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ORIGINAL SCIENTIFIC PAPER

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ENVIRONMENTAL IMPACT ASSESSMENT OF LAND CONSOLIDATION

ABSTRACT: Land consolidation (LC) is an important tool for the improvement of agriculture and rural development, which also includes environmental issues in most of the countries in Europe. This paper presents the most important results of the environmental impact assessment (EIA) of land consolidation, conducted in the municipality of Vršac through a pilot project based on the EU methodology set within the project: “Strengthening Municipal Land Management in Serbia”, supported by the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH. During the summer of 2018, field survey was carried out at 90 locations, documenting the natural, semi-natural and man-made landscape elements of ecological or cultural values, assessing their quality and estimating the potential harmful environmental impacts of the land consolidation. The already existing negative impacts of intensive agriculture were also registered, such as abandonment or overgrazing of pastures and meadows, converting grasslands into arable land, soil erosion and habitat fragmentation. Although the area of LC is without natural forest and extremely poor in semi-natural elements of rural landscape, the existing entities were revealed as refuges for protected species. Some of the grassland fragments belonged to protected habitat types. The final categorization of the landscape elements was conducted in three levels. Category I landscape elements had to remain undisturbed; Category II landscape elements could be removed with obligatory ecological compensation, while the Category III landscape elements could be removed without environmental compensation. Recommendations were given pointing out the possibilities for improving the environmental characters of the area by the land consolidation process.

KEYWORDS: land consolidation, environmental impact assessment, landscape elements, indicator species, Vršac

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INTRODUCTION

Land consolidation (LC) is a legally regulated procedure implemented by the public authorities to redistribute and reallocate parcels of individual agricultural landowners (Veršinskas et al., 2020). In the past, the main goal of LC was an economic effect: increasing agriculture production by ownership defragmentation and improving infrastructure. The landscape changes derived from the agricultural intensification have led to multiple negative effects (Stoate et al., 2010), including soil erosion (Borrelli et al., 2023), pollution (Rodríguez-Eugenio et al., 2018) and decrease of natural pest control (Perrot et al., 2021; Rusch et al., 2016). Hereupon, the rural development component was recognized in LC planning documents (Thomas, 2006) and, according to international initiatives of sustainable development, a set of measures for environment improvement have been included in LC process (Gečaite and Jankava, 2017; Moravcová et al., 2017; Muchová et al., 2016). According to the Veršinskas et al. (2020) definition, the contemporary land consolidation is used to adjust the property structure in rural areas and to achieve a number of other public objectives, including nature restoration and construction of infrastructure. The economic benefits of land consolidation are widely documented through cost-benefit analysis. However, the benefits of environment protection and nature conservation are difficult to calculate and their effects are not immediately visible (De Groot et al., 2010). For these reasons, the environmental component has not been strongly involved in land consolidation so far (Elvestad and Sky, 2019).

Land consolidation changes the landscape structure, providing a great tool to plan environmental issues, climate change adaptation and mitigation, including water management, habitat restoration and creation of nature reserves (Veršinskas et al., 2020). According to Gečaite and Jankava (2017), LC is a mechanism for reducing soil erosion, arranging reclamation facilities, preserving biodiversity, reducing air pollution and improving the landscape structure. This process requires multidisciplinary approach: in addition to professionals in the fields of geodesy, law, economics, agriculture, etc. it is obligatory to consult specialists in environmental sciences.

According to Vasiljević (2019), land consolidation in the Republic of Serbia covered 1.4 million hectares between 1955 and 1990, when the process was discontinued, primarily due to lack of funding. Since 2006, new LC projects have been implemented, predominantly through international cooperation. However, a full legislative framework is still pending and projects are implemented on small areas according to the real needs (Vasiljević et al., 2018).

There is no single umbrella law of land consolidation. Different countries apply different models and follow non-identical objectives (Veršinskas et al., 2020). Serbia lacks a modern legislative framework governing land consolidation (Vasiljević, 2019). According to the existing legislation (*Sl. gl. RS* 62/2006 in version 95/2018), environmental impact assessment is not a mandatory part of the LC documentation in the Republic of Serbia as it is in most of the EU countries (Veršinskas et al., 2020). This paper presents the most important results of the EIA for land consolidation conducted in the municipality of Vršac

(Szabados and Ninkov, 2018), through a pilot project within the project "Strengthening Municipal Land Management, Rural Development: Effective Land Management", supported and coordinated by GIZ (GIZ, 2017).

MATERIALS AND METHODS

General characteristics of the land consolidation area

The investigated land consolidation area includes three cadastral municipalities (CMs) of the municipality Vršac, R. Serbia: CM Vlajkovac (4,962 ha), CM Uljma (6,114 ha) and CM Izbište (4,501 ha). This area, according to Bugarski et al. (1995), includes three geomorphological units. The southern part belongs to the South Banat Loess Plateau with the Dumače hill at the south-western border of the area. The elevation divergence between the hilltop and the plateau margin contributes to the formation of several loess valleys. The north-eastern parts are located on the lake-loess terrace, while the northern part belongs to the Alibunar Depression (Vršački rit).

According to Nejgebauer et al. (1971), in the northern part of the area, Hydromorphic soils, primarily Humoglay – Hydromorphic black soil, were formed. These soils in the northern and north-western part are permeated by halomorphic Solonetz and Solonchakas saline soils along the margin of the Alibunar depression, while the largest part of the land consolidation area is occupied by Chernozem – automorphic soil. Soils with a higher sand content are found at the south of the area, on the highest terrain towards the Deliblato Sands.

Municipality of Vršac has a moderate continental climate (*Lokalni ekološki akcioni plan grada Vršca, 2016). The average air temperature is 11.5 °C, by 0.5 °C higher than the Serbian Autonomous Province of Vojvodina average and the average annual rainfall is 644.1 mm. The municipality is known by its intense winds. The number of days with winds of 6 Beaufort (strong winds) is 167 days and the most common winds are directed from the southeast.

Natural watercourses have been canalized or drained and the groundwater levels of the wider area have been lowered (Bugarski et al., 1995). The first aquifer is quite alkalized, with a high content of dissolved sodium (Bogdanović and Marković, 2005). The investigated area includes one section of the Basic Canal Network and several lower order canals of Danube-Tisa-Danube hydro system. The artificial lakes of the fishpond "Vršački ritovi", with the 712 ha water surface, were formed on the low terrain of Alibunar depression. Surface waters also include the abandoned pits of the brickyard in Uljma, filled with groundwater. Groundwater is the main source of water supply for the residents and the economy, no sanitary sewage system has been built in any of the settlements within the LC area (Lokalni ekološki akcioni plan grada Vršca, 2016).

The potential vegetation, according to Jovanović et al. (1986) on the southern part of the LC area is forest-steppe (communities from the alliances *Festucion*

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* Local ecological action plan by the Vršac town, 2016

rupicolae and *Aceri tatarici-Quercion*). The northern part is a mosaic made from patches of saline meadow-steppe (*Festuco-Puccinellietea*), forest of oak and tatarian maple (*Aceri tatarici-Quercetum s. lat.*), as well as hygrophilous forest of pedunculate oak and broom (*Genisto-Quercetum roboris s. lat.*). From the natural habitats the salt meadows and salt steppes are most noteworthy (Knežević, 1994; Slavnić, 1948).

The main land use type of the investigated area is agriculture with the domination of arable crops. Meadows and pastures cover 5.6–11.6% of the CMs, vineyards located on the Vinogradarski breg occupy below 2% of total area, while orchards are found on negligible areas (**Službeni list*, VR 92/2012). The forests cover of LC area is below 0.5%.

Field work

The main objectives of the field work were set by GIZ. In the phase of planning, the input data (landscape history, geographical features, recent and planned land use, etc.) were collected and the localities foreseen for survey were selected: habitat fragments, semi-natural and man-made landscape elements, environmentally sensitive areas and large units of homogenous arable land. The field work was carried out by the use of printed orthophotos and cadastral maps of land use, in June and July of 2018, at 90 locations of observation. List of detected plant species and photo-documentation were prepared at each site, while the threats to natural resources were estimated visually. Unless otherwise stated, nomenclature follows the Euro+Med Plant Base (2006+). At the observed locations under field crops, the condition of crop cover was also assessed. All the surveyed locations were georeferenced by Trimble GPS receiver, as point and/or shape and transferred on the cadastral map. The software used for the mapping was ESRI ArcGIS Geostatistical Analyst 10. The presence of birds was detected by transects in 6 selected areas containing natural or semi-natural landscape elements.

Landscape and habitat assessment

Landscape and habitat assessment, by descriptive method set by GIZ (Thomas, 2017), was performed in relation to the following criteria:

- Endangerment of soil quality by erosion, pollution or inadequate use;
- Presence of indicator plant species of natural habitat types;
- Presence of strictly protected and protected species or protected habitat types;
- The habitat quality was assessed by floristic diversity and abundance of invasive plant species. At selected localities the presence and abundance of rare/threatened bird species were also used as indicators;

* The Official Paper.

– Evaluation of habitat fragments from the aspect of long-term preservation possibilities was based on the next characteristics: size and shape of the spatial unit, influences of neighbouring plots (land use type, presence of invasive species or human disturbances), as well as possibilities of sustainable use;
– Rarity – elements within a given space which have a higher value.

RESULTS AND DISCUSSION

Landscape elements with natural or cultural values

Natural habitats were presented by heavily fragmented grasslands, in the form of pastures, meadows and grass strips along the roads and canals. Based on the presence of characteristic species (Blaženčić et al., 2005), two habitat types listed as priorities for protection in Serbia (*Službeni glasnik RS*, 35/2010) were revealed. Pannonic loess steppic grasslands were identified by 42 indicator species, such as *Andropogon ischaemum*, *Festuca stricta* subsp. *sulcata*, *Poa angustifolia*, *Asparagus officinalis*, *Centaurea scabiosa*, *Euphorbia nicaeensis* subsp. *glareosa*, *Fragaria viridis*, *Linum austriacum*, *Orlaya grandiflora*, *Salvia nemorosa* and *Thymus pulegioides* subsp. *pannonicus.* Steppe fragments were detected on the slopes of valleys near Uljma and Izbište and along the road between Uljma and Dumača hill, as well as in the grass strips along the hedges and dirt roads of the loess plateau. The fragments of Pannonic salt steppes and marshes, identified by 15 indicator species, such as *Agrostis stolonifera*, *Alopecurus pratensis*, *Festuca pseudovina*, *Puccinellia distans* subsp. *limosa*, *Galatella cana*, *Camphorosma annua*, *Hordeum hystrix*, *Plantago maritima*, including the protected *Tripolium pannonicum* and *Limonium gmelini*, were revealed in the surroundings of Vlajkovac. Non-saline meadows and pastures represented the most fragmented and degraded habitat type, hardly identifiable by national classification, detected on the pastures, in the road verges and canal banks. However, some fragments still preserved some floristic rarities: the population of strictly protected *Iris spuria* was detected in the abandoned meadow near Vlajkovac, while specimens of *Iris spuria* and the protected *Senecio doria* were found on the edge of the dirt road leading from Uljma toward Nikolinci.

The semi-natural landscape elements of the investigated area were deficient in woody vegetation. Only one small, planted forest (named as Memorial Park) and several very small woodlots, dominated by young trees and shrubs were present in the LC area. Tree lines were restricted on the edges of the asphalted roads connecting the settlements (Figure 3), and a few solitary trees were found near the settlements. Field margins were detected only on the steep slopes of the Dumača hill, in the form of narrow grass strips more or less overgrown by bushes. A few detected hedges were scattered in the area, containing 5–7 autochthonous species such as *Acer tataricum*, *Cornus sanguinea*, *Crataegus monogyna*, *Euonymus europaeus*, *Ligustrum vulgare*, *Rhamnus catharticus*, *Rosa* sp. The network of field roads was dominated with narrow dirt roads unfavourable for hosting tree lines or hedges, therefore the grass strips of road verges and canal banks were the most frequent semi-natural landscape elements of the area. Their floristic composition indicated that most of them were remains of natural grasslands.

The most important anthropogenic landscape element with considerable natural values was the network of ameliorative canals. The secondary vegetation of canal beds and banks contained the species natural habitats, in the main canal the protected *Trapa natans* agg. was abundant. The floristic diversity and the large number of observed animal species (dragonflies, butterflies, bugs, beetles, etc.) suggested that they represent an important secondary habitat, in accordance with the literature data from the wider region (Krizmanić et al., 2015; Stojanović et al., 2007; Tölgyesi et al., 2022). The abandoned clay-pits of the brickyard near Uljma represented a habitat mosaic containing wetlands, used as a feeding place for wildlife and a breeding habitat for the strictly protected *Merops apiaster*.

The artificial mounds, noted as archaeological sites (*Prostorni plan Opštine Vršac, 2015; Vršac EIA, 2015), were the only remarkable anthropogenic landscape elements with cultural values.

Detected threats to natural resources

Soil degradation by erosion was observed in the loess valleys in the CM Uljma and CM Izbište. These valleys were covered by grasslands in the past (Arcanum, 2018), but are currently used as arable land. Due to the ploughing, water erosion has washed down the black Chernozem topsoil from the slopes and the yellow loess substratum become clearly visible even on the orthophoto (Figure 1). The ongoing erosion process generated fissures in the soil between the crop rows (Figure 2). Earlier, the precipitation was retained by the stretch of grasslands. In recent years, the high intensity rainfalls form flood waves. The floods damaged the lower parts of the village Uljma in the years 2009, 2014 and 2017. In order to prevent further floods, construction of a retention basin is planned near the settlement (**Hidroprojekt, 2018), without even considering erosion control based on the sustainable use of the valley.

Despite the threat of wind erosion (Lokalni ekološki akcioni plan grada Vršca, 2016), there were no windbreaks in the area. Ploughing the grass strips along the road edges by the users of neighbouring plots has proved to be a widespread practice in all three CMs. Destruction of road edges resulted in the lack of hedges and the scarcity of floristically diverse grass strips. The extremely low number of semi-natural landscape elements refers to the poor quality of the connected ecosystem services. Grass verges provide habitats for pollinators and arthropods (Kütt et al., 2016), including species important for

* Spatial plan of the Vršac town
** Hydroproject

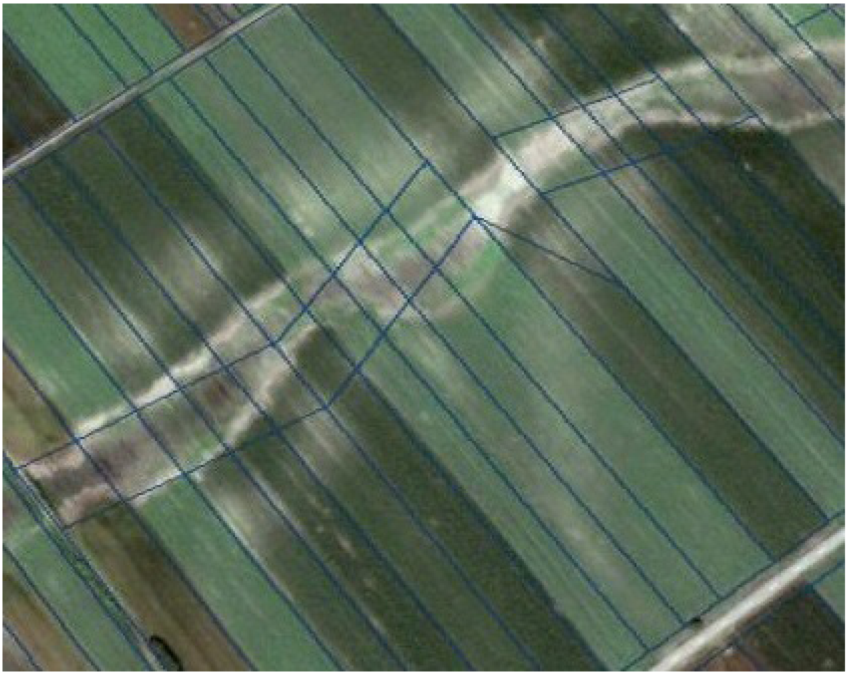

Figure 1. The satellite image shows the erosion on the slopes and the appearance of a light parent loess substrate

Figure 2. Traces of water erosion in the loess valley south of Uljma

the biological pest control (Rusch et al., 2016) and feeding areas for bird and game species (Graham et al., 2018). Hedges perform as windbreaks modifying microclimate and controlling erosion, also representing important habitats for wildlife and pollinators (Graham et al., 2018; Vanneste et al., 2020). Converting grass strips along the banks of ameliorative canals into arable land was also revealed at several localities, decreasing the buffer capacity of the vegetation (Dorioz et al., 2006).

The investigated common pastures near the settlements were mostly overgrazed, while the grasslands located at large distances of the settlement were abandoned, and some of them were even overgrown with woody vegetation. Invasive plant species were detected both at abandoned and overgrazed grasslands. Only small parts of the grasslands were used as hay fields that had been mowed in the period of the fieldwork. Almost at every grassland patch units converted into arable land were detected, or segments showing traces of previous cultivation and abandonment. Communal and construction waste was deposited on the common pastures near the settlements. The improper use has led to degradation of the grasslands, threatening not only their biodiversity (De Groot et al., 2010), but reducing ecosystem services provided by them, such as natural pest control (Perrot et al., 2021) or habitat for wildlife and game species (Stoate et al., 2009).

Invasive plants (a total of 14 species) were detected in all types of the surveyed landscape elements, except the grasslands on the extremely saline soil. The most frequent species of canal network was *Amorpha fruticosa*. Some sections of the canal dikes were covered with *Lycium barbarum* and *Robinia pseudoacacia*, the later forming a dense thicket as a result of illegal coppice. *Ailanthus altissima* and *Asclepias syriaca* were spreading on the overgrazed pastures, while *Celtis occidentalis* was observed in all habitat types.

Evaluation and proposed compensation measures

Despite the low number of natural and semi-natural landscape elements, the surveyed area still preserved a remarkable biodiversity. During the field work a total of 266 plant species were determined, six of them protected by law (*Službeni glasnik RS*, 98/2016). Results of the habitat values assessment show that 23 bird species were detected, but the real number of rare and threatened species is probably higher since the field work was carried out at the end of the breeding period. According to Serbian law (*Službeni glasnik RS*, 98/2016), 16 species were strictly protected, three were protected and four were listed as game species under special condition. According to EU Birds Directive (2009), 11 species were of international importance, out of which six belonged to the category I (species subject of special conservation measures): *Egretta garzetta*, *Tringa glareola*, *Chlidonias hybrida*, *Circaetus gallicus*, *Lanius collurio* and *Anthus campestris*. The presence of some species, such as *Merops apiaster*, *Saxicola rubetra*, *Upupa epops* and *Falco subbuteo* indicated richness of biodiversity and importance of remaining steppic grasslands for their survival.

One of the possible explanations for high biodiversity is the fact that at its southern part the LC area borders with the Special Nature Reserve Deliblato Sands. The dispersing organisms from Nature Reserve can maintain species populations at sites that would not ensure their long-term viability (De Groot et al., 2010).

By processing all collected data, the landscape elements were categorized (Thomas, 2017) in three levels (Figure 3). Category I Landscape elements that remain undisturbed included the following spatial units:

- Grasslands registered as a habitats of protected and strictly protected species (INCVP, 2018) and the abandoned clay-pits as a habitat of strictly protected species proved by the observations during the survey,
- Memorial Park, as the only forest of the area and the mounds already foreseen for protection (Prostorni plan Opštine Vršac, 2015; Vršac EIA, 2015),
- Landscape elements with the role of habitat and/or ecological corridor, including the hedges with grass strips along three roads (Izbište – Ritiševo, at the foot of Dumača hill and near Vlajkovac) and also the grass strip along the road leading from Uljma toward Nikolinci.

Category II Landscape elements that could be destroyed with obligatory ecological compensation include the meadows, pastures and hedges not listed in Category I, as well as the woodlots. Category III Landscape elements, not listed in the previous categories, could be removed without environmental compensation.

Compensation of meadows and pastures destroyed in the process of land consolidation should be done by forming meadows or pastures of the same total area in areas unsuitable for cultivation (muddy soils, saline depressions, slopes endangered by erosion), primarily near isolated habitats or near ecological corridors. The site selection has to be in accordance with habitat conditions and opportunities for sustainable use by mowing or grazing.

The compensation of removed hedges and woodlots has to be carried out within planning the windbreak network and game management. Since the subject area is extremely poorly forested, it is necessary to ensure the necessary area for windbreaks during the land consolidation process, in accordance with the current legislation (*Službeni glasnik RS*, 62/2006 in version 95/2018).

