UNIVERSITY OF PLOVDIV "PAISII HILENDARSKI" • FACULTY OF BIOLOGY
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„POSSIBILITIES FOR SUSTAINABLE MANAGEMENT OF URBAN SOILS THROUGH BUFFER GREEN AREAS"

A B S T R A C T

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The defense of the dissertation will take place on 12.02.2025 from 11:00 a.m. in the 14th auditorium of the Faculty of Biology, University of Plovdiv “Paisii Hilendarski”, 2 “Todor Samodumov” Street, at an open meeting of the scientific jury composed of:

Assoc. Prof. Dilyan Georgiev Georgiev, DSc
Assoc. Prof. Slaveya Tencheva Petrova, PhD
Prof. Neli Hristova Grozeva, DSc
Prof. Georgi Georgiev Beev, PhD
Prof. Mariana Genova Doncheva-Boneva, PhD

The materials for the defense are available to those interested in the Department „Development of the academic staff and doctoral studies“ at the University of Plovdiv “Paisii Hilendarski” and in the Central Library of University of Plovdiv “Paisii Hilendarski”.

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1. INTRODUCTION

Urbanization is one of the main processes causing continuous change of natural landscapes, and its pace is increasingly intensifying. Any such impact that changes the physico-chemical parameters of the environment is inevitably associated with changes in the ecosystems' composition and structure. This disrupts their ecological balance, alters their biodiversity and their self-regulatory ability, and therefore their potential to provide ecosystem services, which ultimately affects human health and well-being.

The lack of regular complex monitoring programs for assessment of the urban soils' status is registered in almost all settlements in our country. The need for detailed soil studies in large cities, including in the city of Plovdiv, is determined by the fact that the soils are subjected to very strong pressure due to the increasing intensity of urbanization, and also the state of the urban green infrastructure is closely related to the soils in which the vegetation develops. Furthermore, the quality of urban soils turns out to be decisive for the ecosystem services provided in the urban environment, and for this reason – for the quality of life in the settlements.

Since soils provide the basis for the development of green infrastructure and are a key element of the urban landscape, the sustainable management of urban soils is of utmost importance for soil conservation, its use and restoration with the aim of long-term improvement of soil quality and living conditions in the settlements.

2. LITERATURE REVIEW

The abundance of pollutant sources in urban areas leads to an increased chemical pressure on the environment, which subsequently has a destructive effect on human and the ecosystems' health (Taylor and Owens, 2009; Wong et al., 2006). The mobilization of heavy metals and other potentially toxic elements in the biosphere as a result of anthropogenic activity is becoming an increasingly significant process in the geochemical cycle of these elements. Urban soils are the main reservoir of pollutants in terrestrial ecosystems, which is why the protection of their quality and their sustainable management are becoming an increasing priority (Li et al., 2013).

Infrastructure development and urbanization is one of the main threats to biodiversity and the disruption and alteration of soil microbial communities. Microorganisms are sensitive to environmental conditions and anthropogenic impact; therefore, they are increasingly used to detect anomalies in the environment. One of the key parameters for ensuring the normal functioning of soils is the amount of microorganisms, the ratios between different functional groups, their enzymatic activities (Dec, 2014), and the presence of indicators of faecal-domestic pollution such as faecal coliforms (FC), faecal enterococci (FE) and *Escherichia coli* (Hitzl et al., 1997; Nogueira et al. 2006; Malik et al., 2016;

Song et al., 2015). The urban soil microbiome is involved in diverse processes such as organic matter decomposition, humus synthesis, nutrient release and nitrogen-fixation (Beare et al., 1995), pollutant disposal, etc., thus strongly influencing soil characteristics and quality (O'Donnell et al., 2001).

Phytoremediation is an effective strategy that is often applied in the integrated approach used in ecological restoration programs for contaminated lands (Zurek et al., 2014; Azimi et al., 2019). This technological solution allows, by using the specificity of the plant genotype, to minimize various stress factors in the soil. It is successfully applied in soils affected by pollution with heavy metals and organic pollutants (Dinev, 2009). The method includes both the fixation of pollutants in the soil and the prevention of erosion processes, as well as the extraction of heavy metals in the plant biomass, the concentration of which in the soil significantly exceeds the sanitary threshold (Dinev, 2009). One of the main advantages of phytoremediation is that it takes place at the site of pollution, but its main disadvantage is that it requires a long-term commitment, since the process is dependent on the ability of plants to grow and develop in an environment, which is not quite suitable for their normal growth.

The creation and use of green areas is seen as an effective, ecological, economically sustainable and cost-effective approach to remove pollutants from contaminated soils (sites), which also has aesthetic benefits (Ahmad et al, 2012; Hussain et al., 2018; Masu et al. al., 2014).

Economically effective and one of the good technological solutions is to use the ability of some plant species to metabolize, accumulate and detoxify heavy metals or other harmful organic or inorganic pollutants accumulated in the soil layer (Besalatpour et al., 2008; Gołda and Korzeniowska, 2016; Langella et al., 2014; Pandey and Singh, 2019; 2020). Important factors for a successful outcome of phytoremediation are the ability of plants to assimilate heavy metals and their phytoavailability (Gul et al., 2018, 2019; Zhang et al., 2018).

The selection of species and varieties of plants used for grassing lawns and buffer zones along intensively loaded urban road arteries is determined by the biological characteristics of the species related to their intensity of growth, biomass synthesis, bioaccumulation of pollutants in the roots and shoots, as well as their biological potential for pollutant detoxification (Akinci et al., 2010; Niknahad et al., 2018; Pandey, 2012; Pandey et al. al., 2015).

The creation and maintenance of green areas is related to compliance with a number of technological solutions and requirements that determine their effective use in grassing and formation of a natural hedge of perennial grasses (Vasilev, 2012). The formation of grasslands is based on many factors: climatic and meteorological features of the given area, soil characteristics, natural vegetation (type and degree of weeding of the areas), species composition of the local biodiversity, as well as the needs for its protection and restoration.

3. AIM AND TASKS

The aim of this dissertation is to explore the possibilities of sustainable management of urban soils through buffer green areas.

The object of research in this dissertation are the soils of the territory of the city of Plovdiv.

The subject of research is the potential of buffer green areas for sustainable soil management in populated areas.

Scientific hypothesis: on the basis of the planned complex studies of urban soils, the selection and testing in real conditions of effective herbaceous plants with bioaccumulation capabilities, through the construction and functioning of buffer green areas around the transport arteries (tested in the conditions of the city of Plovdiv), it can be constructed a model for sustainable management of urban soils leading to long-term improvement of soil quality and living conditions in the city.

The fulfill this aim, the following tasks have been set :

1) Selection of experimental plots within the regulatory boundaries of the city of Plovdiv

2) Selection of methods and plant species for the construction of buffer grass areas

3) Creation and maintenance of buffer green areas in the selected experimental plots

4) Analysis of the physico-chemical parameters of the soils in the experimental plots

5) Analysis of the content of selected chemical elements in the soils and the tested herbaceous species

6) Analysis of the properties and characteristics of the soil communities in the experimental plots

7) Analysis of the bioremediation potential of the tested herbaceous species

8) Analysis of the possibilities for sustainable management of urban soils through buffer green areas.

4. MATERIALS AND METHODS

4.1. Selection of experimental plots within the regulatory boundaries of the city of Plovdiv

For the purposes of the present study, in each of the six administrative regions of the city Plovdiv (Trakiya, Eastern, Southern, Western, Northern, Central) were selected on two plots. One plot is located in a large park area and is interpreted as a background for the area. The other plot is located along one of the main road arteries in the respective area and in it the vegetative green areas, which are the subject of research in the course of the dissertation, were constructed (Fig. 1). In this way, a total of 12 experimental plots were selected, reflecting different types of anthropogenic impacts on the environment, according to the urban gradient, wind rose and other factors.

4.2. Selection of methods and plant species for the construction of buffer grass areas

Taking into account the soil characteristics and climatic features of the region of the city of Plovdiv, the following plant species were selected for the construction of the buffer grass strips: Crested wheatgrass (*Agropyron cristatum* L.) variety "Svezhina", Perennial ryegrass (*Lolium perenne* L.) variety "IFK Harmony", Tall fescue (*Festuca arundinacea* Schreb) variety "Albena" and the perennial leguminous plant Bird‘s-foot trefoil (*Lotus corniculatus* L.) variety "Leo". Seeds from them were purchased from certified producers.

