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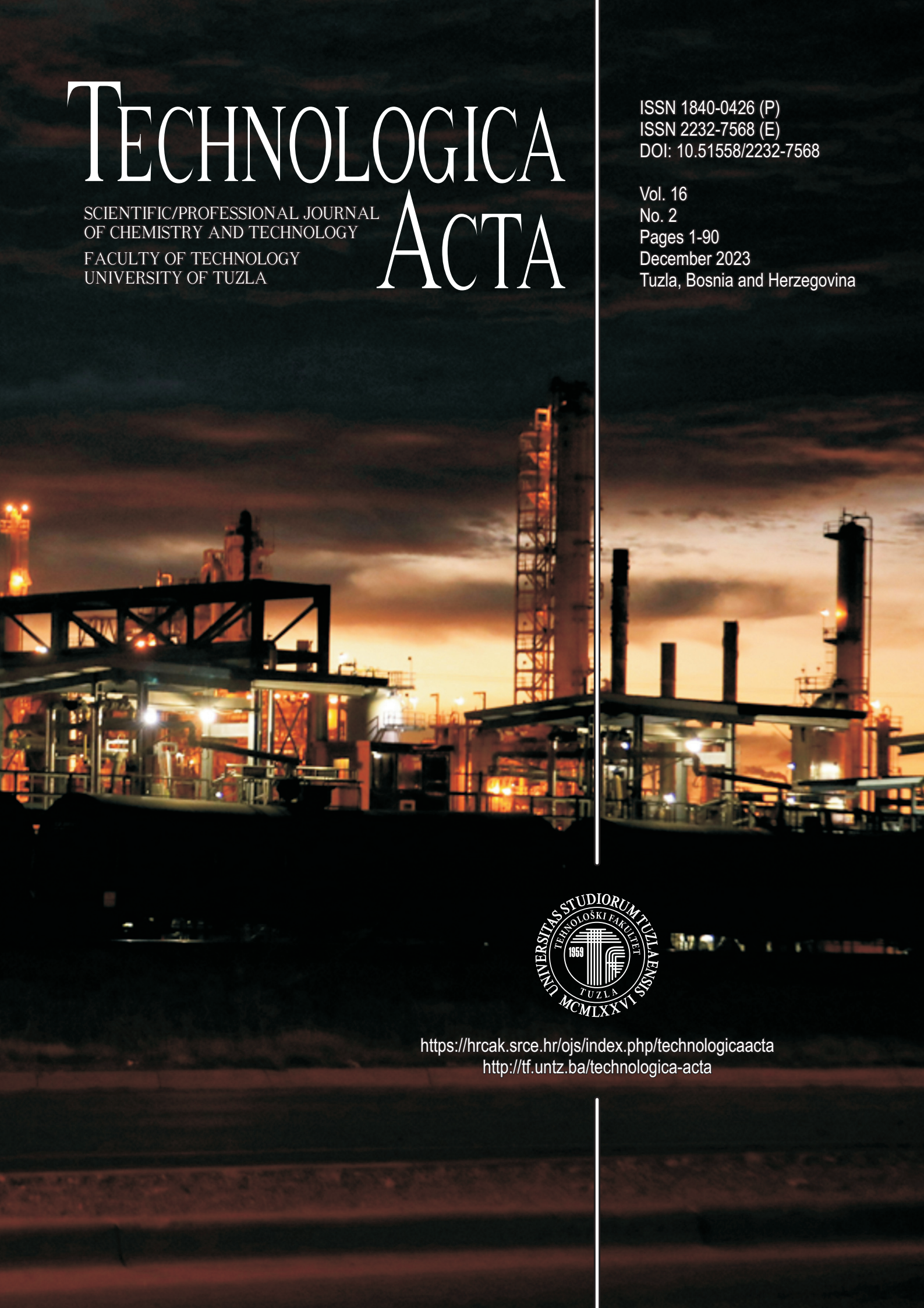

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CONTENT


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ANALYSIS OF THE REPRESENTATION OF EE-DEVICES AS POTENTIAL EE-WASTE IN THE AREA OF THE CITIES BIHAĆ AND KLJUČ

PROFESSIONAL PAPER

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ABSTRACT:
Nowadays, EE-waste represents a big problem, but also a danger for the environment and the population. Many households use various EE-devices that end up as EE-waste after their expiration date. The objectives of the research within this work for the cities of Bihać and Ključ were to determine: the total amount tf EE-devices; percentage representation of the three most represented/leastrepresented devices; total weight of EE-devices as a potential waste; the total amount of EE-waste per household and estimete the annual amount of EE-waste per inhabitant. To realize the goals, survey and comparison method were used. The results of the research showed that in the area of cities of Bihać and Ključ the quantitative representation (about 1 775 pieces); the percentage representation (mobile phones and TV) and the average age (about 5 years) of EE-devices were approximately the same. The differences were in the total weight of EE-devices as potential waste (38 853–39 316.4 kg); the total amount of EE-waste (5 942.9–6 971.7 kg) and the annual amount of potential EE-waste for Bihać which varied 0.12–0.13 kg/inhabitant and for Ključ 1.09–1.21 kg/inhabitant (Ključ municipality from 0.35 to 0.39 kg/inhabitant).

KEYWORDS: EE-devices, EE-waste, representation in cites, Bihać, Ključ

INTRODUCTION

The waste is one of the urgent issues in environmental protection, especially when it comes to waste production, insufficient utilization of waste as secondary raw materials and inadequate waste managment, including landfilling. There are several types of waste, solid waste, liquid waste, gaseous, non-hazardous, hazardous, etc., including the electrical and electornic waste (EE-waste). If is handled inadequately, the EE-waste has a negative impact on the environment. It originates from electrical and elecotrnic equipment (EEO). EE-equipment is equipment whose normal operation depends on electric ccurrent or electromagnetic field and equipment for production, transmission and measurement of electric current or magnetic field, intended for use at voltage up to 1000 V alternating current and 1500 V direct current [1]. Regarding electrical and electronic waste (EE-waste) in Bosnia and Herzegovina, there is a legal regulation at the entity level, i.e.: Federation of Bosnia and Herzegovina, Republic of Srpska and Brčko District. In general, EE-waste exist as a result of the digital revolution and the modern way of life, and at the same time became a potential danger to the environment and the humanity. It is one of the fastest growing types of waste in the EU, and only about 40% is recycled[1].

It is estimated that in 2020 the amount of EE-waste amounted to 10.5 kg/per inhabitant, while the average amount of EE-equipment placed on the market in the period 2017–2019 was about 22.9 kg/per inhabitant[2]. In the EU, this waste is collected, and it is estimated that the rate of its collection is around 45%[3]. EE equipment, which is in 10 categories, i.e. [1]: Large household appliances; Small household appliances; IT and telecommunications equipment; Consumer

[1]Source: https://www.europarl.europa.eu/news/hr/headlines/society/20201208STO93325/e-otpad-u-eu-u-cinjenice-i-brojke-infografika

[2] Source: https://ec.europa.eu/eurostat/statisticsexplained/images/d/d2/EEE_put_on_the_market_in_the_three_preceding_years_%28201720 19%29%2C_waste_EEE_generated_in_2020_and_waste_EEE_collected_in_2020_%28kilograms_per_inhabitant%29_V2.png

[3] Source: https://ec.europa.eu/eurostat/statisticsexplained/index.php?title=Waste_statistics_electrical_and_electronic_equipment#Electrical_and_electronic_equipment_.28EEE.29_put_on_the_market_and_WEEE_collected_by_country

equipment and photovoltaic panels; Lighting equipment; Electrical and electronic tools (with the exception of large-scale stationary industrial tools); Toys, leisure and sports equipment; Medical devices (with the exception of all implanted and infected products); Monitoring and control instruments; Automatic dispensers. In addition to 10 categories, the mentioned EE equipment is also classified according to an additional 6 categories, namely: Temperature exchange equipment; Lamps; Large equipment (any external dimension more than 50 cm) including, but not limited to certain household appliances (This category does not include equipment included in categories 1 to 3); Small equipment (no external dimension more than 50 cm) including, but not limited to certain household appliances (This category does not include equipment included in categories 1 to 3 and 6) and Small IT and telecommunication equipment (no external dimension more than 50 cm). Also in the Federation of Bosnia and Herzegovina, EE-equipment is identically classified according to [2]. In particular, TV and mobile phones are also found in the mentioned categories of EE equipment. Also, it is known that TV and mobile phones and other EE-devices belong to the group of electrical and electronic devices that are considered sources of electric, magnetic and electromagnetic fields [1]. In general, the use of this EE equipment (TV and mobile phones) can have a potentially positive (information, easier communication, availability, rest, etc.) and negative impact (human health, behavior, etc.) on people.

TV AND MOBILE PHONES AND THEIR POTENTIAL INFLUENCE ON PEOPLE

Television (TV) is a global phenomenon that is available to almost every household. The first television program was broadcast in 1928 in the USA. In every household, the TV took a central place, mainly in the living room. In addition to gathering the young and old population, they are also a source of entertainment for the whole family. In most developed countries, watching TV is a very popular (in)activity among children, adolescents and the elderly. It is considered that the time spent sitting and watching television in the elderly, i.e. sedentary behavior is associated with the occurrence of cardiovascular diseases and the frequency of occurrence of type 2 diabetes and obesity [3].

Also, there are studies that indicate the influence of TV on the occurrence of depression and anxiety in children and adolescents [4], as well as the influence on their behavior, physical activity, poorer eyesight [5].

At the beginning of its application, the mobile phone was used for business purposes and not for general social conversation [6]. However, it quickly found widespread use among people, and it is assumed that about two-thirds of the world's population uses mobile phones. According to [7] in 2021, the number of mobile users worldwide stood at 7.1 billion, with forecasts suggesting this is likely to rise to 7.26 billion by 2022. In 2025, the number of mobile users worldwide is projected to reach 7.49 billion. According to data [8], in BH in 2021 the number of mobile phone users is 3 728 775 and the number of TV program distribution users is 885 620

Due to their widespread use, mobile phones have raised concerns about risks to human health [9] and deserve the attention of both the health and public health communities. Each person should fully use the potential of the cell phone as a tool, but take care that it is not used excessively for entertainment or the environment because it causes addiction [10], as well as the presence of depression [11]. According to [12] mobile screen use ≥8 hours/24 hours as well as mobile phone use at least 30 minutes before bedtime is associated with sleep quality. In addition to the occurrence of violence through mobile phones [13], mobile phones affect the daily social life of young people, communication and interaction [14].

EE-WASTE MANAGEMENT

In the EU, according to [1], it is stated that waste electrical and electronic equipment (WEEE) is also one of the target areas that should be regulated in terms of the application of the principles of prevention, recovery and safe disposal of waste.

The mentioned directive complements the general EU legislation on waste management, such as Directive 2008/98/EC on waste, and also refers to general waste management procedures. Earlier parts of waste management (responsibility for waste management; principles of self-sufficiency and proximity; monitoring of hazardous waste; prohibition of mixing hazardous waste; labeling of hazardous waste; hazardous waste generated in households; waste oils; bio-waste) are supplemented with the following parts: Product design, Separate collection, Disposal and transport of collected WEEE, Collection rate, Proper treatment, Permits, Shipments of WEEE, Recovery targets, Financing in respect of WEEE from private households and from users other than private households, Information for users, Adaptation to scientific and technical progress, Penalties, Inspection and monitoring etc.

In the Federation of BH, with the aim of achieving the elements of a circular economy, there is a legal act that regulates the way of managing EE-waste, which include the operation of the entire management system for this type of waste and also the label (Figure 1). For the establishment of a waste management system created from EE-products, the procedure and rules for placing EE-products on the market have been defined. The following activities are defined in the EE-waste management system: collection, processing/recycling, export, temporary storage and other activities of waste management of EE-products and their parts [2]. The following participate in the EE-waste management system (chapter I-general provisions): Federal Ministry of Environment and Tourism; operators of waste electrical and electronic equipment (WEEE) management systems; importers, producers, distributors, traders of EE-equipment; owners of EE-waste; companies dealing with transport and/or temporary storage, processing, recycling and export of waste; utility companies, Fund for environmental protection FBiH; Information system (available on the web: www.otpadfbih.ba) and competent inspection bodies for market and environmental supervision [2]. In [2] there are other chapters related to: Informing and raising awareness of end users about WEEE management; Obligations of the final user; Free collection of WEEE from the household to the final user and other entities; Objectives of collection and collection of WEEE and temporary storage; Obligations of producers, importers and distributors; System operator and Transitional and final provisions.

Figure 1. A sign indicated for the mandatory special collection of waste equipment [2]

In addition to [2], there are other legal acts that touch on the topic/problem of EE-waste, such as: Law on collection, production and trade of secondary raw materials and waste materials (Official Gazette F BH, No. 35/98, 109/12), Guideline on categories of waste with lists (Official Gazette F BH, No. 9/05), Regulation on criteria for calculation and method of payment of fees for products that become packaging and EE-waste after use (Official Gazette F BH, No. 104/22) and others. However, in practice, unfortunately, one of the leading problems in the management of EE-waste is that complete recycling and repurposing is still not carried out within the borders of our country, and that this type of waste often ends up in the environment together with municipal waste.

WASTE MANAGEMENT IN THE CITIES OF BIHAĆ AND KLJUČ

Bihać and Ključ are located in the northwestern part of Bosnia and Herzegovina within the Federation of Bosnia and Herzegovina and are part of the US Canton. The area of the city of Bihać occupies an area of about 900 km^2, it is composed of 35 local communities with a total number of households of 20 650[4]. The municipality of Ključ is smaller with an area of about 358km^2 and has 10 local communities with a total number of households of 5 249, and the city part of Kljuc itself has 5 409[5]. The total population as of 30 June 2022 for Bihać (municipality code 10049) is 54 921 and for Ključ (municipality code 11509) is 15 315 [15]. Also according to [15], the average number of employees in Bihać is 13 512, Ključ is 1 513, while the average net salary for Bihać is about 1 195 KM, and for Ključ about 904 KM.

In the city of Bihać, the company that manages waste (municipal and part of EE-waste) in the territory of the city of Bihać is the Public Utility Company (JKP) „Komrad". This company contains a recycling yard that receives and temporarily stores discarded EE-devices, i.e. EE-waste. In the city of Ključ, the company authorized to manage municipal and partly EE-waste in the area of the city and municipality of Ključ is PUK „Rad".

Both companies provide services of collection, removal and depositing of municipal waste. However, both of these companies are obliged to act in accordance with the current legislation that also refers to the management of EE-waste, ie that part of EE-waste brought to them by the owners (owners) of EE-waste. The amount and composition of collected EE-waste depends on the interest of citizens to take such waste to the receiving station of the utility company for temporary storage.

[4] Source: https://www.bihac.org/mjesne-zajednice

[5] Source: https://opcina-kljuc.ba/kljuc/#

MATERIAL AND METHODS

Two scientific methods were used in this research: the survey method (conducted on the basis of a survey questionnaire[6]) and the comparison method.

The survey was conducted in the period from April 2022 to April 2023. The research convenience sample consisted of 200 households from the area of two cities. Respondents from different households from Bihać (100 households) and Ključ (100 households) participated in the research. The survey questionnaire consisted of a selected tabular review of EE-devices in which respondents entered numerical data on how many of them the household owns. For Bihać, households were surveyed from the area of 7 local communities in the narrower part of the city, namely: Centar, Bakšaiš, Harmani, Hatinac, Luke, Ozimice I and II. From the area of Ključ, households were surveyed from the area of the largest local community of Ključ, which in fact includes the area of the city.

The main objectives of the research within this work were to determine the annual amount of total potential EE-waste in the area of two cities and to estimate the annual amount of potential EE-waste per inhabitant for Bihać and Ključ. For the realization of the set goals, the following research tasks were defined: quantitative representation of EE-equipment; percentage representation (most and least represented) EE-devices per household; average age of EE-devices; total weight of EE-equipment as potential waste.

RESULTS AND DISCUSSION

The results of the research in relation to the surveyed households (a total of 200) in the cities of Bihać and Ključ are presented tabularly (Table 1 – 2) and graphically (Figure 2 – 3).

Table 1. Amount of EE-waste (kg) in the city of Bihać

Device name	1	2	3	4	5
TV	142	16	2272	7	324.6
Washing machine	100	70	7000	5	1400
Clothes dryer	39	50	1950	5	390
Fridge	107	90	9630	5	1926
Computer	56	13	728	4	182
Clothes iron	102	2	204	6	34
Hair straightener	50	0.3	15	4	3.8
Mobile phone	267	0.2	53.4	3	17.8
Tablet	46	0.3	13.8	4	3.5
Laptop	93	2	186	4	46.5
Bluetooth speaker	36	0.5	18	3	6
Vacuum cleaner	116	10	1160	6	193.3
Boiler	112	20	2240	10	240
Electric stove	110	60	6600	9	733.3
Microwave oven	59	15	885	7	126.4
Dishwasher	66	50	3300	5	660
Heater	58	15	870	6	145
Air conditioning	36	40	1440	3	480
Electric kettle	96	0.4	38.4	4	9.6
Shaving machine	47	0.2	9.4	5	1.9
Printer	40	6	240	5	48
Σ	**1778**	**460.9**	**38853**	**5.2**	**6971.7**

1–Quantitative representation of devices in Bihać (pcs); 2–Average device weight (kg); 3–Total weight of the device (kg); 4–Average age (years); 5–Annual amount of waste (kg)

[6] The survey questionnaire in this paper was specially designed for this type of research

Table 2. Amount of EE-waste (kg) in the city of Ključ

Device name	**1**	**2**	**3**	**4**	**5**
TV	167	16	2 672	8	334
Washing machine	103	70	7 210	5	1442
Clothes dryer	33	50	1 650	5	330
Fridge	124	90	11160	10	1116
Computer	64	13	832	4	208
Clothes iron	106	2	212	7	30.3
Hair straightener	71	0.3	21.3	4	5.3
Mobile phone	263	0.2	52.6	2	26.3
Tablet	57	0.3	17.1	4	4.3
Laptop	52	2	104	3.5	29.7
Bluetooth speaker	44	0.5	22	3	7.3
Vacuum cleaner	109	10	1090	5	218
Boiler	107	20	2140	10	214
Electric stove	104	60	6240	9	693.3
Microwave oven	64	15	960	7	137.1
Dishwasher	53	50	2650	5	530
Heater	43	15	645	6	107.5
Air conditioning	35	40	1400	3	466.7
Electric kettle	91	0.4	36,4	4	9.1
Shaving machine	50	0.2	10	5	2
Printer	32	6	192	6	32
Σ	**1772**	**460.9**	**39316.4**	**5,5**	**5942.9**

1–Quantitative representation of devices in Bihać (pcs); 2–Average device weight (kg); 3–Total weight of the device (kg); 4–Average age (years); 5–Annual amount of waste (kg)

The results of the research showed that the quantitative representation of EE-devices for Bihać and Ključ was approximately the same and in the middle it was 1 775 pieces. The average age of EE devices was 5.35 years. The total weight of EE-equipment for Bihać was slightly lower i.e. by 463.4 kg compared to Ključ, while the annual amount of waste for Bihać was higher by 1 028.8 kg compared to Ključ.

Considering the research period, the estimated annual amount of waste was calculated according to the official population census from 2013 and 2023. The estimated annual amount of potential EE-waste per inhabitant for the city of Bihać (6 971 kg/56 261 inhabitants[7]) was 0.12 kg/inhabitant, while for the city of Ključ (5 942 kg/4 898 inhabitants[8]) it was 1.21 kg/inhabitant, which means that for the municipality Ključ (5 942 kg/16 744 inhabitants[9]) was 0.35 kg/inhabitant.

However, according to the official population census from 2023[10], the estimated annual amount of potential EE waste as of June 30, 2022. for the city of Bihać (6 971 kg/54 921 inhabitants) is about 0.13 kg/inhabitant, for the city of Ključ (5 942 kg/5 409 inhabitants) 1.09 kg/inhabitant and for the municipality of Ključ (5 942 kg/15 315 inhabitants) about 0.39 kg/inhabitant.

From the point of view of the percentage representation (Figure 2 – 3) of EE-devices by households in the surveyed cities, they showed that the most represented was in Bihać: mobile phone (267%), TV (142%) and vacuum cleaner (112%), and in Ključ were mobile phone (263%), TV (167%) and refrigerator (124%). Of the three EE-devices with the lowest percentage representation for the city of Bihać were: bluetooth speaker (36%), air conditioner (36%) and clothes dryer (39%). For the city of Ključ, the three least represented EE devices are: printer (32%), clothes dryer (33%) and heater (43%).

Based on the high prevalence of TVs (approximately two TVs per household) and mobile phones (about 2-3 mobile phones per household), it

[7] Source: According to the official population census for the city of Bihać from 2013

[8] Source: According to the official population census for Ključ – a populated place from 2013

[9] Source: According to the official population census for the municipality of Ključ from 2013

[10] Source: Federal Statistical Office (2023). Una-Sana Canton in numbers, Sarajevo 2023

can be determined that their use has a potential impact and risk on both the physical and mental health of people.

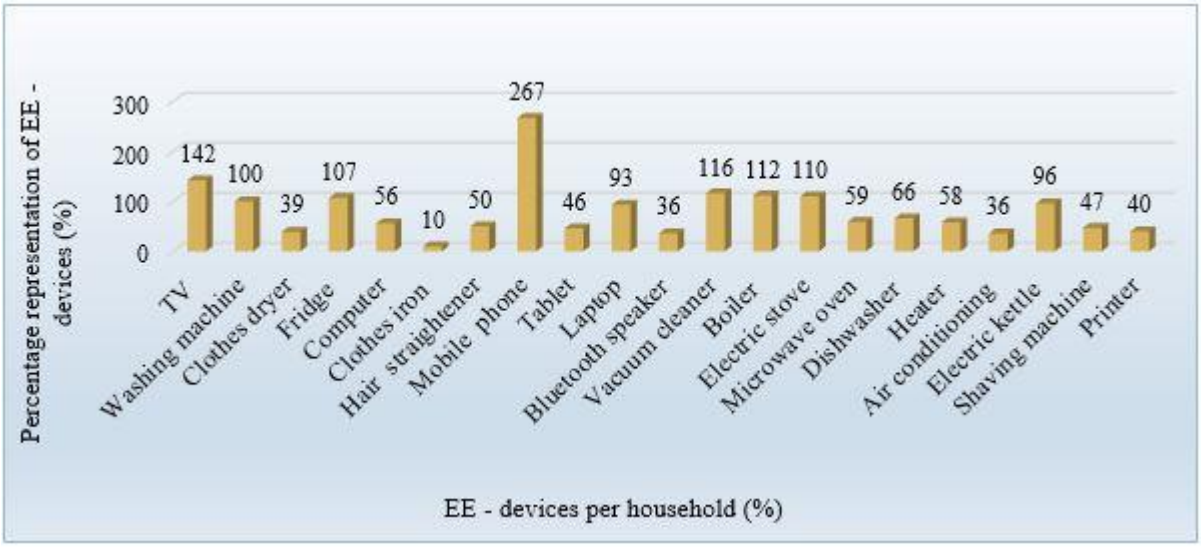

Figure 2. Percentage representation of EE-devices in Bihać (%)

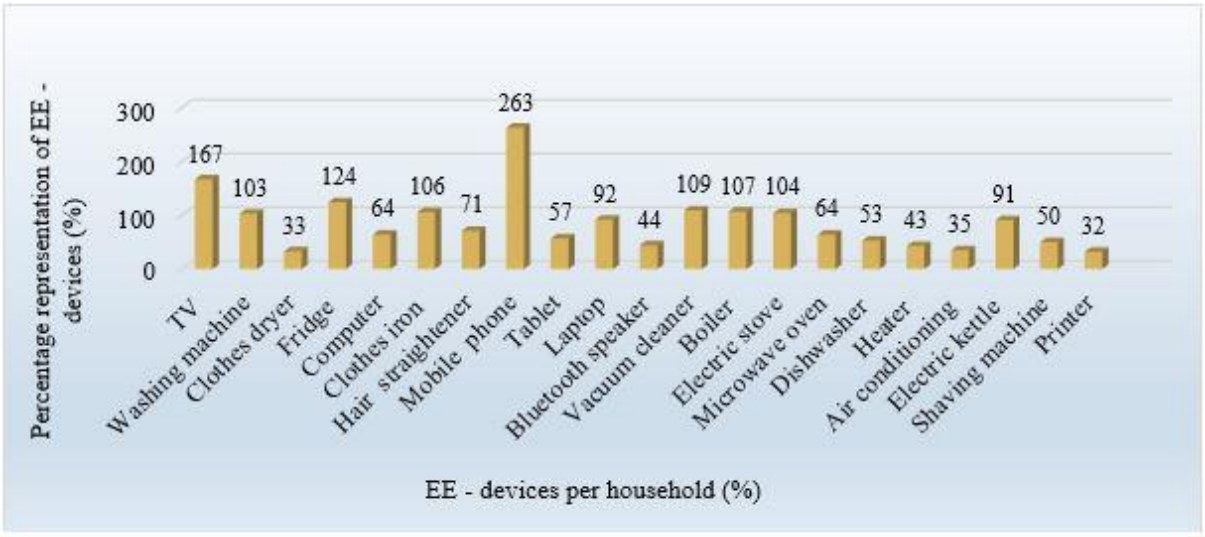

Figure 3. Percentage representation of EE-devices in Ključ (%)

CONCLUSIONS

The quantitative representation of EE-equipment in the middle was 1 775 pieces, including 1 778 pieces for Bihać and 1 772 pieces for Ključ.

In terms of percentage, the most represented EE-devices for Bihać and Ključ were mobile phones (Bihać: 267%; Ključ: 263%) and TV (Bihać: 142%; Ključ: 167%). The least represented EE-devices for Bihać were the bluetooth speaker (36%), air conditioner (36%) and clothes dryer (39%), and for Ključ they were printer (32%), clothes dryer (33%) and heater (43%).

The average age of EE devices was 5.35 years, i.e. 5.2 years for Bihać and 5.5 years for Ključ.

The total weight of EE-equipment as potential waste for Bihać was 38 853 kg, and for Ključ: 39316.4 kg.

The total amount of potential EE-waste in the area of the two cities was 12914.6 kg, for Bihać it was 6971.7 kg, and for Ključ 5942.9 kg.

The calculated values of the amount of potential EE-waste according to the official population census from 2013 and 2023 for the city of Bihać and for the city of Ključ did not vary much.

The values mostly ranged for the city of Bihać from 0.12 to 0.13 kg/inhabitant and for the city of Ključ from 1.09 to 1.21 kg/inhabitant (municipality of Ključ from 0.35 to 0.39 kg/inhabitant).

The management of EE-waste is handled by municipal companies of the cities of Bihać and Ključ. Unfortunately, the EE-waste management system has not yet fully taken off.

Due to the high prevalence of TV and mobile phones, there is a potential impact and risk on human health (mental and physical).

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MICROBIOLOGICAL QUALITY OF BOTTLED WATER IN FREESTANDING DISPENSERS

ORIGINAL SCIENTIFIC PAPER

Snježana Hodžić, Ivana Tešić, Amela Hercegovac, Melisa Šehić, Suada Cipurković✉, Darja Husejnagić, Aldijana Avdić, Suad Širanović

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ABSTRACT:

Nowadays, bottled water has become part of the lifestyle, replacing tap water, and water from freestanding dispensers is increasingly being used. The main goal of this research was to determine the health suitability of bottled water in freestanding dispensers. The research included the microbiological analysis of a total of 100 samples of bottled water from freestanding dispensers. The samples were mostly taken in the wider area of the city of Doboj (doctor's offices, shops, public buildings), and one dispenser in the city of Tuzla. Water samples were taken twice, in the period from April to June 2022. Water samples from 6 different manufacturers (Vivia, Kristal, Nevra, Gora, Aqua doria, Aqua team) were analyzed. 9% of water samples (9/100) were microbiologically correct. Microbiologically defective samples contained a higher total number of bacteria at 22°C and 37°C, as well as a higher number of coliform bacteria than the maximum allowed values. No significant differences in microbiological quality were found between older and/or recently installed water dispensers, as well as in terms of environmental conditions, while visible differences were observed between dispensers that were regularly hygienically maintained.

KEYWORDS: dispensers; bottled water; coliform bacteria; biofilms; total bacteria count

INTRODUCTION

During the past decade, in many regions of the world there has been a significant increase in the consumption of drinking water obtained from various sources, instead of tap water. One of these alternative sources is water from dispensers, which are popular in office buildings, medical offices and commercial shops. Dispensers are often presented as systems that are able to improve some water characteristics and are easy to use and maintain [1]. The quality of such a water source is questioned and has the potential to cause waterborne epidemics, especially in sensitive and immunocompromised individuals [2]. Bacteria present in bottled water can multiply due to high ambient temperatures. It is also possible for pathogens to enter bottled water through contaminated dispensing systems. Freestanding bottled water dispensers are tanks where water is stored in a bottle. As the water from the dispenser is discharged, the bottle is filled with room air that has its own transient microbiota. The volume of potentially contaminated air increases over time. Until the moment of replacement and installation of a new one, the bottle could have up to 19 liters of air [2]. Water contains different nutrients: organic substances such as carbohydrates, fats and proteins, and inorganic substances such as calcium, potassium, magnesium, iron, manganese [3]. Nutrient concentration affects the concentration of microorganisms in water, and the higher their concentration, the higher the concentration of microorganisms [4]. Pathogens can get into bottled water if faucets are not properly cleaned or maintained, which is a major drawback of freestanding dispensers. Various inorganic and organic substances present in bottled water provide favorable conditions for the development of microorganisms. Although water itself is not a rich source of nutrients, bacteria are incredibly resourceful in their ability to use them. Certain types of bacteria, such as *Pseudomonas aeruginosa*, can feed on components found in plastic packaging, as well as on rubber caps present in the dispenser [5], bacteria can attach to the inside of the bottle and multiply. After the contact of bacterial cells with the substrate, changes in gene expression occur, and the extracellular matrix begins to form and biofilms are formed [6]. Biofilms can be defined as highly structured and complex communities of bacteria connected by an extracellular matrix that they secrete themselves [7]. Given that the use of bottled water dispensers is very common, their

improper maintenance can pose a health risk, especially for people with a weaker immune system. It is important to have insight into the suitability of drinking water from freestanding dispensers and thus point out the importance of regular sanitization in order to achieve an optimal state of chemical and microbiological quality of drinking water consumed through dispensers.

MATERIALS AND METHODS

A total of 100 samples of bottled water from 6 different producers Vivia, Kristal, Nevra, Gora, Aqua doria, Aqua tim were microbiologically analyzed, which in the paper are marked with alphabetical letters (A, B, C, D, E, F), from freestanding dispensers in the area of the city of Doboj and its surroundings (doctor's offices, shops, public buildings), as well as a water dispenser installed at the Faculty of Science and Mathematics of the University of Tuzla. Samples for determining microbiological parameters were taken in sterile glass bottles, volume 1L with a metal cap. A cold chain was provided for all collected samples during transport and they were stored in refrigerators at a temperature of ± 4°C. The samples were analyzed within 24 hours. Sampling was carried out twice, in the period from April to June 2022. The first (I) sampling was the day after the installation of the balloon, and the second (II) sampling of the bottled water was performed at the end of the consumption of the water from the balloon. The time period from I and II measurements was 6-12 days. The volume of balloons on freestanding dispensers was 18.0L in all dispensers, except for one, whose balloon volume was 15L. The analysis was carried out in the Laboratory for Genetics and Microbiology of the Faculty of Science and Mathematics, University of Tuzla.

MEMBRANE FILTRATION METHOD

A standard membrane filtration technique was used to estimate the abundance of the bacterial population in water with a low content of suspended particles. Before microbiological testing, the work surfaces were disinfected with 70% ethanol. After each new sample, the funnel of the membrane filtration device was sterilized by immersing it in a beaker with 70% ethanol, after which the inner sides and edges of the funnel and the support for the filter paper were burned with a gas burner. A sample volume of 100 mL was filtered through a sterile membrane filter with a pore diameter of 0.45 μm, and placed on a nutrient medium and incubated at the appropriate temperature. After incubation, the grown colonies were counted and the number of colonies per 1 mL of water sample was calculated.