CONCLUSION

Land consolidation (LC) is an excellent tool for the planned protection and improvement of nature through ensuring coexistence of agriculture and environmental protection. Negative consequences of intensive agriculture have been noticed in the Vršac area of LC, including abandonment or overgrazing of pastures and meadows, converting grasslands into arable land, soil erosion and habitat fragmentation. Although the area of LC is without natural forest and extremely poor in semi-natural elements of rural landscape, the existing entities were revealed as refuges for protected species. Some of the grassland

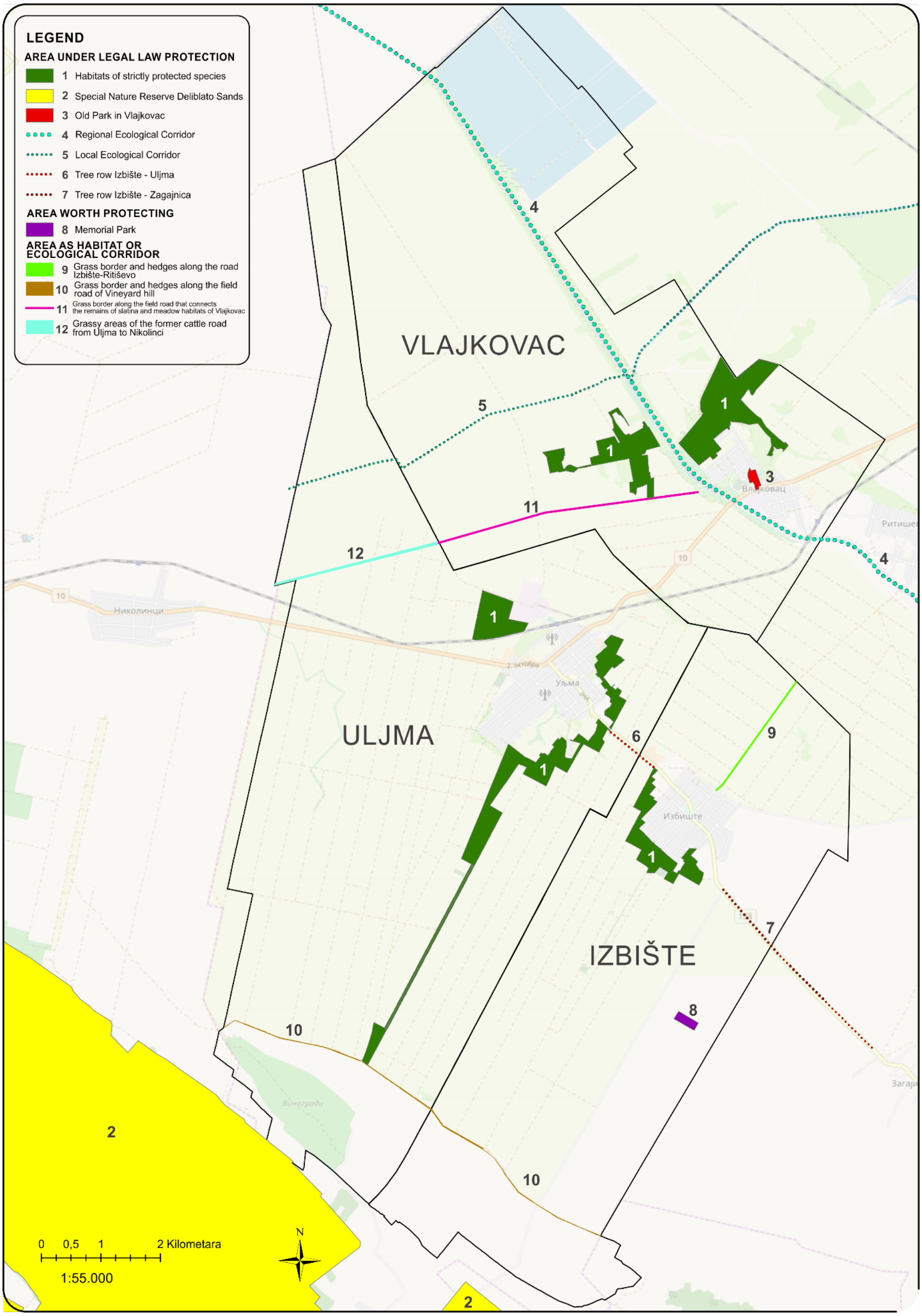

Figure 3. The categorization of the landscape elements

fragments belonged to protected habitat types. The final categorization of the landscape elements was conducted in three levels. Category I landscape elements had to remain undisturbed; Category II landscape elements could be removed with obligatory ecological compensation, while the Category III landscape elements could be removed without environmental compensation. Recommendations were given pointing out the possibilities for improving the environmental characters of the area by the land consolidation process.

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ОРИГИНАЛНИ НАУЧНИ РАД

ПРОЦЕНА УТИЦАЈА НА ЖИВОТНУ СРЕДИНУ У ОКВИРУ КОМАСАЦИЈЕ ЗЕМЉИШТА

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РЕЗИМЕ: У већини земаља у Европи комасација земљишта (КЗ) представља важну меру за унапређење пољопривреде и руралног развоја, укључујући питања животне средине,. У овом раду представљени су најважнији резултати процене утицаја комасације на животну средину (EIA) у Општини Вршац кроз пилот пројекат заснован на методологији ЕУ постављеној у оквиру пројекта: "Strengthening Municipal Land Management in Serbia", уз подршку Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH. Током лета 2018. године спроведено је теренско истраживање природних, полуприродних и вештачких елемената предела, еколошких и културних вредности на 90 локација. Урађена је процена стања ових вредности и процена потенцијалних штетних утицаја комасације на животну средину. Осмотрени су негативни утицаји интензивне пољопривреде, као што су: напуштање или прекомерна испаша пашњака и ливада, њихово претварање у обрадиво земљиште, ерозија земљишта и фрагментација природних станишта. Иако је подручје КЗ без природне шуме и изузетно сиромашно природним елементима руралног пејзажа, постојеће целине су откривене као уточишта за заштићене врсте. Неки од фрагмената травне вегетације припадали су заштићеним типовима станишта. Коначна категоризација елемената пејзажа спроведена је у три нивоа. Елементи предела I категорије – који остају ненарушени (не смеју се уклањати); II категорија – елементи пејзажа неутралног карактера са обавезном еколошком компензацијом; III категорија – елементи пејзажа који се уклањају без еколошке накнаде. Дате су препоруке којима се указује на могућности за побољшање еколошких карактеристика подручја процесом комасације.

КЉУЧНЕ РЕЧИ: комасација, процена утицаја на животну средину, елементи предела, индикаторске врсте, Вршац

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ORIGINAL SCIENTIFIC PAPER

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THE IMPORTANCE OF URBAN VEGETATION IN THE CARBON CYCLE AND OXYGEN RELEASE

ABSTRACT: Urban vegetation affects the environment in several ways. It improves air quality, the height of drainage water, and soil properties, balances temperature variations (temperatures are lower in summer and higher in winter in the presence of urban vegetation), reduces UV radiation, reduces noise, increases relative air humidity and contributes to certain types of social development (ecological, aesthetic, economic aspects of urban development). Urban vegetation especially improves microclimatic conditions in large cities and considerably contributes to the urban increase of oxygen. It is of vital interest to pay attention to its size and quantity in urban areas. To assess CO_2 absorption and O_2 release, the photosynthesis of five tree species and three types of herbaceous plants species was measured. The obtained results show that there is a certain variability in terms of the species that was analyzed. Thus, fast-growing woody deciduous species are more suitable due to the greater and faster growth of biomass, which requires a larger amount of CO_2 compared to herbaceous species. The careful calculation indicates the requirement of two to four trees per person or 30–40 m^2 of a grassy area per person to balance the inhabitant oxygen consumption. For the overall contribution of green vegetation, the multiple qualitative benefits of green areas in urban areas should certainly be considered.

KEYWORDS: urban vegetation, carbon cycle, oxygen release

INTRODUCTION

Urban vegetation increases the quality characteristics of urban habitats in several ways. It contributes to the quality of life and human health in populated

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areas. Primarily, it improves the quality of the air, the height of the drainage water and the soil properties, balances temperature variations (temperatures are lower in summer and higher in winter in the presence of urban vegetation), reduces UV radiation and contributes to certain types of social development (ecological, aesthetic, economic aspects of urban development). There is also a negative impact related to the costs of planting, maintenance of greenery, damage to road pavements, buildings, etc. (Song et al., 2018).

Urban vegetation especially improves the microclimate conditions in large cities, considering the huge amount of anthropogenically released heat that is generated due to the accumulation of solar heat on asphalt, concrete and other artificial surfaces, but also due to the huge use of air conditioners and increased traffic circulation (Erell et al., 2011). Salamanca et al. (2013) state that in the summer months, electricity consumption in the city is increased by 50–65% in the afternoon, because the urban temperature is several degrees higher than the corelated non-urban one in the same region.

Urban greening is a procedure that greatly reduces such problems, bringing a whole range of benefits. Takács et al. (2016) determined that urban trees reduce the temperature by 0.6 K on average in the open urban area, while in the immediate shade of the trees, the canopy blocks up to 90% of short-wave radiation. Kumar and Mahalle (2016) found in India that the so-called green roofs reduce the temperature in the building by 4.5 K. Hoelscher et al. (2016) determined that greening of facades reduces the temperature in buildings by about 1.7 K.

There is also a direct contribution to the improvement of air quality, which is reflected in the fact that in the process of photosynthesis, significant amounts of CO_2 are bound and, consequently, proportional amounts of O_2 are released. Thus in this process, released amounts of O_2 by far exceed the levels of aerobic respiration of the plant itself. So called "daily net photosynthesis" represents the difference between the total gross photosynthesis and respiration of a plant in a day, and it accounts for huge amounts of O_2 being released daily as a surplus, compared to its consumption in aerobic respiration. On an annual level, the contribution to the urban increase in oxygen is simply calculated through the increase in biomass, which is proportional to the absorbed carbon, i.e., the released oxygen (Nowak et al., 2007). Therefore, the species with the largest increase in biomass, which in the urban environment are primarily woody species, have the largest such contribution.

In addition to the above, the green belt in urban areas visibly affects the mood of the population. In urban areas, there are a lot of red and orange colours that have an unsettling effect on people, as well as gray colors that are depressing, therefore green alleys and other green areas have a calming effect (Kastori, 1995). Green areas built around landfills, garbage dumps, and other pollutants prevent the spread of unpleasant odours and the transfer of dust, although settling dust reduces the intensity of plant photosynthesis, but this way they protect people in urban areas. Under the influence of traffic and thermal power plants, a larger amount of SO_2 is released, which plants can absorb by oxidizing it into sulfate or including it in organic compounds. The degree of resistance to SO_2

depends on the plant species, so sophora (*Sophora japonica*) and boxwood (*Buxus sempervirens*) are very resistant, while white pine (*Pinus sylvestra*), spruce (*Picea abies*) etc. are not very resistant. These facts are very important when establishing tree lines in urban areas (Krstić, 2011).

MATERIAL AND METHOD

The net intensity of photosynthesis was measured on many woody and herbaceous species in the urban environment of the city of Novi Sad during the sunny days of September, 2019. To assess the absorption of CO_2 and the release of the correlative amount of O_2, the measurement was performed on ten randomly selected, young, fully formed leaves of five types of trees and three types of herbaceous plants (Table 1). A mobile device – the LCpro-SD Portable Photosynthesis System (ADC Biosystem) – was used to measure photosynthetic intensity.

Table 1. Measured intensities of photosynthesis and estimation of the amount of released oxygen (kg) per tree or per surface of an herbaceous grass plot, with an assessment of the needs of such plants per one able-bodied individual

Species	Intensity of photosynthesis (Range and mean) µmol m-2 s-1	Released O_2 (moles - proportional to moles of assimilated CO_2 when respiratory coefficient = 1)	Daily mO_2 (kg) released O_2 per 10 m tall tree or 10 m^2 plot of land per day in 12 h of daylight	Annual mO_2 (kg)	K	K yr
Silver-leaved linden (*Tilia argentea*)	5.5–10.2 (7.3)	7.3	0.40	74.73	2.90	5.71
Wild chestnut (*Aesculus hypocastanum*)	4.2–7.8 (5.8)	5.8	0.32	59.38	3.65	7.19
Plane tree (*Platanus* spp.)	7.8–12.9 (8.9)	8.9	0.49	91.11	2.38	4.69
European nettle tree (*Celtis occidentalis*)	8.9–14.5 (9.8)	9.8	0.54	100.33	2.16	4.26
Canadian poplar (*Populus* x *canadensis* (*P. nigra x P. deltoides)*	7.9–18.6 (13.2)	13.2	0.73	135.13	1.60	3.16
Bluegrass (*Poa pratensis*)	4.5–6.8 (5.2)	5.2	0.34	62.50	3.46	6.83
Dandelion (*Taraxacum ofiicinale*)	6.8–9.2 (7.8)	7.8	0.51	93.76	2.31	4.55
Wall barley (*Hordeum murinum*)	5.7–10.3 (7.8)	7.8	0.51	93.76	2.31	4.55

K – a coefficient showing how many trees of 10 m height (or an area of 10 m^2) are needed to meet the oxygen needs of one average working-age person. **K yr**. – annual coefficient that shows how many trees with a height of 10 m (or an area of 10 m^2) is needed to meet the annual oxygen needs of one person, considering that there is no photosynthesis in winter, and that photosynthesis is reduced even during cloudy days.
Annual mO_2 – mass of O_2 release per one year (kg)

By extrapolating the measured average values to the surface of the green parts of plants of the whole tree in a certain species or a certain surface of the herbaceous population, an estimate of the amount of released oxygen was made on a daily and annual level, as well as an estimate in relation to the needs of an able-bodied person.

Applied formulas:
Daily mass of O_2 (kg) = (A x 43200 x B (or C) x 0.000032) / 1000, and
Annual mass of O_2 (kg) = daily mass of O_2 x 185 days.

Parameters used for calculations are:
– A – Net intensity of photosynthesis;
– 43,200 – Number of seconds during 12 hours of nursery (the nursery lasts longer than 12 hours during the growing season, but photosynthesis is not at its optimum 1–2 hours after dawn, before dusk, also in periods of midday when there is not enough available water – therefore the number of hours is reduced to 12);
– 185 days – The factor of 185 days was obtained when 365 days were reduced for the period November–March when there is no photosynthetic activity of vegetation in our midday, but also for the number of cloudy hours when photosynthesis is at the level of 10–20% of the optimal;
– B – The surface of a pyramidal canopy with a height of 10 m is approximately 40.03 m^2. Peper and McPherson (2003) measured that the average surface area of *Platanus acerifolia* with a height of 5.23 m^2 was 20.94 m^2; based on this, it was calculated by proportion that the area of the leaves of a tree whose height is 10 m is 40.03 m^2, assuming a linear increase in area with height;
– C – Area of herbaceous grass vegetation. With the device for measuring the leaf area, it was measured that the area of the leaves of the species *Poa pratensis* is 47 cm^2 per 10 cm^2, i.e., the area of the plot is multiplied by a coefficient of 4.7; therefore, on a plot of 10 m^2 of a homogeneous population of *Poa pratensis*, the area of green leaves is about 47 m^2;
– 0.000032 – conversion coefficient µmol O_2 to g O_2;
– 1000 – conversion of g to kg O_2.

RESULTS AND DISCUSSION

The obtained results show that there is some variability in terms of the species analyzed. When it comes to woody species, 59.38–135.13 kg of oxygen is released per tree per year, depending on the species. For herbaceous species, the values vary from 62.5 to 93.76 kg of oxygen per 10 m^2 of herbaceous plot. In accordance with these results, the similar results are given by Nowak et al. (2007). They evaluated the amount of released oxygen per tree in many US settlements and concluded that the values vary in the range of 2.9 kg to 110.3 kg of oxygen per tree, which depends on the species, age, health status and climatic conditions. These authors obtained such results by considering the annual increase in biomass and estimating how much oxygen was released proportionally to the bound carbon in the given biomass. Their estimate of the number of trees per capita is slightly higher and ranges from 17 trees in New Jersey (USA) to 81 trees in a Calgary, Alberta (CAD) neighbuorhood per person (an average of 30 trees per person).

According to Huang et al. (2018), an able-bodied human consumes an average of 1.17 kg of oxygen per day during one day in the growing season. Our results indicate a ratio of three to four trees per person, considering the estimate/assumption that an average tree of 10 m^2 height is of similar leaf area among different species. Such data indicate that urban greening would contribute significantly to this aspect of regulating air quality, bearing in mind that the oxygen concentration, according to Huang et al. (2018), by the year 2100 will decrease from the current 20.946 to 20.825%. Urban vegetation simultaneously contributes to the reduction of CO_2 concentration in the air, as well as to the stabilization of air and soil quality in terms of pollutants (pollutants are adsorbed both in the air and in the soil), increases humidity in the air and increases the water retention capacity of the soil (Streiling and Matzarakis, 2003). If greening is viewed only from the aspect of CO_2 absorption and O_2 release, measures to maintain healthy and functional vegetation must also be considered, as well as the fact that on green areas, soil respiration to a significant extent cancels the effect of green vegetation, due to the significant consumption of oxygen by microbiological processes (Velasco et al., 2016). For the overall contribution of green vegetation, the multiple qualitative benefits of green areas should certainly be considered.

The question arises as to what the daily oxygen needs per inhabitant are. Comparing oxygen consumption in different groups of athletes, Pelemiš et al. (2011) found that maximum oxygen consumption or aerobic capacity (VO_2 max) ranges from 3.7 L/min in judo masters, to 5.7 L/min in kayakers, that is 0.4 kg O_2/h during active sports.

Considering that a person inhales 12–16 times in one minute for 0.5 liters of air containing 21% oxygen, and by converting to moles of oxygen, i.e., grams, i.e., kilograms, it turns out that a person's daily need for oxygen is about 3 kg/day. According to the data of Sziser (2003), about 14% of unused O_2 is released from the air that enters the lungs (air entering lungs 21% O_2), after exhalation (air exiting lungs), which means that about 1.5 kg of O_2 is consumed per day. Certainly, consumption is influenced by various factors, both external and internal, and the concentration of dissolved gas in body fluids depends on its solubility. The difference in gas solubility is important because the amount of gas multiplied by the gas solubility coefficient determines the amount of gas dissolved in body fluids, which affects the rate of gas diffusion through tissues. The solubility of oxygen in body fluids is 1.1 mmol/L. Considering the data of Huang et al. (2018), an adult inhabitant requires approximately 1.17 kg (816 L) of oxygen per day. From everything mentioned in the literature as well as our data, we can calculate that it is necessary to have 2–4 adult trees or 30–40 m^2 of grassy areas per inhabitant in urban areas. To increase the production of oxygen in urban areas, the Vertical Forest on the balconies of the solitaire is increasingly propagated, a kind of symbiosis of plants and buildings, which makes the buildings beautiful and aesthetic, and the plants in this way block dust and gases and protect the building from the wind and the sun (Blanc, 2008).

CONCLUSION

Contribution of urban green cover, especially trees and other woody species is of vital importance for improvement of air quality, soil properties and general climate conditions. Sustainable plant cover also supports important qualities of social human processes in urban settlements. Special emphasis is on the basic plant ability of increasing the amount of oxygen and reducing carbon dioxide, integrating to a protective role in air pollution. Oxygen consumption by one average person can be compensated by 2–4 planted trees or approximately 30–40 m^2 of grassy green area. Thus, the amount of oxygen release can significantly improve breeding air quality but also substantially increase carbon sink, contributing to global initiative to slow down anthropogenic carbon release to the atmosphere. Likewise, an aesthetic role of plant green areas is not to be neglected, because they make the environment more beautiful, providing a piece of habitat for other life forms too. All the aforementioned factors must be considered when planting urban plants.

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ОРИГИНАЛНИ НАУЧНИ РАД

ЗНАЧАЈ УРБАНЕ ВЕГЕТАЦИЈЕ У КРУЖЕЊУ УГЉЕНИКА И ОСЛОБАЂАЊУ КИСЕОНИКА

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РЕЗИМЕ; Урбана вегетација утиче на животну средину на више начина. Она пре свега побољшава квалитет ваздуха, висину дренажне воде и квалитет земљишта, уравнотежује варирање температуре (лети су температуре ниже, а зими више у присуству урбане вегетације), редукује УВ зрачење, смањује буку, повећава релативну влажност ваздуха и доприноси одређеним видовима социјалног развоја (еколошки, естетски, економски аспекти урбаног развоја). Урбана вегетација нарочито побољшава микроклиматске услове у великим градовима и значајно доприноси урбаном повећању кисеоника те је од виталног интереса усмерити пажњу на повећање величине и заступљености зелене вегетације у урбаним срединама. У циљу процене усвајања CO_2 и ослобађања O_2 мерен је интензитет фотосинтезе 5 врста дрвећа и 3 врсте зељастих биљака. Добијени резултати показују да постоји одређена варијабилност у погледу врсте која је анализирана. Брзорастући дрвенасти лишћари погоднији су због већег и бржег прираста биомасе за коју је потребна већа количина CO_2 у односу на зељасте врсте. Из литературе као и наших података можемо закључити да је неопходно имати 2–4 одрасла стабла дрвенастих врста или 30–40 m^2 травнатих површина по становнику у урбаним срединама. За сагледавање општег доприноса зелене вегетације, њене многобројне квалитативне карактеристике и утицаји морају се пажљиво проценити и узети у обзир.

КЉУЧНЕ РЕЧИ: урбана вегетације, кружење угљеника, ослобађање кисеоника

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ORIGINAL SCIENTIFIC PAPER

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HERBARIUM WOLNYANUM: BOTANICAL COLLECTION IN SREMSKI KARLOVCI GRAMMAR SCHOOL

SUMMARY: Herbaria as botanical collections have an interesting historical background. What was considered a traditional old-school field of science in the 20th century has become one of the focal points of interest in new-age science at the beginning of the 21st century, closely related to biodiversity, molecular screening and many other topics. Following the trend of rediscovering botanical collections, the Serbian scientific community invested in the process of acknowledging, renewing and presenting collections of national importance. The herbarium of the Sremski Karlovci Grammar School, protected under the name "Herbarium of Andreas Wolny", is the oldest herbarium collection in Serbia, dating back to the 18th century. It was established as a collection for obvious and practical teaching, but it grew beyond this scope due to the first systematic discoveries of the plant species of Srem and Fruška Gora Mountain. The most famous collector is Andreas Wolny, but besides him important teachers and botanists are Karl Georg Rumy, Grigorije Lazić, Dimitrije Petrović, Teofil Dimić and Jovan K. Borjanović. Although only a few data are of floristic importance (data that include both the location and date of collection), there is no doubt about the botanical and historical importance of this collection. Moreover, this botanical collection provides an overview of the events under which education and the first scientific and museum collections were developed in Serbia during the 18th and 19th centuries, and is thus an important cultural-historical document.

