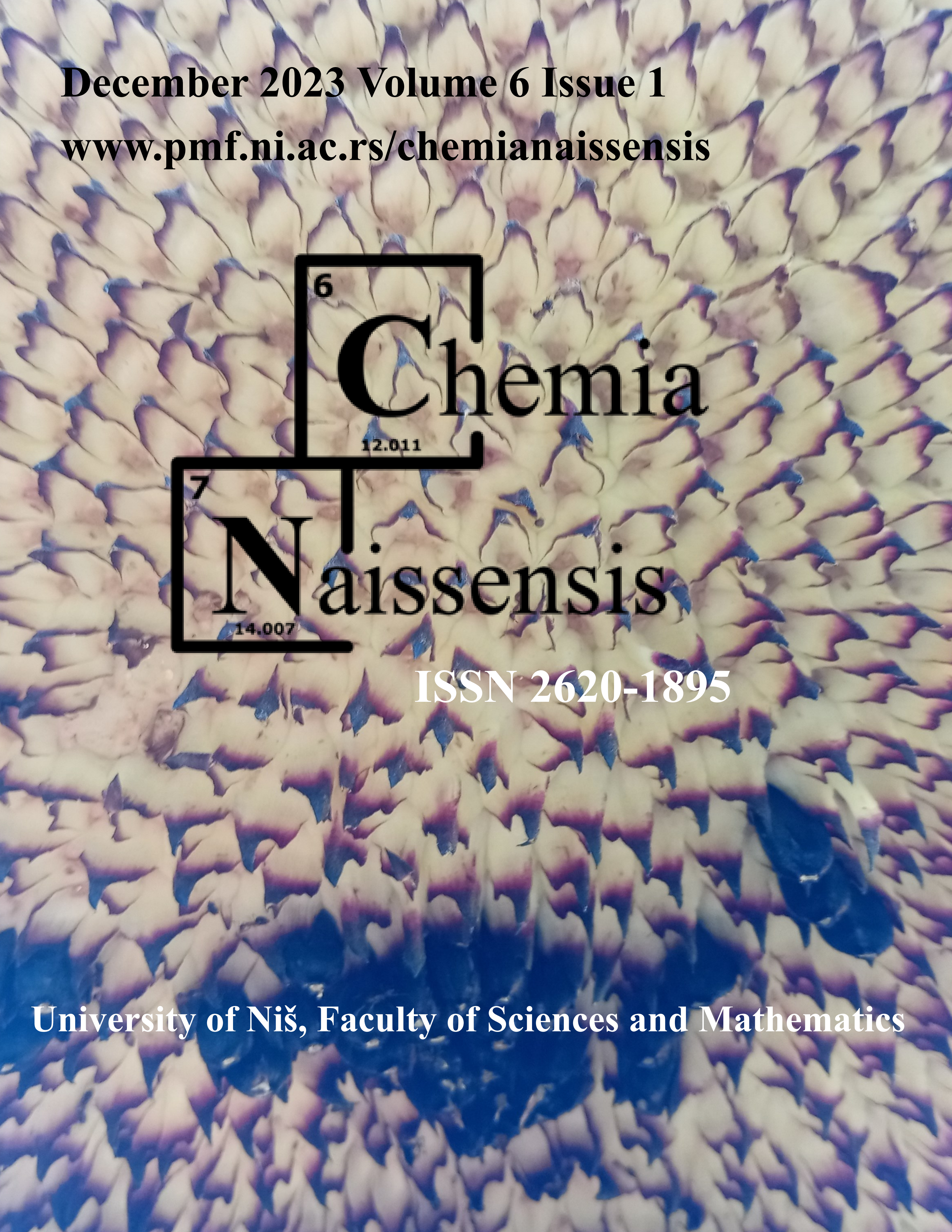

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CONTENT

Mini-review

Marija Vasić Jovev, Aleksandra Krstić, Marjan Ranđelović, Radomir Ljupković, Katarina Stepić, Aleksandra Zarubica

TiO_2-CeO_2 based composite materials and their application in photocatalysis: A short review 1
https://doi.org/10.46793/ChemN6.1.01VJ

Kompozitni materijali na bazi TiO2-CeO2 i njihova primena u fotokatalizi: kratak pregled 18

Matériaux composites à base de TiO2-CeO2 et leur application en photocatalyse : une brève revue 19

Композиционные материалы на основе TiO2-CeO2 и их применение в фотокатализе: краткий обзор 20

Verbundwerkstoffe auf TiO_2-CeO_2-Basis und ihre Anwendung in der Photokatalyse: Ein kurzer Überblick 21

Research articles

Tamara Petronijević, Đurađ Milošević, Ivana Kostić Kokić, Milica Stojković Piperac, Tatjana Anđelković, Tatjana Mihajilov Krstev, Nikola Stanković

The influence of benzyl butyl phthalate on the growth of several phytoplankton species (*Microcystis* sp., *Anabaena variabilis, Chlorella* sp., *Scenedesmus* sp.) in laboratory conditions 22
https://doi.org/10.46793/ChemN6.1.22P

Uticaj benzil butil ftalata na rast nekoliko vrsta fitoplanktona (Microcistis sp., Anabaena variabilis, Chlorella sp., Scenedesmus sp.) u laboratorijskim uslovima 41

L'influence du phtalate de benzylbutyle sur la croissance de plusieurs espèces de phytoplancton (Microcystis sp., Anabaena variabilis, Chlorella sp., Scenedesmus sp.) en conditions de laboratoire 43

Влияние бензилбутилфталата на рост некоторых видов фитопланктона (Microcystis sp., Anabaena variabilis, Chlorella sp., Scenedesmus sp.) в лабораторных условиях 45

Der Einfluss von Benzylbutylphthalat auf das Wachstum mehrerer Phytoplanktonarten (*Microcystis* sp., *Anabaena variabilis, Chlorella* sp., *Scenedesmus* sp.) unter Laborbedingungen 47

Jelena Nikolić, Milica Nikolić, Violeta Mitić, Slobodan Ćirić, Marija Dimitrijević, Milan Mitić, Vesna Stankov Jovanović

Dispersive solid phase extraction for antibiotics analysis 49

https://doi.org/10.46793/ChemN6.1.49N

Ekstrakcija disperzivnom čvrstom fazom za analizu antibiotika 64

Extraction dispersive en phase solide pour l'analyse des antibiotiques 65

Дисперсионная твердофазная экстракция для анализа антибиотиков 66

Dispersive Festphasenextraktion zur Analyse von Antibiotika 67

Vesna P. Stankov Jovanović, Marija D. Ilić, Marija S. Marković, Jelena S. Nikolić, Slobodan A. Ćirić, Marija V. Dimitrijević, Violeta D. Mitić

Heavy Metal Accumulation in *Sideritis montana* near Landfill: A Case Study 68

https://doi.org/10.46793/ChemN6.1.68SJ

Akumulacija teških metala u *Sideritis montani* u blizini deponije: studija slučaja 77

Accumulation de métaux lourds à Sideritis montana près d'une décharge : une étude de cas 78

Накопление тяжелых металлов в Sideritis montana возле свалки: практический пример 79

Schwermetallansammlung in *Sideritis montana* in der Nähe eines Deponiegeländes: Eine Fallstudie 81

Popular scientific article

Milena Nikolić, Dragana Marković Nikolić, Aleksandar Zdravković, Aleksandra Pavlović

Marigold – an ornamental plant with medicinal application 82
https://doi.org/10.46793/ChemN6.1.82N

Neven – ukrasna biljka sa lekovitom primenom 93

Souci – une plante ornementale aux applications médicinales 94

Бархатцы – декоративное растение лекарственного назначения 95

Ringelblume – eine Zierpflanze mit medizinischer Anwendung 96

Chemia Naissensis, Vol 6, Issue 1, MINI REVIEW, 1-17 **DOI: 10.46793/ChemN6.1.01VJ**

TiO_2-CeO_2 Based Composite Materials and Their Application in Photocatalysis: A Short Review

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ABSTRACT

In light of the ever-increasing problem of environmental pollution, photocatalysis represents one of the most promising solutions for the remediation/decomposition of wastewater pollutants. Among the materials used for the photocatalytic degradation of organic pollutants, titanium dioxide has been widely investigated due to its unique and favourable properties. On the other hand, its limitation in absorbing only about 5% of solar light and its relatively wide band gap and rapid recombination of electron-hole pairs restricts its practical application. In order to overcome this drawback and improve photocatalytic ability, various methods have been investigated. Some of the significant methods that have been extensively studied over the past decades involve the preparation of binary oxides, mixed oxide systems, composite materials, etc. This short review provides a comprehensive summary of scientific reports of titania-ceria composite materials and their applications in photocatalytic reactions reported in the literature.

Keywords: *TiO_2-CeO_2, composite materials, photocatalysis*

Introduction

The problem of environmental pollution is continuously increasing due to the constant development of industry in the growing world. Wastewater from industry often contains carcinogenic, toxic and mutagenic compounds/pollutants. These pollutants present a great threat/concern to the environment/ecosystem and all living world (plants, animals and humans) (Kumari et al., 2023; Kusmierek, 2020). One of the most studied methods used to remove environmental pollutants is photocatalysis. It is considered ecologically friendly and can be applied to degrading a broad spectrum of pollutants in water treatments (Kumari et al., 2023; Kusmierek, 2020; Malekkiani et al., 2022).

TiO_2 is one of the most investigated versatile materials in photocatalysis as it is considered a non-toxic, chemically stable, inert, and relatively inexpensive material. It is known for efficiently deleting various organic pollutants from water systems under relatively mild conditions (Kumari et al., 2023; Kusmierek, 2020; Malekkiani et al., 2022). In addition to TiO_2 being characterised by numerous favourable characteristics, the main problem for practical and commercial use of titania is its relatively wide band gap (3.0 to 3.2 eV) and limitation to use only 3-5% of the entire solar radiation spectrum for its activation (Kumari et al., 2023; Malekkiani et al., 2022; Stefa et al., 2020).

Various methods have been used to overcome these limitations- such as metal doping, synthesis of composites (TiO_2-CeO_2, Cu_2O-TiO_2, ZnO-TiO_2, Bi_2O_3-TiO_2) (Bessekhouad et al., 2005; Bian et al., 2008; Malekkiani et al., 2022; Qu et al., 2014; Topkaya 2014; Yang et al., 2007), binary oxides (TiO_2-SiO_2, TiO_2-ZnO, SnO_2-TiO_2 etc.), (Luo et al., 2015; Siwińska-Stefańska et al., 2018; Tricoli et al., 2009), multi mixed oxides (CuO-ZnO-Al_2O_3-ZrO_2) (Kumari et al., 2023; Kusmierek, 2020; Luo et al., 2015; Qu et al., 2014; Siwińska-Stefańska et al., 2018; Velu et al., 2002) etc.

The TiO_2-CeO_2-based composites have been broadly investigated due to ceria's distinct features, especially its Ce^{3+}/Ce^{4+} redox couple, easy transition between these oxidation states, high UV absorption ability, and remarkable oxygen storage capacity (Malekkiani et al., 2022; Qu et al., 2014).

Similar to TiO_2, CeO_2 has found a broad application in the photocatalytic processes of organic pollutants degradation, and it is one of the most investigated materials in literature, following TiO_2 (Kusmierek, 2020). This can be attributed to numerous favourable characteristics possessed by CeO_2, such as high thermal and chemical stability, a high oxygen storage capacity, high hardness, relatively low cost, etc. (Kumari et al., 2023; Kusmierek, 2020; Stefa et al., 2020; Vita 2020). CeO_2 is a rare-earth metal oxide that has a band gap in the range of 2.6 to 3.4 eV and can absorb a somewhat larger portion of the solar spectrum compared to TiO_2 (Kumari et al., 2023; Kusmierek, 2020; Malekkiani et al., 2022; Vita 2020). However, the position of its conduction band (CB) and valence (VB) band restricts its application, as it has low photonic effectiveness of solar energy (Kumari et al., 2023; Kusmierek, 2020).

It has been reported in the literature that titania's ability to absorb in the visible region can be achieved by coupling (integrating) titania with rare-earth metal oxides such as CeO_2 (Kumari et al., 2023; Malekkiani et al.; 2022 Vita 2020). The presence of ceria ions can facilitate the transfer of pollutant molecules onto the surface of the TiO_2 catalyst since Ce ions can form complexes with organic pollutants (Kumari et al., 2023). Furthermore, replacing Ti with Ce ions in the TiO_2 lattice introduces impurity levels in the band gap. This process increases the absorption capability of TiO_2, shifting its absorption towards the visible region, and provides better charge carrier separation (h^+/e^- pairs), thus enhancing the photocatalytic activity/efficiency of the TiO_2 catalyst (Kumari et al., 2023; Malekkiani et al., 2022). Additionally, CeO_2 can store oxygen from a water solution, increasing the amount of chemisorbed oxygen on the TiO_2 surface and consequently enhancing the degradation rate of pollutants, as oxygen plays a key role in oxidation reactions (Malekkiani et al., 2022). It has been reported in the literature that the TiO_2-CeO_2 composite has improved physico-chemical properties compared to pure titania when ceria is incorporated into TiO_2 (Kumari et al., 2023; Moongraksathum & Chen, 2017). In addition to causing the absorption edge to shift toward the visible region of the spectrum, the presence of ceria has an impact on the textural and structural characteristics of titania, stabilises the anatase crystal phase by suppressing the anatase to rutile phase transformation at elevated temperatures, thus consequently improving the photocatalytic activity (Kumari et al., 2023; Moongraksathum & Chen, 2017).

This short review provides a broad summary of scientific reports detailing the different synthesis methods, their optimisation and their influence on the physicochemical properties of prepared TiO_2-CeO_2-based composite materials. Furthermore, it will observe/discuss the impact of these characteristics on the photocatalytic performance investigated in various pollutant degradation reactions published in scientific literature.

Synthesis methods for the preparation of TiO_2-CeO_2-based composite

Various methods for synthesising TiO_2-CeO_2-based composite materials have been reported in scientific literature, including sol-gel (Moongraksathum & Chen, 2017; Qu et al., 2014; Wandre et al., 2016), hydrothermal (Stefa et al., 2020), co-precipitation (Zhang et al., 2018), electrodeposition, flame spray pyrolysis etc. (Kusmierek, 2020; Qu et al., 2014). It is well known that processing parameters and synthesis/preparation routes significantly affect the physicochemical characteristics of catalytic materials (such as textural, structural, morphological, etc.), consequently determining their photocatalytic performances (Kusmierek, 2020). In this paper, selected methods for synthesising titania-ceria composites will be discussed, as well as their influence on physical-chemical properties.

A group of authors, T. M. Wandre et al., reported (Wandre et al., 2016) the simple and economical sol-gel method using a cationic surfactant for preparing a series of TiO_2-CeO_2 composites. Titanium (IV) isopropoxide was used as the titania precursor, while cerium nitrate hexahydrate was used as the ceria precursor. Firstly, CeO_2 nanoparticles (NPs) were prepared and used later in the sol-gel synthesis of the TiO_2-CeO_2 nanocomposite. Cetyl trimethyl ammonium bromide (CTAB) was used as a capping agent, and ammonia facilitated the precipitation of the material. After the precipitation was finished, the precipitate was washed, filtered and dried, after which calcination was performed at a temperature of 500° C. Similarly, the authors prepared pure TiO_2 without adding CeO_2 for comparison (Wandre et al., 2016).

N. Zhang et al. (Zhang et al., 2018) reported the preparation/synthesis of sulfur-resistant TiO_2-CeO_2 composite material via the co-precipitation method. The authors used $TiOSO_4 \cdot 2H_2O$ and $Ce(NO_3)_3 \cdot 9H_2O$ as precursors for TiO_2 and CeO_2, respectively, to obtain TiO_2-CeO_2 catalyst powder with a molar ratio Ti: Ce=9:1. After the precipitation was completed, with the use of $NH_3 \cdot H_2O$ solution, the obtained residue was washed, dried and calcined for 3 hours at a temperature of 500 °C. The authors also prepared pure TiO_2 and CeO_2 using the same procedure (Zhang et al., 2018).

S. Stefa et al. (2020) reported using four different methods to prepare CeO_2/TiO_2 nanostructured oxides/composites. They used the hydrothermal method in one and two steps, precipitation and the Stöber method. The $TiOSO_4$ and $Ti(OBu)_4$, and $Ce(NO_3)_3{\cdot}6H_2O$ were used as a precursors for preparing CeO_2/TiO_2 materials with a Ce/Ti atomic ratio 4. The chemicals used by the authors were of analytical grade. Moreover, they studied the effect of these methods on the physico-chemical properties of as-synthesised materials. Furthermore, the authors used the CO oxidation performance for the catalytic evaluation of prepared catalysts (Stefa et al., 2020).

S. Ameen et al. (2014) synthesised CeO_2-TiO_2 nanocomposite material via facile solution-processed method. They used a Teflon-beaker autoclave, which was kept at 120° C for 48 hours. After the process, the autoclave was rested to cool down to room temperature. The resulting material was filtered, washed, and dried at a mild temperature overnight. This procedure was followed by a calcination process at 450° C (Ameen et al., 2014).

F. K. Dokan and Kuru (Dokan & Kuru, 2021) reported preparing an ultrafine composite material based on TiO_2-CeO_2 using the following procedure. Firstly, TiO_2 microspheres were prepared using the hydrothermal method. Afterwards, CeO_2 nanoparticles were loaded in different ratios/proportions onto the mentioned TiO_2 microparticles via the sol-gel method, using Pluronic 123 surfactant. Titanium (IV) butoxide ($Ti(OBu)_4$) was used as the precursor for TiO_2. The hydrothermal treatment was performed in a stainless steel autoclave at 240 °C for 2 hours. After the reaction, the precipitate was filtered and washed with water and ethyl alcohol. The next step was drying the material at 80 °C, and to obtain microspheres of TiO_2, the calcination was performed at 500 °C for 4 hours. Cerium nitrate (($Ce(NO_3)_3{\cdot}6H_2O$) was used as a precursor for the preparation of ultrafine nano-spherical TiO_2-CeO_2 composites. The preparation of composites was achieved by the surfactant-supported sol-gel method, as mentioned above (Dokan & Kuru, 2021).

The proposed schematic diagram of TiO_2-CeO_2-based material under UV and Visible light

The preparation method of the composite can significantly influence the position of the valence and conduction bands, affecting the photocatalytic performance of the prepared composite materials (Kusmierek, 2020). A heterojunction can form after coupling CeO_2 with TiO_2 (Jiang et al., 2018; Kumari et al., 2023;). Figure 1. (Kusmierek, 2020), presented/reported by E. Kusmierek, shows the possible photo-excitation mechanism of CeO_2/TiO_2-based composites.

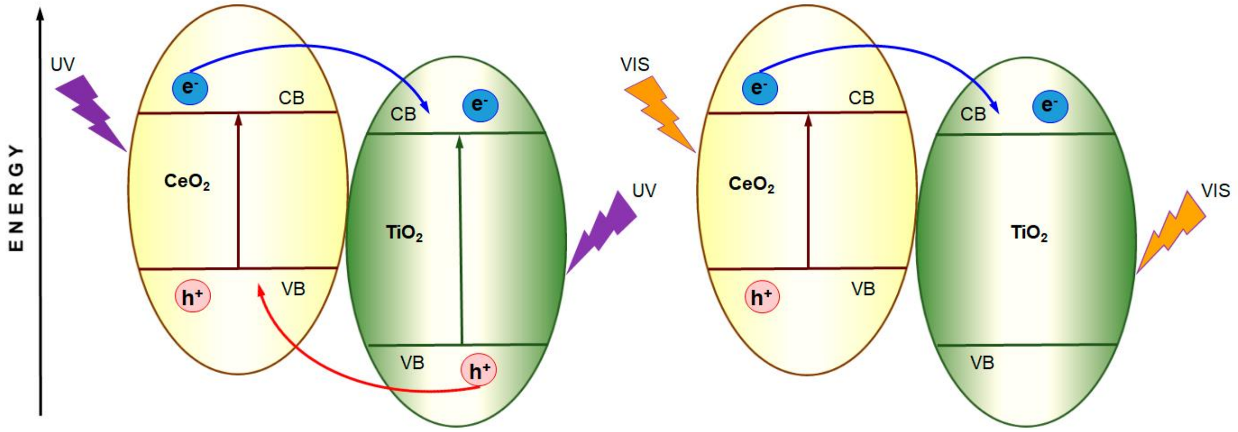

Figure 1. The proposed diagram of CeO_2/TiO_2 composite photo-excitation under UV and Visible light irradiation (Kusmierek, 2020)

After illumination by UV light, a CeO_2/TiO_2-based composite exhibits the formation of electron vacancies (holes) in the valence band (VB) and electrons in the conduction band (CB) of both semiconductors, resulting in the creation of h^+/e^- pairs. Electrons move from the valence band to the conduction band, generating electron vacancies (h^+) in the valence band. This phenomenon is depicted in Fig. 1 and reported by E. Kusmierek (Kusmierek, 2020). Ceria's VB and CB possess higher energy levels compared to titania, initiating photogenerated electrons to transfer from the CB of CeO_2 to the lower energy CB of TiO_2 (Ameen et al., 2014; Kusmierek, 2020; Tuyen et al., 2018). At the same time, photogenerated holes transfer from the VB of Titania to the VB of Ceria. This way, with the presence of O_2 and water, the formation of superoxide anion radicals ($O_2^{\bullet -}$) and hydroxyl radicals ($OH^{\bullet}$) occurs, as presented by the following reaction mechanism (Dokan & Kuru, 2021; Kusmierek, 2020):

$TiO_2 + h\nu \rightarrow e^{-}_{CB} + h^{+}_{VB}$

$Ce^{4+} + e^{-}_{CB} \rightarrow Ce^{3+}$

$Ce^{3+} + O_2 \rightarrow Ce^{4+} + O_2^{\bullet-}$

$H_2O + h^{+}_{VB} \rightarrow OH^{\bullet} + H^{+}$

$O_2^{\bullet-} + 4H^{+} \rightarrow 2OH^{\bullet}$

Ce^{4+} ions scavenge the photoexcited electrons, thus suppressing the recombination of h^{+}/e^{-} species and enhancing the photocatalytic efficiency of the composite materials (Kusmierek, 2020; Tuyen et al., 2018).