METHOD FOR DETERMINING THE TOTAL BACTERIA COUNT IN 1ML AT 22°C AND 37°C

Determination of the total bacteria count (TBC) was performed according to the BAS EN ISO 6222:2010 standard [8]. The method is suitable for microbiological examination of all waters, and refers to the determination of living microorganisms in water by counting colonies formed inside or on the surface of the nutrient medium after aerobic incubation at temperatures of 22°C and 37°C. The number of microorganisms is determined using the standard pouring technique (pour plate) with the use of samples from decimal dilutions. The number of microorganisms was determined by incubating Petri plates at two different temperatures, 22°C and 37°C, for 24 hours. The colonies present on each plate were counted, and the CFU per mL of sample were calculated for each incubation temperature. The number of aerobic heterotrophs, as CFU/mL, is calculated according to the formula:

$$C_s = Z/V_{tot} \; x \; V_s$$

where Cs is the number of formed colonies in the tested volume, Z is the sum of the counted colonies on solid substrates with or without membrane filters resulting from dilution d1, d2, or resulting from special volumes of the test sample (initial sample or dilution), Vs - the volume that is chosen to express the concentration of microorganisms in the sample, Vtot - calculated total volume of the initial, original sample that was seeded on solid substrates and whose colonies were counted.

DETECTION AND DETERMINATION OF THE NUMBER OF COLIFORM BACTERIA AND *E.COLI*

Determination of the presence and abundance of coliform bacteria and *E. coli* using the membrane filtration technique was carried out according to the BAS EN ISO 9308-1:2015 standard [9]. The method is based on membrane filtration of a 100 mL water sample, incubation of the concentrate after membrane filtration, on Coliform Chromogenic Agar (CCA), at a temperature of 37°C, and colony assessment after a confirmatory test. After incubation, all colonies that give a positive β-D-galactosidase and β-D-glucuronidase reaction are counted. The appearance of a dark blue to purple color is understood as the presence of *E. coli*. All colonies that give a positive β-

D-galactosidase reaction (pink to red color) are then counted as likely non-*E.coli* coliforms. To confirm probable coliform bacteria other than *E. coli*, an oxidase test is performed. The number of *E.coli* and coliform bacteria present in 100 mL of the sample is calculated from the total number of confirmed colonies from the membrane filter. The coliform count is the sum of all oxidase negative pink to red colonies plus all dark blue to purple *E.coli* colonies.

DETECTION AND DETERMINATION OF THE NUMBER OF INTESTINAL ENTEROCOCCI

Detection and determination of the number of intestinal enterococci using the membrane filtration technique was carried out according to the standard BAS EN ISO 7899-2:2003 [10]. The detection and determination of the total number of intestinal enterococci is based on the filtration of a 100 mL water sample through a sterile membrane filter with pores of 0.45 μm. The filter is then placed on the surface of SBA (Slanetz Bartley Medium) and incubated (24h at 37°C), after which chestnut, red or pink colonies are counted, as typical colonies. The detection of enterococci is carried out by transferring the filter with colonies to a plate with Bile Aesculin Azide Agar, which is heated to 44°C, and incubating for 24 hours at 44±0.5°C. After incubation, all dark brown or black colonies are counted as enterococci colonies.

DETECTION AND QUANTIFICATION OF *PSEUDOMONAS AERUGINOSA*

Detection and determination of the number of *P. aeruginosa* using the membrane filtration technique was carried out according to the standard BAS EN ISO 16266:2009 [11]. The method is based on membrane filtration of a certain volume of water sample, incubation of the concentrate after membrane filtration on a certain selective medium (*Pseudomonas agar* base/CN - agar), at a temperature of 37°C, and colony assessment after a confirmatory test. After the required incubation (24h), all blue-green colonies are counted as confirmed *P. aeruginosa* colonies. After that, the filter is viewed under a UV lamp and all colonies that fluoresce are counted, as probable *P. aeruginosa* colonies, which are then proven with Acetamide broth. All red-brown pigmented colonies are also counted, as probable *P. aeruginosa* colonies, which are confirmed by Oxidase test, Acetamide broth and King's B medium. The growth is read after 22±2 hours due to the possibility of overgrowth and merging of colonies, which can happen with a reading of 44±4 hours.

RESULTS

During this research, 50 bottles of bottled water from freestanding dispensers, from six different manufacturers, were analyzed through two measurements (100 samples in total). Bacteria from the genus *Enterococcus sp., E. coli and P. aeruginosa* were not isolated in any sample. The presence of aerobic mesophilic and psychrophilic bacteria (total number of bacteria at 22°C and 37°C) and coliform bacteria was recorded in the water samples.

TOTAL NUMBER OF BACTERIA AND COLIFORM BACTERIA IN SAMPLES OF BOTTLED WATER IN FREESTANDING DISPENSERS OF MANUFACTURER A

The measured values of the total bacteria count (TBC) in a milliliter of the sample (CFU/mL) of manufacturer A was from 3.78×10^2 to 5.05×10^2 at a temperature of 22°C, while at a temperature of 37°C these values ranged between 2.25×10^2 and 1.83×10^3. Coliform bacteria were not isolated. The sample analysis indicates that the TBC exceeds the Maximum Allowed Concentration (MAC) specified in the Official Gazette of Bosnia and Herzegovina 30/12. The MAC for TBC is set to 100 colonies at 22°C and 20 colonies at 37°C, with no presence of other coliform bacteria [12]. One sample of manufacturer A was analyzed.

TOTAL NUMBER OF BACTERIA AND COLIFORM BACTERIA IN SAMPLES OF BOTTLED WATER IN FREESTANDING DISPENSERS OF MANUFACTURER B

Determination of microbiological parameters of bottled water in freestanding dispensers of manufacturer B included 7 samples. The dispenser of the mentioned manufacturer was not regularly hygienically maintained. The measured values of the total bacteria count in a milliliter of sample (CFU/mL) of producer B were from 3.1×10^1 to 1.40×10^4 at a temperature of 22°C, while at a temperature of 37°C these values ranged between 1.9×10^1 and 1.95×10^4. Coliform bacteria were not isolated. All analyzed samples show that the TBC is significantly higher compared to the maximum allowed concentrations.

TOTAL NUMBER OF BACTERIA AND COLIFORM BACTERIA IN SAMPLES OF BOTTLED WATER IN FREESTANDING DISPENSERS OF MANUFACTURER C

Determination of microbiological parameters of bottled water in freestanding dispensers of producer C included 11 samples. The dispensers of the mentioned

manufacturer were not regularly hygienically maintained, with the exception of two (samples 14 and 17). The measured values of the total bacteria count in a milliliter of the sample of producer C were from 2.2 x 10^1 to 1.27 x 10^4 at a temperature of 22°C, while at a temperature of 37°C these values ranged between 1.2 x 10^1 and 2.21 x 10^1. Coliform bacteria were detected in two samples and their values ranged from 1.34 x 10^2 to 5.45 x 10^2. Analysis of the samples shows that the TBC and coliform bacteria are significantly higher than MAC except for samples 14 and 17, which are microbiologically correct.

TOTAL NUMBER OF BACTERIA AND COLIFORM BACTERIA IN SAMPLES OF BOTTLED WATER IN FREESTANDING DISPENSERS OF MANUFACTURER D

Determination of microbiological parameters of bottled water in freestanding dispensers of manufacturer D included 10 samples. The dispensers of the mentioned manufacturer were not regularly hygienically maintained, with the exception of four (samples 22, 23, 26 and 29). The measured CFU/mL values of manufacturer D ranged from 6 to 3.80 x 10^4 at a temperature of 22°C, while at a temperature of 37°C these values ranged between 3 and 3.06 x 10^4. Coliform bacteria were detected in two samples and their values ranged from 4 to 4.40 x 10^3. The analysis of the samples shows that the TBC and coliform bacteria are significantly higher compared to the MAC, with the exception of samples 22, 23, 26 and 29, which are microbiologically correct.

TOTAL NUMBER OF BACTERIA AND COLIFORM BACTERIA IN SAMPLES OF BOTTLED WATER IN FREESTANDING DISPENSERS OF MANUFACTURER E

Determination of microbiological parameters of bottled water in freestanding dispensers of producer E included 11 samples. The dispensers of the mentioned manufacturer were not regularly hygienically maintained, with the exception of one (sample 35). The measured values of the total bacteria count in a milliliter of the sample of manufacturer E were from 3.4 x 10^1 to 6.30 x 10^4 at a temperature of 22°C, while at a temperature of 37°C these values ranged between 2 and 4.58 x 10^4. Coliform bacteria were detected in six samples and their values ranged from 1 to 1.08 x 10^4. The analysis of the samples shows that the TBC and coliform bacteria are significantly higher than the MAC, except for sample 35, which is microbiologically correct.

TOTAL NUMBER OF BACTERIA AND COLIFORM BACTERIA IN SAMPLES OF BOTTLED WATER IN FREESTANDING DISPENSERS OF MANUFACTURER F

Determination of microbiological parameters of bottled water in freestanding dispensers of manufacturer F included 10 samples. The dispensers of the mentioned manufacturer were not regularly hygienically maintained, with the exception of two (samples 43 and 45). The measured values of the total bacteria count in a milliliter of the sample from manufacturer F were from 6 to 2.19 x 10^4 at a temperature of 22°C, while at a temperature of 37°C these values ranged between 4 and 5.88 x 10^4. Coliform bacteria were detected in five samples and their values ranged from 1 to 7.10 x 10^3. The analysis of the samples shows that the TBC and coliform bacteria are significantly higher than the MAC, except for samples 43 and 45, which are microbiologically correct.

A SUMMARY OF THE RESULTS OF THE MICROBIOLOGICAL ANALYSIS OF BOTTLED WATER

Table 1. Summary of the number of defective samples of bottled water from freestanding dispensers of different manufacturers.

	First sampling (50 samples)				Second sampling (50 samples)		
Total number of analyzed samples		TBC at 22 °C	TBC at 37 °C	Coliform bacteria	TBC at 22 °C	TBC at 37 °C	Coliform bacteria
Manufacturer A	1	1	1	0	1	1	0
Manufacturer B	7	5	6	0	6	7	0
Manufacturer C	11	7	9	2	9	9	2
Manufacturer D	10	6	6	2	6	6	2
Manufacturer E	11	8	9	5	9	10	6
Manufacturer F	10	7	7	6	8	8	6
Total number of defective water samples	41/50	34	38	12	39	41	16

Out of a total of 100 analyzed samples of bottled water from freestanding dispensers (I and II sampling), 9% of the samples were bacteriologically correct.

Table 1 shows the number of defective samples of bottled water from freestanding dispensers from different manufacturers. The samples showed an increased total bacteria count and coliform bacteria (over MAC).

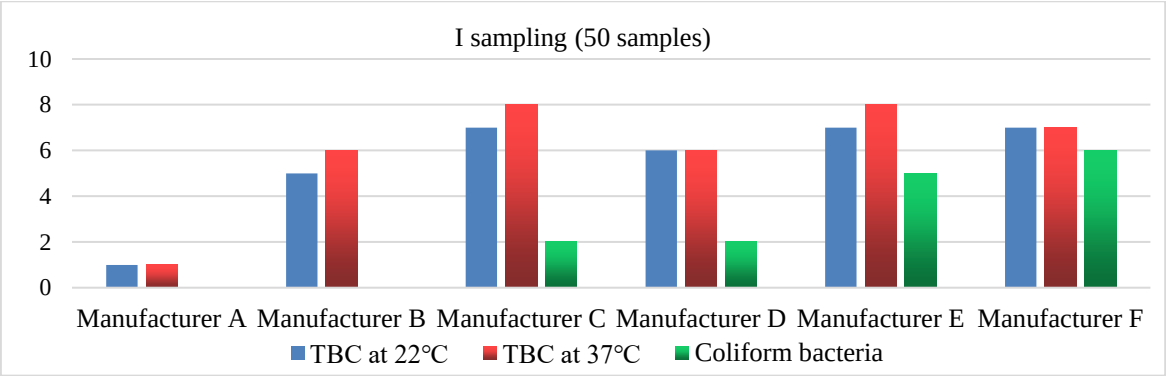

Figure 1. Summary of the number of defective samples of bottled water from freestanding dispensers during the first sampling.

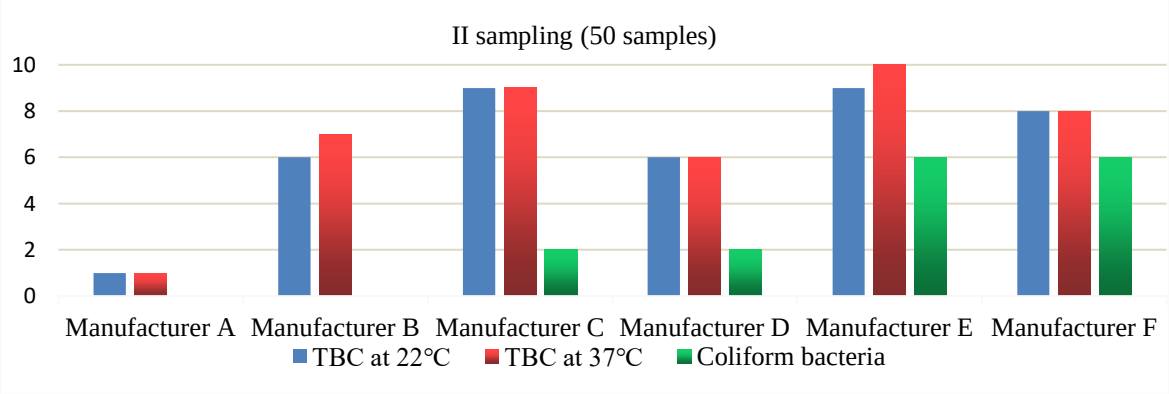

Figure 2.. Summary of the number of defective samples of bottled water from freestanding dispensers during the second sampling.

The first sampling (Figure 1) included 50 samples during which TBC was detected at 22°C significantly higher than MAC from 34 samples, and significantly higher TBC at 37°C from 38 samples, while coliform bacteria were detected in 15 samples. All the mentioned samples were microbiologically defective, i.e. 76% (38/50). The second sampling (Figure 2) included 50 samples during which TBC was detected at 22°C significantly higher than MAC from 39 samples, and significantly higher TBC at 37°C from 41 samples, while coliform bacteria were detected in 16 samples. All the mentioned samples were microbiologically defective, i.e. 82% (41/50)

DISCUSSION

To our knowledge, very few studies have been conducted on the microbiological quality of bottled water from dispensers. Testing of the quality of bottled water in freestanding dispensers began in 2005 by the German Federal Institute for Risk Assessment. After a microbiological analysis of all publicly available dispensers (799), the results indicated that a third of the water samples were contaminated with bacteria [13]. The results in our research show a high percentage of defective samples already after the first sampling (the day after the balloon placement). The bacteriological quality of the water at the end of the balloon was worse compared to the water at the very installation of the freestanding dispenser balloon. Such results were expected considering the initial number of bacteria, as well as the fact that during the discharge of water from dispenser, the bottle is filled with air from the room, which can also be contaminated with various microorganisms. Over time, the volume of potentially contaminated air in the bottle increases. Even running water does not wash away microorganisms, because bacteria in the form of biofilm adhere to the exit openings and can spread even against the direction of flow and continue to

multiply in the water. The results in this research show that the surfaces of the bottle and the dispenser favored the excessive growth of bacteria and the formation of biofilms. In microbiologically defective samples, an increased total bacteria count and coliform bacteria (via MAC) was found. Coliform bacteria are the most suitable group of indicator bacteria for evaluating the hygienic quality of water [3]. *Enterococcus spp., P. aeruginosa* and *E. coli* were not detected in any of the 100 samples of bottled water from freestanding dispensers. Absence of *Enterococcus spp*. and *E. coli*, which are considered to be indicators of fecal contamination, makes the water satisfactory and safe without health implications [13]. Observing the results for the mentioned parameters, regardless of the institution where the dispenser is installed and which manufacturer is represented, it is clearly observed that the concentration of bacterial cells increases with the reduced volume of water in the balloon. Also, when water is used less often from the balloon, there is an increased concentration of bacteria. As an example, we can take sample number 4 of manufacturer C, where a period of 7 days passed from the first to second sampling, and sample number 6 of manufacturer D, where this period was 12 days. No significant differences were found in the number of tested bacteria between older and/or more recently installed water dispensers, as well as in terms of environmental conditions. The environmental conditions of the rooms where the freestanding dispensers were installed (temperature, light) did not affect the number of bacteria. The devices were not placed in illuminated places, and the temperature in all locations was approximately uniform. Visible differences were observed between dispensers that were regularly and those that were occasionally hygienically maintained. Samples that were microbiologically correct were taken from dispensers that are disinfected every 3 to 6 months. Water coolers must be thoroughly cleaned and disinfected to prevent biofilm formation. It has also been proven that disinfection of microfiltered water dispensers with hydrogen peroxide allows obtaining water with TBC levels in accordance with drinking water regulations (≤100 CFU/ml) [14].

CONCLUSION

Out of a total of 100 bottled water samples analyzed, 9 samples were microbiologically correct (9%). In the bottled water samples of all tested manufacturers, the presence of the total bacteria count above the MAC was recorded in 82% and coliform bacteria in 32% of the samples.

Enterococcus sp., Escherichia coli and *Pseudomonas aeruginosa* were not isolated in any water sample. No significant differences were found in the number of tested bacteria between older and/or recently installed water dispensers. Water samples taken from dispensers that are regularly hygienically maintained are microbiologically correct. The results highlight the importance of a periodic disinfection procedure (every 3 to 6 months) of the water cooler monitoring system, in order to keep the level of microbial contamination under control.

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THE ANTIOXIDANT POTENTIAL OF THE CHOKEBERRY (*ARONIA MELANOCARPA L.*) JUICES FROM BOSNIA AND HERZEGOVINA

PROFESSIONAL PAPER

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ABSTRACT:

In addition to their undeniable importance for industry, natural plant products are widely used in the production of functional food, which, in addition to satisfying nutritional properties, also exhibits certain pharmacological and physiological effects on human health. Chokeberry fruit and products are considered to be excellent sources of polyphenolic compounds. A large part of polyphenolic compounds from aronia berries is found in the juice, but the flesh of the berry that remains behind in the juice production process is also rich in these bioactive compounds. The aim of the conducted research was to examine the antioxidant potential of the chokeberry juices from Bosnia and Herzegovina. The antioxidant potential of chokeberry juices in this study was tested by the ferric reducing antioxidant power (FRAP) and total phenol content (FC). Tests were carried out in other juices for the same parameters in order to compare the obtained values. The research results show that the values of FRAP and total phenols in chokeberry juices vary (20-63 mmol Fe II/L; 850-4930 mg GAE/L), but are significantly higher than in other analyzed juices (7-25 mmol Fe II/L; 220–1265 mg GAE/L). By comparing the content of total phenols and FRAP values in pasteurized and unpasteurized chokeberry juice samples, it can be concluded that they are higher in the sample prepared by the pasteurization process. Statistical parameters show that the linear correlation between the total phenols and the FRAP values of chokeberry juices (r=0,964 ; p <0,001) and other analysed juices (r=0,960 ;p <0,001) is statistically significant.

KEYWORDS: chokeberry (*Aronia melanocarpa L.)* juice, antioxidant potential, FC, FRAP

INTRODUCTION

Nowadays, we struggle day by day with diseases related to lifestyle, unhealthy diet and insufficient physical activity. Nature has given us hundreds of different antioxidants that protect our body from the oxidation process, which we can find in fruits, vegetables, grains, etc. In the last few years, the world's scientific community has focused on the research of natural biomolecules. Among the many plant species rich in antioxidants, berries stand out.

Aronia is a genus of deciduous shrubs native to the eastern parts of North America. Indians are also mentioned as the first users, who noticed the therapeutic properties of this plant and allegedly used it to make teas. Its application has spread to the territory of Russia and European countries. Thanks to the medicinal effect of its chemical components, aronia is increasingly used in the food industry in the production of: juices, teas, jams, wine and as a natural food colouring. Researching the chemical composition and biological effects of aronia has become popular in recent years. Numerous studies have shown that moderate consumption of aronia products has a beneficial effect on human health. The chemical composition of chokeberry fruit is directly related to its biological activity and potential use in the therapy of many diseases[1]. These effects are mainly attributed to chokeberry phenolic compounds.

In a study where 143 different plant species were analysed for polyphenol content, the highest proportion was found in chokeberry. The authors associate the content of anthocyanins and organic acids with the type and maturity of the fruit. The authors point out that the anthocyanin content can increase between mid-August and mid-September, which means that the proportion of polyphenolic compounds is influenced by: climate, harvest time and chokeberry type [2]. When determining the concentration of polyphenolic compounds, the chosen method of analysis can also affect it.

In addition to polyphenols, chokeberry fruits also contain other bioactive compounds: vitamins, minerals, carotenoids, pectins, organic acids, proteins

and carbohydrates. Compared to other berries, chokeberry is one of the richest sources of carotenoids. Among these ingredients, there are vitamins with antioxidant activity, vitamins C and E. Aronia berries have a higher content of polyphenols (especially anthocyanins) than most other fruits. The high content of polyphenols is associated with a very high antioxidant activity. Arginine, tyrosine, histidine, lysine, cysteine, α-alanine, asparagine, serine, glutamic acid and threonine are some of the amino acids in chokeberry. Most amino acids, including essential ones, are found in pomace. Chokeberry pomace is a rich source of dietary fiber, which makes up about 70% of the dry matter [3]. Aronia fruits and products are potentially rich sources of: K, Ca, P, Mg, Na, Fe and Zn [4].

Thanks to the presence of numerous bioactive compounds, primarily polyphenolic compounds, the following properties of chokeberry have been proven and confirmed: antioxidant, antimutagenic, anti-inflammatory, antimicrobial, cardioprotective [5-11].

EXPERIMENTAL

MATERIALS

In order to test the antioxidant activity of chokeberry juice, 13 samples from different geographical origins were collected. One sample of juice from the following locations was analysed: Bijeljina, Srebrenica, Tuzla, Livno, Brčko, Gornja Tuzla, Novi Travnik (pasteurized), Novi Travnik (unpasteurized), Sanski Most, Kalesija, Tešanj, Bosanska Gradiška and Dokanj.

In order to compare the total phenols and FRAP values of chokeberry juices with other juices, the following samples were taken for analysis: blackcurrant, blueberry, blackberry, beetroot, tomato and plum. All juices are produced in Bosnia and Herzegovina.

METHODS

DETERMINATION OF TOTAL PHENOLS

To determine the concentration of total phenols in chokeberry juice samples, 20μL of sample solution (10% v/v), 1580μL of distilled water and 100μL of Folin-Ciocalteu reagent were pipetted. After one minute, 300μL of sodium carbonate solution (200g/L) was added to this mixture, and the mixture was then incubated for 2h at room temperature. Absorption was measured spectrophotometrically (Cecil CE 2021 UV-VIS Spectrophotometer) at a wavelength of 765 nm [12].

The results were recalculated according to the calibration curve for Gallic acid:

$$y=0.00189x–0.00929$$

where y is the absorbance at 765 nm and x is the concentration of Gallic acid expressed in mg GAE/L; R^2=0,98646.

DETERMINATION OF FRAP

To determine the reducing antioxidant power of the tested juices, the method of the reducing property of iron was used [13]. For analysis, 200 μL of sample (10% v/v) and 1.8 mL of FRAP reagent were pipetted out. The FRAP reagent was prepared from 6 mL of a solution of TPTZ (10 mmol/L) in hydrochloric acid (40 mmol/L) and 6 mL of a solution of $FeCl_3 \times 6H_2O$ (20 mmol/L) mixed with 60 mL of acetate buffer (300 mmol/L; pH=3.6). The mixture was then incubated for 10 min at 37°C. As a result of the reaction of the antioxidants present in the sample and the FRAP reagent, coloured solutions are obtained. After that, the absorption is measured spectrophotometrically at a wavelength of 593 nm.

FRAP values were calculated according to the calibration curve for $FeSO_4 \times 7H_2O$:

$$y=0.47268x-0.04447$$

where y is the absorbance at 593 nm and x is the concentration of $FeSO_4 \times 7H_2O$ expressed in mmol FeII/L; R^2=0,98939.

STATISTICAL ANALYSIS

The direction and magnitude of corelation between variables was done using analysis of variance (Minitab relase 13,32; 2000; statistical software) and quantified by the correlation factor „r". The p-values less than 0,001 were considered statistically significant.

RESULTS AND DISCUSSION

The highest concentration of total polyphenols was measured in a sample of chokeberry juice from the Dokanj and is 4930.105mg/L GAE. The following are the concentrations measured in samples from Brčko > Tuzla > Bosanska Gradiška > Sanski Most > Srebrenica > Kalesija > Livno > Bijeljina > Novi Travnik (pasteurized) > Gornja Tuzla > Novi Travnik (unpasteurized), while the lowest concentration of total polyphenols was measured in a sample of juice in Tešanj (850.211mgGA/L) (Table 1).

Table 1. Total phenols concentration of chokeberry juice samples

	Geographical origin of the chokeberry sample	Concentration (mg GAE/L)
1.	Bijeljina	2203.121
2.	Srebrenica	3476.137
3.	Tuzla	4087.248
4.	Livno	2614.232
5.	Brčko	4421.111
6.	Gornja Tuzla	1853.386
7.	Novi Travnik (pasteurized)	2054.440
8.	Novi Travnik (unpasteurized)	1484.603
9.	Sanski Most	3520.052
10.	Kalesija	2909.470
11.	Tešanj	850.211
12.	Bosanska Gradiška	3769.259
13.	Dokanj	4930.105

Table 2. Total phenols concentration of other analysed juices

	Sample	Concentration (mg GAE/L)
1.	Black currant	1265.555
2.	Blueberry	1171.904
3.	Blackberry	757.089
4.	Beet	974.550
5.	Tomato	268.730
6.	Plum	227.989

Of all the other analysed juices, the highest concentration of total phenols was measured in black currant juice, followed by: blueberry > beet > blackberry > tomato and plum (Table 2). In an analysis that included different chokeberry varieties from two growing seasons, a wide range of total phenol values was observed, ranging from 8563.8 mg/kg GAE to 12557.7 mg/kg GAE [14]. Some studies also cover different growing seasons and report total phenol values ranging from 8834mg GAE/L to 11093mg GAE/L [15].

The range of different values of the content of total phenols in the literature may be a consequence of differences in sample preparation methods, applied analytical procedures, different storage, but also different chokeberry varieties [16]. It should also be borne in mind that in the case of determining total phenols, different ways and conditions of growing plants must be taken into account. The content of total phenols and individual polyphenolic compounds of different types of berries depends on many factors, such as: growing conditions (location, growing techniques, ripening stage), processing and storage. Large variations and discrepancies in the total content of phenols during different growing seasons are the result of different air temperatures, sunlight and rainfall intensity [17]. By comparing the content of phenols in pasteurized and unpasteurized juice samples, it can be concluded that it is higher in the sample prepared by the pasteurization process.

Heat treatment is the most commonly used conservation technique. Pasteurization is one of the methods of heat treatment and takes place at a temperature of 70-80°C. Although thermal treatments cause some harmful effects, such as the loss of some nutrients (vitamin C), it also exhibits many beneficial effects, such as improving the extraction of total phenols and extending the shelf life. The results of research carried out by Kim et al. show that thermal processing can help preserve nutritional values and slow down changes in the quality of fruits and vegetables related to phenols [18]. Also, some other examples involving the improvement of the overall phenolic composition were published earlier in research [19][20]. According to the research of the mentioned authors, the content of total phenols in the samples increases after pasteurization, which is in accordance with this research. Based on the obtained results, it can be concluded that the growth of bioactive compounds in chokeberry juice occurs due to accelerated extraction by the pasteurization process. An increase in temperature leads to the degradation of cell walls, which accelerates the release of cell contents. By comparing the content of total phenols in chokeberry juice samples with the content of total phenols in other analysed juices, it can be concluded that the content of phenols is significantly higher in favour of chokeberry (Table 2). Data presented by other authors show that chokeberry has a higher concentration of phenols compared to other berries, such as blackcurrants, blackberries and raspberries, which is in accordance with the research conducted [21].

This research included the determination of the ferring reducing antioxidant power (FRAP) of chokeberry juice as well as other juices. The results of the measured values are presented in tables 3 and 4.

The highest FRAP values was measured in a sample of chokeberry juice from the Dokanj and is 63.4947 mmolFeII/L. A slightly lower concentration was shown by the sample from the Brčko (60.1852 mmolFeII/L), while the lowest was measured in the juice sample from Tešanj (20.0721 mmolFeII/L) (Table 3). The differences in the obtained results may be related to the method of cultivation and processing

of chokeberry In order to fully explain the obtained results, further research is needed that focuses on different processing methods, including: crushing, thermal treatments and drying.

Table 3. Antioxidant capacity of chokeberry juices

	Geographical origin of the chokeberry sample	Concentration (mmol FeII/L)
1.	Bijeljina	44.5665
2.	Srebrenica	47.9176
3.	Tuzla	57.6853
4.	Livno	46.5467
5.	Brčko	60.1859
6.	Gornja Tuzla	32.2368
7.	Novi Travnik (pasteurized)	41.6956
8.	Novi Travnik (unpasteurized)	28.4499
9.	Sanski Most	52.4471
10.	Kalesija	47.0777
11.	Tešanj	20.0721
12.	Bosanska Gradiška	56.7502
13.	Dokanj	63.4947

According to research by Jakobek et al., the antioxidant activity of chokeberry juice (DPPH) was stronger than the antioxidant activity of black currant, elderberry, red currant, strawberry, red raspberry and cherry samples [22]. In order to compare the reducing antioxidant power of chokeberry juice with other berry juices, the following juices were analysed: blackcurrants, blueberries and blackberries. It was observed that the majority of chokeberry samples show a much higher FRAP values than the mentioned juices. By comparing FRAP values of chokeberry juice with beet, plum and tomato samples, it was also proven that chokeberry shows a much stronger reducing antioxidant power (Table 4).

Table 4. FRAP values of other analysed juices

	Sample	Concentration (mmol FeII/L)
1.	Black currant	25.5473
2.	Blackberry	19.7040
3.	Blueberry	20.4466
4.	Beet	20.0234
5.	Tomato	9.1810
6.	Plum	7.7424

In order to confirm the relationship between ferring reducing antioxidant power of juices and the content of total phenols, a correlation analysis was performed (Figure 1). It is proved a high correlation between the analysed parameters ($r=0.964$; $p<0,001$). The data are consistent with the results of other researchers and confirm the fact that high concentrations of total phenol contribute to higher reducing antioxidant potential [23].

Correlation analysis of blackcurrant, blackberry, blueberry, beetroot, tomato and plum juice samples also proved a high correlation between FRAP and total phenols content ($r=0.960$; $p<0,01$) (Figure 2).

