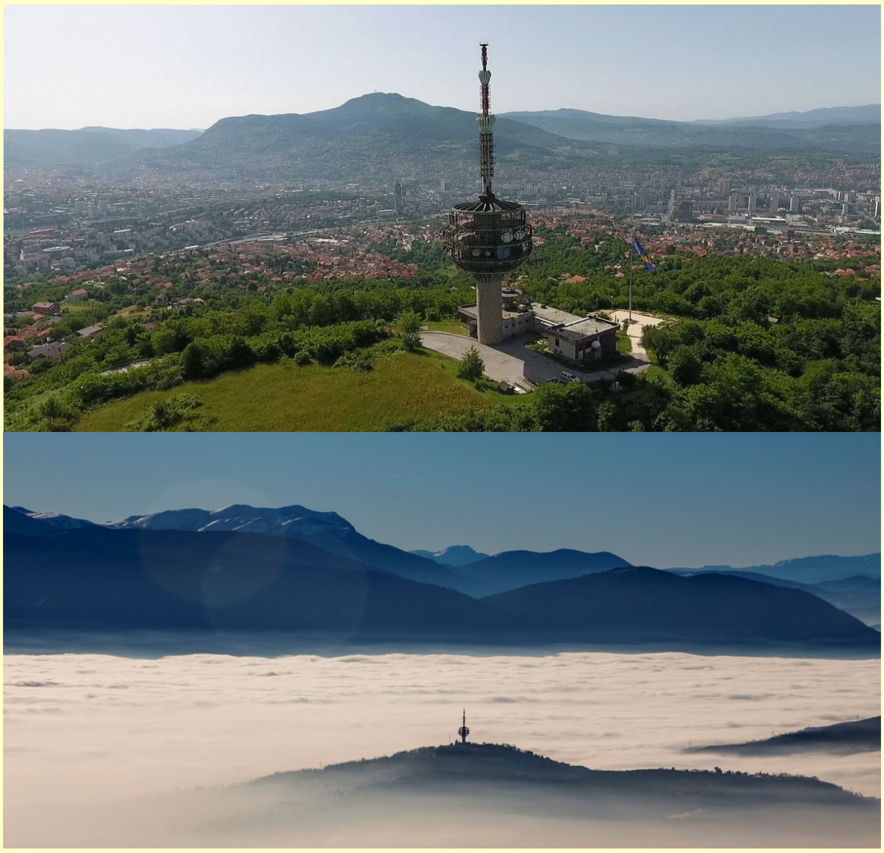
# Glasnik hemičara i tehnologa Bosne i Hercegovine

# Bulletin of the Chemists and Technologists of Bosnia and Herzegovina



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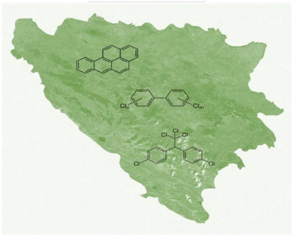
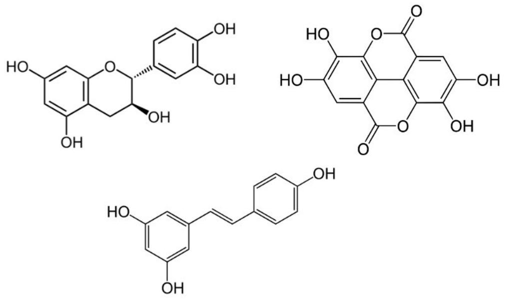
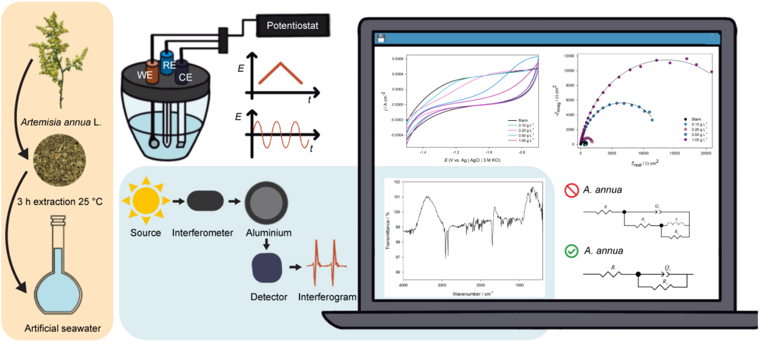
# CONTENT

## ORIGINAL SCIENTIFIC ARTICLES





## *PROCEEDINGS*



**Editorial**

# Decarbonization and the Phase-Out of Coal: A Public Health Imperative and a Development Opportunity

Air pollution remains one of the most serious environmental and public health challenges in Sarajevo Canton and across Bosnia and Herzegovina. Winter pollution episodes characterized by extremely high concentrations of particulate matter ($PM_{2.5}$ and $PM_{10}$), sulphur dioxide, and polycyclic aromatic hydrocarbons clearly indicate the dominant contribution of coal and other solid fuels used in residential heating and the energy sector. In this context, decarbonization should not be viewed as an abstract climate objective, but rather as an immediate measure for protecting public health and improving quality of life.

In recent years, Sarajevo Canton has taken an important step forward by developing strategic documents aimed at the gradual restriction of coal use and the modernization of heating systems. The emphasis on replacing inefficient individual stoves, improving building energy efficiency, and strengthening district heating systems reflects a sound understanding of the local pollution profile. However, the main challenge remains implementation: without stable financing mechanisms, clearly defined timelines, and consistent enforcement, even well-designed strategies risk having limited impact.

At the national level, Bosnia and Herzegovina's energy system remains heavily dependent on coal, particularly in electricity generation. This dependency creates multiple risks—environmental, health-related, and economic—as European climate policies steadily increase the cost of emissions and undermine the long-term viability of coal-fired power plants. At the same time, progress toward renewable energy deployment and cleaner heating solutions has been slower than in most European countries, partly due to institutional fragmentation and the absence of coherent long-term planning.

Within the European context, decarbonization has become the central framework for energy and industrial development. The European Green Deal, emission reduction targets, and the Just Transition mechanism clearly signal that investments in coal no longer have a sustainable development rationale. For countries aspiring to closer integration with the European Union, including Bosnia and Herzegovina, this represents a strong external signal: energy transition is no longer a matter of choice, but a prerequisite for economic competitiveness and political alignment.

The technical and policy solutions to reduce air pollution and carbon emissions are well known. Energy efficiency measures—such as thermal insulation and building retrofitting—often deliver the fastest and most cost-effective benefits. Replacing individual coal-fired stoves with modern heating systems, including heat pumps, district heating, and renewable energy-based solutions, directly reduces local air pollution. In parallel, the expansion of renewable electricity generation must be accompanied by clear and credible plans for the gradual phase-out of coal-based capacity.

The social dimension of the transition deserves particular attention. Coal is not merely an energy source, but also a livelihood for certain communities. Decarbonization policies must therefore incorporate measures to protect vulnerable households, subsidize clean heating alternatives, and support reskilling and employment transition programs for affected workers. Without such measures, the energy transition risks losing public support and exacerbating social inequalities.

The role of the scientific community in this process is crucial. Through robust measurements, source apportionment studies, and assessments of the health and economic benefits of decarbonization, science can provide an evidence-based foundation for policy decisions. Editorials, scientific papers, and interdisciplinary research can help bridge the gap between climate policy objectives and everyday public concerns—cleaner air, reduced health risks, and long-term economic resilience.

Decarbonization in Sarajevo Canton and Bosnia and Herzegovina is therefore not only an obligation to future generations, but a responsibility toward present ones. The era of coal as the backbone of the energy system is drawing to a close; the challenge lies in transforming this inevitability into a development opportunity rather than another missed chance.

**Editors**

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# Occurrence of Selected Persistent Organic Pollutants in Bosnia and Herzegovina: A Systematic Review

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**Abstract:** Persistent organic pollutants (POPs) are compounds that are recognized as significant health and environmental hazards, and their use and emission have been restricted or limited by the Stockholm Convention. Despite the control measures foreseen by the international treaties, the presence in the environment is still evident. Bosnia and Herzegovina is a party to the Stockholm Convention, but regular integrative monitoring of POPs occurrence in the environment has not been established. Data on POPs concentrations reported by various research groups and institutions are scattered in the available publications, making a general assessment difficult. This paper aims to summarize data on the presence and levels of selected POPs in environmental and biological samples from Bosnia and Herzegovina, which can aid in planning further monitoring actions, the assessment of effects of already implemented control measures, and policy decision making. The main findings indicate the presence of organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) in most of the matrices (soil, air, water, sediment, and certain food). Potential ecological or health risks cannot be excluded in respect to heptachlor (Bosna river basin), dichlorodiphenyltrichloroethane (in free-range hen eggs), PCBs (in free-range hen eggs and in Modrac Lake sediment), and PAHs (in agricultural soil in Spreča valley and in sediments from Bosna and Spreča rivers).

## INTRODUCTION

Persistent organic pollutants (POPs) are toxic chemicals that, upon intentional or unintentional release, tend to accumulate and persist in the environment. (Stockholm Convention on POPs, 2023) POPs are compounds of a major concern due to their physicochemical characteristics (semi-volatility, long half-lives, long-range transport, bioaccumulation, and biomagnification) and harmful effects to living organisms (acute and chronic toxicity, endocrine disruption, genotoxicity and carcinogenicity, immunotoxicity, neurotoxicity) (Guillotin, S., Delcourt, N., 2022). Since their clear recognition as significant environmental and health risk, their use and emission have been controlled. Specifically, the Stockholm Convention on POPs lists chemicals which are considered as POPs, some of which were listed for a long time, while others were added on the basis of new evidences (emerging POPs). (Stockholm Convention on POPs, 2023) Despite the control measures foreseen by the international treaties, their presence in the environment is still evident. (Matei, M., Zaharia, R., Petrescu, S. I., et al., 2023), including organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and dioxins, as well as polycyclic aromatic hydrocarbons (PAHs).

POPs are generally grouped into three main categories. The first category, intentionally synthesized for agricultural purposes, includes most organochlorine pesticides (OCPs) whose key representatives are aldrin, dieldrin and dichloro-diphenyl-trichloroethane (DDT). Intentionally synthesized substances for industrial applications, the second category includes hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs), and various perfluorinated and brominated compounds (BFRs). The third category consists of chemicals unintentional produced through incomplete combustion of organic matter, such as polycyclic aromatic hydrocarbons (PAHs), dioxins/polychlorinated dibenzodioxins (PCDDs), and furans/polychlorinated dibenzofurans (PCDFs). (Matei, M., Zaharia, R., Petrescu, S. I., et al., 2023)

*Organochlorine pesticides (OCPs)*

Organochlorine pesticides are defined as organic compounds that contain multiple chlorine atoms. They are synthetic, chemically very stable, and hydrophobic compounds widely applied in various domains - including agriculture, industry, medicine, and domestic settings—not only for pest control but also for the management of insect-borne diseases.

Organochlorine insecticides may be divided to four distinct structural groups:

- chlorinated derivatives of ethane, namely dichlorodiphenyltrichloroethane (DDTs), and methoxychlor,
- chlorinated derivatives of benzene and cyclohexane, namely hexachlorobenzene (HCB), hexachlorocyclohexane (HCH) isomers,
- cyclodienes ("drins": aldrin, dieldrin, endrin; heptachlor, chlordanes, endosulfan) and
- chlorinated camphenes (toxaphene and chlordecone). (Hernández, F., A., 2023)

The chemical structures of key representative compounds belonging to these four major OCP structural groups are presented in Figure 1.

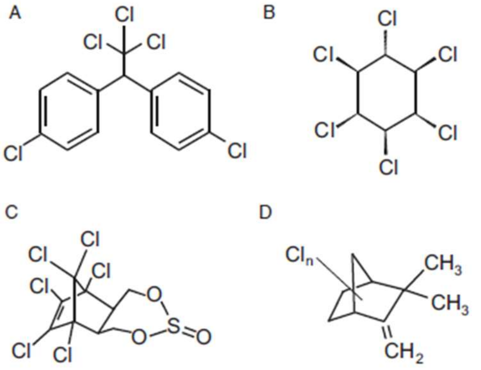

**Figure 1**. Chemical structures of dichlorodiphenyltrichloroethane, DDT (A); endosulfan (B); gamma-hexachlorocyclohexane, γ-HCH (lindane) (C); and toxaphene (D); (Hernández, F., A., 2023)

Since OCPs have been used worldwide in agriculture and the health sector, they can enter in all environmental compartments through various routes such as agricultural runoff, industrial wastewater, and atmospheric transport and deposition. (Ali, U., Syed, J. H., Malik, R. N., et al., 2014) Due to their ability to persist, bioaccumulate and biomagnify through the food chain, they pose a risk to human health and ecological entities. (Sparling, D. W., 2016) Human exposure to organochlorine pesticides and their metabolites is associated with an increased risk of cancer, cardiovascular diseases, diabetes, and both neurological and reproductive disorders. (Marriya, S., Naima, H., Muhammad, J., et al., 2023) They act as endocrine-disrupting chemicals (EDCs), affecting thyroid hormone balance and thereby contributing to metabolic disorders and hormone-related cancers such as breast, prostate, stomach and lung. (Jayaraj, R., Megha, P., and Sreedev, P., 2016). Due to environmental persistence and long-term toxicity to humans and wildlife in recent decades, the agricultural use of these pesticides is banned in the European Union. However, their presence is still reported in many areas of the world, including Europe (López-Benítez, A., Guevara-Lara, A., Domínguez-Crespo, M.A., et al., 2024). Currently, their use (DDT, γ-HCH) is restricted only for very specific purpose in certain world regions.

In the Western Balkans OCPs use is currently not allowed, but the stockpiles and/or previous production (during the last two decades) were recorded in the National Implementation Plans (NIPs) for the Stockholm convention. (Stockholm Convention on POPs, NIP)

According to national regulations (Regulation of FBiH, 2022; Regulation of RS, 2016), the permissible concentration of ∑DDT, ∑HCH, and "drins" in agricultural soils is 0.1 mg/kg. In surface waters of FBiH, the maximum residue levels for OCPs are defined within the range of 0.001–0.3 µg/L (Regulation of FBiH, 2007).

*Polychlorinated biphenyls (PCBs) and dioxins*

Polychlorinated biphenyls (PCBs) are synthetic polyhalogenated aromatic hydrocarbons consisting of two benzene rings with up to ten chlorine substitutions, forming 209 possible isomers and congeners. General chemical structure of PCBs is shown in Figure 2.

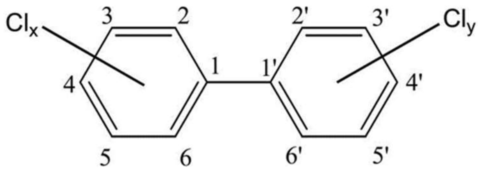

**Figure 2.** General chemical structure of PCBs (ATSDR, 2000)

PCB congeners are divided into two categories: dioxin-like (dl-PCBs) and non-dioxin-like (ndl-PCBs). Dioxin-like PCBs (dl-PCBs) are named due to their shared mechanism of toxicity with dioxins. They possess a planar structure that enables strong binding to the aryl hydrocarbon receptor (AhR), triggering toxic responses similar to dioxins. (Benedetto, A., Brizio, P., Guaraldo, P., et al., 2016) Thus, dl-PCBs have assigned toxic equivalency factors (TEF= Toxic Equivalency Factor) and are included in the total toxic equivalent (TEQ= Toxic Equivalent Quantity) calculation. (DeVito, M., Bokkers, B., van Duursen, M.B.M., et al., 2024). In contrast, ndl-PCBs have a non-planar configuration due to ortho-chlorine substitutions, which prevents strong AhR binding and results in lower toxicity. However, ndl-PCBs are also toxic compounds (FAO/WHO, 2016).

Physicochemical properties of PCBs, such as non-flammability, high boiling point, chemical stability, and insulating capacity, led to extensive use in industry, including applications in electrical equipment, heat transfer and hydraulic systems, as well as in paints, plastics, rubbers, pigments, dyes, and carbonless copy paper. Due to their stability and persistence, PCBs easily enter various environmental compartments, where they bioaccumulate through food chain. In humans, they are primarily stored in adipose tissue and the liver, with elimination half-lives of approximately 10–15 years.

Beyond their persistence, experimental studies demonstrate that PCBs act as endocrine disruptors, contributing to disturbances in reproductive and metabolic functions. (Gupta, R., Kumar, P., Fahmi, et al., 2020).

Dioxins, or polychlorinated dibenzodioxins (PCDDs), are also a group of organic compounds, chemically classified as halogenated aromatic hydrocarbons, which, depending on the number and position of chlorine atoms, can form 75 congeners. Structurally, they consist of two benzene rings conected by oxygen atoms at the "meta" and "para" positions. The basic structure for unsubstituted dibenzo-p-dioxin (showing the carbon numbering scheme that is used to name specific congeners) is shown in Figure 3 (ATSDR, 1998).

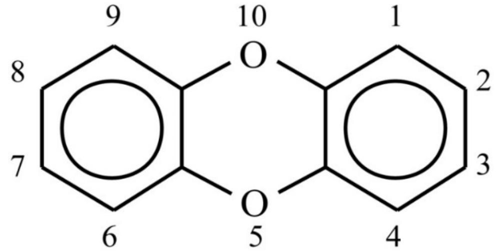

**Figure 3.** Basic structure for unsubstituted dibenzo-p-dioxin

The term „dioxin" is generally used for polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (furans or PCDFs). 2,3,7,8-tetrachloro dibenzo-p-dioxin (2,3,7,8-TCDD) is the most toxic congener used as a reference for the determination of TEFs for other dioxins and dl-PCBs.