Characterisation of TiO_2-CeO_2-based composite materials

Numerous techniques and methods have been used by scientists for thorough characterisation of TiO_2-CeO_2-based composite materials such as X-ray diffraction (XRD), energy dispersive X-ray spectroscopy (EDS), Brunauer–Emmett–Teller (BET) by N_2 adsorption-desorption measurements, Fourier-transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM), photoluminescence (PL) spectroscopy, field emission scanning electron microscopy (FE-SEM), transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS), H_2 temperature-programmed reduction (H_2-TPR), high-resolution transmission electron microscopy (HRTEM) and others (Ameen et al., 2014; Dokan & Kuru, 2021; Stefa et al., 2020; Wandre et al., 2016; Zhang et al., 2018;).

As detailed above, T. M. Wandre et al. reported synthesising and comprehensively characterising TiO_2-CeO_2 nanocomposites (Wandre et al., 2016). Various methods were used for detailed characterisation by the authors, including XRD with EDS attachment, UV–Vis diffuse reflectance spectra, FTIR spectra, PL spectra, XPS, and SEM. In this short review, the XRD and SEM methods will be discussed, as well as the photocatalytic activity of the samples prepared and reported by T. M. Wandre et al. (Wandre et al., 2016).

The authors (Wandre et al., 2016) used the X-ray diffraction method for crystallinity and phase determination. XRD results revealed that in the TiO_2-CeO_2 composite, the transformation from anatase phase to rutile was suppressed. Hence, the presence of ceria secured the stabilisation of the anatase phase. Furthermore, they noted that with an increase in CeO_2 content, the intensity of the peak characteristic for the anatase phase decreased (Wandre et al., 2016).

The authors (Wandre et al., 2016) investigated the morphological properties using SEM imaging. As the authors (Wandre et al., 2016) discussed, SEM micrographs revealed a similar fine particle structure of all samples that differed in particle sizes. They noted and reported that an increase in CeO_2 content led to a decrease in TiO_2 particle sizes; thus, the presence of CeO_2 restricted particle growth. In addition, the authors suggested that even/homogeneous dispersion of CeO_2 nanoparticles onto the TiO_2 surface was observed (Wandre et al., 2016).

N. Zhang et al. (Zhang et al., 2018) reported a thorough characterisation of the composite based on TiO_2-CeO_2 they prepared, as mentioned before. The methods they used were XRD, Raman spectra, BET XPS, H_2-TPR and oxygen storage capacity (OSC) technique. The XRD results reported and presented by the authors N. Zhang et al. are shown in Figure 2. (Zhang et al., 2018).

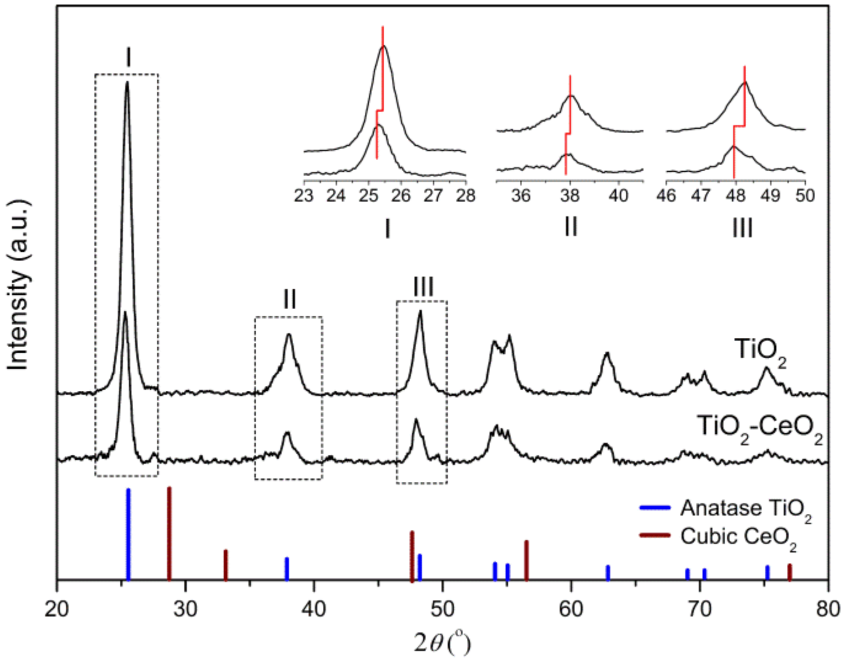

Figure 2. XRD patterns of the TiO_2 and TiO_2-CeO_2 catalysts (Zhang et al., 2018)

The XRD spectra obtained by the authors (Zhang et al., 2018) indicated the presence of the anatase TiO_2 crystal phase in both samples, pure TiO_2 and TiO_2-CeO_2. The authors N. Zhang et al. discussed that the position of peaks characteristic of the anatase TiO_2 crystal phase shifted to smaller angles in the TiO_2-CeO_2 composite compared to pure TiO_2 and suggested that this shift could be due to the possible entry of Ce^{4+} ions into the TiO_2 lattice, so complete CeO_2 crystals were not formed. Moreover, the authors reported the restriction of crystal growth in the TiO_2-CeO_2 composite material (Zhang et al., 2018). Also, no characteristic peaks for CeO_2 were observed. For the catalyst evaluation, the authors studied the oxidation test of the diesel-soluble organic fraction and noted an enhancement in the catalytic performances of the TiO_2-CeO_2 composite (Zhang et al., 2018).

S. Stefa et al. provided an extensive characterisation of the series of the materials they prepared, including BET, XRD, SEM/EDX, TEM, H_2-TPR (Stefa et al., 2020).

The textural characteristics presented by the S. Stefa et al. demonstrated that the sample synthesised by the one-step hydrothermal method (CeO_2/TiO_2-H1) had the highest value for BET surface area, followed by bare CeO_2 nanorods (CeO_2 NRs), CeO_2/TiO_2-S (sample prepared by the Stöber method), CeO_2/TiO_2-H2 (prepared by the two-step hydrothermal method), and the CeO_2/TiO_2-P sample (prepared by the precipitation), which demonstrated the lowest BET surface area value in the series of the prepared materials (Stefa et al., 2020).

S. Stefa et al. examined the structural characteristics of the XRD method (Stefa et al., 2020). Their results revealed the presence of the cubic fluorite structure of ceria in all samples. However, the small intensity of the peak characteristic for the anatase phase at around 25° was hardly noticed only in the sample prepared by the Stöber method (CeO_2/TiO_2- S), which was ascribed to the low loading of titania, as well as its high dispersion, by the authors (Stefa et al., 2020).

The authors discussed the morphological properties obtained by TEM and SEM-EDS methods (Stefa et al., 2020). TEM and HRTEM images obtained by S. Stefa et al. are shown in Figure 3. (Stefa et al., 2020). They noticed that the bare CeO_2 sample displayed a rod-like morphology and the samples prepared by the hydrothermal method in both one and two steps. In contrast, the sample obtained by the precipitation method showed irregular shapes. Furthermore, in the TEM images of the sample prepared by the one-step hydrothermal method, they discussed that no separated TiO_2 nanoparticles were identified. In contrast, in the sample prepared by the two-step hydrothermal method, TiO_2 nanoparticles were clearly observed (Stefa et al., 2020). The sample prepared by the Stöber method (CeO_2/TiO_2- S) displayed a rod-like morphology without separated TiO_2 particles, but the uniform distribution of TiO_2 around CeO_2 nanorods, as discussed by the authors S. Stefa et al. (Stefa et al., 2020).

These results clearly indicated that the choice of the preparation method and synthesis conditions/parameters significantly influenced the physicochemical properties of prepared samples (Stefa et al., 2020).

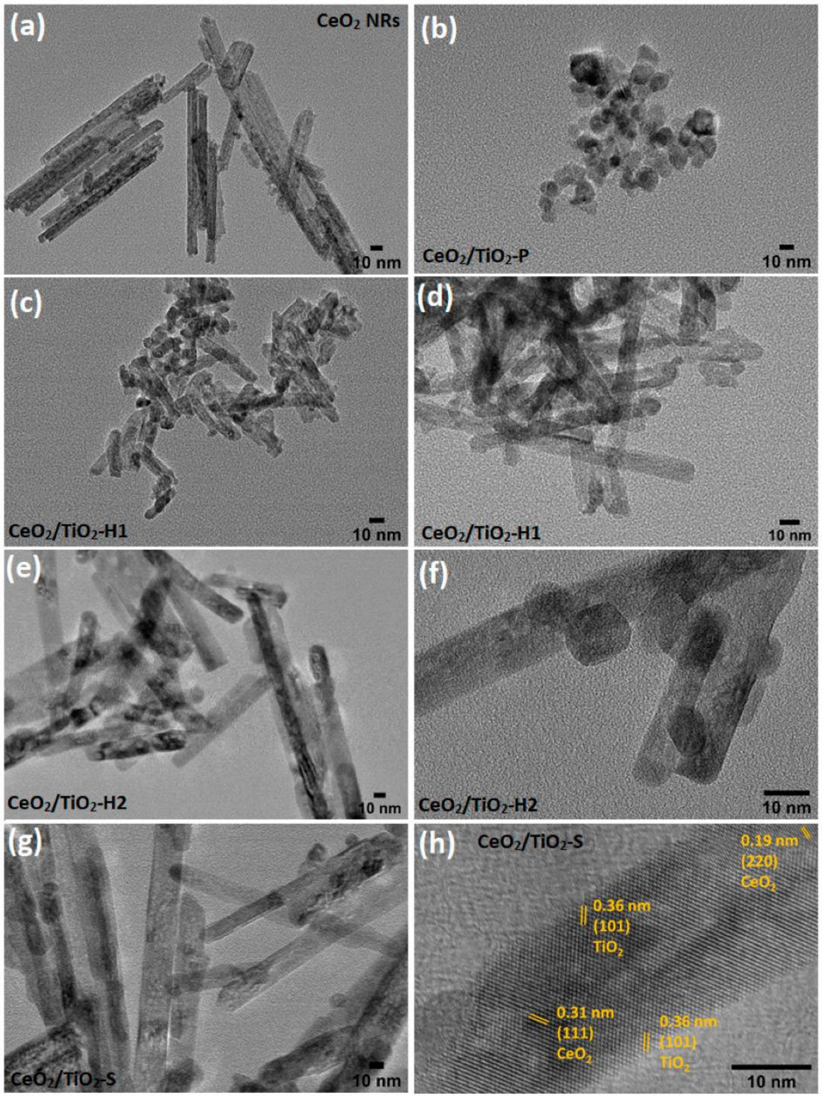

Figure 3. TEM images of the samples: (a) CeO_2 nanorods; (b) CeO_2/TiO_2-P; (c,d) CeO_2/TiO_2-H1; (e,f) CeO_2/TiO_2-H2; (g) CeO_2/TiO_2-S; (h) HRTEM images of CeO_2/TiO_2-S obtained by S. Stefa et al. (Stefa et al., 2020)

S. Ameen et al. (Ameen et al., 2014) published XRD results for structural characterisation, and they used FESEM and TEM for the determination of morphological properties. The FESEM and TEM images obtained by the authors showed that the large CeO_2 particles were uniformly embedded into small TiO_2 nanoparticles, which indicated/proved an excellent interaction and good mixing of CeO_2 into the TiO_2 in CeO_2-TiO_2 composite material (Ameen et al., 2014). Furthermore, the XRD spectra revealed the presence of diffraction peaks characteristic of anatase TiO_2 and cubic fluorite CeO_2 structure at 25,3° and 28,3°, respectively. These results revealed a significant red shift in the CeO_2/TiO_2 composite, indicating a shift to higher wavelength, which indicated the incorporation of Ce cations into the lattice of TiO_2 in TiO_2-CeO_2 composite material as discussed by the authors (Ameen et al., 2014).

F. K. Dokan and Kuru (Dokan & Kuru, 2021) thoroughly characterised the physicochemical properties of the TiO_2-CeO_2 prepared materials described earlier, which included techniques such as XRD, EDX, FE-SEM, BET, FTIR, etc. The BET results provided data on the textural properties of the materials prepared by authors F. K. Dokan and Kuru (Dokan & Kuru, 2021). The results obtained and presented by the authors revealed that the surface area increased with the increase in CeO_2 content (Dokan & Kuru, 2021). It has been reported that a high surface area is a favourable parameter, as it facilitates/promotes the adsorption of pollutant molecules on the catalytic surface, therefore improving/enhancing photocatalytic ability (Dokan & Kuru, 2021, Wang et al., 2013).

Application/testing of (photocatalytic) activity of TiO_2-CeO_2-based composite materials

A wide spectrum of hazardous pollutants has been investigated in photocatalytic degradation by titania-ceria composite materials under UV and/or Visible light.

The T. M. Wandre et al. (Wandre et al., 2016) reported investigating the photocatalytic application in the degradation reaction of methyl orange (MO) under UV irradiation (applied wavelength of 365 nm using a mercury lamp) and sunlight. Before UV irradiation, an adsorption-desorption equilibrium was secured. To estimate the photocatalytic activity, they utilised/used the UV-Vis-NIR spectrophotometer to observe the changes in the concentration of the tested solution.

The authors (Wandre et al., 2016) published that in the series of TiO_2-CeO_2 composite materials, all samples prepared with different ceria content showed improved photocatalytic activity compared to pure TiO_2. They discussed that as the CeO_2 content increased up to 30 %, the degradation rate increased and then decreased when the CeO_2 content was 50 %. It was concluded that the composite sample TiO_2-CeO_2 (7:3) exhibited the highest degradation rate of MO in the observed photocatalytic reactions and also showed outstanding performance in sunlight (Wandre et al., 2016).

S. Stefa et al. studied the CO oxidation performance order to investigate the catalytic activity of the samples they prepared based on CeO_2/TiO_2, CeO_2-NRs, and commercial TiO_2 that was used for comparison (Stefa et al., 2020). Their results showed that the oxygen storage capacity significantly impacted catalytic activity. The authors concluded that with an increase in oxygen storage capacity, the activity was improved. The sample prepared by the Stöber method (CeO_2/TiO_2-S) displayed the highest reaction rate (CO conversion), followed by the sample prepared by the one-step hydrothermal method. Their results clearly indicated that the synthesis method and preparation route significantly impacted the prepared ceria-titania materials' structural, morphological and other properties and that oxygen storage capacity was crucial for their catalytic performance (Stefa et al., 2020).

As detailed above, S. Ameen et al. (2014) reported the results of testing the CeO_2-TiO_2 composite they prepared for the photodegradation of bromophenol (Bph) dye under visible light. The results revealed outstanding efficiency of the CeO_2-TiO_2 composite, with 72% dye degradation after 3 hours, compared to only 6% when bare TiO_2 was used. This was attributed to enhanced charge separation (e-/h+ pairs) and higher adsorption capacity. The authors also provided mass results that indicated the degradation of Bph dye into less harmful chemicals under light illumination (Ameen et al., 2014).

The F. K. Dokan and Kuru (Dokan & Kuru, 2021) examined the photocatalytic activity of TiO_2 and TiO_2-CeO_2 nanocomposites in the photocatalytic degradation of Methylene blue dye under 365 nm UV light. The TiO_2-CeO_2 composite showed a higher degradation rate compared to TiO_2 microspheres. However, the authors discussed a phenomenon that with an increase in CeO_2 content, the surface area increased, and the degradation rate decreased. This decrease resulted from the increased coverage of the TiO_2 surface by CeO_2 microns, making it difficult for UV to stimulate/activate the TiO_2 catalyst surface. Therefore, it was concluded that for photocatalytic efficiency, it was fundamental/crucial to optimise the CeO_2 content, proving that only 0.1% was sufficient for achieving excellent photocatalytic performance (Dokan & Kuru, 2021).

Conclusion

Environmental contamination presents a challenging global issue, with industrial development constantly contributing to increasing the need for the improvement of methods and techniques used in combating pollution. This methods should be environmentally friendly, inexpensive and should not have a harmful impact on the environment during their application.

Recently, composite materials based on TiO_2 and other metal oxides have attracted significant attention from scientists, particularly the TiO_2-CeO_2-based composite materials that was the focus of this short review. The scientific reports discussed clearly indicate the existence of a synergistic effect between TiO_2 and CeO_2 that are reflected on the characteristics of the TiO_2-CeO_2-based composite material and significantly improves its activity. The presence of CeO_2 extended the light absorption towards the visible spectrum, enhanced charge separation and improved the overall photocatalytic performance of TiO_2-CeO_2 composite material compared to pure TiO_2.

Within the scope of this short review, different synthesis methods, characterization methods/techniques, and the catalysts' activities of TiO_2-CeO_2-based materials published in scientific papers were observed. Based on the discussed scientific papers, the synthesis method, together with optimising experimental conditions, has a crucial role in obtaining favourable physicochemical characteristics that further influence these materials' (photo)catalytic activity in the degradation of organic pollutants to less harmful or completely benign to the environment.

The composite materials based on TiO_2-CeO_2 exhibit unique physico-chemical properties that exceed the limitations/restrictions of individual components, thus offering a promising solution to environmental challenges regarding the green chemistry approach.

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None.

References

Ameen, S. Akhtar, M. S., Seo, H.-K., & Shin, H.-K. (2014). Solution-processed CeO_2/TiO_2 nanocomposite as potent visible light photocatalyst for the degradation of bromophenol dye. Chemical Engineering Journal, 247, 193–198.

Bessekhouad, Y., Robert, D., & Weber, J.-V. (2005). Photocatalytic activity of Cu_2O/TiO_2, Bi_2O_3/TiO_2 and $ZnMn_2O_4/TiO_2$ heterojunctions. Catalysis Today, 101, 315−321.

Bian, Z., Zhu, J., Wang, S., Cao, Y., Qian, X., & Li, H. (2008). Self-assembly of active Bi_2O_3/TiO_2 visible photocatalyst with ordered mesoporous structure and highly crystallised anatase. The Journal of Physical Chemistry C, 112, 6258−6262.

Dokan, F. K., & Kuru, M. (2021). A new approach to optimise the synthesis parameters of TiO_2 microsphere and development of photocatalytic performance. Journal of Materials Science: Materials in Electronics, 32, 640-655.

Jiang, H., Li, M., Liu, J., Li, X., Tian, L., & Chen, P. (2018). Alkali-free synthesis of a novel heterostructured CeO_2-TiO_2 nanocomposite with high performance to reduce Cr(VI) under visible light. Ceramics International, 44, 2709–2717.

Kumari, V., Sharma, A., Kumar, N., Sillanpaa, M., Makgwane, P. R., Ahmaruzzaman, Md., Hosseini-Bandegharaei, A., Manju Rani, M., & Chinnamuthu, P. (2023). TiO_2-CeO_2 assisted heterostructures for photocatalytic mitigation of environmental pollutants: A comprehensive study on band gap engineering and mechanistic aspects. Inorganic Chemistry Communications, 151, 110564.

Kusmierek, E. (2020). A CeO_2 Semiconductor as a Photocatalytic and Photoelectrocatalytic Material for the Remediation of Pollutants in Industrial Wastewater: A Review. Catalysts, 10 (12), 1435. https://doi.org/10.3390/catal10121435

Luo, S., Nguyen-Phan, T.-D., Johnston-Peck, A.C., Barrio, L., Sallis, S., Arena, D.A., Kundu, S., Xu, W., Piper, L.F.J., Stach, E.A., Polyansky, D.E., Fujita, E., Rodriguez, J.A., & Senanayake, S. D. (2015). Hierarchical Heterogeneity at the CeO_x−TiO_2 Interface: Electronic and Geometric Structural Influence on the Photocatalytic Activity of Oxide on Oxide Nanostructures. The Journal of Physical Chemistry C, 119, 2669−2679.

Malekkiani, M., Ravari, F., Magham, A. H. J., Dadmehr, M., Groiss, H., Hosseini, H. A., & Sharif, R. (2022). Fabrication of Graphene-Based TiO_2@CeO_2 and CeO_2@TiO_2 Core−Shell Heterostructures for Enhanced Photocatalytic Activity and Cytotoxicity. ACS Omega, 7, 30601−30621.

Moongraksathum, B. & Chen, Y.-W. (2017). CeO_2–TiO_2 mixed oxide thin films with enhanced photocatalytic degradation of organic pollutants. Journal of Sol-Gel Science and Technology, 82, 772–782.

Qu, X., Xie, D., Gao, L., & Du, F. (2014). Synthesis and photocatalytic activity of TiO_2/CeO_2 core–shell nanotubes. Materials Science in Semiconductor Processing, 26, 657–662.

Siwińska-Stefańska, K., Kubiak, A., Piasecki, A., Goscianska, J., Nowaczyk, G., Jurga, S., & Jesionowski, T. (2018). TiO_2-ZnO Binary Oxide Systems: Comprehensive Characterisation and Tests of Photocatalytic Activity. Materials, 11, 841.

Stefa, S., Lykaki, M., Fragkoulis, D., Binas, V., Pandis, P. K., Stathopoulos, V. N., & Konsolakis, M. (2020). Effect of the Preparation Method on the Physicochemical Properties and the CO

Oxidation Performance of Nanostructured CeO_2/TiO_2 Oxides. Processes, 8 (7), 847. https://doi.org/10.3390/pr8070847

Topkaya, E., Konyar, M., Yatmaz, H. C., & Öztürk, K. (2014). Pure ZnO and composite ZnO/TiO_2 catalyst plates: A comparative study for the degradation of azo dye, pesticide and antibiotic in aqueous solutions. Journal of Colloid and Interface Science, 430, 6−11.

Tricoli, A., Righettoni, M., & Pratsinis, S. E. (2009). Minimal Cross-Sensitivity to Humidity During Ethanol Detection by SnO_2-TiO_2 Solid Solutions. Nanotechnology, 20, 315502.

Tuyen, L. T. T., Quang, D.A., Toan, T. T. T., Tung, T.Q., Hoa, T. T., Mau, T. X., & Khieu, D.Q. (2018). Synthesis of CeO_2/TiO_2 nanotubes and heterogeneous photocatalytic degradation of methylene blue. Journal of Environmental Chemical Engineering, 6, 5999–6011.