KEYWORDS: botany, Fruška Gora, Herbarium, Lazić, Kitaibel, Metropolitan Stratimirović, Rumy, Serbia, Sremski Karlovci, Wolny

INTRODUCTION

There are not many scientific documents about the historical development of herbarium collections. From the numerous travelogues documenting the exploration of the New World at the turn of the 14th and 15th centuries, there is

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accurate information about the first dried and pressed plants, *hortus sicuus* or *hortus mortuus*. They were not part of the scientific collections known today, nor were they kept in museums. They were created at a time when explorers and navigators were discovering previously unknown parts of the world, areas far from Europe, North Africa and the Middle East. European emperors and rulers of that time (both church and state leaders) wanted to conquer new territories and their natural wealth as well. Among them, plants, animals, rocks and minerals were of the greatest interest because they were used as drugs, medicines and other important elements of human and animal nutrition and health. They were all an important part of the valuable treasure of these expeditions. Plants, fungi and algae were numerous. To preserve them and transport them to Europe, they were either drawn or pressed and dried and then fixed (sewn) in books. Because of its credibility, the latter method prevailed over time. This gave the books a different appearance, and because of the maintenance they were reduced to a certain number of pages, usually a hundred, and therefore called *Centuria*. "Books with dried plants" designed this way could be easily transported, but also supplemented with notes. Finally, they were used as textbooks for pharmacists and physicians. They contained knowledge not only about human health, but also about the health of animals and livestock, and about the cultivation and production of numerous plants, either as edible species or as sources of materials for fabrics, cosmetics and other products (Manning and Klestinec, 2017). The limited space in these books may have dictated a systematic approach to description. Dried specimens were given names, usually in Latin, which was the official language at most schools and universities at the time. Latin plant names were polynomial, meaning they were composed of several words, epithets, that described the plant in more detail. These polynomials formed the basis for the emergence of the first scientific (binomial) nomenclature. Various vernacular names were also noted. For traders, collectors and breeders, these names were of great importance as they were recognized by the locals (Harris, 2018).

As a result of these expeditions, in the oldest European museums the most important and valuable parts of the collections are exsiccates collected during these events, rather than native plants. The native plants collected at that time were mainly species important for medicine, pharmacy and agriculture. As the material increased, conservation methods had to be changed, and over time institutions – herbaria – were established to preserve and maintain the large collections. These events marked the period from the 14th to the 18th century. During these 400 years, a large amount of knowledge was accumulated, and the conditions were created for the development of systematic revision, which led to the development of scientific disciplines, especially the systematics of plants and animals. There are a large number of such collections in Europe, and today they are a historical part of the largest and most important museum and university herbaria. Italian scientists are considered pioneers in the establishment of herbaria of this type, book-herbaria (Findlen, 2017).

An event that is now considered a turning point in the scientific approach to collecting information about the living world is the publication of the multivolume monographs *Species Plantarum* in 1753 and *Systema Naturae* in 1758 by Carl Linnaeus. Since then, botanical collections, i.e., herbaria have been the scientific documents on living plants. Today there are more than 3,000 herbaria worldwide, with 390 million botanical specimens permanently preserved (Thiers, 2022). These significant changes and recognition of the importance of the information preserved in herbaria were documented by Fosberg (1946), and today more than ever they are used by scientists as primary data and sources of information for the development of modern technologies (Besnard and al., 2018).

HERBARIUM OF ANDREAS WOLNY

Historical perspective

The first scientific expeditions in Serbia were organized in 1800 by Waldstein and Kitaibel, the leading botanists of Central European flora at that time (Gombocz, 1945 a,b). The first published floristic data for Serbia date from this period (Niketić et al. 2018). However, the collection of plant specimens and the preparation of the first "books of dried plants" preceded them. Andreas Wolny, professor of botany at the Sremski Karlovci Grammar School, collected plants in the surroundings of the Fruška Gora Mountain, as was common practice at universities and schools at that time, and in 1797 created a book of 100 plants – Centuria I. This can be considered the beginning of collecting plants in Serbia and preserving them in the form of a herbarium.

Sremski Karlovci Grammar School was founded at the end of the 18th century according to an educational concept clearly defined in the Habsburg Monarchy. This meant that professors gave lectures in German and Latin. Sremski Karlovci Grammar School was the first higher school founded and opened for children of Orthodox Serbs (Petrović, 1991), and in the first two decades the employed professors were mostly Slovaks. Among the professors, Andreas Wolny certainly stood out the most. He came to Sremski Karlovci in 1794 at the invitation of Metropolitan Stevan Stratimirović as a professor of geology, mineralogy, botany, and other school subjects in the field of nature. In addition to his teaching activities, Wolny worked with great dedication to develop a new curriculum, which was in accordance with the state principles, but included a greater proportion of demonstrative and practical teaching in the natural sciences of the time. In order to successfully implement the curriculum, it was necessary to adapt the teaching materials to it. As an already experienced naturalist (geologist and botanist), Wolny realized that the area in which he worked was insufficiently known and researched. Therefore, in parallel with his teaching activities, he started researching the flora and geomorphology of Sremski Karlovci and the wider surroundings of the Fruška Gora Mountain. Very soon after his arrival, already in 1797, Wolny prepared the first (of five) centuria on the flora of Srem, entitled "*Florae Sirmiensis Seu Plantarum in Sirmio sponte nascentium Centuria I. Anno 1797*" (Figure 1). This part of the collection is the best known and several publications are available (Marčetić, 1950; Marčetić, 1952 a,b; Marčetić and Babić, 1954).

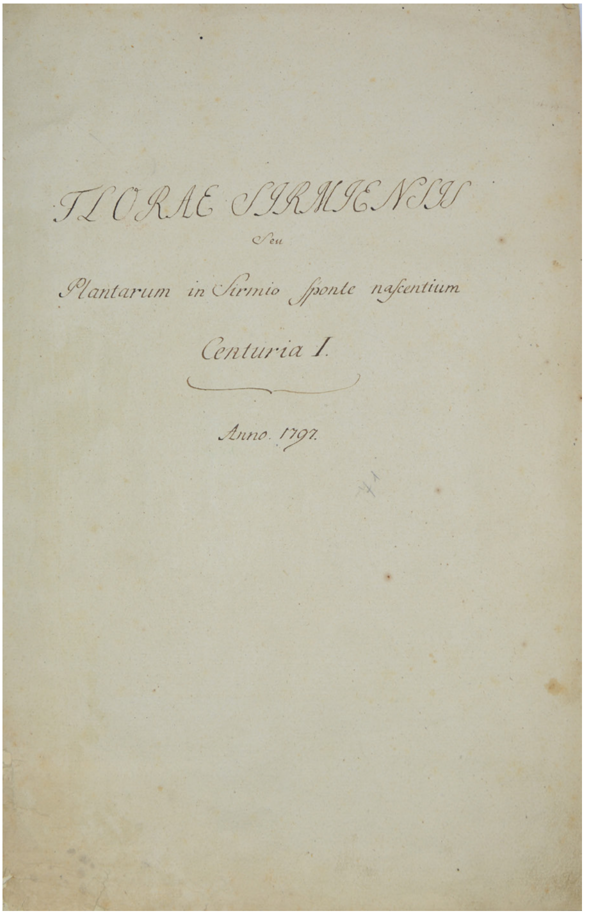

Figure 1. Title page of the first Wolny's Centuria

Conservation and legal protection of the collection

Until the end of the 19th century, the herbarium was continuously supplemented, and almost 7,000 plant specimens were collected and deposited. Only a small part of the material was collected by Wolny himself, an estimated 800 exsiccates. The rest was collected by later professors and probably by students. Among the material, there are specimens collected by Josif Pančić, as well as material obtained by exchange, which came from the Botanical Garden in Vienna. Due to its historical and cultural significance, the collection has been under state protection since 1950 as a cultural monument *Herbarium Andrije Volnija* (Decision No. 279/50, Institute for the Protection of Cultural

Monuments of Serbia). The declaration as a cultural monument was preceded by the process of cataloguing a cultural monument, and a catalogue book and a microfilm of this catalogue were prepared in the following ten years (Perić et al., 2012). The book is kept together with the collection in the Grammar School, while the microfilm is kept in the Institute for Nature Conservation of Vojvodina Province (*pers. comm*. Vida Stojšić).

In the process of legal protection, the herbarium was divided into sub-collections: 1) Ceremonial Herbarium (*свечани хербаријум* in Serbian), 2) Collection of Josif Pančić, 3) Fungi, Lichens, Algae, 4) Bryophytes, 5) Monocotyledons, 6) Dicotyledons, and 7) Material obtained by exchange. These sub-collections are kept in 54 cardboard boxes following this arrangement. The index cards are kept with the collections. However, the exsiccates are sorted differently and divided into two sub-collections: 1) Wolny, and 2) General Herbarium.

Regarding the original collection, Centuria II was not preserved, and there are several assumptions about its disappearance. It is not known whether it was destroyed, or its trace was lost. Part of the material from the herbarium of Andreas Wolny is now kept in the Institute for Nature Conservation of Vojvodina Province (PZZP). These boxes were found in underground premises at the Petrovaradin fortress, which were used as depots, where they were probably kept during the World War II. Due to the age and origin of the material, it was assumed that they also belonged to the collection of the Sremski Karlovci Grammar School (*pers. comm*. Ranko Perić).

Collectors in the Herbarium of Andreas Wolny

Andreas Wolny spent 22 years in Sremski Karlovci, where he worked first as a professor and later as the director of the Grammar school. The importance of his work and his influence on the following generations are undeniable. At the same time, they are a confirmation of his significant contribution to the beginnings of the development of natural sciences among Serbs and the knowledge of the nature of Srem. Wolny was and is one of the most famous professors and collectors. Therefore, it is justified to honour him by naming the collection after him.

Even though the collection of the Grammar School in Sremski Karlovci is named after Andreas Wolny, he is not the only collector, nor the only professor of this institution who made a significant contribution to the herbarium. The first among them to continue his work was his successor in the position of the director of the Grammar School (1816–1821) – Karl Georg Rumy. It is not known whether and how many plants he collected during this period. However, it is known that he was in contact with Metropolitan Stratimirović and visited this place several times. His interest in botany is proven by his published article, although at the end of his life, in 1846, more than 20 years after he had left the Grammar School and Sremski Karlovci (Rumy, 1846). This article consists of botanical data collected by Wolny mainly in the vicinity of Sremski Karlovci and Fruška Gora Mountain. The importance of this article is emphasized by Niketić et al. (2018), who states that it is one of the most important works from the beginnings of the development of botany in Serbia, which is less known until today.

In the same year that Rumy left the Grammar School, Grigorije Lazić became a professor of several natural science subjects. He remained in this position until his death in 1842, during which time he collected plant material and deposited it in the herbarium. In addition, in 1833 he published a special Centuria entitled "*Index vegetabilium sua sponte circa Carlovitium crescentium*" (Lazić, 1833). In this publication he listed 100 plant species from the surroundings of Sremski Karlovci and Petrovaradin, together with their vernacular names in German and Serbian. His total contribution to this collection is unknown to this day, but it is important to emphasize that he was a member of the Botanical Society in Regensburg, as Andreas Wolny had been before him.

The successors of Grigorije Lazić in terms of herbarium and plant collecting, were Dimitrije Petrović and Teofil Dimić. Almost 20 years after them, Jovan K. Borjanović collected exsiccates.

Dimitrije Petrović, together with Wolny and Lazić, collected an important part of the material. Josif Pančić himself, according to the information in the index cards, revisited some of his material. The name Petrović is also found on the labels of Josif Pančić's material (Perić et al., 2012). The year of his death, 1854, also marks the end of the era of collecting plants on Fruška Gora Mountain, 60 years after Wolny had started it.

Teofil Dimić 's contribution to the Herbarium, as far as it is known from the analysis of the index cards, consists of numerous materials obtained by exchange in the middle of the 19th century. He himself brought some of the material from the Botanical Garden in Vienna, while it is assumed that he was in correspondence with other botanists of the time, together with Dimitrije Petrović, and that he received specimens from other parts of Europe and the world. His contribution to botany, as well as to zoology, was recognized outside Sremski Karlovci, and he was a member of the scientific society *Zoologisch-botanische Gesellschaft.*

A few decades after them, in the 1880^{s}, Jovan K. Borjanović collected a significant number of specimens. At this time, it is not apparent how many specimens he collected. However, he published a book that contained a great knowledge in the field of fruit and viticulture and showed his great botanical knowledge (Borjanović, 1887).

The goal beyond creating a scientific collection

According to the dates in the herbarium cards, the plants were deposited in the herbarium of the Grammar School in Sremski Karlovci between 1797 and 1886. In this period almost 7,000 exsiccates were mounted. The specimens were collected in Sremski Karlovci and its surroundings, with many plants collected on Fruška Gora Mountain. Dimić enriched the collection with plant material collected in the Botanical Garden in Vienna. In addition, as was customary at the time, numerous sheets entered the herbarium through material exchange with botanists from all over Central Europe. Part of the material originated from outside Europe.

The composition and structure of the stored plant material suggests that the herbarium was intended primarily as a learning collection. That is, the goal was to enrich the collection with various representatives of different plant families, life forms, habitats and regions. This approach opens the possibility for students to become familiar with many representatives of different groups and regions. This assumption is supported by the information on the labels in Centuria I and II, where information on habitat, use, and flowering time is given, while the exact date is missing. Bunke (1995) points out this consistency with Wolny and adds that he was aware of the importance of giving the exact location and date (this is noted on some labels in the *Herbarium Wolnyanum* at BP), but his greater interest was in passing on knowledge about these plants rather than recording their distribution. It must be emphasized at this point that a system of recording data in collections was established at this time, and museum collections (including herbaria) evolved into scientific collections that served to classify and interpret the world.

The preliminary analysis of the stored material revealed that Wolny and his successors aimed to educate the citizens from the surroundings of Sremski Karlovci, whose main activity was agriculture (viticulture, fruit growing, farming). Therefore, today the collection contains data relevant to modern agriculture, namely traces of old varieties grown in gardens, fields and orchards.

Finally, all the above-mentioned professors and collectors in the herbarium have spent a lot of time collecting vernacular plant names. Considering the fact that the first decades of education were in German and Latin, and the use of Serbian language was forbidden, collecting and publishing these names in a herbarium or in publications is a significant step in the history and culture of the Serbian people in the Habsburg Monarchy and later in the Austro-Hungarian Empire. The multidisciplinary importance of the collection is also reflected in the scientific work, which, in addition to a systematized overview of medicinal plants, also provides an overview of the language fund of the 18th century (Tutuš, 2019).

CONCLUSION

Contemporary natural history collections involve the organization and maintenance of exsiccates in a specific systematic or geographic order, with fulfilled labels integrating the date and the location of sampling. Old historical collections that were part of school inventories usually do not contain all of these data. Nevertheless, their importance cannot be neglected. In most of these collections, such as in the Herbarium of Andreas Wolny in Sremski Karlovci, the first data on the distribution of plants in a given region are found, along with important etymological references. Within them, data can be found that support and enrich the national cultural and historical heritage. Finally, they can be used as a source of knowledge for various fields of study.

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ОРИГИНАЛНИ НАУЧНИ РАД

HERBARIUM WOLNYANUM: БОТАНИЧКА КОЛЕКЦИЈА КАРЛОВАЧКЕ ГИМНАЗИЈЕ

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РЕЗИМЕ: Хербаријуми, као ботаничке збирке, имају занимљив историјски развој. Оно што се у 20. веку сматрало традиционалном и превазиђеном науком, на почетку 21. века постало је центар интересовања науке савременог доба, уско повезано са биодиверзитетом, развојем молекуларних метода и многим другим. Пратећи тренд поновног откривања музејских збирки, међу које спада и хербаријум, као ботаничка колекција, српска научна заједница је улагала у процес упознавања, обнављања и представљања збирки од националног значаја. Хербаријум у Карловачкој гимназији у Сремским Карловцима, заштићен под називом *Хербаријум Андрије Волноī*, најстарија је хербарска збирка у Србији, која потиче из 18. века. Настала је као колекција неопходна у процесу извођења очигледне и практичне наставе, а превазишла је ове оквире, захваљујући подацима које чува, а који су резултат првих систематских открића биљних врста Срема и Фрушке горе. Најпознатији легатор и оснивач колекције је Андреас Волни. Осим што је био професор и директор, он је остао запамћен и као први научник који је сакупљао податке о биљном свету Фрушке горе, али и стена и минерала. Поред њега, значајни наставници, који су истовремено оставили свој траг у развоју хербаријума су Ђорђе Руми, Григорије Лазић, Димитрије Петровић, Теофил Димић и Јован К. Борјановић. Иако је мали број података од флористичког значаја (подаци који укључују и прецизне податке о локалитету и датуму сакупљања), нема сумње да колекција има и ботанички и културно-историјски значај. Проучавањем свеобухватних података који се налазе у овој колекцији, она даје преглед догађаја у оквиру којих се развијала просвета и прве научне и музејске збирке у Србији током 18. и 19. века и као таква представља значајан културно-историјски документ, законом заштићен од 1950. године.

КЉУЧНЕ РЕЧИ: ботаника, Фрушка гора, хербаријум, Лазић, Китајбел, митрополит Стратимировић, Рума, Србија, Сремски Карловци, Волни

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ORIGINAL SCIENTIFIC PAPER

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QUALITY OF GARDEN PEA (*Pisum sativum* L.) PRIMED SEED

ABSTRACT: The objective of this study was to examine the effects of different priming treatments on seed quality and initial growth and development of garden pea. For this purpose, three garden pea cultivars, developed at the Institute of Field and Vegetable crops, Novi Sad were examined. The laboratory experiment was conducted under optimal conditions. Seeds were primed in water (hydropriming), 0.5% KNO_3 solution, and -0.49 MPa PEG solution for 24 hours; non-primed seeds were controls. The results showed that the percentage of germination, shoot and root length, and mean germination rate significantly increased after the tested priming treatments, while the percentage of abnormal seedlings and mean germination time were significantly decreased after the priming treatments compared to the control. The increase of fresh and dry seedling biomass was significant only in cv.1 and cv.2. Despite the genetic diversity of pea cultivars, the results indicated that the examined seed priming treatments enhanced seed quality and vigour of garden pea cultivars.

KEYWORDS: garden pea cultivar, seedling growth and biomass, seed priming, seed quality

INTRODUCTION

Garden pea (*Pisum sativum* L.) is an important legume, rich in proteins, dietary fibres, starch, carbohydrates and micronutrients, including vitamins and

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minerals. With up to 35% of proteins and essential amino acids, such as lysine and tryptophan (Elzebroek and Wind, 2008), the garden pea is an important legume in human nutrition. According to Petrović et al. (2016), together with other legumes, it provides one-third of the required amount of protein in human consumption. The annual global production of green pea seeds is approximately 21.7 million tons (FAOSTAT, 2021), while Serbian production amounts to approximately 38,000 tons and has a rising trend.

Seed germination is the most critical stage in plant growth and development, ultimately determining the success of crop production (Kathare and Hug, 2021; Almansouri et al., 2001). Rapid and uniform seedling emergence is the key factor of crop performance, because slow germination exposes plants to adverse environmental conditions, strong weed competition and soil-borne diseases (Paparella et al., 2015; Vidak et al., 2022). Seed quality has become a priority, while seed priming has proven to be a well-established treatment for seed quality enhancement (Adhikari et al., 2021; Cokkizgin, 2013; Paparella et al., 2015). Seed priming is a water-based technique that allows controlled seed rehydration and triggers metabolic processes normally activated during pre-germinative metabolism, while preventing seed transition towards full germination.

Beneficial effects on seed germination of various seed priming techniques, such as hydropriming and osmopriming, have been reported in many crops. However, information on the effects of these seed pre-treatments on the seed quality of the domestic garden pea is lacking. In this context, the aim of the research was to evaluate the effects of hydropriming and osmopriming on garden pea seed quality and initial development.

MATERIALS AND METHODS

Experimental (Plant) materials

The seeds of three garden pea cultivars were obtained from the Institute of Field and Vegetable Crops, the National Institute of the Republic of Serbia, Novi Sad.

Priming treatments

Before priming, garden pea seeds were disinfected with 5% (w/v) sodium hypochlorite for 5 min and then rinsed thoroughly with distilled water thrice. Seeds were immersed keeping the ratio of seed weight and solution volume 1:5 (w/v) in a 0.5% KNO_3 solution, a polyethylene glycol PEG-6000 solution (-0.49 MPa) and water at 25 °C for 24 h in dark. Thereafter, treated seeds were rinsed with distilled water thrice and dried back near to their original moisture content at room temperature.

The germination test

Working samples consisted of 3 x 100 seeds. Primed and non-primed seeds were placed in plastic boxes 240 x 150 mm with double-layer filter paper moistened with distilled water. The samples were incubated for 8 days in a germination chamber at 20 °C. Germination, abnormal seedlings, as well as shoot and root length, and fresh seedling weight of ten seedlings were determined eight days after seed placement in the germination chamber. To obtain the dry seedling weight, pea seedlings were oven-dried at 80 °C for 24 h. Mean germination time was calculated using the equation of Ellis and Roberts (1981): $MGT=\Sigma Dn/\Sigma n$, where n is the number of germinated seeds on day D and D is the number of days. The mean germination rate was calculated as the reciprocal value of the mean germination time (Ranal et al., 2009).

Statistical analysis

The data were subjected to analysis of variance using Statistica 10 (StatSoft, Inc., 2007) software package. Mean values followed by standard deviation were separated using Duncan's multiple range test at probability level $p<0.05$.