Dioxins are primarily emitted as by-products of human activities (waste incineration, vehicle emissions) or during natural disasters (volcano eruptions, wildfires). PCBs are man-made chemicals which are banned for production and use, but they can be released by improper disposal of PCB-containing products or from hazardous waste sites. (Stockholm Convention on POPs, NIP) National regulations establish permissible concentrations of PCBs and dioxins in food and environmental matrices. According to the Regulation for certain contaminants in food (Regulation of BiH, 2014), the maximum allowable level of ∑dl-PCBs, expressed as TEQ is 40 ng/g of lipid weight (l.w.), while values range from 1 to 300 ng/g for wet weight (w.w.) For dioxins, the permissible concentrations are 0.75–2.5 pg/g l.w. and 0.1–3.5 pg/g w.w. In surface waters of FBiH, maximum residue levels for total PCBs are set between 0.02 and 0.2 µg/L, and for 2,3,7,8-TCDD is 4.5x10-7 µg/L. (Regulation of FBiH, 2007)

For agricultural soils, the threshold value for ∑PCBs is 0.2 mg/kg (Regulation of FBiH, 2022; Regulation of RS, 2016).

*Polycyclic aromatic hydrocarbons (PAHs)*

Polycyclic aromatic compounds (PAH) are a very large group of both natural and man-made chemicals containing two or more aromatic rings, which usually occur as a complex mixture. They are lipophilic, many of them resist degradation and tend to accumulate in environment. Therefore, most of PAHs are POPs.

PAHs natural emission sources (volcanic eruptions, natural forest fire, moorland fire) are less important than the anthropogenic sources (industrial, agricultural, traffic, domestic). They are mostly produced by incomplete combustion of organic materials such as coal, gas, garbage, meat, oil, tobacco, and wood. (Patel, A.B., Shaikh, S., Jain, K.R., et al., 2020; Manousi, N., and Zachariadis, G. A., 2020) PAHs toxicity is mostly attributed to a group of 16 priority compounds which are usually monitored in the environment. (Mumtaz, M. and George, J., 1995) Well-known PAHs are benzo[a]pyrene, fluoranthene, naphthalene and anthracene whose chemical structures are shown in Figure 4.

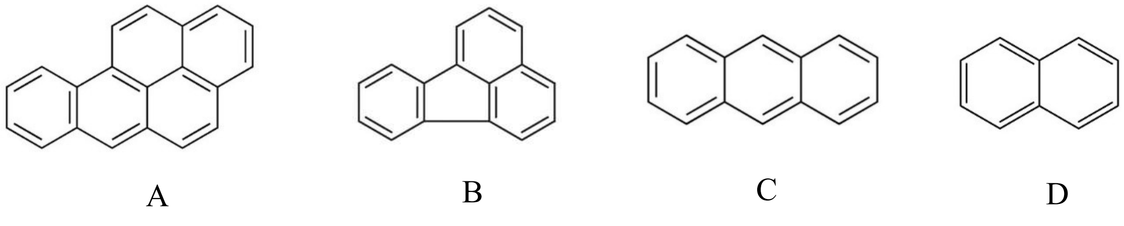

**Figure 4.** Chemical structures of well-known PAHs (benzo(a)pyrene (A), fluoranthene (B), anthracene (C) and napthalene (D)) (Sudip, S.K., Singh, O.V., and Jain, R.K., 2002.)

Numerous adverse health effects, including various types of cancer, respiratory and cardiovascular disorders, reproductive dysfunction, immune suppression, and endocrine disruption, have been associated with chronic exposure to PAHs. (Montano, L., Baldini, G. M., Piscopo, M., et al., 2025)

As with OCPs and PCBs, national regulatory agencies have also established maximum permissible levels for both, individual and total PAHs, in food and various environmental samples. In surface waters, the maximum allowable concentrations for total PAHs range from 0.2 to 1 µg/L. (Regulation of FBiH, 2007) In agricultural soil, the maximum permissible concentrations for individual PAHs range from 0.1 to 0.2 mg/kg, and for total PAHs the range is between 1 and 2 mg/kg. (Regulation of FBiH, 2022; Regulation of RS, 2016) In food, the maximum allowable concentrations for individual PAHs range from 1 to 6 µg/kg, while for total PAHs, the range is from 1 to 35 µg/kg. (Regulation of BiH, 2014)

## Study area

Bosnia and Herzegovina is one of the Western Balkan countries with specific socio-economic, political and environmental challenges. The region's primary economic activities are in climate-related sectors like agriculture, forestry, and tourism.

The main industrial activities in Bosnia and Herzegovina include (Figure 5):

1. Pharmaceutical industry (Sarajevo, Tuzla, Banja Luka region, Travnik),
2. chemical industry (mostly in Tuzla region),
3. metal industry (Goražde, Zenica, Tuzla, Mostar, Sarajevo region)
4. wood industry (Sarajevo, Zenica, Tuzla, Bihać, Mostar, Banja Luka region)
5. ammunition production (Konjic, Goražde, Bugojno, Sarajevo region)
6. textile industry (Sarajevo, Tuzla, Doboj, Zenica, and Banja Luka region, Travnik, Goražde),
7. food industry (all regions)
8. Petroleum industry-refinement and delivery (Modriča and Bosanski Brod)
9. electric power industry (Tuzla, Zenica, and Mostar region)
10. coal mining (Tuzla and Zenica region)

Agricultural production is widespread in the country, but it is mainly characterized by small, inefficient family farms.

**Figure 5**. Map of Bosnia and Herzegovina

According to various indicators of a green transition, the entire Western Balkan region is lagging behind EU countries. (Radovanovic, N., Stevanovic Carapina, H., 2024) In order to implement the Green Agenda, Western Balkan countries are strongly encouraged to enhance monitoring and reporting on pollutants in air, water and soil. For the aforementioned reasons, this review aims to summarize published data on the presence of selected POPs in environment, including air, water, sediment, soil, and biological material. The paper can aid in planning further monitoring actions, the assessment of effects of already implemented control measures, and policy decision-making.

## EXPERIMENTAL

### *Data research strategy*

Literature search was conducted between 01.04.2025. and 16.04.2025. ScienceDirect, Web of Science and Google Scholar were searched (see Table 1), yielding 41 results in total (Figure 6). Further, a grey literature search was conducted as manual searches through reference lists of articles found, by using Google search for the key words in the Bosnian language. This additional search yields another 38 results (Figure 6). After exclusion of duplicates (n=12), records were title- and abstract-screened for eligibility (12 records excluded). One record couldn't be retrieved, and 18 records were excluded after full-text reading (Figure 6). Thus, 36 results were included in this review.

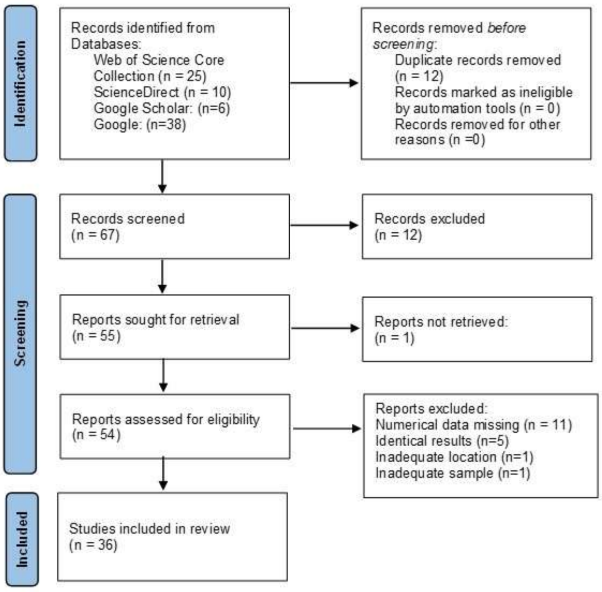

**Figure 6**. PRISMA flow diagram

**Table 1**. Search strategy used for each data source

| Data source | Search strategy |
|---|---|
| Web of Science Core Collection | AB=((POPs OR persistent organic pollutants OR PCB OR PAH OR pesticide OR DDT) AND (Bosnia OR Herzegovina)) |
| ScienceDirect | in title, abstract or keywords: (POPs OR persistent organic pollutants OR PCB OR PAH OR pesticide OR DDT) AND (Bosnia OR Herzegovina) |
| Google Scholar | allintitle: (Bosnia OR Herzegovina) AND (PCB OR PAH OR DDT OR "pesticides" OR "persistent organic pollutants") |

## RESULTS AND DISSCUSION

### *Organochlorine pesticides (OCPs) levels*

Concentrations of OCPs in ambient air were reported in two papers. Klánová et al. (2007) reported on DDT, HCH, and HCB concentrations in air samples from Sarajevo and Tuzla and Lamell et al. (2011) from Banja Luka (Table 2). The concentrations found in soil samples from the same locations were also reported by Lamell et al. (2011) (Table 2). Concentrations for each of the OCPs reported here were within the range of the European background levels. (Helene, L. H., Pernilla, B-N., Sabine, E. et al., 2023)
Data on OCPs in soil were also found for the area of Zenica, Spreča river catchment (municipalities Lukavac, Gračanica, and Doboj Istok), and Olovo area (Table 2). None of the samples exceeded the permissible levels for the agricultural soil according to local regulations (Regulation of FBiH, 2022; Regulation of RS, 2016), although at all sites multiple pesticides were detected. In comparison with the average (0.03 mg/kg for Sum DDT, and 0.01 mg/kg for HCB) and maximum levels (0.31 mg/kg for Sum DDT, and 0.01 mg/kg for HCB) in European agricultural soil, (Silva, V., Mol, H.G.J., Zomer, P., et al., 2019) concentrations found in samples from Bosnia and Herzegovina (Table 2) were significantly lower.
OCP concentrations in air and soil samples are not sufficient for trend analysis since the measurements were temporary and spatially scattered. However, the authors (Lammel, G., Klanova, J., Erić, Lj., et al., 2011) attributed OCP in air primarily to the industrial activities (sawmill) and sporadic waste burning as a local source in the Banja Luka area.

In 2008 and 2009, sediment samples from the Bosna River (Table 2) were analyzed for OCPs (groups of HCH, DDT and its metabolites, and cyclodiene pesticides including "drins" and other: heptachlor and chlordanes). The γ-isomer of HCH (lindane) was detected at all locations. Among all analyzed OCPs, the highest concentration was recorded for heptachlor and its epoxides at all sampling sites, which also exceeded the TEL (Threshold Effect Level) or PEL (Permissible Exposure limit) (Harman, C., Grung, M., Djedjibegovic, J., et al., 2013). Ratio of parent compounds to their metabolites indicated more weathered input of heptachlor in the river Bosna at Doboj, while the opposite was recorded in other sampling sites along the river Bosna. For DDT more recent source was assumed only at the Doboj location on the river Bosna.
OCP concentrations (ng/L) obtained by passive sampling in rivers are shown in Table 2.
OCP detected in the river Neretva in 2007 were HCB (all sampling sites), heptachlor (at 2 of 3 sites), and in one location (Gabela – near delta), in addition to the previous two OCPs, pentachlorobenzene, dieldrin, and p,p'-DDE were also detected. In the river Bosna, most of the analyzed OCPs were detected, with the highest concentrations for heptachlor (among cyclodiens), followed by the sum of DDT/DDD/DDE, endrin (among „drins“), and lindane (γ-HCH). These concentrations were very similar to those reported for surface water at the Central European Biosphere Reserve Krivoklatsko (Kocí, V., Ocelka, T., Dragoun, D. et al., 2007), representing the background levels in the European region.
Concentrations found by repeated measurements in Bosna and Neretva water samples from 2007-2012 didn't differ significantly (Table 2), suggesting that there was no new load within this time period.
In 2008, samples of honey from Bosanska Krajina were analyzed for OCPs (HCH, DDT, heptachlor, and drins). Measured concentration in all analyzed samples was below 0.001 mg/kg. (Mujić, I., Alibabić, V., Jokić, S., et al., 2011)
Concentration of HCB, Sum HCH, and Sum DDT in free range chicken eggs from Tuzla and Zenica region (2014-2015) were <1-2.65 ng/g fat, ND-3.23 ng/g fat (0.02-0.45 ng/g fresh weight), and ND-933.48 ng/g fat (ND-145.44 ng/g fresh weight), respectively. (Petrlík, J., Behnisch, P., 2016) Only DDT concentrations were higher than the EU limit (50 ng/g fresh weight). (EC, 2023; EC, 2017; EC, 2016) Since hens were from the free-range farming, DDT residues found in eggs could not be attributed to animal feed. Thus, the residues are probably accumulated from the soil. However, the soil-to-eggs bioaccumulation factor of DDT can vary greatly (Kesic, R., Elliott, J. E., Fremlin, K. M., et al., 2021) and cannot be used in a reliable assessment of a possible source of exposure. This underlines the need for regular monitoring of hen eggs used for human consumption.

**Table 2.** OCPs concentrations in air, soil, rivers and river sediments from different locations

| AIR | | | | |
|---|---|---|---|---|
| **Sampling area** | **Sampling year** | **Analytes** | **Range *** | **Ref** |
| Sarajevo (gaseous phase)<br>Tuzla (gaseous phase) | 2004 | Sum DDT<br>Sum HCH<br>HCB | 0.012-0.193*<br>0.025-0.054*<br>0.064-0.171* | Klánová *et al*. (2007)<br>*for data from (ng/m$^3$) Klánová *et al*. (2007) |
| Sarajevo (particulate matter)<br>Tuzla (particulate matter) | 2004 | Sum DDT<br>Sum HCH<br>HCB | 0.001-0.101*<br>0-0.002*<br>0.001-0.002* | Klánová *et al*. (2007) |
| Banja Luka<br>(sum of gaseous and particulate matter) | 2008 | Sum DDT<br>Sum HCH<br>HCB<br>PeCB | 27-74**<br>52-147**<br><0.1-48**<br>6-9.9** | Lammel, G., *et al*., 2011<br>**pg/ m$^3$ for data from Lammel *et al*., 2011 |

| SOIL | | | | |
|---|---|---|---|---|
| **Sampling area** | **Sampling year** | **Analytes** | **Range (mg/kg)***** | **Ref** |
| Banja Luka (agricultural soil) | 2008 | Sum DDT<br>Sum HCH<br>HCB<br>PeCB | 0.112-1.222***<br>0.116-0.424***<br>0.124-0.526***<br>ND | Lammel, G., *et al*., 2011<br>***ng/g for data from Lammel *et al*., 2011 |
| Zenica (agricultural soil) | 2011-2015 | Sum HCH<br>Sum DDT<br>Drins | ND-0.0003<br>ND<br>ND-0.0005 | Federal Institute of Agropedology, 2016 |

| | | | | |
|---|---|---|---|---|
| Spreča lower flow (agricultural soil) | 2017 | | | Federal Institute of Agropedology, 2018 |
| Lukavac | | Sum HCH | 0.0046-0.08 | |
| | | Sum DDT | ND | |
| | | Drins | 0.0114-0.0232 | |
| Gračanica | | Sum HCH | 0.006-0.0189 | |
| | | Sum DDT | ND-0.005 | |
| | | Drins | 0.0255-0.0596 | |
| Doboj Istok | | Sum HCH | 0.0057-0.009 | |
| | | Sum DDT | 0.0013-0.0029 | |
| | | Drins | 0.0205-0.035 | |
| Olovo (agricultural soil and roadside soil) | 2020 | Sum HCH | ND-0.003 | Federal Institute of Agropedology, 2021 |
| | | Sum DDT | ND-0.001 | |
| | | Drins | ND-0.065 | |
| Sarajevo (public children's playgrounds) | 2016 | Sum DDT | <0.001 | Sapcanin, A., Cakal, M., Imamovic, B., *et al.*, 2016 |

**RIVERS**

| **Sampling area** | **Sampling year** | **Analytes** | **Range (ng/L)** | **Ref** |
|---|---|---|---|---|
| Neretva | 2007 | Sum 8 OCP | 0.040-0.14 | Djedjibegovic, J., Marjanovic, A., Sober, M. *et al.,* 2010 |
| Bosna | 2008/2009 | Sum 8 OCPs | 2008: 0.028-0.171<br>2009: 0.059-0.195 | Harman, C., *et al.,* 2013 |

| | | | | |
|---|---|---|---|---|
| Bosna<br>Neretva | 2010 | Sum 8 OCP | 0.021-0.133<br>0.011-0.014 | Harman, C., Grung, M., Djedjibegovic, J., *et al*., 2018 |
| Bosna | 2012 | Sum DDT<br>Sum HCH<br>HCB<br>PeCB | 0.0039-0.044<br>0.0059-0.13<br>0.0018-0.02<br>0.0005-0.0033 | Toušová, Z., Vrana, B., Smutná, M., *et al*., 2019 |
| **RIVER'S SEDIMENTS** | | | | |
| **Sampling area** | **Sampling year** | **Analytes** | **Range (ng/g)** | **Ref** |
| Bosna | 2008/2009 | Sum DDT<br>Sum HCH<br>Sum Drins<br>Other cyclodienes | 0.71-4.6<br>0.01-0.10<br>0.04-6.7<br>0.64-9.4 | Harman, C., Grung, M., Djedjibegovic, J., *et al.,* 2013 |