Velu, S., Suzuki, K., Gopinath, C. S., Yoshida, H., & Hattori, T. (2002). XPS, XANES and EXAFS Investigations of $CuO/ZnO/Al_2O_3/ZrO_2$ Mixed Oxide Catalysts. Physical Chemistry Chemical Physics, 4, 1990−1999.

Vita, A. (2020). Catalytic Applications of CeO_2-Based Materials. Catalysts, 10, 576.

Wandre, T. M., Gaikwad, P. N., Tapase, A. S., Garadkar, K. M., Vanalakar, S. A., Lokhande, P. D., Sasikala, R., & Hankare, P. P. (2016). Sol–gel synthesised TiO_2–CeO_2 nanocomposite: an efficient photocatalyst for degradation of methyl orange under sunlight. Journal of Materials Science: Materials in Electronics 27, 825–833.

Wang, L., Wang, X., Cui, S., Fan, X., Zu, B., & Wan, C. (2013). TiO_2 supported on silica nanolayers derived from vermiculite for efficient photocatalysis. Catalysis Today, 216, 95-103.

Yang, H., Zhang, K., Shi, R.; & Tang, A. (2007). Sol−gel synthesis and photocatalytic activity of CeO_2/TiO_2 nanocomposites. Journal of the American Chemical Society, 90, 1370−1374.

Zhang, N., Yang, Z., Chen Z., Li, Y., Liao, Y., Li, Y., Gong, M., & Chen, Y. (2018). Synthesis of Sulfur-Resistant TiO_2-CeO_2 Composite and Its Catalytic Performance in the Oxidation of a Soluble Organic Fraction from Diesel Exhaust. Catalysts, 8 (6), 246. https://doi.org/10.3390/catal8060246

Chemia Naissensis, Vol 6, Issue 1, MINI REVIEW, 18 **DOI: 10.46793/ChemN6.1.01VJ**

Kompozitni materijali na bazi TiO_2-CeO_2 i njihova primena u fotokatalizi: kratak pregled

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SAŽETAK

U svetlu sve većeg problema zagađenja životne sredine, fotokataliza predstavlja jedno od najperspektivnijih rešenja za remedijaciju/razgradnju zagađivača otpadnih voda. Među materijalima koji se koriste za fotokatalitičku degradaciju organskih zagađivača, široko je istražen titan-dioksid zbog svojih jedinstvenih i povoljnih svojstava. S druge strane, njegovo ograničenje u apsorpciji samo oko 5% sunčeve svetlosti, zajedno sa relativno velikim rasponom pojasa i brzom rekombinacijom parova elektron-šupljina, sužavaju njegovu praktičnu primenu. Da bi se prevazišao ovaj nedostatak i poboljšala fotokatalitička sposobnost, istražene su različite metode. Neke od značajnih metoda koje su opsežno istražene tokom proteklih decenija uključuju pripremu binarnih oksida, mešovitih oksidnih sistema, kompozitnih materijala, itd. Ovaj kratak pregled pruža sveobuhvatan rezime naučnih izveštaja o kompozitnim materijalima titan-cer, kao i njihovoj primeni u fotokatalitičkim reakcijama opisanim u literaturi.

Ključne reči: *TiO_2-CeO_2, kompozitni materijali, fotokataliza*

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Matériaux composites à base de TiO_2-CeO_2 et leur application en photocatalyse : une brève revue

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ABSTRAIT

À la lumière du problème toujours croissant de pollution environnementale, la photocatalyse représente l'une des solutions les plus prometteuses pour l'assainissement/décomposition des polluants des eaux usées. Parmi les matériaux utilisés pour la dégradation photocatalytique des polluants organiques, le dioxyde de titane a été largement étudié en raison de ses propriétés uniques et favorables. D'un autre côté, sa capacité limitée à absorber seulement environ 5 % de la lumière solaire, ainsi que sa bande interdite relativement grande et sa recombinaison rapide des paires électron-trou, limitent son application pratique. Afin de remédier à cet inconvénient et d'améliorer la capacité photocatalytique, diverses méthodes ont été étudiées. Certaines des méthodes importantes qui ont fait l'objet d'études approfondies au cours des dernières décennies impliquent la préparation d'oxydes binaires, de systèmes d'oxydes mixtes, de matériaux composites, etc. Cette brève revue fournit un résumé complet des rapports scientifiques sur les matériaux composites titane-cérium, ainsi que leurs applications dans les réactions photocatalytiques rapportées dans la littérature.

Mots clés : *TiO2-CeO2, matériaux composites, photocatalyse*

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Композиционные материалы на основе TiO_2-CeO_2 и их применение в фотокатализе: краткий обзор

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Резюме

В свете постоянно растущей проблемы загрязнения окружающей среды, фотокатализ представляет собой одно из наиболее многообещающих решений по очистке/разложению загрязнителей сточных вод. Среди материалов, используемых для фотокаталитического разложения органических загрязнителей, диоксид титана широко исследуется благодаря его уникальным и благоприятным свойствам. С другой стороны, его в поглощении всего около 5% солнечного света, а также его относительно большая запрещенная зона и быстрая рекомбинация электронно-дырочных пар ограничивают его практическое ограничение применение. Чтобы преодолеть этот недостаток и улучшить фотокаталитические способности, были исследованы различные методы. Некоторые из важных методов, которые широко исследовались за последние десятилетия, включают получение бинарных оксидов, смешанных оксидных систем, композиционных материалов и т. д. Этот краткий обзор содержит исчерпывающее резюме научных отчетов о композиционных материалах на основе диоксида титана и церия, а также об их применении в фотокаталитических реакциях, описанных в литературе.

Ключевые слова: *TiO_2-CeO_2, композиционные материалы, фотокатализ.*

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Verbundwerkstoffe auf TiO_2-CeO_2-Basis und ihre Anwendung in der Photokatalyse: Ein kurzer Überblick

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ABSTRACT

Angesichts des immer größer werdenden Problems der Umweltverschmutzung stellt die Photokatalyse eine der vielversprechendsten Lösungen zur Sanierung/Zersetzung von Schadstoffen im Abwasser dar. Unter den Materialien, die für den photokatalytischen Abbau organischer Schadstoffe verwendet werden, wurde Titandioxid aufgrund seiner einzigartigen und positiven Eigenschaften extensiv untersucht. Andererseits schränkt seine begrenzte Absorption von nur etwa 5 % des Sonnenlichts zusammen mit seiner relativ großen Bandlücke und der schnellen Rekombination von Elektron-Loch-Paaren seine praktische Anwendung ein. Es wurden verschiedene Methoden untersucht, um diesen Nachteil zu überwinden und die photokatalytische Fähigkeit zu verbessern. Zu den bedeutenden Methoden, die in den letzten Jahrzehnten ausführlich untersucht wurden, gehört die Herstellung von Binäroxiden, Mischoxidsystemen und Verbundwerkstoffen etc. Dieser kurze Überblick bietet eine breite Zusammenfassung wissenschaftlicher Berichte über Titand-Ceroxid-Verbundwerkstoffe sowie über ihre Anwendungen in photokatalytischen Reaktionen, wie sie in der Literatur beschrieben werden.

Schlüsselwörter: TiO_2-CeO_2, *Verbundwerkstoffe, Photokatalyse*

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The influence of benzyl butyl phthalate on the growth of several phytoplankton species (*Microcystis* sp., *Anabaena variabilis*, *Chlorella* sp., *Scenedesmus* sp.) in laboratory conditions

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ABSTRACT

Phthalic acid esters (PAEs) are organic compounds extensively used as plasticisers. Their widespread use has resulted in their presence in aquatic and terrestrial ecosystems, making them a high-risk pollutant. PAEs are detrimental to human health as they disrupt the endocrine system and can potentially cause cancer. Although their impact on humans is relatively well-known, more research is necessary to comprehend their effects on phytoplankton. This work aimed to examine the influence of different concentrations (50, 100, 150, 200, 250 µg/L) of benzyl butyl phthalate (BBP) on the growth of several most common phytoplankton species (*Microcystis* sp., *Anabaena variabilis, Chlorella* sp., *Scenedesmus* sp.) in laboratory conditions. Phytoplankton growth was monitored spectrophotometrically to determine the concentration of chlorophyll *a*. The results showed that higher concentrations of BBP significantly inhibited the growth of *A. variabilis* and *Microcystis* sp. Green algae showed a considerably lower sensitivity, especially *Chlorella* sp., where significant growth inhibition was not observed. After the experiment, the detection and quantification of BBP in extract samples were performed using gas chromatography with mass spectrometry (GC-MS). BBP was detected only in the extracted sample with *Scenedesmus* sp., but the detected concentration was insignificant. The results indicate that all tested organisms could probably absorb and metabolize BBP, of which *Scenedesmus* sp. has the least ability.

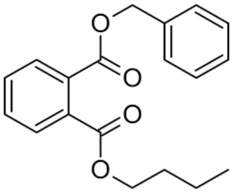
Keywords: *algae, chlorophyll a, GC-MS, pollution*

Introduction

Phthalic acid esters (PAEs) are a class of organic plasticizer compounds widely used as additives in various products, including cosmetics, food packaging, construction supplies, medical equipment, home furnishings, plastic tubing, automotive components, floor tiles, insect repellents (Huang et al., 2021a; Staples, 1997). Due to their wide application, they are increasingly present in aquatic and terrestrial ecosystems, which represents a danger to the environment, considering that they are classified as priority pollutants in the USA, China, and the European Union (Zhang et al., 2016). There are several types of PAEs used for commercial and industrial purposes, of which six (dimethyl phthalate, diethyl phthalate, di-n-butyl phthalate, benzyl butyl phthalate, di (2-ethylhexyl) phthalate, and di-n-octyl phthalate) are classified as primary pollutants (Net et al., 2015). PAEs, as pollutants harm the environment and human health, disrupting the endocrine system, can exhibit anti-thyrogenic, anti-androgenic, anti-progestogenic, and anti-estrogenic properties associated with adverse health effects (Huang et al., 2021b). Given that their production is significant and that they are not tightly bound to the polymer matrix of the final product, they can easily migrate into the environment and thus be found in freshwater and marine systems, drinking water, sediment, air, and aerosols (Net et al., 2015). Once they reach aquatic systems, they can bring many ecological problems (Net et al., 2015). Therefore, to accurately evaluate the effects of PAEs on aquatic ecosystems and the potential ecological hazards they present, closely monitoring the interactions between them is imperative. That is an essential step in ensuring the safety and sustainability of the environment.

Benzyl butyl phthalate (BBP) is often used as a plasticizer for vinyl tiles (CMA, 1999). Apart from these purposes, it is also used to produce automotive components, food packaging, perfumes, cosmetics, adhesives, and artificial leather (Li et al., 2021). It has been reported that BBP can promote breast cancer stem cell expansion (Cao et al., 2023), participate in DNA damage and methylation (Tsai et al., 2014), and induce significant neurodevelopmental damage (Miodovnik et al., 2014). In addition to humans, this pollutant has been shown to have toxic effects on other organisms, including chironomids (Call et al., 2001; dos Santos Morais et al., 2020; Llorente Ortega, 2022; Planello et al., 2011), cladocerans (Li et al., 2021; Wang et al., 2011), mice and rats (Ambe et al., 2019; Agas et al., 2007; Ema et al., 2003; Ema & Miyawaki, 2002; Gray et al., 2000; Piersma et al., 2000), the marine univalve Haliotis diversicolor super-texta (Liu et al., 2009), daphnia (Gledhill et al., 1980), fish (Bhaisare et al., 2022; Gledhill et al., 1980) and algae (Gledhill, 1980). The structure and physiochemical properties of BBP are shown in Table 1.

Table 1: Structure and physicochemical properties of BBP

Properties	Benzyl butyl phthalate	References
Molecular formula	$C_{19}H_{20}O_4$	Staples et al. (1997)
Chemical structure		Bogdanovic (2021)
Molecular weight	302.39	Staples et al. (1997)
Water solubility (mg/L)	2.7	Staples et al. (1997)

Chemically synthesized PAEs are widely used for industrial purposes due to improved product quality. However, recent research has shown that PAEs, apart from chemical means, can also be synthesized naturally by synthesizing some organisms, including algae (Babu et Wu, 2010a). The natural synthesis of PAEs is thought to provide better adaptation to the species that produce them (Huang et al., 2021a). However, in contrast to the biosynthesis of PAEs, in recent times, scientists have paid much greater attention to biodegradation by algae. Since PAEs are not bound to the polymer matrix of the final product, they can easily migrate into the environment and thereby affect the natural inhabitants of that environment (Net et al., 2015). Different biotic and abiotic pathways, including hydrolysis, photolysis, photooxidation, and biodegradation, have been identified as contributing to the degradation of PAEs (Net et al., 2015). The abiotic degradation process for PAEs is inefficient and prolonged, with DMP taking around three years and DEHP taking around 2000 years for aqueous photolysis (Miriyam et al., 2022). Furthermore, hydrolysis is not an effective method for phthalate elimination due to its slow reaction rate (Bogdanović, 2021). In contrast, microbial degradation is a much faster and cost-effective method that is environmentally friendly for eliminating phthalates' primary pathways for the degradation of PAEs (Net et al., 2015). Researchers have conducted multiple studies demonstrating the ability of bacteria present in wastewater to break down a range of PAEs under aerobic, anaerobic, and facultative conditions. In addition to bacteria, microalgae have also been found to biodegrade PAEs. Microalgae exhibit unique capabilities that enable them to function as effective bioremediators of organic contaminants. In contrast to bacteria, microalgae can perform photosynthesis, stimulating heterotrophic bacteria for growth and organic contaminants' degradation. Moreover, microalgae can directly degrade organic contaminants, for example, cyanobacteria *Cyanothece* sp. PCC7822, *Syenchocystis* sp. PCC6803, and *Synechococcus* sp. PCC7942 has been shown to metabolize DMP (Zhang et al., 2016). Similarly, green microalgae, such as *Chlorella pyrenoidosa* and single-celled dinoflagellate *Karenia brevis*, degrade PAEs (Yan et al., 2002; Sun et al., 2019). The proven possibility that microalgae can degrade PAEs is of great importance because these findings highlight the potential of microalgae-based bioremediation strategies for addressing environmental pollution.

Despite the availability of different pathways to eliminate PAEs from freshwaters, their extensive use has resulted in their omnipresent occurrence in the environment. Consequently, researchers have examined the efficiency of diverse physicochemical techniques that could effectively eliminate PAEs from freshwater systems, in addition to the natural processes that occur continually. In a recent study by Miriyam et al. (2022), five primary methods were proposed for eliminating PAEs, including adsorption, photocatalysis, electrochemical and membrane technology, and microbiological degradation. After considering each method's advantages and disadvantages, the authors conclude that phthalate biodegradation is the most efficient and effective way of eliminating these harmful compounds. Gobas et al. (2002) concluded that PAEs are not bioaccumulative substances and pointed out that there is no evidence to confirm this, considering laboratory, field, and mathematical studies. The life of PAEs in water is so short that long-term bioaccumulation (Semenov et al., 2021) and biomagnification in food chains (Gobas et al., 2002) are impossible.

Considering the ever-increasing production of plastic, pollution levels are expected to increase, causing global concern. Although the impact of PAEs on humans is relatively well known, the effect on phytoplankton still needs to be further investigated. In addition to being global oxygen producers, phytoplankton also represent the base of the aquatic food chain. However, these photosynthetic organisms are sensitive to environmental pollution. Data on the effects of PAEs on phytoplankton are scarce and need to be more comprehensive to understand the natural consequences that can occur when exposed to these pollutants. BBP is one of six phthalates that have been characterized as high-risk pollutants. However, its impact on phytoplankton species is almost unknown. To our knowledge, only Gledhill et al. (1980) investigated its effect on several representatives of algae. Also, it is unknown whether phytoplankton has the potential to biodegrade BBP. The main objective of this study is to investigate the effects of different concentrations of the high-risk organic pollutant BBP on two cyanobacteria (*Anabaena variabilis*, *Microcystis* sp.) and two green microalgae (*Chlorella* sp., *Scenedesmus* sp.). To achieve this, the present study monitored the biomass production of the tested phytoplankton species by measuring the concentration of chlorophyll *a*. Subsequently, the investigation aimed to determine the phytoplankton ability to metabolize BBP, evaluate if BBP activity exhibits dose-dependent manners, and identify potential differences in sensitivity among the different species of phytoplankton. In the present study, the impact of this pollutant on *A. variabilis*, *Chlorella* sp., and *Scenedesmus* sp. is described for the first time, as well as their potential for biodegradation.

Experimental

Algal cultures and cultivation conditions

The experiment used BBP in concentrations of 50, 100, 150, 200, and 250 µg/L.

Chlorella sp., *Scenedesmus* sp., and *Microcystis* sp. were isolated from a freshwater pond (43°17'51.9''N 21°47'40.8'' E) in Southeast Serbia and were cultivated at the Department of Biology and Ecology, Faculty of Science and Mathematics in Niš. They are identified using an identification key to the genus level (Burchadt, 2014).

Trichormus variabilis 0441 (Kützing ex Bornet & Flahault) (Komárek & Anagnostidis, 1989) (heterotypic synonym *Anabaena variabilis*) was isolated from the Danube River in the Vojvodina region (Serbia). It was cultivated in the Department of Biology and Ecology laboratory in Novi Sad (NSCCC) (Stankovic, 2020). *A. variabilis* was identified using molecular methods described in the previous study (Stankovic et al., 2022).

All cultures were prepared in 250 ml Erlenmeyer flasks at 24 °C under cool LED lighting for a 16-h photoperiod with constant aeration. Standard BG11(Ripkka et al., 1979) liquid medium was used to cultivate all species except *A. variabilis*, for which modified BG11 medium (without nitrogen source) was used for cultivation.

Exposure conditions to BBP and growth inhibition test

To examine the influence of BBP on several phytoplankton species, the effect of different concentrations of BBP (50, 100, 150, 200, 250 µg/L) on the growth rate of *A. variabilis*, *Microcystis* sp., *Chlorella* sp., *Scenedesmus* sp., within three weeks was monitored. Investigated concentrations of BBP were selected based on the low solubility of BBP in water (0.71 mg/L) (NCBI, 2023). In the experiment, in the sterile glass test tubes was added 10 ml of liquid medium BG11, 2 ml of a culture of each strain individually, and a specific concentration of BBP. Each concentration for each tested strain was set up in triplicate, as were the controls (18 replicates for each species). Controls (without BBP) were monitored for assay validity. All tubes were incubated at 24°C under cool LED lighting for a 16-h photoperiod for 21 days. Algae growth was monitored spectrophotometrically to determine chlorophyll's concentration. During the three weeks of the experiment, 1 ml of the culture, previously vortexed, was placed in cuvettes, and the optical density at 678, 720 and 750 nm was determined spectrophotometrically. The chlorophyll concentration was calculated according to the following formula (Stankovic, 2020): $Chl\ (\mathrm{m}g \times ml\text{-}1) = 14.96 \cdot (OD678 - OD750) - 0{,}616 \cdot (OD720 - OD750)$

BBP extraction

After the inhibition test was completed, the supernatant of all experimental cultures was separated by centrifugation and filtration to prepare a sample for further analysis. Liquid-liquid extraction was applied to extract BBP from the samples. For this purpose, *n*-hexane was used as a non-polar solvent. Detection and quantification of BBP were performed using gas chromatography with mass spectrometry (GC-MS).

Instrumental GC-MS analysis

Analysis was carried out by gas chromatography coupled to a mass spectrometer (Agilent 6890 series GC System with autosampler connected with Agilent 5973 Mass Selective Detector

(Electron Ionization MSD-EI, single quadrupole). The separation was achieved using a non-polar AGILENT DB-5MS column, phenyl arylene-based (30 m × 0.25 mm × 0.25 µm). The oven temperature was programmed from 65 °C (holding time 1 min) to 220 °C (1 min) at a rate of 20 °C min-1, then to 280 °C at a rate of 5 °C min-1 (4 min). Splitless mode was used, and 1 µL of volume was injected. The inlet temperature was 250 °C. MS Quad and MS Source temperatures were 150 °C and 230 °C, respectively. The energy of ionizing electrons was 70 eV. Helium (purity 99.999%) was the carrier gas with a constant flow rate of 1.0 mL min-1. The MSD was used in the single ion-monitoring (SIM) mode at *m/z* 149. The target compound was identified based on the relative retention time, the presence of target ion and relative abundance. The most abundant ion, m/z 149, was chosen for the quantification of BBP, with no qualifier ions, due to the simplicity of the matrix. Ion *m/z* 185 was selected as the representative ion of the DBA internal standard. The dwell time was 100 ms.

Statistical analysis

Univariate analysis of variance – ANOVA was used to test any significant difference in growth parameters between treated and control groups of algae between all concentrations. The statistical analyses were performed using IBM SPSS Statistics. The results were considered significant at the level of $p < 0.05$.

Results and Discussion

Correlations between BBP concentration, exposed species, and chlorophyll production

It was noticed that lower concentrations of BBP (50 µg/L and 100 µg/L) had no significant effect on the growth rate of *Microcystis* sp. When exposed to higher concentrations (150 µg/L, 200 µg/L and 250 µg/L), it was observed considerable growth inhibition ($p < 0.05$). A concentration of 150 µg/L of BBP caused this species' most significant growth inhibition, starting from the beginning of the exposure until the end of the experiment. *A. variabilis* responded to the presence of BBP similarly to *Microcystis* sp. More precisely, BBP concentrations of 200 and 250 µg/L caused a significant inhibition ($p < 0.05$) of the growth of this species. The most significant growth inhibition was observed at a 250 µg/L BBP concentration. When all concentrations are considered, it is observed that the higher the concentration of BBP, the smaller the increase in chlorophyll *a*, so the effect of BBP on *Microcystis* sp. and *A. variabilis* dose-dependently.

Green algae responded significantly differently to the presence of BBP compared to cyanobacteria. More precisely, none of the applied concentrations greatly affected the growth of *Chlorella* sp. On the other hand, on *Scenedesmus* sp., the BBP concentration of 150 µg/L caused a significant inhibition ($p < 0.05$) only on the seventh day of the experiment.