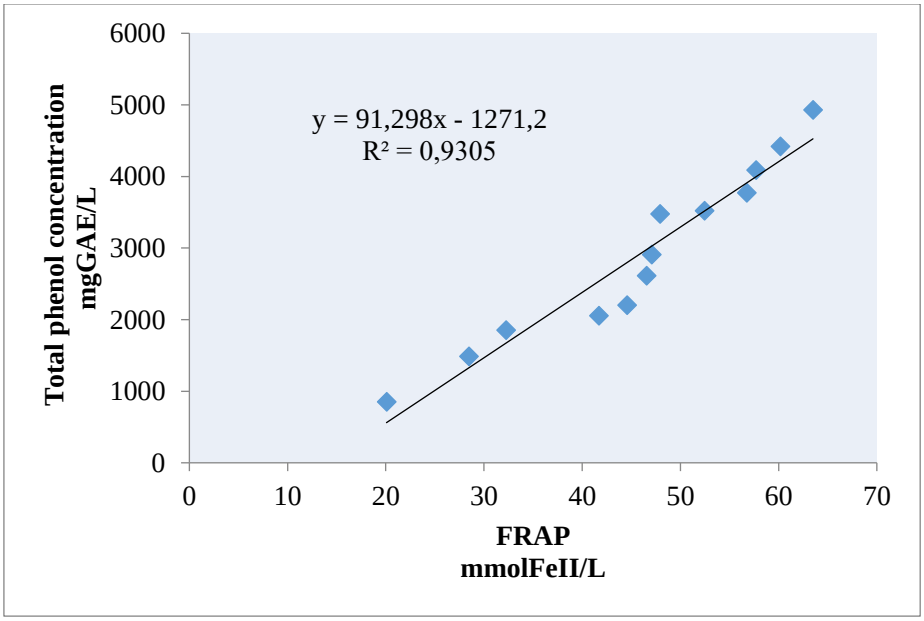

Figure 1. Correlation of FRAP and total phenol concentration of chokeberry juice

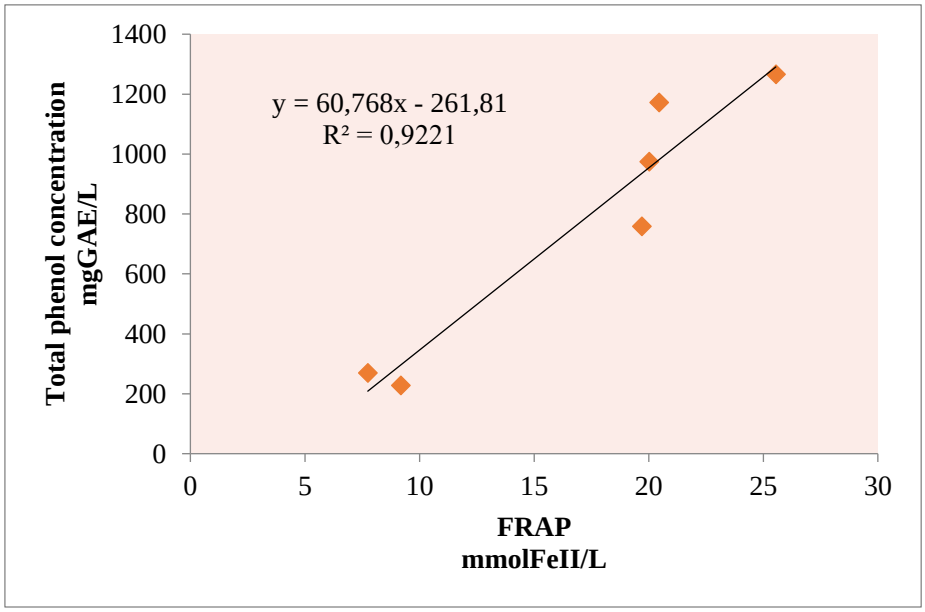

Figure 2. Correlation of FRAP and total phenol concentration of other juices

CONCLUSIONS

The research showed that all chokeberry juices (except for the juice produced in Tešanj) show a much higher antioxidant potential than the other analysed juices (blackcurrant, blueberry, blackberry, beetroot, tomato, plum). No influence of geographical area was observed, but the range of measured concentrations may be due to differences in juice preparation methods and storage methods. In the case of determining total phenols and ferring reducing antioxidant power, different ways and conditions of growing plants, air temperature, sunlight, intensity of precipitation must be taken into account. We are of the opinion that the processing method of chokeberry juice played a crucial role in this research. By comparing the content of total phenols and FRAP values in pasteurized and unpasteurized chokeberry juice, it was observed that the analysed parameters were higher in the sample prepared by the pasteurization process. The growth of bioactive compounds in the juice occurs due to the accelerated extraction of phenols at higher temperatures, which can be explained by the degradation of cell walls, where the increase in temperature accelerates the release of cell contents. There is a highly significant correlation between FRAP values and total phenol content of chokeberry juices and other analysed juices.

The conducted research gives importance to the natural resources of Bosnia and Herzegovina in the area of food quality. Since this kind of research has not been done in chokeberry juice samples from Bosnia and Herzegovina, it undoubtedly represents a contribution to the analysis and promotion of domestic products.

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APPLICATION OF THE PRINCIPLES OF GREEN CHEMISTRY IN THE PLASTIC RECYCLING INDUSTRY: A CASE STUDY

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:
The subject of this research was to verify the feasibility of implementing green chemistry principles within the business company „Omorika Reciklaža" Ltd. situated in Johovac near Doboj, Bosnia and Herzegovina. The objective of this study was to conduct a detailed assessment of the company's facilities and operations, using environmental audits, to identify technological processes (production lines), energy and waste flows, capacities, product range, and other pertinent factors crucial for the application of green chemistry principles. Special emphasis was placed on analyzing each substance that constitutes a raw material, whether used individually or as part of mixtures. The assessment of safety data sheets involved utilizing the CAS registration numbers of substances from the Chemical Abstracts Service, cross-referenced with the ECHA database (European Chemicals Agency). The outcomes, attained through an exhaustive analysis of each substance, were presented as a "chemical inspection" of the company. Through the analysis of all substances and mixtures in the technological process (chemical inspection), as well as the capacity of production flows, energy and resource flows, wastewater, and waste, the potential for enhancing the technological process was identified. This involved reducing dust levels in the workspace, decreasing electricity consumption (utilizing renewable sources), and substituting particularly hazardous chemicals used in the technological process.

KEYWORDS: green chemistry; chemical safety; SVHC; environmental audit

INTRODUCTION

Green chemistry, according to the definition popularized by Paul T. Anastas, one of the pioneers in the field of green chemistry, is the design of chemical products and processes that reduce or eliminate the use and generation of hazardous substances [1]. Green chemistry applies across the life cycle of a chemical product, including its design, manufacture, use, and ultimate disposal. Green chemistry, or the 12 principles of green chemistry, encompasses techniques from chemistry, engineering, and many other scientific fields, and has spurred entirely new research [2]. The principles of green chemistry can be applied in various fields and industries to enhance sustainability and reduce negative impacts on the environment.

According to the European Association, Plastics Europe AISBL, in 2021, 90.2% of the world's plastic production was fossil-based. Post-consumer recycled plastics and bioplastics accounted for 8.3% and 1.5% of the world's plastic production, respectively [3]. From the mentioned data, the importance of plastic recycling and its positive impact on the environment is evident. However, the industrial plastic recycling process itself can be further improved to have a lesser environmental impact. There are many ways in which the principles of green chemistry can be applied in industry to reduce the negative impact on the environment and human health while simultaneously improving the efficiency of production processes.

On the other hand, according to the World Health Organization (WHO), chemical safety is an approach that encompasses all activities aimed at protecting people and the environment from the harmful effects of chemicals, whether of natural origin or manufactured [4]. This includes measures to reduce human and animal exposure to harmful chemicals, as well as measures to mitigate the damage that can be caused by incidents involving hazardous materials. The goal of chemical safety is to reduce the risk of adverse effects of chemicals on human health and the environment. To apply the principles of green chemistry in an industrial facility, it is necessary to be

familiar with the basic principles of green chemistry. In addition to the principles of green chemistry, knowledge in the field of chemical management (chemical safety) is also essential.

Chemical safety in Bosnia and Herzegovina (BiH) is under the jurisdiction of the entities (Republika Srpska and the Federation of Bosnia and Herzegovina) and the Brčko District of Bosnia and Herzegovina, except in the area of reporting to the European Commission (in accordance with the obligations of the Stabilization and Association Agreement) and customs policies, including the control of the import and export of goods subject to special regimes in trade. Regulations governing chemical safety in BiH are not harmonized, and the adoption of EU regulations in this area is not coordinated. As a consequence, different regulations are applied in BiH, and legal entities operating in the entire country do not have equal status.

In October 2020, the Federation of BiH adopted the Law on chemicals [5], which, among other things, aligns the Federation of BiH with EU regulations governing the field of chemicals. However, to this point, Law on biocidal products has not been adopted, and the subordinate regulations foreseen by the Law on chemicals have not been adopted. Therefore, the transposition of the mentioned EU legislation and conventions is at a low level, which has been recognized as the biggest challenge for the institutions of the Federation of Bosnia and Herzegovina in this area. In the Republika Srpska, there is clear legislation regarding chemical management, stemming from the Law on chemicals [6] and the Law on biocidal products [7], under the jurisdiction of the Ministry of Health and Social Welfare of Republika Srpska. The existing regulations governing chemical safety in Republika Srpska are generally, to a greater or lesser extent, aligned with certain conventions (of which BiH is a signatory) or directives and regulations of the European Union (EU).

EU legislation defines centralized procedures within the EU, which are applicable only to member states. Such provisions cannot be transposed into the legislation of entities and the Brčko District of BiH; instead, adjustments and preparatory actions have been undertaken for their future implementation. In 2006, the EU introduced the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulation [8], which requires companies to provide data showing that their products are safe. This regulation ensures not only the assessment of the hazards of chemicals as well as the risks during their use, but also includes measures to ban or restrict/authorize the use of certain substances [9]. The REACH chemicals regulation is considered a very powerful promoter of sustainable innovation and green chemistry. REACH favors innovative new materials and processes by allowing exemptions from registration for five years for substances used in research and development. REACH is a tool used by the EU to encourage the transition to "green chemistry" in line with environmental policies. The Law on chemicals of the Republika Srpska [6] and the Law on chemicals of the Federation of Bosnia and Herzegovina [5] are harmonized with the REACH Regulation. REACH is currently in the process of revision and changes to the existing REACH regulation are expected. One of the most significant results of REACH is the establishment of the European Chemicals Agency (ECHA).

From the above, it is clear that green chemistry and chemical safety are interconnected as they both ensure human health and environmental protection. Legislation in the European Union (EU) plays a crucial role in this field.

The aim of this research was to analyze the production process, applied technologies, waste streams (wastewater, solid waste, waste gases, etc.), raw materials, chemicals and energy flows. A special aspect is devoted to the analysis of raw materials and chemicals, which includes a detailed analysis of safety data sheets (SDS) to identify hazardous chemicals and assess substances contained in given mixtures (chemicals). The research was conducted at the company "Omorika Reciklaža" Ltd. Johovac, Doboj.

MATERIALS AND METHODS

The application of green chemistry begins with an understanding of the 12 principles of green chemistry [2]. These principles form the basis for the development of green chemistry, which is aimed at designing chemical products and processes that are sustainable, less harmful and contribute to environmental protection. In practice, the production process or its stages can rarely satisfy all 12 principles of green chemistry, at least not completely. Sometimes it is one principle, three or four, but it is important to have them as goals to strive for [2].

The application of some of the principles of green chemistry involves a detailed analysis of the production process, i.e., an environmental audit. An environmental audit is a systematic evaluation of the environmental impact of an organization's operations. In this case, the environmental audit is tailored and conducted to assess the feasibility of implementing green chemistry principles in the company "Omorika Reciklaža" Ltd. An environmental audit can be considered a key tool for implementing green

chemistry principles in a business, and by conducting an environmental audit, the company can assess its impact on the environment in accordance with green chemistry principles. The final result of the environmental audit is a report that contains information about the organization's impact on the environment, identified areas for improvement, as well as recommendations (measures) for the implementation of green chemistry principles.

These measures can encompass the establishment of new policies and procedures related to chemical management, recycling, and reducing the use of harmful chemicals and materials, the utilization of renewable energy sources, as well as waste reduction and greenhouse gas emissions reduction. However, this paper will not present measures concerning energy sources and energy flows. "Energy measures" will be presented in a separate research.

As previously mentioned, special attention is devoted to the analysis of raw materials and chemicals. According to the Law on chemicals, a chemical means a substance and a mixture containing a substance. A substance means a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition. While mixture means a mixture or solution composed of two or more substances and a hazardous chemical is a chemical classified within at least one hazard classes [6].

As part of the environmental audit, a so-called "chemical inspection" was conducted, during which SDSs for twenty-six chemicals (mixtures) were thoroughly analyzed. The assessment of SDSs was conducted using the Chemical Abstracts Service (CAS) numbers of substances that are part of each mixture and the ECHA`s database [11]. Each substance included in the composition of the mixtures used in this company was analyzed. By entering the CAS number or name of the substance into ECHA's database, basic data on the substance (*Substance Infocard*) containing *Substance identity*, *Hazard classification & labeling* and *Properties of concern* were obtained. Also, it is possible to view the *Brief profile* of the substance, where the classification of the substance is specified. ECHA uses a system of chemical classification and labeling based on the EU CLP (Classification, Labelling, and Packaging) regulation [12]. This labeling system utilizes several key elements to clearly and precisely identify the hazards and properties of chemicals.

There are the basic labels used according to the CLP regulation. Pictograms are visual icons that depict hazards associated with chemicals. Examples of pictograms include a flame symbol for flammable chemicals, a skull and crossbones for substances that are harmful or toxic, and others. Hazard statements ("H numbers") include words such as "Flammable," "Carcinogenic," "Toxic," "Irritant," and others, describing specific hazards associated with chemicals. For example, "H225" corresponds to the Hazard Statement "Highly flammable liquid and vapor". Each chemical has an associated Signal Word that emphasizes the severity of the hazard. Examples include "Danger" or "Warning." Precautionary statements provide additional information about hazards and precautionary measures to be taken when handling the chemical. This includes information on safe handling, storage, and disposal (for example, precautionary statement "P264": Wash hands thoroughly after handling). Chemicals are categorized into various Hazard Classes and Categories based on their properties. Examples of hazard classes include "Flammable," "Health Hazard," and "Environmental Hazard".

ECHA, in accordance with the CLP [13], publishes and regularly updates the list of classified and labeled chemicals - CL Inventory [14]. This database contains information on the classification and labeling of substances that have been submitted by manufacturers and importers in the EU. This information is valuable for chemical users as well as regulatory bodies enforcing chemical regulations to ensure the safe use of chemicals in the EU and protect the human health and the environment. The CL Inventory is implemented in the Republic of Srpska through the Rulebook on Chemical Inventory [15]. As of September 25, 2023, there are 6.782 chemicals listed in the Chemical Inventory [16], while there are 227.204 chemicals listed on the European CL Inventory [17].

Substances that can have serious consequences for human health and the environment can be identified as Substances of Very High Concern (SVHC). The list of SVHC substances is maintained by the ECHA and is regularly updated to include new substances identified as SVHC. Due to their potentially harmful impact on health and the environment, SVHC substances are subject to strict regulation in the EU, and their use may be restricted or prohibited. To this end, REACH introduces, among other instruments, an authorization regime for SVHC substances listed in Annex XIV of the Regulation [8].

In 2008, ECHA recorded its first chemical that could potentially harm human health and the

environment. This led to the creation of two lists, the Authorization List [18] and the Candidate List [19], which include all hazardous chemicals. To be placed on the market, specific conditions of use must be prescribed for these chemicals. The goal of these procedures is to reduce the presence of SVHCs in products and transition to safer alternatives. The Candidate List has been transposed into the legislation of the Republic of Srpska as the List of substances that are candidates for inclusion in the list of SVHC (Candidate List) and contains 209 substances (updated on December 22, 2020) [20], while the ECHA Candidate List of SVHC now contains 235 entries [19]. The Law on Chemicals [5, 6] stipulates that the supplier is obliged to provide any distributor or user with information about a particular product if it contains a SVHC in a concentration greater than 0.1%. The information should include the name of the substance that the product contains and should be voluntary for the safe use of that product.

Substances listed on the Candidate List can indeed move to the Authorization List (list for issuing approval) according to Annex XIV of REACH [8]. This means that, after a specified date, companies will not be allowed to place SVHC on the market or use them unless they have been specifically authorized to do so. One of the primary goals of authorization is to gradually phase out SVHCs wherever possible [21]. The Authorization List was transposed into the legislation of the Republic of Srpska through the Rulebook on restrictions and bans of chemicals [22]. The list of substances of particular concern includes 43 substances [23], as of September 25, 2023, while 59 substances are on the Authorization List.

ECHA also provides a database (list) of substances classified as persistent organic pollutants (POPs) – "List of substances subject to the POPs Regulation" and chemicals under preparation for their potential inclusion as POPs – "List of substances proposed as POPs". POPs are organic substances that persist in the environment, accumulate in living organisms and pose a risk to our health and the environment. POPs are regulated worldwide by the Stockholm Convention and the Aarhus Protocol [24]. These international treaties are implemented in the European Union by the POPs Regulation [25].

Companies in the EU are increasingly replacing hazardous chemicals and manufacturing processes with safer chemicals and greener technologies. This business approach can bring significant benefits to companies, the environment, and the health of workers and consumers. Additionally, it can have a substantial positive impact on the implementation of a circular economy [26]. In the company "Omorika Reciklaža" Ltd, a "chemical inspection" was conducted as part of a comprehensive environmental audit to verify whether chemicals listed by ECHA are used in the production process. The company mainly recycles PET packaging and produces several types of semi-finished and finished products made from recycled PET, but also recycles other types of plastic such as polyethylene (PE), polypropylene (PP) and polystyrene (PS).

RESULTS AND DISCUSSION

The audit included a specification of the facility and the processes. Only the block diagrams of the processes for the production of crushed flakes of PET (Figure 1) and the production of PET film for thermoforming (Figure 2) are shown here, i.e. only for those processes in which the implementation of the principles of green chemistry is proposed. Other plant parts or processes as well as energy flows will not be considered here. Therefore, the presented results are the analysis of chemicals ("chemical inspection"), water and wastewater streams, as well as the analysis of the generated waste.

As part of the "chemical inspection", a review and analysis of the contents of 26 SDSs for substances and mixtures used in the company was carried out. Here is a brief overview of the research, where commercial names of substances are not listed for reasons of confidentiality. Table 1 provides an overview of the most commonly used chemicals with the amounts consumed in 2021. In the production process, the largest quantities of chemicals are used in the washing process, specifically sodium hydroxide and detergents. In smaller quantities, appropriate additives are used in the facility to enhance certain product properties. Aerosols in the form of pressurized sprays are used as needed for corrosion removal, lubrication (containing concentrated silicone oil), and surface cleaning. All the mentioned aerosols do not contain persistent, bioaccumulative, and toxic (PBT) substances, very persistent and very bioaccumulative (vPvB) substances, SVHC, or contain them at less than 0.1% w/w in their composition.

Sodium dichloroisocyanurate dihydrate (NaDCC) is used as a disinfectant. This substance can be found in the form of white crystals, powder or tablets. NaDCC is a stable salt that contains chlorine and is soluble in water, forming a solution containing hypochlorous acid and isocyanurate acid. This solution is an effective disinfectant used for water disinfection, including drinking water, swimming pools, public bathrooms, and other facilities where maintaining hygiene is crucial. NaDCC has advantages over other disinfectants like chlorine as it

has a milder odor and is less irritating to the skin and mucous membranes. Additionally, NaDCC can be used in smaller quantities compared to other disinfecting agents, which can reduce its environmental impact. Although NaDCC is considered relatively safe, it is essential to handle it with care as it can be irritating to the eyes and skin and harmful to health in larger quantities. NaDCC is harmful if swallowed (H302), causes serious eye irritation (H319), may cause respiratory irritation (H335), and is very toxic to aquatic life with long-lasting effects (H410). Therefore, special precautions are necessary when handling it, following the SDS.

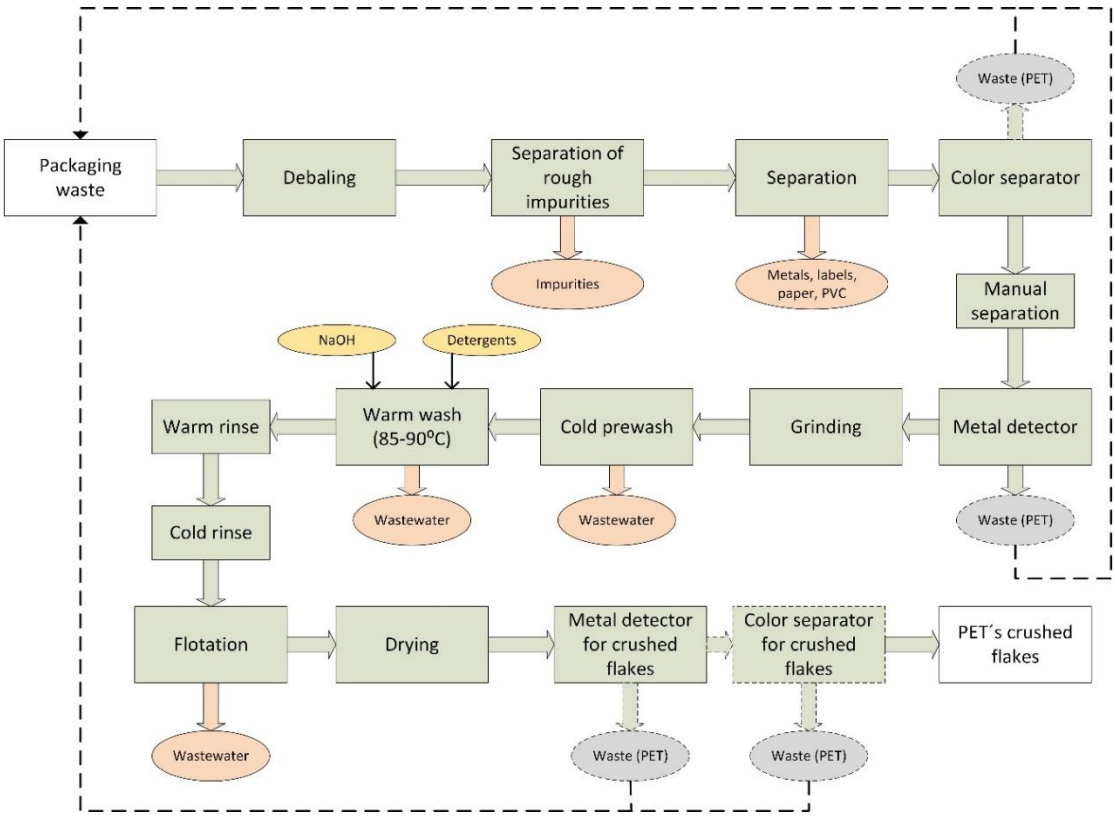

Figure 1. Block diagram of the process for the production of PET´s crushed flakes

To prevent the occurrence of electrostatic electricity in plastic packaging and consumer goods, an effective antistatic agent is used (safe for food contact). It neutralizes the static charge that polymeric materials acquire during production and processing. The antistatic agent can cause severe eye damage (H318) and is toxic to aquatic life (H411). The active ingredient is registered on the positive list for "food grade materials" in accordance with Regulation (EC) No 1935/2004 [27]. To reduce adhesion between surfaces, another additive (antiblock) is used, which does not contain hazardous substances and is safe for use.

The mixture used as an antifoaming agent and flotation aid is a combination of highly efficient substances (nonionic and anionic surfactants) that suppress foam formation and additives that assist in separating PET and HDPE or PP through the flotation process. This mixture is classified as hazardous because it can cause severe eye irritation (H319) and is harmful to aquatic life with long-lasting effects (H412). However, the mixture contains (<0.25% w/w) octamethylcyclotetrasiloxane (D4) (CAS 556-67-2), which is classified as an SVHC according to ECHA due to its PBT and vPvB properties. For this reason, it has been included in the Candidate List for authorization, which means a complete ban on its use can be expected very soon. The same mixture contains (<1% w/w) decamethylcyclopentasiloxane (CAS 541-02-6), known as D5, which is classified as an SVHC substance due to its PBT and vPvB properties and is included on the Candidate List for authorization.

D4 and D5 are also on the List of substances proposed as POPs, which means that they are being prepared for their potential inclusion in the Stockholm Convention. In the Republic of Srpska, D4 and D5 are on the List of substances that are candidates for inclusion in the list of SVHC by Ministry of Health and Social Welfare [20].

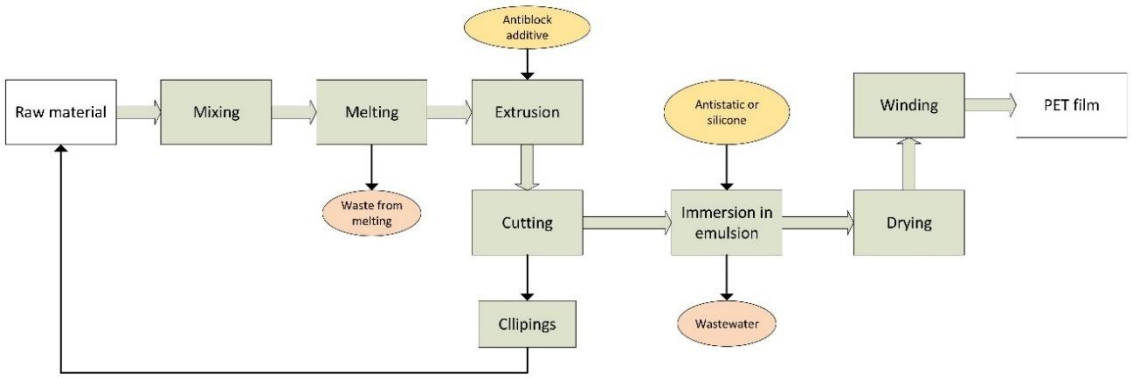

Figure 2. Block diagram of the process for the production of PET film for thermoforming

Table 1. Data on the type and consumption of chemicals

Chemical name	Location of use	Hazard Statement	Consumption in 2021.
Caustic soda	Warm wash process of PET´s crushed flakes (Fig. 1)	Corrosive. Hazards: May be corrosive to metals (H290). Causes severe skin burns and eye damage (H314)	10 - 12 t
Detergent and cleaning agent 1	Warm wash process of PET´s crushed flakes (Fig. 1)	May cause eye damage (H318)	1.44 m^3
Detergent and cleaning agent 2	Warm wash process of PET´s crushed flakes (Fig. 1)	Irritating to skin (H315). Causes eye damage (H318). Harmful to aquatic life (H412)	2 m^3
Antifoam and flotation agent	Flotation (Fig. 1)	Causes severe eye irritation (H319). Harmful to aquatic life (H412). Contains SVHC substances	700 kg
Antistatic	Production of PET foil (Fig. 2)	Causes severe eye damage (H318). Toxic to aquatic life with long-term effects (H411)	0.1 m^3
Antiblock	Production of PET foil (Fig. 2)	It is not a dangerous substance.	100 kg

The cleaning agent is a mixture of nonionic surfactants (ethoxylated isododecanol) and solvents (alcohol) and can cause severe eye damage (H318). Isotridecanolethoxylate (ITO) is a surfactant commonly used in cleaning products and detergents. It is an alcohol with a single side methyl group and a total alkyl chain length of 13 carbon atoms, along with 1-2 polyethylene oxide groups. ITO is a type of surfactant that enhances the detergent's ability to remove impurities from surfaces. This surfactant can be found in various cleaning products and detergents, including laundry, dishwashing, and floor cleaning products. Although it is considered relatively safe, isotridecanolethoxylate can cause eye and skin irritation in concentrations exceeding the recommended dose.

Isotridecanolethoxylates (CAS 69011-36-5) found in the mixture in an amount < 40% m/m are very toxic substances for aquatic life with long-term consequences. They are not on some of the lists that imply imminent authorization. Despite this, some chemical and cleaning product manufacturers are making efforts to replace them with less harmful surfactants. In addition to the mentioned isotridecanolethoxylate, another washing and cleaning agent is also used, which is a mixture of ethoxylates of fatty alcohols, complexing and dispersing agents. It also contains isotridecanolethoxylates in an amount < 40% m/m. The mixture causes skin irritation (H315) and can cause severe eye damage (H318) and is harmful to aquatic life with long-term effects (H412), which requires special care when handling and treating wastewater.

The highest consumption of water is in production of PET's crushed flakes, where water is mainly used for the process of cold and warm washing, rinsing and in the flotation process. By analyzing the wastewater and water streams, special attention was paid to the

washing process in production of PET's crushed flakes (Fig. 1). Table 2 provides an overview of water consumption and the amount of wastewater generated in 2021. Raw water is used in the washing process, without prior treatment. The hardness of raw water and possibly present suspended matter and water turbidity cause inefficient use of chemicals for the washing process, which is why the consumption of chemicals is higher. The proposal is to provide water treatment (demineralization) for the washing process, where a washing mixture containing surfactants is used. In the process of warm washing, caustic soda is added to the water (the final concentration of NaOH is 2%) and three types of detergents (in the final concentration of 1, 3, 5 mg/L). If the water has a high total hardness at a temperature of 85 – 90 °C and this concentration of NaOH, insoluble $CaCO_3$ can precipitate (scale formation) on the walls of the reactor and heaters, which affects the reduction of the efficiency of the heaters and increases energy consumption.

Table 2. Water consumption and the amount of wastewater generated in 2021.

Process	Water consumption	Water type	Wastewater quantity
Washing and rinsing of PET´s crushed flakes (Fig. 1)	5 390 m^3	Raw water	< 5 405 m^3
Flotation (Fig. 1)	1 348 m^3	Raw water	< 1 348 m^3
Immersion of PET foil in emulsion (Fig. 2)	4 m^3	Deionized water	< 4 m^3

During the grinding of waste PET in the production of PET crushed flakes (Fig. 1), there is an emission of PET dust, which is a useful by-product. A part of this dust is collected and sold in the market (approximately 40 tons of PET dust annually), and the rest is waste due to the low efficiency of the dust collection system. Dust collection is carried out through ventilation and bag filters, but this method is not entirely efficient. The audit determined that there is a possibility to improve dust collection by installing new filters on the PET's crushed production line, which could increase the amount of collected dust and reduce the proportion of generated waste.

PROPOSED MEASURES FOR IMPLEMENTING THE PRINCIPLES OF GREEN CHEMISTRY

After the audit was completed, measures were proposed for the implementation of the principles of green chemistry (without measures related to energy flows):

1. Replacement of the mixture used as an antifoam and flotation agent (contains SVHC and substances proposed as POPs),
2. Water treatment (demineralization) for the process of washing PET´s crushed flakes,
3. Improve the dust collection system.

Measure no. 1 represents the fifth principle of green chemistry: Safer solvents and auxiliaries. The average annual consumption of this chemical is 700 kg. By replacing it with a safer alternative, the use of hazards chemicals would be completely avoided, thus protecting human health and the environment. Measure no. 2 represents the first and sixth principle of green chemistry: Waste prevention and Design for energy efficiency. The implementation would reduce the amount of wastewater and the consumption of chemicals for the washing process. The same washing effect would be achieved with a smaller amount of chemicals. Due to the use of NaOH in heated water that has high hardness (raw water), scale deposits occur, which reduces the efficiency of the heater and increases the energy consumption for heating water (85-90 ºC). Measure no. 3 represents the first principle of green chemistry: Waste prevention. It is expected that the emission of waste PET dust into the work area will be reduced by ≈ 50% and the share of dust sold on the market will increase.

The report of the environmental audit was presented to the management of the company Omorika Reciklaža Ltd. who accepted the implementation of the proposed measures. Currently, measures 1 and 3 have been implemented, and measure 2 will be implemented later. With the implementation of measure 1, the antifoam and flotation agent was taken out of use without affecting the quality of the product. The company's staff tested the possibility of performing flotation without this agent and the dangerous mixture was completely phased out by December 2022. This reduced the cost of purchasing the given chemical. By installing new bag filters in

January 2023, measure 3 was implemented. Filters (diameter 20 cm, length 4 m) are installed on the line for the production of PET´s crushed flakes, after the drying process (Fig. 1), where a large amount of PET dust appears. Bag filters contain antistatic, suitable for working in certain explosive zones. So far, it has been observed that the amount of dust in the production facility is lower compared to the period when the old dust collection system was used. Monitoring of this measure continues.