4.3. Creation and maintenance of buffer green areas in the selected experimental plots

In each of the six experimental plots, 5 squares with dimensions of 1 × 1 m were set aside, in which 5 experimental variants were planted, respectively: Variant 1 – Perennial ryegrass (*Lolium perenne* L.) variety "IFK Harmony" (monoculture); Variant 2 – Crested wheatgrass (*Agropyron cristatum* L.) variety "Svezhina" (monoculture); Variant 3 – Tall fescue (*Festuca arundinacea* Schreb) variety "Albena" (monoculture); Variant 4 – Bird‘s-foot trefoil (*Lotus corniculatus* L.) variety "Leo" (monoculture); Variant 5 – mixed cropping of the four species in a ratio of 1:1:1:1.

4.4. Analysis of the physico-chemical parameters of the soils in the experimental plots

Before starting of the experimental work (2019), as well as during the study, regular sampling was carried out twice within each year (2019-2022) – in the spring and in the autumn.

According to a standardized methodology (Fig. 1), representative soil samples were collected from the six background and six experimental plots, in each of which five squares were separately sampled, in order to account for the influence of the planted herbaceous species from the five experimental variants. The samples were placed in sterile polyethylene bags, labeled and transported to the appropriate laboratory (microbiology, agroecology, analytical chemistry, biochemistry) for sample preparation and analysis.

Soil moisture was measured in situ using a Soil Humidity Meter TR 46908 (Turoni, Italy), and in laboratory conditions was determined using the weight method. The response of the soil samples was determined potentiometrically in laboratory conditions using the pHotoFlex Set, 2512000 (WTW-Germany), and the electrical conductivity was determined conductometrically using the Multiset, F340 (WTW-Germany).

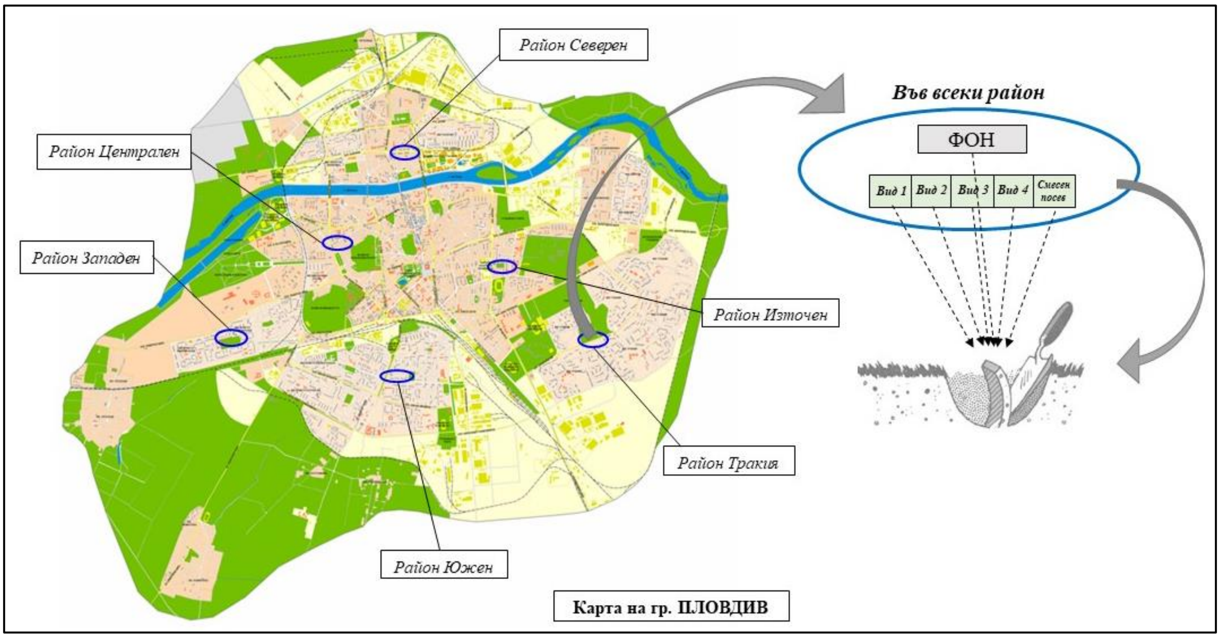

Fig. 1. Map of the city of Plovdiv, locations of the selected experimental plots and sampling scheme

The soil samples preparation for the analysis of physico-chemical indicators was carried out in the laboratory of Agroecology (Faculty of Plant Protection and Agroecology, Agricultural University) according to ISO 11464, where the following analyses have been performed: organic carbon (Angelova et al., 2014), organic matter (calculation method), total nitrogen (BDS ISO 11261:2002), C/N ratio (calculation method), mobile nitrogen (ISO/TS 14256-1:2003), mobile phosphorus and mobile potassium (GOST 26209:1991), soil texture (Đamić et al., 1996).

4.5. Analysis of the content of selected chemical elements in the soils and the tested herbaceous species

As target elements that are of interest from the point of view of potential soil contamination in the city of Plovdiv, as well as for evaluating the effectiveness of soil remediation measures in the urban environment, were selected: P, S, Mg, Fe, Al, Ca, Mn, Na, Cu, Zn, Pb, Cr, Co, Ni, As. The determination of the content of selected chemical elements in soil and plant samples was carried out in a laboratory of Analytical Chemistry (Faculty of Chemistry, University of Plovdiv "Paisiy Hilendarski") using two modern plasma-spectral methods – ICP-OES (optical emission spectrometer with inductively coupled plasma Thermo Scientific iCAP 6300 Duo) and ICP-MS (Inductively Coupled Plasma Quadrupole Mass Spectrometer ICP-MS Agilent 7700 (Tokyo, Japan) with an octopole reaction system). For quality control of the analytical results, a portion of the certified reference material ERM-CC141 is analyzed with each batch of samples.

4.6. Analysis of the properties and characteristics of the soil communities in the experimental plots

Physiological parameters. The intensity of the photosynthesis and transpiration processes, as well as the stomatal conductance of the studied plants, were measured periodically during the growing season using portable photosynthetic system Q-box CO650 (Quibit Systems Inc., Canada).

Photosynthetic pigments. The content of photosynthetic pigments was analyzed in the laboratory of Ecology (Faculty of Biology, University of Plovdiv "Paisii Hilendarski") after their extraction with acetone, and the absorbance was measured using a CamSpec M108 spectrophotometer.

Biochemical parameters. The sample preparation and analyzes were carried out in the laboratory of Biochemistry (Faculty of Biology, University of Plovdiv "Paisiy Hilendarski"). The first step involves the preparation of extracts from the collected plant samples (separately from below- and aboveground biomass) as follows: an initial sample of 0.2 g of fresh plant material is ground in a mortar and quantitatively transferred to a beaker with 10 ml of phosphate buffer (100 mM KH_2PO_4/K_2HPO_4). The extract was centrifuged at 10 000 g for 15 min at 4°C. Then the content of total soluble protein (Bradford, 1976) and free proline (Carillo & Gibon, 2011) were analyzed, as well as the activity of the enzymes catalase (Aebi, 1984), glutathione peroxidase (Wendel, 1990), glutathione reductase (Mavis & Stellwagen, 1968).

4.7. Analysis of the bioremediation potential of the tested herbaceous species

Microbiological analyzes were conducted twice per year during the experimental work in the selected 12 plots. The soil samples were collected in sterile containers and transported in the dark under refrigerated conditions to the laboratory of Microbiology (Faculty of Biology, University of Plovdiv "Paisiy Hilendarski"). The following parameters were analyzed: number of viable microorganisms (ISO 6222:1999), *Escherichia coli* and faecal coliforms (ISO 9308-1:2014), faecal enterococci (ISO 9308-1:2004), actinomycetes (Zhang, 2011), total number of fungi (Pitt & Hocking, 2009), soil respiration (ISO 16072:2001), soil microbial biomass (Alef & Nannipieri, 1995), enzymatic activity of soil communities (Belser, 1979; Hart et al., 1994; von Mersi & Schinner, 1991;), physiological profile (CLPP) of microbial communities (Biolog Inc., Hayward CA, USA), metagenomic analysis (Illumina PE250, Novogene – UK).