*Polychlorinated biphenyls (PCBs) and dioxins levels*

In agricultural soils, PCB concentration (Table 3) was below the regulatory limit. (Regulation of FBiH, 2022; Regulation of RS, 2016) High concentrations of PCBs in soil were found in samples collected at locations close to industrial facilities, and most probably due to local sources (damaged capacitors, coal mines, paper production). Mean concentrations reported by Picer et al. (2005) ranged from 0.1-1.2 mg/kg dry weight, which is significantly higher than the Europe-wide mean concentration in soils (JRC, 2010)

**Table 3.** PCB concentrations in air, soil, rivers, and river sediments from different locations

| **AIR** | | | | |
|---|---|---|---|---|
| **Sampling area** | **Sampling year** | **Analytes** | **Range (ng/m$^3$)** | **Ref** |
| Sarajevo (gaseous phase)<br>Tuzla (gaseous phase) | 2003-2004 | Sum 7 indicator congeners | 0.059-0.172<br>0.06-5.645 | Klánová *et al*. (2007) |
| Sarajevo (particulate matter)<br>Tuzla (particulate matter) | 2003-2004 | Sum 7 indicator congeners | 0.01-0.121<br>0.013-1.648 | Klánová *et al*. (2007) |
| **SOIL** | | | | |
| **Sampling area** | **Sampling year** | **Analytes** | **Range (mg/kg dry weight)** | **Ref** |
| Tuzla | 2003-2004 | Sum 7 indicator congeners | 0.9 (mean) | Klánová, J., *et al*., 2007 |
| Tuzla area<br>Bihać<br>Tešanj<br>Sarajevo area | 2003 | Sum 7 indicator congeners | 0.007-22 890<br>0.004-73.4<br>0.005-43 092<br>0.007-3.5 | Picer, N., Čalić, V., Miošić, N., *et al*., 2005 |
| Zenica (agricultural soil) | 2011-2015 | Sum 7 indicator congeners | ND-0.0012 | Federal Institute of Agropedology, 2016 |
| Spreča lower flow (agricultural soil) | 2017 | | | Federal Institute of Agropedology, 2018 |
| Lukavac<br>Gračanica<br>Doboj Istok | | Sum 7 indicator congeners | 0.0008-0.006<br>ND-0.001<br>ND-0.002 | |

| | | | | |
|---|---|---|---|---|
| Olovo (agricultural soil and roadside soil) | 2020 | Sum 7 indicator congeners | ND-0.008 | Federal Institute of Agropedology, 2021 |
| Sarajevo (public children's playgrounds) | 2016 | Sum 12 congeners (18, 28, 31, 44, 52, 101, 118, 138, 149, 153, 180, 194) | <0.002 | Sapcanin, A., *et al.*, 2016 |
| Banja Luka (Incel factory) | 2020 | Sum 7 indicator congeners | <0.021-18.10 | UNDP/BiH, 2021 |
| **RIVERS (concentrations obtained by passive sampling)** | | | | |
| **Sampling area** | **Sampling year** | **Analytes** | **Range (ng/L)** | **Ref** |
| Neretva | 2007 | Sum 10 congeners (28, 52, 101, 105, 118, 138, 153, 156, 180, 209) | ND-0.12 | Djedjibegovic, J., et al., 2010 |
| Bosna | 2008/2009 | Sum 7 indicator congeners | 2008: 0.123-0.242<br>2009: 0.134-0.169 | Harman, C. et al., 2013 |
| Bosna<br>Neretva | 2010 | Sum 10 congeners (28, 52, 101, 105, 118, 138, 153, 156, 180, 209) | 0.06-0.294<br>0.024-0.195 | Harman, C., et al., 2018 |
| Bosna | 2012 | Sum 7 indicator congeners | 0.026-0.18 | Toušová, Z. et al., 2019 |

| **RIVER'S SEDIMENTS** | | | | |
|---|---|---|---|---|
| **Sampling area** | **Sampling year** | **Analytes** | **Range (ng/g)** | **Ref** |
| Bosna | 2008/2009 | Sum 7 indicator congeners | 0.78-79 | Harman, C. *et al.,* 2013 |

| | | | | |
|---|---|---|---|---|
| Una | 2003-2004 | Sum 7 indicator congeners | 0.35-361 (fish pond Klokot 2) | Čalić, V., Miošić, N., Kodba, C., *et al.*, 2005 |
| Usora and tributary Tešanjka | | | 17.5-90 | |
| Miljacka and Zujevina | | | <0.35-2691 | |
| Buna and Bunica | | | 0.35-4 | |
| Spreča and tributaries (Gostelja, Oskova, Jala) | | | 100-483 | |
| River Bosna tributaries (Krivaja, Lašva, Bioštica) | | | 12.06-24 | |
| Vrbas near Incel | 2020 | Sum 7 indicator congeners | <0.021-0.528 | UNDP/BiH, 2021 |

PCB concentrations in both river Neretva and river Bosna water samples (Table 3) were low, with slightly higher results for river Bosna. Downstream decrease of concentrations was recorded in the river Bosna, most probably due to the geographical location of the river source (vicinity of Sarajevo city). In contrast, on the river Neretva, PCB concentrations increased downstream, which can be attributed to the fact that most of the possible sources (cities Mostar and Čapljina, industrial facilities) are located in the lower river flow. (Harman et al., 2018)

Surface water was also analyzed after active sampling in Lake Modrac near Lukavac, Tuzla region (Marjanović, A., Djedjibegović, J., Omeragić, E., et al., 2021), showing Sum 7iPCBs in the range 3.23-6.19 mcg/L, and Bjelić et al. (2023), where PCBs were not detected. Except for Modrac lake, all other here reported concentrations were similar to the Europe-wide average (JRC, 2010). Sources of PCBs in Lake Modrac include industrial wastewater from nearby plants like coal mines, metal, wood, and plastic industries, as well as household wastewater from settlements lacking adequate sewage treatment.

In comparison to the Europe-wide average, PCB concentrations in sediment samples (Table 3) were similar. (JRC, 2010) The highest concentrations were found in the river Zujevina (2691 ng/g) and Spreča (483 ng/g), most probably due to the vicinity of old PCB-containing equipment, coal mines, waste waters, and damaged military relays. (Čalić et al., 2005)

PCBs in ambient air were analyzed in Tuzla and Sarajevo, 2003-2004 (Table 3). (Klánová, J., et al., 2007) These levels do not indicate higher pollution in comparison to the European average. (JRC, 2010) However, the highest concentrations were found in Tuzla near the fire station of the electrical power plant “Elektrodistribucija” due to leakage from old and damaged capacitors and transformers. (Klánová, J., et al., 2007)

In samples from Sarajevo, dominant congeners in the gaseous phase were PCB28, PCB153, and PCB138, while samples from Tuzla were dominated by PCB28, PCB52, and PCB101. In particulate matter, dominant congeners were PCB153, PCB138, and PCB180 at both locations.

PCBs were found in locally grown food, including free-range chicken eggs from Tuzla and Zenica, cow milk from the Sarajevo area, and fish samples from the Sana river and Bosnian rivers (Neretva tributaries, Una, Bosna, and its tributaries).

Sum 7iPCB in eggs ranged from ND-16.67 ng/g in fat (Petrlík, J., Behnisch, P., 2016), and didn't exceed the maximum residue level set by national regulatory (Regulation of BiH, 2014). However, the TEQ values for the sum of dioxin-like PCBs and dioxins exceed EU food standards, indicating a possible health risk. Recent studies have shown that dioxin and PCB levels in eggs from free-range chickens frequently exceed EU food standards of 2.5 pg TEQ/g fat for PCDD/Fs or 5 pg TEQ/g fat for the sum of PCDD/Fs and dl-PCBs when grazed on soil with PCDD/F or dl-PCB concentrations at levels around 2–4 ng TEQ/kg. (Weber, R., Bell, L., Watson, A. et al., 2019)

Sum 6PCB in cow milk was 10.87-187.72 ng/g in fat (Djedjibegović, J., Turalic, A., Ajdinovic, N., et al., 2019), being higher than the maximum residue level in some of the samples. (Regulation of BiH, 2014) PCB concentrations in fish from Sana River (Djedjibegovic, J., Marjanovic, A., Burnic, S., et al., 2015) and Bosnian rivers (Picer, M., Kovač, T., Picer, N., et al., 2005) expressed as Ar1254 were ND-208 mcg/kg w.w. and 2-73 mcg/kg w.w., respectively.

*Polycyclic aromatic hydrocarbons (PAHs) levels*

PAHs concentration in most samples from the Spreča valley was higher than the regulatory limit for agricultural soil. (Regulation of FBiH, 2022; Regulation of RS, 2016) The increase in concentrations recorded after 2014 was probably due to extreme flooding in this area (Table 4). In these samples, fluoranthene and pyrene derivatives were dominant, in some cases even critically high in comparison to the regulatory limits. (Regulation of FBiH, 2022; Regulation of RS, 2016). Benz(a)pyrene was detected in most samples, mainly in deeper layers. PAH concentrations in crops from this area were also high. (Federal Institute of Agropedology, 2014; 2015; 2016; 2017; 2018; 2019)

PAH concentrations in rivers were analyzed after passive sampling (Table 4). Surface water sampled in 2020 near powerplant Gacko did not contain detectable concentrations of PAHs. (Bjelić, S.L., Ilić, P., Nešković, M.D., et al., 2023) Most samples from Bosna river contained benz(a)pyrene in detectable concentrations.

Data for PAHs in sediment samples were found for two rivers, Bosna and Spreča. In Bosna river Sum 16 EPA PAHs was 0.2-48.973 mg/kg in 2008-2009 (Harman, C., et al., 2013). Risk assessment for 7 carcinogenic PAHs showed HI>1 for sediment from rivers Bosna and Spreča collected in 2018, Spreča beeing couple of times more polluted than Bosna. (Vijdea, A., Alexe, V., Balan, L., et al., 2022)

Benz(a)pyrene was detected in all air samples (table 4). Sum of eight carcinogenic PAHs ranged from 1.02-5.94 ng/m3 (1-2% benz(a)pyrene) in air samples from Sarajevo, and 8.44-22.37 ng/m3 (2-3% benz(a)pyrene) in samples from Tuzla.

The analyzed studies were not designed to determine a specific PAH source(s), and the available data are insufficient for such conclusions. However, known local possible sources of PAH include: traffic emission, industrial sources, household heating in all of the locations (Sarajevo, Tuzla, Zenica, Banja Luka), as well as mining and electricity production in Tuzla region.

Sum 16 PAH concentration in honey from Mostar region collected in 2019 ranged from ND-12.58 mcg/kg. In one sample from location by the road benz(a)pyrene was dominant PAH. In general, samples were safe for consumption. (Kazazic, M., Djapo-Lavic, M., Mehic, E., et al., 2020)

**Table 4.** Sum 16 EPA PAHs concentrations in soil, rivers, and air samples from different locations

| **SOIL** (*mg/L in percolates) | | | |
|---|---|---|---|
| **Sampling area** | **Sampling year** | **Range (mg/kg)*** | **Ref** |
| Sarajevo and Tuzla | 2003-2004 | 0.036-3.9 | Klánová, J., *et al*., 2007 |
| Sarajevo Canton | | | |
| Urban soil | 2009-2015 | 0.314-0.461 | Rešidović, N., Filipović, H., Mrković, A., *et al*., 2016 |
| Agricultural soil | | 0.119-0.216 | |
| Zenica agricultural soil | 2010 | ND-0.67<br>MRL<2 | Federal Institute of Agropedology, 2010 |
| Zenica agricultural soil | 2011 | ND | Federal Institute of Agropedology, 2016 |
| | 2012 | 0.84-2.62 | |
| | 2013 | ND | |
| | 2014 | 0.03-1.4 | |
| | 2015 | 0.1-0.7 | |
| | 2011-2015 average | 0.41-1.03 | |
| Soil percolate | 2013 | ND-0.237 | |
| | 2014 | 0.003-0.098 | |
| | 2015 | 0.001-0.041 | |
| | 2013-2015 average | 0.019-0.092 | |
| Spreča lower flow (agricultural soil) | | | Federal Institute of Agropedology, 2014; 2015; 2016; 2017; 2018; 2019 |
| Lukavac | 2014 | ND-0.14 | |
| | 2015 | 0,06-37.45 | |
| | 2016 | 0.49-102.64 | |
| | 2017 | 0.159-9.773 | |
| | 2018 | 1.196-13.301 | |
| Gračanica | 2014 | 0.03-4.42 | |
| | 2015 | ND-9.72 | |
| | 2016 | 0.30-52.12 | |
| | 2017 | 0.333-11.734 | |
| | 2018 | 0.348-21.116 | |
| Doboj Istok | 2014 | 0.01-0.16 | |
| | 2015 | 0.04-3.19 | |
| | 2016 | 0.76-8.07 | |
| | 2017 | 0.272-1.136 | |
| | 2018 | 0.454-0.757 | |

| | | | | |
|---|---|---|---|---|
| Olovo (agricultural soil and road side soil) | 2020 | | 0.24-0.73 | Federal Institute of Agropedology, 2021 |
| Sarajevo (public children's playgrounds) | 2016 | | 0.184-7.983 | Sapcanin, A, Cakal, M, Jacimovic, Z, *et al.*, 2017) |
| Banja Luka (Incel factory) | 2019 | | 0.13-2.848 | Ilić, P., Markic, D., Stojanović, Lj. 2020; Stojanović Bjelić, L., Nešković Markić, D., Ilić, P., Farooqi, Z. U. R., 2022) |
| **RIVERS (concentrations obtained by passive sampling)** | | | | |
| **Sampling area** | **Sampling year** | | **Range (ng/L)** | **Ref** |
| Neretva | 2007 | | 0.16-4.0 | Djedjibegovic, J., *et al.,* 2010 |
| Bosna | 2008/2009 | | 2008: 17.456-480.444<br>2009: 19.571-98.525 | Harman, C. *et al.,* 2013 |
| Bosna<br>Neretva | 2010 | | 6.527-67.558<br>0.447-6.956 | Harman, C. *et al.*, 2018 |
| Bosna | 2012 | | 1.2-57 | Toušová, Z. *et al.*, 2019 |
| **AIR SAMPLES (ng/m$^3$)** | | | | |
| **Sampling area** | **Sampling year** | **Analytes** | **Concentration (ng/m$^3$)** | **Ref** |
| Lukavac<br>Zavidovići | 2024 | BaP | 16.29-22.50 (PM10)<br>0.16-1.14 (PM10) | OSCE & Ekoforum Zenica, 2024 |
| Sarajevo<br>Tuzla | 2004 | Sum 16 EPA PAHs | 15.15-50.44<br>58.17-121.24 | Skarek, M., Cupr, P., Bartos, T., *et al.*, 2007 |
| Sarajevo | 2017-2018 | Sum 10 PAHs<br>BaP | 64.8 (PM10)<br>7.28 (11% total PAH) | Pehnec, G., Jakovljević, I., Godec, R., *et al.*, 2020 |
| Banja Luka | 2008 | Sum 28 PAHs | 14.5-40.8 | Lammel, G., Klanova, J., Ilić, P., *et al.*, 2010 |

## CONCLUSION

This paper presents data on selected POPs in environmental samples from Bosnia and Herzegovina summarized by thorough literature review. The main conclusions are:

- Although Bosnia and Herzegovina ratified the Stockholm convention in 2010, regular monitoring and other obligations in this respect are inadequate. Most of the available data are from individual studies conducted by research groups and institutions, while official monitoring is insufficient. Thus, spatial or temporal trends cannot be assessed.
- OCP levels in surface water samples are mostly in accordance with the European background levels. Concentrations in agricultural soil samples were within the regulatory limits. Heptachlor concentrations in samples from most sites on Bosna river were higher than the action levels and it is of environmental concern.
- DDT (and PCB/dioxins) levels in free-range chicken eggs were high indicating possible health risk.
- PCB concentration in agricultural soils was acceptable, and higher concentrations are recorded in industrial zones. Concentrations in air, surface water and sediment were similar to the European average. The exception is sediment from Lake Modrac, which can be marked as a local „hot spot“.
- PAH concentrations were critical in agricultural soil in Spreča valley. Benz(a) pyrene was detected in river water, and concentrations in sediments from Bosna and Spreča rivers indicated ecological risk.

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## Summary/Sažetak

Postojani organski polutanti (POP) su supstance prepoznate kao značajne opasnosti za zdravlje ljudi i okoliša pa je njihova upotreba i emisija zabranjena ili ograničena odredbama Štokholmske konvencije. Unatoč kontrolnim mjerama predviđenim međunarodnim ugovorima, prisustvo POP u okolišu i dalje je evidentno. Bosna i Hercegovina je članica Štokholmske konvencije, ali redovan integrativan monitoring prisustva POP u okolišu nije uspostavljen. Podaci o koncentraciji POP publicirani od različitih istraživačkih grupa ili institucija su rasuti u dostupnoj literaturi što otežava generalnu procjenu stanja. Cilj ovog preglednog rada je da sistematizira dostupne podatke o prisustvu i nivoima odabranih POP u biološkim i uzorcima iz okoliša u Bosni i Hercegovini, što može biti od koristi u planiranju budućih aktivnosti praćenja, procjene efekata implementiranih kontrolnih mjera i donošenju regulatornih odluka. Glavni rezultati ukazuju na prisutnost organohloriranih pesticida (OCP), polihloriranih bifenila (PCB) i policikličkih aromatskih ugljikovodika (PAH) u većini uzoraka (tlo, zrak, voda, sediment, određena hrana). Potencijalni ekološki ili zdravstveni rizici ne mogu se isključiti u odnosu na heptahlor (sliv rijeke Bosne), dihlorodifeniltrihloroetan (DDT) (u jajima kokoši iz slobodnog uzgoja), PCB (u jajima kokoši iz slobodnog uzgoja i u sedimentima Modračkog jezera) te PAH (u poljoprivrednom tlu u dolini Spreče i u sedimentima rijeka Bosne i Spreče).