CONCLUSIONS

Green chemistry and the EU REACH regulation, as the main drivers of implementing green chemistry principles in the industry, promote sustainability and the reduction of the harmful impact of chemicals on the environment and human health. Through an environmental audit, the possibility of improving the technological process for this case study in the plastic recycling industry has been identified in terms of applying the principles of green chemistry, with the aim of protecting human health and the environment. The proposed 3 measures result in:

- Ceasing the use of a mixture containing two substances (organosiloxanes) classified as SVHC and substances proposed as POPs.
- Reducing the amount of waste PET dust while simultaneously increasing the production of PET dust for the market (byproduct).
- Reducing the consumption of chemicals in the warm washing process by water demineralization treatment while increasing the efficiency of the heater and increasing energy consumption.

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THE INFLUENCE OF POTASSIUM CHLORIDE AND MAGNESIUM CHLORIDE ON THE COLOR AND SENSORY PROPERTIES OF COOKED CHEESE

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:

The aim of this paper is focused on reducing sodium chloride content by partial replacement with potassium chloride and magnesium chloride in cooked cheese samples. For the production of cheese, standardized cow's milk from a domestic market producer was used, and the cheese was produced by heating the milk to a temperature of 95°C and coagulation with acetic acid. The one salted only with NaCl was designated as the standard sample, and the other samples were salted with combinations of salts in which NaCl reduction was performed: sample A1 had a ratio of 15% KCl:85% NaCl, sample A2 30% KCl:70% NaCl, sample B1 15% $MgCl_2$:85% NaCl and sample B2 30% $MgCl_2$:70% NaCl. The cheese samples were stored at + 4°C and color parameters and sensory properties were analyzed on the 1st, 3rd and 5th days of storage. Based on the performed analyses, it was concluded that it is completely acceptable to replace sodium chloride with potassium chloride in the ratio of 15% KCl:85% NaCl. It is acceptable to replace sodium chloride with potassium chloride in the ratio of 30% KCl:70% NaCl, with the note that on the 5th day of storage there is a gradual deterioration of the sensory properties compared to the samples analyzed on the 1st day of storage. Replacement of sodium chloride with magnesium chloride in the ratios 15% MgCl2:85% NaCl and 30% MgCl2:70% NaCl is not acceptable. As such, it is not recommended in the production of cooked cheeses due to the appearance of a metallic and bitter taste that is present in cheese samples from the 1st to the 5th day of storage.

KEYWORDS: cooked cheese, sodium chloride, potassium chloride, magnesium chloride

INTRODUCTION

Nowadays, cheese is a premium dairy product consumed by all age groups around the world. The cheese market is one of the most dynamic within the dairy sector over 22,17 million megatons of cheese produced in 2022., which corresponds to one third of cow's milk production [1]. Cheese is a dairy product rich in nutrients such as proteins, peptides, fatty acids, vitamins and essential minerals such as calcium. Since it is very nutritious, it has a long history in human nutrition. The high content of fat and protein makes cheese an energy-rich and nutritious food for all age groups [2]. Homemade cooked cow's milk cheese is a representative of traditional cheesemaking and is widely represented in Bosnia and Herzegovina and has the characteristics of a traditional specialty due to the long-standing method of production and traditional composition [3]. It is produced by heating milk to a temperature of 90°C to 95°C, and by directly acidifying it with acid. The obtained curd is salted, placed in molds and pressed. This type of cheese can be consumed immediately after production or for a longer storage period [4]. The addition of salt to cheese is of great importance; in addition to the taste and smell, salt affects the ripening process, reduces the amount of water, increases the durability of the cheese and participates in the formation of the cheese rind [5].

Table salt is the most common ingredient responsible for the salty taste of food; it is the only substance that gives a clean, salty taste. The perception of salty taste is influenced by age, gender, genetics, weight or smoking habit, as well as taking medications. During the aging of the organism, there is a decrease in the response of receptors for salty taste [6]. Sodium is an essential mineral needed in human nutrition. The European Food Safety Agency (EFSA) has established a sodium intake of 2 g/day (corresponding to a salt intake of 5 g/day) for the adult population in Europe [7]. In practice, long-term salt consumption of more than 5 g/day for an adult has a negative effect because it affects the cardiovascular system and increases the risk of hypertension, heart

disease and stroke [8, 9]. High daily salt intake in some countries, 12–16 g/day [10] or 6.8–10.7 g/100 g, which was determined on the basis of urinary excretion [11], causes kidney diseases, and indirectly affects the incidence of stomach cancer [12, 13]. There is strong evidence of a causal relationship between high salt intake and high blood pressure and stroke risk in humans and animals [14, 15]. In contrast, increased potassium intake protects against stroke, high blood pressure, heart problems and kidney failure. Additional use of potassium chloride with partial replacement with sodium chloride could help to reduce the sodium content [16].In blood pressure control, potassium plays an important role as well as sodium, and the balance of these two elements is important. However, the use of potassium chloride is generally limited due to its bitter taste [17, 15]. Magnesium lowers blood pressure because it causes a decrease in intracellular sodium and calcium content. As a natural calcium channel blocker, magnesium blocks sodium binding to vascular smooth muscle cells, increases prostaglandin vasodilation, cooperatively binds potassium, increases nitric oxide, causes vasodilation and lowers blood pressure [16, 15].

Health protection organizations driven by raised awareness of public change and consumer associations recognize a unique strategy for reducing salt in food as a preventive measure, which can be linked to initiatives on the consumption of saturated fat and sugar [18]. The World Health Organization (WHO) has set a global goal of reducing sodium chloride intake by 30% by 2025. [19, 20].

Over the past two decades, research dealing with the reduction of sodium content in cheese has intensified and now represents a multidisciplinary approach to reducing sodium content without compromising the quality and safety of cheeses [21]. In addition to reducing the mass fraction of sodium chloride that is added to the product, there are other approaches to reducing table salt, such as the use of substitutes for sodium chloride (potassium chloride, magnesium chloride, monosodium glutamate, potassium lactate, calcium lactate and monobasic potassium phosphate), the addition of improvers flavors or the use of microparticles of table salt crystals [22, 23].

MATERIAL AND METHODS

In this research work, a partial replacement of sodium chloride with potassium chloride and magnesium chloride was performed in samples of cooked cheese. After production, cheese samples were stored at +4°C for five days. On the first, third and fifth days of storage, sensory analysis and instrumental measurement of color parameters were performed on the samples.

For the production of cheese, cow's milk standardized on milk fat content from a domestic market producer was used, with the following composition that was printed on the declaration: fat 2.8%, proteins 3.4%, carbohydrates 4.6%, salt (from naturally occurring sodium) 0.1%, calcium 120 mg/100 ml. For the production of cheese, the milk was heated to a temperature of 95°C, and the coagulation of the milk was performed with 80% acetic acid in the amount of 0.2%. After forming the curds, the curds were left to rest for about 15 minutes and then transferred to a cheesecloth strainer to drain. After extracting the whey, the curd is salted with table salt or a combination of salts in the amount of 2%. The one salted only with NaCl was designated as the standard sample, and the other samples were salted with combinations of salts, whereby the reduction of NaCl with KCl and $MgCl_2$ was performed as followed: sample A1 15% KCl:85% NaCl, sample A2 30% KCl:70% NaCl, sample B1 15% $MgCl_2$:85% NaCl and sample B2 30% $MgCl_2$:70% NaCl.

Then each sample was transferred to a mold, where additional whey draining was performed under load for 6 hours, and then the cheese was removed from the mold and packed in plastic bags and stored at +4°C.

Instrumental color measurement was performed with a colorimeter LCC-A11 (LABTRON, United Kingdom). The spectrophotometer is equipped with a standard D65 light source and a standard 10° refraction shield. The instrument was calibrated using a white calibration plate. Instrumental color parameters were measured according to the CIE L*, a*, b* system. L* represents the light intensity and ranges from black (0) to white (100). a* indicates red (+ a*) when positive and green (-a*) when negative, while b* indicates yellow (+a*) when positive and blue (-a*) when negative.

Sensory analysis of cheeses was performed by a three-member Commission for Sensory Evaluation at the Biotechnical Faculty of the University of Bihać according to FIL-IDF Standard [24]. The following sensory properties were evaluated: external appearance (max. 2 points), color (max. 2 points), consistency (max. 2 points), cut surface (max. 2 points), smell (max. 2 points), taste (max. 10 points).

The obtained results were analyzed using appropriate mathematical and statistical methods, and the significance of the obtained differences was evaluated. All results are presented as mean of replicates ± standard deviation. In the analysis, the method of analysis of variance with two factors of variability

(ANOVA) with a significance level of 5% was applied, where factor A is the time interval of storage, and factor B is the type of sample.

RESULTS AND DISCUSSION

Table 1. shows the results of instrumental measurement of color parameters on the surface of cheeses.

Table 1. Results of instrumental color measurement (L*, a* and b*)

Day 1			
Sample	L*	a*	b*
Standard	93.52±0.513	0.21±0.415	10.12±0.248
A1	93.34±1.311	0.14±0.398	11.22±0.915
A2	93.44±1.418	0.06±0.916	11.57±0.547
B1	94.76±0.842	0.62±0.383	10.89±0.801
B2	90.08±0.962	-0.63±0.307	2.55±0.903
Day 3			
Sample	L*	a*	b*
Standard	99.87±0.894	4.72±1.715	7.07±0.428
A1	99.72±±0.835	4.93±0.994	6.70±01.934
A2	93.30±±0.486	-0.25±0.275	10.61±00.477
B1	94.23±0.368	0.23±0.305	10.35±00.185
B2	94.53±0.379	-0.45±0.186	9.64±0.346
Day 5			
Sample	L*	a*	b*
Standard	91.77±1.114	-0.30±0.516	12.14±0.577
A1	92.02±0.981	-0.12±0.439	12.47±0.511
A2	92.98±0.599	-0.85±0.268	11.85±0.486
B1	93.57±2.074	-0.60±0.715	10.62±0.544
B2	93.85±±0.768	-0.94±0.445	10.50±0.652
p	p_A=0.0807 p_B=0.695	p_A=0.07872 p_B=0.2615	p_A=0.2571 p_B=0.2571

A1- replacement of NaCl with KCl 15% , A2 – replacement of Na Cl with KCl 30%,
B1 – replacement of NaCl with $MgCl_2$ 15%, B2 – replacement of NaCl with $MgCl_2$ 30%,
results are shown as medium value ±standard deviation of five consecutive measurements

The surfaces of all samples are light in color (L*=100 completely light). The lowest L* value was recorded for sample B2 on the 1st day of storage 90.08, and the highest brightness value was determined for the standard sample on the 3rd day of storage 99.87. For the 3rd day of storage, the measured valuesof brightness L* were higher in all samples compared to the 1st and 5th days of storage, but the analysis of variance with two variability factors for the color parameter L* did not reveal a statistically significant difference ($p>0.05$) between samples for factor A and factor B.

The a* parameter indicates the range of colors from green (-a*) to red (+a*). On the 1st day of storage, all samples except sample B2 had positive valuesof the parameter a*, which means that the surface of sample B2 has a green tone, and the surfaces of the other cheeses have a red tone. For the 3rd day of storage, samples A2 and B2 had negative values, and the surfaces of the cheeses of these samples had a green tone, and the surfaces of the other samples had a red tone. On the 5th day of storage, all cheese samples had negative values for the parameter a*, which means that the surfaces of all cheese samples had a green tone. For the parameter a*, no statistically significant difference was found between the samples ($p>0.05$) in relation to factor A and factor B. Samples in which there were larger amounts of replacement salt yield cheeses with a more pronounced green tone, whereby the replacement of NaCl with 30% should be singled out $MgCl_2$ because the green tone of the surface in cheeses with this replacement amount of salt was present from the 1st to the 5th day of storage (the a* parameter was on the 1st day of storage -0.63; on the 3rd day -0.45 and on the 5th day of storage -0.94).

The b* parameter indicates the range of colors from yellow (+b*) to blue (-b*). The b* values are positive and all samples have a yellow tone, which is

one of the important characteristics of cooked cheeses. Sample A2 on the 1st day of storage had the highest b* value 11.57, and sample B2 had the lowest b* value 2.55. On the 3rd day of storage, sample A2 had the highest value for b* 10.61, and sample A1 had the lowest value 6.70. On the 5th day of storage, sample A1 had the highest b* value of 12.47, and sample B2 had the lowest value of 10.50. No statistically significant difference was found for the parameter b* ($p>0.05$) in relation to A and factor B. Sample A2, where NaCl was replaced by KCl 30%, should be singled out because it had the highest measured valuesof b* for the 1st and 3rd day of storage, and in these samples the surface had a more pronounced yellow tone. In sample B2, where NaCl was replaced by $MgCl_2$ 30%, smaller values were recorded for the parameter b*.

Table 2. Results of the cheese samples sensory analysis

Sample	External appearance	Color	Consistency	Cut surface	Smell	Taste	Total
Day 1							
Standard	1.87±0.06	1.93±0.12	1.87±0.06	1.93±0.12	1.90±0.10	9.63±0.55	19.30±0.44
A1	1.83±0.06	1.97±0.06	1.83±0.12	1.83±0.15	1.93±0.06	9.63±0.15	19.43±0.42
A2	1.83±0.06	1.83±0.15	1,80±0.17	1.77±0.21	1.97±0.06	9.63±0.21	18.83±0.40
B1	1.80±0.10	1.83±0.06	1.77±0.12	1.80±0.17	1.83±±0.12	9.47±0.49	18.50±0.70
B2	1.97±0.06	1.97±0.06	1.90±0.10	1.90±0.10	1.97±0.06	8.77±1.08	18.47±1.36
Day 3							
Standard	1.87±0.577	1.90±0.10	1.97±0.06	1.93±0.06	1.93±0.06	9.90±0.10	19.50±0.17
A1	1.93±0.06	1.97±0.06	1.87±0.15	1.83±0.06	1.97±0.06	9.83±0.06	19.40±0.20
A2	1.87±0.15	1.83±0.15	1,83±0.06	1.80±0.10	1.90±0.10	8.03±1.00	17.27±1.43
B1	1.83±0.15	1.83±0.12	1.70±0.10	1.77±0.12	1.83±±0.15	8.00±1.20	16.97±1.76
B2	1.93±0.12	1.83±0.06	1.80±0.10	1.87±0.15	1.80±0.10	8.40±0.36	17.70±0.56
Day 5							
Standard	1.87±0.06	1.87±0.06	1.83±0.06	1.83±0.06	1.87±0.05	9.80±0.10	19.07±0.25
A1	1.80±0.10	1.80±0.10	1.73±0.12	1.87±0.06	1.93±0.06	9.83±0.21	19.00±0.30
A2	1.70±0.10	1.70±0.10	1,90±0.10	1.63±0.12	1.70±0.10	7.40±1.02	16.07±1.20
B1	1.67±0.06	1.67±0.06	1.60±0.10	1.53±0.12	1.80±±0.17	7.70±1.25	16.03±1.32
B2	1.80±0.10	1.80±0.06	1.73±0.06	1.83±0.05	1.80±0.10	8.50±0.46	17.47±0.58
p	p_A=0.06558 p_B=0.5031	p_A=0.00214 p_B=0.01071	p_A=0.1803 p_B=0.05631	p_A=0.05186 p_B=0.02182	p_A=0.1155 p_B=0.2914	p_A=0.1649 p_B=0.03454	p_A=0.02131 p_B=0.00554

A1- replacement of NaCl with KCl 15%, A2 –replacement of Na Cl with KCl 30%, B1 – replacement of NaCl with $MgCl_2$ 15%, B2 –replacement of NaCl with $MgCl_2$ 30%, results were shown as medium value ± standard deviation for three ratings

In general, cooked cheeses have a homogeneous and pliable dough, without sticking to the blade of a knife, with a specific milky sweet-sour taste combination [25]. Cooked cheeses obtained by coagulation of milk with acetic acid have a good cutting ability, a characteristic pale yellow color, a moderately salty taste and a pleasant aroma [3].

According to the results of the sensory analysis shown in Table 2., on the 1st day of storage, sample A2 had the highest total number of points 19.43, and sample B2 had the lowest number of points 18.47. The evaluators noted that sample A1 had a fairly uniform structure compared to other cheese samples. For the property external appearance, sample B2 was rated best 1.97, and sample B achieved the lowest number of points 1.80. For the color property, samples A1 and B2 had equal numbers of points 1.97, while the lowest number of points was recorded for samples A2 and B1 1.83. On the first day of storage, sample B2 had the best consistency 1.90, and sample B1 achieved the lowest number of points for consistency 1.77. The evaluators noted that sample B2 has a compact dough and does not crumble when cut. For the property of cut surface, the standard sample achieved the highest number of points 1.93, and sample A2 the lowest number of points 1.77. Samples A2 and B2 had the highest number of points for the smell on the first day of storage 1.97, and sample B had the lowest number of points 1.83. On the first day of storage, the highest number of points for taste was recorded for samples standard, A1 and A2 9.63, and the lowest number of points for taste was achieved by sample B2 8.77. The evaluators found that the standard sample had a more pronounced salty taste compared to the other samples.

For sample B2 (replacement of NaCl with $MgCl_2$ 30%), the evaluators found a metallic taste that became more intense during chewing of the sample.

By sensory analysis of the samples on the 3rd day of storage, the highest total number of points was recorded for the standard sample 19.50, and the lowest for B1 16.97. For the standard sample, a slightly higher feeling of saltiness was found compared to the other samples. For the property external appearance, samples A1 and B2 were rated best 1.93 points, and sample B1 achieved the lowest number of points 1.83 . For the color property, sample A1 was the best rated 1.97, and samples A2, B1 and B2 had the lowest number of points 1.83. The standard sample had the best consistency 1.97, and sample B1 achieved the lowest number of points for consistency 1.70. The standard sample had the best cut surface 1.93, and sample B1 had the lowest score 1.77, because the evaluators noticed that the cheese dough crumbles when cutting this sample. The highest number of points for smell was achieved by sample A1 1.97, while the evaluators noted that the standard and A1 retained a fresh smell, and sample B2 had the lowest number of points 1.80. The standard achieved the highest number of points for taste 9.90, while sample B1 had the lowest number of points 8.00. Samples of cheeses where NaCl was replaced with $MgCl_2$ had a more pronounced bitterness and metallic taste compared to the standard.

By sensory analysis of samples on the 5th day of storage, the highest total number of points was recorded for the standard 19.07, followed by sample A1 19.00. Comparing the total number of points on the 5th day of storage in relation to the total number of points recorded for the 1st and 3rd day of storage, the standard and sample A1 were uniformly rated high by the evaluators. In the case of other samples, a trend of reduction in the total number of points was observed for the 5th day of storage compared to the 1st and 3rd days. For the attribute external appearance, the standard was rated best 1.87, and sample B1 achieved the lowest number of points 1.67. The standard had the best score for color 1.87, and sample B1 had the lowest number of points 1.67. Sample A2 had the highest number of points for consistency 1.90, and B1 had the lowest number of points 1.60. For the property of cut surface, sample A1 had the highest number of points 1.87, and sample B1 had the lowest number of points 1.53. Sample A1 had the best score for smell 1.93 points, and sample A2 had the worst score 1.70) Sample A1 and standard had the best taste scores 9.83 and 9.80, and sample A2 scored the lowest number of points 7.40. According to the results of the analysis of variance, no statistically significant difference was found between the samples ($p>0.05$) for external appearance, consistency and smell in relation to factor A and factor B. For cut surface and taste, in relation to factor A (storage time interval) no statistically significant difference ($p>0.05$) was found between the samples, while a statistically significant difference ($p<0.05$) was found for factor B (type of sample). A statistically significant difference ($p<0.05$) was found between the samples in relation to factor A and factor B for color variations, as well as for the overall impression.

Replacement of sodium chloride with compounds of high molecular weight gives sour taste and less saltiness of cheeses [26]. If the replacement of sodium chloride with potassium chloride is more than 50%, then it negatively affects the sensory properties of cheeses, but it is still the best alternative for the substitution of sodium chloride due to the similarity in the chemical structure of KCl and NaCl [27].

Like potassium chloride, salts such as magnesium chloride in combination with sodium chloride cause less salty taste, and increased bitterness of food products to which they are added, creating a metallic taste. In a cheddar cheese production experiment with complete or partial replacement (1:1) of NaCl with $MgCl_2$, $CaCl_2$ and KCl, and after 4 months of ripening at 4°C cheeses in which NaCl was completely replaced with alternative salts were extremely bitter and completely unacceptable, and cheeses with $MgCl_2$ and $CaCl_2$ had the worst firmness, hardness and extensibility. Cheese salted with a combination of NaCl and KCl salts was the only acceptable one and did not differ much from the control cheese sample [28].

Grummer et al. [29] used sodium chloride and sea salt in combination with potassium chloride, modified potassium chloride, magnesium chloride or calcium chloride in the production of cheddar cheeses. Cheeses with calcium chloride and magnesium correlated positively with bitter, metallic, earthy, soapy, impure taste, and negatively with cooked and milky taste. Cheeses that were salted with combinations of NaCl:$MgCl_2$ (1:1) or NaCl:$CaCl_2$ (1:1) had a bitter and metallic taste, and the conclusion is that the use of calcium chloride and magnesium chloride for salted cheeses results in differences in taste that are unacceptable for quality cheese. For cream cheese in which during production sodium chloride was replaced with potassium chloride and magnesium chloride [22], during the sensory evaluation it was determined that in the standard sample (cheese salted only with sodium chloride) the dominant taste is saltiness. In samples where sodium chloride was replaced with magnesium chloride, a significant salty

taste lasted approximately nine seconds. After that, the bitter taste prevailed until the end of the analysis; the taste is also characterized as undesirable and more intense than the bitter taste of potassium chloride.

During the production of Minas fresh cheese [30], sodium chloride was replaced with potassium chloride in the following concentrations: 0, 25, 50 and 75%. The control sample with 0% KCl achieved the highest number of points for all evaluated sensory parameters. Samples with 50% and 75% KCl achieved a lower number of points for taste and texture, which is related to the concentration of potassium chloride, because the perception of bitter taste occurs. Sodium chloride can mask the resulting unpleasant aroma as long as potassium chloride is present in smaller concentrations. The conclusion is that potassium chloride can be a successful replacement for sodium chloride in the production of cheeses with a reduced level of taste and smell, such as fresh cheese that has a shelf life of up to 20 days.

CONCLUSION

According to the results of measuring the color parameter L*, the surfaces of the cheese samples are light in color. By measuring the color parameter a*, samples in which higher concentrations of substitute salts were used result in the color of cheeses with a more pronounced green surface tone, while the sample in which sodium chloride was replaced with magnesium chloride in the amount of 30% should be singled out. The replacement of sodium chloride with potassium chloride in the amount of 30% results in the color of cheeses with a more intense yellow tone of the surface, as shown by the results for the color parameter b*. If sodium chloride is replaced with magnesium chloride in the amount of 30%, the color of the surface of the cheese is lighter, pale yellow.

Samples of cheeses in which sodium chloride is partially replaced by magnesium chloride have generally worse sensory properties compared to the standard sample and samples in which sodium chloride is partially replaced by potassium chloride, especially in terms of taste. It is completely acceptable to replace sodium chloride with potassium chloride in the amount of 15%, because the quality of the samples is uniform during the observed storage period. It is also acceptable to replace sodium chloride with potassium chloride in the amount of 30%, with a note that during storage, the quality of the cheese gradually decreases, which is indicated by a decrease in the total number of points. If there is a greater replacement amount of magnesium chloride salt present in the cheese, the metallic taste and bitterness are more pronounced, which become more pronounced starting from the 1st to the 5th day of storage.

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NATURAL RUBBER COMPOSITES WITH HYDROCHAR AS A PARTIAL FILLER: INVESTIGATION OF KINETIC PARAMETERS

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:

Reducing the environmental impact of the rubber industry has emerged as a major challenge, and one potential solution that has garnered significant attention is incorporating hydrochar as a partial filler in natural rubber composites. Hydrochar was obtained through hydrothermal carbonization treatment of hardwood waste biomass, and it has been found to have potential as a sustainable alternative to traditional filler carbon black. The aim of this study was to investigate the effect of varying hydrochar and carbon black content in natural rubber composites, while keeping the total filler amount constant at 50 phr. The study's findings indicated that higher hydrochar content resulted in a greater curing activation energy, facilitating the manufacture of larger natural rubber vulcanizates that required extended curing periods at reduced temperatures. The rubber industry's stringent environmental regulations have created a pressing need for sustainable alternatives, and incorporating hydrochar as a partial filler could offer a promising solution by repurposing waste materials into a valuable component for rubber composites.

KEYWORDS: hydrochar, hydrothermal carbonization, natural rubber composites, kinetic parameters

INTRODUCTION

The fossil fuels increasing consumption and huge dependence on them have become the pressing issues of 21st century [1]. The measures are taken in order to reduce the dependence on fossil fuels, where the first one is exploring renewable industrial products, and second is recycling and reusing waste. Products made from natural rubber find widespread use in a diverse range of applications, and typically, they are strengthened using nanofillers, with carbon black being a common choice [2]-[5]. Carbon black is widely used in rubber products due to its high purity and low cost, although its production is highly dependent on fossil fuels [6], [7]. As public awareness and pressure to reduce reliance on fossil fuels has intensified in recent decades, bio-sourced materials have gained attention as eco-friendly substitutes for petroleum-based reinforcing fillers [8]-[11]. Using waste as a source for production of hydrochar obtained by hydrothermal carbonization (HTC), for partially replacing the carbon black, as a traditionally used filler in rubber composites, is preferred. HTC presents a means of carbonizing biomass with high water content at lower temperatures than pyrolysis, resulting in the production of hydrochar, which is similar to carbon black in its properties and structure. Hydrothermal carbonization has emerged as a green, non-toxic, cheap, and environmentally benign process, which is simple to implement and does not require pre-treatment of biomass. Hydrochar is expected to degrade the rubber products properties since it has larger particles and there is a necessity for its milling [12]. Based on literature review, it was revealed that hardwood waste was most used for obtaining hydrochar, due to lower ash and higher carbon content, comparing to other feedstocks [13], [14].

In this study, the natural rubber samples with different hydrochar and carbon black content were prepared, and the total amount of the filler was 50 phr, while the amount of other additives was kept constant. Rheological curves of all prepared samples were obtained, in order to investigate the samples' kinetic parameters. The fitting procedure from previously published paper enabled calculating the kinetic parameters of all prepared samples, i.e., curing and reversion energy activation, Arrhenius pre-exponential factor and curing and reversion rate constants. The kinetic parameters provided valuable insights into the potential applications of the prepared samples in the production of large-scale natural rubber composites.

MATERIALS AND METHODS

MATERIALS

Natural rubber, standard Vietnamese rubber SVR CV60, crosslinking agent sulfur (S), carbon black (CB) and hydrochar (HC) as fillers, N-cyclohexylbenzothiazol-2-sulfenamide (CBS) as accelerator, crosslinking activators zinc oxide (ZnO) and stearic acid, as well as antioxidant N-isopropyl-N'-phenyl-p-phenylenediamine (IPPD), were obtained from Edos (Zrenjanin, Republic of Serbia). All raw materials used in the work, except for hydrochar, are commercially used in the rubber industry.

Hydrochar was obtained by hydrothermal carbonization of waste biomass from hardwood, which was conducted at 300 °C and 86.6 bar, during 30 min [15]. The obtained hydrochar was milled in a Fritch planetary mill at 200 revolutions per minute, for 5 min. The ground biochar particles were sieved from 500 to 800 μm, and washed with warm deionized water, until the dark leachate stopped separating [16]. In the rubber industry, the relative composition of the rubber mixture is expressed by considering that the basic ingredient of the mixture is the amount of natural rubber and is denoted by 100, while the amount of other components is represented in relation to the amount of rubber, and thus, obtained unit is denoted by phr (parts per hundred rubber).

In order to examine the possibility of partially replacing carbon black with an environmentally friendly filler, carbon black and hydrochar were mixed together in the rubber mixture, and the content of the two fillers was varied, where the total filler content was 50 phr. The content of the other components was not varied, and the samples were labelled as presented in Table 1.

Table 1. Samples labelling

Samples label	Description
VCB10	Vulcanized sample with 10 phr carbon black and 40 phr hydrochar
VCB20	Vulcanized sample with 20 phr carbon black and 30 phr hydrochar
VCB30	Vulcanized sample with 30 phr carbon black and 20 phr hydrochar
VCB40	Vulcanized sample with 40 phr carbon black and 10 phr hydrochar
VCB50	Vulcanized sample with 50 phr carbon black and 0 phr hydrochar

The recipe for obtaining samples of the rubber mixture is shown in Table 2, as explained in detail in previously published work [15]. The presented values of components are determined ensuring that the filling factor of the mixing chamber is 0.75, where the filling factor of the chamber represents the ratio of the volume of the mixture to the volume of the empty mixer chamber.

Table 2. Recipe for obtaining rubber mixture samples

Components	phr
NR	100
CB/HC	50*
ZnO	4
Stearin	1
IPPD	1
Sulphur	2.5
CBS	0.5

* The amount of CB and HC was varied according to Table 1, and the total amount of the filler was 50 phr.

The components shown in Table 2 are usually divided into inactive and active, where carbon black, hydrochar, ZnO, stearic acid and IPPD are inactive, and sulphur and CBS are active components.

RUBBER MIXING COMPOUND

A HAAKE Rheomix mixer (model 600) with a Rheocord EU-5 drive unit and "CAM" type mixers, manufactured by HAAKE, Germany, was used to mix the components of the rubber mixture. In order to ensure isothermal mixing, it was necessary to heat the mixer for 10 minutes, before adding the components, as well as a constant temperature of 90 °C in all zones, with the included air supply that ensures cooling and temperature maintenance.

Mixing was described in detail in previously published paper [17]. The first phase is idle, i.e., the mixer is set on rotation mixer rotor speed of 30 s^{-1}. After first part, the measured natural rubber is added to the chamber, the rotor speed is increased to 100 s^{-1} during three minutes, then the speed is reduced to 60 s^{-1} during three minutes. During the second and third phase, when only the rubber is mixed, its mastication occurs, which enables a greater dispersion of the mixture components during the following stages. In the fourth phase, at the same rotor speed, inactive components of the rubber mixture are added, i.e., fillers (CB and HC), zinc oxide, stearin and IPPD, and mixed for five minutes. In the last mixing phase, the active components, sulphur and CBS, are added and

mixed for 2 min, at the same rotor speed. The specified mixing procedure enables optimal dispersion of all components and distribution of fillers in the polymer matrix. The resulting mixture is cooled and stored in a cold place until vulcanization is performed, at least 24 h, in order to ensure conditioning [18].

RHEOLOGICAL PROPERTIES

The rheological properties of the rubber mixtures were monitored using an oscillatory rheometer MDR-A Rotorless Rheometer, manufactured by Beijing Rade Instrument co., Ltd. The rheological properties of prepared samples were tested at 130-180 °C, with a step of 10 °C, in order to examine samples reversion phenomena. An oscillatory rheometer enables vulcanization monitoring by placing a rubber mixture sample between two disks, which oscillate with a small rotational amplitude causing material shear deformation. The torque that is necessary for the oscillation of the disc depends on the resistance to deformation, i.e., of the rubber shear modulus, and is monitored by an oscillating rheometer as a function of time.

FITTING PROCEDURE

The fitting procedure was explained in detail in research [19]. Crosslinking and reversion are considered as two parallel phenomena of complex vulcanization, where curing and reversion degree are calculated as following:

$$\alpha_c = 1 - \frac{1}{1 + (k_c t)^n} \quad (1)$$

$$\alpha_r = x(1 - e^{-k_r t}) \quad (2)$$

Where α_c and α_r are the curing and reversion degree, k_c and k_r are curing and reversion constants (s^{-1}), respectively, n is the reaction order, t is vulcanization time and x is the maximal reversion degree, temperature-dependent parameter. The curing and reversion constants have an Arrhenius dependence on temperature, whereas the temperature increases, the rate of the chemical reaction also increases, as shown by the following equation:

$$k_{c,r} = A_{c,r} \exp\left(\frac{-E_{ac,r}}{R_g T}\right) \quad (3)$$

Where $A_{c,r}$ represents the pre-exponential factor, $E_{ac,r}$ is the activation energy ($Jmol^{-1}$), for curing and revesion, respectively, R_g is the universal gas constant (8.314 $Jmol^{-1}K^{-1}$) and T is the absolute temperature (K).