4.8. Analysis of the possibilities for sustainable management of urban soils through buffer green areas

Two factors, namely Bioaccumulation Factor (BAF) and Translocation Factor (TF), were used to evaluate the effectiveness of herbaceous species for phytoremediation. They have been recommended as particularly suitable for such purposes because phytoremediation technology exploits the potential of heavy metal bioaccumulation in plants to clean up heavy metal-contaminated areas (Baker & Walker, 1990; Schnoor, 2002).

The **Bioaccumulation Factor (BAF)** of the investigated potentially toxic elements (PTEs) is calculated by the ratio of the level of the element in plant roots to the level of the same element in the soil, using the formula of Yoon et al. (2006):

$$\boldsymbol{BAF = \frac{PTE\ root}{PTE\ soil}}$$

Values of BAF > 1 indicate that the given element accumulates in the roots of plants from the soil.

The **Translocation Factor (TF)** is used as a measure of the transport of the element from the underground organs to the aboveground organs of the plants and is calculated by the formula of Yoon et al. (2006):

$$\boldsymbol{TF = \frac{PTE\ shoot}{PTE\ root}}$$

When TF>1 it is evident that the element is efficiently transported from the roots to the aboveground plant organs.

4.9. Mathematical and statistical data processing

All data obtained in the course of physiological measurements and analyzes of physico-chemical, biochemical and chemical parameters were subjected to mathematical processing using the statistical package Statistica 7.0. (StatSoft Inc., 2006) and SPSS Ver.22 (Microsoft Com, 2022). Analyzes of enzyme activities and physiological profiles of microbial communities were performed with Primer 6 (E-Primer), Statistica v.10 (StatSoft) and Microsoft Excell 2016 with additional statistical package XLSTAT (Addinsoft).

5. RESULTS AND DISCUSSION

5.1. Creation and maintenance of buffer green areas in the selected experimental plots

Six experimental plots with buffer green areas were successfully constructed, located in close proximity along ones of the busiest road arteries in the city of Plovdiv (for each one of the six administrative regions). Each experimental plot includes five squares – one with a monoculture of the respective tested species (Variant 1, Variant 2, Variant 3 and Variant 4) and one with a mixed crop of the four tested species in a ratio of 1:1:1:1 (Variant 5).

Photos of the experimental plot in the Northern region 25 days after sowing (04/05/2019), 3 months after sowing (06/19/2019) and 1 year later (04/29/2020) are presented in Fig. 2.

Fig. 2. Experimental plot with buffer green areas in the Northern region of the city of Plovdiv

SUMMARY

The technological model for the construction of buffer grass strips proposed in the dissertation was developed and validated in the conditions of the city. Plovdiv, but can be easily adapted to other settlements according to local conditions.

5.2. Analysis of the physico-chemical parameters of the soils in the experimental plots

The reaction of the studied soil samples from the experimental plots ranges from moderately acidic – 5.57 (Trakiya region, background) to slightly alkaline – 7.62 (Trakiya region, experiment). This is the region with the greatest difference in pH between the background and the experimental site ($p < 0.05$). In the remaining 5 regions, the measured pH values are close and in the range of slightly acidic soil reaction. The humidity of the studied soils is in the range of 56.9% (Southern region, experiment – Variant 2) to 69.5% (Eastern region, background), and the obtained results determine the degree of humidity as good. The soils of the studied plots in all areas have low electrical conductivity. Statistically reliable differences in the studied soil parameters (pH, humidity and electrical conductivity) between the tested variants in the individual squares within the boundaries of one plot have not been found.

The analysis of the soil stock with biogenic elements was made by region with a view to taking into account local specifics in the soil conditions.

Trakiya region. The soil used as background was very high in organic C, total nitrogen and organic matter (OM). The C:N ratio is low. The content of mobile nitrogen and mobile phosphorus is average, and the supply of mobile potassium is very strong. The soil on which the experiment is based in the same area is on average stocked with org. C, total nitrogen and OM. The C:N ratio is low. The content of mobile nitrogen is insufficient, of mobile phosphorus – very good. The storage of mobile potassium is very strong.

Central region. The soil used as background is high in org. C and OM, with an average stock of total nitrogen (0.159%). The C:N ratio is average. The mobile nitrogen content is insufficient. The reserve with mobile phosphorus is very good, and with mobile potassium – very strong. The soil on which the experiment is based in the area is also high in organic C and OM, with an average stock of total nitrogen. The C:N ratio is average. Stocking with mobile nitrogen is average, with mobile phosphorus – very good, with mobile potassium – very strong.

Eastern region. The soil in the background plot is very high in org. C and OM, high storage capacity with total nitrogen (0.259%). The C:N ratio is average. The availability of mobile nitrogen is insufficient, with mobile phosphorus – very large, with mobile potassium – very strong. The reserve of the soil, on which the experiment is based, is high in terms of org. C and OM, for total nitrogen – medium (0.151%), and the C:N ratio is high. The content of mobile nitrogen is also average, of mobile phosphorus – very good, of mobile potassium – very high.

Northern region. The soil used as background in the area is high in org. C and OM, moderately stocked with total nitrogen (0.169%) and mobile nitrogen, with a high C:N ratio. The reserve with mobile phosphorus is very good, and with mobile potassium – very strong. The soil on which the experiment is based has a very large supply of org. C and total nitrogen – 0.302%, high OM content and low C:N ratio. It is moderately stocked with mobile nitrogen, with a very high content of mobile phosphorus and mobile potassium.

Western region. The soil used as background is high in org. C, total nitrogen and OM. The C:N ratio is very low. The soil is insufficiently stocked with mobile nitrogen, very well stocked with mobile phosphorus and has a very strong stock of mobile potassium. The soil on which the experiment is based has a very high content of org. C, with a high content of total nitrogen – 0.221% and OM, medium availability of mobile nitrogen, very good availability of mobile phosphorus and very strong availability of mobile potassium. The C:N ratio is average.

Southern region. The soil used as background is very rich in org. C and OM. The total nitrogen content (0.256%) is high and the C:N ratio is medium. The soil is moderately stocked with mobile nitrogen, has a very high content of mobile phosphorus and mobile potassium. The soil on which the experiment is based is on average stocked with org. C, OM, total nitrogen (0.149%) and mobile nitrogen, very well stocked with mobile phosphorus and mobile potassium. The C:N ratio is low.

SUMMARY

The content of organic C and total nitrogen in the soils in which the buffer green areas were constructed varies from medium to very high, and that of organic matter (OM) from medium to high. The C:N ratio is low in some areas, medium in others, and high in others. In almost all areas where the experimental plots are set, the content of mobile nitrogen is average, and that of mobile phosphorus and mobile potassium is very high.

An analysis of the soil texture from the selected plots was made only in the first year of the study (2019), because due to its specifics, this parameter is not expected to change over time. All soils are high in sand and low in clay, which can potentially increase contaminant mobility. On the other hand, the high content of organic C, organic matter and mobile phosphorus creates relatively good opportunities for hindering the mobility of pollutants that have entered the soil.

5.3. Analysis of the content of selected chemical elements in the soils and the tested herbaceous species

In general, the Mn content of the studied urban soils ranged between 333 mg/kg and 813 mg/kg, Zn ranged between 49 mg/kg and 179 mg/kg, Ni ranged from 14 mg/kg to 44 mg/kg, while Cu and Pb varied in the range 16–55 mg/kg and 22–103 mg/kg, respectively (Fig. 3–5). The remaining potentially toxic elements also demonstrated significant variability in the studied urban soils as follows: Co ranged from 5.6 mg/kg to 11 mg/kg, Mo from 0.19 mg/kg to 0.92 mg/kg, As from 2 .8 mg/kg to 5.9 mg/kg, Cd from 0.21 mg/kg to 0.77 mg/kg and U from 1.7 mg/kg to 2.8 mg/kg. The statistical evaluation revealed that there are strong dependencies and synergism between the studied elements in the soils ($p < 0.05$).

In the plant biomass of the examined herbaceous species, the macroelement Ca (0.61–1.08%) has the highest content, followed by Fe (0.12–0.77%), and their amount is significantly higher in the aboveground organs (Fig. 6). Mn, Zn, V, Cr, U and Cd preferentially accumulate in underground organs, and their levels are higher in perennial wheat grasses (Fig. 7–8).