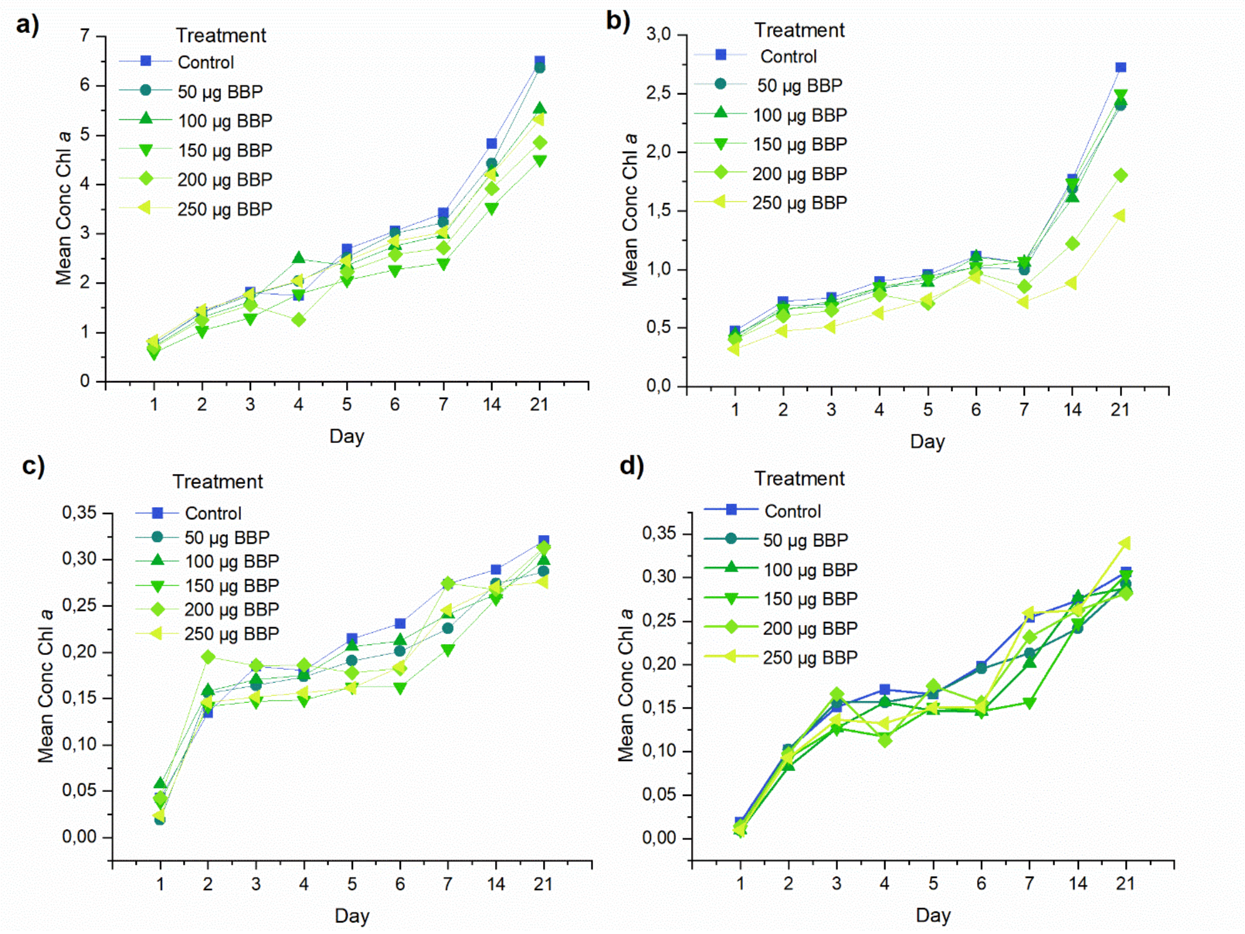


Figure 1. Spectrophotometric comparison of the growth of a) *Microcystis* sp., b) *Anabaena variabilis*, c) *Chlorella* sp., and d) *Scenedesmus* sp. in the presence of BBP, compared to the growth of the same strains in control conditions (without BBP).

There are a limited number of algae on which the effects of BBP have been tested. Gledhill et al. (1980) investigated the effect of BBP on aquatic organisms, and the research included five species of algae (*Selenastrum capricornatum*, *Navicula pelliculosa*, *Dunaiela tertiolecta*, *Sceletonema costatum, and Microcystis aeruginosae*). The results showed a significant inhibitory effect on all tested species except for *M. aeruginosa*, which showed resistance. A document published by the World Health Organization (1999) showed that the most sensitive species is *Selenastrum*, with EC_{50} = 110 g/L. Given that the influence of BBP on phytoplankton is almost unexplored, this research represents the first report on its impact on all species we examined, except for *Microcystis* sp.

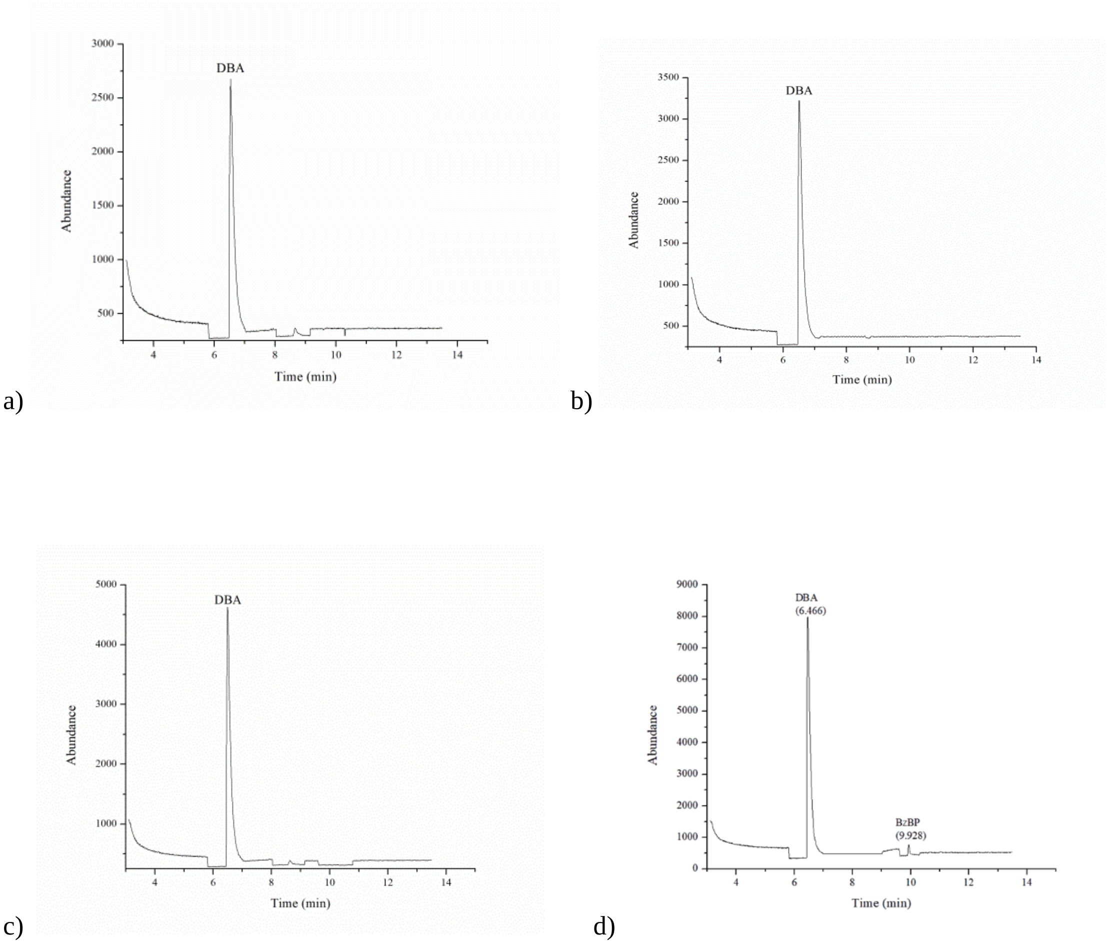
PAEs may have little to no effect on phytoplankton growth at low concentrations. However, as the concentration increases, the effect becomes more pronounced (Kuang et al., 2003). At some point, the concentration of PAEs may reach a level where it becomes toxic to the phytoplankton, inhibiting growth and potentially causing mortality. We noticed that this is the case with the cyanobacteria whose growth we monitored, where only higher concentrations of BBP caused a significant inhibition of growth. On the other hand, lower doses had an insufficiently substantial effect on the production of chlorophyll *a*. In general, the dose-dependent response of phytoplankton to PAEs is influenced by various factors, including the species of phytoplankton, the duration of exposure, and the environmental conditions in which the organisms live. We observed that the response of cyanobacteria to the presence of BBP was dose-dependent, especially in the case of *Microcystis* sp., considering that the higher the concentration of BBP, the lower the chlorophyll a production. Also, it can be noted that only in the second phase of the experiment, after the first week, was a noticeable difference in the production of chlorophyll a between controls and treatments observed for most concentrations. This indicates that prolonged exposure to BBP is necessary to observe a significant impact on growth.

Studies have shown that the sensitivity to PAEs can vary among phytoplankton species. Some species may be more sensitive than others, and certain species may even be able to tolerate high concentrations of PAEs. In this study, green algae showed greater tolerance to the presence of BBP in the growth medium in comparison to cyanobacteria. It can be assumed that the potential reason for their tolerance is their capability to detoxify or metabolize phthalates through physiological or biochemical mechanisms. Certain strains of green algae can even produce enzymes that efficiently decompose phthalates. Another possible reason is that some strains of green algae may have adaptations that allow them to manage the stress caused by exposure to phthalates. While BBP did not significantly impact the growth of green algae, it did cause a slight inhibition of chlorophyll production at most concentrations. This suggests that the pollutant did affect green algae to some extent, although the inhibition was not considered significant. As previously stated, this study revealed that cyanobacteria exhibit greater sensitivity to BBP toxicity than green algae. Their metabolic and morphological properties differ, which is probably the main reason for the differences in response to BBP influence. The difference in green microalgae and cyanobacteria sensitivity to various pollutants has been discussed before. Several authors have reported that cyanobacteria are more sensitive, suggesting that their prokaryotic structure is probably the main reason (Mao et al., 2017; Perron et Juneau, 2011; Drábková et al., 2007). Unlike green algae, the photosynthetic apparatus is not enclosed in the chloroplast in cyanobacteria. Still, photosynthesis takes place in the cytoplasm, so toxic substances can more easily affect chlorophyll production because they do not have to penetrate through the double membrane (Mao et al., 2017).

Scientists researching the effects of PAEs on mammalian tissue have discovered a connection between induced oxidative stress and damage to various tissues (Chen et al., 2019; Chen et al., 2022; Wang et al., 2023). That raises the question of whether phthalates can also cause oxidative stress in phytoplankton species, which could lead to reduced production of biomass and chlorophyll *a*. The researchers have provided an answer and reported that PAEs, such as BBP, induce oxidative stress in phytoplankton species. In a study by Drábková et al. (2007), the impact of H2O2 on green algae and cyanobacteria was examined, with the conclusion that several factors contribute to the greater sensitivity of cyanobacteria to this compound. The main reason is a less developed enzymatic oxidative pathway than green algae. Differences in their photosynthetic apparatus and response to oxidative stress may explain the varying sensitivity levels to BBP between cyanobacteria and green algae.

BBP elimination from the growth medium - do phytoplankton biodegrade BBP?

After the experiment, the detection and quantification of BBP in extract samples did not show the presence of BBP, except in the extract of the sample with *Scenedesmus* sp., but the detected concentration was not significant. The results indicate that probably all organisms could absorb, metabolize, and eliminate BBP from the aquatic environment, of which the strain *Scenedesmus* sp. has the least ability.



a) b) c) d)

Figure 2. HPLC chromatogram of a) *Microcystis* sp., b) *A. variabilis*, c) *Chlorella* sp. and d) *Scenedesmus* sp. extracts

The action of phytoplankton is considered one of the most effective ways to detoxify the water environment, considering that phytoplankton species, including green microalgae and cyanobacteria, have developed mechanisms to eliminate organic pollutants. The mechanisms by which they act include the processes of biosorption, bioaccumulation, biomineralization, biotransformation and biodegradation (Touilibah et al., 2022). At the end of the experiment, BBP was not detected in the samples, which indicates that the tested algae eliminated it from the medium by one of these detoxification methods. Considering that long-term bioaccumulation of PAEs by algae has not been proven (Gobas et al., 2002), it is assumed that the tested phytoplankton species in the experimental conditions bioaccumulated PAEs in the short term and then biodegraded them. Recently, scientists have focused on the biodegradation processes of PAEs by algae, and several studies have reported that phytoplankton species can biodegrade them (Babu & Wu, 2010b; Zang et al., 2016). Most isolates capable of biodegradation are anaerobes or facultative aerobes (Liang et al., 2008), which include bacteria, fungi, and green microalgae. Babu and Wu (2010b) investigated the ability of PAEs biodegradation by cyanobacteria (*Anabaena flos-aquae* G. S. West (strain 4054), *Microcystis aeruginosa* (Ku¨tz.) Ku¨tz. (strain 2396 and strain SM) and reported that the examined cyanobacteria can biodegrade PAEs using them as carbon sources for growth.

However, to our knowledge, previous studies have not reported that phytoplankton species can biodegrade BBP. It has been reported that BBP can be degraded by pure bacterial cultures, mixed bacterial cultures, and fungi (Chatterjee et al., 2003). This study provided insight that the phytoplankton species which are tested can eliminate BBP, which is of great importance because microalgae and cyanobacteria are known as promising candidates for the biodegradation of a variety of pollutants in comparison to bacteria and fungi, which require carbon input, energy, nutritional sources, and other supplements to remove pollutants (Touilibah et al., 2022).

The present study's findings revealed that the tested phytoplankton species can remove BBP from their growth medium. However, it is essential to consider the potential consequences of this process on the species. This study found that the production of chlorophyll *a* in all species was affected to some degree. This research begins a deeper investigation into the interaction between this endocrine disruptor and phytoplankton species. Our future research will focus on understanding the mechanisms behind the reduction in microalgae growth.

Conclusion

Due to the increasing use of BBP, they have become one of the world's most serious environmental challenges, which is why monitoring its impact on other organisms is of great importance. The effect of this toxic pollutant still needs to be investigated, especially on phytoplankton. This research provides new information on the interactions between BBP and four phytoplankton species (*Microcystis* sp., *A. variabilis, Chlorella* sp., *Scenedesmus* sp.). It reveals the potential of BBP to affect the growth of phytoplankton and the significantly greater susceptibility of cyanobacteria strains to this pollutant. It can be assumed that the main reason is their prokaryotic structure and insufficiently built defence system, unlike green microalgae. In addition, this research showed that the tested species eliminated phthalates from the growth medium. In future studies, we will try to discover the mechanisms through which BBP acts and processes occur during the elimination of BBP from the medium. This work represents the initial research on interactions between BBP and phytoplankton.

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Conflict-of-Interest Statement

None.

References

Ambe, K., Sakakibara, Y., Sakabe, A., Makino, H., Ochibe, T., & Tohkin, M. (2019). Comparison of the developmental/reproductive toxicity and hepatotoxicity of phthalate esters in rats using an open toxicity data source. The Journal of Toxicological Sciences, 44, 245-255.

Agas, D., Sabbieti, M. G., Capacchietti, M., Materazzi, S., Menghi, G., Materazzi, G., & Marchetti, L. (2007). Benzyl butyl phthalate influences actin distribution and cell proliferation in rat Py1a osteoblasts. Journal of cellular biochemistry, 101, 543-551.

Babu, B., & Wu, J. T. (2010a). Production of phthalate esters by nuisance freshwater algae and cyanobacteria. Science of the total environment, 408, 4969-4975.

Babu, B., & Wu, J. T. (2010b). Biodegradation of phthalate esters by cyanobacteria 1. Journal of Phycology, 46(6), 1106-1113.

Bhaisare, L. Y., Zade, S. B., Nagwanshi, A. M., Netam, A. K., & Chaudhary, D. D. (2022). Impacts of Benzyl Butyl Phthalate on Histo-Architecture of Gonads of African Catfish Clarias gariepinus (Burchell, 1822). Polish Journal of Environmental Studies, 31.

Bogdanovic, S. D. (2021). Phthalates food contamination due to their migration from plastic packaging. Doctoral dissertation. University of Niš, Faculty of Mathematics and Natural Sciences.

Burchadt, L. (2014). Key to identification of phytoplankton species in lakes and rivers. Guide for laboratory classes and field research. W. Szafer Institute of Botany. Polish Academy of Sciences, Kraków.

Call, D. J., Markee, T. P., Geiger, D. L., Brooke, L. T., VandeVenter, F. A., Cox, D. A., & Mount, D. R. (2001). An assessment of the toxicity of phthalate esters to freshwater benthos. 1. Aqueous exposures. Environmental Toxicology and Chemistry: An International Journal, 20, 1798-1804.

Cao, W. S., Zhao, M. J., Chen, Y., Zhu, J. Y., Xie, C. F., Li, X. T., Geng, S. S., Zhong, C. Y., Fu, J. Y., & Wu, J. S. (2023). Low-dose phthalates promote breast cancer stem cell properties via the oncogene ΔNp63α and the Sonic hedgehog pathway. Ecotoxicology and Environmental Safety, 252, 114605.

Chatterjee, S., & Karlovsky, P. (2010). Removal of the endocrine disrupter butyl benzyl phthalate from the environment. Applied microbiology and biotechnology, 87, 61-73.

Chen, Y., Li, C., Song, P., Yan, B., Yang, X., Wu, Y., & Ma, P. (2019). Hepatic and renal tissue damage in Balb/c mice exposed to diisodecyl phthalate: the role of oxidative stress pathways. Food and Chemical Toxicology, 132, 110600.

Chen, J., Yang, S., Ma, B., Wang, J., & Chen, J. (2022). Di-isononyl phthalate induces apoptosis and autophagy of mouse ovarian granulosa cells via oxidative stress. Ecotoxicology and Environmental Safety, 242, 113898.

Chemical Manufacturers Association. (1999). Comments of the Chemical Manufacturers Association phthalate esters panel in response to request for public input on seven phthalate esters. Chemical Manufacturers Association.

dos Santos Morais, G., Vieira, T. B., Santos, G. S., Dolatto, R. G., Cestari, M. M., Grassi, M. T., & da Silva, M. A. N. (2020). Genotoxic, metabolic, and biological responses of Chironomus sancticaroli Strixino & Strixino, 1981 (Diptera: Chironomidae) after exposure to BBP. Science of The Total Environment, 715, 136937.

Drábková, M., Matthijs, H. C. P., Admiraal, W., & Maršálek, B. (2007). Selective effects of H2O2 on cyanobacterial photosynthesis. Photosynthetica, 45, 363-369.

Ema, M., & Miyawaki, E. (2002). Effects on development of the reproductive system in male offspring of rats given butyl benzyl phthalate during late pregnancy. Reproductive Toxicology, 16, 71-76.

Ema, M., Miyawaki, E., Hirose, A., & Kamata, E. (2003). Decreased anogenital distance and increased incidence of undescended testes in fetuses of rats given monobenzyl phthalate, a major metabolite of butyl benzyl phthalate. Reproductive Toxicology, 17, 407-412.

Huang, L., Zhu, X., Zhou, S., Cheng, Z., Shi, K., Zhang, C., & Shao, H. (2021a). Phthalic acid esters: Natural sources and biological activities. Toxins, 13, 495.

Huang, S., Qi, Z., Ma, S., Li, G., Long, C., & Yu, Y. (2021b). A critical review on human internal exposure of phthalate metabolites and the associated health risks. Environmental Pollution, 279, 116941.

Komárek, J. & Anagnostidis, K. (1989). Modern approach to the classification system of Cyanophytes 4 - Nostocales. Algological Studies 56: 247-345.

Kuang, Q. J., Zhao, W. Y., & Cheng, S. P. (2003). Toxicity of dibutyl phthalate to algae. Bulletin of environmental contamination and toxicology, 71, 0602-0608.

Gobas, F. A., Mackintosh, C. E., Webster, G., Ikonomou, M., Parkerton, T. F., & Robillard, K. (2003). Bioaccumulation of phthalate esters in aquatic food-webs. Series Anthropogenic Compounds, 201-225.

Gledhill, W. E., Kaley, R. G., Adams, W. J., Hicks, O., Michael, P. R., Saeger, V. W., & LeBlanc, G. A. (1980). An environmental safety assessment of butyl benzyl phthalate. Environmental Science & Technology, 14, 301-305.

Gray Jr, L. E., Ostby, J., Furr, J., Price, M., Veeramachaneni, D. R., & Parks, L. (2000). Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicological Sciences, 58, 350-365.

Li, J., Li, H., Lin, D., Li, M., Wang, Q., Xie, S., Zang, J., & Liu, F. (2021). Effects of butyl benzyl phthalate exposure on Daphnia magna growth, reproduction, embryonic development and transcriptomic responses. Journal of Hazardous Materials, 404, 124030.

Liu, Y., Guan, Y., Yang, Z., Cai, Z., Mizuno, T., Tsuno, H., & Zhang, X. (2009). Toxicity of seven phthalate esters to embryonic development of the abalone Haliotis diversicolor supertexta. Ecotoxicology, 18, 293-303.

Liang, D. W., Zhang, T., Fang, H. H., & He, J. (2008). Phthalates biodegradation in the environment. Applied microbiology and Biotechnology, 80, 183-198.

Llorente Ortega, L. (2022). Characterisation of environmental stress biomarkers in Prodiamesa olivacea (Diptera) and their analysis and comparative evaluation in two chironomid species for ecotoxicity studies in natural scenarios.

Mao, F., He, Y., Kushmaro, A., & Gin, K. Y. H. (2017). Effects of benzophenone-3 on the green alga Chlamydomonas reinhardtii and the cyanobacterium Microcystis aeruginosa. Aquatic Toxicology, 193, 1-8.

Miodovnik, A., Edwards, A., Bellinger, D. C., & Hauser, R. (2014). Developmental neurotoxicity of ortho-phthalate diesters: review of human and experimental evidence. Neurotoxicology, 41, 112-122.

Miriyam, I., Anbalagan, K., & Magesh Kumar, M. (2022). Phthalates removal from wastewater by different methods–a review. Water Science and Technology, 85, 2581-2600.

National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 2347, Benzyl butyl phthalate. Retrieved October 5, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/Benzyl-butyl-phthalate.

