L of diesel/t of collected waste. The highest consumption was observed in the seldom populated regions of Denmark (Larsen, Merrild, Christensen, 2009). In urban areas with a high population density, diesel consumption is 3.2 L/t of collected waste, while in areas with a lower population density, the diesel consumption increases to approximately 14.6 L/t of collected waste (Nguyen and Wilson, 2010). Diesel consumption, which accounts for emptying containers and traversing the distance between them, ranges from 3.7 to 4.6 L/t of waste (Eisted, Larsen, Christensen, 2009).

The waste transport is a process that includes loading, transport (as well as reloading), and unloading of waste (Official Gazette of the Republic of Srpska no. 111/13, 106/15, 16/18, 63/21, 65/21). One of the most crucial factors in the transport of communal waste is the density and degree of waste compression, as this can affect the amount of waste collected before unloading. Metal and scraps, which have a high density, are less compressible than materials such as plastic or paper that have lower density (Yaman, Anil, Jaunich, *et al.,* 2019). The vehicles used in the City of Zvornik transport the collected waste to the landfill situated approximately 15 km from downtown. The total annual diesel consumption for this task amounts to 12,200 L. Given that the quantity of waste transported in 2020 was 8,220 t, it implies that transporting of 1t of waste requires 1.48 L of diesel.

Waste deposit refers to any procedure or method that involves the permanent disposal of waste following the D-list, which is a set of regulations that outlines the categorization, testing, and classification of waste (Official Gazette of the Republic of Srpska no. 111/13, 106/15, 16/18, 63/21, 65/21). Waste deposited on the landfill undergoes complex biochemical and physical processes of decomposition, resulting in the production of leachate and landfill gas (Vaverková, Toman, Kotovicová, 2012). If not properly managed, the emission of these substances can pose significant environmental challenges and health risks to humans (Kotovicová, Toman, Vaverková, *et al.,* 2011). Additional issues related to the landfills include the risk of fires and explosions, damage to vegetation, unpleasant odors, surface contamination, and air pollution (Calvo, Moreno, Zamorano, *et al.,* 2005).

Waste deposit remains the most common method of waste treatment worldwide (Aljaradin and Persson, 2012; Ismail and Manaf, 2013).

The decomposition processes of waste, particularly organic waste under anaerobic conditions, generate significant quantities of landfill gases. These gases tipically consist of CH_4 (40-60%), CO_2 (35-50%), N_2 (0-20%), O_2 (0-1%) and H_2S (70-280 mg/m^3), along with trace amounts of compounds such as *n*-aliphatic and aromatic hydrocarbons and halogen compounds (total concentration of 2,000 mg/m^3) (Vaverková, Toman, Kotovicová, 2012). Landfill gas should be collected as much as possible through a degasification system and then either flared or utilized for other purposes. The waste of the City of Zvornik is disposed of at the "Crni vrh" landfill according to all regulations that define waste disposal, but the landfill gas generated there is released directly into the atmosphere without prior treatment.

MSW management in the future in Zvornik–Scenario 2027

Compared to the quantity value of waste in the year 2020 (8,220 t), it is estimated that the quantity of communal waste in 2027 will be increased by about 40%, whereas the quantity of waste in 2027 would be 11,644 t. The increase in waste quantity includes parameters such as an increase in the degree of collected waste, a rise in the Gross domestic product (GDP), and an increase in the generated quantity of waste per inhabitant of the City of Zvornik.

The waste management system in Zvornik should undergo certain improvements up to the year 2027: (1) separate collection of packaging and biodegradable waste, treatment of that waste (including separating, recycling, and composting), and collection and depositing of the remaining waste; (2) improvement of the transportation fleet used for collecting and waste transportation, and (3) construction of a degasification system for treatment of landfill gas, which should include gas collection and flaring.

By the year 2027, the separation of 10% of secondary raw materials for recycling is planned. Out of the generated waste that is projected in the quantity of 11,644 t, 10% of the waste would be separated, apropos 1,164 t of secondary raw material (by primary or secondary recycling). Of this separated quantity, it is estimated that 284 t would be paper, 568 t of plastic,121 t of metal, and 191 t of glass.

The quantity of biodegradable waste expected be collected and composted separately is projected to be around of 987 t. The quantity of waste that would be deposited at the “Crni vrh” landfill, in this case, would be around 9,494 t.

The waste flow in Scenario 2027 is presented in Figure 2.

The proposal for upgrading the waste collection system in Zvornik introduces a “two bins” system. In the first bin/container, all the fractions of so-called “dry” waste are collected, apropos secondary raw material: plastic (PET, plastic foil, plastic bags), paper and cardboard, metal, rubber, and glass. In contrast, the second bin/container is meant for all “wet” fractions of the remaining communal waste.

For the separate collection of secondary raw materials, it is planned to collect packaging waste in a single container. This means citizens would separate paper, cardboard, plastic, metal, and glass into one container. The parameters taken into consideration when estimating the number of these containers/bins include:(1) the density of packaging waste (185 kg/m^3), (2) the degree of fullness of the container (80%), (3) the volume of the container (1.1m^3) and (4) collection once per week. Up to the year 2027, it is necessary to purchase and place 138 containers with a volume of 1.1m^3.

Separate collection of biodegradable waste in Zvornik should be organized for individual households, especially in rural areas, so that each household has one composter at its disposal. It is necessary to obtain, in total, 6,766 wooden composters with a volume of 380 L by2027 (UNDP, Environmental Protection and Energy Efficiency Fund of the Republic of Srpska and the City of Zvornik, 2022).

In the system of waste collection and transport, it is planned to reduce diesel consumption by approximately 18%. This will be achieved by procuring newer trucks that have smaller capacity and use less diesel fuel (UNDP, Environmental Protection and Energy Efficiency Fund of the Republic of Srpska and the City of Zvornik, 2022).

One of the most important upgrades in this landfill management system is the construction of a degasification system located at the newly built sanitary landfill “Crni vrh”. Furthermore, this will be achieved by collecting about 60% of the landfill gas and treating it in a flare.

In the year 2027, the quantity of waste that will be recycled and composted is projected to be 2,150 t, of which approximately 18.46% will be directed to other flows. As a result, the quantity of waste deposited should be reduced by this percentage.

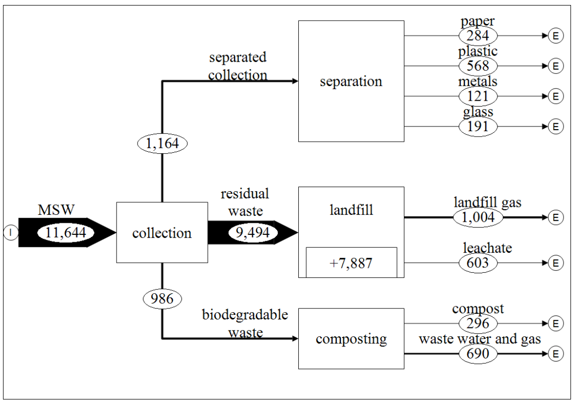

Figure 2. MSW management in Zvornik in Scenario 2027

RESULTS AND DISCUSSION

Status Quo

Based on the data related to the length of the collection route and the number of trips during a day and a week, the calculation of GHG for 2020 has been done (Figure 3).

The total emission of GHG for the collection process in the City of Zvornik amounts to100.52 t $CO_{2\text{-}eq}$/year.