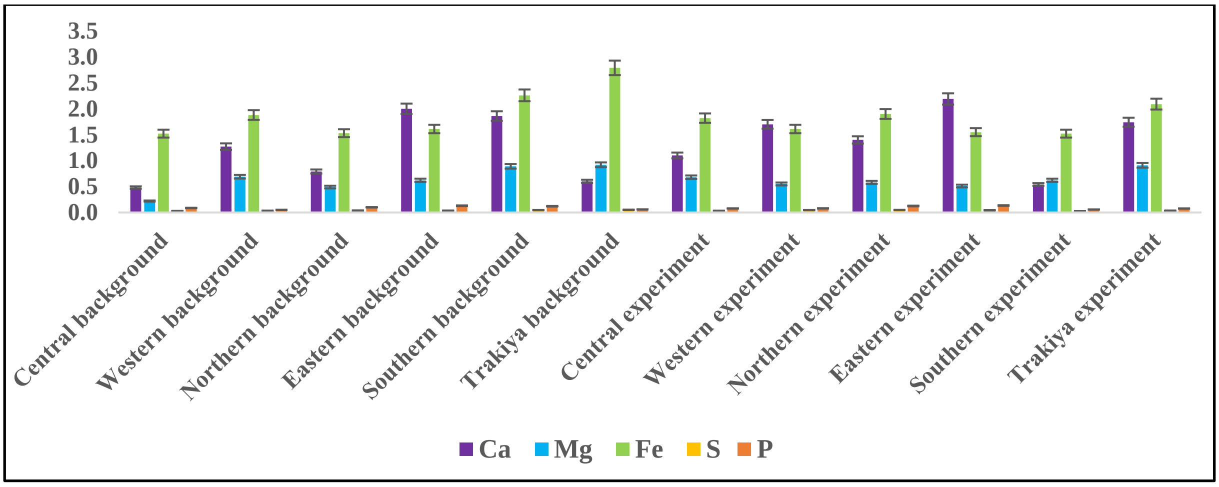

Fig. 3. Content of macroelements in studied soils, %

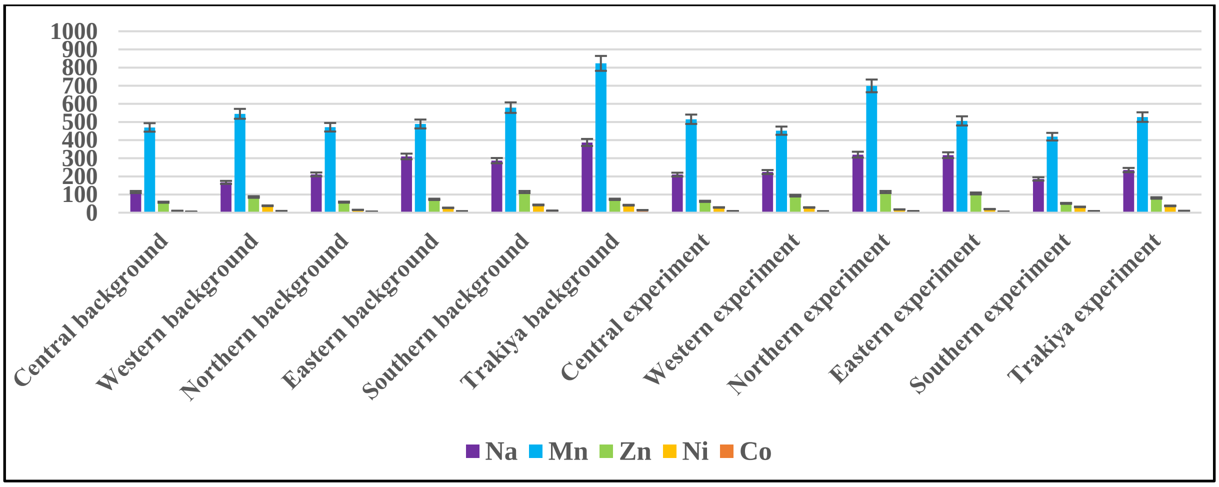

Fig. 4. Content of microelements in studied soils, mg/kg

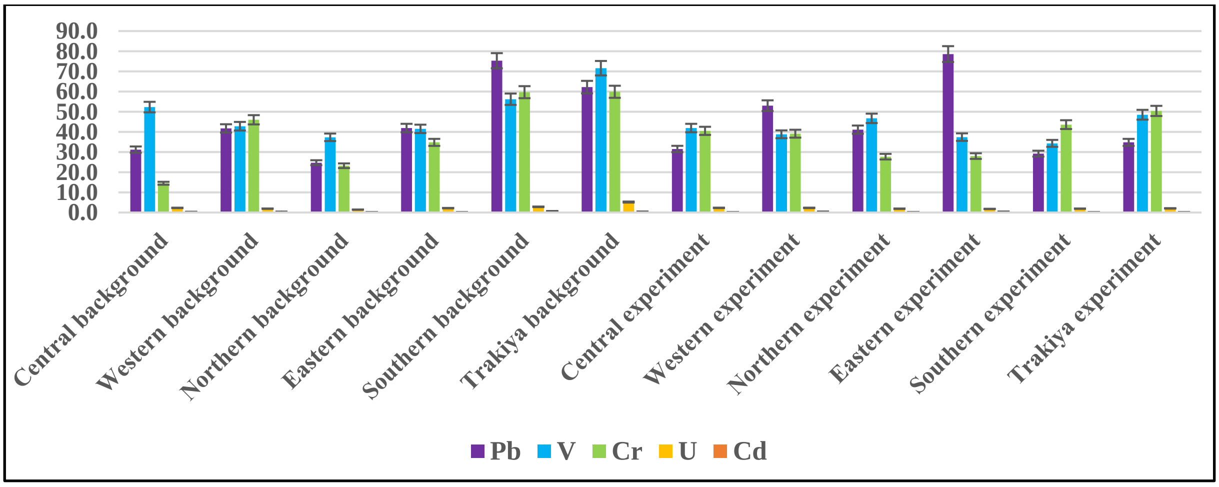

Fig. 5. Content of potentially toxic elements in studied soils, mg/kg

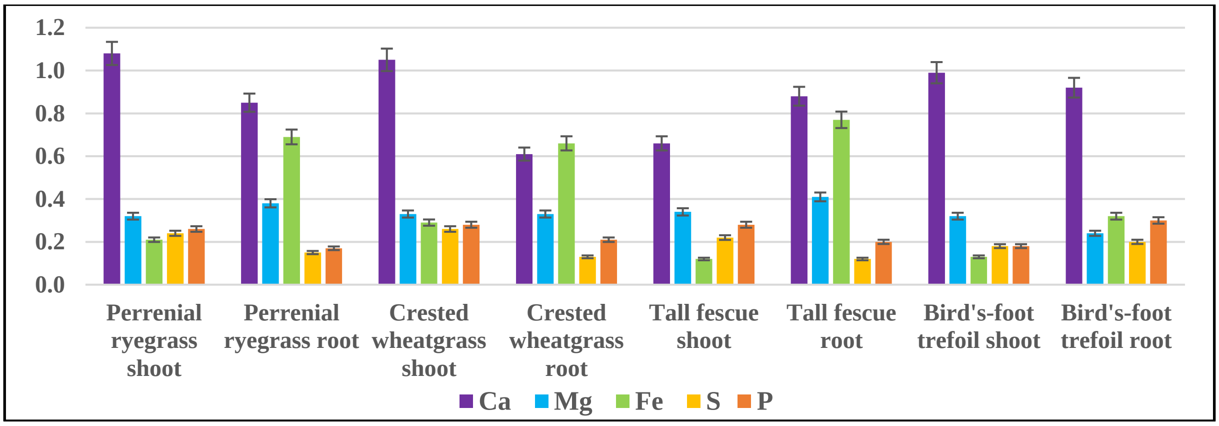

Fig. 6. Content of macroelements in plant biomass, %

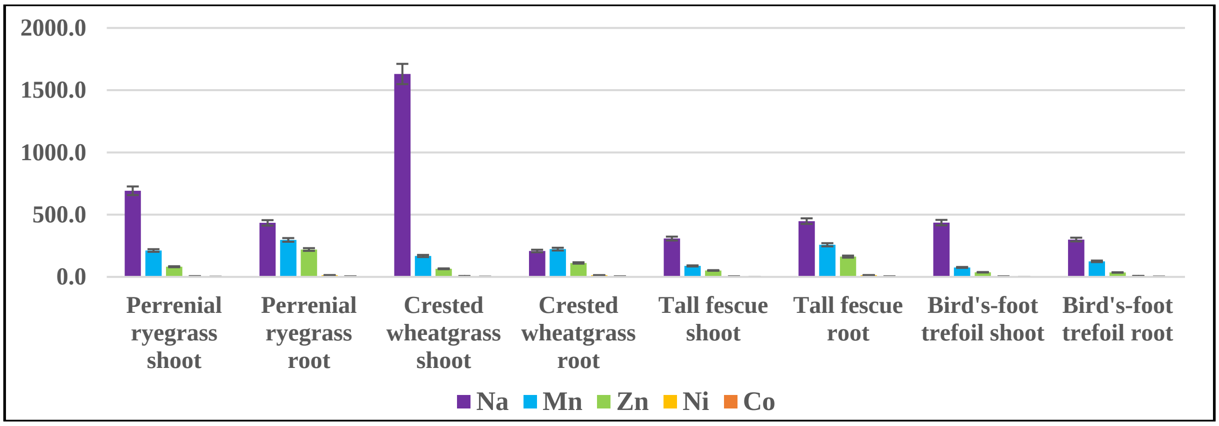

Fig. 7. Content of microelements in plant biomass, mg/kg

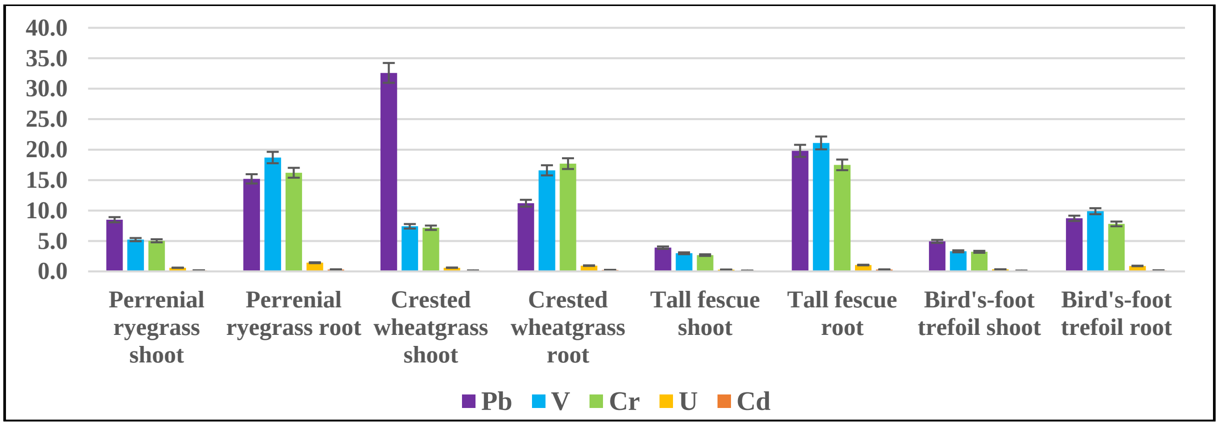

Fig. 8. Content of potentially toxic elements in plant biomass, mg/kg

Based on the average values of the content of potentially toxic elements in their underground and aboveground organs, the studied plant species can be arranged in the following descending order:

Mn – Crested wheatgrass > Tall fescue > Perrenial ryegrass > Bird's-foot trefoil

Zn – Tall fescue > Perrenial ryegrass > Crested wheatgrass > Bird's-foot trefoil

Ni – Crested wheatgrass > Tall fescue > Perrenial ryegrass > Bird's-foot trefoil

Cu – Tall fescue > Crested wheatgrass > Bird's-foot trefoil > Perrenial ryegrass

Pb – Tall fescue > Crested wheatgrass > Perrenial ryegrass > Bird's-foot trefoil

Co – Crested wheatgrass > Tall fescue > Perrenial ryegrass > Bird's-foot trefoil
Mo – Bird's-foot trefoil > Perrenial ryegrass > Tall fescue > Crested wheatgrass
As – Crested wheatgrass > Tall fescue > Perrenial ryegrass > Bird's-foot trefoil
Cd – Perrenial ryegrass = Crested wheatgrass = Tall fescue > Bird's-foot trefoil
U – Crested wheatgrass > Tall fescue > Bird's-foot trefoil > Perrenial ryegrass

SUMMARY

Regarding the content of potentially toxic elements, the status of the studied urban soils can be defined as satisfactory, i.e. at the moment, it does not pose a risk of disturbance of soil functions and dangers regarding the environment and human health. However, attention should be paid to elevated levels of lead, chromium, nickel, copper, etc., when compared to the target and intervention concentrations introduced in some European countries (VROM – Circular on target values and intervention values for soil remediation. Annex A, 2000)

5.4. Analysis of the properties and characteristics of the soil communities in the experimental plots

Analysis of the abundance of the main taxonomic groups