RESULTS AND DISCUSSION

Analysis of variance showed that the germination percentage of cv.1 was significantly influenced by all the tested seed priming treatments (Figure 1). However, other tested pea cultivars responded differently to priming treatments. Germination of pea cultivar cv.2 was significantly increased by priming with KNO_3 and PEG solution up to 7.28% and 4.41%, respectively. In cv.3, a beneficial effect on germination percentage was observed after hydropriming (74.6%) and priming with PEG solution (88.0%) compared to the control (72.3%). Garden pea cultivars also responded differently to priming treatments in terms of abnormal seedling percentage. In cv.1, no significant difference in the percentage of abnormal seedlings was observed between primed seeds and control. Hydropriming significantly increased the percentage of abnormal seedlings (4.7%) of cv.2 compared to control, while all the tested treatments led to a significant decrease of abnormal seedlings in cv.3 compared to control. The results are in agreement with the findings of Yanglem et al. (2016) and Kuar et al. (2015). Sachan et al. (2016) also found that hydropriming significantly increases pea seed germination. However, a higher percentage of abnormal seedlings in cv.2 after hydropriming could be due to a higher rate of radical protrusion and imbibitional injury, and a rapid inflow of water into the fast-absorbing legume seed embryonic cells, which led to physical disruption of the cell membrane (Powell and Matthews, 1977; Sachan et al., 2016). Moreover, similar increases in the germination of alfalfa (Mouradi et al., 2016a), soybean (Miladinov et al., 2018), wheat (Baque et al., 2016), and rice (Ruttanaruangboworn

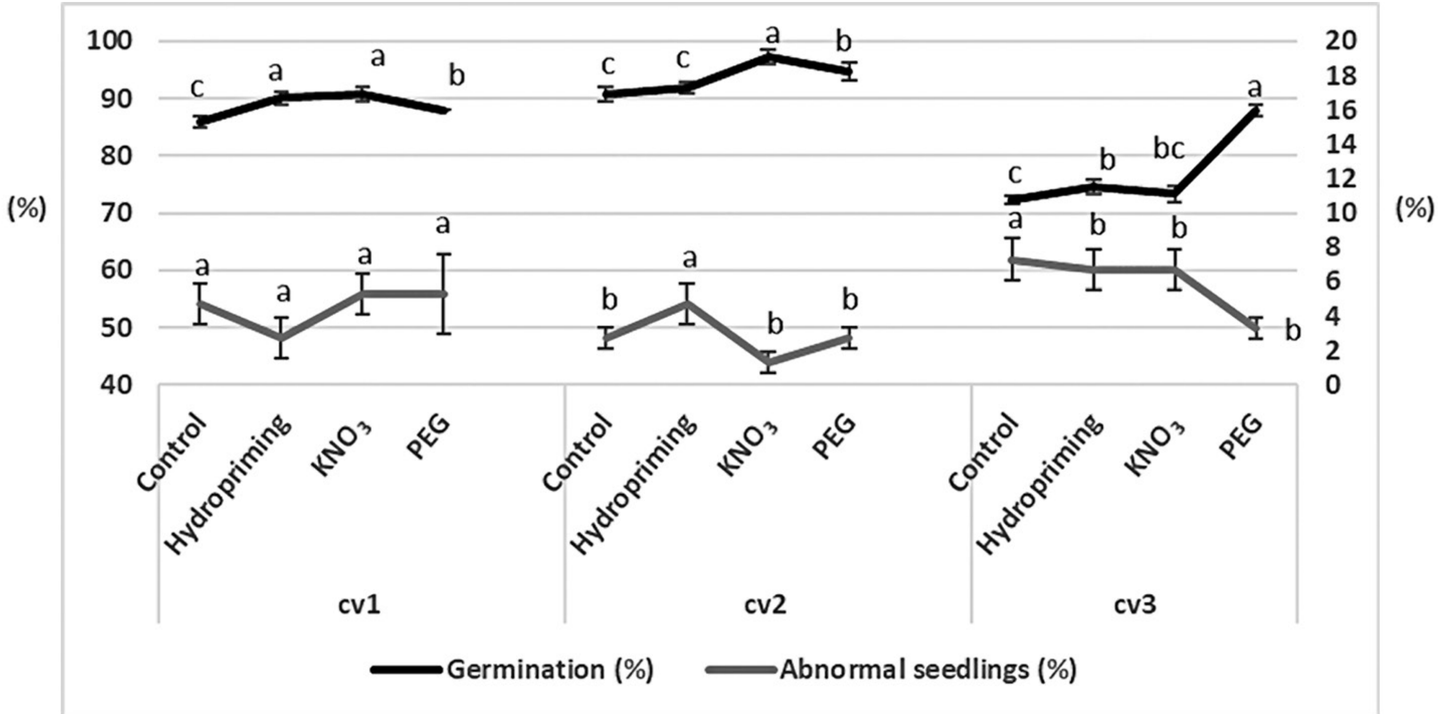

Figure 1. The effects of seed priming with water, KNO_3 solution and PEG solution on germination percentage (%) and abnormal seedlings (%) of garden pea cultivars. Mean values with the same letter within pea cultivar are not statistically different at $p \leq 0.05$.

et al., 2017) through priming with water, KNO_3 and PEG were reported in previous research. Seed priming most likely contributes to the repair of membrane damage caused by deterioration, which results in better germination and higher vigour level compared to non-primed seed (Jisha et al., 2013).

The shoot length of pea seedlings varied among the pea cultivars, ranging between 25.9 mm and 29.5 mm in control (Figure 2). However, the shoot length of pea cultivars was significantly affected by all the tested seed priming treatments. The results revealed the maximum shoot length (41.6 mm) in pea cultivar cv.1 primed with water (hydropriming). Pea cultivars cv.2 and cv.1 had a similar performance after the seed priming treatments, while the shoot length of cv.3 was increased up to 16.3% by priming with PEG. Beneficial effects of hydropriming and PEG priming on shoot length were also reported by Cokkizgin (2013) and Yanglem et al. (2016) on pea, and Baque et al. (2016) on wheat. Furthermore, the root length of cv.1 and cv.2 was significantly improved after all the tested seed priming treatments compared to the control, while cv.3 responded positively only to priming with KNO_3. According to Yanglem et al. (2016), a significant improvement in shoot and root length in primed seeds might be due to the involvement of priming agents in cell elongation or cell division and meristem growth.

However, pea cultivars responded differently to priming treatments in terms of fresh and dry biomass accumulation (Figure 3). All the tested pea cultivars had similar fresh and dry seedling weights in control. A significant increase in fresh and dry seedling weight was recorded in cv.1 after all the seed priming treatments, and in cv.2 after hydropriming, while no significant differences were observed in cv.3 compared to the control. Maximum values of the fresh and dry weight of seedlings were observed in cv.1 primed with KNO_3. The results are in accordance with the findings of Ali et al. (2021), who confirmed KNO_3 effectiveness in improving the fresh and dry weight of seedlings. Furthermore, a positive effect of hydropriming on fresh and dry seedling biomass was also observed in chickpea (Sarwar et al., 2006), sunflower (Catiempo et al., 2021) and other plant species. Contrary to our results obtained in cv.3, where the increase in fresh and dry seedling weight was not statistically significant compared to the control, Barique et al. (2016) reported that the maximum dry weight of seedlings was recorded in seeds primed with PEG solution. These results are in accordance with the results of Mouradi et al. (2016b), obtained on alfalfa under optimum conditions.

Analysis of variance showed that MGT and MGR were significantly affected by the seed priming treatments (Figure 4). In general, all the seed priming treatments significantly decreased MGT and increased MGR in all the tested pea cultivars compared to the control. The reduction in MGT ranged between 19.4% (cv.3 primed with PEG) and 46.7% (cv.3 primed with KNO_3) depending on the pea genotype and the seed priming treatment. A similar pattern of increase in MGR following a priming treatment was observed. Osmopriming also caused MGT reduction in soybean (Sadeghi et al., 2011), wheat (Abnavi and Ghobadi, 2012), sugar beet (Hosseini and Koocheki, 2007), and maize (Ahammad et al., 2014). Seed priming with KNO_3 and PEG has

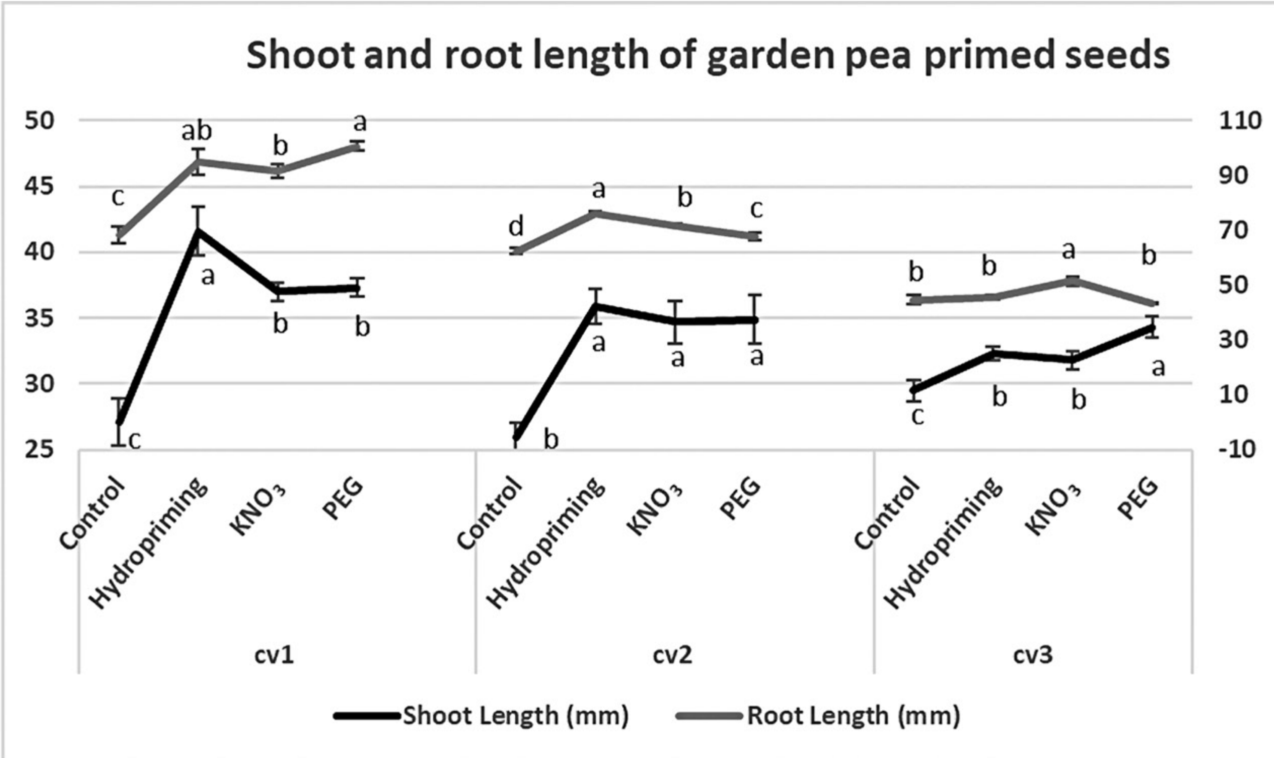

Figure 2. The effects of seed priming with water, KNO_3 solution and PEG solution on a shoot (mm) and root length (mm) of garden pea cultivars. Mean values with the same letter within the pea cultivar are not statistically different at $p \leq 0.05$

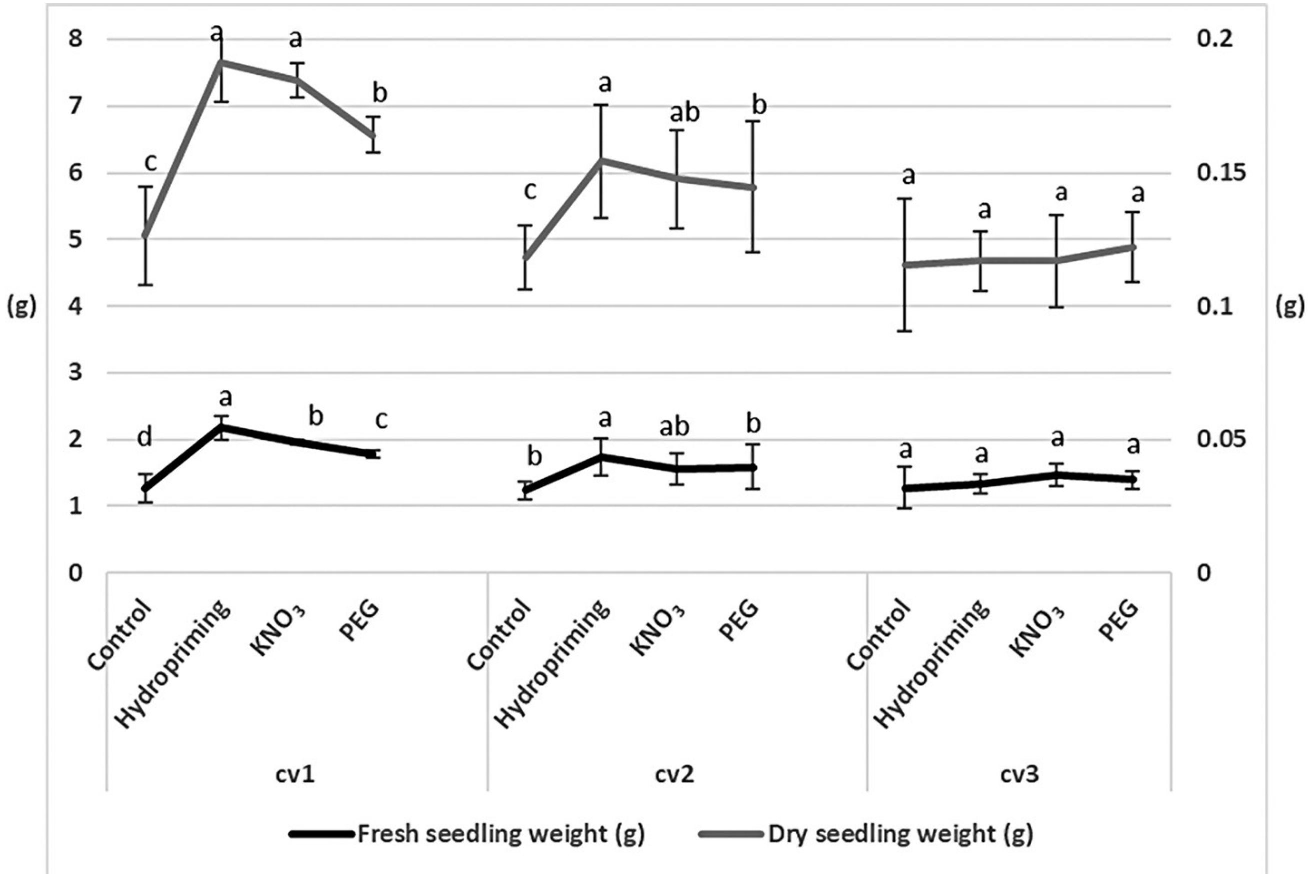

Figure 3. The effects of seed priming with water, KNO_3 solution and PEG solution on fresh (g) and dry seedling weight (g) of garden pea cultivars. Mean values with the same letter within the pea cultivar are not statistically different at $p \leq 0.05$

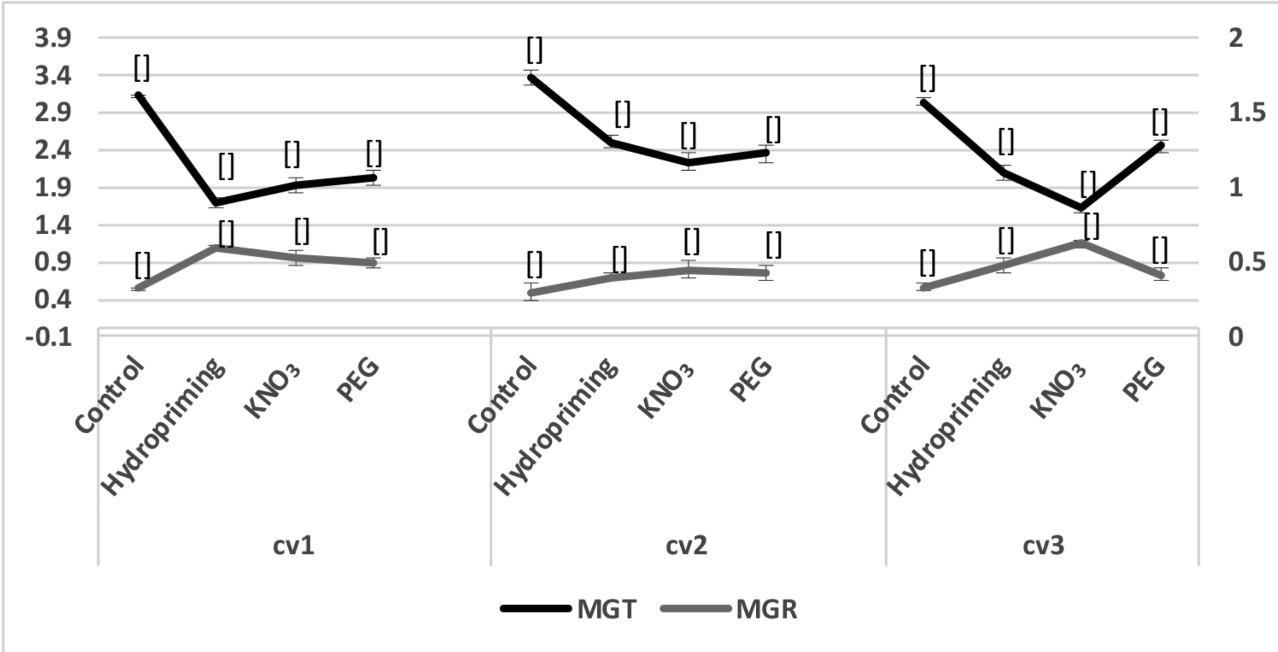

Figure 4. The effects of seed priming with water, KNO_3 solution and PEG solution on mean germination time (MGT) and mean germination rate(MGR) of garden pea cultivars. Mean values with the same letter within the pea cultivar are not statistically different at $p \leq 0.05$

beneficial effects on germination speed and uniformity (Ruttanaruangboworn et al., 2017), as well as supporting the early stages of the germination process by the mediation of cell division in germinating seeds (Nasri et al., 2011), which in turn results in positive effects on MGT and MGR. Moreover, seed priming changes the activity of enzyme α-amylase (Farooq et al., 2006) and other hydrolytic enzymes (Szopińska and Politycka, 2016) in primed seeds, which leads to better germination and seedling growth.

CONCLUSION

The obtained results confirmed the positive effects of the tested seed priming treatments on seed germination and initial growth of garden pea cultivars. The positive effect of the tested seed priming treatments on biomass accumulation was less pronounced in cultivar 3 compared to the other garden pea cultivars. The findings indicate that priming seeds with a solution of KNO_3 and PEG could efficiently improve the quality of garden pea seeds and initial plant development.

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ОРИГИНАЛНИ НАУЧНИ РАД

КВАЛИТЕТ СЕМЕНА ПОВРТАРСКОГ ГРАШКА (*Pisum sativum* L.) НАКОН ПРАЈМИНГА

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РЕЗИМЕ: Циљ овог истраживања био је да се испитају ефекти различитих третмана прајминга на квалитет семена и почетни раст и развој повртарског грашка. У ту сврху испитане су три сорте повртарског грашка створене у Институту за ратарство и повртарство у Новом Саду. Лабораторијски оглед је изведен у оптималним условима. Семе је потапано у воду (хидропрајминг), 0,5% раствор KNO_3 и -0,49 MPa раствор PEG током 24 сата; нетретирано семе је било контрола. Резултати су показали да су се проценат клијања, дужина надземног дела и корена изданака, као и средња стопа клијања значајно повећали у испитиваним третманима прајминга, док су се проценат атипичних изданака и средње време клијања значајно смањили у третманима прајминга у поређењу са контролом.

Повећање свеже и суве биомасе изданака било је значајно само код сорти cv.1 и cv.2. Упркос генетској разноликости сорти грашка, ови резултати су показали да су испитивани третмани прајминга семена ефикасне методе у смислу повећања квалитета и вигора семена сорти повртарског грашка.
КЉУЧНЕ РЕЧИ: сорте повртарског грашка, раст и биомаса биљака, прајминг семена, квалитет семена

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REVIEW SCIENTIFIC PAPER

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RARE EARTH ELEMENTS IN ENVIRONMENT AND EFFECTS ON PLANTS – A REVIEW SCIENTIFIC PAPER

ABSTRACT: Rare earth elements (REEs) are widely distributed in low concentrations in all parts of the biosphere. REEs are not at all rare, their concentration in the earth's crust is close to 0.015%. REEs include the elements scandium, yttrium and the lanthanides from lanthanum to lutetium. REEs show similar physical and chemical properties. Today REEs are applied in industries and technologies, in agriculture as microfertilizers and feed additives and they are also used in medicine. REEs are dispersed especially as accessory minerals in pegmatites, granites and associated metamorphic volcanic rocks. Out of more than 250 kinds of minerals containing REEs, only bastnaesite and monazite are of economic importance. Their concentration in the soil varies widely and depends on their presence in parental materials, soil texture, organic matter content, pedogenetic processes and anthropogenic activities. REEs are found in small concentrations in surface stagnant and flowing waters as well as in underground waters. They are found in water in both suspended and dissolved form. REEs enter the atmosphere from various sources, largely owing to human activities. The majority of REEs in the atmosphere are carried by microscopic particles. The uptake and consequently accumulation of REEs in plants are affected by numerous biotic factors, such as plant species and genotype, and abiotic factors such as their concentration in the soil and some chemical and physical soil characteristics. They can enter plants via both root and foliage tissues. There are plant species called hyperaccumulators that are able to accumulate significant amounts of REEs without adverse consequences. RREs are not biogenic for higher plants or for other living organisms, but can influence their life processes. Experiments show that REEs can be beneficial for growth, yield and biochemical composition of cultivated plants. They can also alleviate some ecological stress in plants. It is not clear enough how they affect human and animal health. The wide-spread application of REEs in different industries as well as in agriculture lead to a constant increase of the concentrations of these elements in the environment. Therefore, studies on the uptake, accumulation, distribution of REEs in cultivated plant species and their entrance into the food chain as well as their stimulating or toxic effect on living organisms, can be very significant in the future.