Based on the calculated GHG emissions from all seven vehicles and the quantity of waste collected in 2020, the GHG emissions per ton (t) of collected waste amount to 12.23 kg $CO_{2\text{-}eq}$/t of waste. A study conducted in Denmark in 2009, which focused on the calculation of GHG emissions during the collection of waste from residential blocks, reported a GHG emission range 5.0-5.4 kg of $CO_{2\text{-}eq}$/t of collected waste (Larsen, Vrgoc, Christensen, *et al.,* 2009). The total GHG emissions from transporting vehicles in 2020 amounted to 32.87 t of $CO_{2\text{-}eq}$/year. The average GHG emissions per t of transported waste was calculated to be 4.0 kg $CO_{2\text{-}eq}$/t of waste. When considering both the collection and transportation of waste in Zvornik, the total GHG emissions reached 133.39 t of $CO_{2\text{-}eq}$/year. Recalculating it per t of waste, the GHG emissions amounted to 16.23 kg $CO_{2\text{-}eq}$/t of waste. In many studies, the GHG emissions generated by collecting and transporting waste vary from 7.7 kg of $CO_{2\text{-}eq}$/t of waste (Korkut, Yaman, Küçükağa, *et al.,* 2018), to 9.3-9.9 kg $CO_{2\text{-}eq}$/t of waste (Larsen, Vrgoc, Christensen, *et al.,* 2009). The reason for the high values of GHG generated by vehicles, as stated in the literature, is the type of waste treatment, whether it is local or regional.

Based on the waste composition analysis (morphological composition of the waste obtained through a standard for separate components of waste in RS), the quantity of waste deposited in 2020 was approximately 8,220 t. Additionally, the method of waste deposition, characterized by direct release of landfill gas into the atmosphere without prior treatment, necessitated the calculation of GHG emissions.

The total GHG emissions from the Zvornik landfill amount to 11,356 t of $CO_{2\text{-}eq}$/year, apropos 1.38 t of $CO_{2\text{-}eq}$/t of deposited waste.

In their study, Manfredi, Tonini, Christensen, *et al.* (2009) made different calculations of GHG emission for different types of landfills and obtained the following results: (1) dumpsites: >1 t of $CO_{2\text{-}eq}$/t of deposited waste, (2) conventional landfill: 0.3 t of $CO_{2\text{-}eq}$/t of deposited waste, (3) landfill with a low content of carbonite: 0.07 t of CO_2-eq/t of deposited waste, (5) landfill using the landfill gas for producing the energy: from 0.07 to 0.030 t of $CO_{2\text{-}eq}$/t of deposited waste.

The primary GHG emitted from landfills is CH_4. The GWP of the CH_4 depends on its conversion to CO_2 through various processes such as combustion in flares, utilization in gas engines, or by microbial oxidation in the soil top cover of the landfill. It is important to note that when CH_4 is converted to CO_2, its contribution to GWP does not account, as CO_2 is a less potent greenhouse gas than CH_4 (Maria, Góis, Leitão, 2020).

Based on the recalculated emission for the reference year 2020, it can be concluded that in the waste management system in Zvornik, which involves waste collection, transportation, and depositing waste, the highest GHG emissions from the landfill (98.84%) is attributed to the uncontrolled release of landfill gas without prior treatment. The process of waste collection contributes to a mere 0.87% of GHG emissions, while transportation accounts for approximately 0.29%.

The landfill is still the most dominant factor in total GHG emissions. By improving the landfill operation, the emissions into the environment can be reduced significantly, even up to 50-70%. The greatest benefit for the environment from the GHG emission aspect is the exploitation of landfill gas for obtaining energy (heat and/or electric energy).

Scenario 2027

In the foreseeable future, the enhancement of waste collection and transportation of waste in the City of Zvornik should have as a priority the reduction of diesel consumption. The European Commission adopted a strategy for reducing diesel consumption and CO_2 emissions from heavy-duty vehicles in 2014 (European Commission, 2014).

According to this strategy, upgrading of technology in heavy-duty vehicles can lead to reductions in diesel consumption and CO_2 emission. Significant reductions in diesel consumption and emission of CO_2 in heavy-duty vehicles can be achieved through various technological improvements, such as motor improvements (including heat recuperation), transmission upgrades, improvements in aerodynamics, wheels optimization, and the use of additional equipment. Furthermore, reducing vehicle mass can also contribute to efficiency gains. Additionally, efficiency can be increased through better management of the vehicle fleets, and staff training (European Commission, 2014).

The proposed solution to reduce the diesel consumption associated with activities like waste collection and transportation in the City of Zvornik is a novelty in the vehicle fleet.

By procuring the vehicles with Euro V or Euro VI motors (capacity<7.5 t), with an average diesel consumption of approximately 0.25 L/km instead of 16.400 L consumed by two old vehicles, the diesel consumption would be reduced to 7.500 L for the new vehicles. This transition to newer vehicles would result in cost savings and lead to a reduction in diesel consumption generated by the collection and transport of the waste by up to 18% annually.

Furthermore, if these measures of vehicle fleet improvement and collection system enhancement were implemented, the total GHG emissions resulting from waste collection would amount to 81 t of $CO_{2\text{-eq}}$/year. The GHG emissions from waste transport would amount to 26 t of $CO_{2\text{-eq}}$/year. Consequently, the GHG emissions from waste collection in 2027 would be reduced by 20% and from waste transportation by 15%, compared to the current waste collection practices. By improving the fleet, GHG emissions would decrease from 12.23 kg $CO_{2\text{-eq}}$/t of waste (Status Quo) to 9.19 kg $CO_{2\text{-eq}}$/t of waste (Scenario 2027). The estimated GHG range from 9.4 to 368 kg $CO_{2\text{-eq}}$/t of waste, depending on factors such as the collection method, capacity and selection of transport equipment, as well as the travel distances involved (Eisted, Larsen, Christensen, 2009).

The negative values of GHG represent savings, i.e. benefits in the environment, and the positive values represent ballast or pollution of the environment.

In Figure 3, it is evident that recycling and composting processes have negative values, indicating their positive environmental benefits within the waste management system. In contrast, activities such as waste collection and transportation have positive values, indicating their negative impact on the environment.

The process of separating and recycling secondary raw materials enables the reuse of materials and the production of new products using recycled materials. This directly reduces the need to extract and exploit natural resources, which minimizes negative impacts on the environment. In addition, the separation of secondary raw materials reduce the amount of waste that is deposited of, which further contributes to the reduction of waste and benefits for the environment. The recovery of high-frequency materials such as LDPE, PET, textiles, steel cans, and aluminum cans has resulted in significant avoided GHG emissions. This emphasizes the crucial role of effective source-segregated recycling of these key waste materials in reducing the GHG impacts associated with waste management (Turner, Williams, Kemp, 2015).

The process of composting, which involves generating the compost of high quality for agriculture use, results in negative GHG emissions, and thus provides environmental benefits. Instead of depositing the biodegradable waste in the landfill, which is currently practiced and represents a negative impact on the environment due to the significant GHG generation, composting enables the production of a valuable product known as compost.

Composting presents a high potential for GHG reduction by avoiding chemical fertilizer production since the compost product is used as an agricultural fertilizer to replace the chemical alternative. One ton of compost product can supply the soil with nutrients of 7.1 kg of nitrogen, 4.1 kg of phosphorus, and 5.4 kg of potassium (Thanh, Yabar, Higano, 2015).