One of the widely used indicators for assessing the degree of soil loading with organic matter is the total number of heterotrophic microorganisms, determined by cultivation at 22°C temperature (TVC22). The data showed that the number of heterotrophic microorganisms was higher in all experimental variants compared to the background plots. The highest number was observed in Variant 5, which is a mixture of all herbaceous species – between 6×10^6 and 279×10^6 cfu.g^{-1}.

Another essential element of the soil microbiome are fungi, actively involved in the cycling of substances and energy in soil ecosystems (De Ruiter et al., 1993). In 2020 the data for the individual variants show that Variant 1 (Perrenial ryegrass) has a lower value of fungi ($15–69 \times 10^3$ cfu.g^{-1}) and the highest value is reported for Variant 3 (Tall fescue) and Variant 5 mixed crop (between 105×10^3 cfu.g^{-1} and 24×10^3 cfu.g^{-1}).

The analysis of the number of soil actinomycetes in the different experimental plots shows an increase compared to the background plots, with the most numerous being in Variant 2 (Crested wheatgrass) and Variant 3 (Tall fescue). Their number varies between 25×10^3 and 299×10^3 cfu.g^{-1}. The analysis of the experimental plots showed a dominance of fungi over actinomycetes by several orders of magnitude (between 2 and 20 times) compared to the background soils, which brings the experimental soils very close to the forest soils in this characteristic.

Activity of soil microbiota

Nitrogen mineralization is generally considered a key process in terrestrial ecosystems. The study conducted did not show significant differences in the nitrification potential of the communities both between the study plots and between the individual variants in the thus constructed experimental setting (Fig. 9A and Fig. 9B). The values found were an average concentration of 76 µg N-NO_3^-/g DW and 5 µg N-NO_2^-/g DW. Significant differences were reported only for 1h ($p=0.0488$) for the nitrite concentration in the variants with grassing. As time progresses, the values of the indicator remain high compared to the background levels in a park environment, but lacking the statistical significance ($p > 0.05$).

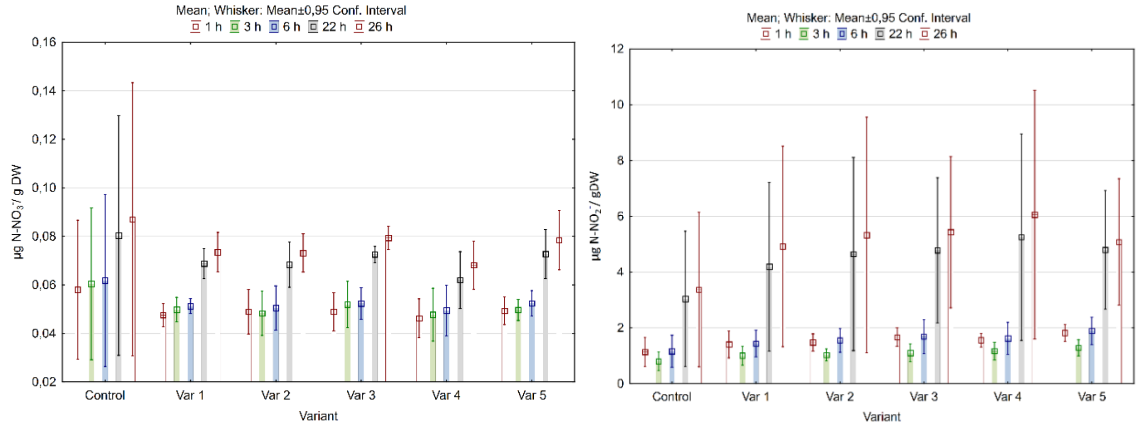

Fig. 9. Dynamics of the concentration of nitrates (A) and nitrites (B) in studied soils from different areas of the city of Plovdiv.