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# The Impact of Selected School Experiments on Pupils' Attitudes Toward Chemistry

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**Abstract:** School experiments, as a teaching method, represent one of the visual ways of explaining relevant natural phenomena to pupils. The application of experiments in chemistry teaching is often interpreted as an excellent way to achieve good learning outcomes. The impact of conducted school experiments on pupils was examined using psychological instruments, administered before and after performing the selected school experiments in front of the pupils. Feedback from 143 respondents, pupils from primary and secondary schools in Bosnia and Herzegovina, was collected. Adequate statistical analysis was performed. Two key measures were identified: Affinity towards Chemistry and positive experiment experience. A slight but significant increase in the affinity scores was noted after demonstrating school experiments, showing a mildly positive impact on students' attitudes toward chemistry. Positive Experiment Experience scores were high, indicating a favorable perception of the demonstrations. The data suggest that students' initial affinity towards chemistry is the best predictor of their attitudes after the experiment, suggesting that pre-existing attitudes are key. No evidence was found for a "magic" intervention that significantly changes students' affinity. These findings highlight the importance of continuous engagement over isolated demonstrations, which alone cannot replace the impact of ongoing work with students.

## INTRODUCTION

In recent years, chemistry education in Bosnia and Herzegovina has encountered a range of systemic challenges, including outdated curricula, inadequate laboratory infrastructure, and limited opportunities for professional development among teachers. Compared to neighboring countries such as Croatia, Serbia, and Slovenia, BiH has made slower progress in adopting modern, inquiry-based approaches and integrating digital tools into science and chemistry teaching (Agency for Pre-primary, Primary and Secondary Education of BiH, 2019). Chemistry teaching often presents educators with certain challenges that require innovative approaches compared to traditional teaching methods (Hajrić et al., 2012). Regional studies indicate that both elementary and high school pupils often perceive themselves as least efficient in mastering chemistry (Brković et al. 1998). Experts often emphasize the importance of practical experiments in chemistry education and their potential to enhance pupils' engagement with the subject (Hofstein & Lunetta, 2004).

Despite the recognized benefits of school experiments, research on how selected school experiments affect pupils' affinities toward chemistry remains limited in Bosnia and Herzegovina. The lack of relevant studies makes effective lesson planning for chemistry teachers challenging and disrupts a comprehensive understanding of educational phenomena in the region. This study aims to address how selected school experiments affect pupils' affinities toward chemistry. We seek to investigate how these experiments influence pupils' overall attitudes and affinity towards chemistry, contributing to both educational practice and theory in Bosnia and Herzegovina. The primary goal of research is to measure the impact of selected school experiments on pupils' attitudes towards chemistry. More specifically, the general aim of the research is to investigate the extent to which the selected school experiments influence changes in pupils' overall attitudes and affinities towards chemistry.

## MATERIALS AND METHODS

Adequate school experiments were selected for demonstration based on the specific criteria. The selected school experiments needed to be short, cost-effective, and safe for both pupils and the experimenter. They also had to demonstrate a chemical change in a way that the audience could easily observe and understand. Additionally, experiments should effectively demonstrate chemical changes, emphasizing visual indicators such as color changes or other relevant stimuli. The following school experiments were chosen for research: Red Cabbage Anthocyanin Extract as a pH Indicator, Neutralization of Hydrochloric Acid Using Antacid and The Effect of Reactant Concentration and the Presence of a Catalyst on the Rate of a Chemical Reaction.

Prior to the demonstration, the substances used in the first experiment were prepared: Anthocyanin Extract (solution prepared by soaking shredded red cabbage in warm water at 40°C, concentration unknown, 100 *mL* prepared), Hydrochloric Acid ($c$=1 *mol/dm*$^3$, $V$=20 *mL*), Acetic Acid (aqueous solution, $c$=1 *mol/dm*$^3$, $V$=20 *mL*), Sodium Bicarbonate (aqueous diluted solution, $V$=20 *mL*), Sodium Hypochlorite (aqueous diluted solution, $V$=20 *mL*), Sodium Hydroxide (aqueous diluted solution, V=20 *mL*), and Distilled Water. For the second experiment, the substances used were: Anthocyanin Extract (solution prepared as described above, 100 *mL* prepared), Hydrochloric Acid ($c$=0.1 *mol/dm*$^3$), and an antacid of choice (granules based on a basic substance). In the third experiment, the substances used were: Hydrochloric Acid (two solutions, $c$=0.1 *mol/dm*$^3$ and $c$=1 *mol/dm*$^3$), Calcium Carbonate, Manganese IV Oxide, and Hydrogen Peroxide. Standard Laboratory Equipment was used for preparing these school experiments. Preparation of experiments is done in a previously established order: First, preparation of required solutions is conducted using a standard laboratory procedure. Following that, laboratory glassware is strategically positioned with prepared solutions, equipment, and schemes related to color-pH changes. After the preparation, participants were allowed to enter the laboratory where school experiments would be demonstrated. After the preparation, participants were allowed to enter the laboratory where school experiments were demonstrated. Psychological measuring instruments were chosen and designed to evaluate the possible impact of selected school experiments on pupils' attitudes toward chemistry. The appropriate instruments were selected. CAEQ questionnaire on attitudes and experiences with chemistry (Coll et al., 2002), an adapted translation from Cvjetković's (2019) research, was picked for the research. The selected instrument contains items on a seven-point scale, where the endpoints are defined as "1 = harmful to people" and "7 = beneficial to people", with intermediate values (2 to 6) representing varying degrees between these two extremes. The following instruments were designed: A constructed scale was used with items rated on a five-point Likert scale, where response options ranged from 1 = not true, 2 = somewhat true, 3 = moderately true, 4 = mostly true, to 5 = completely true., designed for measurement affinities and attitudes towards chemistry before and after the demonstration of selected school experiments. Also, evaluation sheets are designed to measure the experience of experiments after each of the three demonstration chemical reactions. Each of the three evaluation sheets contains three five-point Likert scale items, with responses ranging from "1=not true" to "5=completely true". The purpose of the selected instruments was to verify the reliability and validity of the designed instruments. Research is done in the predetermined order.

After the preparation, participants were introduced to the laboratory. Distribution of sheets with psychological instruments to participants was conducted. After that, clear instructions were given to participants regarding questionnaire filling. Participants were instructed to fill out the first questionnaire related to pupils' attitudes towards chemistry before the demonstration of selected school experiments. After that, the experimenter started the demonstration of the first selected school experiment, Red Cabbage Anthocyanin Extract as a pH Indicator.

During the first experiment, pupils were shown how Anthocyanin Extract changes color based on the pH of the tested substance. After the experiment demonstration, participants were asked to fill out the second questionnaire related to measuring the experience of the first selected school experiment. For the next two experiments, the procedure is similar and includes the demonstration of selected school experiments and filling out the questionnaire related to measuring the experience of the specifically selected school experiment.

During the second experiment, pupils were shown how the color of the indicator, in our case, Anthocyanin Extract, changes during the neutralization process.

In the third experiment, pupils were shown how a different concentration of hydrochloric acid affects the rate of the chemical reaction in a way that is easily visible to participants. This is achieved by positioning the rubber balloons on the Erlenmeyer flasks where the reactions are taking place, at the same time. Pupils noticed a difference in the level of inflation of the rubber balloon since more carbon dioxide will be produced in the reaction with a higher concentration of the reactant, hydrochloric acid. It is also shown how manganese(IV)-oxide affects the rate of the hydrogen-peroxide composition: the reaction is vigorous. After the demonstration of all three experiments, participants were instructed to fill out the last questionnaire related to measuring their attitudes towards chemistry after the demonstration of selected school experiments. In the end, the experimenter thanked the pupils for participating and gave them appropriate gifts. After the research procedure, filled instruments were properly labeled, statistically processed, and stored. Several methods and analyses were used in the research:

### Exploratory Factor Analysis (EFA)

Used to explore the latent structure of attitudes towards chemistry and the intensity of experiment experiences.

### Parallel Analysis

Utilized to determine the number of factors to retain based on eigenvalues extracted from actual data compared to random data.

**Confirmatory Factor Analysis (CFA)**
Employed to confirm the factor structure identified through EFA.

**Bivariate Correlation Analysis**
Conducted to assess relationships between variables such as Affinity towards Chemistry and experiment experiences.

**Linear Regression Analysis**
Used to predict Affinity towards Chemistry scores after experiments using predictors like Positive Experiences of Experiments.

**Moderation Analysis**
Applied to explore if certain variables, such as school level (elementary vs. high school), moderated the relationships between experiment experiences and Affinity towards Chemistry.

**Mediation Analysis**
Examined to understand the indirect effects of experiment experiences on Affinity towards Chemistry through potential mediators. These methods collectively provided insights into the structure of attitudes towards chemistry, the impact of experiment experiences, and how these relationships varied across different groups of students.

## RESULTS AND DISSCUSION

Feedback from 143 participants, pupils from primary and secondary schools in Bosnia and Herzegovina, was collected. The sample included 143 students from primary (N=74, or 52%) and secondary (N=69, or 48%) schools, of which 66% (N=95) were girls and 34% (N=48) were boys. A prerequisite for the main analyses necessary to achieve the research goals was the determination of the dimensionality and the justification of the scoring method for the dependent variable, in this case, the students' attitudes and affinities towards chemistry. Nine items measuring attitudes towards chemistry were subjected to exploratory factor analysis (EFA) (JASP Team, 2024), separately for the measurements before and after the experiments were conducted. The factor analysis was based on a matrix of polychoric correlations and the minimum rank factor analysis (MRFA) extraction method. The parallel analysis procedure, which is recommended for selecting the optimal number of factors to retain and is based on comparing factor eigenvalues from the actual correlation matrix with simulated values (Subotić, 2013), suggested retaining one latent factor in both cases (Table 1). This factor had a functionally identical vector structure (congruence coefficient of φ≈1.00; Lorenzo-Seva & Ten Berge, 2006), and was named Affinity towards Chemistry. In both cases, the factor explained over 50% of the common variance of the items (with two-thirds of factor loadings exceeding .71, which is conventionally considered the threshold for excellent loadings; Tabachnick & Fidell, 2013, p. 702) and had relatively high reliability (internal consistency). These findings justify the use of a single general score for students' attitudes and affinity towards chemistry and allow for a direct comparison of this measure before and after the experiments.

The latent structure and convergent validity of the measure of attitudes toward chemistry were examined. The summative scores of the Affinity towards the Chemistry dimension indicate an average expression slightly above the theoretical scale mean of 3.0, both for the measurement before (t(142)=5.41, p<.001, d=0.45) and after (t(142)=6.24, p<.001, d=0.52) the experiments demonstration in front of the pupils.

**Table 1**: Exploratory factor analysis of attitudes towards chemistry

| No | Items | Factor Saturations | |
|---|---|---|---|
| | | $\Lambda_{before}$ | $\Lambda_{after}$ |
| 7. | I like chemistry. | .91 | .94 |
| 2. | I would gladly volunteer to participate in chemistry lab experiments. | .84 | .86 |
| 3. | I enjoy lab work. | .80 | .82 |
| 1. | I would be happy to study chemistry. | .80 | .83 |
| 6. | I want to conduct chemistry experiments in the future. | .78 | .80 |
| 4. | Attending lab experiments motivates me to independently search for more information about chemistry online. | .71 | .78 |
| 5. | Chemistry is useful in everyday life. | .53 | .61 |
| 9. | Learning chemistry is difficult. (R) | .52 | .58 |
| 8. | I will need chemistry for further education. | .51 | .57 |
| | Characteristic Root | 4.97 | 5.46 |
| | % of Explained Common Variance | 50.7 | 56.6 |
| | McDonald's ω (reliability) | .87 | .90 |
| | *M (summative scores)* | 3.40 | 3.49 |
| | *SD (summative scores)* | 0.88 | 0.94 |

For convergent validation of the measures used, i.e., verifying their correlation with other similar measures, the summative scores of the Affinity towards Chemistry dimension (before and after the experiments) were correlated with the total summative score of selected subscales from the CAEQ questionnaire of attitudes and experiences with chemistry (Coll et al., 2002). The correlations are shown in Table 2. Firstly, it can be observed that the correlations between the scores of Affinity towards Chemistry before and after the experiments are very high. Squaring the obtained correlation coefficient indicates that initial and outcome attitudes toward chemistry share approximately 83% of the variability. In the context of the convergent validity of these scores, it can be noted that they are related to CAEQ scores to a relatively high extent of around .6, sharing 32% and 40% of variability with them, respectively. This, combined with previously demonstrated clear unidimensional factor structure and a good level of reliability, supports the justification for using the Affinity towards Chemistry scores to test the main hypotheses of the study.

**Table 2.** Correlations of Affinity towards Chemistry Dimension with CAEQ Score

| Variables | (1) | (2) | (3) |
|---|---|---|---|
| (1) CAEQ | 1.00 | | |
| (2) Affinity for Chemistry – before experiments | .57 | 1.00 | |
| (3) Affinity for Chemistry – after experiments | .63 | .91 | 1.00 |

*Note: All correlations are statistically significant at p<.001.*

Additionally, the latent structure and intensity of experiences during experiments were examined. Exploratory factor analysis was conducted on three items related to the experience of each experiment, separately for each of the three presented demonstration experiments. The technical specifications of the procedure were identical to those used for analyzing Affinity towards Chemistry. In the case of experiences during all three experiments, it was justified to aggregate all three items into a single dimension (Table 3), with adequate reliability and explaining approximately 50-60% of the common variance of the items. The factor exhibited functional equivalence, with congruence coefficients of $\varphi \geq .98$. This dimension was labeled as Positive Experience of Experimentation, primarily determining interest, followed by comprehensibility, and to a lesser extent, the perceived usefulness of the experiment.

The summative scores for experiences across all three experiments were nearly identical and indicated a relatively high level of positivity in the experiences. The experiences from all three experiments were relatively highly correlated: rE1~E2=.61, rE1~E3=.58, rE2~E3=.52 (all p<.001).

**Table 3.** Factor Structures of Experiences in Three Experiments

| No. | Items | Factor Saturations | | |
|---|---|---|---|---|
| | | $\Lambda_{E1}$ | $\Lambda_{E2}$ | $\Lambda_{E3}$ |
| 3 | This experiment is interesting to me. | >.99 | >.99 | .96 |
| 1 | I can understand this experiment. | .67 | .68 | .77 |
| 2 | I learned something useful from this experiment. | .35 | .58 | .60 |
| | Characteristic Root | 1.76 | 2.01 | 2.19 |
| | % of Explained Common Variance | 49.9 | 57.5 | 62.5 |
| | McDonald's ω (reliability) | .79 | .80 | .78 |
| | *M (summative scores)* | 4.33 | 4.33 | 4.32 |
| | *SD (summative scores)* | 0.73 | 0.76 | 0.81 |

Experiences across all three experiments show low to moderate bivariate correlations with Affinity towards Chemistry scores, ranging from .28 to .40 (Table 4). Positive Experience in Experiment 3 is the most strongly associated with Affinity towards Chemistry—both before and after the experiments.

**Table 4**. Bivariate Correlation of Experiment Experiences with Affinity towards Chemistry

| Variables | Affinity for Chemistry before experiment | Affinity for Chemistry after experiment |
|---|---|---|
| Positive Experience E1 | .28 | .29 |
| Positive Experience E2 | .30 | .35 |
| Positive Experience E3 | .40 | .39 |

*Note: All correlations are statistically significant at p<.001.*

Additional insight into the level of experience in each experiment was gained through responses to open-ended questions, from which appropriate categories were coded. The frequency of these categories is shown in Table 5. These categories indicate a more pronounced differentiation in the experiences of the experiments compared to the summative scores of assessments. Specifically, Experiment 2 is characterized as somewhat more useful in daily life, with respondents noting that they learned more new things compared to the other two experiments. Experiment 3 is described as more enjoyable/interesting than the other two.