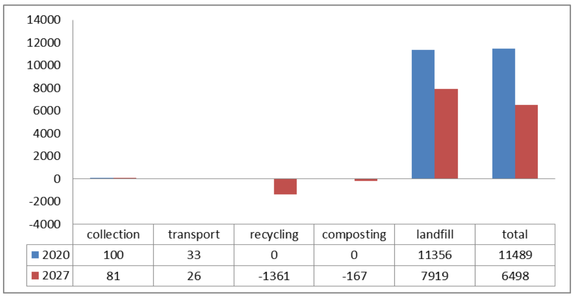

Figure3. GHG emissions (in a t of $CO_{2\text{-eq}}$) in scenario Status Quo and Scenario 2027

The largest GHG emission in 2020 was from the landfill. One of the most important upgrades in the landfill management system is the construction of a degasification system to treat the gas on a flare. It is expected that the construction of the degasification system will be completed in 2023, which will enable the collection and treatment of landfill gas at approximately 60% capacity by 2024. This system is anticipated to significantly reduce GHG emissions from the landfill by 2027. Instead of directly releasing landfill gas directly into the atmosphere, the gas will be flared, producing CO_2, which has a lower greenhouse effect (about 25 times less potent). This results in a direct decrease in GHG emissions by approximately 40% annually.

The calculations for the GHG emissions from the waste management system in Zvornik include separate processes of waste collection, transport, recycling, composting, and landfill deposition, as presented in Figure 4. It can be concluded that the GHG emissions per t of waste generated in Scenario 2027 is significantly lower compared to the Status. In 2020, the GHG emissions per t of MSW were measured at 1,400 kg of $CO_{2\text{-eq}}$. However, it is anticipated that by 2027, this value will significantly decrease to approximately 560 kg of $CO_{2\text{-eq}}$/t of MSW, resulting in a reduction of about 60% in GHG emissions. This significant decrease can be attributed to the upgrade of landfill, which plays a crucial role in reducing GHG emissions per t of waste. Depending on the type of landfill, the GHG emissions from the landfilling of waste have been calculated to range from −145 to 1,016 kg $CO_{2\text{-eq}}$/t of wet waste (Friedrich and Trois, 2013).

Composting and recycling processes, as well as improved waste collection and transportation, lead to a reduction in GHG emissions (Eisted, Larsen, Christensen, 2009; King and Gutberlet, 2013).

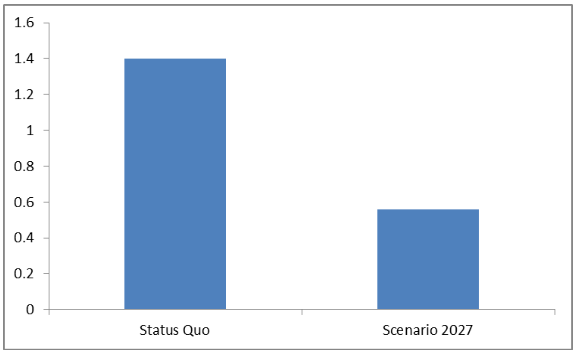

Figure 4. Total GHG emission (in kg/t of MSW) in the scenario Status Quo and Scenario 2027

CONCLUSION

The current system of waste management in the City of Zvornik (Status Quo) consists of the following processes: (1) collection of the mixed communal waste, (2) transportation of the mixed communal waste to the landfill, and (3) deposition the waste at the landfill. The city faces several challenges, including a very old vehicle fleet, low level of waste collection, and a lack of separate waste collection.

At present, the highest GHG emissions originate from the sanitary landfill, which lacks a degasification system and proper landfill gas treatment.

By constructing a degasification system and implementing landfill gas treatment until 2027 (Scenario 2027), a significant reduction in GHG emissions can be achieved. Additionally, a substantial GHG emissions reduction can be achieved by implementing a separate collection system for packaging and biodegradable waste, optimizing collection routes, and updating the waste collection vehicles.

This study can provide valuable insights for numerous local communities struggling with waste management issues. It can serve as a guiding resource for upgrading and improving their current waste management systems.

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Summary/Sažetak

Svaki korak u procesu upravljanja čvrstim komunalnim otpadom (MSW) stvara gasove staklene bašte (GHG). Stoga je imperativ fokusirati se na komunalni otpad od izvora do konačnog odlaganja otpada kako bi se smanjio negativan utjecaj na okoliš. Ova studija ima za cilj izračunavanje emisija stakleničkih plinova u sadašnjem trenutku (Status Quo) za upravljanje otpadom kao i za poboljšano upravljanje komunalnim otpadom koje bi trebalo implementirati u ovoj lokalnoj zajednici do 2027. godine (Scenario 2027). Za vizualizaciju tokova otpada u ova dva scenarija korišten je softver STAN 2.5, a za proračun emisija GHG u Gradu Zvorniku korišten je softver IWM-2. Status quo upravljanja komunalnim otpadom u osnovi karakteriše sakupljanje komunalnog otpada i njegovo odlaganje na deponiju bez sistema za degasizaciju i tretman deponijskog gasa. Smjernice i preporuke za poboljšanje upravljanja komunalnim komunalnim otpadom, Scenarij 2027, predlažu uspostavljanje odvojenih sakupljanje sekundarnih sirovina i biorazgradivog otpada, kao i poboljšano sakupljanje i tretman deponijskog gasa na lokaciji deponije. Implementacija ovih mjera rezultirala bi smanjenjem emisija stakleničkih plinova za približno 40% u odnosu na status quo. Najveći uticaj bi se ostvario na životnu sredinu zbog sakupljanja i tretmana deponije.

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Cyclosporine A Concentrations in Blood Measured with the Immunoassays on Roche e601® and ADVIA Centaur XP® Analysers-What is the Extent of the Agreement?

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Abstract: Monitoring of cyclosporine A (Cs A) concentrations is inevitable for efficient and safe immunosuppression. Currently, immunoassays are the most often used method. The study compared the Cs A concentrations in EDTA-blood samples of 50 patients, measured on Roche e601® and ADVIA Centaur XP® analyzers. The Cs A concentrations on e601® were between 30.00 and 573.00 ng/mL. On Centaur XP® they were in the range 30.2-395.2 ng/mL. For all data the correlation coefficient (95% confidence interval (CI)) was 0.98 (0.97-0.99), while in the groups with concentrations below and above 100 ng/mL it was 0.90 (0.74-0.93) and 0.98 (0.94-0.99), respectively. The slope (95% CI) in the Passing-Bablok analysis on all results was 0.73 (0.67-0.83), and the intercept (95% CI) was 12.53 (6.66-17.78). In the group with results below 100 ng/mL, the slope was 0.92 (0.77-1.12) and the intercept 3.05 (from -8.45 to 12.09). For the Cs A concentrations above 100 ng/mL the slope was 0.71 (0.64-0.84) and the intercept 9.31 (from -8.86 to 24.27). The proportional and systematic errors were present in a wide range of Cs A concentrations measured on the evaluated analyzers. The concordance was satisfactory for concentrations below 100 ng/mL.

INTRODUCTION

Cyclosporine A (Cs A) is an immunosuppressant drug belonging to the family of calcineurin inhibitors (Tapia *et al*., 021). It has a narrow range between the pharmacologic and toxic doses. If overdosed, the common adverse effects include hypertension and nephrotoxicity (Pollard 2004; Damiano *et al.* 2015). Also, the extensive metabolism makes the pharmacokinetics of Cs A highly unpredictable (Survase *et al*.,2011; Soldin et al., 2010).
Therefore, monitoring Cs A concentrations in blood is inevitable in achieving efficient and safe immunosuppression in transplanted patients.
The commercial immunoassays represent usual methods for measuring the Cs A concentration in a majority of the routine clinical laboratories (Vogeser *et al*., 2014). Their main advantages are simple sample preparation and automatization (Zhang and Zhang, 2018). Nevertheless, caution is necessary when interpreting the results because the Cs A metabolites can interfere with the assays (Seger *et al*.,2016).