Legend: Control – city park; Var 1 – Perennal ryegrass (*Lolium perenne* L.), Var 2 –Crested wheatgrass (*Agropyron cristatum* L.), Var 3 – Tall fescue (*Festuca arundinacea* Schreb.), Var 4 – Bird's-foot trefoil (*Lotus corniculatus* L.), Var 5 – mixed crop.

The levels of soil respiration, dehydrogenase activity and microbial biomass values are characterized by significant spatial differences for the same types f grassing within the boundaries of the city of Plovdiv (Fig. 10), with average levels varying from 33% for soil biomass to 52% for dehydrogenase activity of microbial communities.

In particular, the soil respiration ranges from 3.47 µgCO_2.g^{-1}.day^{-1} to 6.61 µgCO_2.g^{-1}.day^{-1} with an average value of 4.68 µgCO_2.g^{-1}.day^{-1}. Dehydrogenase activity levels ranged from 1.49 до 7.25 µg INTF.g^{-1}.h^{-1} with a mean of 4.87 µg INTF.g^{-1}.h^{-1}. Both parameters are significantly lower than the values (36 µgCO_2.g^{-1}.day^{-1}), observed for natural grasslands and forests (Trasar-Cepeda et al., 2008; Gomez-Brandon et al., 2022), but they are in the lower range of agricultural lands (5.5–32.8 µgCO_2.g^{-1}.day^{-1}), which is indicative of low levels of biological activity in the studied soil samples from the city of Plovidv.

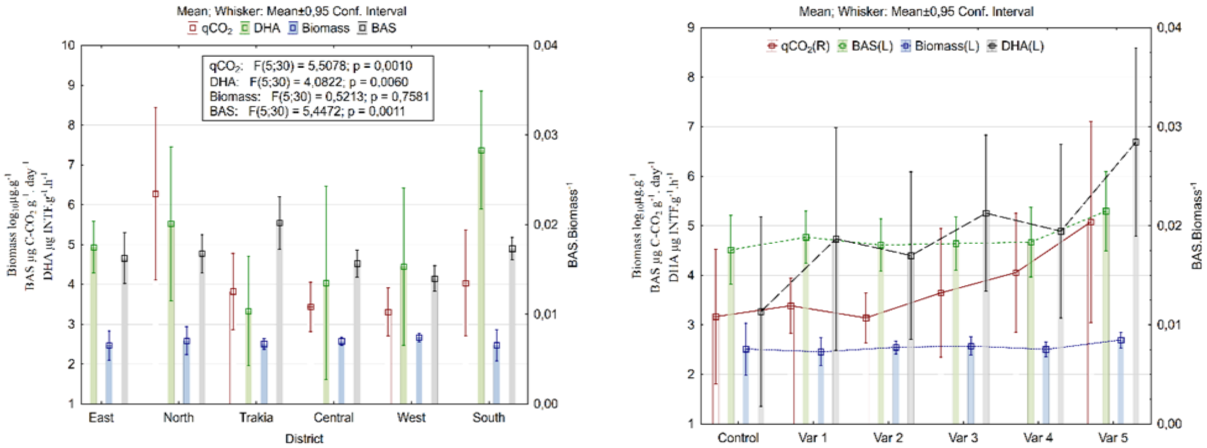

Fig. 10. Levels of soil respiration, dehydrogenase activity and microbial biomass by study area (A) and experimental variant (B).

For the studied period, the levels of total biological activity in the background soil samples differed reliably from the experimental variants ($p < 0.05$). Cluster analysis generated on the basis of a correlation matrix of the total biological activity confirmed the significant spatial variations (Fig. 11A), clearly separating the control areas and Var 5 into independent clusters, while all other variants showed no significant differences (Fig. 11B).

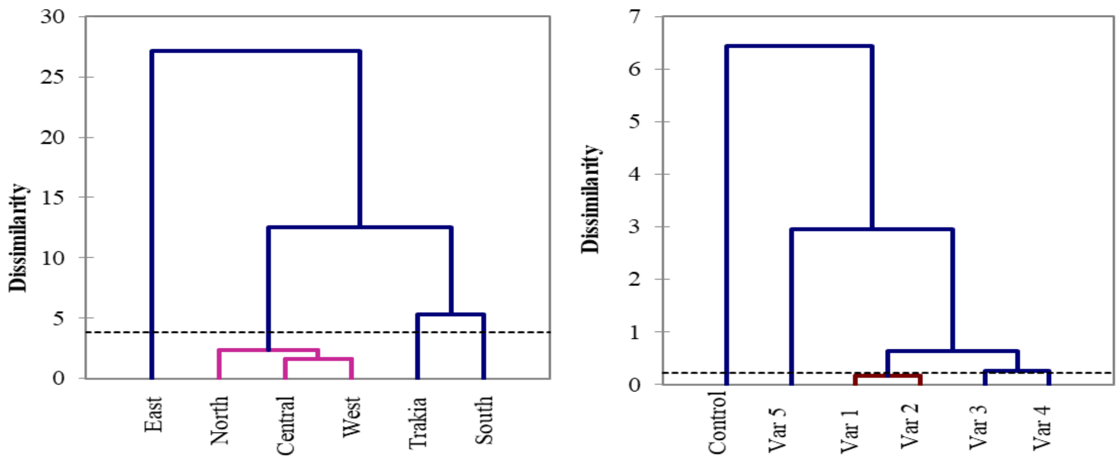

Fig. 11. Agglomerative hierarchical cluster analysis reflecting the similarity (squared Euclidean distances) between communities based on the biological activity of the microbiome by areas (A) and variants (B) of study.

Metabolism of soil communities

The Community Level Physiological Profile (CLPP) was prepared based on the metabolic activity of the communities and their ability to assimilate specific carbon sources (Gomez et al., 2004). The obtained kinetic curves reveal differences in the rate of uptake of substrates between the experimental variants. The geometric mean threshold value $AWCD_{0.8}$ for the individual variants selected as the baseline for the statistical analyzes of physiological profiles according to Garland et al. (2001), is reached within 72 h (for Var 1, Var 3, Var 4 and Var 5), up to 120 h (for the background samples and Var 2). The levels of assimilation by classes of substrates by the soil microbial communities in the different variants of grassing are presented in Fig. 12.

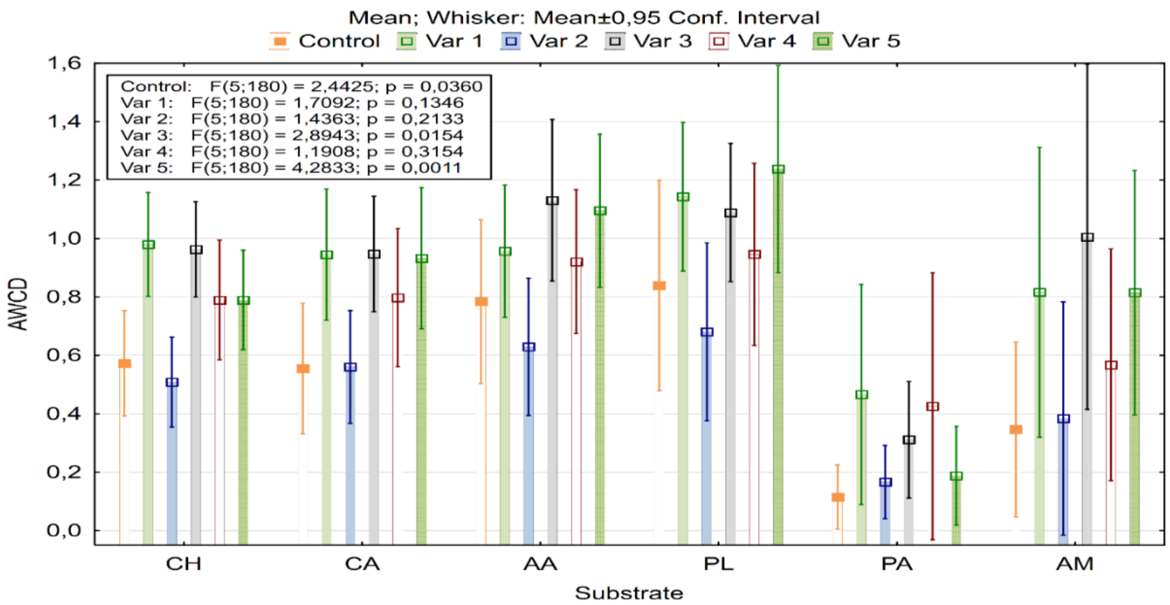

Fig. 12. Level of assimilation of the analyzed carbon sources, expressed as a percentage of the total metabolic activity, by substrate categories: amines (AM); amino acids (AA); phenolic acids (PA); carboxylic acids (CA); carbohydrates (CH); polyhydric alcohols (PL).