**Table 5**. Frequency of Categories Based on Open-Ended Responses

| Category | Experiment | Frequency of Response | |
|---|---|---|---|
| | | Frequency | % |
| Something new was learned. | E1 | 17 | 12 |
| | E2 | 27 | 19 |
| | E3 | 18 | 13 |
| Nothing particularly new was learned. | E1 | 9 | 06 |
| | E2 | 8 | 06 |
| | E3 | 4 | 03 |
| The experiment was interesting, fun, or enjoyable. | E1 | 53 | 37 |
| | E2 | 53 | 37 |
| | E3 | 75 | 52 |
| The experiment was not interesting, fun, or enjoyable. | E1 | 15 | 10 |
| | E2 | 15 | 10 |
| | E3 | 13 | 09 |
| The knowledge demonstrated by the experiment is useful for everyday life. | E1 | 3 | 02 |
| | E2 | 32 | 22 |
| | E3 | 4 | 03 |

Regarding changes in attitudes towards chemistry after the experiments, the Affinity towards Chemistry scores were statistically significantly higher after conducting the three experiments (M=3.49, SD=0.94), compared to the initial scores before the experiments (M=3.40, SD=0.88): F(1, 142)=7.67, p=.006, $\eta^2$=.051. The magnitude of change was very low, with scores increasing by $\Delta M$=0.09, corresponding to a change of just over a quarter of a standard deviation (d=0.23), or approximately 5% of the score variance attributable to the experiments. At the level of individual items, the most notable effects were observed in the increase of average scores for the statements: "I want to conduct chemical experiments in the future." (M_after=3.47 > M_before=3.20; t(142)=-3.40, p<.001, d=-0.28) and: "I would be glad to study chemistry." (M_after=2.67 > M_before=2.43; t(142)=-3.09, p=.002, d=-0.26). When considering the participants' school levels (primary vs. secondary), there was a significant interaction with changes in Affinity towards Chemistry scores: F(1, 141)=4.61, p=.033, $\eta^2$=.002, suggesting a larger increase in scores among primary school students (ΔM=0.16) compared to secondary school students, where the increase was functionally marginal (ΔM=0.02). However, introducing this interaction of changes in Affinity towards Chemistry scores in relation to school level and exposure to experiments explains only about 0.2% of the variance in scores across the total sample, while the main effect of experiment exposure additionally explains another 0.2% of the variance (F(1, 141)=7.44, p=.007, $\eta^2$=.002). When analyses are purposefully conducted separately on subsamples of primary and secondary school students, a significant effect is found in the primary school subgroup: F(1, 73)=10.33, p=.002, $\eta^2$=.124, suggesting an increase in Affinity towards Chemistry scores, with a change size of approximately d=0.37 standard deviations, or about 12.4% of explained variance. The effect on the secondary school subgroup is not significant, suggesting functionally identical Affinity towards Chemistry scores before and after the experiments: F(1, 68)=0.21, p=.652, $\eta^2$=.003, indicating a change in scores of only d=0.05 standard deviations, or just 0.3% of explained variance attributable to the presentation of experiments to students. From the obtained low but significant acute changes towards increased Affinity towards Chemistry, attributed to the presented experiments, it is not possible to determine which individual experiment is responsible or to what extent each is responsible for the score changes. This can only be indirectly tested by examining how the introduction of experiences from each experiment affects the outcome scores of Affinity towards Chemistry. Including these individual experiment experiences in the model shows that, in competition with the other two experiments, only the Positive Experience of Experiment 3 (E3) achieves a significant positive contribution to Affinity towards Chemistry scores, explaining about 2.4% of the variance: F(1, 139)=10.67, p=.001, $\eta^2$=.024. The effects (statistically non-significant) of experiences from the other two experiments are F(1, 139)<0.001, p=.986, $\eta^2$<.001 for E1 and: F(1, 139)=2.77, p=.098, $\eta^2$=.006 for E2. The effect of E3 experience persists even when including the school level of participants in the model, reducing the percentage of explained variance from 2.4% to 1.9%: F(1, 138)=10.68, p=.001, $\eta^2$=.019. Due to the observed trend of differences in changes in Affinity towards Chemistry scores depending on whether the participant is in primary or secondary school, as well as the established primary relevance of Experiment 3 experiences in explaining changes in affinities, additional regression-moderation-mediation analyses were conducted to gain deeper insight into the obtained statistical trends.

Firstly, it was checked whether the bivariate correlation between Affinity towards Chemistry scores before and after the experiments (r=.91, p<.001) significantly differed among primary (r=.89, p<.001) versus secondary school students (r=.92, p<.001). The difference between these two correlation coefficients was not statistically significant: Δr=-.03, Δp=.375. Additionally, this relationship did not significantly differ based on the Positivity of the experience of any of the three experiments, regardless of whether the participants were primary or secondary school students. Thus, the experiences of the experiments themselves and the interactions between the experiences of the experiments and the school level of the participants did not statistically moderate this relationship. Next, it was examined to what extent the Positive Experience of each of the three experiments uniquely predicted Affinity towards Chemistry scores after conducting the experiments. This was done using linear regression analysis, conducted without (Table 6, Step 1) and with control for initial Affinity towards Chemistry scores (Table 6, Step 2).

**Table 6**. Positive Experiences of Experiments as Predictors of Affinity towards Chemistry after experiments.

| Steps | Predictors | Criterion: Affinity for Chemistry after experiments | |
|---|---|---|---|
| | | β | *p* |
| Step 1: $R_1^2$=.161 | Positive Experience E1 | -.002 | .989 |
| | Positive Experience E2 | .20 | .046 |
| | Positive Experience E3 | .28 | .004 |
| Step 2: $R_2^2$=.826 ($\Delta R^2$=.647, Δ*p*<.001) | Positive Experience E1 | -.01 | .897 |
| | Positive Experience E2 | .09 | .044 |
| | Positive Experience E3 | -.02 | .725 |
| | Affinity for Chemistry before experiment | .89 | <.001 |

In the first step, when only Positive Experiences of experiments are considered as predictors, it is possible to explain 16.1% of the variance in Affinity towards Chemistry after Experiment, where Experiments 3 and 2 make statistically significant contributions, without a relative contribution from Experiment 1. None of these correlations were statistically moderated by the school level of the participants, meaning that regression correlations did not significantly differ based on whether the participant was in primary or secondary school (E1: Δβ=.02, Δp=.974; E2: Δβ=.33, Δp=.113; E1: Δβ=.09, Δp=.674). However, although the difference between the heights of these regression correlations was not statistically significant, the relationship between the Positive Experience of Experiment 2 and Affinity towards Chemistry after Experiment was slightly higher and statistically significant only in the case of primary school students: βE2; Primary=.37, p=.002, compared to the coefficient obtained for the secondary school subgroup, which was not statistically significant: βE2; Secondary=.04, p=.829. In the second step, when the initial level of Affinity towards Chemistry is included in addition to the experiences of the three experiments, the prediction of Affinity towards Chemistry after experiments increases to as much as 82.6% (with a statistically significant increase in prediction compared to the first step of 64.7%), while the partial contributions of Experiments 1 and 3 disappear, and only a minor relative contribution of the Positivity of Experiment 2 remains statistically significant. This suggests that the best predictor of Affinity towards Chemistry after experiments is the intensity with which Affinity entered, and that, beyond this, only to a lesser extent, how positively they experienced E2 additionally predicts an increase in initial Affinity scores towards chemistry. None of these regression correlations was statistically moderated by the school level of students.

The change in relative contributions of experiment experiences in predicting Affinity towards Chemistry after experiments was also considered in relation to categories of experiment experiences obtained from open-ended responses. In no case was any statistically significant moderation effect obtained (for categories where the prerequisites for these analyses were met).

## CONCLUSION

The results of the analysis of responses from 143 elementary and high school students in Bosnia and Herzegovina have been presented. Two distinct measures were identified: Affinity towards Chemistry and Positive Experiment Experience. A slight but statistically significant increase in Affinity toward Chemistry scores was observed after demonstrating school experiments to students, indicating that conducting selected demonstration experiments has a mildly positive impact on students' attitudes (affinity) towards chemistry. Scores for Positive Experiment Experience were relatively high, suggesting a positive perception of the experiment demonstrations themselves. The effect of increasing Affinity toward Chemistry scores was primarily localized to elementary school students, with no statistical significance observed among high school students. It was inconclusive which experiment had the greatest impact on the slight increase in Affinity towards Chemistry scores, although there are indications that the third experiment, involving the effect of solution concentration and catalyst presence on the speed of chemical reactions, had the most significant influence. Additionally, the second experiment, neutralizing hydrochloric acid using antacids, showed a significant indication of increasing Affinity towards Chemistry. The first experiment could potentially be omitted from the demonstration without a significant impact on students' affinity toward chemistry. Experiment experiences approximate the direct effect of experiments. The data suggest that the best predictor of Affinity towards Chemistry after the experiment is the intensity of affinity with which students entered, indicating that students' pre-existing attitudes best predict their attitudes after viewing selected school experiments. The existence of a "magic" short intervention or shortcut that fundamentally changes students' affinity towards chemistry was not confirmed. The above findings underscore the importance of continuity in teachers' work. It is noted that isolated demonstrations do not have as intense an effect as continuity in working with students. It is concluded that demonstrating school experiments as an isolated component cannot substitute for continuity in working with students. Given the nature and complexity of chemistry as a science, demonstration experiments represent an indispensable teaching method in modern education. The research has shown a mild positive impact of selected school experiments on students' affinity toward chemistry. The degree of confirmation of the hypothesis is low. Certain indications and dilemmas have emerged, which can serve as topics for future research aimed at improving educational processes and chemistry teaching. The method of demonstrating and presenting school experiments alone is not powerful enough to significantly influence students' affinity towards chemistry. Isolated demonstrations of school experiments to students cannot replace continuous teacher-student interaction.

To increase students’ interest in chemistry, demonstration experiments should be incorporated as part of a sustained and comprehensive teaching approach rather than isolated activities. Future efforts should emphasize continuous teacher-student interaction, the selection of engaging and relevant experiments, the development of supportive instructional materials, and the provision of professional training for teachers to deliver effective and stimulating demonstrations. Additionally, creating a positive classroom atmosphere where students feel encouraged to participate is crucial for maintaining and enhancing students’ enthusiasm for chemistry.

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## Summary/Sažetak

Školski eksperimenti, kao nastavna metoda, predstavljaju jedan od vizuelnih načina objašnjavanja relevantnih prirodnih pojava učenicima. Primjena eksperimenata u nastavi hemije često se tumači kao izuzetno efikasan način za postizanje dobrih ishoda učenja. Uticaj izvođenja školskih eksperimenata na učenike ispitan je primjenom psiholoških instrumenata, koji su primijenjeni prije i nakon izvođenja odabranih školskih eksperimenata pred učenicima. Prikupljeni su odgovori 143 ispitanika – učenika osnovnih i srednjih škola u Bosni i Hercegovini. Izvršena je odgovarajuća statistička analiza. Identifikovane su dvije ključne mjere: afinitet prema hemiji i pozitivno iskustvo s eksperimentima. Zabilježen je blag, ali statistički značajan porast rezultata afiniteta prema hemiji nakon demonstracije školskih eksperimenata, što ukazuje na umjereno pozitivan uticaj na stavove učenika prema hemiji. Rezultati pozitivnog iskustva s eksperimentima bili su visoki, što ukazuje na pozitivnu percepciju demonstracija. Podaci sugerišu da je početni afinitet učenika prema hemiji najbolji prediktor njihovih stavova nakon izvođenja eksperimenata, što ukazuje na to da su prethodno formirani stavovi od ključnog značaja. Nisu pronađeni dokazi o postojanju „magične“ intervencije koja bi značajno promijenila sklonost učenika prema hemiji. Ovi rezultati naglašavaju značaj kontinuiranog angažmana u radu s učenicima, jer pojedinačne demonstracije same po sebi ne mogu zamijeniti efekat dugotrajnog i sistematskog rada s učenicima.

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# Phenolic Profile and Antioxidant Activity of Selected Premium Wines from Herzegovina Region

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**Abstract:** Red wines are rich in antioxidants, which have anti-inflammatory activity and beneficial effect on human health. The aim of this research was to determine the content of bioactive compounds, i.e. total phenols, anthocyanins and tannins, as well as antioxidant activity in red wines from the Herzegovina region. Four wines from two different harvest years, i.e. 2019 and 2021, were analysed. Phenolic compounds are important wine quality parameters that affect color, taste and aroma. The content of total phenols varied from 1733.87 ± 10.22 to 2382.09 ± 21.42 mg GAE/L. The content of total anthocyanins, which directly affect the color of red wines, was determined by the pH differential method and the results obtained ranged from 13.64 ± 0.19 to 21.76 ± 0.35 mg CGE/L. Total tannins were determined using spectrometric method, and values ranged from 9.00 ± 0.09 to 11.42 ± 0.21 g/L. The antioxidant activity was assessed using two different methods, FRAP and ABTS. The total phenolic content of the tested wines was positively correlated with their antioxidant activity. The content of total phenols, anthocyanins, tannins and antioxidant activity were influenced by the year of harvest as well as the aging methods used.

## INTRODUCTION

The red wines of Herzegovina occupy a special place in the oenological tradition of Bosnia and Herzegovina, recognized for their specificity, richness of taste and unique character. This region, which stretches through the southern part of Bosnia and Herzegovina, is characterized by a Mediterranean climate and rich limestone soil, which is a key factor in the cultivation of vines (Kojić, Sefo and Delić, 2013). Blatina is an autochthonous Herzegovinian grape variety. The Blatina variety is special because it contains a functional female flower, therefore it is planted together with Trnjak or Merlot. In addition to enological and cultural aspects, red wines are also known for their positive effects on human health when consumed in small doses, reducing the risk of cancer, inflammatory and cardiovascular diseases (Hrelia, Di Renzo, Bavaresco et al., 2023). Numerous studies have shown that grapes and red wines are rich in bioactive compounds, and contain high levels of phenolic and antioxidant compounds, anthocyanins and tannins (Tutino, Gigante, Milella et al., 2020; Sabra, Netticadan, and Wijekoon, 2021). Polyphenols pass from grapes into must and wine, influencing organoleptic properties, ripening process, aging, wine stability , and nutritional efficacy (Zoričić, 1998). Anthocyanins, the most abundant flavonoids in grape berries, also exhibit antioxidant properties. Research shows that the antioxidant activity of berries is directly proportional to the concentration of anthocyanins in them (Heinone, Meyer and Frankel, 1998). Tannins in wine act as a natural preservative, allowing the product to age gracefully. During the aging process, tannins disperse, enhancing the wines complexity by contributing to its fullness of flavour, bitterness and astringency (Zhang, Wei, Han et al., 2023). The aim of this study was to determine the content of bioactive compounds and antioxidant activity in red wines of the Blatina variety from the Herzegovina region, produced from different harvest years (2019 and 2021) and aged using different methods.

## EXPERIMENTAL

### *Chemicals*

All solutions were prepared using analytical-reagent grade substances and distilled water.

Gallic acid, Folin-Ciocalteu reagent, sodium acetat-3-hydrate were purchased from Semikem (BiH). Compound 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) and 2,2’-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma-Aldrich (Germany). Potassium peroxysulfate and potassium chloride were purchased from Lach Ner (Czech Republic) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) from Acros Organics (USA). All other chemicals were purchased from Kefo d.o.o. (BiH).

*Wine Samples*

Four different red wines were selected for this study (Table 1). The wines were produced from grapes harvested in two different years (2019 and 2021). Two wines were stored in stainless steel tanks, while the other two wines aged for three years in barrique barrels. All four wines are from the winery Carski Vinogradi Mostar with a controlled geographic origin, commercially available and widely consumed. The winery maintains a temperature between 20°C and 22°C, with a relative humidity ranging from 65% to 75%, while the barrels, which are not new, have a medium toastiness. The wines were stored at room temperature in a dark environment until analysis. All wines were diluted with distilled water at a ratio 1:10. All analyses were performed in July 2024 in triplicates (three samples from the same bottle). Absorbance measurements were conducted using UV Shimadzu spectrophotometer.

**Table 1**: Description of analysed red wine samples

| Sample | Harvest year | Aging |
|---|---|---|
| **1** | 2019 | stainless steel |
| **2** | 2021 | stainless steel |
| **3** | 2019 | barrique barrels |
| **4** | 2021 | barrique barrels |

*Determination of content of total phenols*

The content of total phenols was determined by the Folin-Ciocalteu method described by Singelton and Rossi (1965) and later modified by Keskin-Šašić, Tahirović and Topčagić (2012). 0.4 mL of sample was added to 2 mL (1/10 dilution) of Folin-Ciocalteu reagent. After 10 minutes, 1.6 mL of 7.5% sodium carbonate ($Na_2CO_3$) was added. The samples were left at room temperature for 30 minutes. After that, the absorbances were read at 743 nm. The calibration curve was created using different concentrations of gallic acid (50, 100, 150, 250 and 350 mg/L). The content of total phenols was expressed as mg of gallic acid equivalent (GAE) per liter of sample.

*Determination of total anthocyanin content*

Total anthocyanin content was determined using the pH differential method described by Zhishen, Mengcheng and Jianming (1999). This method is based on the structural difference of anthocyanins at different pH values of the medium. Two test tubes were used for each sample. 1 mL of the prepared sample was added to each test tube. Then, 4 mL of pH 1.0 buffer (potassium chloride, 0.025 M) was added to one tube, and 4 mL of pH 4.5 buffer (sodium acetate, 0.4 M) to the other. The content of total anthocyanins was expressed as mg of cyanidin-3-*O*-glucoside equivalent (CGE) per liter of sample.

*Determination of antioxidant activity using Ferric Reducing Antioxidant Power method*

Determination of the antioxidant activity with FRAP reagent was carried out according to Benzie and Strain (1996). This method is based on the reduction of the colourless $Fe^{3+}$-TPTZ to $Fe^{2+}$-TPTZ of intense blue colour. The FRAP reagent was prepared by mixing 200 mL of acetate buffer pH 3.6 (300 mM), 20 mL of TPTZ (2,4,6-tris(2-pyridyl)-*s*-triazine) solution (10 mM) in 40 mM hydrochloric acid, 20 mL of $FeCl_3$ (20 mM) and 24 mL of distilled water. The calibration curve was created using different concentrations of $FeSO_4$ (0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mM). 200 μL of sample was added to 3800 μL of freshly prepared FRAP reagent. The mixture was left for 4 minutes at room temperature. After that, the absorbances at 593 nm were read. The results were expressed as mM $FeSO_4$ equivalent per liter of sample (mmol $Fe^{2+}$/L).