Roche® diagnostics and Siemens Healthcare® developed competitive immunoassays to measure the Cs A concentration in the whole blood (Soldin *et al*., 2010; Vogeser *et al*., 2014). The Roche assay employs precipitation with zinc sulfate and extraction with methanol for sample preparation and electrochemiluminescence for quantification of Cs A (Vogeser *et al*., 2014). The Siemens assay uses a single-step precipitation-free extraction for sample preparation and direct chemiluminescent technology on the ADVIA Centaur for quantification (Soldin *et al*., 2010). For both assays, the validation included a comparison with the liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) (Soldin *et al*., 2010; Vogeser *et al*., 2014), the "gold standard" for quantitation of the immunosuppressants (Seger *et al*., 2016). Nevertheless, no study has compared the results of these two immunoassays. This study aimed to evaluate the agreement between the Cs A concentrations in blood measured using the immunoassays on Roche e601® and Siemens Healthcare ADVIA Centaur XP® analyzers.

EXPERIMENTAL

Samples

We used the surplus of samples from 50 patients on therapy with Cs A. Between October 2014 and January 2015, we collected venous blood samples into vacuum tubes with K_2EDTA (BD Vacutainer). After the manual pretreatment, the samples were analyzed within 6 hours from sampling, simultaneously on both analyzers, according to the manufacturer's instructions.

Immunoassays

In Elecsys Cs A assay (Roche Diagnostics GmbH, Mannheim, Germany), the pre-treated sample was incubated with a Cs A-specific biotinylated antibody and a Cs A derivative labeled with the ruthenium. Both the sample analyte and the ruthenium-labeled hapten interacted with the binding site on the labeled antibody. In the next step, the streptavidin–coupled paramagnetic particles on the solid phase bounded the entire complex. The intensity of the electrochemiluminescence signal was reciprocal to the Cs A concentration. The measuring range was 30-2000 ng/mL, and the within run coefficient of variation (CV) is 2.0-4.1 %. Concentrations of 1-hydroxy cyclosporine (AM1) and 4-N-desmethyl cyclosporine (AM4N) less than 2000 ng/mL showed a cross-reactivity of 2%. The analogous concentrations of 1, 9-dihydroxy cyclosporine (AM1,9) and 1- hydroxy-1-tetrahydrofuryl cyclosporine (AM1c) did not produce detectable cross-reactivity (Vogeser *et al*., 2014).

In Advia Centaur XP Cs A assay (Siemens Healthcare Diagnostics) Cs A from the pre-treated sample competed with the acridinium ester-labeled Cs A for binding to a biotin-labeled monoclonal anti-Cs A antibodies binding sites. In the next step, biotin-labeled anti-Cs A antibodies bound to the magnetic particles coated with streptavidin. The intensity of the generated chemiluminescent signal is inversely proportional to the concentration of Cs A. The measuring range was between 30 and 1500 ng/mL and the within run CV from 3.8 to 4.6%. The cross-reactivity with AM1, AM1c, AM4N, and AM1,9 in concentrations below 1000 ng/mL was less than 5 % (Soldin *et al*., 2010).

3. Statistical analysis

Statistical evaluation included Pearson correlation, Passing-Bablok, and Bland-Altman analyses, all performed in MedCalc® Statistical Software Version 12.5.0.0.

RESULTS AND DISCUSSION

Table 1 presents the obtained results. For all data, the correlation coefficient was 0.98 (0.97-0.99). In the groups with concentrations below and above 100 ng/mL was 0.90 (0.74-0.93) and 0.98 (0.94-0.99), respectively.

Our results indicate a close correlation between the wide range of Cs A concentrations measured on Roche e601® and ADVIA Centaur XP® analyzers. The previous articles reported the correlation between the concentrations obtained with the evaluated methods and LC-MS/MS (Soldin et al., 2010; Vogeser et al., 2014). The correlation was also present with the other immunoassays like chemiluminescent microparticle immunoassay (Soldin *et al*., 2010; Vogeser *et al*., 2014) and antibody-conjugated magnetic immunoassay (Vogeser *et al*.,2014).

The Bland-Altman analysis on all 50 samples identified only one point beyond the limits of agreement (Figure 1A). In the group with Cs A values below 100 ng/mL, two out of 32 points did not fit into the limits of the agreement (Figure 1B). Finally, for the samples with the concentrations of Cs A above 100 ng/mL, the Bland-Altman analysis showed one out of 18 points exceeding the limits of agreement (Figure 1C).

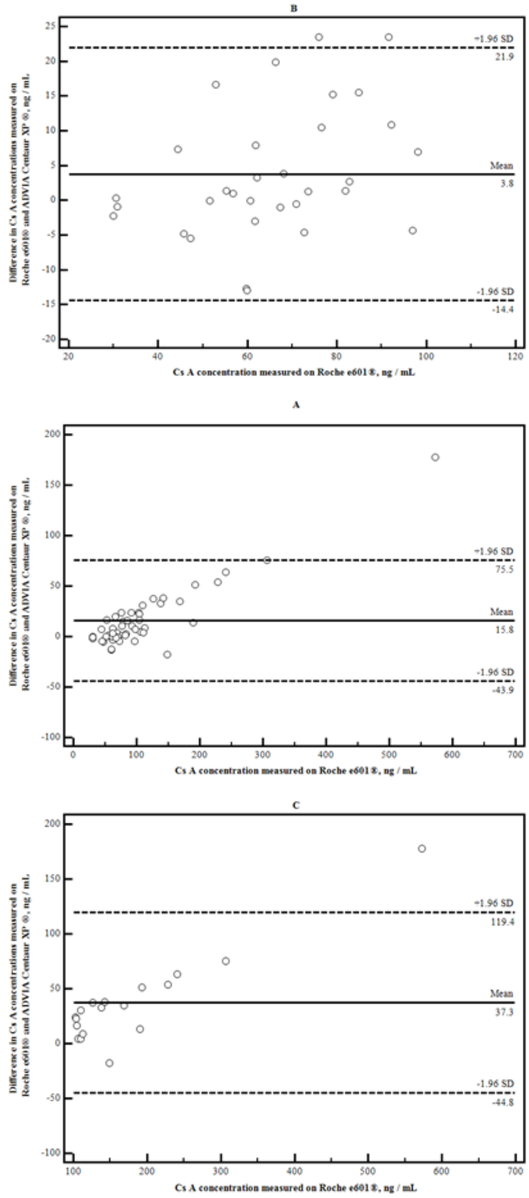

Figure 1. Bland-Altman analysis for (A) all samples, (B) samples with Cyclosporine A (Cs A) concentrations lower than 100 ng/mL, and (C) samples with Cs A concentrations higher than 100 ng/mL.