Cluster analysis demonstrated that the rate of assimilation of carbon sources was habitat determined rather than biochemically determined (Fig. 13A). Three main clusters were differentiated in the variant analysis (Fig. 13B). The first cluster includes soil samples with low overall physiological activity and low uptake levels of the tested substrates, pointing out the Var 2 (Crested wheatgrass *Agropyron cristaum* L.) as the least suitable for bioremediation under the tested conditions. The second cluster includes only the mixed crop variant (Var 5) and is close in activity and metabolic profile to the third cluster (Var 1, Var 3 and Var 4). The results confirm that with combined sowing, the three species (Perennial ryegrass (Lolium perenne L.), Tall fescue (*Festuca arundinacea* Schreb.) and Bird's-foot trefoil (*Lotus corniculatus* L.) manage to create suitable conditions for increasing microbial biodiversity.

The PERMANOVA-generated design indicated that a substantial part of the variation in metabolic profile was the result of spatial differences (p(perm) < 0.001), with high levels of variation not allowing to reliably confirm the reported differences between individual grassing variants (p(perm) = 0.436).

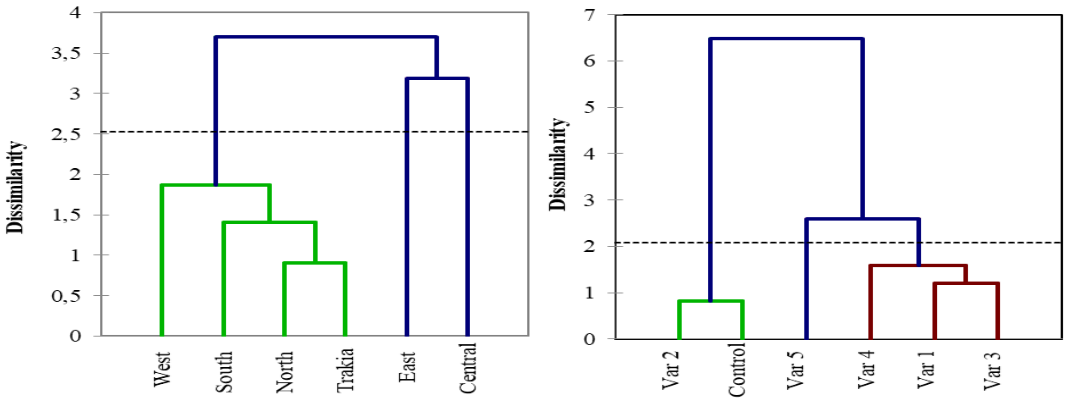

Fig. 13. Cluster analysis reflecting the grouping of the analyzed samples according to the levels of assimilation of carbon sources by zones (A) and variants (B) of study spatial differences (p(perm) < 0.001), the high levels of variation not allowing to reliably confirm the reported differences between individual weeding variants (p(perm) = 0.436).

Metagenomic analysis

In continuation of analyzes to test the hypothesis that tillage and planting of grass species directly affects the soil microbiome in relation to soil "health" and increases plant-relevant bacterial species, two-fold massively parallel sequencing of the hypervariable region V4-V5 of the 16S rRNA gene on an Illumina PE250 platform. Individual sequences were obtained that were reduced by 3% after removal of low-quality sequences. After a filtering step, they were assigned to 3612 operational taxonomic units (OTUs). The assigned OTUs were grouped into a total of 853 taxa at the genus level. As a result of the analyses, the obtained OTUs could be assigned to a total of 44 taxa at the level of Division, Class – 124 taxa, Family – 476 taxa and Genus or genus candidate – 1708 taxa.

The total number of OTUs obtained was used to calculate alpha-diversity (within-community) and beta-diversity (between-community diversity) indices. The comparison of the microbial communities using PERMANOVA indicates an identical structure of the dominant complex and the minor taxa in the soils of all the studied areas of the city of Plovdiv (p(perm) = 0.197). Taxonomic composition is relatively constant across variants (p(perm) = 0.602). This indicates that the differences between individual samples are rather random in nature and due to a change in the numerical composition of individual taxa than to extinction or emergence of new species. The similarity in the structure of the communities is also confirmed by the conducted principle component analysis.

The dominant complex in the artificially grassed variants was formed by only 3.3% of the total number of identified taxa and included 36 bacterial genera represented by > 1% (Fig. 14). They form an average of about 42% of the total abundance and are present both in all planting options and in the urban park samples. The qualitative analysis of the presented species does not show significant differences between the communities in the studied soil samples $p > 0.05$).

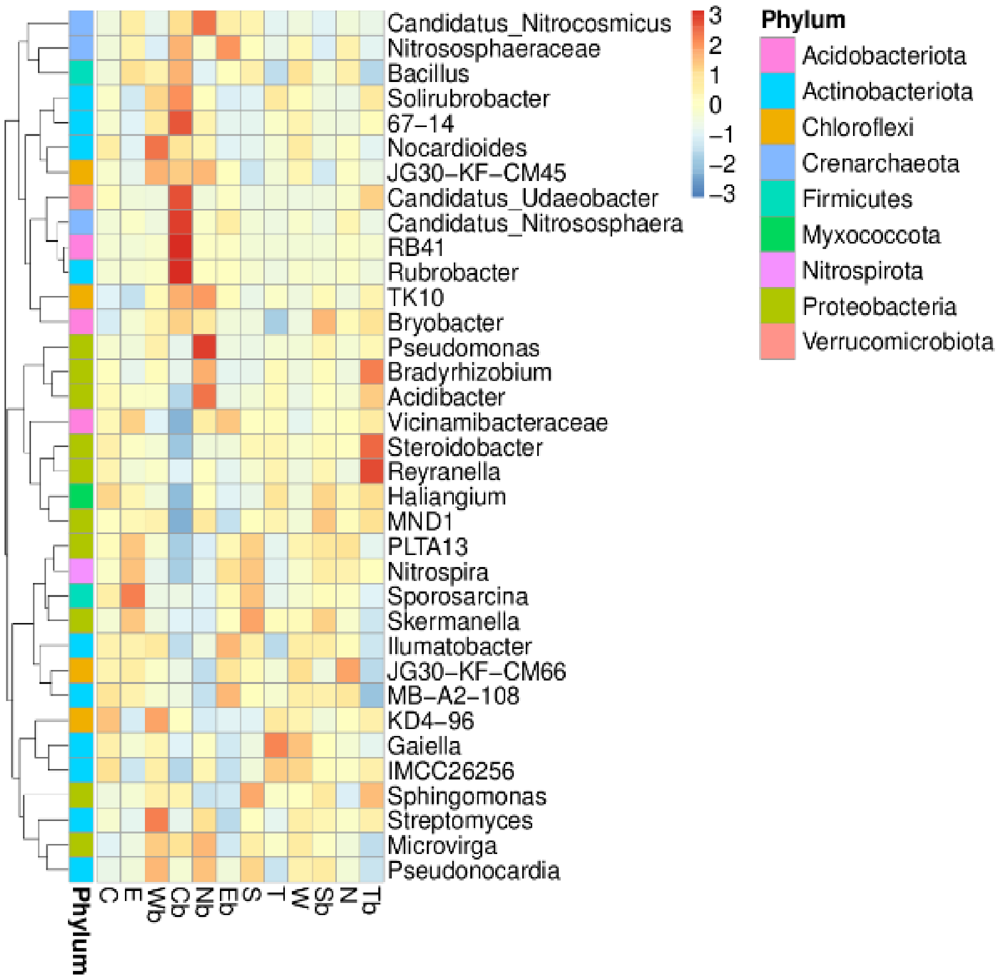

Fig. 14. Heatmap – analysis of bacterial communities at the level of genus (genera) constituting > 1% of the total count

SUMMARY

Data on the taxonomic composition and physiological activity of the microbiome in the urbanized soils from the city of Plovdiv are presented for the first time in the country, taking into account the possibility of modeling the microbial communities by changing the purpose of the territories and building new green areas. The study is focused on the surface soil layer, as this zone is most often subject to anthropogenic impact. The assessment of diversity indices was performed under similar conditions for all variants, which is key in analyzing the effect of spatial variation and landscaping activities performed (Chen et al., 2017). The results revealed high levels of diversity indices, although the study of physiological profiles (CLPP) and next-generation sequencing did not show statistically significant seasonal differences in the structure and functions of the soil microbiome. This is also confirmed by the multivariate analyses. A tendency to maintain the levels of diversity indices in the newly designated buffer green areas is reported, and the lack of reliability of the results may be due to the short period of the study.