*Determination of antioxidant activity using ABTS method*

The determination of antioxidant activity using ABTS radical was carried out according to Re, Pellegrini, Proteggente et al. (1999). This method is based on the reduction of ABTS radicals, which are formed either by chemical or enzymatic oxidation of the solution of ABTS. ABTS cation radical was prepared by dissolving 19.5 mg of ABTS and 3.3 mg of potassium persulfate in 7 mL of distilled water. The prepared solution was left to stand in the dark for 12-16 hours at room temperature before use. This solution was then diluted with ethanol (96%) to a final concentration of 1%, resulting in an absorbance of 0.70 ± 0.02 at 734 nm. A calibration curve was constructed using different concentrations of Trolox (0.1, 0.2, 0.4 and 0.6 mg/mL). 40 μL of sample was mixed with 4 mL of the ABTS radical solution and the absorbance was measured after 6 minutes at 734 nm. The results were expressed as mM of Trolox equivalent (TE) per liter of sample.

*Determination of total tannin content*

Total tannin content was determined using the method described by Ribéreau-Gayon, Glories, Maujean and Dubourdieu (2006). Two test tubes (A and B) were prepared for each sample. 1 mL of distilled water, 3 mL of hydrochloric acid (12 M) and 2 mL of sample were added to each test tube. One test tube (A) was heated in a water bath at 100°C for 30 minutes. 0.5 mL of ethanol (96%) was added to the other test tube (B) and kept in the dark at room temperature. After 30 min, test tube A was also placed in the dark until it had partially cooled, and then 0.5 mL of ethanol was added. After cooling, the absorbance for each prepared sample (A and B) was measured at 470, 520 and 570 nm. The concentration of total tannins was calculated according to the following formulas (1-3):

$$\Delta A_{520} = 1.1 \times \Delta A_{470} \tag{1}$$

$$\Delta A_{520} = 1.54 \times \Delta A_{470} \tag{2}$$

$$\gamma_{tannins} = 15.7 \times minimum_{\Delta A_{520}} \tag{3}$$

The content of total tannins was expressed as g/L.

*Statistical analysis*

All measurements are expressed as mean ± standard deviations. Statistical analysis was performed using analysis of variance (ANOVA). The statistical differences are considered significant at $p < 0.05$.

## RESULTS AND DISCUSSION

The results of the content of total phenols, anthocyanins and tannins are presented in Table 2.

**Table 2:** Content of total phenols, anthocyanins and tannins in wine samples

| Sample | Total phenols (mg GAE/L) | Total anthocyanins (mg CGE/L) | Total tannins (g/L) |
|---|---|---|---|
| **1** | 1733.87 ± 10.22$^{a}$ | 15.47 ± 0.59$^{c}$ | 9.46 ± 0.11$^{b}$ |
| **2** | 1828.19 ± 87.87$^{b}$ | 21.76 ± 0.35$^{d}$ | 9.00 ± 0.09$^{a}$ |
| **3** | 2382.09 ± 21.42$^{d}$ | 13.64 ± 0.19$^{a}$ | 11.42 ±0.21$^{d}$ |
| **4** | 2051.60 ± 16.06$^{c}$ | 14.08 ± 0.54$^{b}$ | 9.57 ± 0.15$^{c}$ |

Different letters within the same column represent a statistically significant difference by ANOVA with Tukey's test (p<0.05)

The results of this study on premium red wines indicate that the content of total phenols ranged from 1733.87 ± 10.22 to 2382.09 ± 21.42 mg GAE/L. The highest value was observed in sample 3, a wine aged in barrique barrels, while the lowest was observed in sample 1, a wine that was not aged and was directly bottled from stainless steel vessels. The statistically significant difference between the results indicates that oak barrels influence the increase in the content of total phenols. Marković and Talić (2013) analysed nine wines from the Herzegovina region, from different vintage years, using the same method as us for determining the content of total phenols. Blatina from the Stolac region showed lower values compared to ours. Similarly, Radeka, Rossi, Bestulić et al. (2022) analysed red wines from Croatia, comparing young wines with those aged in barrique barrels using the Folin-Ciocalteu method. Their content of total phenols varied between 1527.12 and 3936.21 mg GAE/L, with wines aged in barrique barrels exhibiting significantly higher concentrations than young wines, which is consistent with our results. The increased concentration of phenols is attributed to the release of hydrolysable tannins from oak barrels during aging, as confirmed by Waterhouse, Sacks and Jeffery (2016). According to Table 2., the total anthocyanin content ranged from 13.64 ± 0.19 to 21.76 ± 0.35 mg CGE/L. The highest content was found in sample 2, a wine stored in a stainless steel vessel from the 2021 vintage, while sample 3 had the lowest value. These results confirm that the total anthocyanin content decreases with aging due to copigmentation and polymerization reactions (Boulton, 2001). Wines aged in barrique barrels exhibited lower anthocyanin levels, supporting the notion that aging in oak barrels leads to anthocyanin degradation. A study by Aleixandre-Tudo and du Toit (2020) on 82 red wines from South Africa supports these findings. They proved that the content of total anthocyanins decreases with time in both barrique barrels and bottled wines. The differences between their results and ours may be attributed to variations in aging periods, geographical regions, climatic conditions, grape varieties and harvest years. Similarly, Guld, Racz, Tima et al. (2019) investigated three grape varieties typical of Hungary, monitoring changes in anthocyanin content during aging in barrique barrels. They also applied the pH differential method for determination, reporting a significant decrease in anthocyanin concentration with time, which is consistent with the findings of this study.

The determination of the total tannins content yielded values ranging from 9.00 ± 0.09 to 11.42 ± 0.21 g/L. Sample 2, representing a young wine, exhibited the lowest values, while sample 3, aged in barrique barrels, displayed the highest content. Both samples aged in barrique barrels (samples 3 and 4) exhibited a higher total tannins content compared to samples 1 and 2. These results confirm that aging in barrique barrels leads to an increase in total tannins content. American oak was used for aging the tested wines, which, due to its lower porosity, allows for lower oxygen permeability compared to French oak (Martínez-Gil, Del Alamo-Sanza and Nevares, 2022). It was shown that the concentration of tannins released from barrique barrels ranges from 2 to 4 g/L, and that these tannins are hydrolysable. Such increased concentrations contribute significantly to the total tannin content, particularly when the aging period is extended to several years (Smith, McRea and Bindon, 2015).

**Table 3:** Content of antioxidant activity in wine samples

| Sample | ABTS (mmol TE/L) | FRAP (mmol Fe$^{2+}$/L) |
|---|---|---|
| **1** | 16.97 ± 0.23$^{a}$ | 20.65 ± 0.84$^{a}$ |
| **2** | 18.14 ± 0.35$^{b}$ | 20.94 ± 0.20$^{c}$ |
| **3** | 19.81 ± 0.62$^{d}$ | 25.04 ± 0.70$^{d}$ |
| **4** | 19.07 ± 2.54$^{c}$ | 20.87 ± 0.70$^{b}$ |

Different letters within the same column represent a statistically significant difference by ANOVA with Tukey's test (p<0.05)

FRAP and ABTS methods were used to determine the antioxidant activity. According to the results shown in Table 3, the values obtained by the FRAP method ranged from 20.65 ± 0.84 mM Fe$^{2+}$/L to 25.04 ± 0.70 mM Fe$^{2+}$/L. The results obtained by the ABTS method ranged from 16.97 ± 0.23 to 19.81 ± 0.62 mM TE/L. Both methods confirm that the red wine from 2019, which was aged in barrique barrels for three years, had the highest antioxidant activity, while the wine from the same year from stainless steel vessels showed the lowest activity. Kesić, Zaimović, Ibrišimović-Mehmedović et al. (2018) conducted a study on 5 red wines, including Blatina from the Čapljina area. Using FRAP method, Blatina showed a value of 2427 µM Fe$^{2+}$/L. The significant difference between the results of our study and those reported by Kesić, Zaimović, Ibrišimović-Mehmedović et al. (2018) can be attributed to the development of various Blatina varieties over the years in different regions of Herzegovina, which significantly affects the yield and quality of the grapes (Kojić, Blesić, Delić et al., 2010). Stasko, Brezova, Mazur et al. (2008) determined the antioxidant capacity of red wines from Austria and Slovakia using the ABTS method. The results ranged from 7.84 to 13.59 mmol TE/L. Stratil, Kuban and Fojtova (2008) also analysed red and white wines from the

Czech Republic using the ABTS method. Their results for red wines ranged from 4.92 to 13.94 mmol TE/L. Based on these results, we can conclude that the geographical area and grape varieties significantly influence the results of the analyses. Crippen and Morrison (1986) explained in their study that grapes that are more exposed to sunlight and higher temperatures contain higher concentrations of phenolic compounds and monomeric anthocyanins, which ultimately affects the antioxidant activity of red wines.

The correlation coefficient between the total phenolic content and antioxidant activity was determined. The results show a strong positive correlation between total phenols content and the antioxidant activity determined by FRAP ($r = 0.995$) and ABTS method ($r = 0.994$). Statistical analysis was performed using analysis of variance (ANOVA) revealing a statistically significant difference between harvest years.

## CONCLUSIONS

The red wines analysed in this study showed high levels of total phenols, anthocyanins, tannins and antioxidant activity. A statistically significant difference in the concentrations of bioactive compounds was found between wines from two different harvest years. The results obtained in this study also demonstrate that the content of bioactive compounds in the wines is significantly influenced by the technological process used. The wine samples aged in oak barrels showed higher concentrations of total phenols, total tannins and antioxidant activity compared to the wines from stainless steel vessels from the same harvest year. At the same time, we showed that the total anthocyanin content decreased over time in oak-aged wine. These findings suggest that wine aged in oak barrels may be more beneficial for health-related applications due to its stronger antioxidant properties. However, this study had a limited number of samples and focused on a single grape variety. Future studies should include a larger and more diverse sample set, explore different aging techniques, and assess sensory characteristics and bioavailability to better understand the health and quality implications of wine.

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## Summary/Sažetak

Crna vina bogata su antioksidansima koji imaju protuupalno djelovanje i blagotvoran efekat na ljudsko zdravlje. Cilj ovog istraživanja bio je utvrditi sadržaj bioaktivnih spojeva, tj. ukupnih fenola, antocijanina i tanina, kao i antioksidativnu aktivnost u crnim vinima sa područja Hercegovine. Analizirana su četiri vina iz dvije različite godine berbe, tj. 2019. i 2021. Fenolni spojevi važni su parametri kvalitete vina koji utječu na boju, okus i aromu. Sadržaj ukupnih fenola varirao je od 1733.87 ± 10.22 do 2382.09 ± 21.42 mg GAE/L. Sadržaj ukupnih antocijanina, koji direktno utječu na boju crnih vina, određen je pH diferencijalnom metodom pH, a dobiveni rezultati kretali su se od 13.64 ± 0.19 do 21.76 ± 0.35 mg CGE/L. Ukupni tanini određeni su spektrometrijskom metodom, a dobivene su vrijednosti u rasponu od 9.00 ± 0.09 do 11.42 ± 0.21 g/L. Antioksidativna aktivnost procijenjena je dvjema različitim metodama, FRAP i ABTS. Ukupni sadržaj fenola u ispitivanim vinima pozitivno je korelirao s njihovom antioksidativnom aktivnošću. Na sadržaj ukupnih fenola, antocijanina, tanina i antioksidativnu aktivnost utječu godina berbe kao i korištene metode odležavanja vina.

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# Electrochemical Behaviour of Aluminum in Artificial Seawater in the Presence of *Artemisia annua* L.

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**Abstract:** This study investigated the use of *Artemisia annua* L. aqueous extract as a corrosion inhibitor for aluminum in artificial seawater using cyclic voltammetry (CV), potentiodynamic polarization (PP) and electrochemical impedance spectroscopy (EIS) measurements. The surface film formed on the Al in the presence of *A. annua* was analyzed using Fourier-transform infrared (FTIR) spectroscopy. Electrochemical results showed that *A. annua* is a mixed type inhibitor of Al corrosion with predominantly anodic action and is changing the electrochemical behavior of Al in artificial seawater by inhibiting dissolution processes. The inhibition efficiency of ~91% was attributed to the formation of a homogenous multilayer composed of phenolic acids. According to the results of FTIR analysis, the adsorption was established through the carboxyl group of phenolic acids, where stabilization of the adsorption layer was achieved through interactions between aromatic rings and the aliphatic chains. The adsorption mechanism obeyed the Freundlich isotherm model and El-Awady kinetic-thermodynamic models and referred to physical adsorption ($\Delta G_{ads}$ = 18.83 ± 2.34 kJ $mol^{-1}$).

## INTRODUCTION

Aluminum is a widely used metal in the production of marine structures and the shipbuilding industry for its resistance to corrosion and low density, which improves performance and reduces the fuel consumption of ships (Ashkenazi, 2019). The resistance to corrosion results from the formation of a thin, stable oxide layer on the surface, which acts as a protective barrier against further oxidation of the aluminum and thereby reduces the rate of corrosion processes (Ashkenazi, 2019, Vargel, 2020). However, in the presence of chlorides that can specifically adsorb on the charged metal surface and penetrate the surface film Melchers, 2014), localized change in the potential can occur resulting in localized corrosion known as pitting. From ecological and economic aspects, localized corrosion is a significant threat to various industries as it can lead to structural damage, injuries, and the potential release of harmful substances into the environment (Jia, Unsal, Xu et al, 2019). This raises another concern associated with the protective measures against corrosion since any materials contributing to the pollution of the marine environment complicate the application of chemical substances, including those used in marine coatings and corrosion inhibitors.

European Union Water Framework Directive (2000/60/EC) emphasizes the need to reduce pollution from hazardous substances, which can include certain chemicals used in coatings and corrosion inhibitors (http-1). For example, tributyltin (TBT), once commonly used in antifouling paints, has been banned due to its toxic effects on marine organisms (Amara, Miler, Ben Slama et al, 2018). The shift toward sustainable practices includes the use of non-toxic corrosion protection methods, such as the use of natural inhibitors that do not leach harmful substances into the environment (Jia, Unsal, Xu et al, 2019; Zlatić, Martinović. Pilić et al, 2023). Plants are the richest source of natural antioxidants with polyphenolics being the most prevalent (Pratt, 1992, Kaur, Daksh, Saxena, 2024). These compounds can be found throughout all parts of the plant and can offer a base for the development of renewable natural inhibitors of corrosion processes (Kaur, Daksh, Saxena, 2024). In our earlier studies, the aqueous extraction of *Artemisia annua* L. was carried out at room temperature, intentionally avoiding the use of excessive energy and solvents that typically produce a significant amount of waste by-products in plant extract preparation (Zlatić, Martinović. Pilić et al, 2023; Zlatić, Martinović. Pilić et al, 2024). The extract showed 80% inhibition efficiency toward corrosion of aluminum alloy in both abiotic (Zlatić, Martinović. Pilić et al, 2023) and biotic simulated marine environment (Zlatić, Martinović. Pilić et al, 2024) which was attributed to the physical adsorption of caffeic acid derivatives on the alloy surface, as well formation of a protective $Al_2O_3$. *A. annua* grows naturally on the North hemisphere in moderate climates and poses extraordinary antibacterial, antioxidant and antiviral properties (Zlatić, Martinović. Pilić et al, 2024, Zlatić, Martinović. Pilić et al, 2023b) wherefore can replace hazardous chemicals used in protection measures against corrosion. Taking into account the wide application of aluminum and that protecting existing constructions can reduce waste and promote sustainability, an investigation of the influence of *A. annua* on aluminum corrosion can contribute to the development of effective, environmentally friendly protective measures against corrosion. So the purpose of the investigations was twofold; (1) characterization of the electrochemical properties of aluminum in the artificial seawater without and with the addition of *A. annua* extract and (2) characterization of the adsorption layer using empirical models and Fourier transform infrared (FTIR) spectroscopy.

## EXPERIMENTAL

The electrochemical measurements were executed in a standard three electrode cell using an Autolab PGSTAT320N potentiostat that was controlled by Nova 1.5 software. Electrochemical cell was composed of aluminum (99.99%) as a working electrode, platinum sheet as a counter electrode and the Ag | AgCl | 3 M KCl reference electrode. The working electrolyte was artificial seawater with the following chemical composition (g/L): 4.1575 $Na_2SO_4$, 11.1211 $MgCl_2x6H_2O$, 0.79023 KCl, 1.5877 $CaCl_2x2H_2O$, 24.9772 NaCl, 0.0587 $NaHCO_3$ (Zlatić, Martinović. Pilić et al, 2023a, Zlatić, Martinović. Pilić et al, 2024, Zlatić, Martinović. Pilić et al, 2023b, Dickson, 1990). The *A. annua* aqueous extract was prepared according to the procedure described previously (Zlatić, Martinović. Pilić et al, 2023a, Zlatić, Martinović. Pilić et al, 2024, Zlatić, Martinović. Pilić et al, 2023b), using the same batch of plant material. The phytochemical profile of *A. annua* extract, including HPLC (Zlatić, Martinović. Pilić et al, 2023a) and FTIR (Zlatić, Martinović. Pilić et al, 2024) analyses, is available in previously published studies. Briefly, 1.0 g of dried plant was mixed with 1.0 L of working electrolyte and set aside in dark for 3h. The sample was then filtered using 0.45 µm filter paper and diluted up to 1.0 L. Prior each measurement aluminum was abraded by SiC emery paper (360-1000 grades), degreased in ethanol using an ultrasonic bath and polarized at –1.60 V for 2 minutes.