KEYWORDS: rare earth elements, environment, plants, physiological processes, growth

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INTRODUCTION

Rare earth elements (REEs) are trace metals, natural parts of the environment, which demonstrate similar chemical and physical properties. There are over 250 minerals which contain REEs. They are widely used in various industries and agriculture. Global production of REEs has increased exponentially in the recent decades. Their economic, biological and ecological influence is thus gaining importance. Numerous research papers, reviews on REEs, were published during the last decades (Kastori et al., 2010; Haneklaus et al., 2015; Aide, 2019; Kovariková et al., 2019; Kotelnikova et al., 2021). REEs include the lanthanides group with 14 elements from cerium to lutetium in the periodic system: cerium (Ce), praseodymium (Pr), neodymium (Nd), promethium (Pm), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb) and lutetium (Lu). REEs or *terrae rarae* (TR), beside lanthanides, comprise also lanthanum (La), yttrium (Y) and scandium (Sc). Lanthanum is associated with the REEs because of its periodic table position and its trivalent chemical affinity. Yttrium is frequently associated with the REEs because of its small ionic radius (Aide, 2019). The lanthanides are divided into two groups (Walters and Lusty, 2011): the light rare earth elements (LREEs) with atomic numbers from 57 to 63 (La to Eu) with a lower mean atomic mass than 153 and a larger effective radius than 95 pm and the heavy rare earth elements (HREEs) with atomic numbers from 64 to 71 (Gd to Lu) and with a higher atomic mass than 153 and a lower effective ion radius than 95 pm. Some authors separately consider the middle rare earth elements (MREEs) comprising the elements with medium atomic mass and ion radius, from Sm to Dy. This subgroup is rarely well defined (Tyler, 2004). Compounds containing light lanthanides are more soluble than compounds containing heavier ones. Yttrium has properties more similar to the HREEs and is often included in this group.

REEs demonstrate an affinity for oxygen and they occur in nature predominately in +3 valence, except for Ce and Eu that have variable valences (Ce^{4+} and Ce^{3+}; Eu^{2+} and Eu^{3+}) in the environment. The lanthanides electron configuration determines their interaction with other elements. Complexation of the REE elements involves coordination with primarily anionic species. Common inorganic complexing species with RRE^{3+} include: NO_3^-, Cl^-, F^-, SO_4^{2-}, CO_3^{2-} and HPO_4^{2-} (Aide, 2018). The lanthanides were named after the element lanthanum, with which they are placed together in special section of the periodic table. Lanthanides are often denoted by the common symbol Ln. They are very similar in chemical and physical properties. Lanthanides are called rare elements, which is not completely true. Namely, the amount of Ce in the Earth's crust is slightly higher than the amount of lead, copper and zinc, and the amount of Lu and Tm is higher than cadmium and selenium. However, lanthanides are rarely found concentrated in one place.

Rare earth elements have found wide application in various fields of industry, especially in the production of modern electronic devices, energy technologies and in agriculture (Balaram, 2019). In the production of REEs, China plays a dominant role with approximately 95% of global production (Jaireth et al., 2014). Their largest deposits are in China and the total reserve there is estimated 43 million tons (Hu et al., 2004). The annual global consumption of rare earth oxides is estimated at 150,000 t (Ganguli and Cook, 2018), and the growth for the period between 2017 and 2021 is 13.7%. Among other things, REEs are used in the production of electric motors, wind turbines, car catalysts, solar panels, mobile phones, digital cameras, energy-efficient lighting, batteries, laptops, lasers, fiber-optics magnetic resonance tomography, new drugs (Kovariková et al., 2019), in agriculture as plant fertilizers (Ren et al., 2016) and in medicine (Balaram, 2019). Their application as microfertilizers in plant production is also constantly growing, and they are also used as feed additives (Tommasi et al., 2021). The increased use of REEs in industry and in some regions in agriculture in order to increase the productivity of cultivated species increases their entry into the biosphere. Bearing in mind the abovementioned, these elements are receiving increasing attention from the point of view of application and thus from the scientific and ecological aspects.

RARE EARTH ELEMENTS IN THE ENVIRONMENT

REEs in parental material

The concentration of REEs in soil depends on their presence in parental materials, soil texture, organic material contents, pedogenic processes and anthropogenic activities. The average REEs concentration in the Earth's crust is close to 0.015% and amounts to 189 mg/kg, and is present in smaller quantities near the Earth's surface (Dobransky, 2013), since REEs are concentrated into mineable ore deposits. Taylor and McLennan (1985) state the following values for the content of certain light lanthanides in the earth's crust (in mg/kg): La 30, Ce 64, Pr 71, Nd 26, Sm 4.5, Eu 0.8, Ce 66, Nd 40, La 35 and Tm 0.5, and Y 24 (Wedepohl, 1995). REEs are found in concentrated form in phosphorites as well as argillaceous sediments (Alina and Henryk, 1984). Most REE parent material compositions range from 0.1 to 100 mg/kg (Aide, 2019). REEs are widely dispersed especially as accessory minerals in pegmatites, granites and associated metamorphic volcanic rocks (Tyled, 2004). More than 250 sorts of minerals containing REEs are known, among which the best known are: bastnaesite, monazite, xenotime, loparite, euxenite and parisite. Only bastnaesite and monazite are of economic importance (Saveleva, 2011).

REEs often include elements from the scandium group: Y, La and Sc. Yttrium is known to be incorporated mainly as Y^{3+} in several minerals, of which silicate, phosphate and oxide forms are the most frequent ones. Yttrium is naturally present in minerals gadolinite and xenotime, mostly in the form of YPO_4. Lanthanum is not a rare element. There is more of it in the Earth's crust than lead. The main natural source of La is the mineral monazite. It is a phosphate that, in addition to La, contains different proportions of light lanthanides

(Ce, La, Nd, Pr) also in the form of phosphates. Sc is usually considered to be one of the rarest elements. Scandium occurs in the lanthanide minerals monazite and gadolinite, and in the very rare silicate thortvetite. REE transfer from bedrock to soil depends on the chemical properties of the rocks. According to Brioschi et al. (2013) for the limestone, the average soil/bedrock transfer factors were about 2 for most REE and for the granite about 1.

REEs in the soil

REEs enter the soil mainly from parent materials and anthropogenic sources, primarily from phosphoric mineral fertilizers and atmospheric deposition. The content of REEs in the soil depends first of all on the characteristics of the parent material. REEs content in parent material decreases in the following order: granite > basalt > purple sandstone > red sandstone (Zhu and Liu, 1988). Soils originating from lime rock, loess, sand-shell stone, neutral endogenous rock, purple sandstone demonstrate a lower REE concentrations, between 137 and 190 mg/kg. Acid endogenous rock, sediment rock and shale, laterite, basic igneous rock and sandstone tend to contain higher concentrations of REE, between 196 and 219 mg/kg (Liu, 1996). Most often, the lowest concentrations of REEs are reported for ultramafic and calcareous rocks (Kabata-Pendias, 2000).

According to Bohn et al. (1985), content of REEs in the soil is in the range 30–700 mg/kg. Hu et al. (2006) stated, on the basis of the results of the examination made by a number of authors, that in the various soil types of China, in the examined 1,225 samples, the concentration of REEs was on average 181 and ranged within wide limits from 68 to 629 mg/kg. The same authors state that in soils of different origin from the examined REEs, the most abundant elements were Ce and Nd, as well as that the presence of LREEs was significantly higher than HREEs. Most geological processes lead to the redistribution of REEs, changing the LREE/HREE ratio. Redling (2006) states that the share of individual REEs elements in the soil has the following sequence: Ce > La > Y > Sc > Nd > Pr > Sm > Gd > Dy > Er > Yb > Eu > Tb > Ho > Tm > Lu (Redling, 2006).

Depending on the physico-chemical properties and biological activity, REEs are found in different chemically bound forms in the soil (Zhu and Xing, 1992a; Rogova et al., 2022). Adsorption and desorption of REEs in the soil depends on the physical and chemical properties of the soil and especially on the cation exchange capacity (CEC). The following fractions of REEs were found by extraction with different solvents: water soluble, exchangeable, carbonate bound and adsorbed, bound to organic matter, bound to Fe/Mn oxide, bound in crystalline Fe-oxides, amorphous Fe-oxide bound, bound to no-labile organic matter/sulphides. Zhu and Xing (1992b) state the following average representation of certain fractions of REEs in different soil types of China (%): water soluble 0.2, exchangeable 1.3, carbonate and specifically adsorbed 9.2, organic matter bound 4.5, Fe-Mn oxide bound 15.0, residual 69.9. It is clear from the above data that the water soluble and exchangeable fractions are by far the least represented. The water-soluble fraction is directly accessible to plants and microorganisms and undergoes translocation processes in the soil. Representation of the exchangeable fraction of REEs, depending on the type of soil, varies within wide limits. Calcium minerals (apatite, augite, hornblende, fluorite) can bind most REEs in the Earth's crust through hydatogenetic and magmatism processes, therefore a significant part of REEs in the soil is in the form of carbonates. Calcium oxyphosphate fertilizers and apatite change the speciation and bioavailability of exogenous REEs in the soil-plant system (Wu et al., 2001). REEs bound in colloidal form as Fe-Mn oxides are not fully accessible to plants. In reducing conditions, their reduction and release may occur. REEs can be bound in organic matter in the form of chelates or organic sulfides. They can be released from these forms only under severe oxidizing conditions (Hu et al., 2006). Common organic complexes include lower molecular weight organic acids (oxalic acid, malic acid) and humus components contain fulvic and humic acids. Humic and fulvic acids mainly accumulate LREEs (Aide and Aide, 2012). The residual form of REEs is not accessible to plants, it is bound in the lattices of minerals.

The vertical distribution of certain rare earth elements in the soil is different due to their different adsorption and thus leaching from the soil (Mihajlovic et al., 2014, Aide, 2019). Certain types of soil and minerals are characterized by different REEs adsorption capacity (Ladonin, 2019). Soil organic matter and its pH value play a significant role in the adsorption of REEs. As the pH value increases, their adsorption increases as well (Hu et al., 2006). Fodotov et al. (2019) state that metal-organic complexes are sink for REEs in the soil.

REEs in water bodies

The wide application of REEs in various branches of industry and agriculture leads to a constant increase in their concentration in the environment, which, among other things, leads to endangerment of water systems (Balaram, 2019). Numerous research results indicate that REEs can be significantly mobilized during weathering, alteration and diagenesis and thereby be included in geochemical cycling (Haley et al., 2014; Crocket et al., 2018). According to Brioschi et al. (2013) the REEs concentration in soil water was mainly controlled by solubility and not by the REEs concentrations in the soil. REEs are found in water in both suspended and dissolved form. They are easily attached to suspended colloidal material, especially to Fe-Mn-oxyhydroxide, and the dissolved part is in the form of free ions or as complexes. Part of the REEs is dissolved in river water and suspended material is included in the REEs cycle of seawater and in the continental crust (Goldstein and Jacobsen, 1988). The solubility of REEs is strictly controlled by pH. At a pH lower than 6, the total concentration of REEs in ground waters is higher than at a higher pH. Citing the results of other authors, Smedley (1991) states that: in close to neutral to alkaline conditions, complexing of REEs with carbonate or bicarbonate anions is significant; in acidic water, sulphate ligands are significant; in waters rich in organic matter, complexing with organic matter is dominant; in hydrothermal systems complexing with CO_3^{2-}, Cl^-, SO_4^{2-} and F^- ligands. Heavy rare earth elements form more stable complexes with inorganic and organic ligands than the light rare earth elements (Ding et al., 2006). Most of the lanthanides have +3 valence. When dissolved, some of them are in +2 and +4 oxidation states (Walters and Lusty, 2011).

According to investigations by Smedley (1991), the concentration of REEs in groundwater was in many cases below the detection level, although in some cases it reached 229 µg/L. The concentration of REEs in groundwater depends on the pH value and increases when it decreases to pH <6. According to Wang et al. (2001) in the suburb of Beijing, China, the concentration of soluble REEs was 0.69 µg/L in rainwater, 5–7 µg/L in surface runoff, as well as 1–4 µg/L in soil water. Foliar application of different doses of REEs did not affect their concentration in the mentioned waters. Annual input of REEs by rain to the experimental plot was 4 g/ha, which is significantly less than the amount used annually in agriculture (160 g/ha). The concentration of REEs in seawater is significantly lower than in river water and is present in the pH range (Nozaki, 2001). According to Al-Qutob and Al-Rimawi (2016) the concentration of REE III in harvested rainwater in Gaza Strip was 32.27 µg/L, and in Ross See was detected to be 10.3 ng/L (Turetta et al., 2017). According to Zicari et al. (2018) *Lemna minor* is potentially a useful tool for biomonitoring of Ce-polluted freshwater.

REEs in the atmosphere

REEs enter the atmosphere from numerous sources and anthropogenic activities. Due to their increasing application around the world, their emission into the atmosphere is increasing. REEs from the atmosphere reach the soil, surface water and plants through precipitation. The production of phosphorus fertilizers can be a significant pollutant of the atmosphere with REEs, and snow is a perfect depositing medium. The concentration of the REEs – La, Ce, Nd, Sm, Eu, Tb, Dy, Yt and Lu in snow in the environment of the phosphorus fertilizer production factory was 1,333.7 mg/L. The majority of REEs is carried by microscopic particles up to 8 µm in size (Volokh et al., 1990). According to Wang et al. (2000) in atmospheric particulate matter in the western part of the Netherlands, the total REEs concentration, the light-REEs (LREE) and heavy-REEs (HREE) were 0.22–33.0, 0.21–30.68 and 0.01–2.32 ng/m^3 respectively. The variation in the concentration of REEs depended on the direction of the wind, and especially on meteorological conditions and anthropogenic activities. The uptake of REEs from atmospheric deposition is positively related to a high ionic charge and atomic mass. The influence of the application of REEs on their concentration in the atmosphere is indicated by the research by Wang et al. (2001). During the foliar application of 16, 32 and 64 mg/m^2 REEs, the concentration of REEs in air particles with a diameter <10 µm increased from 35.7 ng/m^3 before application to 93.5 ng/m^3 after application of REEs in the area surrounding the plots. The concentration of REEs in air particles depends on their size and finer particles have lower concentrations of REEs (Wang et al., 2000).

PHYSIOLOGICAL EFFECTS OF RARE EARTH ELEMENTS ON PLANTS

Uptake and long-distance transport of REEs in plants

REEs can enter plants via both root and foliage tissues. REEs uptake by plant roots from soil solution is not only controlled by the plants themselves, but depends on their concentration, exchangeable fractions and solubility in soil, soil water pool and texture, organic matter content, anthropogenic activities, presence of other elements, mycorrhizis, and some chemical and physical soil characteristics (Brioschi et al., 2013). From the point of view of bioavailability of REEs, redox potential, organic matter content, soil texture, and pH value are particularly important. Absorption of REEs by plants tends to be higher at a low pH. Organic and inorganic ligands play a significant role in the uptake of REEs. Humic, fulvic, malic, and citric aids or ethylene diamine tetra acetic acid (EDTA) change bioaccumulation of REEs in plants. Organic ligand EDTA increases the uptake of REEs by promoting their desorption from soil particles. Amino acids as chelators may have a positive effect on the uptake of La and Y (Wu et al., 2013).

Plants mainly absorb REEs from soil water in the form of ions and less often as a soluble complex. The concentration of REEs is higher in small roots, which is in agreement with the hypothesis that REEs predominantly accumulate in the primary cell wall during the early phase of root cell growth (Brioschi et al., 2013). After uptake, the transport of REEs in the root takes place in two ways: by apoplastic or symplastic pathway. By apoplastic pathway, REEs move into the cell wall passively based on the diffusion gradient. The symplastic way comprises passing through the plasma membrane and entering the cytoplasm of the cell. For the uptake and movement of REEs towards the central cylinder of the root, the endoderm of the root represents a barrier due to the existence of the ion-selective cell wall, the Casparian strip (Hu et al., 2004; Brioschi et al., 2013). The affinity of plasma membrane affects uptake of individual REEs elements (Hu et al., 2004). According to Wang et al. (2014) REEs can activate endocytosis in plant cells and facilitate their deposition. Arabinogalactan protein-rare earth element complex activates plant endocytosis (Wang et al., 2019). However, the known role of ion channels, carriers and pumps located in plant cell membranes in the transport of REEs through membranes is insufficient.

Organic ligands contribute to the long upward transfer of REEs in xylem. The transpiration stream also influences the acropetal transport of REEs. REEs elements differ by their mobility in plants. In the xylem saps of the *Phytolacca americana*, citric acid facilitates the transport of HREEs rather than LREEs (Yuan et al., 2017). In the xylem sap of soybean, the majority of REEs are combined with certain ligands (Ding et al., 2007). A significantly higher concentration of REEs in the roots than in the aerial parts of plants indicates their inefficient ascending translocation, probably caused by their efficient filtering by the endoderm, owing to the Casparian strip (Biroschi et al., 2013). Some REEs were immobilized during transport in xylem mainly by phosphate particles and cell wall absorption.

As mentioned earlier, certain REEs are characterized by different mobility in plants. The transfer factor represents the relationship between the accumulation of an element in the plant and its concentration in the external environment. It depends on its concentration in the external environment, the mobility of the element, plant organ and plant species. For example, transfer factor of Y in sunflower was much higher in root than in stems and leaves, since its uptake from the external environment by the root is more intense than translocation from the root to the aerial part (Maksimović et al., 2012). In citrus, the leaf/soil ratio of La was 0.62–1.09 (Turra et al., 2013). Translocation factor of REEs (leaves/root) in *Boehmeria nivea* was lower at a concentration of 1.6–80 µmol/L REEs (0.01–0.09) than when applying 160–800 µmol /L (0.08–0.16) (Liu et al., 2022).

DISTRIBUTION, ACCUMULATION AND INTERACTION OF REES WITH OTHER ELEMENTS

The distribution is specific for certain elements in plants. Knowledge on the distribution of elements in plants is important from an ecological point of view, since their accumulation in the edible part of cultivated plants can allow their entrance in the food chain and thus may have adverse health consequences. The largest part of REEs (68%) is found in the form of REE-pectin complex in the cell wall of plants. It can be generally said that distribution of RREs in plant organs is as follows: root > leaf > stem > flower > fruit. The large accumulation of REEs in the root probably is not the result of the life activity of the root tissue, but primarily of their adsorption onto the surface of the root system. For example, yttrium concentrations in young maize plants increased with the increase in Y concentration in nutrient solution. The largest was found in the root, then in the leaf and the smallest in the shoot, according to Maksimović et al. (2014). A similar distribution was found when applying Nd. Application of Nd in rapeseed indicated distribution in the following order: root > stem > leaf (Wei, 2001). According to Wu et al. (2002), after application of REEs containing fertilizer, the concentration of individual REEs in maize decreased in the following order: root > leaf > stem > grain. Roots of maize plants can accumulate approximately 60% more La than shoots (Durate et al., 2018). The Ce concentrations in rice were higher in roots than in shoots (Ramirez-Olivera et al., 2018).

Plants can also take up REEs through aerial organs. The method of their application affects their distribution in plants. According to Liang et al. (2005) in the case of foliage-dressing wheat with REEs, their greater accumulation was found in the leaves and roots, while their presence was not found in the grain. According to Rodrigues et al. (2020), the La applied to the leaf surface of soybean was translocated to seeds. The content of La in the grains depended on the dose, but the translocation rate was not linear. Only 3.2% of total La applied was translocated form leaf to grains in the case of 2000 mg La/L and 2.5% at 200 mg La/L. Cerium foliar application at 20 mg/L prevents UVB-induced stress in soybean seedlings (Turra, 2018). According to Tyler and Olsson (2006) the uptake of REEs from atmospheric deposition is positively related to a high ionic charge and atomic mass.

Plant species that are able to accumulate significant amounts of REEs – hyperaccumulators – have the ability to accumulate REEs without undesirable consequences. These include, among others, *Dicranopteris dichotoma* (Wang et al., 2003), *Pronephrium simplex* (Lai et al., 2006), *Phytolacca americana* (Wu et al., 2013; Yuan et al., 2017), *Boehmeria nivea* (Liu et al., 2022). They can be used for phytoremediation of REEs-contaminated soils.

In numerous experiments, the interaction of REEs with other elements via antagonistic or synergistic mechanisms was established. Elements with similar chemical properties can compete with each other for the same binding site, e.g. Ca and REEs. With the increase in the concentration of Ca^{2+} and EDTA, a change in the concentration of HREE and LREE in leaves was recorded (Ding et al., 2007). It is thought that Eu can replace Ca in plants especially in soils with increased Eu/Ca ratio (Shtangeeva and Ayrault, 2007). According to Lian et al. (2019), treatment with $LaNO_3$ improves phosphorus use efficiency under P-deficiency and alleviates negative effects of P-deficiency on chlorophyll content and photosynthesis in *Vigna angularis* seedlings. Wu and Wang (2007) REEs improved P-uptake and corn yield. The addition of REEs, especially under 80–800 μmol/L, decreases the concentrations of Ca, Mn, K, Mg, Cu and Zn, and increases the concentration of P. in *Boehmeria nivea* L. (Liu et al., 2022). Increasing the doses of nitrogen and potassium fertilizers increased the uptake of REEs and the application of phosphate decreased the uptake of REEs (Hu et al., 2004).

REEs and water relations

The water regime of plants includes the uptake, transport and release of water. The mentioned processes depend on the development of the roots and shoots, their structure and physiological activity. REEs, depending on their concentration, can have a favourable or inhibitory effect on certain elements of water regime. The root has a significant role in the uptake of mineral substances and water as well as in the upward transport of the absorbed water. REEs can favourably influence root growth. Cerium application in common bean plants alleviated drought-induced stress and increased water use efficiency.