The Bland–Altman plots suggested a satisfactory agreement between Roche e601® and ADVIA Centaur XP® platforms regardless of the Cs A concentration in the sample. In the initial evaluation study of the ADVIA Centaur XP®, the Bland–Altman analysis revealed the close agreement with the LC-MS/MS, the substantial specificity for Cs A, and the absence of the significant interference of the Cs A metabolites (Soldin *et al*., 2010; Vogeser et al., 2014). Similar findings occurred during the multicentric evaluation of Roche e601 (Vogeser et al., 2014), although a mild bias, acceptable according to the consensus recommendations (Wallemacq (2004), raised the possibility of the eventual cross-reactivity with the Cs A metabolites.In the Passing-Bablok regression (Figure 2A), the slope (95 % confidence interval) was 0.73 (0.67-0.83) and the intercept 12.53 (6.66-17.78). Also, the slope 0.92 (0.77-1.12) and the intercept 3.05 (from -8.45 to 12.09) were the results of the Passing-Bablok analysis (Figure 2B). The slope and the intercept calculated in the Passing-Bablok analysis (Figure 2C) were 0.71 (0.64-0.84) and 9.31 (from -8.86 to 24.27).

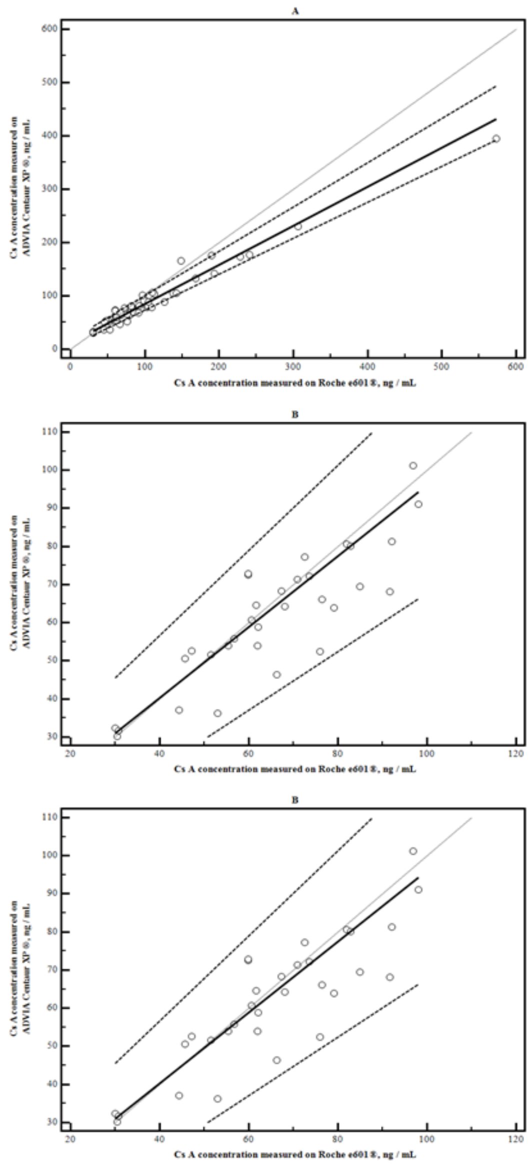

Figure 2. Passing-Bablok regression analysis for (A) all samples, (B) samples with Cyclosporine A (Cs A) concentrations lower than 100 ng/mL, and (C) samples with Cs A concentrations higher than 100 ng/mL.

The Passing–Bablok analysis revealed the concentration-dependent disparity between the evaluated methods. In the wide range of concentrations, the combination of negative proportional and positive systematic error appeared after the comparison. The errors were absent when the analysis included only samples with a Cs A concentration less than 100 ng/mL. On the contrary, the negative proportional error persisted for the blood samples with a Cs A concentration higher than 100 ng/mL. The validation studies reported that in comparison with the LC-MS/MS, ADVIA Centaur XP® had shown the negative proportional error (Soldin *et al*., 2010), while for Roche e601®, there had been both positive proportional and systematic error (Vogeser *et al*., 2014). The negative proportional error characterized both ADVIA Centaur XP® and Roche e601® in comparison with the other immunometric platforms (Soldin *et al*., 2010; Vogeser *et al*., 2014), while the negative systematic error was present for Roche e601®
The observed disparity presumably has at least two causes. One could be the cross-reactivity with the Cs A metabolites. They can occur in concentrations between 5 and 150% relative to the Cs A concentration (Seger *et al*., 2016). The findings of the validation studies showed the neglectable interference from metabolites in ADVIA Centaur XP® assay (Soldin *et al*., 2010). For the Roche e601®, they ranged between 2 and 6% (Vogeser *et al*., 2014), and although they characterized the concentrations much higher than those measured in this study, the fact that we confirmed a concentration-dependent proportional error supports their potential significance.

Also, the disparity could result from the difference in the sample preparation between the evaluated methods. Approximately 50% of Cs A in the blood is in the erythrocytes, and the rest is tightly bound to proteins (Seger *et al*., 2016). Therefore, the manual pre-treatment of the sample makes the Cs A accessible for the antibodies in both assays (Oellerich *et al*., 1995). For ADVIA Centaur XP® the pre-treatment had included single-step Cs A extraction (Soldin *et al*., 2010), instead of the precipitation and extraction, as it had been for Roche e601® (Vogeser *et al*., 2014). Notwithstanding that the single-step Cs A extraction had been technically more convenient, the advantage of precipitation could be the removal of the potentially interfering heterophilic antibodies (Bartoli *et al*., 2010).
A relatively small number of participants may represent a limitation of the study. However, the results contribute to the evaluation of the equivalence between the Cs A concentrations measured with different immunometric platforms. Therefore, they serve as a reliable starting point for future larger studies.

CONCLUSION

In a wide range, the proportional and systematic errors were present after the comparison of the Cs A concentrations measured on Roche e601® and ADVIA Centaur XP ® analyzers. The concordance is satisfactory for Cs A concentrations less than 100 ng/mL.

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Summary/Sažetak

Praćenje koncentracija ciklosporina A (Cs A) u krvi je neizostavan element postizanja efikasnog i bezbjednog imunosupresivnog efekta. Trenutno se u tu svrhu najčešće primjenjuju imunohemijske metode. U ovom istraživanju, poređene su koncentracije Cs A u uzorcima EDTA krvi 50 pacijenata, izmjerenih na analizatorima Roche e601 i ADVIA Centaur XP. Interval koncentracija izmjerenih na Cobas e601 analizatoru kretao se od 30.00 do 573.00 ng/mL. Na Centaur XP analizatoru taj interval bio je od 30.2 do 395.2 ng/mL. Uzevši u obzir sve podatke, koeficijent korelacije (interval pouzdanosti 95% (CI)) iznosio je 0.98 (0,97-0,99), dok je u grupama sa koncentracijama Cs A iznad i ispod 100 ng/mL iznosio 0.90 (0.74-0.93) i 0.98 (0.94-0.99). Passing-Bablok analizom ukupnih podataka (95% CI), dobijena je vrijednost nagiba od 0.73 (0.67-0.83), dok je vrijednost odsječka iznosila 12.53 (6.66-17.78). Za grupu podataka sa koncentracijama Cs A manjim od 100 ng/mL, nagib i odsječak iznosili su 0.92 (0.77-1.12) i 3.05 (8.45 - 12.09). Za koncentracije Cs A iznad 100 ng/mL vrijednost nagiba bila je 0.71 (0.64-0.84), a odsječka 9.31 (8.86-24.27). Proporcionalne, kao i sistemske greške bile su zastupljene u širokom području koncentracija Cs A izmjerenih na ispitivanim analizatorima. Pri koncentracijama nižim od 100 ng/mL, slaganje metoda je zadovoljavajuće.