The electrochemical properties of aluminum without and with the addition of *A. annua* were studied using cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and potentiodynamic polarization (PP) measurements. Cyclic voltamograms were taken in the potential range from –1.60 to –0.70 V with the scan rate of 30 $mVs^{-1}$. EIS was carried out at the open circuit potential (EOCP) that was measured until the stationary state was attained which was approximately 60 minutes. The spectra were taken in the frequency range from 10 kHz to 5 mHz using alternating voltage of 10 mV. Right after EIS measurements, PP curves were obtained at a scan rate of 1 $mVs^{-1}$ in the potential range of ± 0.20 V vs. EOCP.

The presence of functional groups of *A. annua* extract on the Al surface was investigated using a Fourier transform infrared (FTIR) spectrometer IRAffinity-1S (Shimadzu, Japan) coupled with single reflection type attenuated total reflection (ATR) attachment (GladiATR10, Shimadzu, Japan). For this purpose, working electrodes were immersed in working electrolyte with and without the addition of 1 $gL^{-1}$ *A. annua* extract for 24h, after which the electrode surfaces were dehydrated with the series of ethanol solutions (v/v: 50%, 75%, and 96%). FTIR spectrum was taken directly from the surfaces in the wavelength range between 400 and 4000 $cm^{-1}$.

## RESULTS AND DISCUSSION

### Cyclic voltammetry

Cyclic voltammograms of aluminum recorded in artificial seawater with and without the addition of *A. annua* are shown in Figure 1. It can be seen that the cyclic voltammogram recorded without *A. annua* show current shoulder at around −1.3 V associated with the accumulation of cations and anions on the Al surface (Hasenay and Šeruga, 2007). Anodic current densities around this potential decreased with the increasing concentration of *A. annua*. Another anodic peak was observed around −0.8 V, but only in the cyclic voltammogram recorded with the addition of 0.5 g $L^{-1}$ *A. annua*, and corresponds to the formation of $Al_2O_3$ (Zlatić, Martinović. Pilić et al, 2023a, Hasenay and Šeruga, 2007). A decrease in the diffusion rate of aluminium ions from the electrode surface on the electrode/electrolyte interface, and the formation of an oxide film with protective properties are typical features of anodic inhibitors that decrease anodic active sites of metal (Martinović, Zlatić, Pilić et al., 2023). A decrease in the active area of the metal is achieved either by the adsorption of inhibitor molecules onto the charged metal surface or by promoting the formation of protective films (Salci, Yukel and Solmaz, 2022). From the presented cyclic voltammograms (Figure 1) the total amount of anodic charge, QA per unit of the Al surface was determined by integrating the area under the anodic current. The obtained values were then used in the calculation of the surface coverage ϴ of the working electrode with the inhibitor using Equation 1 (Ma, Ammar, Kumar et al, 2022):

$$\Theta = \frac{Q_A - Q'_A}{Q_A} \qquad (1)$$

Here, QA and Q'A refer to anodic charges determined from cyclic voltammograms of aluminum in artificial seawater without and with the addition of *A. annua*, respectively. As the amount of adsorbed inhibitor on the alloy surface is quantitatively related to the inhibition efficiency, *A. annua* inhibition efficiency (IE) toward Al corrosion in artificial seawater was calculated with the following equation:

$$IE = \Theta \cdot 100 \qquad (2)$$

Table 1 shows results of CV analysis. The amount of anodic charges (QA) decreased with the increasing concentration of *A. annua*. When 1 $gL^{-1}$ of *A. annua* was added to the artificial seawater, almost the entire aluminium surface was covered with inhibitor resulting in a maximum IE of 99%.

**Table 1.** The values of the parameters obtained by analyzing the experimental results from Figure 1.

| *γ* (g L$^{-1}$) | *Q*$_A$ (μC cm$^{-2}$) | *θ* | *IE* (%) |
|---|---|---|---|
| 0 | 71 | - | - |
| 0.10 | 65 | 0.084 | 8.42 |
| 0.25 | 55 | 0227 | 22.77 |
| 0.50 | 11 | 0.838 | 83.83 |
| 1.00 | 0.013 | 0.999 | 99.98 |

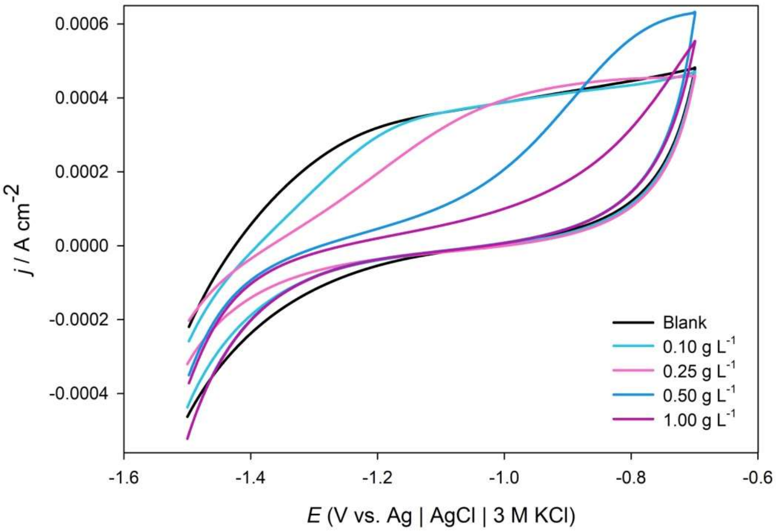

**Figure 1**. Cyclic voltammograms recorded on aluminum in artificial seawater at a scan rate of 30 mV/s, without and with the addition of different concentrations of *A. annua* (marked in the figure).

Potentiodynamic Polarization

Figure 2 shows potentiodynamic polarization (PP) curves recorded in artificial seawater without and with the addition of different concentrations of *A. annua*. Analyzing the PP curves provided insight into the corrosion kinetic parameters such as the Tafel slopes (bA, bC), corrosion potentials (Ecorr), and corrosion current densities (jcor). These values are useful for assessing the influence of inhibitors on the electrochemical behavior of metals, allowing the calculation of the working electrode's surface coverage Θ by the inhibitor using the corrosion current densities (eq. 3) Martinović, Zlatić, Pilić et al., 2023).

$$\Theta = \frac{j^0_{\mathrm{cor}} - j_{\mathrm{cor}}}{j^0_{\mathrm{cor}}} \qquad (3)$$

Where $j^0_{\mathrm{cor}}$ and $j_{\mathrm{cor}}$ are corrosion current densities deduced from PP curves recorded without and with the addition of *A. annua*. The values of kinetic parameters obtained by analyzing the results from Figure 2 are given in Table 2 along with the inhibition efficiencies and surface coverage calculated using Equations 2 and 3.

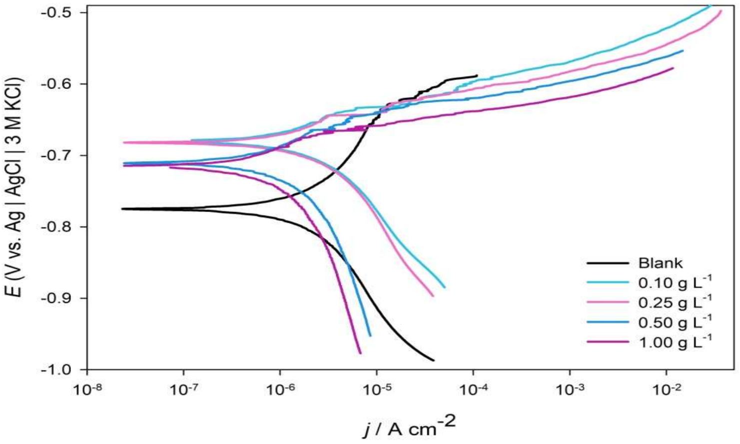

**Figure 2**. Potentiodynamic polarization curves recorded on aluminum in artificial seawater at a scan rate of 1 mV s$^{-1}$, without and with the addition of different concentrations of *A. annua* (marked in the figure)

**Table 2.** The values of the corrosion parameters obtained by analyzing the experimental results from Figure 2.

| $\gamma$ (g L$^{-1}$) | b$_a$ (V dec$^{-1}$) | b$_c$ (V dec$^{-1}$) | $E_{corr}$ (V) | $j_{corr}$ (μA cm$^{-2}$) | $\theta$ | *IE* (%) |
|---|---|---|---|---|---|---|
| 0 | 0.052 | 0.052 | −0.775 | 0.567 | | |
| 0.1 | 0.032 | 0.029 | −0.681 | 0.405 | 0.285 | 28.5 |
| 0.25 | 0.028 | 0.024 | −0.683 | 0.360 | 0.365 | 36.5 |
| 0.50 | 0.035 | 0.038 | −0.710 | 0.242 | 0.572 | 57.2 |
| 1.00 | 0.024 | 0.023 | −0.716 | 0.130 | 0.771 | 77.1 |

As can be seen from Figure 2, adding *A. annua* to artificial seawater resulted in a positive shift in the corrosion potential of aluminum. The anodic reaction rate with the addition of *A. annua* was controlled by charge transfer, as recognized by an increase in the reaction rate with increasing potential (Fig. 2). Also, both anodic and cathodic Tafel slopes slightly decreased after the plant extract was added to the artificial seawater, indicating that *A. annua* acts as a mixed type corrosion inhibitor for Al (Salci, Yukel and Solmaz, 2022), with a predominant anodic action recognized by a positive shift of corrosion potential after the addition of *A. annua* to the artificial seawater (Zlatić, Martinović. Pilić et al, 2023a, Martinović, Zlatić, Pilić et al., 2023). An increase in the concentration of the inhibitor resulted in a decrease in jcor. Withal, the inhibition efficiency of *A. annua* toward Al corrosion in artificial seawater increased with the increasing concentration of the extract, reaching a maximum of 77% at 1 gL$^{-1}$ (Table 2).

### Electrochemical Impedance Spectroscopy

Impedance spectra of Al electrodes recorded at open circuit potential in artificial seawater without and with the addition of extract in different concentrations are presented by Nyquist and Bode plots in Figure 3. The obtained data showed the capacitive behavior of the electrode in the middle-frequency range for all examined conditions. Simultaneously, an inductive loop was observed on the Nyquist plot in the low-frequency region for aluminum immersed in artificial seawater and in the presence of 0.10 g L$^{-1}$ of the extract. This feature, also visible in Bode plots as a positive phase shift at low frequencies, has been previously linked to the dynamic dissolution of the surface oxide film and the participation of adsorbed intermediates in the electrochemical process (Yang, Yuan, Liu et al, 2017, Ma, Lis Chen et al, 2002). According to the model proposed by H. Ma et al. (Ma, Lis Chen et al, 2002), the occurrence of inductive behavior can arise in systems involving sequential reaction steps with adsorbed intermediates. Specifically, the inductive loop appears when the rate-determining step shifts between adsorption and desorption of the intermediate, i.e., when the ratio of adsorption/desorption rate constants changes as a function of electrode potential.

The disappearance of the inductive loop at higher concentrations of *A. annua* extract ($\geq$ 0.25 g L$^{-1}$) suggested a shift in surface reaction dynamics, likely caused by the formation of a more stable organic layer that suppressed the formation or participation of such intermediates. This is in line with the "chemical inductor" framework described by J. Bisquert and A. Guerrero (2022), where inductive elements in electrochemical systems arise from coupling between fast charge transfer processes and slower surface phenomena such as intermediate formation, surface restructuring, or inhibitor reorganization. As seen from Figure 3, the radius of the capacitive semicircle increased with increasing the inhibitor's concentration. These observations indicate that the adsorbed organic molecules at higher extract concentrations inhibit the dynamic processes responsible for inductive behavior, resulting in a more ideal capacitive response. This supports the interpretation that *A. annua* acts as a surface-blocking inhibitor, stabilizing the electrode interface and shifting the mechanism away from dynamic dissolution toward surface passivation. The results of EIS measurements were interpreted using electrical equivalent circuits (EEC) presented in Figure 4. Values of EEC elements are given in Table 3.

EEC presented in Figure 4a was assigned to EIS results recorded in artificial seawater without and with the addition of 0.10 g L$^{-1}$ *A. annua*. The electrochemical behavior of Al during the addition of higher concentrations of *A. annua* (0.25-1.00 gL$^{-1}$) was described with a simplified Randless cell (Figure 4b). In these EECs, R represents the ohmic resistance of the working electrolyte, R1 and Q1 charge transfer resistance, and constant phase element (CPE) associated with the double-layer capacitance. R2 and L refer to resistance and inductance of the modified surface layer (Yang, Yuan, Liu et al, 2017, Ma, Lis Chen et al, 2002, Bisquert and Guerrero, 2022). Compared with bare electrolyte, charge transfer resistance R1 in the presence of *A. annua* (0.25-1.00 g L$^{-1}$) notably increased. Meanwhile, the CPE associated with the capacitance of the electrical double-layer decreased (Table 3). This was due to a decrease in local dielectric constants of the surface layer and/or the formation of a thicker electrical double-layer as a consequence of the adsorption of inhibitors on the electrode surface that protected aluminum from further corrosion (Martinović, Zlatić, Pilić et al., 2023). The addition of *A. annua* to artificial seawater also decreased surface heterogeneity as recognized by an increase in n values (Table 3). For the evaluation of the surface coverage Θ by extract, the total resistance to polarization Rp was taken into account and calculated according to Equation 4 (Zlatić, Martinović. Pilić et al, 2023b, Chellouli, Chebabe, Dermai et al, 2016):

$$\Theta = \frac{R'_{P} - R_{P}}{R'_{P}} \qquad (4)$$

Here, R_P and 〖R'〗 _P are the polarization resistances recorded without and with the addition of *A. annua*. RP represented a sum of R1 and R2 for the results described with two-time constants whereas for a one-time constant model RP = R1 (Zlatić, Martinović. Pilić et al, 2023b, Chellouli, Chebabe, Dermai et al, 2016) Surface coverage deduced from EIS data was also used in determining the inhibition efficiency (IE) of the extract using Equation 2. As presented in Table 3, IE increased with an increase in *A. annua* concentration ranging from 29-97%.

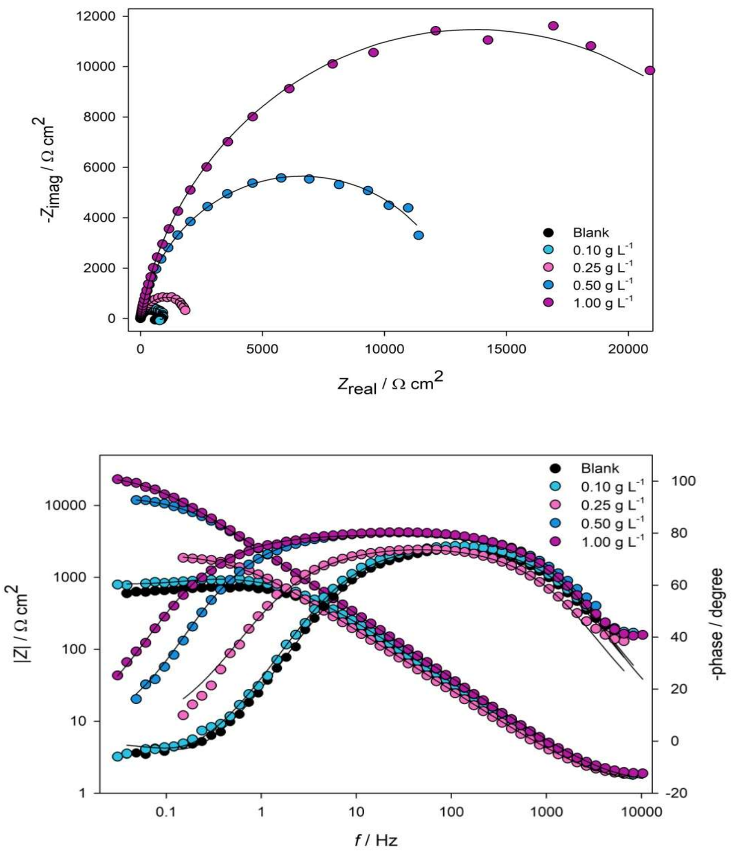

**Figure 3**. Nyquist and Bode plots of impedance spectra recorded on aluminum in artificial seawater without and with the addition of different concentrations of *A. annua* (marked in the figure).

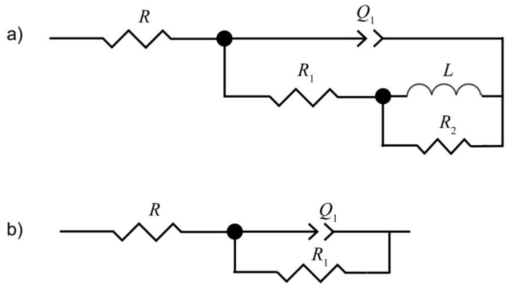

**Figure 4**. Equivalent electrical circuits used in modeling the impedance spectra presented in Figure 3, where $Q_1$ represents the constant phase element (*CPE*) associated with the capacitance of the electrical double layer.