Experimentally obtained rheological data of the torque depending on time are available, for the purposes of fitting the experimental data, there is a necessity to define the form for calculating curing and reversion degree, as presented:

$$\alpha_c = \frac{M_c - M_0}{M_{Max} - M_0} \quad (4)$$

$$\alpha_r = \frac{M_{Max} - M_r}{M_{Max} - M_0} \quad (5)$$

Where M_c and M_r are the curing and reversion torques (dNm), respectively. Equation (5) is an indication that the rubber mixture vulcanization is defined as partially reversible, due to M_r not equal to M_0.

When the Equations (4) and (5) are included in (1) and (2), respectively, the integral forms of the model, modified for fitting the rheological data, can be obtained:

$$M_c = M_{Max} - \frac{M_{Max} - M_0}{1 + (k_c t)^n} \quad (6)$$

$$M_r = M_{Max} - (M_{Max} - M_{Rev})(1 - e^{-k_r t}) \quad (7)$$

As curing and reversion reactions take place simultaneously, and the following general equation can be written for the vulcanization reaction:

$$M = M_c - M_r \quad (8)$$

Where M is the vulcanization torque (dNm). In order to define the final dependence of the torque on time, the Equations (6) and (7) were included in the Equation (8), and the following integral form of the equation describing the complete vulcanization can be obtained:

$$M = M_{Max} - \frac{M_{Max} - M_0}{1 + (k_c t)^n} - (M_{Max} - M_{Rev})(1 - e^{-k_r t}) \quad (9)$$

The vulcanization data was divided into two separate fitting sets, where curing include part before experimentally obtained maximal torque and reversion part after it. The first step represented fitting the reversion with the Equation (6), which resulted in three parameters, i.e., maximal model torque (M_{Max}), model torque asymptote (M_{Rev}) and reversion rate constant (k_r). The initial values for the fitting parameters M_{Max}, M_{Rev}, and k_r were M_{MaxExp}, M_{RevExp}, and $\ln\left(\frac{1}{2}\right)/t_{hr}$, respectively. The initial fitting parameters were selected as experimentally obtained values, where M_{RevExp} was the experimentally obtained final torque, M_{MaxExp} was experimental maximal torque and t_{hr} was the time required to reach half of the experimentally obtained reversion value. After fitting the reversion and determining the model maximal torque, the model was fitted using Equation (5), where the reaction order (n) was the only adjustable fitted parameter, and the value 1 was taken as its initial

value, while the initial torque (M_0) and the curing rate constant (k_c) were fixed parameters, obtained directly from experimental data [19]. The fitting of the vulcanization curve was performed by the least squares method.

DATA ANALYSIS AND PROCESSING

The collection, processing, analysis and display of results were performed using the computer program MATLAB (The Math Works Inc. License number 1108951). The quality of fitting is determined by statistical methods, i.e., using two methods, the mean absolute percentage error (*MAPE*), and coefficient of determination (R^2). The mean absolute percentage error and the coefficient of determination are calculated according to the equations:

$$MAPE = \frac{1}{m}\sum_{i=1}^{m}\left|\frac{y-\hat{y}}{2}\right| \quad (10)$$

$$R^2 = 1 - \frac{\sum(y-\hat{y})^2}{\sum(y-\bar{y})^2} \quad (11)$$

Where m is the number of fitted points, y is the actual (experimental) value, $\hat{y}$ is the predicted value, and $\bar{y}$ is equal to $\frac{\sum y}{m}$.

RESULTS AND DISCUSSION

RHEOLOGICAL PROPERTIES OF RUBBER MIXTURES WITH HYDROCHAR AS A FILLER

Rheometric vulcanization curves are presented at 160 °C for 2 h, in order to compare the effect of hydrochar on crosslinking and reversion (Figure 1).

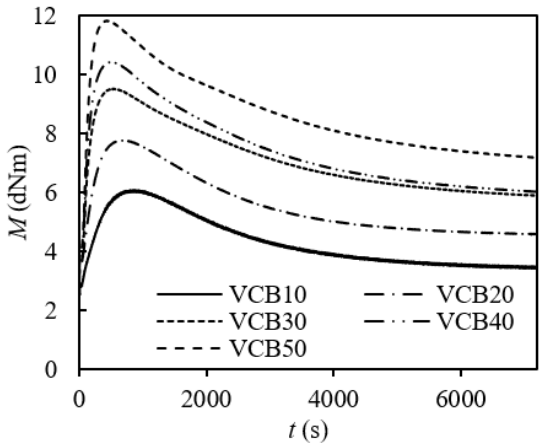

Figure 1. Vulcanization rheometric curves of rubber samples at 160 °C

Slower crosslinking with a higher hydrochar content can be seen in Figure 1. Sample VCB10, which consists 40 phr of hydrochar, reaches a maximum torque of 6.37 dNm, while in contrast, the sample without hydrochar (VCB50) reaches a value of 12.31 dNm, which is almost twice as high. Additionally, it can be noted that the filler type does not significantly affect the reversion of rubber compounds. The rheometric curves for samples containing hydrochar were recorded at all tested vulcanization temperatures for a duration of 2 hours to determine the kinetic parameters. However, only at 160 °C were presented for simplified explanation.

FITTING OF RUBBER COMPOUNDS WITH HYDROCHAR AS A FILLER

The described fitting procedure for rubber compounds was applied to determine the vulcanization parameters of rubber compounds with the addition of different hydrochar content, and the results and quality of fitting are presented in Table 3-

Table 3. Fitting quality

Sample	Parameter	*A*	R^2	*MAPE*
VCB10	k_u	2.653E08	0.9998	6.173
	k_r	7.86E14	0.9874	10.281
VCB20	k_u	1.75E8	0.998	4.819
	k_r	5.705E14	0.9985	9.581
VCB30	k_u	2.281E8	0.9997	2.18
	k_r	4.625E14	0.9997	3.918
VCB40	k_u	1.56E8	0.9998	1.994
	k_r	4.463E14	0.9784	7.971
VCB50	k_u	2.357E8	0.9997	2.004
	k_r	5.166E8	0.9796	7.653

The curing and reversion activation energies of prepared samples with different hydrochar content are presented in Figures 2 and 3.

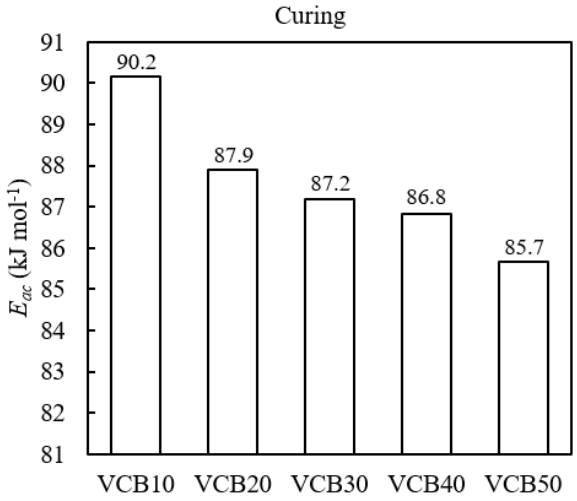

Figure 2. Effect of hydrochar content on curing activation energy

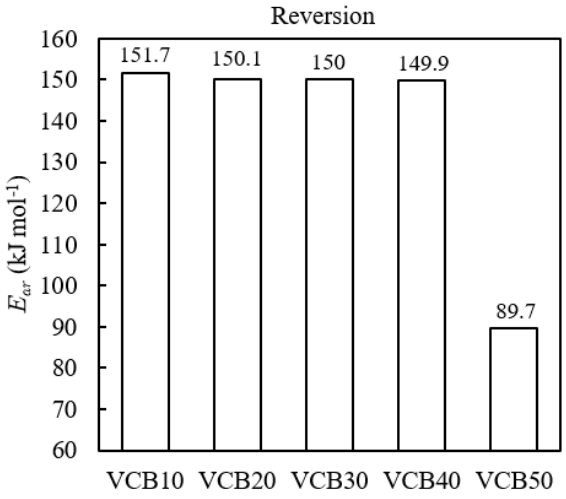

Figure 3. Effect of hydrochar content on reversion activation energy

The crosslinking and reversion rate constants were obtained by fitting Equation 7. As a high quality of crosslinking and reversion parameters was obtained, the results for all tested samples are shown in Figures 4-8.

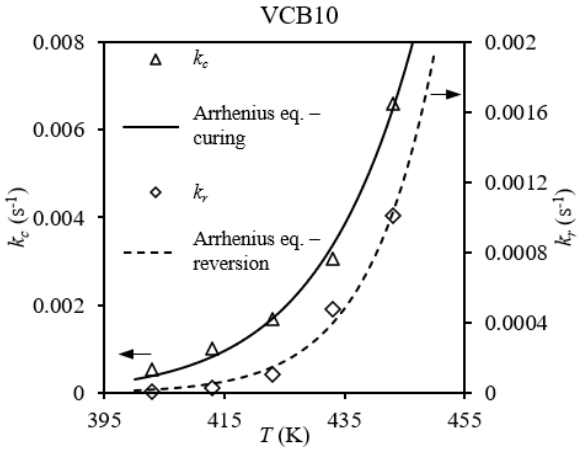

Figure 4. Dependence of curing and reversion rate constants on temperature, for the sample VCB10

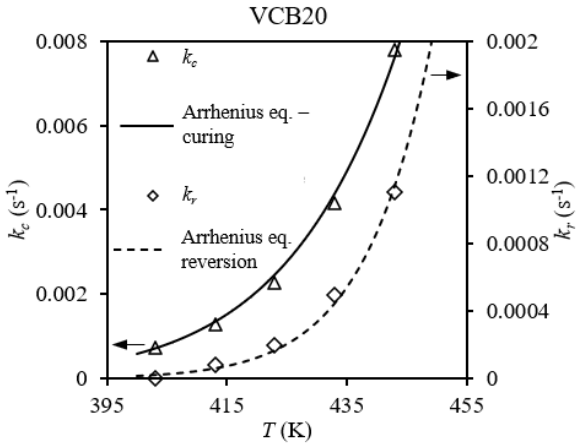

Figure 5. Dependence of curing and reversion rate constants on temperature, for the sample VCB20

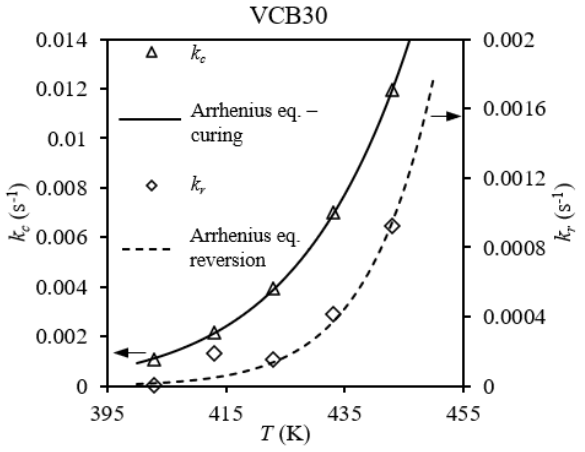

Figure 6. Dependence of curing and reversion rate constants on temperature, for the sample VCB30

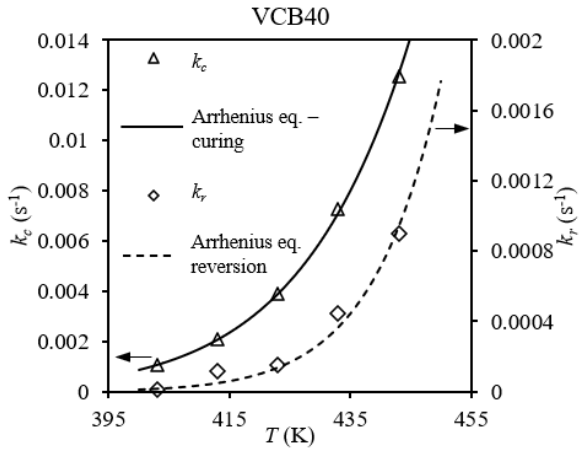

Figure 7. Dependence of curing and reversion rate constants on temperature, for the sample VCB40

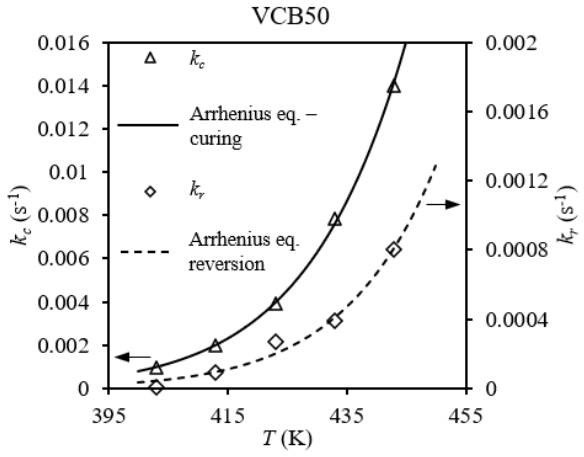

Figure 8. Dependence of curing and reversion rate constants on temperature, for the sample VCB50

Commercially available fillers used in the rubber industry reduce the amount of rubber necessary to obtain quality products, and therefore lower the price of final products. Fillers can be used to affect the course of vulcanization and the properties of the final product, and these are called active fillers, due to the functional groups on the surface of their particles. Active fillers increase the vulcanization rate and reduce the curing activation energy, thereby reducing the energy barrier for the beginning of the process [20]. Therefore, active fillers participate in the creation of polysulfide bridges in the rubber structure, contributing to the formation of a three-dimensional network. Inactive fillers can have the opposite effect on vulcanization, by increasing the activation energy and reducing the vulcanization rate, which can also be a consequence of the filler particles size [21]. In this research, a commercially available filler, activated carbon black and hydrochar obtained by hydrothermal carbonization of hardwood waste biomass were used. Observing the rate constants of products with different hydrochar content, it can be seen that the hydrochar addition reduces the curing rate constant, while it has no significant effect on the reversion rate constant. The curing activation energy increases with increasing hydrochar content, where the product without hydrochar has the lowest curing activation energy (Figure 2). The reversion activation energy is significantly lower for the rubber mixture without hydrochar, compared to products containing bio-filler (Figure 3). It is observed that the curing activation energy values are lower than the reversion activation energy for the same rubber samples, which is in accordance with literature data [21]. The lower curing activation energy of rubber compounds with the addition of hydrochar does not necessarily represent their negative property. Early start of vulcanization is often a problem in the rubber industry, which occurs even before the rubber mixture is distributed in the mould. Higher activation energy values delay the vulcanization beginning, and the hydrochar addition to the rubber mixture can reduce the risk of beginning the curing reaction before the mixture has the proper form in the mould, for which curing retarders are used. Postponing the beginning of curing is especially important for large-sized products, where curing of the surface next to the mould occurs, as well as reversion, while the interior is not yet cured [20]. A delayed start of vulcanization and a "stretched" shape of the process S-curve was observed in the sample with the highest hydrochar content (Figure 1), which makes this sample suitable for the production of large rubber products. The possibility of using hydrochar as a partial filler in the rubber mixture opens up the possibility of a gradual transition to bio-fillers, which can lead to a reduction of the problem of gases with the greenhouse effect, but at the same time the dependence on fossil fuels is also reduced, using sustainable bio-fillers.

CONCLUSIONS

Hydrochar and carbon black-based natural rubber mixtures were prepared, to investigate the influence of filler type and content on the kinetic parameters. A comparison of vulcanization parameters revealed that the bio-filler has impact on the vulcanization kinetic, in the way to lower the maximal vulcanization torque, while does not affect reversion phenomena. Furthermore, the increment of hydrochar content led to higher curing activation energy, presenting the products with hydrochar suitable composite material for production of large rubber products that needs longer time at low temperatures to be cured. On the other hand, an increase in the curing activation energy with the addition of hydrochar as a filler would

represent a negative property during the production of rubber products of smaller dimensions.

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THE ROLE OF FISHING PORTS IN THE SUSTAINABLE BLUE ECONOMY

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:
Fisheries and aquaculture play an important economic, environmental and social role. The development of fisheries and aquaculture depends on many factors, including adequately equipped ports and landing sites. The aim of this paper is to highlight the role of fishing ports in the sustainable blue economy. Fishing ports support the sustainable development of fisheries and aquaculture by promoting best practises for environmental protection, maintaining food quality, creating fair prices, supporting workers' rights, integrating local communities and ports, and more. The Food and Aquaculture (FAO) Blue Ports Initiative aims to strengthen the role of ports as drivers of sustainable development in coastal communities and promote the achievement of several key Sustainable Development Goals (SDGs), including SDG 1 (No Poverty), SDG 2 (Zero Hunger), SDG 3 (Good Health and Well-being), SDG 5 (Gender Equality), and SDG 14 (Life Below Water). However, ports are also encouraged to adopt a blue economy approach to management to achieve long-term benefits for local communities. Port management, in collaboration with scientists, policy makers, the private sector, and civil society, should focus on inclusivity, competitiveness, greening, and process efficiency, and support collaborative actions to improve the quality of life of local communities.

KEYWORDS: fishery and aquaculture; ports; sustainability; blue economy; marine environment

INTRODUCTION

Since the 1960s, fishery and aquaculture have provided an important source of food, jobs, and income for millions of people, when shortages of aquatic organisms and rising demand stimulated especially aquaculture production. According to the Food and Agriculture Organization (FAO) of the United Nations (UN) [1], in 2020, fisheries and aquaculture produced 214 million tonnes of aquatic food valued at about $424 billion, and employed 58.5 million people (21% of whom were women), 35% of whom worked in aquaculture, a 60% increase in production since the 1990s. In 2020, global capture fisheries production was 90.3 million metric tonnes, with an estimated value of USD 141 billion, including 78.8 million metric tonnes from marine waters and 11.5 million metric tonnes from inland waters [1]. In 2020, global aquaculture production reached 122.6 million tonnes, with a total value of USD 281.5 billion [1]. According to the same source, in 2020, per capita aquatic food consumption was 20.2 kg, more than doubling since the 1970s. FAO forecasts that annual per capita consumption will increase from 20.2 kg in 2020 to 21.4 kg in 2030.

Aquatic foods are very important for food security and nutrition, not only as a source of protein in developing countries, but also as a highly diverse source of omega-3 essential fatty acids and micronutrients in developed countries. As demand for protein from marine organisms continues to grow and many stocks are overexploited, aquaculture remains important for feeding the world effectively, equitably, and sustainably [2]. Despite significant efforts and progress toward achieving the 2030 Sustainable Development Goals (SDGs), the world is still far from achieving many of the 17 SDGs, and fishery and aquaculture could promote the achievement of several key SDGs, such as SDG 1 (No Poverty), SDG 2 (Zero Hunger), and SDG 14 (Life Below Water) (UN, 2015). The United Nations Sustainable Development Goals (SDGs) set a clear target on fisheries (SDG Target 14.4): By 2020, effectively regulate harvesting and end overfishing, illegal, unreported and unregulated (IUU) fishing and destructive fishing practices and implement science-based management plans, in order to restore fish stocks in the shortest time feasible, at least to levels that can produce maximum sustainable yield as determined by their biological characteristics [3].

According to [4], in 2019, fishery stocks within biologically sustainable levels decreased to 64.6 %, while stocks fished at biologically unsustainable levels increased to 35.4 %. Biologically sustainable stocks consist of the maximally sustainably fished (57.3 % in 2019) and underfished stocks (7.2 % in 2019).

According to the International Labour Organization (ILO) [5], fishing is among the most dangerous occupations of all, and decent working conditions are very important to prevent unsustainable practices. Illegal, unreported and unregulated (IUU) fishing is usually associated with modern slavery, forced labour, and other abuses, as well as inadequate working conditions, lack of social protection, social security or health care, and absence of formal employment relationships [4] [5].

In 1988, FAO introduced a definition of aquaculture that reduces confusion with capture fisheries: Aquaculture is the farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants. Farming implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc. Farming also implies individual or corporate ownership of the stock being cultivated. For statistical purposes, aquatic organisms which are harvested by an individual or corporate body which has owned them throughout their rearing period contribute to aquaculture, while aquatic organisms which are exploitable by the public as a common property resources, with or without appropriate licenses, are the harvest of fisheries [6]. Mariculture or marine aquaculture occurs in species that rely on wild seed from the sea throughout the cycle in the sea or only during the rearing phase when a species is raised in a hatchery on land and sometimes in freshwater [4]. Offshore aquaculture is the rearing of marine organisms in water more than 50 m deep, at least 2 km from shore [7].

Fishing ports play an important social and economic role in the environment and are an important link for fisheries and aquaculture stakeholders. Because fishing ports can significantly support the sustainable development of fisheries and aquaculture by reducing pollution, promoting good practices to maintain food quality, and helping to create fair prices and integrate local communities and ports, FAO has launched the Blue Fishing Ports initiative (BFP initiative). The BFP initiative was launched following the 33rd Committee on Fisheries (COFI) Session in 2018.

The aim of this paper is to demonstrate the role of fishing ports in the sustainable blue economy.

BLUE TRANSFORMATION

Fisheries and aquaculture face numerous challenges, including economic, environmental, and social challenges. Following the 2021 Committee on Fisheries (COFI) Declaration for Sustainable Fisheries and Aquaculture [8], FAO launched the Blue Transformation roadmap, a priority programme under the FAO Strategic Framework 2022-2031. The Blue Transformation roadmap will help maximize the contribution of aquatic food systems to the SDGs in order to support employment, economic growth, social development and environmental recovery by efficient, inclusive, resilient and sustainable aquatic food systems for better production, better nutrition, a better environment, and a better life, leaving no one behind [4].

By 2030, aquatic food production will increase by a further 15 %, mainly by intensifying and expanding sustainable aquaculture production. Intensifying and expanding sustainable aquaculture production will require further technical innovation (digitalization), policy support, and incentives along the entire value chain, including access to water, optimization of carrying capacity, designation and allocation of aquaculture zones, licensing procedures with good environmental practices and monitoring, trained and skilled labour, production of high-quality seed and feed, regulation of chemical and antibiotic use, and strict biosecurity protocols [1]. The focus should be on genetic improvements in breeding programmes, feed, biosecurity, and disease control, along with coherent measures and appropriate incentives throughout the value chain.

According to [1], the Blue Transformation has three core objectives:

Sustainable expansion and intensification of aquaculture - to support global food security goals and meet global demand for nutritious aquatic food and equitable distribution of benefits.

Effective management of all fisheries to create healthy stocks and secure livelihoods.

Upgraded value chains - to ensure the social, economic, and environmental viability of aquatic food systems and to ensure food security.

THE BLUE PORTS INITIATIVE (BPI)

The Blue Growth Initiative (BGI) is an FAO model for the sustainable development of fisheries and aquaculture [9], that takes a strategic, innovative approach to aquatic resource use and increases social, economic, and environmental benefits to communities (FAO, 2021b). To meet the requirements of sustainable fishing and commercial ports, including

environmental protection, social equity, and economic growth, fishing ports should adopt the blue economy approach as a strategy [10].

In 2019, FAO launched the Blue Ports Initiative (BPI) with the aim of strengthening the role of ports as drivers of sustainable development in coastal cities and communities and promoting positive and sustainable socio-economic growth, while reducing environmental impacts and poverty and supporting the enforcement of workers' rights and gender equality. The BPI has launched specific actions in collaboration with 20 port authorities, fisheries sector administrations and international organizations, including the Intergovernmental Oceanographic Commission of United Nations Educational, Scientific and Cultural Organization (IOC-UNESCO), the World Bank, the ILO and the International Maritime Organization (IMO). This international consultation process with fishing ports consisted of three workshops that sought to better understand the role of ports in coastal development and identified the need for guidelines on how a port can become blue.

The BPI supports the sustainable development of coastal areas through Blue Ports Operations, which have the following objectives [10] [11]:

- Establish and operate the Blue Ports Network - an operational programme, baseline, and guidance to become a Blue Fishing Port.
- Capacity building for the management of Blue Ports as hubs for innovation and sustainable development - through workshops, seminars and training.
- Develop and implement innovative tools for knowledge management in fishing ports, including a digital platform and a data observatory.
- Designing and implementing port strategies and measures under a blue growth approach.
- Measuring the actual impact of ports in their region.

FAO promotes technology, innovation, data and complements (governance, human capital and institutions) through its projects. The BPI enables ports to become a source of added value for local development, and the commercial activities of ports should also be improved in terms of volume and revenues [11]. However, ports are also encouraged to implement a blue economy approach to management to achieve long-term benefits for the local community. Port management should include inclusiveness, competitiveness, greening, and process efficiency in collaboration with scientists, government agencies, the private sector, and civil society, and joint actions aim to improve the quality of life of the local community [11].

STRENGTHENING THE ROLE OF BLUE FISHING PORTS IN MARINE SPATIAL PLANNING

The MSP roadmap, a Joint Roadmap to accelerate Maritime/Marine Spatial Planning processes worldwide adopted by IOC-UNESCO and the Directorate-General for Maritime Affairs and Fisheries of the European Commission (DG MARE), was implemented under the MSP global Initiative project, which ended in October 2021. In 2021, IOC-UNESCO and FAO launched a programme Strengthening the role of Blue Fishing Ports in Marine Spatial Planning [12] with the goal that port authorities incorporate marine spatial planning (MSP) as part of strategic and operational processes. According to [13], MSP is a public process of analyzing and allocating the spatial and temporal distribution of human activities in marine areas to achieve ecological, economic and social objectives that have been specified through a political process. MSP is a practical way to rationally use marine space, balance the demands of development with the need to protect the environment, and achieve social and economic outcomes in an open and planned manner [13]. The programme also contributes to the goals of the UN Decade of Ocean Science for Sustainable Development (2021-2030), a common framework for marine research to achieve the Sustainable Development Goals (SDGs) of the 2030 Agenda.

THE ROLE OF FISHING PORTS IN COMBATING IUU FISHING

Illegal, unreported and unregulated (IUU) fishing is a well-organized business with lower operating costs and higher profits, often associated with substandard vessels and unfair working conditions. Losses in the global economy due to IUU fishing amount to approximately USD 23.5 billion [14]. Therefore, combating IUU fishing can contribute to achieving the SDGs and a sustainable blue economy. Port States can help eliminate IUU fishing by detecting substandard vessels and unfair working conditions. Vessel monitoring is easier in ports than at sea, and therefore Port States can monitor the port activities of vessels and crew, especially for foreign-flagged vessels. Port States can deny port entry and access to port services to known or suspected IUU fishing vessels, identify high-risk vessels, establish systems to reduce the risk of corruption, and improve maritime governance [14].

In 2016, the FAO Agreement on Port State Measures to Prevent, Deter and Eliminate Illegal,

Unreported and Unregulated Fishing (PSMA), the first binding international agreement to combat IUU fishing, entered into force [15]. In 2017, the International Labour Organization (ILO) C188 Work in Fishing Convention entered into force to regulate the minimum age for working on a fishing vessel, medical standards, working arrangements, occupational safety and health, and social security [16]. In 2012, the International Maritime Organization (IMO) adopted the Cape Town Agreement (CTA) to contribute to safe, legal, and sustainable shipping, but it has not yet entered into force [17].

Through these three international instruments, FAO, IMO, and ILO seek not only to combat IUU, but also to improve vessel safety and labor standards through stricter port procedures and to increase benefits for legal operators. Cooperation between port authorities, maritime authorities, fisheries inspectors, maritime police, customs, immigration, labor authorities, the Coast Guard, and the Navy is also very important to curb IUU fishing [14].

CONCLUSION

Fishing ports support the sustainable development of fishery and aquaculture by promoting best practices for environmental protection, maintaining food quality, creating fair prices, supporting workers' rights, integrating local communities and ports, and more. The FAO Blue Ports Initiative aims to strengthen the role of ports as drivers of sustainable development in coastal communities and promote the achievement of several key Sustainable Development Goals (SDGs), including SDG 1 (No Poverty), SDG 2 (Zero Hunger), SDG 3 (Good Health and Well-being), SDG 5 (Gender Equality), and SDG 14 (Life Below Water). However, ports are also encouraged to implement a blue economy approach to management to achieve long-term benefits for local communities. Port management, in collaboration with scientists, decision makers, the private sector, and civil society, should emphasize inclusiveness, competitiveness, greening, and process efficiency, and support joint actions to improve the quality of life of local communities.

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THE POTENTIAL OF BRYOPHYTES IN PHYTOFILTRATION OF HEAVY METAL CONTAMINATED WATER

PRELIMINARY COMMUNICATION

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ABSTRACT:
Bryophytes are a group of plants vital to many ecosystems and biogeochemical cycles and are well known bioindicators and biomonitoring tools. However, they have been deemed industrially less important than vascular plants and their potential for applications other than as biomonitoring tools has been overlooked. In recent years, however, bryophytes, and in particular mosses, are starting to gain attention as viable phytoremediation agents. Studies indicate that some moss species have the ability to uptake heavy metals such as Pb, Cd, Zn, Cu, As, and Cr from contaminated water. Tested mosses could both adsorb and absorb significant amounts of specific heavy metals without adverse effects on the plant. Results suggest that moss biomass, either dry or wet, could be used as a bio-sorbent in filtration of heavy metals. The review of available literature shows a promising prospect of moss to be used in phytofiltration of heavy metals. Existing knowledge on this topic could be the basis for further research which is needed.

KEYWORDS: moss; heavy metal; phytofiltration; bio-sorbent

INTRODUCTION

Bryophytes are a group of plants vital to many ecosystems and biogeochemical cycles. They were the first plants to evolve from water plants, namely green algae, to terrestrial plants and pave the way for other plants to develop in terrestrial environments. There are three major gorups of bryophytes: mosses, liverworts and hornworts. This paper will focus on mosses. Although they are deemed industrially less important and often overlooked in favor of vascular plants, there are several strong points of mosses that indicate that these plants may be a usefull tool in environmental engineering.

Mosses inhabit a variety of ecosystems and microecosystems, in many of which vascular plants can not survive. Mosses adapt well to nutrient poor environments and harsh conditions. The key difference between mosses and bryophytes in general and vascular plants is that the former lack a root system, meaning they absorb nutrients through their whole surface. Moss leaves also lack a cuticle layer which makes their cell walls easily accessible to metal ions and in turn facilitates ion-exchange over a large surface [1]. These characteristics allow moss species to absorb and accumulate large amounts of heavy metals and other pollutants both inside and outside of their cells. The extracellular pollutant concetration shows the current pollution levels, while the intracellular fraction shows an average pollution over a period of time [2]. Beacause of this, mosses proved to be valuable tools for in situ biomointoring and have been used as such for decades. Their potential for applications other than air pollution monitoring, however, has been overlooked up until recently. Recent studies have been conducted researching mosses for applications other than in biomonitoring and this paper aims to concisely present the promising results of those studies.

DISCUSSION

The ability of mosses to accumulate pollutants from air is well known, but scientists are proposing that this ability could be applied to other media as well. For example, Nduka and Umeh [3] showed that a non-specified terrestrial moss displayed the ability to uptake heavy metals from aqueous solutions. Precisely, moss could absorb ions Pb^{2+}, Cd^{2+}, Zn^{2+} and Ni^{2+} and showed a particular tolerance to Pb^{2+} and Cd^{2+}, theorizing that the use of moss could be feasible for waste water treatment.

Some moss species, like *Bryum capillare, Funaria hygrometica* and *Ceratodon purpureus,* are known hyperaccumulators for heavy metals, specially for Fe, Pb, Cu, Mn and Zn [4]. Vukojevic et al [4] also showed that mosses are able to accumulate heavy metals from coal ash landfill and showed potential for remediation of ash deposit sites because they bind surface ash and prevent it from being blown away. Mosses do not absorb nutrients from soil since they lack a root system, but they do facilitate growth of vascular plants on soils which would not likely sustain their growth otherwise [4].