Net, S., Sempéré, R., Delmont, A., Paluselli, A., & Ouddane, B. (2015). Occurrence, fate, behavior and ecotoxicological state of phthalates in different environmental matrices. Environmental Science & Technology, 49, 4019-4035.

Perron, M. C., & Juneau, P. (2011). Effect of endocrine disrupters on photosystem II energy fluxes of green algae and cyanobacteria. Environmental Research, 111, 520-529.

Piersma, A. H., Verhoef, A., te Biesebeek, J., Pieters, M. N., & Slob, W. (2000). Developmental toxicity of butyl benzyl phthalate in the rat using a multiple dose study design. Reproductive Toxicology, 14, 417-425.

Planelló, R., Herrero, O., Martínez-Guitarte, J. L., & Morcillo, G. (2011). Comparative effects of butyl benzyl phthalate (BBP) and di (2-ethylhexyl) phthalate (DEHP) on the aquatic larvae of Chironomus riparius based on gene expression assays related to the endocrine system, the stress response, and ribosomes. Aquatic toxicology, 105, 62-70.

Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M., & Stanier, R. Y. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Microbiology, 111, 1-61.

Semenov, A. A., Enikeev, A. G., Babenko, T. A., Shafikova, T. N., & Gorshkov, A. G. (2021). Phthalates-a strange delusion of ecologists. Теоретическая и прикладная экология, (1), 16-21.

Stankovic, R. N. (2020). Phytoplankton influence on benthic macroinvertebrates of freshwater ecosystems in multistress conditions: laboratory testing of the toxic effect of cyanobacteria and green microalgae on individuals of the species Chironomus riparius. Doctoral dissertation. University of Niš, Faculty of Mathematics and Natural Siences.

Stanković, N., Jovanović, B., Kokić, I. K., Piperac, M. S., Simeunović, J., Jakimov, D., Dimkić, I., & Milošević, D. (2022). Toxic effects of a cyanobacterial strain on Chironomus riparius larvae in a multistress environment. Aquatic Toxicology, 253, 106321.

Staples, C. A., Peterson, D. R., Parkerton, T. F., & Adams, W. J. (1997). The environmental fate of phthalate esters: a literature review. Chemosphere, 35, 667-749.

Sun, C., Zhang, G., Zheng, H., Liu, N., Shi, M., Luo, X., Chen, L., Li, F., & Hu, S. (2019). Fate of four phthalate esters with presence of Karenia brevis: uptake and biodegradation. Aquatic Toxicology, 206, 81-90.

Touliabah, H. E. S., El-Sheekh, M. M., Ismail, M. M., & El-Kassas, H. (2022). A review of microalgae-and cyanobacteria-based biodegradation of organic pollutants. Molecules, 27, 1141.

Tsai, C. F., Hsieh, T. H., Lee, J. N., Hsu, C. Y., Wang, Y. C., Lai, F. J., & Kuo, P. L. (2014). Benzyl butyl phthalate induces migration, invasion, and angiogenesis of Huh7 hepatocellular carcinoma cells through nongenomic AhR/G-protein signaling. BMC cancer, 14, 1-13.

Wang, Z., Hu, F., Song, W., Guo, J., He, W., & Ding, F. (2011). Chronic toxic effect of three estrogens to algae (Scenedesmus obliquus). In Proceedings 2011 International Conference on Human Health and Biomedical Engineering (pp. 877-880). IEEE.

Wang, Q., Yao, X., Jiang, N., Zhang, J., Liu, G., Li, X., Wang, C., Yang, Z, Wang, J., Zhu, L., & Wang, J. (2023). Environmentally relevant concentrations of butyl benzyl phthalate triggered oxidative stress and apoptosis in adult zebrafish (Danio rerio) liver: Combined analysis at physiological and molecular levels. Science of The Total Environment, 858, 160109.

World Health Organization. (1999). Butyl benzyl phthalate.

Yan, H., Pan, G., & Liang, P. L. (2002). Effect and mechanism of inorganic carbon on the biodegradation of dimethyl phthalate by Chlorella pyrenoidosa. Journal of Environmental Science and Health, Part A, 37, 553-562.

Zhang, X., Liu, L., Zhang, S., Pan, Y., Li, J., Pan, H., Xu., S., & Luo, F. (2016). Biodegradation of dimethyl phthalate by freshwater unicellular cyanobacteria. BioMed research international, 2016, 5178697.

Chemia Naissensis, Vol 6, Issue 1, RESEARCH ARTICLE, 41-42 **DOI: 10.46793/ChemN6.1.22P**

Uticaj benzil butil ftalata na rast nekoliko vrsta fitoplanktona (Microcistis sp., Anabaena variabilis, Chlorella sp., Scenedesmus sp.) u laboratorijskim uslovima

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SAŽETAK

Estri ftalne kiseline (PAEs) pripadaju klasi organskih jedinjenja i često se koriste kao plastifikatori. Zbog svoje velike primene su sve prisutniji u vodenim i kopnenim ekosistemima što predstavlja opasnost za životnu sredinu jer su klasifikovani kao visoko rizični zagadjivači. Štetan uticaj ftalata na zdravlje ljudi se ogleda kroz izazivanje smetnji u radu endokrinog sistema, ali mogu imati i kancerogeni i teratogeni efekat. Iako je njihov uticaj na ljude relativno poznat, uticaj na fitoplankton još uvek nije dovoljno istražen. Cilj ovog rada je bio ispitivanje uticaja razlicitih koncentracija (50, 100, 150, 200 250 µg/L) benzil butil ftalata (BBP) na rast nekoliko fitoplanktonskih vrsta (*Microcystis* sp., *Anabaena variabilis, Chlorella* sp., *Scenedesmus* sp.) u laboratorijskim uslovima. Rast fitoplanktonskih vrsta je praćen spektrofotometrijski u cilju utvrdjivanja koncentracije hlorofila *a*. Rezultati su pokazali da su visoke koncentracije BBP-a značajno inhibirale rast cijanobakterija (*Microcystis* sp., *A. variabilis*). Zelene alge su pokazale značajno manju osetljivost, narocito *Chlorella* sp., kod koje značajna inhibicija rasta nije zabeležena. Nakon eksperimenta, detekcija i kvantifikacija BBP-a izvršene su pomoću gasne hromatografije sa masenom spektrometrijom (GC-MS). Rezultati su pokazali da u uzorcima ekstrakta nije detektovan BBP, osim u ekstraktu uzorka sa *Scenedesmus* sp., ali i u ovom slučaju detektovana koncentracija nije značajna. Ovi rezultati ukazuju na to da su verovatno svi organizmi bili sposobni da apsorbuju i metabolišu BBP, od kojih soj *Scenedesmus* sp. ima najmanju sposobnost.

Ključne reči: *benzil butil ftalat, fitoplankton, hlorofill a, GC-MS, rast*

Chemia Naissensis, Vol 6, Issue 1, RESEARCH ARTICLE, 43-44 **DOI: 10.46793/ChemN6.1.22P**

L'influence du phtalate de benzylbutyle sur la croissance de plusieurs espèces de phytoplancton (Microcystis sp., Anabaena variabilis, Chlorella sp., Scenedesmus sp.) en conditions de laboratoire

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ABSTRAIT

Les esters d'acide phtalique (PAE) sont des composés organiques largement utilisés comme plastifiants. Leur utilisation généralisée a entraîné leur présence dans les écosystèmes aquatiques et terrestres, ce qui en fait un polluant à haut risque. Les PAE sont nocifs pour la santé humaine car ils perturbent le système endocrinien et peuvent potentiellement provoquer le cancer. Bien que leur impact sur les humains soit relativement bien connu, des recherches supplémentaires sont nécessaires pour comprendre leurs effets sur le phytoplancton. Ce travail visait à examiner l'influence de différentes concentrations (50, 100, 150, 200, 250 µg/L) de phtalate de benzyle et de butyle (BBP) sur la croissance de plusieurs espèces de phytoplancton les plus courantes (Microcystis sp., Anabaena variabilis, Chlorella sp. ., Scenedesmus sp.) en conditions de laboratoire. La croissance du phytoplancton a été surveillée par spectrophotométrie pour déterminer la concentration de chlorophylle a. Les résultats ont montré que des concentrations plus élevées de BBP inhibaient de manière significative la croissance d'A. variabilis et de Microcystis sp. Les algues vertes ont montré une sensibilité significativement plus faible, en particulier Chlorella sp., pour laquelle aucune inhibition significative de la croissance n'a été observée. Après l'expérience, la détection et la quantification du BBP dans les échantillons d'extraits ont été réalisées par chromatographie en phase gazeuse avec spectrométrie de masse (GC-MS). Le BBP a été détecté uniquement dans l’échantillon extrait avec Scenedesmus sp., mais la concentration détectée était insignifiante. Les résultats indiquent que tous les organismes testés pourraient probablement absorber et métaboliser le BBP, dont Scenedesmus sp. a le moins de capacité.

Mots clés : *algues, chlorophylle a, GC-MS, pollution*

Chemia Naissensis, Vol 6, Issue 1, RESEARCH ARTICLE, 45-46 **DOI: 10.46793/ChemN6.1.22P**

Влияние бензилбутилфталата на рост некоторых видов фитопланктона (Microcystis sp., Anabaena variabilis, Chlorella sp., Scenedesmus sp.) в лабораторных условиях

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Резюме

Эфиры фталевой кислоты (ПАЭ) представляют собой органические соединения, широко используемые в качестве пластификаторов. Их широкое использование привело к их присутствию в водных и наземных экосистемах, что делает их загрязнителями высокого риска. ПАЭ вредны для здоровья человека, поскольку нарушают работу эндокринной системы и потенциально могут вызвать рак. Хотя их влияние на человека относительно хорошо известно, необходимы дополнительные исследования, чтобы понять их влияние на фитопланктон. Целью данной работы было изучение влияния различных концентраций (50, 100, 150, 200, 250 мкг/л) бензилбутилфталата (ББФ) на рост нескольких наиболее распространенных видов фитопланктона (Microcystis sp., Anabaena variabilis, Chlorella sp. ., Scenedesmus sp.) в лабораторных условиях. Рост фитопланктона контролировали спектрофотометрически для определения концентрации хлорофилла a. Результаты показали, что более высокие концентрации BBP значительно подавляли рост A. variabilis и Microcystis sp. Зелёные водоросли показали значительно более низкую чувствительность, особенно Chlorella sp., у которых не наблюдалось существенного торможения роста. После эксперимента обнаружение и количественное определение ББФ в пробах экстракта проводили с помощью газовой хроматографии с масс-спектрометрией (ГХ-МС). ББФ был обнаружен только в выделенном образце с Scenedesmus sp., но обнаруженная концентрация была незначительной. Результаты показывают, что все протестированные организмы, вероятно, могли поглощать и метаболизировать BBP, из которых Scenedesmus sp. имеет наименьшие способности.

Ключевые слова: *водоросли, хлорофилл а, ГХ-МС, загрязнение*

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Der Einfluss von Benzylbutylphthalat auf das Wachstum mehrerer Phytoplanktonarten (*Microcystis* sp., *Anabaena variabilis, Chlorella* sp., *Scenedesmus* sp.) unter Laborbedingungen

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ABSTRACT

Phthalsäureester (PSE) sind organische Verbindungen, die weitgehend als Weichmacher eingesetzt werden. Ihre weit verbreitete Verwendung hat dazu geführt, dass sie in aquatischen und terrestrischen Ökosystemen vorkommen, was sie zu einem hochriskanten Schadstoff macht. PSEs sind schädlich für die menschliche Gesundheit, da sie das endokrine System stören und möglicherweise Krebs verursachen können. Obwohl ihre Auswirkungen auf den Menschen bekannt sind, ist weitere Forschung erforderlich, um ihre Auswirkungen auf Phytoplankton zu verstehen. Ziel dieser Arbeit war es, den Einfluss verschiedener Konzentrationen (50, 100, 150, 200, 250 µg/L) von Benzylbutylphthalat (BBP) auf das Wachstum einiger der am häufigsten vorkommenden Phytoplanktonarten (*Microcystis* sp., *Anabaena variabilis*, *Chlorella* sp., *Scenedesmus* sp.) unter Laborbedingungen zu untersuchen. Das Phytoplanktonwachstum wurde mittels Spektrophotometer überwacht, um die Konzentration von Chlorophyll *a* zu bestimmen.

Die Ergebnisse zeigten, dass höhere BBP-Konzentrationen das Wachstum von *A. variabilis* und *Microcystis* sp. signifikant hemmten. Grünalgen zeigten eine deutlich geringere Empfindlichkeit, insbesondere *Chlorella* sp., bei der keine signifikante Wachstumshemmung beobachtet wurde. Nach dem Experiment erfolgte der Nachweis und die Quantifizierung von BBP in den Probeextrakten durch Gaschromatographie mit Massenspektrometrie (GC-MS). BBP wurde nur in der extrahierten Probe mit *Scenedesmus* sp. nachgewiesen, die nachgewiesene Konzentration war jedoch unbedeutend. Die Ergebnisse deuten darauf hin, dass wahrscheinlich alle getesteten Organismen BBP absorbieren und verstoffwechseln könnten, von diesen hat *Scenedesmus* sp. die geringste Fähigkeit dazu.

Schlüsselwörter: *Algen, Chlorophyll a, GC-MS, Schadstoffe*

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Dispersive solid phase extraction for antibiotics analysis

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ABSTRACT

Antibiotics are widely used to prevent diseases and promote growth in food - producing animals. Their usage may result in the presence of antibiotics in food and environmental samples. Antibiotics analysis in complex samples, such as food and environment samples, require sample pretreatment.

Application of activated carbon, C18 and florisil and the influence of the amount of applied sorbents on their effectiveness in dispersive solid phase extraction (dSPE) for chloramphenicol and tetracycline analysis was examined. Activated carbon showed the lowest efficiency in the extraction of antibiotics (29% when analyzing samples containing chloramphenicol when 0.05 g of sorbent was added). When analyzing samples containing chloramphenicol, florisil showed an equal efficiency for all three sorbent amounts (92%), so extraction efficiency when using florisil does not depend on the mass of applied sorbent. Octadecyl silica (C18) shows high efficiency when analyzing chloramphenicol and tetracycline (96% in samples containing chloramphenicol, and 102% in samples containing tetracycline), so it can be applied in chloramphenicol and tetracycline analysis.

Keywords: *Antibiotic, sorbents, dSPE*

Introduction

Antibiotics are specific products of the metabolism of microorganisms that have a high physiological activity towards other groups of microorganisms (bacteria, molds, protozoa), preventing their growth and destroying them. In addition to natural antibiotics, obtained by microbial biosynthesis, there are also semi-synthetic and synthetic antibiotics (Spahija., 2020).

Apart from their origin, antibiotics also differ in their chemical composition and action on organisms. These drugs belong to a group of useful antimicrobial compounds that are widely used in human and veterinary medicine. Veterinary drugs, especially antibiotics are among the most important associations related to fodder production. Approximately 80% of animals used in food production are treated with antibiotics and other veterinary drugs in a certain period, or throughout life (Pavlov et al., 2008). The main use of antibiotics in animals is in the treatment and prevention of diseases such as mastitis, arthritis, respiratory infections, gastrointestinal and others bacterial infections (Darwish et al., 2013). Veterinary antibiotics were originally used in animals to disease treat and prevention, but today they are gradually added to food for reproduction control cycles and improvement of animal traits and as growth promoters, which goes far beyond their use as therapeutics for animals.

There is often excessive use of antibiotics and their misuse, which results in the appearance of increasing resistance to microorganisms, but also environmental pollution. Most antibiotics are incompletely metabolized after consumption and are excreted in unchanged form into the environment. In the environment they can accumulate and pollute the environment, but also enter food chain. Researchers have shown that antibiotics produced by humans most often reach the environment from the production facilities of the pharmaceutical industry, through excretion after use or discarding unused antibiotics. In a few cases, concentrations of antibiotic residues exceed 1 mg L^{-1} in treated industrial waters, which is much higher than the concentrations that are regularly detected as a consequence of antibiotic excretion (Larsson, 2014).

The degradation of drugs in the environment depends on the chemical properties of the active substance (Robinson et al., 2007). Many drugs are lipophilic and easily pass through cell membrane and bioaccumulate in aquatic animals. In the aquatic environment they are transformed by abiotic and biotic processes. Some antibiotics, such as penicillin's, are easily degraded, while others are significantly more persistent, for example tetracyclines and fluoroquinolones, which allows them to retain and spread in the environment and accumulate to higher concentrations (Larsson, 2014; Li et al., 2008a, Li et al., 2008b).

Residues of these drugs can lead to various toxic effects such as allergy, immunopathological effects, nephropathy, hepatotoxicity, mutagenicity, carcinogenicity, effects to reproductive health and even anaphylactic shock in humans (Darwish et al., 2013; Nisha, 2008). Antibiotics used in veterinary medicine can come into contact with soil, and by washing the soil, they reach the underground water (Heberer, 2002).

Analysis of antibiotics in complex samples requires sample pretreatment and is a challenge for researchers, because of the low concentrations of antibiotics in the samples, but also similar analytical signal given by the individual components. One of the newer techniques, which is more often used in sample preparation is QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) technique. It consists of extracting the analyte with a suitable solvent and purification of the obtained extract by dispersed solid phase extraction (dSPE).

Dispersive solid phase extraction is an improved version of solid phase extraction due to the direct addition of the sorbent to the sample solution, thereby increasing the surface of the contacting phases. dSPE consists of adding a solid sorbent, usually silica or polymer based directly to the sample solution, whereby the dispersion process increases the contact area between the sorbent and the solvent. Sorbents used for the determination of antibiotic residues in samples are solid substances that have undergone chemical modification by the addition of some chemical compounds, which increases their selectivity, longer contact with the analyte and minimal matrix interference during analysis (Xiong et al., 2015; Barrado and Avila, 2019). The selection of sorbents for dSPE is a challenge for researchers, due to the large number of sorbents that their selectivity towards analyte and co-extracted impurities.

In this paper, the possibility of using florisil, C18 and activated carbon for analysis of chloramphenicol and tetracycline in samples was examined. After appropriate treatment, efficiency was determined, in order to point out which sorbent can be used in dSPE. The samples were analyzed by high-resolution liquid chromatography, which achieves efficient separation of analyzed antibiotics on the HPLC column.

Experimental

Chemicals and reagents

Chloramphenicol (HPLC grade), tetracycline (HPLC grade), methanol (HPLC grade), acetonitrile (HPLC grade) were purchased by Sigma Aldrich, Germany. Boric acid (HPLC grade), hydrochloric acid, distilled water, activated carbon for the HPLC were purchased by Merck, Darmstadt, Germany, whereas C18 (octadecylsilane) was purchased by United Chemical Technologies UCT, Horsham, USA.

Preparation of the solution

Solutions of chloramphenicol and tetracycline in methanol are prepared in concentration of 5 mg mL^{-1}.

The mobile phase for HPLC analysis is prepared from boric acid aqueous solution (20 g L^{-1}) and acetonitrile (60 + 45 mL). The pH of the solution was adjusted up to 3 with 2 mol L^{-1} HCl solution.

Samples pretreatment for HPLC analysis

C18, florisil and activated carbon (0.05 g; 0.1 g and 0.2 g) are weighted into QuEChERS tube.

1 mL of the antibiotic solution is transferred to each of them with and mixtures were shaken for 1 minute and centrifuged at 8000 rpm for 10 minutes (Cvetković, 2016). The supernatant was filtered through a membrane filter (0.45 µm) directly into the vial for HPLC analysis.

Figure 1. Sample pretreatment

HPLC analysis

The analysis was performed using the Agilent 1200 Technologies HPLC apparatus which is equipped with a vacuum degasser, a quaternary pump, a sample injector, a column and UV detector, controlled by Agilent software.

Separation of sample components is achieved on a Zorbax Eclipse plus C18 (150 x 4.6 mm, 5 µm) analytical column, at a temperature set at 35 °C, with gradient elution of the mobile phase.

Solvents are mixed in a gradient starts with 20% acetonitrile, which is held for 2 minutes, and then increases linearly to 50% over 12 minutes. After that, the initial conditions were held for 5 minutes to flush the system before the next sample injection. The analysis is performed at a mobile phase flow rate of 1 mL min^{-1}. The absorption wavelength was measured at 220 nm. (Pietron, 2014) Antibiotic concentration was calculated using calibration curve (Table 1), and after that was calculated:

$$\text{Efficiency (\%)} = \frac{C_A}{C_s} \times 100\%$$

C_A - antibiotic concentration after treatment

Cs – antibiotic concentration before treatment

Table 1. Identification and quantification parameters

Antibiotic	Rt (min)	Calibration curve equation	r^2
Chloramphenicol	3,83	y=1,635*x+0,0002	0,99
Tetracycline	1,913	y=1,766*x +0,0001	0,99

Results and Discussion

Choosing the proper sorbent for dSPE is of crucial importance for the entire analytical procedure. In this paper, the possibility of using C18, florisil and activated carbon and appropriate amount of sorbent for the analysis of chloramphenicol and tetracycline was examined.

Efficiency was calculated by comparing the concentrations obtained after sample pretreatment by dSPE and initial concentrations of antibiotics.