Plants treated with 8.1 μmol/L Ce^{3+} displayed an increase of about three-fold in root dry matter in relation to the control and an increase of about eight times in water use efficiency (Salgado et al., 2020). It is assumed that Ce^{3+} increases the water content in cells under stressful conditions by protecting the plasma membrane from the harmful effects of reactive oxygen species by reducing their formation under stressful conditions (Wu et al., 2014). The favourable effect of REEs on the growth of root, an important organ in supplying plants with water, is also explained by their influence on the increased translocation of products of photosynthesis to the roots. According to Ramirez-Olivera et al. (2018) the concentration of total sugars increased in the roots in rice with the application of 50 μM Ce. The favourable effect of REEs on root growth was determined in different plant species by other authors in sunflower (Bai Bao-Zhang et al., 1990), soybean (Kastori et al., 1990), *Arabidopsis thaliana* (Ruiz-Herrare et al., 2012), rice (Liu et al., 2013), *Pseudostellaria heterophylla* (Ma et al., 2017). The development, morphological and anatomical structure of the leaf also has a significant effect on water relations, above all on water release through transpiration and its upward transport. Application of a lower concentration of Sc and lanthanide groups increased the leaf area of young sunflower plants (Bai Bao-Zhang et al., 1990) and soybean (Kastori et al., 1990). Total leaves area, water content in roots and leaves of young sunflower were significantly reduced in the presence of 10^{-4} mol/L Y. (Maksimović et al., 2012). The presence of Y in young maize plants reduced their leaf area, transpiration, stomatal conductance of water vapour, length and width of stomata and increased stomatal density (Maksimović et al., 2014).

Increase in stomatal density and reduction of their size are xeromorphic changes induced by water deficiency. Shan and Zhao (2015) reported an improvement of water capacity after lanthanum application on *Lilium longiflorum* cut flowers. According to Liu et al. (2008) under conditions of calcium deficiency, application of Ce in spinach increased water uptake. REEs may influence gas exchange, especially stomatal conductance. Zhou et al. (2011) concluded that by increasing La concentrations, there was higher stomatal conductance and transpiration in *Salvia miltiorrhiza*. According to Durate et al. (2018) the La concentrations of 25 and 50 μM led to an increase in both maize biomass and chlorophyll index, although increases in photosynthetic rate, transpiration and somatal conductance were not verified.

Effects of REEs on photosynthesis

The influence of REEs on the growth and organic production of plants is primarily based on their influence on photosynthesis. Numerous works point to the positive effect of REEs on photosynthesis. It is believed that REEs can act through different mechanisms on the regulation of photosynthetic rate by chlorophyll formation, chloroplast development, increasing the light absorption efficiency, regulating the excitation energy distribution of FSI and FSII, promoting Hill reaction, as well as activity of Rubisco and alleviating different stresses (Liu et al., 2012; Salgado et al., 2020; Ma et al., 2022). Their effect primarily depends on the applied concentration, the features of a particular REEs and the method of application. The influence of REEs on photosynthesis was mostly studied by Chinese researchers. Based on a large number of papers published in Chinese, Hu et al. (2004) state that the application of REEs increases photosynthesis intensity and net photosynthetic rate by 11.5–31.2%. The effect of La on photosynthesis has been particularly intensively studied. Lanthanum is known to affect different aspects of photosynthesis. Zeng et al. (2010) state that the application of a suitable concentration of La^{+3} can affect the activity of photoelectron transport in the chloroplasts of cucumber leaves. Lanthanum can increase the enzymatic activity of Rubisco in tobacco (Chen et al., 2001), and in spinach it can induce the formation of a complex of Rubisco and Rubisco activase (Hong et al., 2005).

A number of authors have recorded the positive effect of lower and unfavourable effect of higher concentrations of La on the content of photosynthetically active pigments (Nicodemus et al., 2009; Ma et al., 2017; Durate et al., 2018; Cui et al., 2019). Similar results were found when applying Y (Maksimović et al., 2014) and Ce (Hong et al., 2002; Wang et al., 2012; Salgado et al., 2020). Pre-sowing treatment of sunflower (Bao-Zhang et al., 1990) and soybean seeds (Kastori et al., 1990) with low concentrations of Sc and lanthanides groups increased chloroplast pigments content in leaves. Ramirez-Olvera et al. (2018) state that the addition of 100 µM Ce decreased the chlorophyll *a* and *b* by over 60% in rice. Zicari et al. (2018) also reported a decrease in the content of chlorophyll and carotenoids when applying a higher concentration of Ce 0.5 and 1 mM in *Lemna minor*. An increase in the concentration of photosynthetically active pigments in most cases was accompanied by an increase in photosynthesis. Lanthanides could enter the chloroplast and bind easily to chlorophyll and substitute Mg^{2+} ion in the chlorophyll molecule and coordinate the porphyrin ring in pheophytin to form a lanthanide – chlorophyll (Chl)-a complex (Rezanka et al., 2016). According to Wang et al. (2003) in hyper accumulator species *Dicranopteris dichotoma* 8% of the total REEs content in leaves was located in chloroplast, one half located in the chloroplast membrane and the other half in thylakoids. The chloroplast has gained more rounded instead of ellipsoidal shape, and the thylakoids were disorderly arranged in the rice leaves when exposed to 1.0 mM Ce^{3+} (Liu et al., 2012).

Effects of REEs on plant enzymes and hormones

The regulation of enzyme activity represents one of the most important pathways of metabolic activity of the cell. Therefore, the metabolic potential of plants can best be monitored by studying their enzymes. It has been established that REEs can influence the activity of numerous enzymes and thus metabolic processes, organic production and chemical composition of plants. Hu et al. (2004), citing the results of research by Chinese authors in their paper, state the influence of REEs on the activity of sucrose-transform-enzyme in sugar beet leaves as well as nitrate reductase in soybean and cotton leaves. Rico et al. (2013) indicated that cerium oxide nanoparticles modify the antioxidative stress enzyme activities in rice seedlings and that the 62.5 mg/L nanosized cerium oxide reduced the H_2O_2 generation in roots; 125 mg/L enhanced lipid peroxidation and 500 mg/L increased the H_2O_2 concentration in roots. According to Liu et al. (2012) effects of Ce^{3+} on the growth and antioxidant metabolism were found in the roots and shoots of rice. The addition of $LaCl_3$ was found to increase the activities of antioxidants, including superoxide dismutase (SOD), catalases (CAT) and peroxidases (PX) in maize plants (Cui et al., 2019). Lanthanum (III) was found to be an efficient inducer of the enhancement of laccase activity in mycellium cultures of *Shiraia bambussicola* (Wang et al. 2022). Luo et al. (2008) found that Nd^{3+} influences the activity of indole-3-acetic acid oxidase and cytokinin oxidase during enhanced adventitious rooting of *Dendrobium densiflorum* shoot cuttings. REEs increase the photosynthetic rate of spinach through the increase in carboxylation activity of Rubisco, and activities of Mg^{2+}-ATPase and Ca^{2+}-ATPase (Ze at al., 2009). Numerous biotic and abiotic factors can cause oxidative stress in plants, i.e. disturbance in the balance that exists between prooxidative processes and the antioxidant system of plants. According to Liang et al. (2006), Ce^{3+} improved photosynthesis, growth and antioxidant enzyme system including SOD and CAT in rape seedlings under UVB radiation. Under potassium deficiency, salt stress and combined stress of potassium deficiency and salt stress addition of Ce^{3+} could significantly promote seedling growth, and alleviate morphological and structural damage of leaf, decrease oxidative stress and increase antioxidative capacity in maize leaves (Hong et al., 2017). Application of REEs can alleviate cadmium toxicity in rice seedlings by increasing light utilization efficiency, improving photosynthesis, suppressing accumulation of oxidative species and elevating an adequate antioxidant enzymes (Wu et al., 2014). The results of Wang et al. (2009) indicated that La^{3+} alleviated the oxidative damage induced by UVB radiation in soybean plants by reacting with reactive oxygen species (ROS) directly or by improving the defense system of plants. Lanthanum nitrate treatments in tomato plants induced an increase in some antioxidant systems, but this stimulation does not improve the plant responses to the drought stress (Ippolito et al., 2011).

Hormones are biologically active compounds that regulate growth and development and influence the direction and intensity of plant metabolic processes. Hence, their content and ratios between different types of hormones in plants are very important. Numerous papers indicate that REEs can affect hormone activity. It is considered that these influences are particularly related to the synergistic action of REEs with hormones. Sheng and Zhang (1994) reported an increase in the content of endogenous IAA in wheat when La was applied. Lanthanum increases IAA content by stimulating tryptophan, which is precursor for IAA synthesis, and/or by inhibiting the activity of enzymes that degrade IAA (Hu et al., 2004). Lanthanum can increase the resistance of soybean to UVB radiation by increasing IAA and gibberellic acid content and decreasing ABA and acetic acid oxidase activity, so as to alleviate damage to plants under UVB stress (Peng and Zhou, 2009). Lanthanum (III) could also decrease the ROS content by regulating the ethylene content in the soybean seedlings (Yang et al., 2014). According to Ciu et al. (2019) content of gibberellic acid and indoleacetic acid increased while malondialdehyde and abscisic acid contents markedly decreased upon $LaCl_3$ application in maize.

Effects of REEs on germination and growth

In plants that are propagated by seeds, growth begins by germination of seeds. Effects of REEs on germination were established through measures of total percent germination and speed of germination of selected crops and native plant species (Thomas et al., 2014). However, the results of studies on the influence of REEs on seed germination are often contradictory. Seed treatment with REEs did not affect germination and germination energy of soybean and sunflower seeds. Exceptions were the treated soybean seeds which exhibited a significant increase in germination in the accelerated growth test (Zhang et al., 1988). Fashui (2002) and Fashui et al. (2003) reported positive effects of Ce and La on germination of aged rice seeds. Pre-soaking of *Triticum durum* seeds with low concentrations of La and REEs had no effect on seed germination, whereas higher concentrations significantly decreased seed germination (d'Aquino et al., 2009). Ramirez-Olvera et al. (2018) reported that Ce stimulated rice seed germination.

Numerous studies have established the beneficial effect of REEs on growth. The growth and physiological activity of the roots directly affect the growth and development of the whole plant. REEs stimulate the growth of the root system by promoting the formation of adventitious roots and affecting cell differentiation and root morphogenesis (Zhang et al., 2013). Seed treatment with REEs favourably affected the accumulation of dry matter and growth of the above-ground parts and roots of soybean, sunflower, and sugarbeet seedlings (Zhang et al., 1988). When $La(NO_3)_3$ (1.0–3.0 μM) and $Eu(NO_3)_3$ (2.0–3.0 μM) were added to the rooting medium of *Eriobotrya japonica*, rooting rate and root fresh weight *in vitro* significantly increased and root elongation was promoted (Zhang et al., 2013). Cerous nitrate at 1–15 mg/L promoted callus growth and formation of adventitious roots on the stem of *Dioscorea zingiberensis* (Wang et al., 2010). According to Lou et al. (2008), Nb^{3+} significantly increased the rooting rate of stem of *Dendrobium densiflorum* which was possibly induced by an increase in the level of endogenous IAA. A large number of authors did not record the beneficial effect of REEs on the vegetative growth of plants. Diatloff et al. (2008) did not observe a favourable effect on the growth of corn and mungbean treated with lanthanum and Ce^{3+}. According to El-Ramady, no difference was found in the growth of *A. thaliana* treated with 0.5 to 50 μmol/L $Ce(NO_3)_3$. Salgado et al. (2020) also did not observe a significant effect on the growth of common bean when applying increasing doses of $Ce(NO_3)_3$. Application of Y in concentrations of 10^{-5}, 10^{-4} or 10^{-3} mol/L reduced shoot and root length and total leaf area of young maize plants (Maksimović et al., 2014).

Hu et al. (2014) cite numerous results, primarily by Chinese authors, on the beneficial effect of REEs on the yield of cultivated species. Based on the results of the research on the influence of REEs on the growth and organic production of plants, it can be concluded that it can be ineffective, stimulating or inhibitory depending on the type of element, the applied concentration, the plant species as well as on the method of their application and environmental conditions. Further research is needed to gain a better insight into the mechanisms of action of REEs on the life processes in plants and thus on their growth and development.

CONCLUSION

Rare earth elements (REEs) are a group of chemical elements that exhibit similar chemical and physical properties. They are widely distributed in nature in low concentration. Those concentrations in the Earth's crust are close to 0.015%, in the parent material from 0.1 to 100 mg/kg, and in soils 30–700 mg/kg, but most often <100 mg/kg. REEs concentration in water bodies is in the μg/L range, and in the atmosphere it is in the ng/m^3 range. There is no indication that REEs might be essential for any form of life. Their effects on plants range from growth stimulation to inhibitory effects depending on their concentration, some ecological factors and plant species. The REEs do not have a highly toxic effect on plants. Plants can take up REEs both through roots and shoot parts. The intensity of uptake depends on numerous internal factors linked to the plant species, genotype, age and on ecological conditions, physical and chemical properties of the soil, primarily on their concentration in the soil and its pH, as well as on the content of organic matter and the presence of some other elements. The concentration of REEs in plant organs follows the order: roots > leaves > stems > grain. Owing to low accumulation of RREs in the grain, the consumption of products deriving from grains may contribute very little to the entering of REEs into food chain. In the group or individually, REEs at certain concentrations can favorably influence certain physiological processes of plants like enzyme activity and hormone content. In addition, they may stimulate antioxidant systems and thereby increase the tolerance of plants to environmental stress conditions; they can stimulate photosynthesis and germination of seeds, or influence water relations in plants. Due to the abovementioned, their stimulating effect on the yield of cultivated species, as well as their influence on the chemical composition of plant products, was recorded in numerous experiments. REEs are used both in medicine and as feed additives. There are plant species called hyperaccumulators that are able to accumulate REEs to a greater extent without adverse consequences. They can be used for phytoremediation at REEs contaminated soils. The exploitation and application of REEs in various areas of industry and agriculture have been growing rapidly in recent decades, which increases the risk of their accumulation in various parts of the biosphere and thereby entering the food chain. In order to better understand the impact of REEs on the life processes of living organisms and thus the effect of their application in agriculture, the impact on human and animal health and ecosystems, further fundamental research is needed in various areas. Therefore, an increased interest of researchers lately for this group of elements is understandable.

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ПРЕГЛЕДНИ НАУЧНИ РАД

ЕЛЕМЕНТИ РЕТКИХ ЗЕМАЉА И ЊИХОВО ДЕЈСТВО НА БИЉКЕ – ПРЕГЛЕДНИ НАУЧНИ РАД

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РЕЗИМЕ: Елементи ретких земаља (ЕРЗ) широко су распрострањени у природи. Њихова концентрација у Земљиној кори износи око 0,015%. У елементе ретких земаља убрајају се скандијум, итријум и лантаниди (од лантана до лутецијума). Они су по хемијским и физичким особинама веома слични. ЕРЗ нашли су примену у различитим гранама привреде, посебно у електроиндустрији, пољопривреди (као микро-ђубриво), медицини и додацима крмиву или сточној храни. Њихова концентрација у земљишту креће се у широким границама од 30 до 700 mg/kg, најчешће <100 mg/kg и зависи од њиховог присуства у матичном супстрату, структуре зељишта, садржаја органске материје, педогенетских процеса и активности човека. ЕРЗ се у малим концентрацијама налази у површинским стајаћим и текућим водама, подземној води као и атмосфери. Биљке могу да усвајају ЕРЗ преко корена и надземних органа. Интензитет усвајања зависи од унутрашњих чинилаца, пре свега од биљне врсте, генотипа и старости, али и од еколошких услова. Осим тога зависи и од физичких и хемијских особина земљишта, њихове концентрације у земљишту, pH вредности, као и од садржаја органске материје и присуства неких других елемената. Постоје биљне врсте – хиперакумулатори – које се одликују интензивним накупљањем ЕРЗ без негативних последица. Оне могу да се користе у циљу фиторемедијације загађених станишта елементима ретких земаља. ЕРЗ се у различитој мери накупљају у појединим органима биљака, у највећој мери у корену (корен > листови > стабло > зрно). Захваљујући томе не доспевају у већој мери у ланац исхране. Скупина ЕРЗ као и њихови поједини елементи при одређеним концентрацијама и начинима примене могу повољно да утичу на поједине физиолошке и биохемијске процесе, а тиме и на раст биљака. Они могу да утичу на активност појединих ензима, садржај хормона, усвајање елемената, да подстичу образовање антиоксидантних система и тиме да повећају толерантност биљака према еколошким стресним условима, могу да подстичу фотосинтезу и клијање семена, да утичу на водни режим биљке. Захваљујући наведеном, у бројним огледима је утврђено њихово повољно дејство на принос гајених врста, као и њихов утицај на хемијски састав биљака. Експлоатација и примена ЕРЗ

у различитим областима индустрије и пољопривреди последњих деценија нагло расте, чиме се повећава опасност од њиховог улажења у ланац исхране. Они су у поређењу са неким тешким металима мање токсични. На основу досадашњих сазнања може се закључити да ЕРЗ могу да утичу на животне процесе живих организама, али да нису неопходни. Да би се боље разумео утицај ЕРЗ на животне процесе живих организама и тиме ефекат њихове примене у пољопривреди, утицај на здравље људи и животиња и екосистема, потребна су даља фундаментална и примењена истраживања, како би се боље сагледао механизам њиховог деловања.

КЉУЧНЕ РЕЧИ: елементи ретких земаља, животна средина, биљке, физиолошки процеси, раст

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ORIGINAL SCIENTIFIC PAPER

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WHEAT STRAW DELIGNIFICATION BY *Bjerkandera adusta* (Willd.) P. Karst. 1879: THE EFFECT ON ENZYMATIC HYDROLYSIS

SUMMARY: The use of lignocellulosic materials in the production of biofuels and biochemicals holds a huge prospect since wood and agricultural residues represent the most abundant global source of renewable biomass. However, delignification is an inevitable step in lignocellulose pre-treatment rendering the cellulose and hemicellulose more exposed to enzymatic saccharification. The aim of this study was to assess the potential of different *Bjerkandera adusta* strains to enhance the efficiency of enzymatic saccharification of wheat straw after solid-state culturing. Three white-rot fungal strains of *Bjerkandera adusta* (Willd.) P. Karst. 1879, (BEOFB1601, BEOFB1602 and BEOFB1603) were used for partial delignification of wheat straw during solid-state cultivation. Activity of ligninolytic enzymes were measured spectrophotometrically while wheat straw residues were used for determination of hemicelluloses, cellulose and lignin contents. Enzymatic hydrolysis of pre-treated wheat straw was conducted using commercial cellulase in loadings of 60 U g^{-1} of solid substrate. The content of reducing sugars was measured calorimetrically using 1,4-dinitrosalycilic acid. Enzymes predominantly responsible for lignin degradation by tested fungal strains were peroxidases. The highest rate of lignin degradation was noticed in samples pre-treated with the strain BEOFB1601 (42.3 ± 3.7%). The highest reducing sugars yield (8.6 ± 0.3 gGE L^{-1}) was achieved after enzymatic saccharification of samples pre-treated with the strain BEOFB1601, as the most selective lignin degrader. The obtained results suggest that fungal culturing as a biological pre-treatment method can be significantly strain specific. A key mechanism which enhances convertibility of carbohydrates is selective lignin degradation of the biomass.

KEYWORDS: *Bjerkandera adusta*, Ligninolytic enzymes, Wheat straw, Fungal pre-treatment, Saccharification

INTRODUCTION

The increase in consumption and reduction of fossil fuels lead to a higher demand for alternative renewable energy sources. Bioethanol, biogass and

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several other biochemicals can be considered as a possible solution when obtained from lignocellulosic residues such as rice and wheat straw, sugar cane, bamboo, cotton stem as well as other residues from forestry, waste material from paper industry or as a part of communal waste (Pauly and Keegstra, 2008; Ummalyma et al., 2019).

Lignocellulosic material represents a complex of three main structural components: lignin (10%–24%), cellulose (40%–60%) and hemicellulose (20%–40%) which are interconnected into plant cell walls (Putro et al., 2016). Therefore, the production of bioenergy from lignocellulosic biomass feedstock requires biorefinery processing chain composed of pre-treatment, hydrolysis, fermentation and separation. Two crucial operations in this process are delignification as an inevitable step in lignocellulose pre-treatment rendering the cellulose and hemicellulose more exposed to enzymatic saccharification, and bioconversion from cellulose/hemicellulose into fermentable hexoses and pentoses, then into ethanol by fermenting microorganisms (Kapsokalyvas et al., 2018).

Recently, several biorefinery concepts have been established for converting renewable biomass to ethanol, in which the pre-treatment step still remains as physical, physico-chemical and chemical ways. These methods are not successfully applied in the biorefinery demonstration and even considered as the major barriers for commercialization of ethanol production from lignocellulose biomass (Silveira et al., 2015). Biological pre-treatment method is a new concept and deserves attention as it is safe, environmentally friendly and less energy intensive (Ding et al., 2019). This approach is based on the usage of microorganisms, such as fungi and bacteria and their extracellular enzymes (Sun and Cheng, 2002). Among them, white-rot fungi are the most important because they can degrade all components of lignin, hemicellulose and cellulose to a lesser or greater extent (Al-Haj Ibrahim, 2012; Sarkar et al., 2012; Rajala et al., 2015). However, the rate of pre-treatment process and the consequent hydrolysis reaction is relatively low using the biological pre-treated biomass. A great improvement on biological pre-treatment method is required before it is practically used for biorefinery process technology. Biological pre-treatment method and the consequent hydrolysis and fermentation will create a basis for further development of commercial production of the second-generation bioethanol from lignocellulose material to achieve higher tolerance, productivity and yield (Maurya et al., 2015). Thus, the aim of this study is to focus towards achievement of better delignification with minimum holocellulose loss using three *Bjerkandera adusta* isolates during solid state cultivation on wheat straw as carbon source.

MATERIALS AND METHODS

Organism

Cultures (three) of studied *Bjerkandera adusta* isolates BEOFB1601 (KP794073, 100%), BEOFB1602 (MW175891, 98.09%) and BEOFB1603 (MH605076, 100%) were obtained from the Culture collection of the Institute of Botany, Faculty of Biology University of Belgrade, Serbia (BEOFB) and maintained on Malt agar medium (MA) at +4 °C. Identification of fungal isolates was confirmed by *ITS* gene sequencing and the PCR amplification of this region has been done according to Savković et al. (2021). Gene bank accession numbers and homology percentages are given in brackets for each fungal isolate.