Aquatic moss biomass from the species *Taxiphyllum barbieri* showed capacity to accumulate heavy metals Pb, Cd, Zn, Cu, As, and Cr [5]. *T. barbieri* was especially suitable for the phytofiltration of Pb (>100 g/kg DW in 6 h), Cd (about 4.3 g/kg DW in 6 h) and Cr (about 19.4 g/kg DW in 6 h) from contaminated water. It was noted that lighting conditions while growing moss influenced the hue of moss and also the absorbancy efficiency for specific metals. In the same study, Papadia et al [5] asserted that due to their resistant thalli, mosses could also provide some mechanical filtration and support the growth of desirable bacteria. It is important to note that the interrelationship of multiple heavy metals in one medium and its effect on removal pathways in moss are unclear.

Although the application of mosses for phytofiltration of contamintated water still is a very under-researched topic, there are several studies implicating its potential. For example, one study [6] indicated that *Warnstorfia fluitans* removed up to 82% of As from the water in the form of arsenate without toxic effect on the plant biomass, which was comparable to As accumulation capacity of A-hyperaccumulating vascular plant *Pteris vittata*. The same study found that of the As taken up, over 90% was bound to the tissue, and arsenic was both absorbed and adsorbed by the moss, and twice as much As was found in living parts than in dead moss tissue. These results propose that *W. fluitans* could be suitable for use in the phytofiltration of As-contaminated waters.

The same moss species, *W. fluitans* also exhibited the ability to remove nitrogen from polluted water, especially when used in conjuction with other materials such as woodchip [7]. In a hybrid bioreactor combining moss and woodchip, 48% of dissolved inorganic nitrogen on average was removed from mine-influenced water in cold climates. The hybrid bioreactor showed significantly higher removal rates than bioreactors using solely woodchip or moss [7].

Another study showed that the moss species *Bryum muehlembeckii* and *Sphagnum perichaetiale* presented great efficiency in removing Fe and Cr in dry biomass form, both removing at least 95% of Fe and Cr present in aqueous solution [8], comparable to removal rates of Cr by activated carbon [9] which is commonly used for contaminant removal.

Another recent study even presented evidence of the abitility of moss *Sphagnum palustre* L. to retain polystyrene nanoparticles [10].

Itouga et al [11] report that protonema of the moss *Funaria hygrometica* adsorbed Pb with a maximum adsorption capacity of 74.1%, mainitaining stable adsorption within a pH range from 3 to 9 of test solutions. Adsorption of Au, Cr and Tl by *F. hygrometica* was also efficient [11]. Therefore authors suggested that *F. hygrometrica* could be a biomaterial for the bio-sorbent filtration of metals.

Aforementioned studies show that the tested moss species in dry or wet biomass forms can be applied as phytoremediation agents and bio-sorbents of metals from contaminted water. Although existing methods of heavy metal removal from contamintated water using vascular plants are efficient, the presented studies show that other phytogenic options could be auspicious as well.

DISPOSAL OF PHYTOREMEDIATION PLANTS

Since vascular plants are established in phytoremediation, all existing methods of disposal and utilization methods of phytoremediation plants pertain to vascular plants. Theorizing that the end product of phytoremediation is biomass regardless of whether the phytoremediation agent was a vascular plant or a bryophyte, disposal methods applied to vascular plants could also be applied to mosses.

FUTURE DIRECTIONS

Although there are a number of studies reporting on various moss species‘ ability to uptake and retain metals, the extact mechanims through which this is achieved are little understood. In general, it is accepted that the lack of cuticle on moss leaves allows easier ion-exchange and due to the lack of root systems, moss adsorb metals through their whole surface. However, specific characteristics and genes involved in heavy metal uptake and resistance need further research. Itouga et al [11] propose that the high adsorption capacity for Pb of *F. hygrometica* could be linked to phosphoglyceric acid (PGA) found in *F. Hygrometica* cell walls, PGA being found to have similar adsorption capacity to chitosan [12].

Mentioned experiments studied short-term uptake of mosses. With mosses being slow growing plants, long-term uptake and uptake in different growth

phases of the plant should also be investigated. Research is also needed on behavior of moss in presence of multiple contaminants, as well as to what degree a moss bed could provide mechanical filtration to wastewater.

Since it was shown that different lighting conditions during moss growth influence heavy metal absorption capacity [5], studies optimizing moss cultivation conditions to maximize uptake would be beneficial.

It was also proposed that moss species could be genetically modified to further enhance heavy metal uptake capacity and minimize negative effects on the plant [13], which could be a possible.

Development of microplastic removal methods from soil and water is imperative and the possibility to use moss for this purpose is compelling.

Comparison to established biosorbents and phytofilters taking into consideration environmental impact is also necessary.

Since the potential of bryophytes for phytofiltration of heavy metal contaminated water has only recently been noticed, existing research is scarce but promising.

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INTERACTIVE EDUCATION ON SUSTAINABLE USE OF ELECTRICAL DEVICES

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:

Education in GREEN electrical energy and production usage is essential for young people. Every consumer can do a lot if they use electrical devices wisely. This paper will present the design, intention and use of a didactical learning exhibit for middle and high school students during technical days on faculty and faculty promotions and for educational purposes. Our main goal is to introduce students to how some everyday electrical devices work, how much energy they use and how they can save as users. The most significant emphasis will be electromagnetic devices such as elevators and devices we use daily. We will use a professional approach and design for that purpose, but we will focus on presenting it on an interactive touchscreen and small examples of those devices. The content will be in graded expertise levels, from simple picture and animation calculations to the advanced picture and mathematical model presentations. The exhibit will be designed modularly so that content or presentation can change quickly and be made from recycled or sustainable materials as much as possible.

KEYWORDS: Sustainable; Educational; Electricity; Electromagnetic devices; Interactive; Elevator; Exhibit

INTRODUCTION

Before creating a didactical learning exhibit, the most important thing is setting goals for its functionality and target population. Our leading group are younger population but as high school and faculty students. Because of that, it should be physically assembled robustly and look attractive to them. That is why we chose a capacitive touchscreen, which is robust and has no mechanical commands or buttons. With that approach, we can extend usage life because it can be programmed with actual problems in the future without changing hardware. With every part of the design, we are looking to use sustainable components and materials which are environmentally friendly. We found a teaching platform for electromagnetics [1]. Platform designed for teaching the theory of electromagnetics, our work will present teaching practical examples. We use data on elevator energy usage from the publication Advancing Elevator Energy Efficiency [2]. The paper includes three sections. The Materials and Methods section describes the conceptual idea, exhibit design, calculations and simulations. Results, screen configurations and preservations on exhibit are included in the section Results and Discussion. In the final section, Conclusion, we will discuss about further development.

MATERIAL AND METHODS

The main component of the exhibit is a touchscreen display, so we did market research. Our requirements were a size of around 10 inches, capacitive touch, colourful, integrated screen driver hardware, acceptable price, good graphic editor, and an option to connect outside microcontroller. We found Nextion display NX1060P101-011C-I with a 10.1-inch display, 1024x600 pixel resolution. It can also connect over UART communication with the microcontroller and integrated audio driver with an equalizer. In Fig. 1, is presented the selected touchscreen display.

Figure 1. Touchscreen display used for exhibit

For designing the 3D model of the exhibit, we used Designspark Mechanical software. The most straightforward solution will be to 3D print the

touchscreen display housing or mount it in some electrical dose. However, there will be used plastic, which is harder to recycle. Because of that, we propose to use medium-density fiberboard (MDF) from waste material used in carpenter production. Only display will not attract the target population so much, so we should add mechanical devices. Because we are focused on presenting the working of electromagnetic devices, we add three devices of this type: direct current electrical motor, speaker and electric relay. That means we will present some smaller real devices combined with theory and working principles displayed on the touchscreen display. In Fig. 2, you can see the conceptual idea.

Figure 2. The conceptual idea of the display housing

Our faculty organizes many events, presentations and workshops on our work and education about technology to scholars and students. They are focused on teaching and transferring knowledge, but little focus is on ecology and sustainable use. We will present our two proposals and approach to include more of that part. Many people use elevators daily, especially in commercial and residential buildings. For example, exact numbers were not essential to our calculations or measurements, so we used existing data and presented it in Table 1 [2].

Table 1. Elevator energy usage in low-rise buildings [2]

	Residential apartments	Commercial office
No. of trips per day	176	589
Running power (W)	4900	8500
Idle power (W)	1326.45	208
Nonrunning power (W)	1645	409.8
Daily energy consumption (kWh)	5.35	29.68
Annual energy consumption (kWh)	1656.48	6422.88
Reduced daily energy consumption (kWh)	4.55	25.23
Reduced annual energy consumption (kWh)	1408.01	5459.45

Data shows the main difference between residential and commercial use and the type of elevator. The residential is more decorated and better looking, as well as its lighting and control panel. This impacts idle power, which is more than six times bigger than in Commercial ones. The difference is in running power because Residential elevators are mainly constructed to transport people and lighter loads.

We calculated if we lower consumption by 15 % from the presented data, which means lower usage time and less consumption in nonrunning time with more efficient lighting and control units. From equation (1)-(4) you can see calculations [2]:

$$E_{drr} = E_{dr} \cdot 0.85 = 5.35 \cdot 0.85 = 4.55\ kWh \quad (1)$$

$$E_{arr} = E_{ar} \cdot 0.85 = 1656.48 \cdot 0.85 = 1408.01\ kWh \quad (2)$$

$$E_{drc} = E_{dc} \cdot 0.85 = 29.68 \cdot 0.85 = 25{,}23\ kWh \quad (3)$$

$$E_{arc} = E_{ac} \cdot 0.85 = 6422.88 \cdot 0.85 = 5459.45\ kWh \quad (4)$$

Where E_{dr}, E_{dc} daily energy consumption, E_{ar}, E_{ac} annual energy consumption of the elevator system in Residential and Commercial buildings.

After considering 15% lower energy usage where E_{drr}, E_{drc} reduced daily energy consumption, E_{arr}, E_{arc} reduced annual energy consumption of the elevator system in Residential and Commercial buildings. We can see some differences where we can save energy, and users will make something for their health because they will use the staircase instead of the elevator.

For the second proposal, we will be focused on synchronous electric motors with permanent magnets. The number of applications which use that type highly increases. Also, many elevators use that type of electric motor. Its actual theme is to educate how these devices work and why they are more sustainable than other types. If we compare these types over direct

current motors with carbon brushes, those devices have a longer lifespan and higher efficiency. They are also more silent and have fewer vibrations because they have no carbon brush, which is user and environmentally friendly. We make two and three-dimensional motor models of the motor used in the exhibit. Simulations and equations will be presented on display screens. For example, you can see in Fig. 3 the model and magnetic flux of the electric motor used in the exhibit.

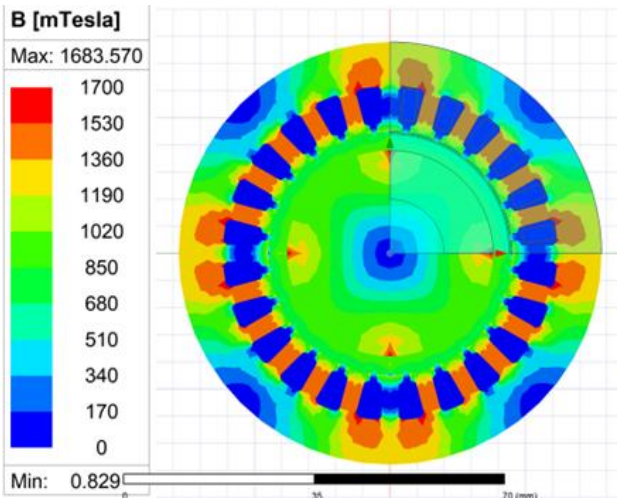

Figure 3. Simulation of magnetic flux density in exhibit electric motor

RESULTS AND DISCUSSION

In the previous section, we present our proposal, which will go into assembly. After the assembly, we will present it with the test screens below. Presented screens are for testing purposes to see how people will accept transferring information that way. After that, it will be graphically improved. The range of presented applications can be extended to more examples and proposals on how to save energy. Fig. 4 presents two screen pictures for presenting elevator energy saving. The upper screen presents an introduction, and the lower presents a calculated example.

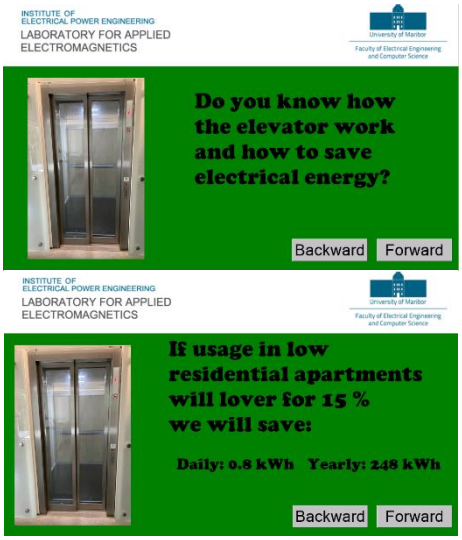

Figure 4. Example of two display screens for presentation elevator energy-saving

Continuing with the expertise level, we presented the magnetic flux density distribution in the exhibit electric motor. As mentioned, it is a permanent synchronous electric motor with permanent magnets. Fig. 5 presents two screen pictures of a computer design of a model for electromagnetic calculation. The upper screen presents a calculation of the magnetic field in the used electric motor.

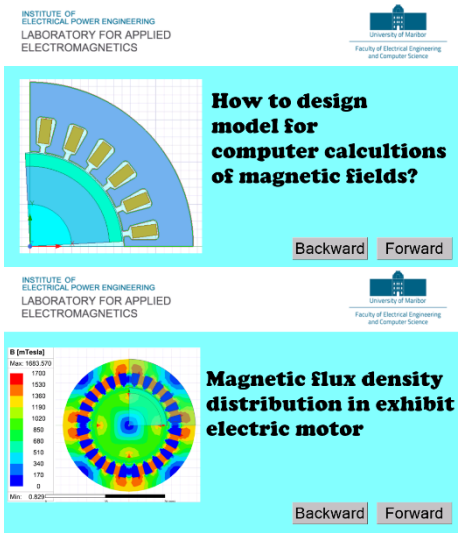

Figure 5. Example of two display screens for presentation electromagnetic calculations

CONCLUSION

Our goal will be fulfilled if we change their mind and turn them to thinking greener when using electrical devices. To educate on reducing energy usage, users must also understand the fundamental aspects of how devices work. Because natural energy resources are closely connected, if we reduce the

consumption of one, we also affect others. Further development will be assembly and evaluation. The evaluation will be in two parts, testing the working of the exhibit and user's experiences.

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PERCEPTION OF FACULTY OF TOURISM AND RURAL DEVELOPMENT STUDENTS ON SLOW FOOD CONCEPT

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:
The Slow Food movement, originated in Italy in 1989, is a movement that defines a respectful attitude towards food. It includes the protection of local food, food culture and traditions. The movement advocates the cultivation of food that is carefully prepared and consumed with pleasure. It encourages the improvement of relations between producers of ecologically produced food, cooks and consumers. Slow Food represents resistance to fast food and modern fast consumption, which has led to the abandonment of local gastronomic traditions and a decrease in people's interest in authentic food. The aim of this paper was to investigate the attitudes of students of the Faculty of tourism and rural development in Požega about Slow Food. Using the online survey method, the research has been conducted on a sample of 38 students of Enogastronomy and Tourism. The results of the research showed that the Slow Food concept is not recognizable among students because only 30% have heard about it. Enjoying the food they consume is extremely important for almost all students. Half of the respondents had heard of Slow Food, while only a quarter declared that they had consumed food presented as Slow Food. According to the respondents, Slow Food is best described by the attributes "natural", "ecological", "healthy", "conscious food consumption" and "enjoying eating".

KEYWORDS: Slow Food, student, organic food, perception, consumption

INTRODUCTION

Since 1986 when Carlo Petrini together with a like-minded group preformed demonstration against Mc Donald's and globalization of food, Slow Food movement has grown into complex approach to food and international organization spread worldwide [1]. According to [2] Slow Food has been perceived as organization, movement, ideology and lifestyle. However, it is important to see it as an alternative type of food consumption which gathers local production, culture, tradition, preparation of food and in the end its consummation. There are three main principles of Slow Food: good, clean and fair, meaning that food should be tasty, produced in authentic and natural way related to certain geographic and cultural region, and also produced in environmentally and socially sustainable ways [3]. Pleasure occupies an important place in the Slow Food philosophy, perceiving food beyond just functional role, whereby pleasure implies consummation of food as well as production and preparation [4]. Dunlap [5] stated that Slow Food as movement is tightly related with leisure. In recent decades, consumer demands regarding food quality and safety have changed significantly. Consumers have become better educated and aware that the way they eat significantly affects their health, which is becoming an increasingly important criterion when choosing food products. In that light, Slow Food principles could become very interesting for hospitality and tourism sector [6] because Slow food tourism can be considered as an important part of the cultural tourism market, attracting tourists who are looking for authentic experiences and the consumption of local food and beverages [7].

The share of tourism in the gross domestic product (GDP) of the Republic of Croatia is about 20%, however it is characterized by distinct seasonality [8]. According to research Institute for Tourism [9]. Basic motives for tourist arrivals in Croatia are sea and nature which explains seasonality. Only 6.6 % of respondents stated that gastronomy is their motive for visiting Croatia. However, Drpić and Vukman [10] stated that gastronomy tourism in Croatia grows every year, assisted by promotion of its gastronomic riches, taking in consideration the fact that in such a small space there is an offer of many different types of food. Skryl et al. [11] conducted a survey among 30

Croatian restaurants about contemporary culinary trends, including Slow Food. 83.3% of the surveyed restaurants answered that they offer food that belongs to the Slow Food movement i.e use fresh ingredients, purchase from small producers, use whole grains, and food processing itself follow traditional ways. However, only few of restaurants in Croatia are officialy recognised as Slow Food restaurants. Gastronomic tourism is an opportunity for Croatia to expand its tourist season throughout the year and contribute to the fact that catering facilities in Croatia's seaside do not close during the winter months.

According to official Slow Food web page [12], there are only 4 convivia and 1 community in Croatia. Convivia is a local Slow Food chapter. It organizes events and activities at the local level, ranging from simple dinners and tastings, where members come together to share the everyday joys of food; visits to local producers and farms, conferences and discussions for adults and children. Food community is a group of small-scale producers and others, united by the production of a particular food and closely linked to a geographic area. Food community members are involved in small-scale and sustainable production of quality products. Çelebi and Genç [13] and Voinea et al. [14] investigated the perception of youth towards Slow Food in Turkey (Gastronomy and Culinary arts students) and Romania (Quality Management, Expertise and Consumer Protection). As they pointed out - students of such specific study programs will be the bearers of gastronomic trends, the developers of tourism in local communities and above all sustainability guardians. It is crucial that they are familiar with the potential of Slow Food principles so they could apply them in the future. To the best of our knowledge, there hasn't been similar research in Croatia or any other research among the student population in the research region (continental Croatia – Slavonia) involving gastronomy. Vuksanović et al. [15] also mentioned scarcity of studies and research gaps about gastronomy and food in the continental Croatia area and surrounding countries, pointing local producers as drivers of rural development and tourism progress, which is in consistency with Slow Food concept. As we already mentioned, there are only few Slow Food facilities in Croatia, so the primary goal of this paper is to find a perception of Tourism and Enogastronomy students towards Slow Food concept in order to get a clearer picture of the current knowledge of this very important topic, which would direct us to future research and projects.

MATERIALS AND METHODS

Students of the Faculty of tourism and rural development in Požega, Croatia, have been trained as experts who will contribute to enrichment of the tourist offer with their knowledge. It is very important that they know the latest trends in gastronomy, which is an integral part of every tourist destination. Main goal of this survey is to reveal the Slow Food perception of Enogastronomy and Tourism students of Faculty of tourism and rural development in Požega, Croatia. The research sample consisted of 38 students of all study years. Survey questions were adapted and inspired from similar research papers [13, 14]. Questionnaire was conducted on line (Microsoft Forms questionary *via* Merlin platform, which is obligatory platform for communication between students and professors on Faculty for tourism and rural development. After uploding the questionary on the Merlin platform, all students got notification as "pending assignment"). Survey consisted of 12 questions (2 questions for demographic and 10 questions for food in general and Slow Food perception). At the time when the survey was conducted, during 2023., 1st and 2nd year of Enogastronoy programe and the 1st year of Tourism programe were in progress, because those were new study programes at the Faculty. The city of Požega is located in eastern continental Croatia, Slavonia region (Figure 1.) The majority of students come from this region although there are few from other parts of Croatia like north Croatia and south Croatia.

After the respondents' answers were collected, the answers were presented in tables and graphs.

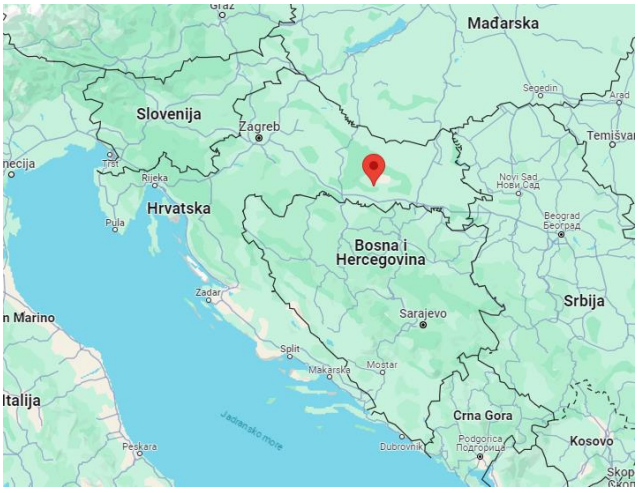

Figure 1. Geographical position of Požega city [16]

RESULTS AND DISCUSSION

The survey is based on a sample of 38 respondents. Their demographic profile is shown in Table 1. 11 (29%) men and 27 (71%) women participated in the research. The age of the participants varies between 19 and 51. Majority of participants were 19 (37%) and 20 (32%) years old.

Table 1. Demographic profile of students

		Frequency (n)
Gender (n=38)	Male	11
	Female	27
Age (n=38)	19	14
	20	12
	21	9
	27	1
	30	1
	51	1

The students' answers to the questions about cooking skills and eating habits shown in Table 2, give an insight into their commitment to food preparation and consumption. Considering the young age of the respondents, result of even 76% respondents who know how to cook is very interesting, but expected because students of Enogastronomy were also part of the research sample. Among male respondents, 27% answered that they cook "a little bit", while 22% of female respondents gave the same answer. The majority of respondents eat their meals at home or in the student canteen, and multiple answers to this question were possible.

Table 2. Cooking and eating habits of students (n=38)

		Frequency (n)
Do you cook?	Yes	29
	No	0
	A little bit	9
Where do you consume food?	Home	34
	Restaurant	1
	Fast food	1
	Student canteen	22

Table 3. shows the answers regarding the experience with the Slow Food concept. A larger number of respondents have never heard of Slow Food (n=22). Accordingly, as many as 76% of them declared that they have never tasted food labeled as Slow Food. However, more than half of the respondents (60%) agreed that Slow Food should be included more often in the gastronomic offer, while 34% declared that they do not know. As previously stated in the Introduction part, there are only few officially recognized Slow Food facilities (farms, restaurants) in Croatia, so students didn't have a chance to meet this concept very often. Unlike our findings, Çelebi and Genç [13] presented that all students who participated in survey regarding Slow Food in Turkey (n=20), had Slow Food experience. Voinea et al. [14] interviewed 50 Romanian students in regard to Slow Food, and 66% of them have heard of it, but 83% of them haven't consumed Slow Food products in the year before survey was conducted. Beside, 80% of respondents didn't know any of Romanian Slow Food products although there are many products on the market. Some students also equalized homemade food with natural ingredients and Slow Food.

Table 3. Experience and attitude of students about Slow Food (n=38)

		Frequency (n)
Have you heard of the Slow Food movement?	Yes	16
	No	22
Have you consumed food with the label Slow Food?	Yes	9
	No	29
Do you think that the slow food movement should be more applicable in the gastronomic offer?	Yes	23
	No	1
	I do not know	13

Table 4. shows the features that the respondents arbitrarily included in the description of the Slow Food concept. For almost half (40%) of the respondents, the Slow Food concept means food that tastes good, is nutritionally rich, is served in small portions, and comes from organic farming. 10% of respondents consider the Slow Food concept to be conscious consumption of food and enjoyment of the moment. 15% of respondents believe that Slow Food is food that is produced in a traditional way and prepared according to traditional recipes. Still, 18% of students didn't know how to describe Slow Food, which is expected since 60% of them previously answered that they had never heard of the concept. However, some of respondents still gave their opinion according to their expectations what Slow Food should be, although they didn't know it from earlier. Turkish

students decribed Slow Food as "healthy", "conscious consumption" and "local tastes" [13].

Table 4. Description of Slow Food according to students (n=38)

	Frequency (n)
Good-tasting, nutritionally rich food, served in smaller portions, and comes from organic farming.	**15**
Conscious consumption of food and enjoyment of flavors.	4
Food produced in a traditional way and prepared according to a traditional recipe for a specific region.	6
Eating less food.	2
Low energy food.	2
Eating more cooked food than fast food.	2
I do not know	7

Characteristics which describe Slow Food movement, according to respondents, are shown in Figure 2. For the vast majority of respondents (n=27), the concept of Slow Food means "natural". For 26 respondents, it means "good quality", that is, "conscious consumption of food", "ecologically acceptable" and "enjoying eating" (n=25). Students could choose multiple answers from offered possibilities. Students from already mentioned survey by Çelebi and Genç [13] mostly used terms "ecofriendly", "sustainability" and "pleasure".

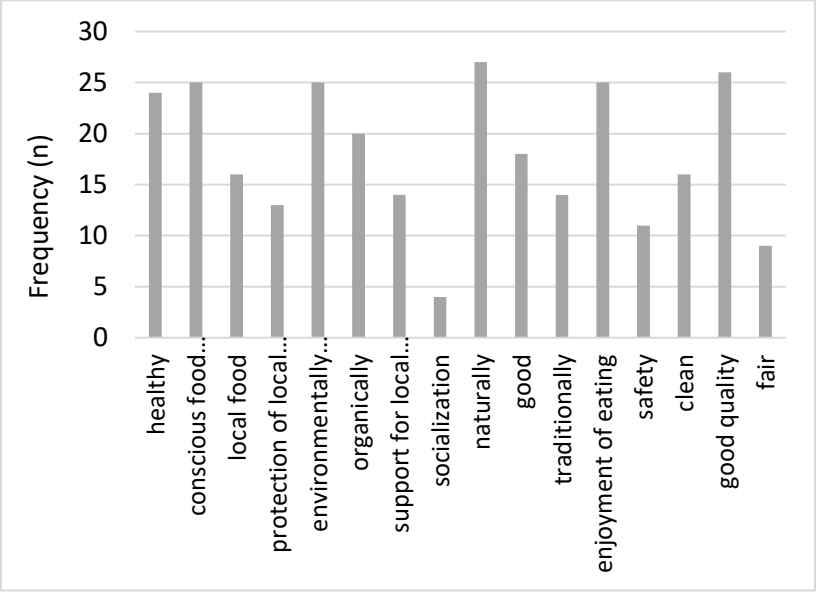

Figure 2. Terms that describe Slow Food movment the best, according to students (n=38)

Students were offered to rate the importance of the origin of the food they consume, enjoyment of the food they consume, and ecologically produced food they consume. They assigned the least important feature a score of 1, and the most important feature a score of 5. Results are presented in Figures 3 - 5.

The origin of the food they consume is not so important according to the attitudes of the respondents shown in Figure 3. For only a quarter of the respondents this feature is the most significant; the share among male and female respondents is approximately equal, 27% and 26%, respectively. On the other hand, only 3 students (8%) answered this question with a grade of 1 or 2, and all of them were female.

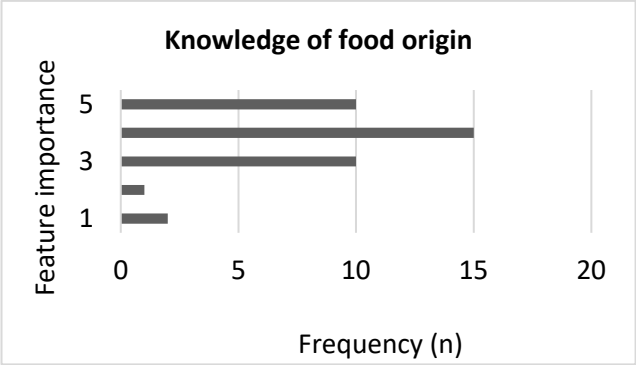

Figure 3. Importance of the origin of the food that students consume (n=38; 1- not important; 5 - extremely important)

Out of the three offered features, the most important for students is the enjoyment of the food they consume (Figure 4). 85% of respondents rated this feature with the highest rating, while consummation of organic food (Figure 5) is less important to them. Only 28% of respondents considered this feature to be extremely important, while 13 of them (34%) gave score 3, and 8% scored it with 1 and 2 (the same as in the previous question regarding the origin of the food).

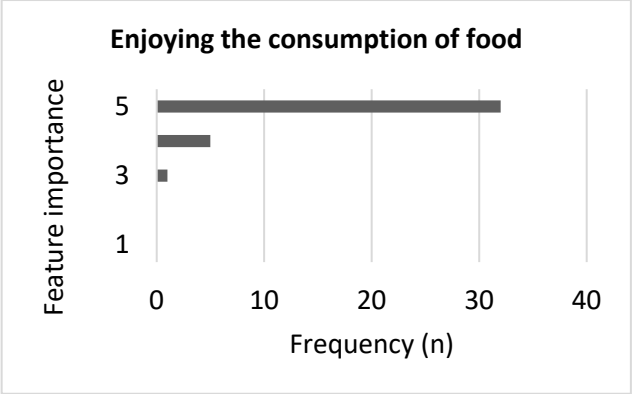

Figure 4. The importance of enjoying the food consumed for students (n=38; 1- not important; 5 - extremely important)

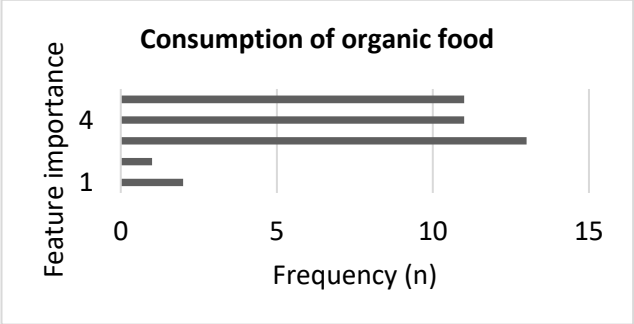

Figure 5. Importance of consuming organic food for students (n=38; 1- not important; 5 - extremely important)

CONCLUSION

Slow Food concept supports local producers, sustainable way of production and pleasure of preparation and consummation of food. Gastronomy as integral part of cultural heritage is important factor for tourism development. Croatia as touristic destination has extremely seasonal character and it is very important do develop selective forms of tourism which attract visitors longer than a summer period. Students of Enogastronomy and Tourism will soon become professionals in this field and responsible for sustainability of this industry.

Research showed that Slow Food as a concept is not recognizable enough among students (only 30% of students have heard about Slow Food, and only 23% tasted Slow Food products). Considering that the origin of the food and organic food were extremely important for less than 30% of respondents, it is crucial to upgrade their knowledge about gastronomy and food in a sustainable perception not only by regular curriculum, but also by involving them in different events like seminars or fairs of local products. It is also necessary to take into account the fact that the oldest students who participated in this research have just finished the third semester of their studies, and they still lack professional knowledge related to these topics, since their first year of study covers mostly general subjects. It would be good to repeat the survey among the students at the final years of their study. Survey should be extended to other target groups like households (persons who procure groceries in the family), farmers, and restaurants to get the larger picture about Slow Food and similar concepts in eastern Croatia.