**Table 3.** Values of the EECs for aluminum in artificial seawater without and with the addition of *A. annua*, obtained by analyzing the experimental results from Figure 3.

| γ | *R* | *CPE* x 10$^4$ | $n_1$ | $R_1$ | $R_2$ | *L* | *θ* | *IE* |
|---|---|---|---|---|---|---|---|---|
| g L$^{-1}$ | Ω cm$^2$ | Ω$^{-1}$s$^n$ cm$^{-2}$ | | Ω cm$^2$ | Ω cm$^2$ | H cm$^2$ | | % |
| 0 | 1.2 | 1.21 | 0.857 | 629.6 | 103.40 | 132.3 | | |
| 0.10 | 1.2 | 1.12 | 0.986 | 803.1 | 235.90 | 131.6 | 0.29 | 29.4 |
| 0.25 | 1.3 | 1.58 | 0.848 | 1765.8 | - | - | 0.58 | 58.5 |
| 0.50 | 1.2 | 0.77 | 0.901 | 11229.0 | - | - | 0.93 | 93.5 |
| 1.00 | 1.3 | 0.81 | 0.898 | 21005.0 | - | - | 0.96 | 96.5 |

A comparison with our previous study (Zlatić, Martinović. Pilić et al, 2023a) highlights both consistencies and novel observations. In both works, the addition of *A. annua* extract led to an increase in polarization resistance and a decrease in double-layer capacitance, consistent with the formation of a protective surface layer. However, in the present study, electrochemical impedance spectra recorded at lower extract concentrations (0.10 $gL^{-1}$) revealed a low-frequency inductive loop (Fig. 3), which was not observed in our previous work (Zlatić, Martinović. Pilić et al, 2023a). Consequently, equivalent circuit modelling in this study required two-time constant elements at low extract concentration, whereas in earlier study a single-time constant (Randles-type) circuit applied for all conditions. This distinction suggests that the lower concentrations of the extract in this study allow for transient processes at the electrode interface, such as the formation and desorption of adsorbed intermediates, which contribute to the observed inductive behavior (Ma, Lis Chen et al, 2002, Bisquert and Guerrero, 2022). At higher extract concentrations, these processes are likely suppressed due to more complete surface coverage, resulting in a capacitive response similar to that observed in our earlier work (Zlatić, Martinović. Pilić et al, 2023a).

### Adsorption mechanism

All electrochemical results showed an increase in the inhibition efficiency of *A. annua* against aluminum corrosion in artificial seawater with increasing extract concentration. The latter was associated with the adsorption of *A. annua* extract on the aluminum surface, so the surface coverage (Θ) calculated using Equations 1, 2, and 4 were utilized in the assessment of the adsorption mechanism. Therefore, different adsorption isotherms were tested to get insight into interactions between extract molecules and aluminum surface. The best fitting isotherm model that reflects the adsorption process of *A. annua* on Al surface in artificial seawater was the Freundlich adsorption isotherm (Figure 5) that can be expressed with the Equation 5 (Khamis, Bellucci, Latanision et al, 1991, Al-Ghouti and Da'ana, 2020):

$$\log \Theta = \log K_{\text{ads}} - \frac{1}{n}\log \gamma \tag{5}$$

Here, Θ is the surface coverage; γ is the concentration of extract in g $L^{-1}$, while parameters K_ads and 1/n indicate constant and the intensity of adsorption and are dependent on the temperature. 1/n determines the intensity of adsorption, that is, surface heterogeneity, which indicates the relative energy distribution and heterogeneity of adsorbate sites (Al-Ghouti and Da'ana, 2020). The Freundlich isotherm model describes a heterogeneous surface assuming that adsorption processes occur in a multilayer way and that each inhibitor molecule has a different adsorption potential (Ituen, Akaranta and James, 2017). The formation of a multilayer can also be discussed in terms of the El-Awady kinetic-thermodynamic model (El-Awady, Abd-El-Nabey, Azis, 1992, ,Martinović, Pilić, Zlatić et al, 2023, Thabet, EL-Moselhy, Azooz et al, 2024):

$$\log\left(\frac{\Theta}{1-\Theta}\right) = \log K + a\log \gamma \tag{6}$$

Here K is the constant, and 1/a is the number of the active sites occupied by one inhibitor molecule. If the multilayer of the inhibitor was formed on the metal surface, a value of 1/a is less than 1. If it's above 1, the inhibitor occupies more than one active site ,(Martinović, Pilić, Zlatić et al, 2023). From parameters obtained by the El-Awady kinetic model (Figure 6), the value of the adsorption constant, Kads can be calculated using the following equation (El-Awady, Abd-El-Nabey, Azis, 1992,(Martinović, Pilić, Zlatić et al, 2023, Thabet, EL-Moselhy, Azooz et al, 2024):

$$K_{ads} = K^{1/a} \tag{7}$$

The adsorption mechanism is determined by the free energy of adsorption ΔGads that can be calculated using corresponding Kads and the following equation:

$$\Delta G_{\text{ads}} = -RT\ln(K_{\text{ads}}c_{\text{w}}) \tag{8}$$

Where R is the universal molar gas constant (8.314 J $K^{-1}$ $mol^{-1}$), T the temperature (298K) and cw is the mass concentration of water in the working electrolyte (1000 g $L^{-1}$) (Zlatić, Martinović. Pilić et al, 2023a, (Zlatić, Martinović. Pilić et al, 2023b, Chellouli, Chebabe, Dermai et al, 2016, (Martinović, Pilić, Zlatić et al, 2023). Values of Kads and ΔGads obtained from all applied models are presented in Table 4.

**Table 4**. Values of the parameters obtained by analyzing the experimental results from Figures 5 and 6.

| Model | Method | $R^2$ | $1/n$ | $1/a$ | $K_{ads}$ (L $g^{-1}$) | - $\Delta G$ (kJ $mol^{-1}$) |
|---|---|---|---|---|---|---|
| Freudnlich isotherm | CV | 0.945 | 1.152 | | 1.26 | 17.69 |
| | PP | 0.973 | 0.447 | | 0.75 | 16.43 |
| | EIS | 0.916 | 0.539 | | 1.13 | 17.43 |
| El-Awady | CV | 0.838 | | 0.409 | 3.28 | 20.07 |
| | PP | 0.936 | | 1.06 | 3.01 | 19.85 |
| | EIS | 0.955 | | 0.513 | 5.99 | 21.56 |

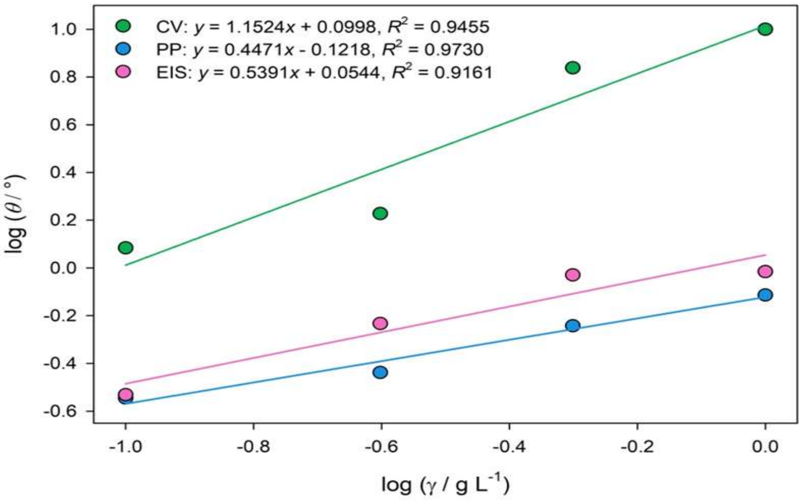

**Figure 5**. Freundlich adsorption isotherm of the adsorption of *A. annua* on the surface of an aluminum electrode in artificial seawater.

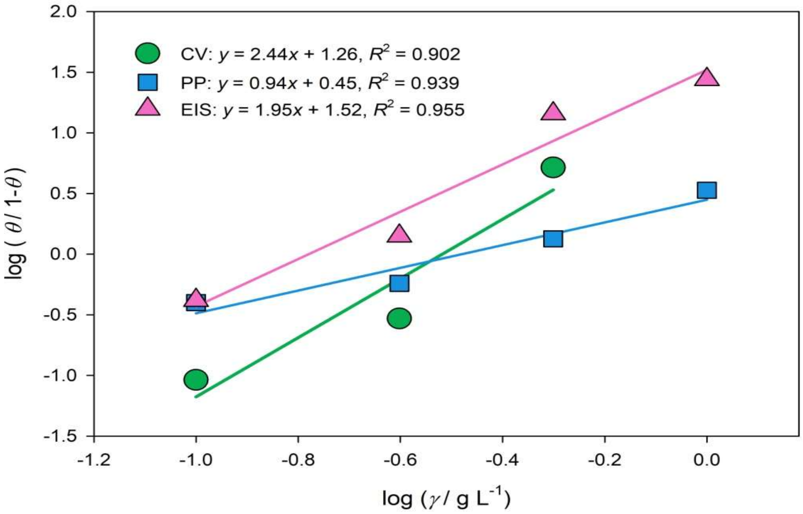

**Figure 6**. El-Awady kinetic–thermodynamic model of *A. annua* adsorption on the Al surface in artificial seawater

When describing the adsorption mechanism of the *A. annua* extract on the aluminum surface, the validity of selected thermodynamic models was proven by the achieved linearity (Figures 4 and 5) and further supported by the R2 values. As seen in Table 4, values of 1/a are equal to or less than 1, indicating that the inhibitor formed a multilayer on the Al surface (Martinović, Pilić, Zlatić et al, 2023). This is consistent with the assumptions proposed by the Freundlich isotherm model. The surface of the aluminum is assumed to have sites with different adsorption energies (Al-Ghouti and Da'ana, 2020). This heterogeneity can be due to the presence of oxides, defects, and other surface irregularities. Because the isotherm does not assume uniform free energy of adsorption, it is well-suited to describe systems where the free energy of adsorption decreases as the surface coverage increases, meaning high-energy sites are occupied first, and as more sites are filled, the remaining available sites have lower binding energies (Ituen, Akaranta and James, 2017).

From the values of the free energy of adsorption ΔGads calculated using Kads obtained from Freundlich isotherm model (−17.18 ± 0.66 kJ mol$^{-1}$, Table 4), as well as El-Awady model (−20.49 ± 0.93 kJ mol$^{-1}$, Table 4), it can be concluded that the inhibitor molecules are bounded to the charged Al surface by van der Waals forces, and the process corresponds to physical adsorption (Zlatić, Martinović. Pilić et al, 2023a, (Zlatić, Martinović. Pilić et al, 2023b, Chellouli, Chebabe, Dermai et al, 2016, El-Awady, Abd-El-Nabey, Azis, 1992, Martinović, Pilić, Zlatić et al, 2023) .

Fourier transform infrared spectroscopy

The FTIR spectra of the Al surface recorded after immersion in artificial seawater with and without the addition of 1 g L$^{-1}$ *A. annua* extract are presented in Figure 7.

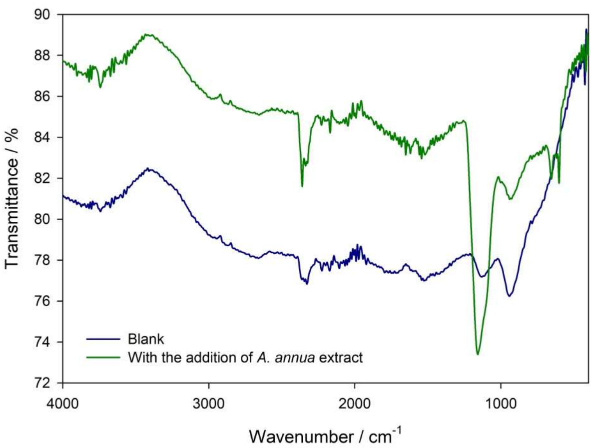

**Figure 7**. FTIR spectrum of the Al surface after immersion in the artificial seawater without and with the addition of 1 g L$^{-1}$ *A. annua* extract.

A broad absorption band between 3800 and 3200 cm$^{-1}$ was observed in both samples, corresponding to O–H stretching vibrations of adsorbed water and/or Al–OH surface groups. A band at approximately 1100 cm$^{-1}$ was also present, and was attributed to surface Al–O (Zlatić, Martinović. Pilić et al, 2023a, (Zlatić, Martinović. Pilić et al, 2023b).

In contrast to the blank sample, the spectrum of the treated aluminum surface revealed several distinct peaks that were attributed to organic species adsorbed from the extract. A broad band in the range 3300–2500 cm$^{-1}$ corresponding to the O–H stretching vibrations, likely originated from –OH groups present in caffeic and chlorogenic acid, as confirmed by our previous HPLC (Zlatić, Martinović. Pilić et al, 2023a, and FTIR (Zlatić, Martinović. Pilić et al, 2023b) analyses. A distinct band at approximately 1100 cm$^{-1}$ was significantly more intense in the treated sample compared to the blank and was attributed to C–O stretching vibrations (Zlatić, Martinović. Pilić et al, 2023b, Iglesias, Garciade-Saldana, Jaen, 2001), confirming the presence of adsorbed organic molecules. Furthermore, two sharp bands appeared at approximately 655 cm$^{-1}$ and 600 cm$^{-1}$, which were absent in the blank sample and were not observed in the FTIR spectrum of the extract itself (Zlatić, Martinović. Pilić et al, 2023b). These bands are characteristic of out-of-plane bending vibrations of aromatic C–H groups (Iglesias, Garciade-Saldana, Jaen, 2001), and their emergence only after exposure to the treated solution suggested surface-associated organization of adsorbed aromatic systems. The absence of a well-defined carbonyl stretching band around 1700 cm$^{-1}$, despite its presence in the extract (Zlatić, Martinović. Pilić et al, 2023b), suggested that the adsorption of phenolic acids occurred primarily via van der Waals forces, or π–π interactions rather than through coordination via carboxylate groups. This observation aligns with electrochemical data and calculated Gibbs adsorption energies (ΔGads = 18.83 ± 2.34 kJ mol$^{-1}$, Table 4), which point towards physisorption as the predominant interaction mechanism (Zlatić, Martinović. Pilić et al, 2023a, Chellouli, Chebabe, Dermai et al, 2016, El-Awady, Abd-El-Nabey, Azis, 1992, (Martinović, Pilić, Zlatić et al, 2023).

## CONCLUSION

Plant extracts are affordable and eco-friendly corrosion inhibitors that could help transition to more sustainable protection measures of metals and alloys against corrosion failures. This work focused on the study of the electrochemical properties of Al in artificial seawater in the presence of *A. annua* using CV, PP, and EIS techniques. The surface of aluminum after treatment with *A. annua* was examined using ATR-FTIR spectroscopy. Electrochemical results showed that the addition of *A. annua* (0.25-1.00 g L$^{-1}$) to artificial seawater changed the electrochemical behavior of Al. This resulted in a decrease in corrosion current densities, as well as an increase in charge transfer resistance compared to bare electrolyte. According to corrosion kinetic parameters obtained from PP and CV measurements, *A. annua* extract acts as mixed type inhibitor of Al corrosion in artificial seawater with prevalent anodic action. Enhanced electrochemical properties were attributed to the adsorption of extract molecules onto the Al surface, as confirmed by the FTIR analysis. The adsorption mechanism was determined using the Freundlich adsorption isotherm and kinetic-thermodynamic model proposed by El-Awady et al. The estimated value of free energy of adsorption (ΔGads = 18.83 ± 2.34 kJ mol$^{-1}$) indicated physical adsorption of *A. annua* molecules on the charged Al surface. *A. annua* extract formed a multilayer and decreased the Al surface heterogeneity. This adsorbed layer played a role in modifying the electrochemical properties of the aluminum and enhancing its corrosion resistance.

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## Summary/Sažetak

Primjena vodenog ekstrakta *Artemisia annua* L. kao inhibitora korozije aluminija u umjetnoj morskoj vodi istražena je uz pomoć cikličke voltametrije (CV), potenciodinamičke polarizacije (PP) i elektrokemijske impedancijske spektroskopije (EIS). Površinski film formiran na Al u prisutnosti A. annua analiziran je korištenjem infracrvene spektroskopije s Fourierovom transformacijom (FTIR). Elektrokemijski rezultati pokazali su kako je *A. annua* mješoviti inhibitor Al korozije s pretežno anodnim djelovanjem koji mijenja elektrokemijsko ponašanje Al u umjetnoj morskoj vodi inhibicijom procesa otapanja aluminija. Učinkovitost inhibicije od ~91% pripisana je formiranju homogenog višesloja sastavljenog od fenolnih kiselina. Prema rezultatima FTIR analize, adsorpcija je uspostavljena preko karboksilne skupine fenolnih kiselina, gdje je stabilizacija adsorpcijskog sloja postignuta interakcijama između aromatskih prstenova i alifatskih lanaca. Mehanizam adsorpcije slijedio je Freundlichov model izoterme i El-Awadyjev kinetičko-termodinamički model te se odnosio na fizikalnu adsorpciju ($\Delta$Gads = 18.83 ± 2.34 kJ mol$^{-1}$).

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