Lignocellulosic material and inoculums preparation

Wheat straw was washed with warm distilled water (t=50 °C), dried in a heating oven at 65 °C, to constant weight, grounded in the laboratory mixer and sieved to obtain pieces of 0.5–2.0 cm.

Fungal inoculum was prepared in synthetic medium and was incubated in a rotary shaker as described by Knežević et al. (2017). Obtained biomass was further used for inoculation of wheat straw.

Cultivation conditions, determination of ligninolytic enzyme activities and polymer contents

Fungal solid-state cultivation was carried out at 25°C in 250 mL flasks containing 6.0 g of wheat straw and 30.0 mL of the modified synthetic medium (without glucose). Samples were harvested after 5, 10, 15 and 19 days of cultivation and further used for ligninolytic enzyme extraction and determination of enzyme activities spectrophotometrically, as described by Knežević et al. (2017). Enzyme activity was determined for Mn-dependant peroxidises (MnP), Mn-independent peroxidise (MnIP) and laccase (Lac). The residues were used for determination of hemicelluloses, cellulose and lignin contents as described by Kneževic et al. (2013).

Enzymatic hydrolysis and reducing sugars content determination

Solid phase remained after the extraction was dried in a drying oven at 65 °C to constant weight and sieved to separate fraction of 0.2–0.5 mm. Saccharification was carried out in flasks containing 2 g of pre-treated wheat straw in 60 mL of acetate buffer (pH 4.8) and commercial cellulase (Cellulase from *Trichoderma reesei*, C2730-50ML; Sigma-Aldrich) in loading of 60 U g^{-1} of substrate, (180 rpm, 50 °C, 72 h) as defined by Lu et al. (2012). Immediately after hydrolysis, samples were immersed in boiling water for 5 min to inactivate the enzymes and were further used for determination of reducing sugar content calorimetrically, using 1,4-dinitrosalycilic acid, according to the method of Miller (1959). Reducing sugars were expressed as g of glucose equivalent (gGE) per L of hydrolysate, using equation of calibration curve for glucose.

Chemical pre-treatment of wheat straw, as a positive control, was performed with 1% (w/v) NaOH at 121 °C for 90 min and the solid phase was further used for enzymatic hydrolysis Han et al. (2012). Autoclaved untreated wheat straw was used as a negative control.

Statistical analyses

The assays were carried out in three replicates and results are expressed as mean ± standard error. One-way analysis of variance (ANOVA) was performed to test any significant differences among means and statistical significance was declared at $p < 0.01$.

RESULTS AND DISCUSSION

During 19 days of solid-state cultivation on wheat straw as substrate, all the tested *Bjerkandera adusta* isolates demonstrated ability to produce ligninolytic enzymes at various levels, depending on the period of cultivation and studied isolate (Figure 1). Isolate *B. adusta* BEOFB1601 exhibited continuously high ligninolytic enzyme activity during studied period of cultivation, in comparison with two other strains. The highest level of MnP activity was detected in *B. adusta* BEOFB1601 (1678.5 ± 102.2 UL^{-1}) after ten days of cultivation while the highest level of MnIP activity was noticed in *B. adusta* BEOFB1603 (1305.0 ± 164.7 UL^{-1}) after 19 days of cultivation on wheat straw. The maximum of laccase activity was noticed in *B. adusta* BEOFB1602 (1023.9 ± 247.49 UL^{-1}) after 19 days of cultivation (Figure 1).

The lowest potential of MnP and MnIP production characterized *B. adusta* BEOFB1602 while the weakest producer of laccase was *B. adusta* BEOFB1603.

Although it has been shown that *Bjerkandera adusta* is a good producer of ligninolytic enzymes, many factors affect its synthesis and the activity, such as type of ligninolytic material, nutrient composition, culturing conditions, type of strain etc. (Gómez et al., 2012; Fang, 2013; Rodríguez-Couto, 2017). Rodríguez-Couto (2017) points out that the ability of enzyme production varies between different strains of the same species under the same conditions, as it was shown in this study. The weak laccase activity of *B. adusta* during cultivation on lignocellulosic substrate was also documented in previous studies (Dinis et al., 2009). Based on the values of Mn-oxidizing peroxidases in this study, ranged between 600–1600 UL^{-1}, all studied strains can be characterized as good peroxidase producers, that was in accordance with results of Sánchez (2009) and Pinto et al. (2012).

The contents of three main polymers in wheat straw used for fungal cultivation were as follows: lignin (9.0 ± 0.4%), hemicelluloses (32.8 ± 0.6%) and cellulose (47.6 ± 1.1%), and proportions for all of them in pretreated samples varied after 19 days of fungal culturing (Table 1). Delignification occurred continuously during fungal culturing, with the highest delignification rates after 19 days, in all tested isolates. The same trend was observed for hemicelluloses and cellulose. The highest lignin degradation was noticed after pretreatment

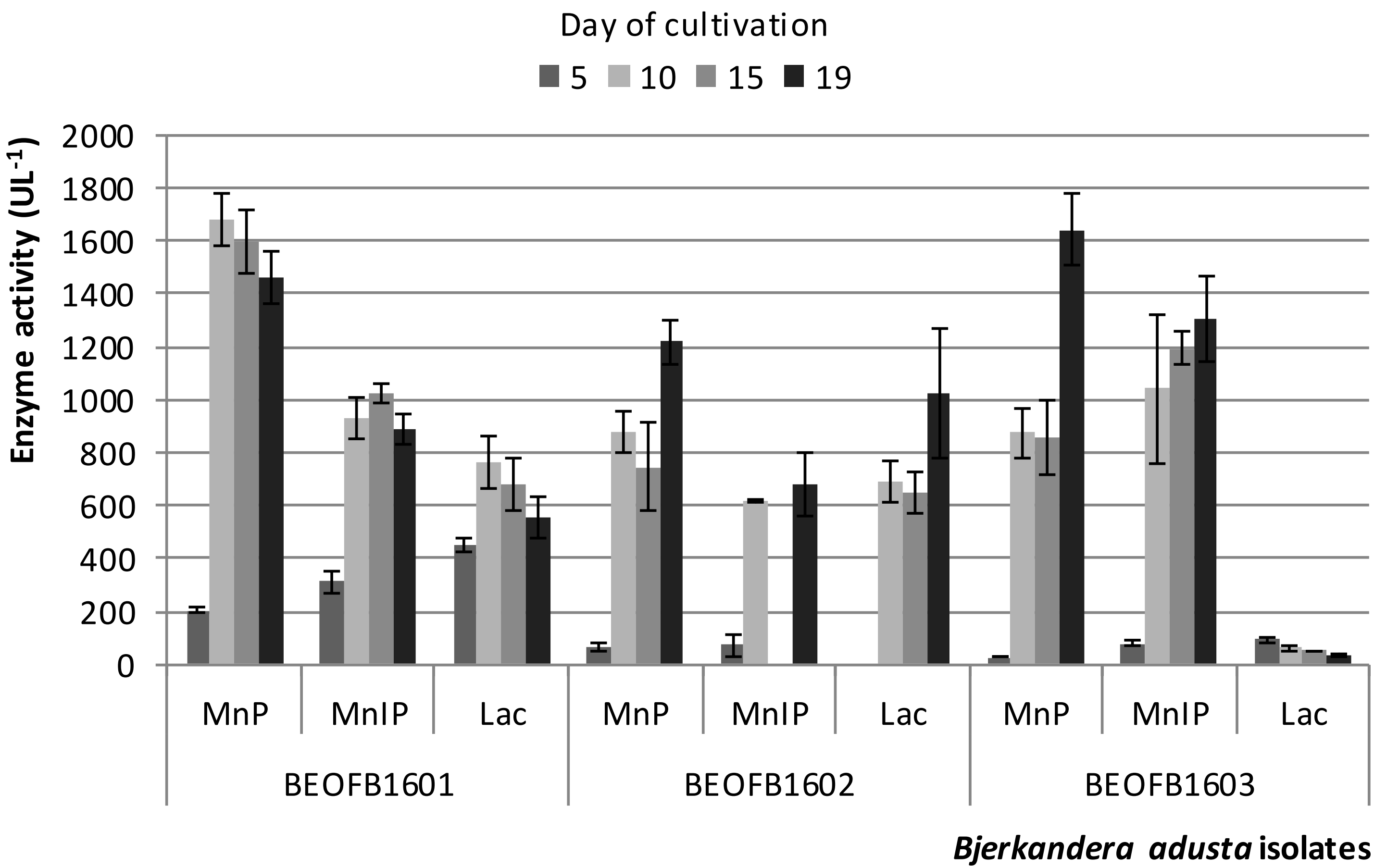

Figure 1. Ligninolytic enzyme activity in *Bjerkandera adusta* isolates

with *B. adusta* BEOFB1601 (47.9 ± 0.5%) still keeping the loss of hemicelluloses (41.9 ± 0.9%) and cellulose (34.3 ± 1.1%) at the lowest level comparing to other two strains (Table 1).

Unlike biological pretreatment, chemical pretreatment with NaOH was significantly more efficient in lignin removal (57.1 ± 0.9%) but the loss of hemicellulose was very high (68.8 ± 0.5%). The minimal loss of cellulose was achieved after pretreatment with NaOH (6.0 ± 0.7%).

Table 2. Effect of biological pretreatment with *Bjerkandera adusta* isolates on degradation of wheat straw polymers

Species/ Sample	Code of strain	Period of cultivation (day)	Total dry matter weight loss after pretreatment (%)	Degraded fibers (%)		
				Lignin	Hemi-cellulose	Cellulose
Bjerkandera adusta	BEOFB1601	5	9.6 ± 0.2	20.9 ± 1.3	12.6 ± 1.5	19.8 ± 0.8
		10	16.0 ± 0.5	26.7 ± 1.9	29.8 ± 1.5	34.6 ± 1.5
		15	19.6 ± 0.6	37.3 ± 2.2	40.3 ± 3.3	34.9 ± 1.3
		19	20.0 ± 0.5	47.9 ± 0.5	41.9 ± 0.9	34.3 ± 1.1
	BEOFB1602	5	11,4 ± 1,6	7,5 ± 1,9	37,5±1,7	9,9 ± 1,7
		10	17,8 ± 0,4	12,6 ± 1,8	48,1±1,0	16,9 ± 1,1
		15	21,1 ± 1,6	37,1 ± 2,5	39,5±6,2	36,1 ± 1,9
		19	26,6 ± 0,5	37,3 ± 4,3	50,8±1,0	37,5 ± 1,2
	BEOFB1603	5	10,1 ± 0,9	3,7 ± 0,9	40,0 ± 3,0	8,3 ± 1,4
		10	16,9 ± 0,4	16,2 ± 1,4	46,4±2,3	17,1 ± 1,0
		15	21,7 ± 0,5	18,3 ± 1,1	37,5±2,3	34,5 ± 0,0
		19	25,7 ± 0,8	24,8 ± 2,9	47,8±1,4	34,9 ± 1,8
Untreated wheat straw		–	–	–	–	–
NaOH pretreated wheat straw		–	36.0 ± 0.0	57.1 ± 0.9	68.8 ± 0.5	6.0 ± 0.7

Results of this study showed that tested *B. adusta* isolates were good lignin degraders but the selectivity of lignin degradation was lower than in chemical pretreatment. Previous researches showed that the selection of species and strains that degrade lignin selectively can be performed in order to keep holocellulose at the low level of degradation (Nazarpour et al., 2013). Dinis et al. (2009) in their work indicated that different white rot fungal species can degrade lignin between 2% and 65% during cultivation. Similar results to these, with slightly increased cellulose degradation in wheat straw using *B. adusta*, were obtained in study of Salvachúa et al. (2011). Finally, Fernandes et al. (2020) clearly demonstrated high delignification capacity of *B. adusta* correlated with enzyme activity.

Enzymatic hydrolysis of wheat straw samples showed that the concentration of released reducing sugars increased during the period of hydrolysis, with maximum values recorded after 72 h (Figure 2).

The concentration of reducing sugars after hydrolysis of untreated wheat straw as a control sample was 6.0 ± 0.1 g L^{-1}, at the end of the reaction. Comparing to this, samples pretreated with *B. adusta* BEOFB1602 and *B. adusta*

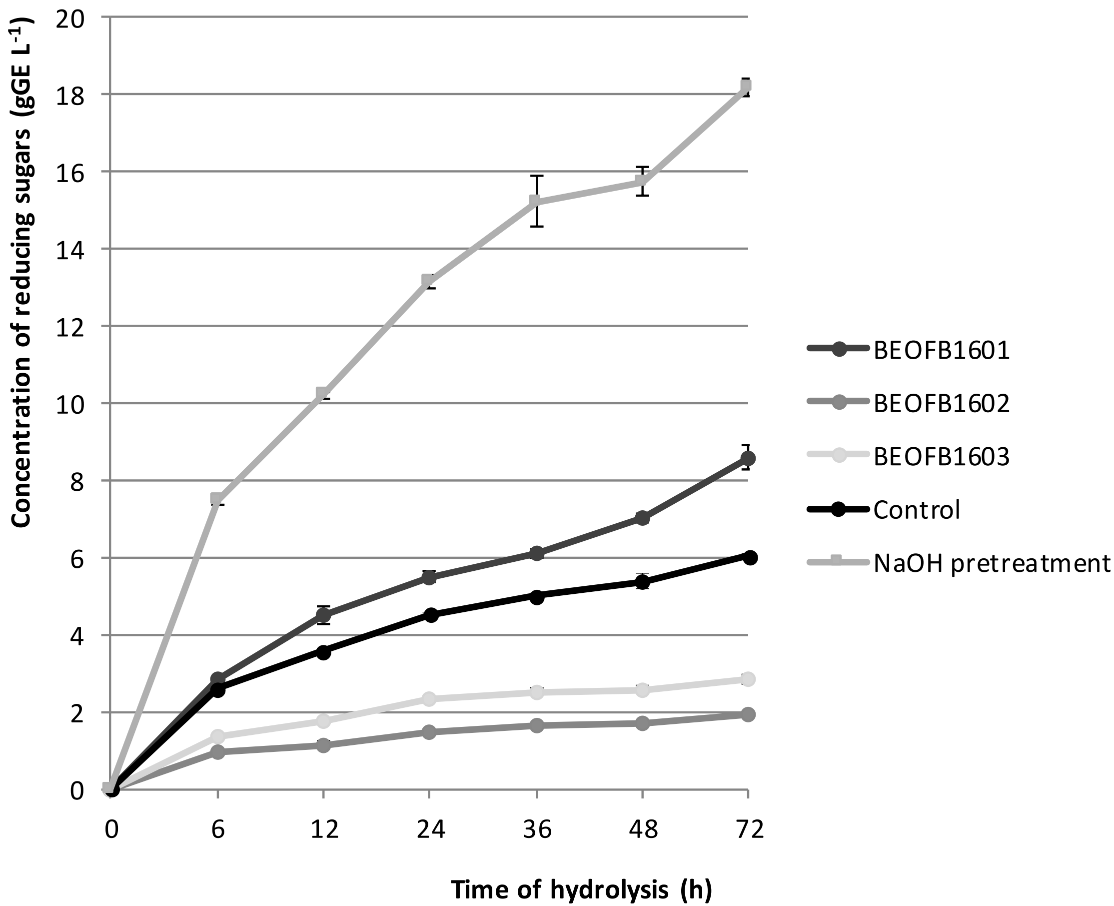

Figure 2. Time course of reducing sugars release from wheat straw during enzymatic saccharification

BEOFB1603 isolates released lower amounts of reducing sugars, 1.2 and 2.9 gGE L^{-1}, respectively. However, the amount of released sugars after enzymatic hydrolysis of samples pretreated with *B. adusta* BEOFB1601 was higher than in the control (8.5 ± 0.1 gGE L^{-1}), which clearly suggests that the highest delignification rate and relatively lower holocellulose loss is directly correlated with more efficient hydrolysis process. Comparing to this, chemical pretreatment of wheat straw was the most beneficiary for the level of sugars release (18.2 ± 0.2 gGE L^{-1}).

Enzymatic saccharification was directly affected by fungal pre-treatment and studied fungal isolates exhibited low effectiveness in increasing saccharification, except in the case of *B. adusta* BEOFB1601. This probably happened due to different physiological response of strains (Viniegra-González et al., 2003). However, the positive effect of pre-treatment with white-rot fungi has been observed by other authors (Dias et al., 2010; Pinto et al., 2012). The explanation for the increased hydrolysis yields after fungal delignification is a better accessibility of polysaccharides. One of the reasons for more successful saccharification after chemical pretreatment could be the partial holocellulose consumption during fungal culturing on lignocellulosic substrate (Salvachúa et al., 2011).

CONCLUSION

Result showed that fungal culturing as a biological pretreatment can be effective in terms of higher sugar yields released during enzymatic saccharification of wheat straw, compared with untreated samples. This process is strain specific and with lower efficiency in comparison with chemical pretreatment.

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ОРИГИНАЛНИ НАУЧНИ РАД

ДЕЛИГНИФИКАЦИЈА ПШЕНИЧНЕ СЛАМЕ ИЗОЛАТИМА *Bjerkandera adusta*: ЕФЕКАТ ПРЕТРЕТМАНА НА ЕНЗИМСКУ ХИДРОЛИЗУ

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РЕЗИМЕ: Употреба лигноцелулозних сировина у производњи биогорива и хемикалија пружа велику перспективу, пошто дрво и пољопривредни отпад на глобалном нивоу представљају најзаступљенији извор обновљиве биомасе. Међутим, делигнификација представља неизбежан корак у претретману лигноцелулозе, чиме се постиже боља доступност целулозе и хемицелулозе процесима ензимске сахарификације. Циљ овог истраживања била је процена потенцијала различитих изолата *Bjerkandera adusta* да побољшају ефикасност ензимске сахарификације пшеничне сламе након култивације у чврстим условима. Изолати *Bjerkandera adusta* (BEOFB1601, BEOFB1602 и BEOFB1603) коришћени су за парцијалну делигнификацију пшеничне сламе у току чврсте култивације. Активност лигнинолитичких ензима је одређивана спектрофотометријски, док су за мерење садржаја хемицелулозе, целулозе и лигнина коришћени остаци пшеничне сламе. За ензимску хидролизу претретиране пшеничне сламе коришћена је комерцијална целулаза у концентрацијама од 60 U g^{-1} чврстог супстрата. Садржај редукујућих шећера

одређиван је колориметријски, употребом 1,4-динитросалицилне киселине. Код тестираних изолата гљива пероксидазе су биле доминантно заслужне за разградњу лигнина. Највећа стопа деградације лигнина забележена је у узорцима третираним изолатом BEOFB1601 (47,9 ± 0,5%). Највећи принос редукујућих шећера (8,6 ± 0,3 gGE L^{-1}) постигнут је након ензимске сахарификације узорака третираних изолатом BEOFB1601, који је био најселективнији у разградњи лигнина. Добијени резултати указују на то да је култивација гљива, као метод биолошког претретмана, условљена коришћеним изолатом. Кључни механизам који повећава конверзију угљених хидрата из биомасе је селективна разградња лигнина.
КЉУЧНЕ РЕЧИ: *Bjerkandera adusta*, лигнинолитички ензими, пшенична слама, претретман гљивама, сахарификација

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ORIGINAL SCIENTIFIC PAPER

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PHOTOLYSIS OF FUMONISIN B_1 UNDER SIMULATED SOLAR IRRADIATION IN AQUATIC ENVIRONMENT

ABSTRACT: Given that the presence of fumonisin as a potentially carcinogenic compound in the aqueous medium was confirmed, it is very important to find a method for its effective removal. In this study, the degradation efficiency of fumonisins in aqueous media was investigated by direct and indirect photolysis under simulated solar irradiation (SSI). The initial pH value had a significant effect on the kinetics of fumonisin B_1 (FB_1) degradation, with the highest efficacy observed at pH 4.0 (88%), and the lowest at pH 10.0 (21%) during the 180 min of irradiation. Under these experimental conditions, FB_1 photolysis in the first degradation period follows pseudo-first-order kinetics. In comparison to direct photolysis, indirect photolysis using H_2O_2 had an inhibitory effect on the degradation of FB_1. Namely, 24% of FB_1 was degraded during 180 min of irradiation at pH 8.0, while 74% was degraded by direct photolysis for the same period of time. In the case of the application of indirect photolysis using $S_2O_8^{2-}$ at pH 4.0, the degradation efficiency of FB_1 (91%) was similar as in the case of direct photolysis (88%), at the same pH, as well as for the same period of time. Considering the degradation efficiency, it was concluded that in both cases only direct photolysis was performed, probably because SSI does not contain suitable wavelengths for sulfate radical ($SO_4^{\bullet-}$) formation. Based on this, we can conclude that direct photolysis at pH 4.0 is practically the most suitable treatment for FB_1 removal under SSI.
KEYWORDS: advanced oxidative processes, mycotoxins, photodegradation, water

INTRODUCTION

The B-series fumonisins produced by *Fusarium verticillioides* and *Fusarium proliferatum*, cause different toxicological effects in both humans and animals (Soriano and Dragacci, 2004). Consumption of feed contaminated with

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fumonisins causes a number of mycotoxicoses, including leukoencephalomalacia in horses (Gelderblom et al., 1988), pulmonary oedema in pigs (Harrison et al., 1990; Haschek et al., 2001) and exhibits cytotoxic and nephrotoxic effects (Gelderblom et al., 1988). Fumonisins, due to their high structural similarity to sphingosine and sphinganine, affect sphingolipid metabolism by inhibiting the key enzyme ceramide synthase during sphingolipid biosynthesis (Norred et al., 1997; Solfrizzo et al., 2004). The structural formula of fumonisin B_1 (FB_1) is shown in Figure 1. Although FBs are soluble in water, research on their presence in natural water samples is rare. According to literary data, the possibility of fumonisins production in the water matrix was confirmed (Oliveira et al., 2018), as well as their presence in different types of water (Waśkiewicz et al., 2015). Waśkiewicz et al. (2015) were the first to report results on the presence of FB_1 in various aquatic systems. The highest concentration of FB_1 was detected in the postharvest season (September to October) at 48.2 ng/dm^3, and the lowest in winter and spring at 21.9 ng/dm^3. FB_2 and FB_3 in water samples were not detected.