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DETERMINATION OF PHYSICAL AND CHEMICAL PARAMETERS OF WATER BEFORE AND AFTER ION EXCHANGE TREATMENT

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:

In accordance with consumer requirements, the water must be adequately purified, and the corresponding parameters within the defined values. Various methods are used for this purpose, of which the ion exchange method can be highlighted as the simplest, most efficient and economically profitable. Ion exchange is a reversible process of ion exchange between a solid phase and an electrolyte solution. The ion exchanger is a macromolecular insoluble material that has chemically bound electrified groups and mobile, oppositely charged ions that compensate for this electrification. Ion exchangers are usually used in the form of compact or granular beds that fill the column through which the solution with the ions to be exchanged flows. They usually contain phenolic, carboxylic, sulfonic amino and other groups, which is why the treatment also results in decarbonization, softening, demineralization and denitrification of water. As the assessment of water quality is based on the most significant physico-chemical parameters, the aim of the work is the analysis of drinking water before and after treatment with an ion exchanger. For this purpose, organoleptic parameters such as smell, taste and color were first analyzed. After that, physico-chemical parameters were analyzed: pH values, electrical conductivity, m-alkalinity, p-alkalinity, water hardness, organic matter content, chloride content, iron and manganese content. An ion exchanger based on resin was used, which after use was regenerated by washing with NaCl solution. The analysis of the water sample, before and after the ion exchange treatment, showed that the treatment process was effective and that the decarbonization and softening of the water was carried out, whereby the water was categorized as soft water (water <9 °dH). The analyzed water is tasteless, odorless and colorless before and after treatment. The results of the analysis showed that all the values of the analyzed physico-chemical parameters are in accordance with the Rulebook on the Healthiness of Drinking Water (Official Gazette of Federation of Bosnia and Herzegovina No. 40/10) are below the maximum allowed values.

KEYWORDS: water, ion exchange, physical-chemical parameters; water treatment

INTRODUCTION

Water is one of the most important substances in nature and an irreplaceable resource for households, agriculture, industry, etc. [1]. The exponential growth of humanity is paralleled by the exponential growth of water consumption, whereby the importance of natural sources of quality water is increasing. Following consumer requirements, the water quality parameters should have suitable values. Therefore, different types of water can be treated with the same technologies, and most often combinations of different treatment processes are applied in water technology. One of the simplest and most effective methods is ion exchange, that is, the membrane method with ion exchange.

Namely, the process of ion exchange results in the exchange of mobile ions in a stoichiometrically equivalent amount with the corresponding ion charge from the solution. The management of the process itself is simple, and the installation of the ion exchanger does not require significant reconstruction, and the desired water quality can easily be achieved by combining certain types of ion exchangers. Ion exchange is carried out in a column filled with an ion exchanger, and after the saturation of active groups, the ion exchanger is regenerated with a solution of an elution agent, which converts the exchanger back into its initial ionic form. Ion exchangers are insoluble macromolecular polyelectrolytes that show the ability to exchange ions. Each exchanger consists of a skeleton or lattice, which is held together by covalent bonds to which active groups that dissociate in water are firmly attached and are therefore electrically charged, so the exchange is carried out at exactly fixed places within the lattice. If those groups are acidic, the lattice is externally charged with a negative charge,

and if they are basic, it is charged with a positive charge. This charge is compensated by a moving ion of the opposite charge so that each particle of the exchanger is electrically neutral to the outside [2,3].

The technology of water treatment using ion exchangers is most often used for the purpose of water decarbonization (dealkalization), water softening, water softening with prior decarbonation, water demineralization, and removal of nitrites (denitrification) and organic substances from water [4,5].

Water quality is determined by various regulations and must be followed. Therefore, water analysis is one of the main measures to prevent and control infectious diseases. It is determined by the size of certain indicators (chemical, physical and biological) that speak about the composition, concentration and properties of certain substances present in the water. The goal of this work is to determine the physico-chemical and organoleptic properties before and after treatment in order to define the water quality and determine the concentrations of the analyzed parameters.

MATERIALS AND METHODS

A water sample from the tap, which is fed from the source of Cerik, Tuzla, was used for analysis before and after treatment with an ion exchanger. Physico-chemical parameters (pH values, electrical conductivity, m-alkalinity, p-alkalinity, water hardness, organic matter content, chloride content, iron and manganese content) as well as organoleptic parameters (odor, taste and color) of tap water sample were analyzed. In accordance with standard methods (APHA, 2000 and the Law on Water Official Gazette of the Federation of Bosnia and Herzegovina No. 70/06), a water sample was analyzed for the analysis of physical and chemical parameters, after which the water was subjected to treatment with an ion exchange device (ion exchange resins). The ion exchange device is used for softening, decarbonizing and demineralizing water. After the treatment, the analysis of the mentioned parameters was repeated. A column of the Italian brand Monostand M-100 was used - Resinex Kw-8 type of ion exchange resin, with a capacity of 5 m^3/h, which is filled with resin based on sodium ions with the possibility of regeneration.

Water analysis before and after treatment was done at the Faculty of Technology of the University of Tuzla. Analytical methods and detection limits for all tested parameters are shown in table 2, and more detailed descriptions of the applied analytical method itself and a discussion of the results are shown below the table. The obtained values were compared according to the Rulebook on the Healthiness of Drinking Water (Official Gazette of the Federation of Bosnia and Herzegovina No. 40/10)

PHYSICAL PARAMETERS OF WATER QUALITY

Temperature is a physical indicator of drinking water quality. In ideal cases, the water temperature is constant or with small deformations (8-14ºC). Increased water temperature values are a consequence of thermal pollution, and it depends on the depth, thermal conductivity of the rocks, the lithological structure of the rocks and the proximity of magmatic bodies [6]. Water temperature before and after treatment was measured with a thermometer (in ºC).
Electrical conductivity represents the total amount of dissolved salts or ions in water and depends on the ions present in the water, concentration, mobility and charge of ions, temperature, etc. It is carried out with the aim of determining the degree of water mineralization and filtration residue (total soluble matter in the water sample), and was carried out by measuring on a Mettler Toledo conductometer. The device was first calibrated using standard calibration solutions (conductivity of 814 μS/cm and 1413 μS/cm), and then the measurement was performed [7-10].

ORGANOLEPTIC PARAMETERS

Table 1. Scoring system for intensity of taste and smell of water [11]

Number of points	The strength of the smell	Description
0	Without	Absence of smell, it cannot be noticed
1	Very weak	The smell can be determined by a specially trained person
2	Weak	Odors do not attract the attention of water users. But it can be noticed if it is pointed at.
3	Noticeable	The smell is easy to detect and causes hesitation to use water
4	Significantly	The smell that is immediately felt and causes attention, and makes the water unsuitable for drinking.
5	Very strong	The smell is so strong that it makes the water unsuitable for drinking

The smell and taste of water originate from the presence of organic and inorganic contaminants, from biological sources and processes, contamination with synthetic chemicals, corrosion or as a result of water treatment. The smell of water was determined organoleptically at room temperature and at 40 ºC, and was defined descriptively according to the table provided by the author [11]. The taste test was performed by heating the sample to 40°C and holding it in the mouth for several seconds in order to come into contact with the receptors in the mouth.

THE COLOR OF THE WATER

The color of water is an optical property, and it is the result of the absorption and reflection of light of a certain wavelength. Water color is a parameter that is not always related to toxicity or pathogen contamination, but it is on the list of aesthetic parameters and the recommended value in water is 15 color units.

CHEMICAL PARAMETERS OF WATER QUALITY

pH value

The pH value is a very important indicator of water quality because a large number of water cleaning procedures depend on the pH value. The required optimal pH value of the water varies in different sources according to the composition of the water, but it is most often from 6.5 to 8.0. It is necessary that the pH value of drinking water be neutral to slightly alkaline (pH=7.0-7.4) because acidic waters corrode water pipes, while alkaline waters create sediment [11]. The pH value before and after the ion exchange treatment was determined using a pH meter Mettler Toledo 220.

Alkalinity of water

Alkalinity of water is a factor of the capacity of water to receive H+ ions. It is determined by acid titration of the sample and is expressed as total alkalinity (m-alkalinity) determined with methyl orange as an indicator that induces a change at pH 4.3, and phenolphthalein alkalinity (p-alkalinity) as a quantitative measure of alkalinity up to pH 8.3.

$$MA = 10 \cdot m \quad (1)$$

$$PA = 10 \cdot p \quad (2)$$

where is:

m- volume of HCl (ml) used for titration with methyl orange as an indicator;

p- volume of HCl (ml) used for titration with phenolphthalein as an indicator.

Water hardness

Water hardness represents the total concentration of calcium and magnesium ions expressed as $CaCO_3$. It is determined according to the amount of calcium and magnesium ions, by titration with EDTA with the indicator erichrome black, d.s. Considering the total hardness of the water, it can be divided into: soft water (up to 9 ºnj), moderately hard water (9 - 18 ºnj), hard water (18 - 26 ºnj), very hard water (above 26ºnj) [2].

$$\mathrm{UT} = \frac{\mathrm{V_{EDTA}} \cdot \mathrm{c_{EDTA}} \cdot \mathrm{M_{CaO}}}{\mathrm{V_{sample}}} \cdot 1000 \qquad \left(\mathrm{mg}\frac{\mathrm{CaO}}{\mathrm{L}}\right) \quad (3)$$

where is:

V_{EDTA}- volume of EDTA (ml) used for sample titration;

C_{EDTA}-concentration of EDTA;

M_{CaO}-molar mass of CaO;

V_{sample}- volume of sample.

The content of organic matter

The content of organic matter in water was determined by the Kubel-Tiemann method, i.e. by titration with a standard solution of $KMnO_4$ in an acidic medium. Based on the $KMnO_4$ content, the oxygen content in the water was also determined [12].

The chloride content

The chloride content was determined by titration with a standard $AgNO_3$ solution in the presence of potassium chromate as an indicator [2].

The content of Iron and Manganese

Iron content was determined spectrophotometrically using a JENWAY model 7300 spectrophotometer, while manganese content was determined using a Perkin Elmer AAnalyst 200 atomic absorption spectrophotometre.

RESULTS AND DISCUSSION

The analyzed values of physical and chemical parameters of the sample before and after treatment are given in the Table 2

Table 2. Values of physical and chemical parameters before and after ion exchange treatment

Parameters		**The value before treatment**	**The value after treatment**	**Limit values**	**Analytical method**
Color		Clear	Clear	-	Organoleptic
Smell and taste		Acceptable to consumers	Acceptable to consumers	-	Organoleptic
Temperature		19ºC	15ºC	-	
pH value		6.77	6.59	≥6.5 – ≤9.5	Potentiometric,ISE
Electrical conductivity		266.333 μS/cm	253.33 μS/cm	2500 μS/cm na 20ºC	Conductometric
Alkalinity	p-alkalinity	0	0	-	Titration with HCl
	m-alkalinity	28	25	-	
Water hardness		25.2 mg/L 2.5 ºnj	2.8 mg/L 0.28 ºnj		Titration with EDTA
The content of organic matter		1.89 mg/L	2.212 mg/L	5 mg O_2/L	Consumption of $KMnO_4$, by cooking in an acidic medium; Titration according to Kubel-Tiemann
The content of O_2		0.472	0.553		
The chloride content		7.1 mg/L	21.3 mg/L	250mg/L	Titration with $AgNO_3$
The content of Iron		0.002 mg/L	0.003 mg/L	200 μg/L	Spectrophotometrically
The content of Manganese		0	0	50 μg/L	Atomic absorption spectrophotometry

The results of the analysis showed that all values are within the permitted limits defined by the Rulebook on the Healthiness of Drinking Water Official Gazette of Bosnia and Herzegovina No. 40/10.

The basic requirement of water quality is that it should be odorless, tasteless and colourless. The presence of an odor most often indicates a qualitative defect in the water because the odor comes from dissolved organic and inorganic substances in the water [13]. The taste of water is most often a sign of faulty water, and is determined by the mineral composition, gas content and temperature, while water turbidity is caused by suspended and colloidal particles in the water (clay, silt, fine, small organic and inorganic matter, dissolved, colored organic matter , microscopic living organisms and plankton), the presence of aquatic organisms and undissolved air bubbles and represents the optical property of water.

The color of the water is the result of the presence of colloidally dissolved substances of plant origin and is very difficult to remove. It originates from substances of different origins, and the intensity of the color (yellow or red) depends on the presence of oxygen. Most often, the color of water is affected by the content of organic matter (yellow color, tea color). Although color as a parameter does not belong to the toxic parameters, it is on the EPA (Environmental Protection Agency) list of secondary (aesthetic) parameters and affects the appearance and sometimes the smell of water [14]. When it comes to the analyzed water and parameters, it has been proven that the water before and after treatment meets the requirements of drinking water, i.e. it is odorless, tasteless and colourless.

The pH value in natural waters is regulated by the balance of carbon dioxide and carbonate and is between 4.5 and 8.5 [15]. The pH is affected by humus substances that change the carbonate balance, the biological activity of plants, and in some cases salts that can be hydrolyzed, which is why waste and polluted waters can have lower or higher pH values. The pH value before and after the treatment showed that it is natural water whose value depends on the content of CO_2, carbonates and hydrogen carbonates, and that the pH value before and after the treatment did not change significantly. The electrical conductivity value also showed that the water is of approximately natural spring water quality. The small difference in values before and after treatment is attributed to the relatively short treatment time. High consumption of oxygen, as an indicator of organic pollution, indicates a high content of compounds subject to oxidation. Analysis of the sample before and after treatment showed a small consumption of

$KMnO_4$, which indicates the absence of organic matter.

Water alkalinity, which is a factor of water's capacity to receive H^+ ions, showed 0 values for p-alkalinity, and 28 for m-alkalinity for the sample before treatment and 25 for the sample after water treatment, which clearly shows that carbonates and bicarbonates are present in the water. The analysis also showed that the total hardness was reduced from 25.2 mg CaO/L before the treatment to 2.8 mg CaO/L after the treatment, respectively from 2.5 ºdH to 0.28 ºdH, which further shows that the process of softening and decarbonization has been fully carried out and of the water classified as soft water (water <9 ºdH).

Metals in water are formed as a result of soil leaching and mineral dissolution. The increased amounts are the result of the discharge of wastewater from various branches of industry, agriculture and households. Water containing iron is not suitable for technological processes in the textile industry, in the leather and paper industry, and also above the upper permissible values in the food industry as well as for drinking [19]. Iron and manganese reach the water by dissolving and passing through the layers of the soil in water poor in dissolved oxygen, and if a concentration higher than the permitted level is observed, measures of deferrization or demanganization are carried out. When it comes to these metals, the analysis of tap water before and after treatment showed minimal values of these metals in drinking water.

CONCLUSION

The quality of drinking water is determined by numerous rules and must be constantly monitored. In order to monitor, it is mandatory to carry out analyzes and compare them with the Rulebook on permitted values. The analysis showed that the ion exchange process was successfully carried out, i.e. that the decarbonization and softening of the water was carried out, whereby the water after treatment was classified as soft water (water <9 ºdH).

The analyzed water is tasteless, odorless and colorless before and after treatment. The pH value and the electric value did not change significantly during treatment.

The analysis also showed that the content of chloride, as well as the content of iron and manganese in the water sample before and after treatment are below the maximum allowed values. The increase in the chloride value after the treatment compared to the analyzed sample before the ion exchange treatment can be attributed to the regeneration of the resin with NaCl ions.

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DETERMINATION OF THE BIOMETHANE POTENTIAL OF DIFFERENT TYPES OF MANURE FROM EXTENSIVE ANIMAL FARMING

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:
Livestock production is a potential environmental pollutan because of the high concentartions of animals on the small area. Production of biogas with anaerobic degradation from organic waste is one of the pledge alternative energetic solutions, especially from organic manure made from animal farming and other residuals of agricultural production. The aim of this paper was to determine posibillity of using manure different origin from extensive animal breeding for production of biogas in labaratory conditions. The results obtained will be based on posibillity of use waste streams from extensive animal husbandry as basic substrate for anaerobic digestion. In this regard, cosubstrates were formed as mixture 1 chicken excrement; mixture 2 sheep manure; mixture 3 cow manure; all three basic substrates were in mixture with the sludge from the wastewater treatment plant. The results showed production of 71,5 ml CH_4/gVS for mixture 1, 68,65 ml CH_4/gVS for mixture 2 and 48,68 ml CH_4/gVS for mixture 3.

KEYWORDS: extensive breeding, manure, anaerobic digestion, biogas, biomethane potentional

INTRODUCTION

One of the main environmental protection problems of modern society is the continuous increase in the generation of organic waste. In many countries, sustainable waste management, which includes preventing its creation and reducing new quantities, has become a major political priority and an important part of joint efforts to reduce environmental pollution and greenhouse gas emissions in order to mitigate global climate change.

Biodegradable organic waste, due to its specificity and continuous flow of production from industrial and agricultural production as well as households and wastewater treatment plants, represents a potential danger to human health and environmental protection. In addition to the fact that the legal regulation prescribes a reduction in the amount of disposed biodegradable organic waste, a smaller amount of waste in landfills directly reduces the emission of methane, which is created by the spontaneous anaerobic decomposition of waste in landfills [1].
It has long been known that animal husbandry generates large amounts of nutrient-rich organic waste that is used to condition agricultural land. Manure treatment would improve the physico-chemical characteristics of the waste and thereby reduce its toxicity [2].

It is considered that the production of biogas by anaerobic digestion is the optimal process for treating animal excrement, as well as a wide range of organic waste, since it transforms these substrates into a renewable energy source and, often, an environmentally friendly fertilizer in agriculture [3].
Biogas is a cheap and CO_2-neutral renewable energy source, which provides the possibility of processing and recycling various agricultural residues and by-products in a sustainable and environmentally friendly way. At the same time, biogas entails numerous socio-economic benefits for society as a whole, but also for actors involved in its production and exploitation [4].

MATERIAL AND METHODS

For research purposes, glass eudiometric tubes "Šurlan-Medulin" were used, placed on glass bottles with a volume of 500 ml. Achieving anaerobic conditions was done by blowing nitrogen, which squeezes air out of the reactor, while due to the required constant temperature of the reactor system,

heating was done in a water bath with water circulation. At the top there is a shelf with bottles to level the level and catch excess liquid.

Using eudiometric tubes, biogas production is easily read, because the produced gas moves the liquid level, and the liquid returns to the receiving bottles, as shown in Figure 1. A 22% NaCl solution was used as the liquid, because the constituents of biogas are difficult to dissolve in to the solution prepared in this way, with the addition of three drops of concentrated sulfuric acid and methyl orange indicator so that the solution takes on a color and is more noticeable.

The pressure and temperature of the surrounding air were measured using a baro-thermohygrometer placed next to the eudiometric tubes, and these values were used to recalculate the volume of the obtained biogas to normal conditions. Mixing was done mechanically using magnetic stirrers.

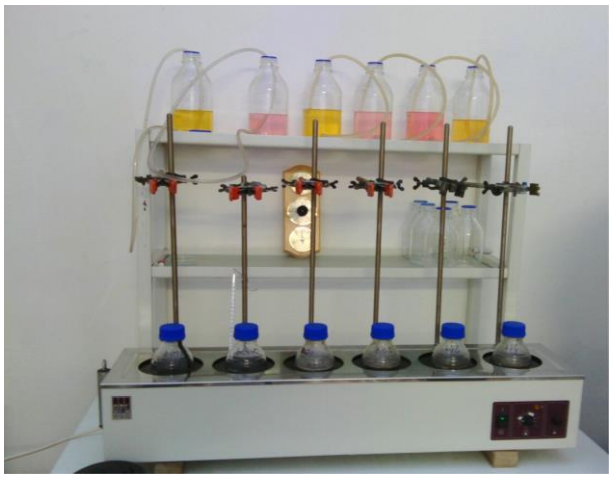

Figure 1. Reactor system

Gas sampling was done using a glass aspirator, with valves on both sides of the cylinder. At one end, the aspirator is connected with a rubber hose to a level bottle containing a 22% NaCl solution.

Gas composition analysis was performed on a Clarus 500 gas chromatograph (Perkin-Elmer, India), equipped with a thermal conductivity detector (TCD-R) and a gas analyzer model 4016 Arnel TotalChrom Work-station software. Helium was used as carrier gas (flow rate 34 ml/min).

A gas mixture consisting of CO, CO_2, CH_4 and O_2 (Messer, Germany) was used to calibrate the device. By connecting the free part of the aspirator to the inlet of the chromatograph and raising the level of the bottle, the flow through the chromatograph column is enabled, which is necessary for determining the biogas composition.

After a twelve-minute analysis, the display shows the composition of the analyzed gas sample in volume percentages.

Three types of manure from extensive farming were used as the basic substrate, chicken (sample 1), sheep (sample 2) and cow (sample 3), and in order to achieve biogas production, sludge from the municipal wastewater treatment plant in Živinice town was used as inoculum.

In order to obtain the necessary parameters, it was necessary to characterize the materials used in the experiment, by determining:

- dry matter content,
- content of volatile organic matter,
- nitrogen content according to Kjeldahl,
- total phosphorus content,
- chemical oxygen demand.

The dry residue means the proportion of dry matter in the sample obtained after the determined drying procedure. It is expressed as a percentage or in grams per kilogram. Determination of dry matter content was performed according to Method 2540-Solid B. Standard Methods for the Examination of Water and Wastewater 21st edition. APHA, Washington, DC (2005). All the analyzed samples were dried in an oven at a temperature of 105 °C to a constant mass. To calculate the dry residue, the difference between the mass before and after the drying process is taken.

Volatile-volatile substances (VS) are those substances that are lost when the sample is heated 550 °C. Volatile organic matter content was analyzed according to Method 2540-Solid E. Standard Methods for the Examination of Water and Wastewater 21st edition. APHA, Washington, DC (2005). The ash content was determined in an incineration furnace for six hours at a temperature of 550 °C. Volatile organic matter content was determined as the difference between dry matter content and ash content in the samples.

The electrometric measurement of the pH value was carried out by direct measurement of the value on a METTLER TOLEDO FE 20/EL 20 pH meter. Before each measurement, the internal control of the device was carried out with certified reference materials with a pH value of 4.01; 7.01; 10.01.

The Kjeldahl nitrogen content was determined according to Method 4500-NorgB. Standard Methods for the Examination of Water and Wastewater 20th edition. APHA, Washington, DC (1998). The method consists of three stages: digestion at a temperature of 340 °C (boiling temperature of H_2SO_4) in the presence of concentrated sulfuric acid and Kjeldahl catalyst with selenium; distillation in the presence of NaOH

where the distillate is accepted in a solution of boric acid and titration with 0.1 M HCl in the presence of the bromine cresol green indicator. The determination was made on a Gerhardt Kjeldahl apparatus, which consists of three blocks: a cleaning block, a digestion block and a distillation block.

When determining the content of total phosphorus in the substrates, the sample was prepared for analysis by weighing a sample mass of about 1 g, transferring it to a beaker with distilled water, and then digesting it in the presence of $K_2S_2O_8$ and 4.5 M sulfuric acid heated on a stove. After digestion, the samples were transferred to a measuring flask of 1 l, supplemented with distilled water, and then filtered. A certain amount of the filtrate was used as a test sample to determine the total phosphorus in the substrate. According to the instructions for the formation of the base diagram, the samples are also treated, and the concentration of total phosphorus is determined through the absorbance, which was read on a Shimadzu UV 1800 spectrophotometer at a wavelength of 880 nm.

A modified method according to BAS ISO 6060:2000 was used to determine COD. A mass sample of 0.03g was diluted with distilled water to 10 ml in an Erlenmayer flask with a ground neck. After that, 20 ml of concentrated sulfuric acid with $AgSO_4$ and 10 ml of $K_2Cr_2O_7$ were added to the sample. After connecting the neck of the flask to the return cooler, reflux is ensured, as the sample is boiled for two hours at a temperature of 148 °C. After cooling, the sample was titrated with a standard ferroammonium sulfate solution with the FEROIN indicator. In parallel with the sample, a blank test was done in an identical manner, as well as one sample without cooking for the purpose of determining the ferroammonium sulfate factor.

Based on the results of the physico-chemical characterization of the substrate, mixtures with an optimal content of dry matter were formed for carrying out the anaerobic digestion process. For all mixtures that were formed and subjected to the process of anaerobic decomposition, two tests were performed and the results were expressed as mean values.

RESULTS AND DISCUSSION

Anaerobic digestion experiments were carried out in mesophilic conditions of different types of manure from extensive animal husbandry with the addition of waste sludge from municipal wastewater treatment plants.

Before the formation of the mixtures, it was necessary to carry out a physical and chemical characterization of the substrates used in the process of anaerobic digestion in order to optimize the parameters in the mixtures.

The results of the analysis of physical and chemical parameters of different types of manure are shown in table 1.

Table 1. Results of physico-chemical parameters of used manures

Parameters	Unit	1	2	3
TS	%	27.0	24.8	17.5
VS	%	15.9	21.2	14.8
VS/TS	-	0.59	0.85	0.84
TP	g/kg	4.45	2.22	0.31
COD	g/kg	144.83	154.56	86.4
TKN	g/kg	10.73	7.63	3.27

TS – total solid; VS – volatile solids; COD – chemical oxigen demand; TKN – total Kjeldahl nitrogen

As can be seen from the table, all the substrates that were analyzed are potential raw materials for biogas production due to the favorable content of VS (volatile matter VS) and COD, which represent the amount of organic matter that is transformed into biogas/methane during anaerobic processes.

In addition, the favorable ratio of COD : TKN (from 13.5:1 to 26:1) in potential raw materials corresponds to the ratios recommended in the literature for substrates undergoing anaerobic decomposition [5],[6],[7],[8].

Generally, it is known that sludge is a carrier of anaerobic microorganisms, but it was also used for to optimize the content of dry and organic matter, i.e. to achieve optimal conditions related to the TS content of less than 20% for the so-called "wet" process of anaerobic digestion [9].

Based on the content of dry matter in the raw materials, which were the subject of research, manure and waste sludge, mixtures were formed through which the biomethane potential was determined.
The composition of the formed mixtures based on the optimal proportion of dry matter is shown in Table 2, while Table 3 shows the most important physical and chemical parameters of the formed co-substrates.

Table 2. Composition of formed cosubstrates expressed in mass percentages

Mixture	Manure mass %	Sewage sluds %	H_2O %
1	26.0	74.0	0
2	35.1	19.29	45.61
3	44.12	16.18	39.70

Table 3. Physical and chemical characteristics of the co-substrates formed

Parameters	Unit	1	2	3
TS	%	5.24	11.92	9.14
VS	%	4.47	10.18	8.10
VS/TS	-	0.85	0.85	0.88

Mixture 1 has a significantly lower content of TS (dry matter) compared to the other two mixes due to the structure of chicken excrement. For easier control and handling of the process (primarily mixing), the mixture was formed with a lower TS content. A smaller share of TS leads to a smaller share of VS in mixture 1 because the parameters are mutually dependent.

Excluding the first mixture, the dry matter content is in the optimal range because it was the parameter on the basis of which the co-substrates were formed. The ratio of organic and dry matter in all three mixtures is in the ideal ratio, which according to literature data ranges from 70-90% [10].

The diagrams (Figure 2 and Figure 3) show the production of biogas per day and the cumulative yield of biogas for all three mixtures that were used in the research.

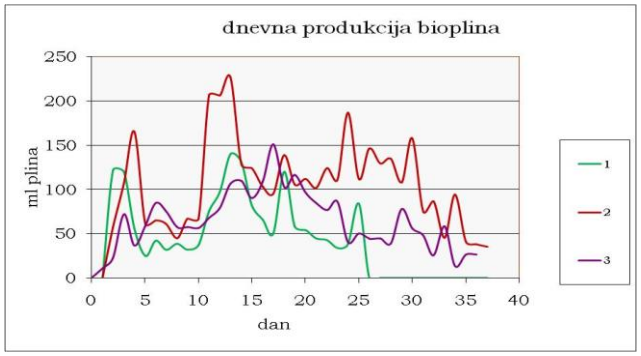

Figure 2. Diagram of daily biogas production

It can be seen from the diagram that in mixture number 2 (sheep manure with sludge) the largest amount of biogas was produced on a daily basis, and on the twelfth day the maximum production of biogas was recorded when it reached a value of 226.8 ml.

After the twenty-sixth day, biogas production was completed in mixture 1, while the process of anaerobic digestion continued until the thirty-sixth day in the remaining two mixtures.

The diagram of cumulative biogas yield (Figure 3) confirms the stages of adaptation and the beginning of hydrolysis in the first days of the process. A significantly higher production of biogas is clearly visible in mixture number 2 compared to mixtures number 1 and 3.

Figure 3. Diagram of cumulative biogas production

In addition, the aforementioned confirmation that the process in mixture number 1 was completed on the twenty-sixth day was confirmed.

The process in mixtures number 2 and 3 lasted ten days longer, and therefore the cumulative production of biogas in those mixtures was slightly higher.

Comparing the obtained results of this research with the results obtained in previously conducted research under the same or similar conditions, it can be concluded that the production of biogas on a daily basis in mixtures 1 and 3 (62.42 ml/day and 65.65 ml/day) is significantly higher compared to the results obtained in the research [11] who obtained a biogas yield of 33.20 ml/day and 23.80 ml/day, respectively, for 40 days of experimental research.

Based on the share of methane in the produced biogas, the composition of the formed co-substrates and the production of biogas, the daily, cumulative and specific production of methane was calculated (Figure 4, Figure 5 and Figure 6) for all three mixtures.

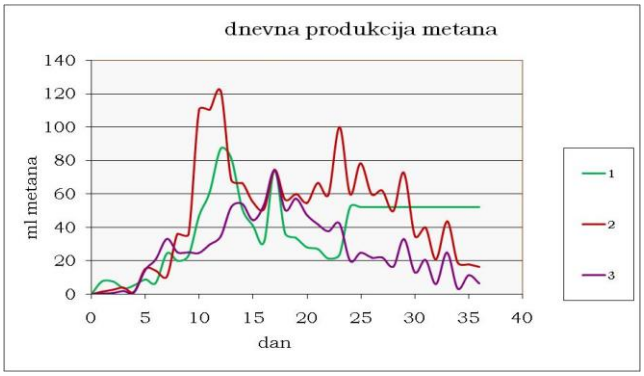

Figure 4. Diagram of daily methane production

The diagram of daily methane production shows discontinuous methane production in all mixtures. The diagram of daily methane production indicates the fact that the production from the second mixture had the highest daily methane yields, and the maximum daily methane production was achieved on the twelfth day in mixture 2 and was 121.38 ml of methane.

The results of daily methane production are largely consistent with the results of daily biogas production.

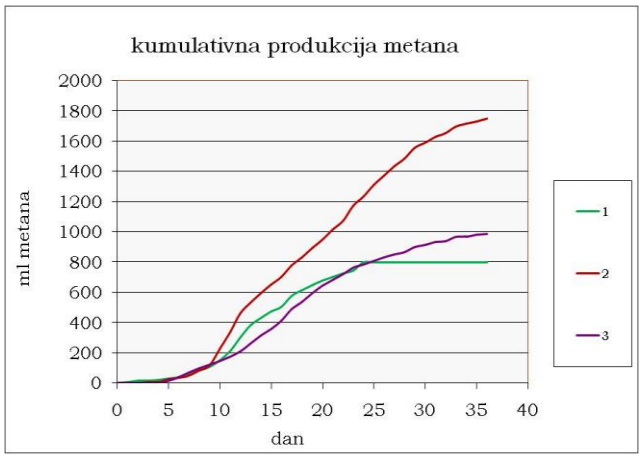

Figure 5. Diagram of cumulative methane production

Identical as in the case of cumulative biogas production, the production of methane from mixture 2 is significantly higher compared to the production of methane in mixtures 1 and 3. Thus, the cumulative production of methane from mixture 2 was almost 1800 ml, the production from mixture 3 was approximately 1000 ml, while methane production from mixture 1 was the smallest and amounted to 800 ml.