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Leachate of Landfill Smiljevići (Sarajevo, B&H) and their Environmental Status

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Abstract: Leachates are produced as a filtrate from waste landfills as a result of highly polluted waters. Organic substances that are mostly present are: phenolic compounds, halogen organic substances, oils and fats. The presence of nitrogen substances is significant, followed by phosphoric substances, sulfates, chlorides, and heavy metals. Sanitary landfill "Smiljevići", created 60 years ago, is placed on the hill area of Novi Grad municipality, Sarajevo. In that time area around landfill was very sparsely populated. Following the legislative, the leachate from the Smiljevići landfill does not have a good environmental status. On the other hand, according to global - typical values, contaminant concentrations are much closer to low than average values. The content of contaminants with long retention in nature and tendency to bioaccumulation is within legal limits. Most parameters that exceed the permitted values include usually biodegradable compounds that the stream of the river Bosnia can absorb better than the small ecosystem of the Lepenički creek. The problem is further aggravated bz the fact that the area surrounding the creek is now relatively densely populated, the stream has a small water capacity, especially in the summer, and the contaminants are mostly volatile. Finally, this problem also can be attributed to irresponsible activity of urban planning.

THE ORIGIN AND HAZARDOUS OF LEACHATE

The problem of human-caused waste dates back to early civilizations. In the Bible (Old Testament) and Ibn Khaldun's writings (14th century) the problems of waste disposal and resulting hazard (Morling, 2007) are mentioned. Accordingly, in the ancient time and today, the most common method to handle solid waste made by human action has been and still is to collect and place it in landfill. An inevitable problem created by the disposing the solid waste is the leachate left on the landfill that could causing significant water pollution. Leachate is produced as a filtrate from a municipal waste disposal site, which is created by the action of various factors, often combination of several of them. They are mostly caused by the passage of external water, most often by precipitation, through the layers of soil where the waste is deposited. Part of the water can evaporate, depending on the air temperature and the intensity of solar radiation. The share of individual sources certainly varies among different landfills, and often changes within the same landfill during the year, depending on the hydrological situation. Figure 1 presents a schematic picture of the water balance in a landfill (Bozkurt, 2000).

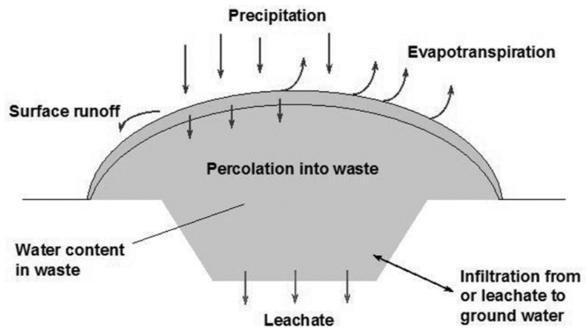

Figure 1. Scheme of leachate generate, (Bozkurt, 2000).

Unlike clearly visible waste that locally contaminates the soil with its presence, or an unpleasant odor from burning/evaporation that contaminates the air, landfill leachate is less noticeable and can contaminate a wider

Kolanka, 2013; Zornoza, Moreno-Barriga, Acosta et al., 2016). These compounds are formed by aerobic decomposition on the surface or anaerobic decomposition in deep deposits of waste in the soil that come into contact with water and create a leachate. Organic substances are mostly present: aromatic compounds (mainly phenolic compounds), halogen organic substances, oils and fats. The presence of nitrogen substances is significant, primarily and mostly in the form of ammonia compounds, then phosphoric substances, sulfates, chlorides and heavy metals (Reinhart and Grosh, 1998; Vaverkova, Elbl, Koda et al., 2020). Waste is decomposed in several stages, so the composition of the leachate is also affected by which stage of decomposition the waste contaminated the water. Factors of landfill water contamination include: method of deposition, depth of deposition, geological characteristics of the area where the waste is deposited, temperature, hydrological situation of the area such as precipitation, alluvium,and presence of groundwater (Christensen, Kjeldsen, Bjerg et al., 2001). The composition and concentration of substances present in leachate also changes with the age of the deposited materials. As landfills age, degradable organic substances slowly undergo anaerobic degradation, which results in leachate from older landfill having a more stable chemical composition and lower concentrations of harmful substances, especially organic substances. Young leachate mainly contains low molecular weight organic compounds often with oxygenated functional groups (e.g. carboxyl and alcoholic groups). Old leachate has organic compounds with a wide range of molecular weight, more complex structures with nitrogen, sulfur,and oxygen functional groups. Generally, leachate contamination gradually increases for the first 5 to 10 years and then decreases (Cheremisinoff, 1997; Calace, Liberatori, Petronio et al., 2001).

Leachates are one of the major problems in attempts to protect the population and the environment from the harmful effects and consequences of waste disposal. They directly threaten surface and underground water in the landfill area and surround (Wiesmann, Choi, Dombrowski, 2007). In order to eliminate or alleviate this problem, it is necessary to process the leachate to the quality of the effluent prescribed by legal acts, before discharging it into the natural recipient. Legislation is mainly based on EU directives regarding waste management. EU directives set strict requirements for the degree of purification and the maximum allowed discharge of harmful substances into natural watercourses. Application of modern purification methods (microfiltration, ultrafiltration, nanofiltration and reverse osmosis) are gaining more and more importance (Brennan, Healy, Morrison et al., 2016; Directive EU, 2018). In Bosnia and Herzegovina, the level of purification is determined according to the source of water discharge, and the allowed concentrations of certain harmful substances are prescribed by the "Regulation of the conditions for the discharge of waste water into the environment and public sewage systems" (Official Gazette FBiH, 2020). Regarding leachate from waste landfills, the contamination parameters prescribed by law that have to be determined are: pH, suspended substances, BOD (biological oxygen demand), COD (chemical oxygen demand), ammonia and total nitrogen, organic halogens, total organic carbon, total phosphorus, fats and oils and heavy metals.

SMILJEVIĆI LANDFILL

Sanitary landfill "Smiljevići", created in the 1960s, is placed on the locality of Buća Potok, municipality of Novi Grad, City of Sarajevo. All the waste that is collected in Sarajevo Canton is deposited at this landfill, and that includes: municipal waste, industrial waste, hospital waste, sewage sludge, waste as a result of traffic, slag and ash from waste incinerators and other waste. The area of the landfill is 122400 m². In the wider area of the Smiljevići landfill, the drainage of surface water and seepage takes place through the Lepenički creek, which flows directly into the Bosna river. Due to the configuration of the soil and tributaries, water level of the creek often varies during the year (KJKP Rad, 2013).

Leachate and rainwater produced in the area of the landfill must be treated in a treatment plant, thus achieving the required level of purification, and then discharged as a purified effluent of satisfactory quality into the natural recipient - Lepenički creek, in accordance with current regulations (Official Gazette FBiH, 2020). In the Smiljevići landfill, there is a pool infrastructure and there is a membrane bioreactor as a water purifier, but it has been out of order for a long time, so the leachate is discharged into the Lepenički creek without any treatment. Considering that the leachate from the Smiljevići landfill has not undergone any treatment, it can be considered as a general picture of the leachate of any landfill, regardless of the differences in the landfills (deposited material, soil structure and composition, environmental conditions, etc.).

THE STATUS OF LEACHATE FROM THE SMILJEVIĆI LANDFILL COMPARED TO OTHER LANDFILLS

In addition to the legal obligation of water purification, which is not carried out, landfill managing also includes monitoring water pollution parameters, which the landfill manager KJKP Rad performs regularly and publishes the results publicly on the website. Tables 1 and 2 show a cross-section of analysis results for the period of 2021.