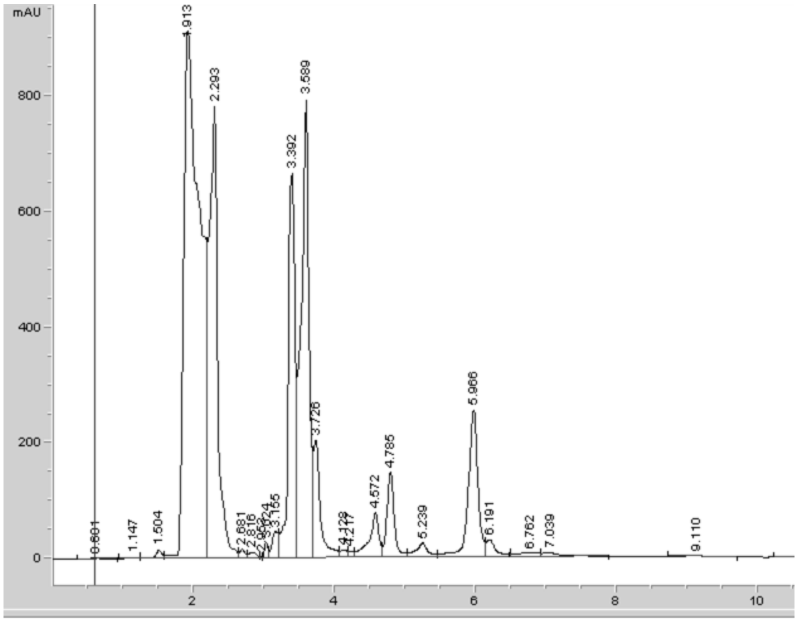


Figure 2. Chromatogram of tetracycline

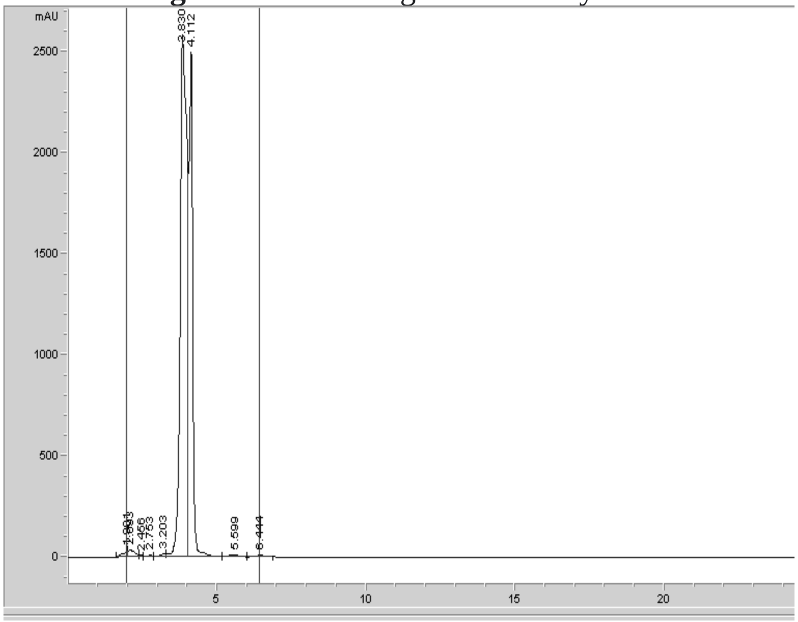
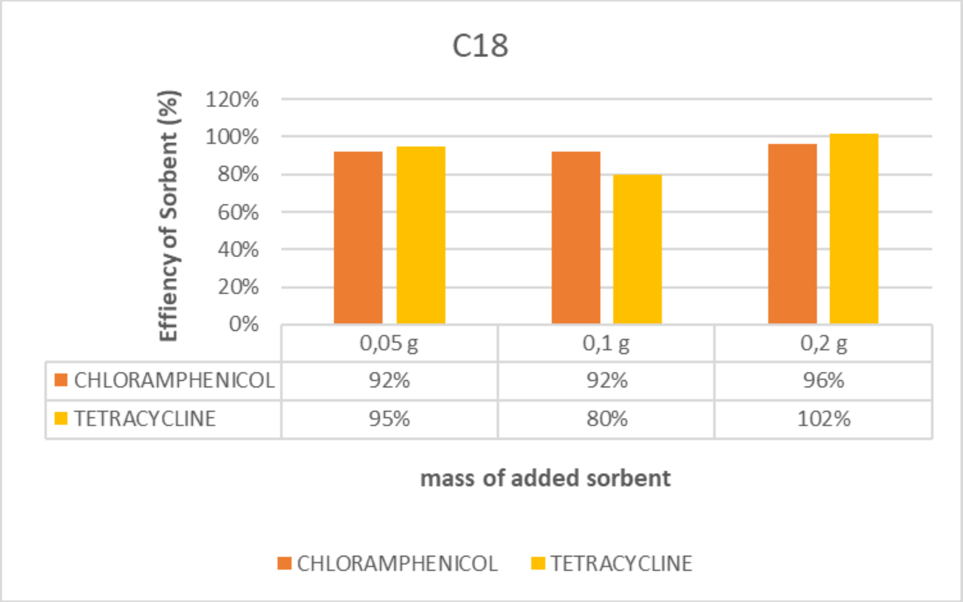


Figure 3. Chromatogram of chloramphenicol

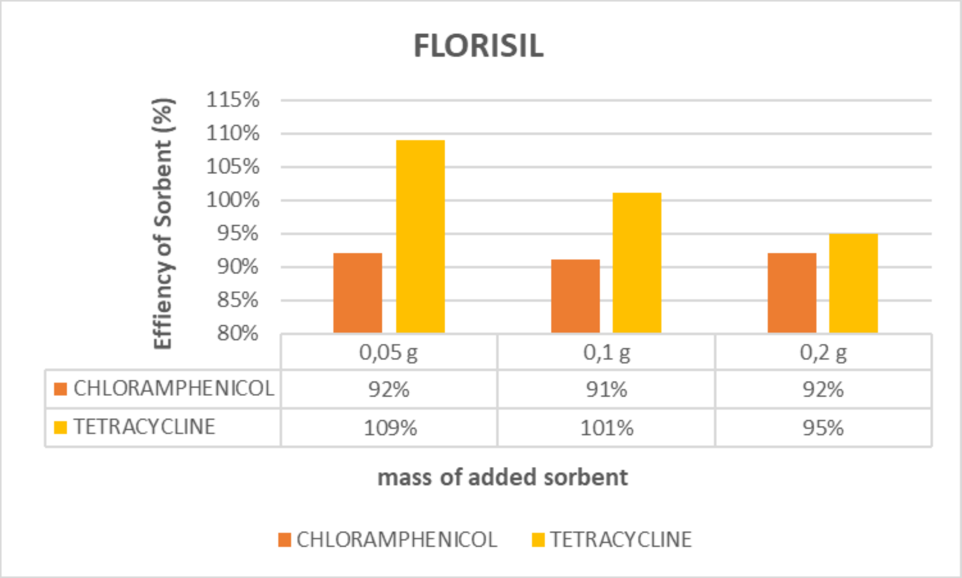
C18 is one of the most commonly used sorbents in solid phase extraction and has been used so far in the analysis of chloramphenicol by solid phase extraction (Śniegocki et al., 2017). C18 shows high efficiency when analyzing chloramphenicol and tetracycline. Efficiency values obtained for chloramphenicol analysis, with the increasing amount of sorbent were: 92, 92 and 96%, respectively. These results fall within the acceptable range of extraction efficiency prescribed by good ISO (Thompson et al., 2002).



	0,05 g	0,1 g	0,2 g
CHLORAMPHENICOL	92%	92%	96%
TETRACYCLINE	95%	80%	102%

Figure 4. The influence of the mass of C18 as a sorbent on the extraction efficiency of antibiotics

When analyzing tetracycline, there are some deviations in the results. At the amount of 0.05 g of sorbent, the efficiency is 95%, at the amount of 0.1 g, it is 80%, while 0.2 g of the sorbent gives efficiency of 102%. Based on these results, it can be seen that there is a difference in efficiency when using different amounts of sorbent.



	0,05 g	0,1 g	0,2 g
🟧 CHLORAMPHENICOL	92%	91%	92%
🟨 TETRACYCLINE	109%	101%	95%

Figure 5. The influence of the mass of florisil as a sorbent on the extraction efficiency of antibiotics

The influence of the mass of florisil as a sorbent on the extraction Florisil is mostly used to retain fat and other non-polar compounds (Zhang et al., 2015). Nagata and Saeki (1986) have used florisil columns in the clean-up of ampicillin from fish tissues When analyzing samples containing chloramphenicol, florisil showed an equal efficiency for 0.05, 0.1 and 0.2 g of sorbent (92%), so extraction efficiency when using florisil does not depend on the mass of applied sorbent. When analyzing samples containing tetracycline, there is a decrease in extraction efficiency with an increase in the mass of florisil in the solution, with a difference in efficiency of 14% when applying 0.05 g and 0.1 g of sorbent. Based on these results, it can be seen that there is a big difference in efficiency when using different amounts of sorbent and in relation to its efficiency with chloramphenicol, and it is desirable to repeat the analysis under other conditions, for example by changing the length of extraction or centrifugation in order to further analyze the application of this sorbent in the analysis tetracycline and studies of the influence of other factors on the results of the analysis. Xu et al. (2021) examined usage of Primary Secondary Amine (PSA), C18, Graphitized Carbon Black (GCB), florisil, and ZrO_2 for were for the determination of 2 lincosamides and 13 macrolides in honey samples. Their results for extraction efficiency when using florisil as dSPE sorbent were between 40 and 100%, depending on antibiotic type.

When activated carbon was used as a sorbent, the results show that efficiency for tetracycline analysis was about 40% when the amount of added sorbent is 0.05 and 0.1g. For 0.1 g of added sorbent, the efficiency is 37%, which shows that the amount of added activated carbon does not affect its efficiency much.

Activated carbon shows a lower efficiency in the analysis of chloramphenicol, according to the results shown in figure 6.

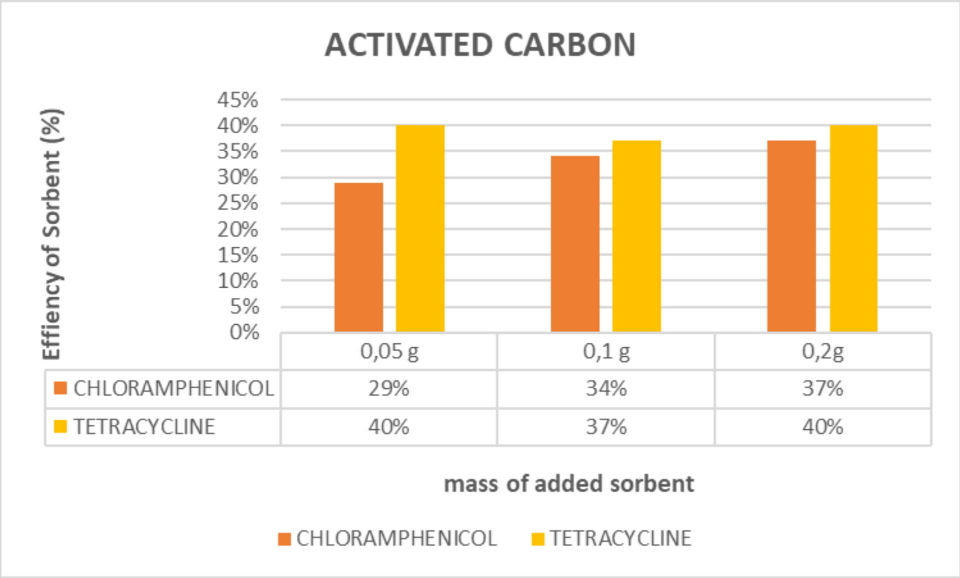


Figure 6. The influence of the mass of activated carbon as a sorbent on the extraction efficiency of antibiotics

The lowest efficiency (29%) is in case when 0.05 g of sorbent is added. By increasing the mass added sorbent, the efficiency also increases, i.e. with the addition of 0.1 g and 0.2 g of activated carbon, sorbent efficiency is 34 and 42%, respectively. It can be concluded that activated carbon is not suitable for sample preparation, because of its binding affinity for analyzed antibiotics. However, it can be used for tetracycline and chloramphenicol removal from various matrices. Lach (2019) analyzed the adsorption of chloramphenicol on commercially available activated carbon and came to the conclusion that activated carbon can be used to remove chloramphenicol from a water sample. Also, Zhao et al. (2020) used newly synthesized activated carbon to remove tetracycline from water samples and concluded that this activated carbon can be successfully used to remove this antibiotic from water.

Conclusion

In this paper the efficiency of different sorbents for dSPE extraction of antibiotics was examined. Chloramphenicol and tetracycline are chosen because of their most common application both for human treatment and in food production. These antibiotics are often found in the environment as a result of inadequate use, and their analysis is of great importance.

C18 shows high efficiency when analyzing chloramphenicol and tetracycline. Efficiency values obtained for chloramphenicol analysis, with the increasing amount of sorbent were: 92, 92 and 96%, respectively. When analyzing tetracycline, there are some deviations in the results, so it can be seen that there is a difference in efficiency when using different amounts of sorbent.

When using florisil in the analysis of chloramphenicol, the obtained results show that the efficiency does not depend on the mass of added sorbent, while in the analysis of samples containing tetracycline, the efficiency decreases with the increase in the mass of the added sorbent.

In this experiment, activated carbon showed poor extraction efficiency of tetracycline and chloramphenicol, it binds a significantly larger amount of antibiotics compared to other sorbent, which was expected considering earlier studies of this sorbent, whereby binds chloramphenicol more than tetracycline. Lowest value for efficiency of extraction was obtained for activated carbon as a sorbent, in the analysis of chloramphenicol (29%). The activated carbon used in this experiment can be used for removal chloramphenicol and tetracycline from the samples, and with potential modifications it is possible improve the removal efficiency of these two antibiotics.

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Conflict-of-Interest Statement

The author did not declare any conflict of interest.

References

Barrado Esteban, E., & Rodríguez Ávila, J. Á. (2019). Magnetic Materials in Separation Science, Encyclopedia of analytical science, 63-66.

Cvetković, J. S., Mitić, V. D., Stankov-Jovanović, V. P., Dimitrijević, M. V., & Stojanović, G. S. (2016). The evaluation of different sorbents and solvent mixtures in PAH sample preparation for GC/GC-MS analysis. *Advanced Technologies*, *5*(1), 31-38.

Darwish, W. S., Eldaly, E. A., El-Abbasy, M. T., Ikenaka, Y., Nakayama, S., & Ishizuka, M. (2013). Antibiotic residues in food: the African scenario. *Japanese Journal of Veterinary Research*, *61*(Supplement), S13-S22.

Heberer, T. (2002). Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicology letters*, *131*(1-2), 5-17.

Lach, J. (2019). Adsorption of chloramphenicol on commercial and modified activated carbons. Water 11: 1141

Larsson, D. J. (2014). Antibiotics in the environment. *Upsala journal of medical sciences*, *119*(2), 108-112.

Li, D., Yang, M., Hu, J., Ren, L., Zhang, Y., & Li, K. (2008a). Determination and fate of oxytetracycline and related compounds in oxytetracycline production wastewater and the receiving river. *Environmental Toxicology and Chemistry: An International Journal*, *27*(1), 80-86.

Li, D., Yang, M., Hu, J., Zhang, Y., Chang, H., & Jin, F. (2008b). Determination of penicillin G and its degradation products in a penicillin production wastewater treatment plant and the receiving river. *Water Research*, *42*(1-2), 307-317.

Nagata T, Saeki M. (1986) Determination of ampicillin residues in fish tissues by liquid chromatography. J Assoc Off Anal Chem. May-Jun;69(3):448-50. PMID: 3722092.

Nisha, A. R. (2008). Antibiotic residues-a global health hazard. *Veterinary world*, *1*(12), 375.
Pavlov, A., Lashev, L., Vachin, I., & Rusev, V. (2008). Residues of antimicrobial drugs in chicken meat and offals. *Trakia J Sci*, *6*(1), 23-25.

Pietron, W. J., Woźniak, A., Pasik, K., Cybulski, W., & Krasucka, D. (2014). Amphenicols stability in medicated feed–development and validation of liquid chromatography method. *Journal of Veterinary Research*, *58*(4), 621-629.

Robinson, I., Junqua, G., Van Coillie, R., & Thomas, O. (2007). Trends in the detection of pharmaceutical products, and their impact and mitigation in water and wastewater in North America. *Analytical and Bioanalytical Chemistry*, *387*, 1143-1151.

Śniegocki, T., Gbylik-Sikorska, M., & Posyniak, A. (2017). Analytical strategy for determination of chloramphenicol in different biological matrices by liquid chromatography-mass spectrometry. Journal of Veterinary Research, 61(3), 321.

Spahija, M. (2020). *Određivanje ostataka makrolidnih antibiotika u uzorcima mišićnog tkiva životinja primjenom LC-MS/MS metode* (Doctoral dissertation, University of Zagreb. Faculty of Food Technology and Biotechnology. Department of Biochemical Engineering. Laboratory for Antibiotic, Enzyme, Probiotic and Starter Cultures Technology).

Xiong, L., Gao, Y. Q., Li, W. H., Yang, X. L., & Shimo, S. P. (2015). Simple and sensitive monitoring of β2-agonist residues in meat by liquid chromatography–tandem mass spectrometry using a QuEChERS with preconcentration as the sample treatment. Meat Science, 105, 96-107.

Xu, J., Yang, M., Wang, Y., Yang, Y., Tu, F., Yi, J., & Chen, D. (2021). Multiresidue analysis of 15 antibiotics in honey using modified QuEChERS and high performance liquid chromatography-tandem mass spectrometry. Journal of Food Composition and Analysis, 103, 104120.

Zhao, C., Yin, W., Xu, J., Zhang, Y., Shang, D., Guo, Z., & Kong, Q. (2020). Removal of tetracycline from water using activated carbon derived from the mixture of phragmites australis and waterworks sludge. ACS omega, 5(26), 16045-16052.

Zhang, H., S. Bayen and B.C. Kelly, (2015). Co-extraction and simultaneous determination of multi class hydrophobic organic contaminants in marine sediments and biota using GC-EI-MS/MS and LC-ESI-MS/MS. Talanta. 143: p. 7-18.

Thompson M., Ellison S. L., Wood R., (2002). Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report). Pure App. Chem. 74: 835-855.

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Ekstrakcija disperzivnom čvrstom fazom za analizu antibiotika

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SAŽETAK

Antibiotici se široko koriste za prevenciju bolesti i podsticanje rasta životinja koje se koriste u proizvodnji hrane. Njihova upotreba može dovesti do prisustva antibiotika u uzorcima hrane i životne sredine. Analiza antibiotika u složenim uzorcima, kao što su uzorci hrane i uzorci iz životne sredine, zahtevaju pripremu uzorka. Ispitivana je primena aktivnog uglja, C18 i florisila i uticaj mase primenjenih sorbenata na njihovu efikasnost u dSPE za analizu hloramfenikola i tetraciklina. Aktivni ugalj je pokazao najmanju efikasnost u ekstrakciji antibiotika (29% pri analizi uzoraka koji sadrže hloramfenikol kada je dodato 0,05 g sorbenta). Kada se analiziraju uzorci koji sadrže hloramfenikol, florisil je pokazao jednaku efikasnost za različite mase sorbenta (92%), tako da efikasnost ekstrakcije pri upotrebi florisila ne zavisi od mase primenjenog sorbenta. C18 pokazuje visoku efikasnost pri analizi hloramfenikola i tetraciklina (96% u uzorcima koji sadrže hloramfenikol i 102% u uzorcima koji sadrže tetraciklin), te se može koristiti u analizi hloramfenikola i tetraciklina.

Ključne reči: *antibiotik, sorbenti, dSPE*

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Extraction dispersive en phase solide pour l'analyse des antibiotiques

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ABSTRAIT

Les antibiotiques sont largement utilisés pour prévenir les maladies et favoriser la croissance des animaux destinés à l'alimentation. Leur utilisation peut entraîner la présence d'antibiotiques dans des échantillons alimentaires et environnementaux. L'analyse des antibiotiques dans des échantillons complexes, tels que des échantillons alimentaires et environnementaux, nécessite un prétraitement des échantillons. L'application de charbon actif, C18 et fleuriste et l'influence du nombre de sorbants appliqués sur leur efficacité en dSPE pour l'analyse du chloramphénicol et de la tétracycline ont été examinées. Le charbon actif a montré la plus faible efficacité dans l'extraction des antibiotiques (29 % lors de l'analyse d'échantillons contenant du chloramphénicol lorsque 0,05 g de sorbant a été ajouté). Lors de l'analyse d'échantillons contenant du chloramphénicol, le florisil a montré une efficacité égale pour les trois quantités de sorbant (92 %), de sorte que l'efficacité de l'extraction lors de l'utilisation du florisil ne dépend pas de la masse du sorbant appliqué. Le C18 montre une grande efficacité lors de l'analyse du chloramphénicol et de la tétracycline (96 % dans les échantillons contenant du chloramphénicol et 102 % dans les échantillons contenant de la tétracycline), il peut donc être appliqué dans l'analyse du chloramphénicol et de la tétracycline.

Mots clés : *Antibiotique, absorbants, dSPE*

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Дисперсионная твердофазная экстракция для анализа антибиотиков

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Резюме

Антибиотики широко используются для профилактики заболеваний и стимулирования роста сельскохозяйственных животных. Их использование может привести к присутствию антибиотиков в пробах продуктов питания и окружающей среды. Анализ антибиотиков в сложных образцах, таких как образцы продуктов питания и окружающей среды, требует предварительной обработки образцов. Изучено применение активированного угля, C18 и флорисила и влияние количества применяемых сорбентов на их эффективность при дТФЭ для анализа хлорамфеникола и тетрациклина. Наименьшую эффективность при экстракции антибиотиков показал активированный уголь (29% при анализе проб, содержащих хлорамфеникол, при добавлении 0,05 г сорбента). При анализе проб, содержащих хлорамфеникол, флорисил показал одинаковую эффективность для всех трех количеств сорбента (92%), поэтому эффективность экстракции при использовании флорисила не зависит от массы нанесенного сорбента. C18 показывает высокую эффективность при анализе левомицетина и тетрациклина (96% в пробах, содержащих хлорамфеникол, и 102% в пробах, содержащих тетрациклин), поэтому его можно применять при анализе левомицетина и тетрациклина.