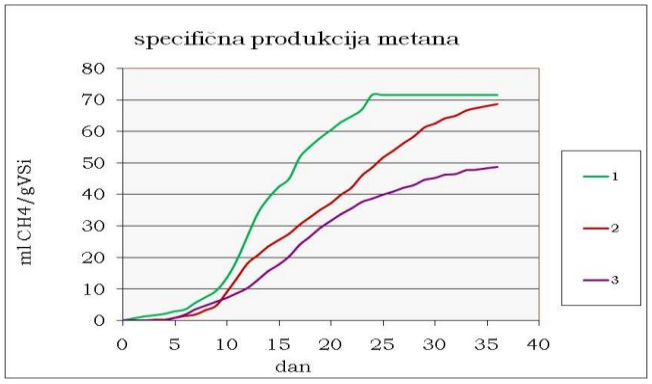

Figure 6. Diagram of specific methane production

On the diagram of specific methane production, it is clear that the biogas potential of chicken manure is the highest, because in the shortest time it has the highest specific yield of methane [mlCH_4/g VSi] compared to the other tested samples. It should be emphasized here that the first mixture (chicken excrement with waste sludge) had a significantly lower content of dry and organic matter compared to the remaining two mixtures, which directly affected the result of specific methane production.

This research result provides a very good basis for further research aimed at optimizing the content of dry and organic matter in different mixtures of raw materials, which especially applies to mixtures containing animal manure.

Comparing the obtained results of specific methane production in relation to the input content of organic matter from mixture 1 (71.5 mlCH_4/gVSi) of this research with the results obtained from the research [12] who conducted an experimental study of dry anaerobic decomposition of chicken manure in mesophilic conditions.

The research results, expressed as specific methane production, amount to 31 mlCH_4/gVSi, and indicate that the production of methane obtained by anaerobic digestion from mixture 1 of this research is significantly higher compared to the previously mentioned research.

Due to the fact that after the anaerobic (co)digestion process is completed, the digestate is left behind, in table 4 the characteristics of the digested residue (digestate) representing the UXO product, as well as the final results of the production of biogas and methane are given.

If the content of dry and volatile organic matter is compared before (table 3) and after (table 4) the process, it is easy to conclude that a certain part of the matter was transformed into gas products.

Table 4. Final results of batch anaerobic digestion after the implementation experiment

Parameters	Unit	1	2	3
TS	%	2.83	9.65	7.95
VS	%	1.86	7.22	5.8
pH	-	7.62	6.64	6.33
V_{biogas}	ml	1623	3869.98	2363.54
$V_{methane}$	ml	800	1747.16	985.8
$W_{methane}$	%	0.49	0.45	0.41
spec.methane yield	mlCH_4/gVS_i	71.5	68.65	48.68

The greatest reduction of TS and VS was recorded in mixture 1, while mixture 2 had an approximate value of reduction of TS and VS. Mixture 3 recorded the lowest reduction of dry matter and organic matter compared to the first two.

Regardless of the slightly lower content of organic matter in the digestate compared to manure and mixtures, the digestate has a useful value in agriculture for the needs of soil conditioning, and the higher water content favors easier handling when applying it to the soil.

After the anaerobic digestion process, the mixtures had different pH values. The pH value of the obtained digestate from mixture 1 was in a slightly basic environment and was 7.62. The remaining two mixtures produced a digestate with a slightly acidic pH value of 6.64 and 6.33, respectively, which means that it will not have a major impact on soil acidity if the digestates are used in agriculture.

If we take into account the fact that agricultural soils with low pH value prevail in our area, the properties of the soil in terms of acidity can be improved by applying digestate.

The result of the research, which is of particular importance, is the possibility of producing energy (biogas) and fertilizer (digestate) from livestock waste streams, because it is generally known that agriculture is a significant consumer of energy.

In this way, the production of energy and useful products from waste streams and their use at the point of origin of waste realizes one of the basic principles of a circular economy, but also of sustainable agricultural production.

CONCLUSIONS

The development and implementation of a system of renewable energy sources such as biogas from anaerobic digestion, based on national resources, will increase the stability of the national energy supply and reduce dependence on energy imports.

The production and use of biogas from anaerobic digestion has a positive effect on the environment and socio-economic benefits for society as a whole, but also for the involved farmers as end users

It is generally known that livestock waste has suitable characteristics for anaerobic processing, so the results of the analysis showed that the substrates used in this research represent potential raw materials for biogas production due to the favorable content of VS and COD, which represent the amount of organic matter from which it is obtained methane.

Due to the relatively high content of dry matter in all three types of manure that represented the basic substrates, waste sludge from the wastewater treatment plant was added in order to optimize the TS to optimal values for the wet digestion process. In addition, waste sludge also plays the role of inoculum because it contains a large number of anaerobic microorganisms.

In mixture 1, the process of anaerobic decomposition lasted twenty-six days, and in the remaining two mixtures, the process lasted ten days longer.

Cumulative production of biogas in mixture 2 (3869.98 ml) is significantly higher compared to the other two mixtures and especially compared to mixture 1 (1623 ml), which is the result of the duration of the anaerobic decomposition process.

Mixture 2 (sheep manure with sludge) produced the highest amount of biogas and methane on a daily basis, and the maximum production was recorded on the twelfth day of the experiment.

The mixture of chicken excrement and sludge has the highest biogas potential because in the shortest time it had the highest specific yield of methane (71.5 $mlCH_4/gVSi$) compared to the other tested samples (68.65 and 48.68 $mlCH_4/gVSi$).

The reduction of dry and organic matter is directly proportional to the specific production of methane and had the highest value in mixture 1, where the highest specific production of methane was recorded.

The production of energy (biogas) and fertilizer (digestate) from animal husbandry waste streams through anaerobic digestion, and their use at the point of origin of waste, is one of the basic principles of circular economy and sustainable agricultural production.

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ANTIMICROBIAL AND CYTOTOXIC ACTIVITIES OF ESSENTIAL OIL FROM THE AERIAL PARTS OF *PULICARIA DYSENTERICA* (L.) BERNH. (ASTERACEAE)

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:
The chemical composition of *Pulicaria dysenterica* (L.) Bernh. aerial parts essential oil (EO), growing wild in Bosnia and Herzegovina, was presented in the study. In addition to the EO composition, antimicrobial and cytotoxic activities were also tested. The aerial parts of *P. dysenterica* contained 0.3% of yellow, liquid, fragrant EO. The 51 components identified accounted for 81.09% of the oil. The EO was characterized by the presence of a high concentration of oxygenated sesquiterpenes 51.83% while oxygenated monoterpenes constituted 15.57%, sesquiterpene hydrocarbons 9.32% and non-terpene compounds presented 4.37% of the EO. The dominant compounds were the sesquiterpenes caryophyllene oxide, (*E*)-nerolidol, *β*-caryophyllene and monoterpene nerol. The antimicrobial activity of EO was tested against selected ATCC strains of microorganisms, the bacteria *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*, and the fungus *Candida albicans.* The results showed that the investigated EO inhibited the growth of all selected ATCC strains of microorganisms. The best result was obtained against *Escherichia coli* bacteria with MIC value of 1 mgmL^{-1}. The cytotoxicity of EO was measured against the HeLa cell line using the MTT method with IC_{50} of 188.52 µgmL^{-1}. This study has provided scientific baseline data on the therapeutic properties of *P. dysenterica.*

KEYWORDS: Pulicaria dysenterica, essential oil, antimicrobial activity, citotoxicity

INTRODUCTION

Pulicaria dysenterica (L.) Bernh. syn. *Inula dysenterica* L. is a perennial plant growing up to 100 cm tall. The leaves are alternate. It is reproduced by roots. The flowers are yellow. It is widespread in humid areas of Europe, Anatolia, Iraq, Iran, Afghanistan, Pakistan and North Africa. *P. dysenterica* is found in the flora of Bosnia and Herzegovina [1]. The decoction of the aerial parts of this plant is used in traditional Iranian medicine for the treatment of diarrhoea and dysentery. The plant is also used to treat dysentery in the United Kingdom. It also has an insecticidal property [2], [3]. The chemical constituents of *P. dysenterica* include flavonoids, acetylenes, sesquiterpene lactons, isocomene and essential oil [2].

Previous studies on the essential oil of the aerial parts of *P. dysenterica* are rare and related to the determination of its composition and content. Studies on the essential oil of *P. dysenterica* aerial parts from Greece and Iran confirmed the well-known fact that the chemical composition of the essential oil depends on various parameters such as environmental conditions [4].

The essential oils and extracts from genus *Pulicaria* have been found to possess considerable biological activities such as antioxidant, antimicrobial, cytotoxic, antitumor, insecticidal [5], [6], [7]. A

limited number of studies have been carried out on the biological activities of *P. dysenterica* extracts [4]. To the best of our knowledge, no studies have been carried out on the biological activities of the essential oil of *P. dysenterica* aerial parts.

The aim of this study is to present the chemical composition of *P. dysenterica* aerial parts essential oil from Bosnia and Herzegovina, its antimicrobial activity on selected ATCC strains of microorganisms and cytotoxic activity on HeLa cell line.

It has already been mentioned that essential oils from the genus *Pulicaria* have antimicrobial properties. Sesquiterpenes, among other compounds, contribute to their activity [8]. The mechanism of antimicrobial activity of the essential oils is not yet fully explained. Essential oils and their constituents have an important property of hydrophobicity, which allows them to be distributed between the lipids of the cell membrane and the mitochondria of the bacterial cell, thereby disrupting the structure and making them more permeable. A change in the permeability of the cell membrane also affects the control of osmotic loss of the cell, which is considered the basic principle of the antibacterial effect of the essential oils [9], [10].

The citotoxicity of essential oils from the genus *Pulicaria* has also been described. In generally, the cytotoxicity of essential oils is mainly related to the presence of phenols, alcohols, and monoterpene aldehydes. As lipophilic mixtures, essential oils are able to cross the cell membrane and degrade and permeabilise the layers of polysaccharides, phospholipids and fatty acids. Cytotoxicity appears to involve such membrane damage. This permeabilization of the outer and inner membranes leads to cell death by apoptosis and necrosis [10].

EXPERIMENTAL

PLANT MATERIAL

Aerial parts of *P. dysenterica* were collected at the site Paklenik, Karaula, Olovo Municipality (Bosnia and Herzegovina) (N44°10'13.6" E18°39'07.2") during the flowering season in July 2020. The plant material was identified by the authors according to Flora Croatica (Domac, 1994). The voucher specimen was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, University of Tuzla. The plant material was cleaned, cut, and air-dried.

REAGENTS AND CHEMICALS

All analyses were performed using analytical grade chemicals and reagents, with the exception of sample preparation solvents for GC-FID/MS analysis, which were MS grade. *P*-iodonitrotetrasolium chloride and streptomycin were purchased from Sigma-Aldrich, while the antibiotic ampicillin was purchased from Panfarma, Belgrade, Serbia and ketoconazole was purchased from Zorka farma, Šabac, Serbia. Minimum Essential Medium Eagle (MEM), 2 mM glutamine, 1 % nonessential Amino Acids, 10% heat inactivated fetal bovine serum (HI FBS), penicillin and Thiazolyl Blue Tetrazolium Bromide (MTT) cell viability reagent were also purchased from Sigma-Aldrich. Double-destilled deionized water or culture medium were used for solution preparations and dilutions.

ISOLATION OF ESSENTIAL OIL

The dried aerial parts of *P. dysenterica* (200g) were crushed several times and subjected to hydrodistillation with 4 litres of distilled water for 3 h using a Clevenger-type apparatus. The essential oil obtained was separated, dried over anhydrous sodium sulfate, and stored at -20 °C until analysis.

GC-FID/MS ANALYSIS

Volatile compounds were determined by the GC-FID/MS analyses using an Agilent 6890N GC system coupled with an Agilent 5975 MSD, FID, and equipped with a HP-5 MS capillary column (30 m x 0.25 mm, film thickness 0.25 μm). The oven temperature was programmed with a linear increase from 60 to 280ºC at 3º min^{-1} followed by isotherm at 280ºC for 5 min; injector 200ºC; FID 300ºC; transfer-line 250ºC; carrier gas He (1.0 mL min^{-1}, constant flow mode); injection volume 1 μL of essential oil dissolved in ethanol; split ratio 10:1. EI Mass spectra (70 eV) were acquired over the m/z range of 35-550. The identification of the individual compounds was based on the comparison of their retention times (tR), retention indices (RIs), and mass spectra with those obtained from authentic samples and/or listed in the NIST, Wiley mass spectral libraries, and the literature. The relative area percentages obtained by FID were used for quantification.

DETERMINATION OF ANTIMICROBIAL ACTIVITY

The antimicrobial activity (AMA) of *P. dysenterica* essential oil was tested against three strains of bacteria, i.e. Gram-positive *Staphylococcus aureus* (ATCC 6538) and Gram-negative *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 35210), as well as against the fungal strain *Candida albicans* (ATCC 10231). The microorganisms were obtained from the Mycology Laboratory, Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia.

The antibacterial assay was performed by the microdilution method (Cazella et al., 2019) using 96-well microtiter plates to determine the minimum inhibitory concentration (MIC) and minimum bactericidal /fungicidal concentration (MBC/MFC). The inoculum was cultivated in a solid medium to verify the absence of contamination and for validation. The nutrient media used were Tryptic Soy Broth (TSB) for bacteria and Sabouraud Dextrose Broth (SDB) for fungus. Microbial suspensions (inocula) were adjusted with sterile saline to a concentration of 1.0×10^5 CFU mL^{-1}. The inoculum was prepared daily and stored at 4 °C until use. The essential oil was added to the nutrient medium for the growth of microorganisms. The microbial inocula were then added and the plates were incubated for 24 h at 37°C for bacteria and 72 h at 28°C for fungus. The lowest concentration without visible growth of microbial biomass under a binocular magnifying glass was defined as the MIC. Determination of the absence of microbial growth, i.e. determination MBC/MFC, was performed by serial reinoculation of 10 µL of inoculated medium from wells with no microbial growth into 100 µL of sterile nutrient medium and reincubation for 24 h at 37°C for bacteria and for 72 h at 28°C for fungus. Results were confirmed by adding 40 µL of purple *p*-iodonitrotetrasolium chloride, microbial growth indicator solution (0.2 mg mL^{-1} distilled water) to each well and icubating for 30 minutes at 37°C [11]. Comparison of color intensity was made with control wells in which microorganisms were allowed to grow unhindered, and commercial antimicrobial agents streptomycin, ampicillin, and ketoconazole were used as positive controls. Inoculated medium without added essential oil was used as the negative control. The antimicrobial tests were performed in triplicate.

DETERMINATION THE CYTOTOXICITY

In vitro culture of the cell lines

The HeLa (cervical cancer) cell line was cultured in MEM supplemented with 2 mM glutamine, 1% non-essential amino acids, 10% HI FBS and 1% penicillin/streptomycin antibiotics. Cells were maintained in humidified atmosphere containing 5% CO_2 at 37°C. For each experiment cells were grown to 80% confluence in cell culture flasks.

MTT Cell Proliferation Assay

The cytotoxic effects of *P. dysenterica* essential oil were assessed using the MTT assay. For each experiment cells were seeded ($2x10^4$ cells/well) in 96 well plates and incubated overnight. The next day, the cells were treated with increasing final concentrations of *P. dysenterica* essential oil and incubated for further 48 h. After incubation of the cells, MTT solution 0.5 $mgmL^{-1}$ was added to each well, and the plates were incubated for another 4 hours at 37°C in a humidified atmosphere containing 5% CO_2. The MTT containing medium was then removed and the remaining MTT-formazan crystals were dissolved by adding 200 µL DMSO to each well with continuous gentle shaking for 15 minutes. The absorbance was read using a microplate reader at a wavelength of 570 nm.

The experiment was repeated three times and each experiment was performed in triplicate. Untreated cells were used as negative control and cells treated with 30% DMSO in culture medium were used as positive control. Samples were dissolved in 10% DMSO and diluted in culture medium. The final concentration of DMSO in treated samples did not exceed 0.1%. Prepared stock solutions of extracts were sterilized by filtration through 0.2 µm sterile syringe filters. The concentration of the extracts that resulted in 50% of viability (IC_{50}) was determined from graph plots of the dose response curve.

RESULTS AND DISCUSSION

RESULTS OF ISOLATION AND GC-FID/MS ANALYSIS OF ESSENTIAL OIL

The aerial parts of *P. dysenterica* contained 0.3% (v/w) of yellow, liquid, fragrant essential oil (EO), and the identified 51 components, accounting for 81.09% of the oil, are listed in Table 1. The EO of aerial parts of *P. dysenterica* was characterized by the presence of a high concentration of oxygenated sesquiterpenes (51.83%). The main compounds were caryophyllene oxide (14.89%), (*E*)-nerolidol (9.18%), *epi*-*α*-cadinol (3.58%). Nerol (5.92%) was the main representative of the oxygenated monoterpenes, which made up 15.57% of the oil. Sesquiterpene hydrocarbons made up 9.32% of the oil with the dominant compound being *β*-caryophyllene (4.75%). Non-terpene (other) compounds represented 4.37% of the EO.

Table1. Chemical composition of essential oil from *P. dysenterica* aerial parts

No.	Ret. time[a]	Compound	RIE[b]	EO[c] (%m/m)
1	16.674	*α*-terpineol	1192.5	0.28
2	18.236	nerol	1229.3	**5.92**
3	22.814	presilphiperfol-7-en	1337.1	0.33
4	25.599	italicen	1402.6	0.35
5	26.383	*β*-caryophyllene	1422.6	**4.75**
6	27.165	(*Z*)-*β*-farnesene	1442.0	0.85
7	27.639	geranyl acetone	1454.0	0.50
8	27.71	*α*-humulene	1456.0	0.29
9	28.63	*γ*-curcumen	1478.6	0.37

10	28.772	*ar*-curcumen	1482.3	**1.65**
11	29.019	(E)-*β*-jonone	1488.8	0.87
12	29.153	neryl isobutanoate	1492.1	**3.60**
13	29.484	3-(1,1-dimethylethyl)-4-methoxy-phenol	1500.5	0.16
14	29.484	*α*-muurolene	1500.5	0.15
15	29.919	*β*-curcumene	1511.6	0.58
16	30.07	10-*epi*-italicene ether	1515.8	0.33
17	30.809	silphiperfol-5-en-3-ol B	1534.6	0.27
18	31.517	italicene epoxide	1552.7	0.37
19	32.053	(*E*)-nerolidol	1566.4	**9.18**
20	32.533	neryl isovalerate	1579.1	**2.01**
21	32.6	*ar*-tumerol	1580.3	**1.58**
22	32.945	caryophyllene oxide	1589.7	**14.89**
23	33.045	helifolen-12-al A(syn-anti-anti)	1592.4	0.50
24	33.226	fokienol	1597.0	0.80
25	33.631	(*Z*)-sesquilavandulol	1607.7	**3.11**
26	33.795	humulene epoxide II	1612.4	0.72
27	34.032	helifolen-12-al C (anti-syn-syn-)	1620.3	0.60
28	34.452	eremoligenol	1630.1	0.36
29	34.488	murola-4,10(14)-dien-1-*β*-ol	1631.2	0.34
30	34.543	1-*epi*-cubenol	1633.0	0.25
31	34.676	caryophylla-4(12),8(13)-dien-5-*α*-ol	1636.3	0.26
32	34.882	*epi*-*α*-cadinol	1642.0	**3.58**
33	35.138	himachalol	1649.1	0.50
34	35.329	vulgarone B	1654.7	**1.58**
35	35.473	*α*-cadinol	1658.1	**1.65**
36	35.923	14-hydroxy-(*Z*)-caryophyllene	1670.5	**1.64**
37	36.107	14-hydroxy-9-*epi*-(*E*)-caryophyllene	1675.6	**2.30**
38	36.125	*β*-bisabolol	1675.8	**1.46**
39	36.443	germacra-4(15), 5,10(14)-trien-1-*α*-ol	1684.5	**1.94**
40	36.692	8-cedren-13-ol	1690.4	**1.76**
41	37.953	(*Z*)-nuciferol	1727.3	0.49
42	38.196	zerumbone	1734.2	0.92
43	38.711	fukinone	1749.1	0.45
44	40.123	(8)-cedren-13-ol acetate	1789.7	0.33
45	41.513	(*Z*)-nuciferol acetate	1830.8	**2.11**
46	42.027	hexahydrofarnesyl acetone	1846.5	0.92
47	44.41	(5*E*,9*E*)-farnesyl acetone	1919.0	0.36
48	47.374	13-*epi*-mannol oxide	2013.0	0.79
49	50.427	(*E*)-phytol	2114.3	0.51
50	60.949	*n*-pentacosane	2498.9	**1.16**
51	65.819	*n*-heptacosane	2698.3	0.42
Total				**81.09**
Oxygenated monoterpenes				**15.57**
Sesquiterpene hydrocarbons				**9.32**
Oxygenated sesquiterpenes				**51.83**
Others				**4.37**

[a]Retention time (min)
[b]RIE – experimental retention indices
[c]EO – essential oil

The content of essential oils from the aerial parts of *P. dysenterica* from Greece and Iran, obtained by hydrodistillation, ranged from 0.24% to 0.4%. In the present study the content of EO from the aerial parts of *P. dysenterica* from Bosnia and Herzegovinawas in this range. Dominant compounds in the EO from Greece were sesquiterpene lactones *β*-caryophyllene, caryophyllene oxide, [(*E*),(*Z*)]-nerolidol [11]. Dominant compounds in the EO from Iran were *ar*-curcumin, *epi*-*α*-cadinol and (*E*)-coniferyl alcohol [2].

In the EO of *P. dysenterica* aerial parts from Turkey, obtained by the petroleum ether extraction, the main compound was identified as terpinolen [13]. In the present study the dominant compounds were the sesquiterpenes caryophyllene oxide, (*E*)-nerolidol, *β*-caryophyllene and the monoterpene nerol, which were also the dominant compounds in the essential oil of *P. dysenterica* aerial parts from Greece.

RESULTS OF ANTIMICROBIAL ACTIVITY

The antimicrobial activity of EO was determined *in vitro* using the microdilution method. The results are in the form of minimum inhibitory concentrations (MICs) and minimum bactericidal/fungicidal concentrations (MBCs/MFCs) by the bacterial strains already mentioned. They are presented in the Table 2.

Table 2. Antimicrobial activity of essential oil from *P. dysenterica* aerial parts expressed as MIC and MBC

Strain	ATCC	MIC ($mgmL^{-1}$)	MBC/MFC ($mgmL^{-1}$)
Staphylococcus aureus	6538	4	7
Pseudomonas aeruginosa	27853	3	4
Escherichia coli	35210	1	7
Candida albicans	10231	20	30

MIC - minimum inhibitory concentration
MBC/MFC - minimum bactericidal concentration/minimum fungicidal concentration

The results obtained showed that EO inhibited the growth of all selected ATCC microorganism strains. The best result was obtained against Gram-negative bacteria *Escherichia coli* ATCC 35210 with MIC value of 1 $mgmL^{-1}$.

There are no previous data on the antimicrobial activity of *P. dysenterica* EO.

The antimicrobial activity of the dominant compounds in the *P. dysenterica* aerial parts EO has been reported in the literature. Schmidt et al. tested caryophyllene oxide, nerolidol, *β*-caryophyllene against selected ATCC strains of microorganisms in

the concentration range from 0.001 to 1 mgmL^{-1}. The MICs of caryophyllene oxide against Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. coli* were 0.06 mgmL^{-1}. The MIC of caryophyllene oxide against the yeast *C. albicans* was below 0.06 mgmL^{-1}, while no inhibition was observed against the Gram-negative bacteria *P. aeruginosa*. The MIC of nerolidol against *S. aureus* was below 0.06 mgmL^{-1} while inhibition against *E. coli*, *P. aeruginosa* and *C. albicans* was not detected. The MIC of *β*-caryophyllene against *S. aureus* was 0.06 mgmL^{-1}. MIC of *β*-caryophyllene against *E. coli* was 0.6 mgmL^{-1} while inhibition against *C. albicans* was not detected [14].

Moura et al. tested nerolidol against selected ATCC strains of microorganisms in the concentration range from 0.25 to 4 mgmL^{-1}. Nerolidol inhibited the growth of *S. aureus* (MIC = 1 mgmL^{-1}) and *P. aeruginosa* (MIC = 0.5 mgmL^{-1}), whereas it showed no activity against the Gram-negative bacteria *E. coli* ATCC [15].

The results of the antimicrobial activity of the *P. dysenterica* aerial parts EO against *E. coli* and *C. albicans* are in an accordance with the literature data on the antimicrobial activity of dominant constituents of the essential oil. Individual, dominant compounds showed effective antimicrobial activity against *S. aureus* in contrast to the weak antistaphylococcal activity of *P. dysenterica* aerial parts EO. The antimicrobial activity of an EO is not a simple sum of the activities of its constituents. It is a mixture of all components with a unique activity [16].

RESULTS OF CITOTOXICITY FOR THE ESSENTIAL OIL

The cytotoxic activity of *P. dysenterica* aerial parts EO was evaluated on the human cervical adenocarcinoma cells (HeLa) using the MTT test. According to the results shown in Figure 1, the oil showed moderate cytotoxicity against HeLa cell line using MTT method with an IC_{50} of 188.52 µgmL^{-1}. Extracts with IC_{50} value in the range of 100 to 500 µgmL^{-1} are classified as extracts with moderate cytotoxicity [17].

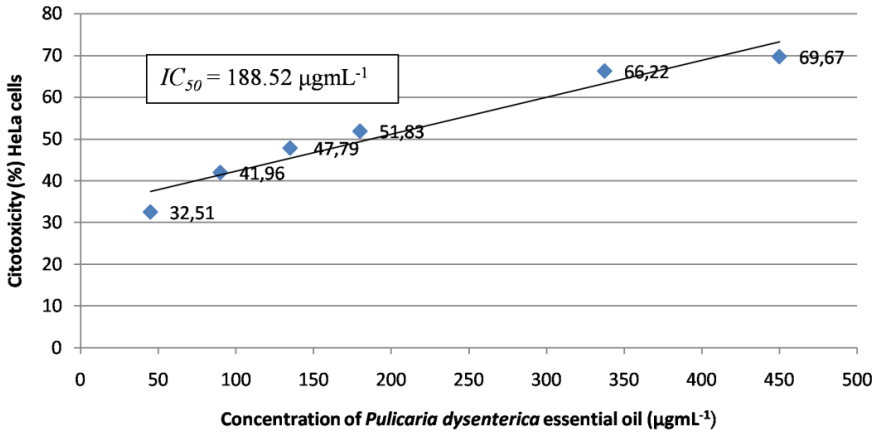

Figure 1. Influence of *Pulicaria dysenterica* essential oil on cell growth in HeLa cell lines

The essential oil induced a dose-dependent inhibition of cell proliferation in tested tumor cell line (Figure 1). There are no published data on the citotoxicity of *P. dysenterica* aerial parts EO.

The cytotoxicity some of the dominant compounds in the EO has been reported in the literature. Caryophyllene oxide showed potent cytotoxic activity against HeLa with IC_{50} values of 13.55 µgmL^{-1}. The results showed that caryophyllene oxide exhibited cytotoxicity in both a dose and time-dependent manner [18]. Nerolidol isomers (*trans*-nerolidol, synthetic nerolidol and *cis*-nerolidol) also showed significant cytotoxicity against HeLa cells. The IC_{50} value of *cis*-nerolidol against HeLa cells was 16.5 µgmL^{-1} ± 6.7 [19], [20]. *β*-Caryophyllene has also been described as cytotoxic compound against HeLa cells [21].

The results of cytotoxic activity of the *P. dysenterica* aerial parts EO are in accordance with the literature data of cytotoxic activity dominant components in the EO.

CONCLUSION

The chemical composition of *P. dysenterica* aerial parts EO growing wild in Bosnia and Herzegovina was

determined. It is characterized by the presence of a high concentration of oxygenated sesquiterpenes. To the best of our knowledge, this study is the first to describe the antimicrobial and cytotoxic activities of *P. dysenterica* aerial parts EO. All microorganisms tested were sensitive to the EO. The best result was obtained against Gram-negative bacteria *E. coli* ATCC (MIC = 1 mgmL^{-1}). The cytotoxic activity was evaluated on the human cervical adenocarcinoma cells (HeLa) with an IC_{50} of 188.52 µgmL^{-1}. This study has provided scientific baseline data on the therapeutic properties of *P. dysentrica.*

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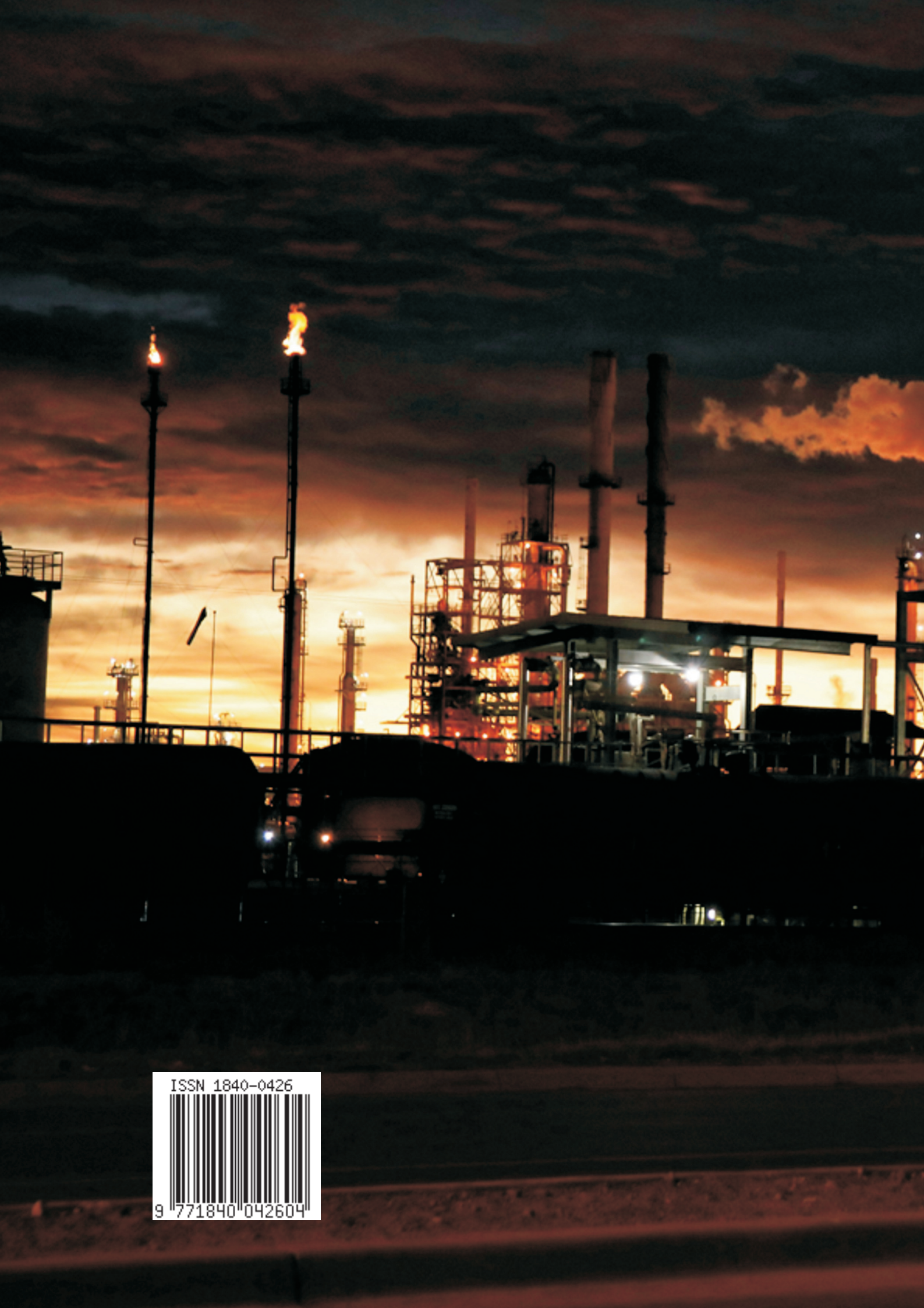

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