Table 1. Contents of the main parameters of contamination of leachate from the Smiljevići landfill during 2021 (KJKP Rad, 2022), legal emission limit values and characteristic values with regard to the age of the landfill (Renou, Givaudan, Poulain et al., 2008; Knežević, Cukut, Dunović, 2012; Oreščanin, 2014; Mukherjee, Mukhopadhyay, Hashim et al., 2015; Brennan, Healy, Morrison et al., 2016).

Parameter	Landfill Smiljevići			Typical or global average values			Official Gazette FBiH 26/20, 96/20 emission limit values
	annual average	annual max.	annual min.	average	young landfill*	old landfill*	
pH	8.0	8.6	7.6	7.8	5.6 - 9.1	7.0 - 11.5	6.5 - 9.0
COD mg/L	1922	2640	1066	13000	1400 - 79000	100 - 10000	187.5**
BOD_5 mg/L	363	480	280	6000	90 - 27000	3 - 800	37.5**
ammonia mgN/L	541	609	399	1000	10 - 13000	1 - 1600	15.0**
total nitrogen mg/L	563	624	422	1000	70 - 13000	5 - 1700	22.5**
total phosphorus mg/L	4.7	7.4	3.3	30	5 - 100	5 - 10	3.0**
TOC mg/L	511	1830	210	6000	1500 - 20000	80 - 160	45.0**
conductivity µS/cm	11330	15570	7660	15000	3000 - 28000	2600 - 10500	-
flow m^3/day	318	380	260				
temperature °C	14.6	18.5	10.8				

* Renou et al. 2008 classify a young landfill as still active or closed max. 5 years ago, closed over 10 years ago is considered old landfill, closed 5 to 10 years ago is intermediate.
** values are according Appendix 19 and Article 22. of Official Gazette FBiH 26/20

Compared to the legal provisions (Official Gazette FBiH, 2020), the leachate from the Smiljevići landfill is considered very polluted because most of the parameters from Table 1 are above the permitted values, which is recorded in the report of the company that manages the landfill (KJKP Rad, 2022).

Compared to the typical - usual values of most landfills, the contamination parameters of the Smiljevići landfill are not high, they are mostly in the range of values of older landfills. Total phosphorus values are mostly below typical minimum values while pH is within average, with small oscillations within one pH unit, that is acceptable. Slightly higher values for total organic carbon (TOC) were detected. The values for all parameters, with the exception of conductivity, do not show large oscillations during the year, showing the characteristic of well-managed sanitary landfills. Conductivity is otherwise a parameter that could oscillates even regardless of the content of conducting substances, so change of temperature can change the conductivity of the same solutionIn addition change of pH has significant influence on conductivity.

The values of chemical oxygen demand (COD) and biochemical oxygen demand (BOD), as parameters that are the most important for assessing the contamination of landfill leachate, exceed the values established by country regulations (Official Gazette FBiH, 2020). However, they are below the average of typical values and are within the limits of older landfills. The reason for that is certainly the sanitary management of waste at Smiljevići landfill. The data from the table are at the global level where many landfills, especially in underdeveloped countries, are not subject to sanitary disposal measures, and the values of contaminants can be extremely high, especially COD and BOD. In addition to BOD and COD values, their ratio is an important parameter in assessing the landfill status, and it is a frequently used indicator of the degree of waste decomposition, i.e. stable values of leachate contaminants. Stable landfills are considered the ones with a BOD/COD ratio below 0.1 (Pohlard and Harper, 1986; Ehrig, 1989; Reinhart and Grosh, 1998). Hovewer this is a necessary but not sufficient parameter to consider that the waste is well decomposed, i.e. the content of contaminants in the leachate is stable (Barlaz, Rooker, Kjeldsen et al., 2002). In the case of the Smiljevići landfill, based on the annual average, the value is 0.19, so it cannot be considered that waste is well-degraded, regardless of the fact that the parameters values do not fluctuate significantly during the year. Otherwise according to Kjeldsen, Barlaz, Rooker et al. (2002) values of the BOD/COD ratio range from 0.02 to 0.8.

According to some authors ammonia compounds are the most significant long-term pollutants of leachate. The reason is the poor degradability of ammonia compounds in anaerobic - methanogenic conditions, which results in accumulation in leachate (Robinson, 1995; Burton and Watson-Craik, 1998; Christensen, Kjeldsen, Bjerg et al., 2001). Interestingly, in unregulated landfills, the ammonia content in leachate can be lower than usual because ammonia compounds are faster degradable in contact with air meaning that significant part of the ammonia evaporates into the atmosphere. Inversely, in the conditions of sanitary landfills potential-total ammonia has a low degree of elimination through air, mostly below 10%, depending on climate, temperature and general weather conditions (Barlaz, Rooker, Kjeldsen et al., 2002). In the leachate of the Smiljevići landfill, ammonia and total nitrogen showed lower concentrations compared to the global average or typical values. Both parameters are within the limits of leachate from older landfills, however they are much above the values allowed by regulations (Official Gazette FBiH, 2020) When compared to the typical values they are higher in concentrations and among the pollutants with the higher value compared to other pollutants. th. The ammonia/total N ratio in landfill leachate in the European Union is on average 0.75 (Brennan, Healy, Morrison et al., 2016). In the case of the Smiljevići landfill, that ratio is higher, and amounts is about 0.9 (Table 1), probably due to possible higher presence of quaternary ammonium compounds, which have been found to inhibit biological processes and prolong staying of ammonia, even in the treatment process of waste water (Tezel, 2009). The situation is similar with total phosphorus in both cases, when comparing with the typical values and legal provisions.

Hazardous phenolic compounds and organic halogens appear in landfill leachate mainly through the decomposition of deposited plastic materials. The share of these materials in the total balance of waste can be large, especially in landfills where sorting and recycling of plastic waste is not carried out. In recent years, the public company that manages the Smiljevići landfill has a program for separating plastic waste and sending it for recycling, but this partly depends on the conscience of the citizens who use the landfill. Otherwise, the content of these substances is increased in older landfills compared to younger ones, which was expected and recorded (Gibbons, Dolan, May et al., 1999), the reason being of course the slow decomposition of plastic substances. The content of phenols in landfill leachates listed in the literature ranges from 0.6 μg/L to 1200 μg/L, the same data for organic halogens ranges from 200 μg/L to 5000 μg/L (Albaiges, Casado, Ventura, 1986; Gintautas, Daniel, Macalady, 1992; Oman and Hynning, 1993; Robinson, 1995; Reitzel and Ledin, 2002; Baun, Ledin, Reitzel et al., 2004). Organic halogens in the leachate of the Smiljević landfill range from 370 to 690 μg/L, i.e. an annual average of 534 μg/L, while phenols are in the range of 30 μg/L to 80 μg/L, with an average of 45 μg/L (KJKP Rad, 2022). Both parameters have relatively low and stable values, i.e. they are much closer to the minimum values given in the literature as typical. Although the content of these substances in the leachates of most of the examined landfills is at the level of μg/L, it is slowly but steadily decreasing due to the increase in the recycling trend (Kjeldsen, Barlaz, Rooker et al., 2002).It is understood that the same trend will be with the Sarajevo landfill.