Ключевые слова: *антибиотик, сорбенты, дТФЭ*

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Dispersive Festphasenextraktion zur Analyse von Antibiotika

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ABSTRACT

Antibiotika werden weitläufig zur Prävention von Krankheiten und Wachstumsförderung bei Tieren, die der Lebensmittelerzeugung dienen, eingesetzt. Deren Verwendung kann dazu führen, dass Antibiotika in Lebensmittel- und Umweltproben vorkommen können. Die Analyse von Antibiotika in komplexen Proben, wie z. B. Lebensmittel- und Umweltproben, erfordert eine Probenvorbehandlung. Untersucht wurde der Einsatz von Aktivkohle, C18 und Florisil sowie der Einfluss der Anzahl der verwendeten Sorbenten auf deren Wirksamkeit in dSPE für die Analyse von Chloramphenicol und Tetracyclin. Aktivkohle zeigte die geringste Wirksamkeit bei der Extraktion von Antibiotika (29% bei der Analyse von Chloramphenicol enthaltenden Proben mit Zugabe von 0,05g Sorbent). Bei der Analyse von Proben, die Chloramphenicol enthielten, zeigte Florisil für alle drei Sorbentenanteile die gleiche Effizienz (92%), so dass die Effizienz der Extraktion bei Verwendung von Florisil nicht von der Menge des verwendeten Sorbents abhängt. C18 zeigt eine hohe Effizienz bei der Analyse von Chloramphenicol und Tetracyclin (96% in Proben, die Chloramphenicol enthalten und 102% in Proben, die Tetracyclin enthalten), so dass es bei der Analyse von Chloramphenicol und Tetracyclin eingesetzt werden kann.

Schlüsselwörter: *Antibiotikum, Sorbente, dSPE*

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Heavy Metal Accumulation in *Sideritis montana* near Landfill: A Case Study

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ABSTRACT

The Gornje Polje Landfill, like many waste disposal sites, raises concerns about the potential environmental contamination of nearby ecosystems. This study investigates the accumulation of selected priority pollutants -heavy metals: mercury (Hg), arsenic (As), and cadmium (Cd) in a potential medicinal species - *Sideritis montana* populations, located near the Gornje Polje Landfill. Our research explores the levels of these priority heavy metal pollutants in *S. montana*. Elevated levels of Hg, As, and Cd are observed compared to samples collected at the control site distant from the landfill. There is a potential influence of landfill activities on heavy metal bioavailability and uptake by *S. montana*. In addition to quantifying selected heavy metal concentrations, the ICP-OES method was employed, and determined concentrations for Hg, As, and Cd were 0.019, 0.109 and 0.025 ppm, respectively and are not for safe plant use. This study provides quantitative data of ICP-OES-determined selected priority pollutants heavy metals in *the Sideritis montana* population plant near the Gornje Polje Landfill, offering a case study that contributes to the broader understanding of environmental health and ethnobotanical considerations in proximity to waste disposal sites.

Keywords: *Sideritis montana, heavy metals, mercury, arsenic, cadmium, environmental contamination*

Introduction

This study focuses on the assessment of mercury (Hg), arsenic (As), and cadmium (Cd) accumulation in Sideritis montana, a plant species commonly found near a landfill site. Heavy metal contamination of any ethnobotanical significant plant can have cascading effects on the food web, highlighting the need for rigorous investigation and monitoring for potential medicinal or proven medicinal plants.

Plant species from the genus *Sideritis* (*S. scardica*, *S. reiserii*), known under the name *Mountain Tea*, are well known for their various traditional and medicinal uses and originate from the region of southern Europe, primarily the Mediterranean and Balkan areas. *Mountain Tea* has a rich history of traditional use in various cultures. The dried leaves and flowers of plants of the genus *Sideritis* are commonly used to prepare herbal teas that are used as a soothing drink and are believed to have several medicinal properties. Traditional practitioners have used these plants to relieve mild respiratory and gastrointestinal inflammation, and their aromatic leaves can be added to salads and used as a spice in cooking (Pljevljakušić et al., 2011; Pieroni et al., 2013).
In Turkey, an infusion of the aerial parts of the plant is used against stomach ailments (Bulut et al. 2017). In Central Macedonia, Greece, *Sideritis montana* is used for decoction against respiratory tract inflammation and cough (Tsioutsiou et al. 2019).

Koleva et al. 2003 and Firuzi et al. 2010 determined that the extracts show high antioxidant activity.

Toth et al. (2015) investigated the neuronal and smooth muscle effects of a methanol extract (prepared from the air-dried flowering aerial parts of *Sideritis montana*)for *in vitro* investigations of Guinea-pig ileum.

Tóth et al. 2017 isolated and identified new abietane diterpenoids from *S. montana*, and their antiproliferative properties were established, and remarkable activities were detected.

Miladinović et al. 2012 concluded that the essential oil of *S. montana* has a high antibacterial potential and should be further studied to apply it.

The essential oil showed noteworthy inhibition on tumour cells (Venditti et al. 2016).

The Serbian flora recognises only *Sideritis montana* L., a perennial herbaceous plant from the genus *Sideritis* (Diklić, 1974). According to Koleva et al. 2003, this plant is not used in traditional medicine due to its potential pro-oxidant properties. Extract from the *S. montana* plant has an intense antioxidant activity (Alexandre et al., 2013), which can qualify this species as a potential medicinal plant species.

Experimental

Analytical grade nitric and perchloric acid were used as reagents for the wet digestion of samples. Ultra-scientific ICP multi-element standard solutions were used as a stock solution for calibration. All analyses were carried out on aniCAP 6000 inductively coupled plasma optical emission spectrometer (ThermoScientific, Cambridge, United Kingdom), which uses an Echelle optical design and a Charge Injection Device (CID) solid-state-detector.

Sideritis montana was collected from an area near Gornje Polje landfill at Kosovo and Metohija, Serbia, in May 2017 (the flowering stage) and from the control site Kravlje, near the City of Niš, Serbia, in June 2017, far from the potentially contaminated sites. Voucher specimens *S. montana* from different locations (No 13889 and No 13890) were deposited in the Herbarium of the Department of Biology and Ecology, Faculty of Science and Mathematics (HMN), University of Niš.

Before the analysis, aerial vegetative parts (leaf and flower) were dried at room temperature. The dried samples were powdered in a stainless-steel mill and kept in polypropylene pouches for analysis. The wet digestion method was adopted to enable the measurement of the metal concentrations. The metal content in the plant material was determined after the acidic treatment. First, concentrated HNO_3 was added to the sample and heated up in the open glass to a small volume (until red vapours originating from NO_2 were removed); then, digestion was continued with 70% $HClO_4$ and again evaporated to a low volume. Finally, the solutions were transferred to standard vessels and diluted to a volume of 25 mL (Ilić et al. 2021).

This research investigates the levels of mercury (Hg), cadmium (Cd), and arsenic (As) in *Sideritis Montana* collected from an area near the Gornje Polje landfill and from the control site far from the potential ecotoxicological contamination. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was utilised to determine heavy metal concentrations accurately and sensitively. At the same time, acid/peroxide digestion was used as a sample preparation method preceding ICP-OES analysis. The study aims to assess the impact of landfill proximity on heavy metal contamination in the plant, offering insights into potential environmental and health risks.

Results and discussion

The results presented in this study were presented and contributed to the Second conference about medicinal and wild growing edible plants Pirot. Serbia (Stankov Jovanović et al., 2023). This study provides results of the accumulation of heavy metals, including mercury (Hg), arsenic (As), and cadmium (Cd), in *the Sideritis montana* population located near the Gornje Polje Landfill, at Kosovo and Metohija, in Serbia. Our research contributes to the literature that provides the levels of these priority heavy metals in *S. montana* or other medicinal or potential medicinal plants. It assesses the ecological and human health implications in the context of landfill proximity. Elevated levels of Hg, As, and Cd are observed compared to samples collected at the control site distant from the landfill. According to the results obtained, landfill activities potentially influence heavy metal bioavailability and uptake by *S. montana*. The ICP-OES method was employed to quantify selected heavy metal concentrations, and determined concentrations for Hg, As, and Cd were 0.019, 0.109 and 0.025 ppm, respectively, higher than in *S. montana* collected at a control location and higher than the officially prescribed limits for safe use of the plant.

Table 1. ICP-OES determined concentrations of Heavy Metals in *Sideritis montana*

Sideritis montana	Hg [ppm]	As [ppm]	Cd [ppm]
Landfill Site (Gornje Polje, Serbia)	0.019±0.020	0.109±0.010	0.025±0.003
Control Site (Kravlje, Serbia)	not detected	0.060±0.001	0.007±0.001

Conclusion

The study highlights the impact of landfill proximity on heavy metal contamination in *Sideritis montana*. Contaminated plants can have ecological implications, potentially affecting the ecosystem. This research utilised ICP-OES to determine the concentrations of mercury (Hg), cadmium (Cd), and arsenic (As) in *Sideritis montana* near an active landfill. The results suggest that the plant exhibits increased heavy metal contamination in an area close to the landfill, and it is not for safe use. Continued monitoring and safety measures are essential to assess the environmental and health risks associated with plants from such locations.

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Conflict of Interest Statement

The authors did not declare any conflict of interest.

References

Alexandre, A. M. R., Rosa, E. A. S., Santos, P. F., Silvestre, A. J. D., Duarte, M. F., Castilho, P. C. (2013). In vitro culture of Sideritis montana: effect of explant type and culture medium on growth and phenolic production. *Industrial Crops and Products*, 45, 431-438.

Bulut, G., Haznedaroğlu, M. Z., Doğan, A., Koyu, H., Tuzlacı, E.. (2017). An ethnobotanical study of medicinal plants in Acipayam (Denizli-Turkey), *Journal of Herbal Medicine 10*, 64–81.

Caf, F., Kiliç, Ö., Algül S. (2018). Evaluation of total antioxidant status, total oxidant status and oxidative stress index of some economically important plants from Turkey, *Progress in Nutrition 20 (1)*, 145-152, doi: 10.23751/pn.v20i1-S.6125.

Diklić, N. (1974). Rod *Sideritis* L. U: M. Josifović (ur.) *Flora SR Srbije 4*, Beograd, Srpska akademija nauka i umetnosti, odeljenje prirodno-matematičkih nauka, 371-372.

Firuzi, O., Javidnia K., Gholami M., Soltani M., Miri R. (2010). Antioxidant Activity and Total Phenolic Content of 24 Lamiaceae Species Growing in Iran. *Natural Product Communications, 5(2),* 261- 264.

Ilić, M. D., Mitić ,V. D., Tošić, S. B., Pavlović, A. N., Marković, M.S., Stojanović, G. S., Stankov Jovanović V. P. (2021). Mineral Composition of Herbaceous Species *Seseli rigidum* and *Seseli pallasii*: a Chemometric Approach, *Acta Chimica Slovenica, 68,* 709–717.

Koleva, I., Linssen, J. P. H., van Beek, T. A., Evstatieva, L. N., Kortenska, V., Handjieva, N. (2003). Antioxidant activity screening of extracts from Sideritis species (Labiatae) grown in Bulgaria. *Journal of the Science and the Food Agriculture 83(8),* 809-819. doi: 10.1002/jsfa.1415

Miladinović, D. L., Ilić, B. S., Mihajilov-Krstev, T. M., Nikolić, N. D., Milosavljević, V. N., Nikolić D. M. (2012). Antibacterial potential of the essential oil from *Sideritis montana* L. (Lamiaceae), *Hemijska industrija. 66 (4)*, 541–545.

Pieroni, A., Quave, C. L., Santoro, R. F., & Folk, L. R. (2013). Sideritis montana L. subsp. montana. *In Ethnobotany of Mountain Regions,* 183-204. Springer

Pljevljakušić, D., Šavikin K., Janković, T., Zdunić, G., Ristić, M., Godjevac, D., Konić-Ristić, A. (2011). Chemical properties of the cultivated Sideritis raeseri Boiss. & Heldr. subsp. raeseri, *Food Chemistry*, *124*(1), 226-233. doi: 10.1016/j.foodchem.2010.06.023

Tóth, B., Bartho, L., Vasas, A., Sándor, Z., Jedlinszki, N., Pinkec, G., Hohmanna, J. (2015). Dual Excitatory and Smooth Muscle-relaxing Effect of Sideritis montana Extract on Guinea-pig Ileum, *Natural Product Communications*, *10(3)*, 487 – 490.

Tóth, B., Kúsz, N., Forgo, P., Bózsity, N., Zupkó, I., Pinke, G., Hohmann, J., Vasas, A. (2017). Abietane diterpenoids from *Sideritis montana* L. and their antiproliferative activity, *Fitoterapia 122*, 90–94.

Tsioutsiou, E. E., Giordani P., Hanlidou E., Biagi M., De Feo V., Cornara L. (2019). Ethnobotanical Study of Medicinal Plants Used in Central Macedonia, Greece, *Evidence-Based Complementary and Alternative Medicine*, *2019*, 1-22

Venditti, A., Bianco, A., Frezza, C., Serafini, M., Giacomello, G., Giuliani, C., Bramucc,i M., Quassinti, L., Lupidi, G., Lucarini, D., Papa, F., Maggi, F. (2016). Secondary Metabolites, Glandular Trichomes and Biological Activity of *Sideritis montana* L. subsp. m*ontana* from Central Italy, *Chemistry & Biodiversity, 13,* 1380 – 1390.

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Akumulacija teških metala u *Sideritis montani* u blizini deponije: studija slučaja

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SAŽETAK

Deponija Gornje Polje, kao i mnoga odlagališta otpada, izaziva zabrinutost zbog mogućeg zagađenja životne sredine obližnjih ekosistema. Ova studija istražuje akumulaciju odabranih prioritetnih zagađivača – teških metala: žive (Hg), arsena (As) i kadmijuma (Cd) u potencijalnoj lekovitoj vrsti – populaciji *Sideritis montana*, koja se nalazi u blizini deponije Gornje Polje. Naša studija istražuje nivoe ovih prioritetnih zagađivača teških metala u *S. montani*. Uočeni su povišeni nivoi Hg, As i Cd u poređenju sa uzorcima prikupljenim na kontrolnom mestu udaljenom od deponije. Postoji potencijalni uticaj aktivnosti deponije na biodostupnost teških metala i njihovo usvajanje od strane *S. montana*. Pored kvantifikacije odabranih koncentracija teških metala, korišćena je ISP-OES metoda, a određene koncentracije za Hg, As i Cd bile su 0,019, 0,109 i 0,025 ppm, i nisu za bezbednu upotrebu. Ova studija pruža kvantitativne podatke odabranih prioritetnih zagađivača teških metala populaciji *Sideritis montana* u blizini deponije Gornje Polje, nudeći studiju slučaja koja doprinosi širem razumevanju zdravlja životne sredine i etnobotaničkih razmatranja u blizini odlagališta otpada.

Ključne reči: *Sideritis montana, teški metali, živa, arsen, kadmijum, zagađenje životne sredine*

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Accumulation de métaux lourds à *Sideritis montana* près d'une décharge : une étude de cas

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ABSTRAIT

La décharge de Gornje Polje, comme de nombreux sites d'élimination des déchets, suscite des inquiétudes quant à la contamination potentielle des écosystèmes voisins. Cette étude examine l'accumulation de polluants prioritaires sélectionnés - métaux lourds : mercure (Hg), arsenic (As) et cadmium (Cd) dans une espèce médicinale potentielle - les populations de *Sideritis montana*, situées à proximité de la décharge de Gornje Polje. Notre recherche explore les niveaux de ces polluants de métaux lourds prioritaires chez *S. montana*. Des niveaux élevés de Hg, As et Cd sont observés par rapport aux échantillons prélevés sur le site témoin éloigné de la décharge. Il existe une influence potentielle des activités de mise en décharge sur la biodisponibilité des métaux lourds et leur absorption par S. montana. En plus de quantifier certaines concentrations de métaux lourds, la méthode ICP-OES a été utilisée et les concentrations déterminées pour Hg, As et Cd étaient respectivement de 0,019, 0,109 et 0,025 ppm et ne conviennent pas à une utilisation sûre en usine. Cette étude fournit des données quantitatives sur les métaux lourds, polluants prioritaires sélectionnés déterminés par l'ICP-OES, dans la population de *Sideritis montana* près de la décharge de Gornje Polje, offrant une étude de cas qui contribue à une compréhension plus large de la santé environnementale et des considérations ethnobotaniques à proximité des sites d'élimination des déchets.

Mots clés : *Sideritis montana, métaux lourds, mercure, arsenic, cadmium, contamination environnementale*

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Накопление тяжелых металлов в Sideritis montana возле свалки: практический пример

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Резюме

Свалка Горне-Поле, как и многие другие свалки отходов, вызывает обеспокоенность по поводу потенциального загрязнения окружающей среды близлежащих экосистем. В данном исследовании изучается накопление отдельных приоритетных загрязнителей - тяжелых металлов: ртути (Hg), мышьяка (As) и кадмия (Cd) в потенциально лекарственных видах - популяциях *Sideritis montana*, расположенных вблизи свалки Горне Поле. Наше исследование изучает уровни этих приоритетных загрязнителей тяжелых металлов в железнице горной. Наблюдаются повышенные уровни Hg, As и Cd по сравнению с пробами, собранными на удаленном от полигона контрольном участке. Существует потенциальное влияние деятельности на свалках на биодоступность тяжелых металлов и их поглощение S. Montana L. В дополнение к количественному определению концентраций отдельных тяжелых металлов был использован метод ICP-OES, и определенные концентрации Hg, As и Cd составили 0,019, 0,109 и 0,025 частей на миллион соответственно и не подходят для безопасного использования на предприятиях. В этом исследовании представлены количественные данные по отдельным приоритетным загрязняющим веществам, определенным с помощью ICP-OES, тяжелым металлам в популяции растения *Sideritis* *montana* возле свалки Горне Поле, предлагая тематическое исследование, которое способствует более широкому пониманию гигиены окружающей среды и этноботанических исследованиий или знаний в непосредственной близости от мест захоронения отходов.

Ключевые слова: *Sideritis montana, тяжелые металлы, ртуть, мышьяк, кадмий, загрязнение окружающей среды.*

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Schwermetallansammlung in *Sideritis montana* in der Nähe eines Deponiegeländes: Eine Fallstudie

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ABSTRACT

Die Deponie Gornje Polje wirft, wie viele andere Mülldeponien, Bedenken hinsichtlich einer möglichen Umweltverschmutzung der umliegenden Ökosysteme auf. Diese Studie untersucht die Anreicherung ausgewählter vorrangiger Schadstoffe – Schwermetalle: Quecksilber (Hg), Arsen (As) und Cadmium (Cd) in einer potenziellen medizinischen Spezies – Populationen von *Sideritis montana*, die sich in der Nähe der Deponie Gornje Polje befinden. Unsere Forschung untersucht die Konzentrationen dieser vorrangigen Schwermetallschadstoffe in *S. montana*. Im Vergleich zu Proben, die an der Kontrollstelle fern der Deponie entnommen wurden, werden erhöhte Werte von Hg, As und Cd beobachtet. Es besteht ein potenzieller Einfluss von Deponieaktivitäten auf die Bioverfügbarkeit und Aufnahme von Schwermetallen durch *S. montana*. Zusätzlich zur Quantifizierung ausgewählter Schwermetallkonzentrationen wurde das ICP-OES-Verfahren eingesetzt und die ermittelten Konzentrationen für Hg, As und Cd betrugen jeweils 0.019ppm, 0.109ppm und 0.025ppm demnach können die Pflanzen nicht sicher verwendet werden. Diese Studie liefert quantitative Daten über vorrangige Schadstoffe (Schwermetalle), die mittels ICP-OES bestimmt wurden, in der *Sideritis montana* Pflanzenpopulation nahe der Deponie Gornje Polje. Sie stellt eine Fallstudie dar, die zum umfassenderen Verständnis von Umweltgesundheit und ethnobotanischen Überlegungen in der Nähe von Mülldeponien beiträgt.

Schlüsselwörter: *Sideritis montana, Schwermetalle, Quecksilber, Arsen, Cadmium, Umweltverschmutzung*

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Marigold – an ornamental plant with medicinal application

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ABSTRACT

Marigold (*Calendula officinalis* L.) is a widespread ornamental and medicinal plant with a long history of use in traditional medicine. *Calendula officinalis* contains many secondary metabolites that benefit human health and relieve skin ulcers and wound pain. Its essential oil is rich in monoterpenes and sesquiterpenes. Specific amounts of geraniol, limonene, thujene, and pinene in essential oil made it desirable in the cosmetics and perfume industries. Ethanolic extracts *of Calendula officinalis* contain flavonoids – substances with potent antioxidant properties. Due to carotenoids in flowers, Calendula officinalis extracts can be used as natural food colourants. The newest studies pointed out the potential of *Calendula officinalis* extracts as anti-inflammatory, anti-cancer, antibacterial and antifungal agents.

Keywords: *Calendula officinalis, essential oil, terpenes, carotenoids, cytotoxicity*

Introduction

Marigold (lat. *Calendula officinalis* L., belonging to the *Asteraceae* family) originates from India and can be found in the Mediterranean, Central Europe, and North America. *Calendula officinalis* is an annual, self-seeded or cultivated ornamental plant with no demanding growth and bloom conditions. Stem and leaves are densely covered in glandular hairs. Flower heads are slightly aromatic, sweet-scented, and single or double-flowered and appear from bright yellow to intensive orange (Figure 1).

Calendula officinalis has been used in traditional medicine for centuries. People have combined flower petals with vegetable oils to prepare balms, salves and skin lotions. Numerous plant secondary metabolites in *Calendula officinalis* (steroids, terpenoids, glycosides, volatile oil, amino acids, flavonoids) provide anti-inflammatory, antioxidant, antibacterial, antifungal, and wound-healing activities (Efstratiou et al., 2012; Rigane et al., 2013; Yalgi and Bhat, 2020; Vinola et al., 2021; Shahane et al., 2023).

Figure 1. *Calendula officinalis* (photo taken from https://unsplash.com/)

The chemistry of marigold – diversity in action

Aerial parts of the plant – flowers and leaves- have similar chemical composition regarding individual phenolic compounds. Gallic acid, rutin, scopoletin-7-*O*-glucoside, quercetin-3-*O*-glucoside and isorhamnetin-3-*O*-glucoside were identified in different quantities in leaves and flowers by using HPLC and LC/MS analysis (Rigane et al., 2013). In another research, flavonoids such as quercetin-rutinoside and quercetin-neohesperidoside, as well as calendoflavosides - isorhamnetin-neohesperidoside and isorhamnetin-rutinoside, were also identified (Ukiya et al., 2006). The presence of the mentioned compounds contributes to the antioxidant activity of *Calendula* e *officinalis* extracts.

The extracts obtained from the flowers *Calendula officinalis* contained a high quantity of polyphenolic compounds and exhibited good antioxidant activity. Antioxidant activity correlated significantly with the content of polyphenols and flavonoids (Veličković et al., 2014).