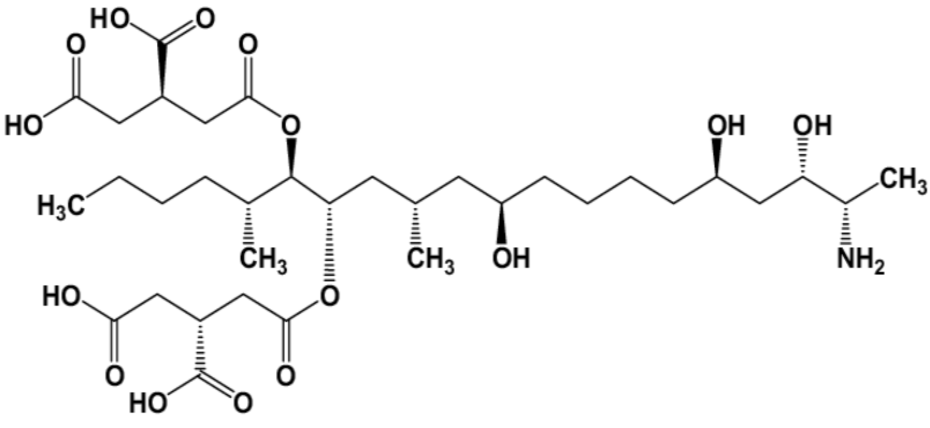

Figure 1. Structural formula of fumonisin B_1

Previous studies on mycotoxins have mainly focused on their production and presence in cereals (Streit et al., 2013; Fromme et al., 2016). Recently, more attention is addressed to studying fungi in drinking water, where mycotoxins and their metabolites have been increasingly regarded as hazardous pollutants, changing its taste and smell (Hedayati et al., 2007; Al-gabr et al., 2014). In the field of improving water treatment, advanced oxidative processes (AOPs) have been developed for the degradation of organic pollutants (Chiron et al., 2000). Several AOPs methods were used to remove mycotoxins from the aquatic environment: direct photolysis, photocatalysis using simulated solar and UV radiation, photolysis using pulsed and γ-radiation and electrochemical oxidation (Abramović et al., 2017; Jevtić, 2022).

Solar irradiation is ecologically and economically acceptable method for the removal of pollutants (Tawfik et al., 2022). The study of the efficiency of solar irradiation in laboratory conditions is usually performed using different types of simulated solar sources. Solar degradation may occur either by direct or indirect photolysis (Emídio et al., 2017). Direct photolysis occurs when the pollutant directly absorbs photons of a certain energy simulated solar irradiation (SSI), causing its degradation (Chowdhury et al., 2010). Furthermore, the application of SSI may lead to self-sensitization via reactive oxygen species (ROS) (Ma et al., 2014). Namely, it was reported that certain organic pollutants absorbed photons and transferred energy as electrons to other chemicals (i.e., O_2) with the formation of ROS, which subsequently oxidized and degraded the pollutants. On the other hand, indirect photolysis occurs via light absorption by photosensitizers, some of the most important being dissolved organic matter nitrate/nitrite ions, and Fe(III)/Fe(II)-organic substance complexes and humic acid (Zhan, 2009; Zhang et al., 2022; Yang et al., 2022). These compounds pass into an excited state by absorbing SSI and then generate free radicals that further degrade pollutants (Chowdhury et al., 2010).

This paper focuses on the removal of FB_1 from the aquatic environment using SSI. The aim of the present study was to investigate the efficiency of direct and indirect photolysis in the degradation of FB_1 in an aqueous medium. The influence of initial pH on the photodegradation efficiency was investigated. Even though, according to previous research, fumonisins were found in waters in small quantities, long-term exposure can negatively affect human health. To the best of our knowledge, this is the first study of the photochemical behavior of FB_1 in an aquatic environment under SSI.

MATERIALS AND METHODS

Chemicals and solutions

A stock solution of FB_1 was prepared by dissolving 1 mg of solid FB_1 (approx. 98% TLC, Sigma-Aldrich, St. Louis, MO, USA F1147, from *F. moniliforme*) in 100 cm^3 of ultrapure water at a concentration of 100 μg/cm^3. Working solutions were prepared by dilution of the toxin stock solutions with ultrapure water to a concentration of 1.39×10^{-6} mol/dm^3 for FB_1. This aqueous solution of FB_1 was found to be stable for more than 500 days in the dark at a temperature from 4 °C to 8 ºC (Jevtić et al., 2021). Methanol (HPLC gradient grade, Fisher Scientific, Merelbeke, Belgium) and 0.1 mol/dm^3 NaH_2PO_4 (extra pure, Merck KGaA, Darmstadt, Germany) were used as components of the mobile phase, where the pH 3.35 was adjusted with H_3PO_4 (p.a., Centrohem, Stara Pazova, Serbia). To prepare the derivatization agent, o-phthaldialdehyde (OPA; min. 99%, Sigma-Aldrich, St. Louis, MO, USA), 0.1 mol/dm^3 $Na_2B_4O_7$ (p.a., Zorka, Šabac, Serbia), and 2-mercaptoethanol (MCE; min 99%, Serva, Haidelberg, Germany) were used. H_2O_2 (30%, p.a., Sigma-Aldrich, St. Louis, MO, USA) and $(NH_4)_2S_2O_8$ (≥ 95%, Merck-Alkaloid, Skopje, North Macedonia) were also used. The solutions used to adjust the pH of the irradiation solution were obtained by dilution of 70% $HClO_4$ (99.999% trace metals basis, Merck-Alkaloid, Skopje, North Macedonia), and 30% NaOH (suprapur, Sigma-Aldrich, St. Louis, MO, USA). All chemicals were used without further purification.

Photodegradation and analytical procedures

Degradation of FB_1 was performed in a photochemical cell (total volume about 40 cm^3, liquid layer thickness 35 mm) with a plain window on which the light beam was focused (Abramović et al., 2011). In order to examine the efficiency of the indirect photolysis process, as well as the influence of H_2O_2 and $S_2O_8^{2-}$ on the efficiency of fumonisins degradation, an appropriate volume of $H_2O_2/(NH_4)_2S_2O_8$ solution was added to the reaction mixture. The photochemical cell was placed on a magnetic stirrer (with continuous stirring in a stream of O_2, 3.0 cm^3/min) and thermostated at 25.0 ± 0.5 ºC. As a source of solar radiation, we used a halogen lamp (Philips, Amsterdam, Netherlands, 50 W) intensity 63.85 mW/cm^2 in the visible area and 0.22 mW/cm^2 in the UV area. The radiation energy fluxes were measured using a Delta Ohm HD 2102.2 (Padova, Italy) radiometer fitted with the LP 471 UV (spectral range 315–400 nm) and LP 471 RAD (spectral range 400–1,050 nm) sensors.

To adjust the pH values, the aqueous solution of NaOH or $HClO_4$ (0.10 mol/dm^3) was added before photodegradation. Changes in the pH during the degradation were monitored by using a combined glass electrode (pH-Electrode SenTix 20, WTW, Thermo Fisher Scientific, MA, USA) connected to the pH-meter (pH/Cond 340i, WTW).

Aliquots of the reaction mixture (0.4 cm^3, allowed volume change up to 10%) were taken before the start of irradiation and at certain time intervals during irradiation to monitor the photodegradation kinetics of FB_1 by liquid chromatography with fluorescence detection (HPLC−FLD). For fumonisin derivatization prior to HPLC–FLD determination, an aliquot of 0.1 cm^3 of the reaction mixture was mixed with 0.1 cm^3 of OPA–MCE reagent, at room temperature, with stirring for 1 min. The derivatized solution was filtered through a PTFE syringe filter (ESF-PT-04-022; 0.22 µm; 4 mm; Kinesis Ltd. Cambridgeshire, UK) directly into a 200 µl insert vial (Supelco, Bellefonte, USA). Ten µl of the derivatized solution were injected into the HPLC−FLD system. Use was made of an HPLC Dionex UltiMate 3000 Series system with a FLD 3100 (Thermo Scientific, Germany), consisting of an autosampler WPS-3000, degasser, quaternary pump, and Hypersil GOLD column (150×3 mm, particle size 3 µm). The system was controlled by Chromeleon® 7 software (Thermo Scientific). The mobile phase was MeOH−0.1 mol/dm^3 NaH_2PO_4 (75+25, *v/v*) adjusted to the pH 3.35 with H_3PO_4 (Jakšić et al., 2015).

The calibration curve for determining of FB_1 concentration during photodegradation is shown in Figure 2 together with the straight-line equation. As can be seen, the correlation coefficient was 0.998.

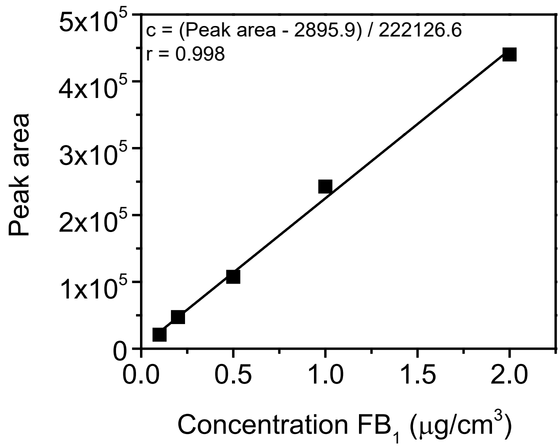

Figure 2. Calibration curve for the determination of the OPA derivative of FB_1 by the HPLC–FLD method and the straight-line equation. Mobile phase composition: MeOH–0.1 mol/dm^3 NaH_2PO_4 (77:23, *v/v*), pH 3.35; flow rate 0.8 cm^3/min.

RESULTS AND DISCUSSION

Figure 3 shows the chromatograms obtained during the photolysis of FB_1 for the first 180 min irradiation using SSI at pH 4.0. As can be seen, the application of this treatment results in the degradation of FB_1, but even after 180 min of irradiation, the degradation of FB_1 was not complete.

The effect of initial pH on the rate of FB_1 degradation using SSI was examined at pH 4.0; 8.0, and 10.0 (Figure 4). Examining the effect of pH in the interval from 4.0 to 10.0 on the photolysis efficiency of FB_1, the highest efficiency was observed at pH 4.0, while the lowest was at pH 10.0. At pH 4.0 and 8.0, the values of the apparent first-order degradation rate constants were 0.011 and 0.007 min^{-1}, respectively, with a correlation coefficient higher than 0.99 for the first 60 min of irradiation in both cases. The apparent first-order degradation rate constant decreased significantly at pH 10.0 and was 0.0009 min^{-1}, while the linear correlation coefficient was 0.959. The change in pH value was also monitored during the direct photolysis of FB_1 and it was found that the pH had no significant changes, except at pH 10.0, when the pH value decreased by about one pH unit during 180 min of irradiation.

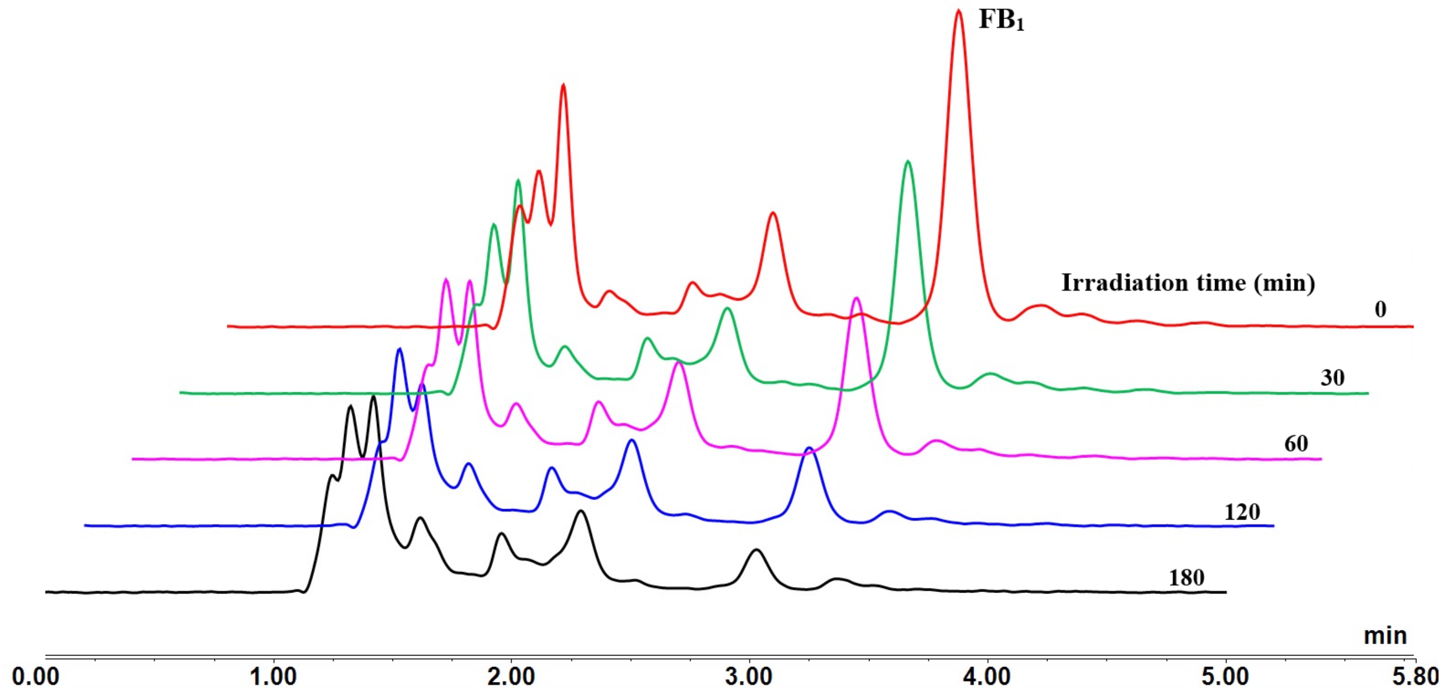

Figure 3. Chromatograms of photolysis FB_1 (1.39×10^{-6} mol/dm^3) for the first 180 min using SSI at pH 4.0

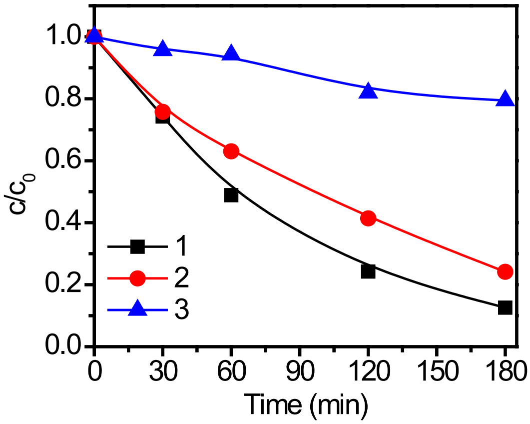

Figure 4. Effect of initial pH on the efficiency of direct photolysis of FB_1 using SSI: 1) pH 4.0; 2) pH 8.0; 3) pH 10.0.

To test the effectiveness of SSI/H_2O_2 treatment, 0.278 mmol/dm^3 concentration of H_2O_2 solution was used, since this concentration proved to be optimal under UV radiation (Jevtić et al., 2021). To simulate natural conditions, the pH value used in this treatment was about 8.0, which is usually the pH of natural waters. Figure 5 shows the kinetic curves of direct photolysis of FB_1 (curves 1 and 2), as well as indirect photolysis with the addition of H_2O_2 (curve 3) and $S_2O_8^{2-}$ (curve 4) obtained using SSI. As can be seen, in the presence of H_2O_2, the efficiency of FB_1 degradation was lower compared to direct photolysis. Namely, 74% of FB_1 was degraded in 180 min by direct photolysis, while with the addition of H_2O_2 only 24% of this fumonisin was degraded. The inhibitory effect of the presence of H_2O_2 on the efficiency of FB_1 degradation was unexpected. Since UV/$S_2O_8^{2-}$ treatment has been shown to be very effective in the degradation of FB_1 (Jevtić et al., 2021), the efficacy of SSI/$S_2O_8^{2-}$ treatment with the addition of optimal concentration of $S_2O_8^{2-}$ was also examined (0.140 mmol/dm^3; Figure 5, curve 4). By comparing the obtained results, it can be concluded that the degradation efficiency was higher at pH 4.0, regardless of whether it was direct (Figure 5, curve 2) or indirect (Figure 5, curve 4) photolysis. Efficiency of indirect photolysis was slightly higher in the presence of $S_2O_8^{2-}$ (Figure 5, curves 3 and 4). During the first 30 min, the same percentage of degradation was observed (about 25%), and after 180 min of irradiation the difference in efficiency was very low (3%). Namely, after 180 min of irradiation in direct photolysis using SSI at pH 4.0, 88% of FB_1 was degraded, while using SSI/$S_2O_8^{2-}$ treatment efficiency was 91% (Figure 5, curves 2 and 4). When comparing direct and indirect photolysis, SSI/H_2O_2 treatment proved to be the least effective (Figure 5, curve 3). The obtained results of indirect photolysis were expected, since H_2O_2, and $S_2O_8^{2-}$ were not subject to photolysis in the visible part of the spectrum, and in both cases only direct photolysis was performed because SSI did not have the appropriate wavelengths that would lead to the formation of radicals.

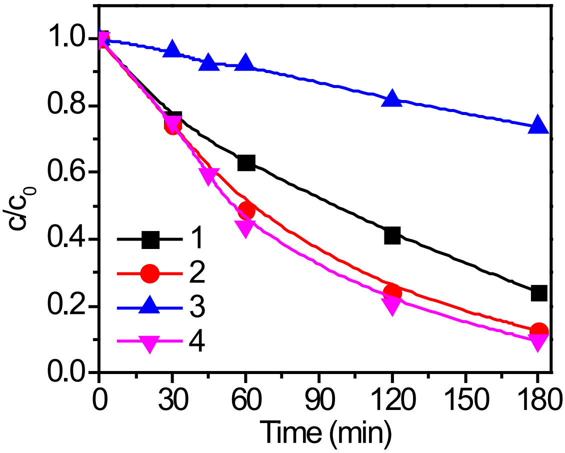

Figure 5. Effect of different treatments on the degradation efficiency of FB_1 (1.39×10^{-6} mol/dm^3): 1) SSI, pH 8.0; 2) SSI, pH 4.0; 3) SSI/H_2O_2, $c(H_2O_2)$ = 0.278 mmol/dm^3, pH 8.0; 4) SSI/$S_2O_8^{2-}$, $c(S_2O_8^{2-})$ = 0.140 mmol/dm^3, pH 4.0.

CONCLUSION

Given the potential danger of fumonisins presence in water, it is very important to pay attention to the development of methods for their removal. AOPs have proven to be efficient processes for the purification of waters that contain pollutants, most often with their complete mineralization. Based on the obtained results, we can conclude that FB_1 is most efficiently degraded using SSI at the pH 4.0 during the indirect (SSI/$S_2O_8^{2-}$) or direct photolysis. The obtained results can be significant for the development of technological methods for water purification from fumonisins as possibly carcinogenic contaminants. Moreover, these results allow us to conclude that degradation by solar irradiation can be possible way of removal of FB_1 in natural waters.

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ОРИГИНАЛНИ НАУЧНИ РАД

ФОТОЛИЗА ФУМОНИЗИНА Б$_1$ ПОМОЋУ СИМУЛИРАНОГ СУНЧЕВОГ ЗРАЧЕЊА У ВОДЕНОЈ СРЕДИНИ

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САЖЕТАК: Појава фумонизина у воденој средини представља актуелни проблем загађења животне средине. Фумонизини у водену средину могу доспети спирањем са контаминираних житарица у пољу, а могу их продуковати и плесни у воденом матриксу. Пошто су фумонизини стабилни у воденом раствору, важно је пронаћи најефикасније методе за њихово уклањање. Напредни процеси оксидације имају широку примену у пречишћавању вода које садрже органске загађујуће материје јер углавном доводе до њихове потпуне минерализације. У овој студији, ефикасност разградње фумонизина у воденој средини је испитивана директном и индиректном фотолизом помоћу симулираног сунчевог зрачења (ССЗ). Почетна pH-вредност имала је значајан утицај на кинетику разградње фумонизина $Б_1$ ($ФБ_1$), при чему је највећа ефикасност забележена при pH 4,0 (88%), а најнижа при pH 10,0 (21%) током 180 минута озрачивања. У овим експерименталним условима, фотолиза $ФБ_1$ у првом периоду разградње прати кинетику псеудо-првог реда. У поређењу са директном фотолизом, индиректна фотолиза уз додатак H_2O_2 имала је инхибиторски ефекат на разградњу $ФБ_1$. Наиме, при pH 8,0 свега 24% $ФБ_1$ је разграђено током 180 минута озрачивања, док је директном фотолизом за исто време разграђено 74% овог микотоксина. У случају примене индиректне фотолизе уз додатак $S_2O_8^{2-}$ при pH 4,0, ефикасност разградње $ФБ_1$ (91%) је слична као у случају директне фотолизе (88%), при истом pH и за исто време озрачивања. С обзиром на ефикасност разградње, закључено је да се у оба случаја врши практично само директна фотолиза, вероватно зато што ССЗ не садржи одговарајуће таласне дужине за формирање сулфатног радикала ($SO_4^{\bullet-}$). На основу овога може се закључити да је директна фотолиза на pH 4,0 практично најпогоднији третман за уклањање $ФБ_1$ применом ССЗ, иако је и при pH 8,0 ефикасност задовољавајућа без потребе за подешавањем pH. Добијени резултати могу бити значајни за развој технолошких метода за пречишћавање вода од фумонизина као могућих канцерогених загађујућих материја које могу бити присутне у воденој средини.

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