The content of heavy metals in the leachate of the Sarajevo landfill compared to typical values is much closer to the minimum values (Table 2), it is within the legal provisions (Official Gazette FBiH, 2020). Heavy metals could be very hazardous contaminants and their presence in landfill leachate must be investigated, but they rarely exceed the limit values in sanitary landfills, especially in areas where there are no large fluctuations in precipitation during the year, e.g. areas of the continental climate (Barlaz, Rooker, Kjeldsen et al., 2002). More often, high concentration of heavy metals appears in arid regions with sudden periods of precipitation, e.g. in subtropical areas (Siddiqi, Al-Mamun, Sana et al., 2022). Similar to the content of phenols and organic halogens, materials that are waste sources of heavy metals are more susceptible to recycling day by day, so it is to be expected that in the future there will be no significant hazardous concentrations of metals in the leachate of the Smiljevići landfill. On the other hand, the content of heavy metals can be influenced by several external factors: pH, the presence of complexing or precipitating substances, oxygen that oxidizes anions into more soluble forms of metal salts (e.g. sulfide into sulfate) etc., so there is always a possibility of changing the content, regardless of the quantity of deposited material, which are sources of heavy metal contamination.

Table 2. Typical values (global range) of content of heavy metals (Lin and Chang, 2000; Silva, Dezotti, Sant'Anna, 2004; Morling, 2007; Li, Yun, Li, et al., 2008; Salem, Hamouri, Djemaa et al., 2008; Kulikowska and Klimiuk, 2008; Oreščanin, 2014), phenols and organic halogens (Albaiges, Casado, Ventura, 1986; Gintautas, Daniel, Macalady, 1992; Oman and Hynning, 1993; Robinson, 1995; Reitzel and Ledin, 2002; Baun, Ledin, Reitzel et al., 2004) in leachate, values from leachate of Smiljevići landfill during 2021 (KJKP Rad, 2022) and legal emission limit values.

Parameter	Landfill Smiljevići (mg/L)			Global range	Official Gazette FBiH 26/20 emission limit values
	annual average	annual max.	annual min.		
As (mg/L)	0.021	0.03	0.01	0.001-0.38	0.05
Cd (mg/L)	0.017	0.03	0.01	0.015-0.13	0.05
Pb (mg/L)	0.043	0.06	0.03	0.002-3.49	0.10
Cr (mg/L)	0.07	0.10	0.02	0.012-0.748	0.15
Hg (mg/L)	<0.001	<0.001	<0.001	0.0002-0.012	0.005
organic halogens (μg/L)	534	690	370	200-5000	1000
phenols (μg/L)	45	80	30	0.6-1200	100

The leachate from the Smiljevici landfill consists of three waters:
1. Shallow drainage, the first collector, collects leachate from the multi-barrier protection through layers of mostly fresh disposal waste;
2. Deep drainage, the second collector, collects water from deep layers of waste under the multi-barrier protection, it is old waste that was not disposed of according to sanitary landfill rules.
3. Calota drainage, the third collector, leachate collected at the lowest points of the landfill, underground water that stream through the tunnels of the landfill;
The company that manages the landfill does not have the obligation to investigate individual waters, but the collective water that flows into the ecosystem of the Lepenički creek, but in the report of Prazina, Đug, Mahmutović et al. (2022) stated that water from shallow drainage from fresh waste is the most polluted. The same water is dominant in volume compared to the other two, and it is the main source of pollution of the creek. This is expected considering that it is known that fresh waste is the main source of landfill leachate pollution (Mukherjee, Mukhopadhyay, Hashim et al., 2015). Only nitrates are close in values in all three waters, and this is usual for this parameter when it is about leachates of young and older waste (Knežević, Cukut, Dunović, 2012).

FINAL CONSIDERATIONS

Leachates belong to highly polluted waters and represent a great danger to the environment. They can be environmentally more harmful than industrial waters because they have a variable and unpredictable quantitative and qualitative chemical composition, even in highly regulated waste management systems. A number of measures are implemented in order to protect the environment from the hazardous impact of landfills and its leachate: sanitary method of disposal, recycling, composting or incineration of biodegradable waste and treatment of leachate. The application of these measures in waste management in the Sarajevo region is not complete, hence the unsatisfactory status of leachate in the scope of legislative. Parameters whose concentration exceeds legal limits (COD, BOD, ammonia, total nitrogen, TOC, total phosphorus) are mainly generated in leachate from the biodegradable waste. Burning and/or composting this type of waste, which is a trend in the European Union, the concentration of these parameters in the leachate of the Smiljevići landfill would be significantly reduced, probably under legal limit values even without treatment of water. Compounds which make up these parameters mainly are biodegradable so the greater ecosystem of river Bosna is able to accept those without serious hazardous consequences. However, the hazard of these parameters is strongly expressed in the area of the small ecosystem of the Lepenički creek, which has become densely populated in the last 20 years, so the responsibility for the exposure of the population to contaminants can also be attributed to the municipality authorities that allowed the urbanization of this area. Parameters that have a high retention in nature and the potential for bioaccumulation in the living organism (heavy metal and organic halogens) are within the limits of legal provisions, which probably indicates good management in that segment of measures that are applied - sanitary waste disposal and recycling. This is positive data, as these substances are potentially the most dangerous contaminants of leachate for the local and wider ecosystem, and their elevated presence in leachate is difficult and long term solvable, in the short term they cannot be eliminated without the use of a water treatment system. On a global scale, the parameters of the leachate contamination of the Smiljevići landfill are closer to the lowest limits than the average values, even those parameters with the highest values that are above the limit values prescribed by law. But this is certainly not a reason for lack of concern and passivity because the leachate from the Smiljevići landfill flows through a relatively large settlement that is not built illegally but urbanized and the population is partially agriculturally active. Another reason which enhance the mentioned problem is small capacity of the Lepenički creek, whose water mass is significantly contributed by the leachate of the landfill. This problem especially increases during the summer period, with high temperatures and drought the water mass of creek becomes significantly reduced, even the air in this area becomes polluted due to the evaporation of contaminants.
Certainly, the best solution is to close the existing Smiljevići landfill because it is quite old, around 60 years. Another reason for the need to close the landfill is the fact that when the landfill was put into use, the surrounding of the landfill and the surrounding of the leachate stream were very rarely populated, today it is the opposite.

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Summary/Sažetak

Procjedne vode koje nastaju kao filtrat s odlagališta komunalnog otpada, spadaju u vrlo onečišćene vode. Najviše su zastupljene organske tvari: fenolni spojevi, halogene organske tvari, ulja i masti. Značajna je prisutnost azotnih tvari, najvećim dijelom u obliku amonijačnih spojeva, zatim fosfornih tvari, sulfata, hlorida i teških metala. Sanitarna deponija "Smiljevići", nastala prije oko 60 godina, nalazi se na brdskom području općine Novi Grad, Sarajevo, koje je u tom periodu bilo vrlo rijetko naseljeno. U okviru zakonskih akata koji propisuju maksimalne koncentracije zagađujućih tvari, procjedne vode deponije Smiljevići nemaju dobar okolinski status. S druge strane, u svjetskim okvirima (tipične vrijednosti), koncentracije kontaminanata su mnogo bliže niskim nego prosječnim vrijednostima. Sadržaj kontaminanata sa dugom retencijom u prirodi i sklonosti bioakumulaciji (teški metali, organski halogeni i sl.) su u zakonskim granicama. Parametri koji prelaze dozvoljene vrijednosti uglavnom čine biorazgradive komponente koje širi ekosistem rijeke Bosne uglavnom može absorbovati, no hazardne su za okolinu primarnog toka - područje Lepeničkog potoka. To je tok malog kapaciteta vode pa je problem dodatno pogoršan, osobito ljeti jer su kontaminanti obično volatilni. Ovo područje je sada relativno gusto naseljeno, stanovništvo je djelimično poljoprivredno aktivno pa se ovaj problem može pripisati i lošoj i neodgovornoj djelatnosti iz oblasti urbanizma.

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