Flowers contained nearly twice as much total phenols and flavonoids than leaves (Rigane et al., 2013). Based on the results of the spectrophotometric determination of flavonoids in their study, Raal et al. (2016) stated that there is no clear relation between the colour of the flowers of *Calendula officinalis* and the total flavonoid content. The authors concluded that the content of flavonoids depended on either the place of cultivation or the cultivar of *Calendula officinalis* (Raal et al., 2016).

However, the plant pigments are responsible for the attractive colour of flowers. Flower petals of *Calendula officinalis* are exceptionally rich in carotenoids and contain flavoxantin, rubixantin, α-carotene, β-carotene, lycopene, and lutein and its derivatives (Kishimoto et al., 2005). The cultivars with higher carotenoid content usually have orange, dark-orange or even brownish flower petals (Raal et al., 2009). According to the results of HPLC analysis, there were 19 identified peaks in chromatograms of orange-coloured and only 10 identified peaks in yellow-coloured flowers, clearly indicating a more significant number of different carotenoids in the orange-coloured cultivars of *Calendula officinalis* (Kishimoto et al., 2005).

Calendula officinalis was recognised as an essential oil-bearing plant. The oil is dominantly accumulated in the flowers and is rich in terpenoids. However, the amount of oil is vegetation-dependent, with the maximum at the full-flowering period and the minimum at the pre-flowering period (Okoh et al., 2007).

Steam distillation is the standard procedure for determination of the content of essential oils in plant material. However, this way, only the "free" essential oil accessible to steam is extracted. A highly efficient alternative to steam distillation is high-pressure solvent extraction (Kajeh et al., 2004; Petrović et al., 2007; Yousefi et al., 2019). For instance, the amount of essential oil obtained from the subcritical and supercritical CO_2 extracts was more than two times higher than that obtained from the steam distillation (Petrović et al., 2007). One of the reasons may be the presence of waxes, fatty oil, and resins in *Calendula officinalis*, where some of the essential oil is dissolved. Subcritical and supercritical CO_2 extraction provides not only the extraction of free essential oil but also the extraction of fatty oil, waxes and resins containing dissolved essential oil (Petrović et al., 2007).

The major component of essential oil is sesquiterpene α-cadinole (Figure 2), followed by τ-cadinole, γ-cadinene and viridiflorol (Petrović et al., 2007; Raal et al., 2016).

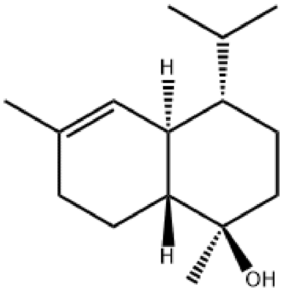

Figure 2. The structural formula of α-cadinole

According to the results of GC/MS analysis, an essential oil also contained several dozen compounds contributing to characteristic aroma: τ-muurolol, hexadecanoic acid, carvacrol, carvenone, *trans*-β-ocimene, α-terpeneol, α-thujene, α-pinene, β-pinene, limonene, geraniol, and many other volatiles (Okoh et al., 2007; Petrović et al., 2007; Sahingil, 2019). τ-cadinol and α-cadinol were reported to suppress the nitric oxide production induced by lipopolisaccharides and possess significant anti-inflammatory activity (Tung et al., 2011). α-pinene and β-pinene are fungicidal agents but are also intensively used as flavours, fragrances, and antiviral and antimicrobial agents (Salehi et al., 2019).

Promising alternative to antibiotics against pathogens

Since the extensive use of antibiotics and chemical agents has caused increasing bacteria resistance to these substances, there is an urgent necessity for efficient substitutes, and the solution could be found in the plant kingdom. In a recent study conducted by Vinola et al. (2021), the antibacterial and antifungal efficiency of the methanolic extract of Calendula officinalis has been compared to the widely used disinfectant chlorhexidine (a common constituent of mouthwash solutions). Even though chlorhexidine was found to be more potent due to its inability to dissolve pulp tissue or remove debris, its use as a routine irrigant has certain limitations. On the other hand, the extract of

Calendula officinalis exhibited lower antibacterial and antifungal activity but can be used as an effective alternative irrigant, safely applied daily (Vinola et al., 2021).

According to the newest *in vivo* study (Yalgi & Bhat, 2020) conducted on *Streptococcus mutans* as a teeth root canal irrigant, treatment with Calendula officinalis caused inhibition of growth or decrease in a number of colonies forming units (CFU). Therefore, *Calendula officinalis* was recognised as a promising medicament for bacterial elimination in teeth root canals (Yalgi & Bhat, 2020). Efstratiou et al. (2012) concluded that methanolic and ethanolic extracts of *Calendula officinalis* petals exhibited antifungal activity against several *Candida* and *Aspergillus* strains. In the same study, the antibacterial activity of both types of extracts was observed. However, methanolic extracts showed slightly higher antibacterial activity against various *Klebsiella, Pseudomonas, Staphylococcus* and even ampicillin-resistant *Escherichia coli* strains (Efstratiou et al., 2012).

Antibacterial activity is a consequence of the very complex composition of essential oil. Due to lipophilic properties, essential oils can pass through the cellular wall of the bacteria. The latter interaction with phospholipids and polysaccharides causes the permeabilisation of the bacteria membranes, further associated with loss of ions and membrane potential reduction (Bakkali et al., 2008). Furthermore, according to the results of molecular docking, α-cadinol showed binding affinity for three antibacterial target proteins, namely topisomerase IV subunit B, DNA gyrase subunit B, and penicillin-binding protein 2X, which are common in most of the bacterial species (Singh et al., 2018).

Exploring cytotoxic potential: Is it present here?

Depending on the part of the plant, extraction solvent, and the cancer cell line used for the study, the results of the cytotoxic efficiency of *Calendula officinalis* extracts varied significantly.

According to Matysik et al. (2005), the cytotoxic activity of the nonpolar heptane extract was due to the presence of different terpenes, while flavonoid glycosides were assigned for the cytotoxic activity of the polar, methanolic extract. Triterpene glycosides (methyl and butyl esters of calenduloside) in the methanolic extract of *Calendula officinalis* showed cytotoxic activity *in vitro* towards melanoma cell line, lung cancer, breast cancer, ovarian cancer and renal cancer (Ukiya et al., 2006). α-Cadinol was reported to be cytotoxic against human hepatocellular cancer cells, lung adenocarcinoma, melanoma, and leukaemia cells (Su & Ho, 2013).

One of the previously mentioned carotenoids found in aerial parts of *Calendula officinalis* – lutein exhibited selective cytotoxic activity towards breast cancer cell lines *in vitro* (Behbahani, 2014). A significant anticancer effect of geraniol was noticed by inducing apoptosis and DNA damage in colon cancer cells (Qi et al., 2018) and prostate cancer cells (Kim et al., 2011). Encapsulation of geraniol increased its cytotoxic activity towards lung cancer cells (Rodenak-Kladniew et al., 2023),

Based on those mentioned above, it is evident that the scientific community's interest in studying *Calendula officinalis* remains the same. This precious plant represents an actual reservoir of natural compounds with diverse biological activities, and many of them are yet to be investigated.

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References

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils-a review. Food and Chemical Toxicology, 46, 446-475.

Behbahani, M. (2014). Evaluation of in vitro anticancer activity of Ocimum basilicum, Alhagi maurorum, Calendula officinalis and their parasite Cuscuta campestris. PublicLibraryof Science One, 9, e116049.

Efstratiou, E., Hussain, A.I., Nigam, P.S., Moore, J.E., Ayub, M.A., &Rao, J.R. (2012). Antimicrobial activity of Calendula officinalis petal extracts against fungi, as well as Gramnegative and Gram-positive clinical pathogens. Complementary Therapies in Clinical Practise,18, 173-176.

Kajeh, M., Yamini, Y., Sefidkon, F., &Bahramifar, N. (2004). Comparison of essential oil composition of Carum copticum obtained by supercritical carbon dioxide extraction and hydrodistillation methods.Food Chemistry, 86, 587-591.

Kim, S. H. Bae, H. C., Park, E. J., Lee, C., Kim, B-J., Lee, S., Park, H. H., Kim, S-J., So, I., Kim, T-W., Jeon, J-H. (2011). Geraniol inhibits prostate cancer growth by targeting cell cycle and apoptosis pathways. Biochemical and Biophysical Research Communications, 407, 129-134.

Kishimoto, S., Maoka, T., Sumitomo, K., & Ohmiya, A. (2005). Analysis of Carotenoid Composition in Petals of Calendula (*Calendula officinalis* L.). Bioscience, Biotechnology, and Biochemistry, 69, 2122–2128.

Matysik, G., Wójciak-Kosior, M., & Paduch, R. (2005). The influence of Calendulae officinalis flos extracts on cell cultures, and the chromatographic analysis of extracts. Journal of Pharmaceutical and Biomedical Analysis, 38, 285–292.

Okoh, O.O., Sadimenko, A., & Afolayan, A. J. (2007). The Effects of Age on the Yield and Composition of the Essential Oils of Calendula officinalisJournal of Applied Sciences, 7, 3806-3810.

Petrović, L., Lepojević, Ž., Sovilj, V., Ad. amović, D., & Tešević, V. (2007). An investigation of CO2 extraction of marigold (Calendula officinalis L.). Journal of Serbian Chemistry Society, 72, 407–413.

Qi, F., Yan, Q., Zheng, Z., Liu, J., Chen,Y., & Zhang, G. (2018). Geraniol and geranyl acetate induce potent anticancer effects in colon cancer Colo-205 cells by inducing apoptosis, DNA damage and cell cycle arrest. Journal of BUON, 23, 346-352.

Raal, A., Kirsipuu, K., Must, R., & Tenno, S. (2009). Content of total carotenoids in Calendula officinalis L. from different countries cultivated in Estonia. Natural Product Communication, 4, 35-38.

Raal, A., Orav, A., Nesterovitsch, J., & Maidla, K. (2016). Analysis of Carotenoids, Flavonoids and Essential Oil of Calendula officinalis Cultivars Growing in Estonia. Natural Product Communication, 11, 1157-1160.

Rigane, G., Younes, S., Ghazghazi, H., & Salem, R.B. (2013). Investigation into the biological activities and chemical composition of Calendula officinalis L. growing in Tunisia. International Food Research Journal, 20, 3001-3007.

Rodenak-Kladniew, B., Gambaro, R., Cisneros, J., Huck-Iriart, C., Padula, G., Castro, G., Chain, C., & Islan, G. (2023). Enhanced anticancer activity of encapsulated geraniol into biocompatible lipid nanoparticles against A549 human lung cancer cells. Journal of Drug Delivery Science and Technology, 80, 104159.

Sahingil, D. (2019). GC/MS-Olfactometric Characterization of the Volatile Compounds, Determination Antimicrobial and Antioxidant Activity of Essential Oil from Flowers of Calendula (Calendula officinalis L.). Journal of Essential Oil Bearing Plants, 22, 1571-1580.

Salehi, B., Upadhyay, S., Erdogan, I., Kumar, A., Jayaweera, S., Dias, D., Sharopov, F., Taheri, Y., Martins, N., Baghalpour, N., Cho, WC., & Sharifi-Rad, J. (2019). Therapeutic Potential of α- and β-Pinene: A Miracle Gift of Nature. Biomolecules, 9, 738.

Shahane, K., Kshirsagar, M., Tambe, S., Jain, D., Rout, S., Ferreira, M.K.M., Mali, S., Amin, P., Srivastav, P.P., Cruz, J., & Lima, R.R. (2023). An Updated Review on the Multifaceted Therapeutic Potential of *Calendula officinalis* L. Pharmaceuticals, 16, 611.

Singh, D., Bhardwaj, S., Kumar, A., Kaushik, V., Mahajan, S., & Satija S. (2018). Antimicrobial Activity and Molecular Docking Studies of a Sesquiterpenoid Alcohol from Leaf Solvent Extracts of Juniperus communis L. International Journal of Green Pharmacy, 12, 22-28.

Su, Y-C., & Ho, C-L. (2013). Composition, in-vitro Anticancer, and Antimicrobial Activities of the Leaf Essential Oil of Machilus mushaensis from Taiwan. Natural Product Communications, 8, 273-275.

Tung, Y.T., Huang, C.C., Ho, S.T., Kuo, Y.H., Lin, C.C., Lin, C.T., & Wu, J.H. (2011). Bioactive phytochemicals of leaf essential oils of Cinnamomum osmophloeum prevent lipopolysaccharide/D-galactosamine (LPS/D-GalN)-induced acute hepatitis in mice. Journal of Agriculture and Food Chemistry, 59, 8117-8123.

Ukiya, M., Akihisa, T., Yasukawa, K., Tokuda, H., Suzuki, T., & Kimura, Y. (2006). Anti-Inflammatory, Anti-Tumor-Promoting, and Cytotoxic Activities of Constituents of Marigold (Calendula officinalis) Flowers. Journal of Natural Products, 69, 1692–1696.

Vinola, S.M., Sekar, M., Renganathan, S., & Dhiraviam, S. (2021). Comparative evaluation of Calendula officinalis and 2% chlorhexidine against Enterococcus faecalis and Candida albicans. Journal of Interdisciplinary Dentistry, 11, 119-123.

Veličković J.M., Dimitrijević D.S., Mitić S.S, Mitić M.N., & Kostić D.A. (2014). The determination of the phenolic composition, antioxidative activity and heavy metals in the extracts of *Calendula officinalis* L. Advanced technologies, 3, 46-51.

Yalgi, V.S., & Bhat, K.G. (2020). Compare and evaluate the antibacterial efficacy of sodium hypochlorite and Calendula officinalis against Streptococcus mutans as a root canal irrigating solution: An in vivo study. Journal of International Oral Health,12, 74-79.

Yousefi, M., Rahimi-Nasrabadi, M., Pourmortazavi, S., Wysokowski, M., Jesionowski, T., Ehrlich, H., & Mirsadeghi, S. (2019), Supercritical fluid extraction of essential oils, Trends in Analytical Chemistry, 118, 182-193.

https://unsplash.com/

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Neven – ukrasna biljka sa lekovitom primenom

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Sažetak

Neven (lat. *Calendula Officinalis* L.) je ukrasna i lekovita biljka koja se vekovima koristi u narodnoj medicini. Neven sadrži veliki broj sekundarnih metabolita koji ispoljavaju blagotvorno dejstvo na ljudsko zdravlje i olakšavaju tegobe kod čireva i rana na koži. Etarsko ulje ove biljke je bogato monoterpenima i seskviterpenima, a sadrži i komponente kao što su geraniol, limonen, tujen i pinen, čineći ga dragocenom sirovinom u kozmetičkoj i parfemskoj industriji. Etanolni ekstrakti nevena sadrže flavonoide, koji su veoma efikasni antioksidanti. Cvetovi nevena su bogati biljnim pigmenitima - karotenoidima, što čini njihove ekstrakte prirodnim bojama u prehrambenoj industriji. Najnovija naučna istraživanja ističu potencijalnu primenu nevena u stomatologiji i medicini zbog protivupalnog, anti-kancerogenog, antibakterijskog i antimikotičnog dejstva.

Ključne reči: *Calendula officinalis, etarsko ulje, terpeni, karotenoidi, citotoksičnost*

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Souci – une plante ornementale aux applications médicinales

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ABSTRAIT

Le souci (*Calendula officinalis L.*) est une plante ornementale et médicinale très répandue, utilisée depuis longtemps en médecine traditionnelle. Le *Calendula officinalis* contient de nombreux métabolites secondaires qui sont bénéfiques pour la santé humaine et soulagent les ulcères cutanés et les douleurs des plaies. Son huile essentielle est riche en monoterpènes et sesquiterpènes. Des quantités spécifiques de géraniol, de limonène, de thujène et de pinène dans l'huile essentielle l'ont rendue souhaitable dans les industries des cosmétiques et des parfums. Les extraits éthanoliques de *Calendula officinalis* contiennent des flavonoïdes – des substances dotées de puissantes propriétés antioxydantes. Grâce aux caroténoïdes contenus dans les fleurs, les extraits de *Calendula officinalis* peuvent être utilisés comme colorants alimentaires naturels. Les études les plus récentes ont souligné le potentiel des extraits de *Calendula officinalis* comme agents anti-inflammatoires, anticancéreux, antibactériens et antifongiques.

Mots clés : *Calendula officinalis, huile essentielle, terpènes, caroténoïdes, cytotoxicité*

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Бархатцы – декоративное растение лекарственного назначения

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Резюме

Бархатцы (*Calendula officinalis* L.) — широко распространенное декоративное и лекарственное растение, имеющее давнюю историю использования в народной медицине. Календула лекарственная содержит множество вторичных метаболитов, которые приносят пользу здоровью человека в лечении кожных язв и облегчают боли в ранах. Его эфирное масло богато монотерпенами и сесквитерпенами. Особое содержание гераниола, лимонена, туйена и пинена в эфирном масле сделало его желательным для использования в косметической и парфюмерной промышленности. Этаноловый экстракт календулы лекарственной содержит флавоноиды – вещества с мощными антиоксидантными свойствами. Благодаря каротиноидам, содержащимся в цветах, экстракты календулы лекарственной можно использовать в качестве натуральных пищевых красителей. Новейшие исследования указали на потенциал экстракта календулы лекарственной как противовоспалительного, противоракового, антибактериального и противогрибкового средства.

Ключевые слова: *Calendula officinalis, эфирное масло, терпены, каротиноиды, цитотоксичность.*

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Ringelblume – eine Zierpflanze mit medizinischer Anwendung

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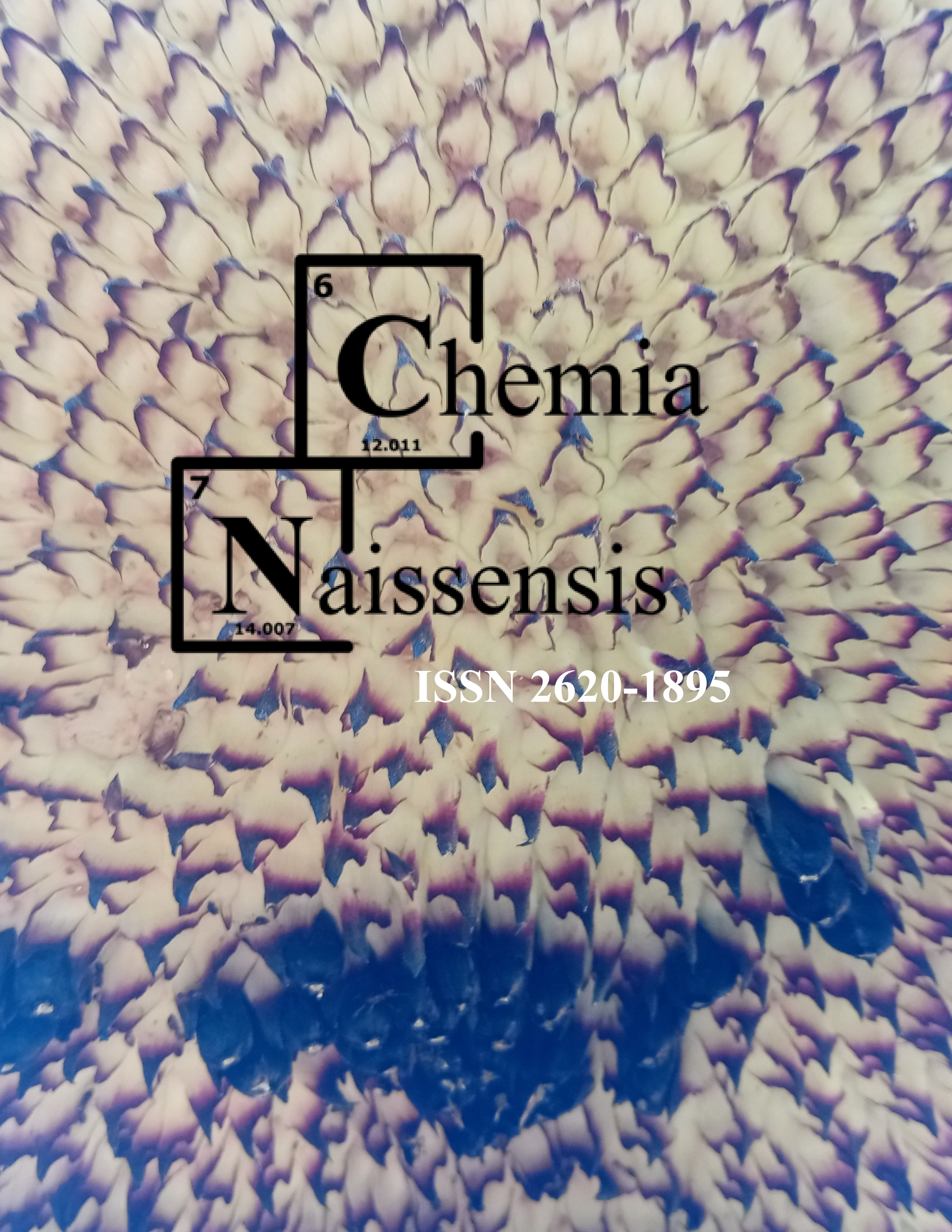
ABSTRACT

Die Ringelblume (*Calendula officinalis* L.) ist eine weit verbreitete Zier- und Heilpflanze mit langjähriger Verwendung in der traditionellen Medizin. *Calendula officinalis* enthält viele sekundäre Metaboliten, die der menschlichen Gesundheit zugutekommen und Hautgeschwüre sowie Wundschmerzen lindern. Ihr ätherisches Öl ist reich an Monoterpenen und Sesquiterpenen. Insbesondere die Anteile an Geraniol, Limonen, Thujen und Pinen im ätherischen Öl haben es in der Kosmetik- und Parfümindustrie beliebt gemacht. Ethanolische Auszüge von *Calendula officinalis* enthalten Flavonoide – Substanzen mit starken antioxidativen Eigenschaften. Aufgrund der Carotinoide in Blüten können Extrakte aus *Calendula officinalis* als natürliche Lebensmittelfarbstoffe verwendet werden. Neueste Studien haben das Potenzial von *Calendula officinalis*-Auszügen als entzündungshemmende, krebsbekämpfende, antibakterielle und antimykotische Wirkstoffe hervorgehoben.

Schlüsselwörter: *Calendula officinalis, ätherisches Öl, Terpene, Carotinoide, Zytotoxizitäta*



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