5.5. Analysis of the bioremediation potential of the tested herbaceous species

Assessment of bioaccumulation potential

The phytoextraction process usually requires the movement of heavy metals from the soil into the roots and from the roots to easily harvested plant parts (stems and leaves). By comparing BAF and TF, we can compare the ability of different plants to take up chemical elements from soils and transport them to aboveground plant organs. It should be noted that the accumulation factors for all investigated potentially toxic elements were significantly higher for roots than for shoots, with the exception of molybdenum. The highest BAF (2.16-15.69) and TF (0.69-1.42) values were calculated for this element.

The bioaccumulation potential of each of the four tested herbaceous species is presented on Figs. 15–18, with respect to the chemical elements that were found to be present in higher amounts in urban soils.

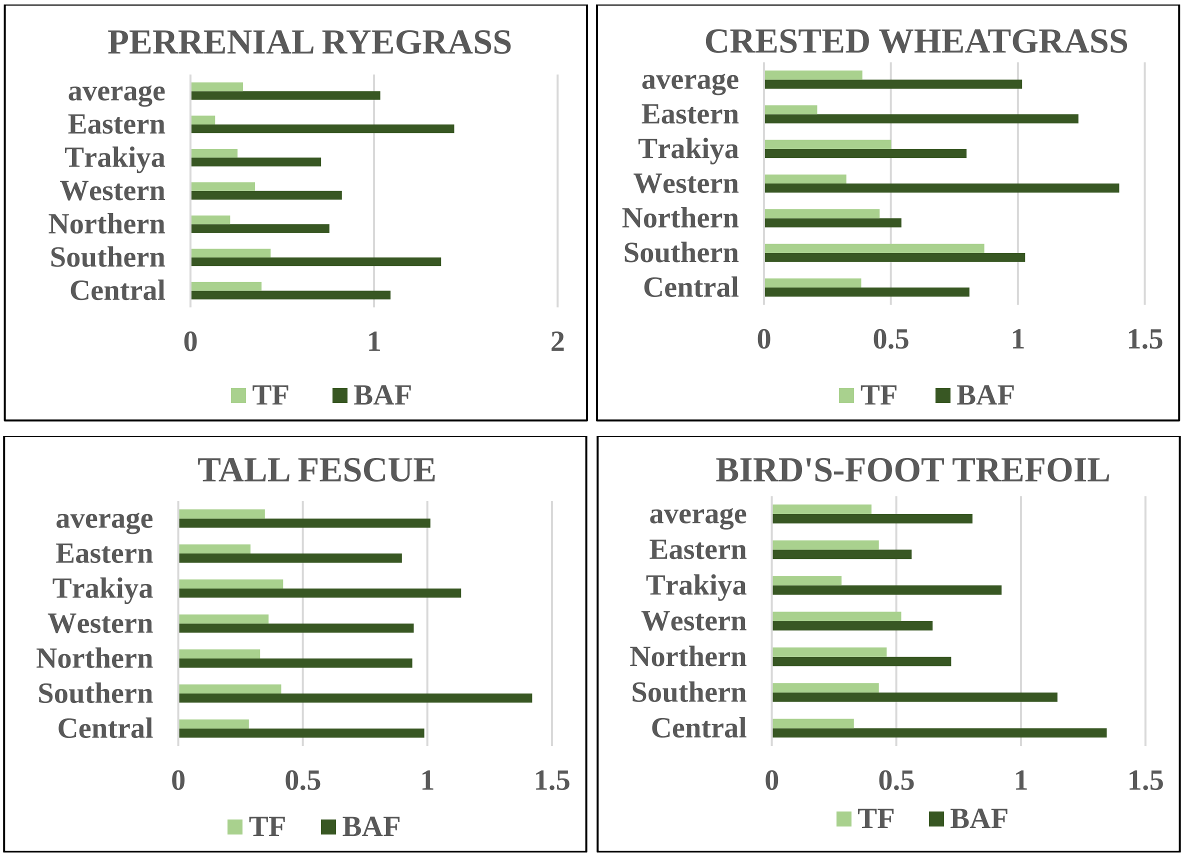

Fig. 15. Bioaccumulative potential for lead

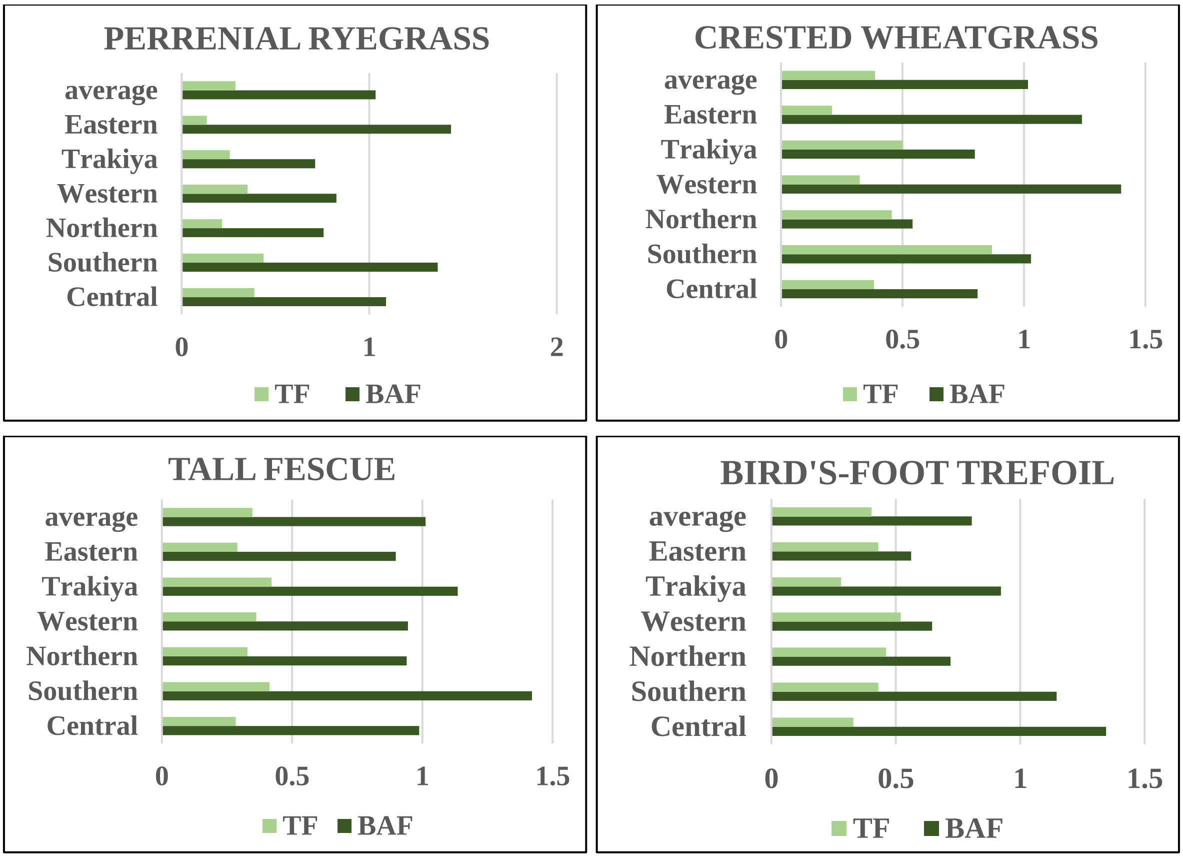

Fig. 16. Bioaccumulative potential for zinc

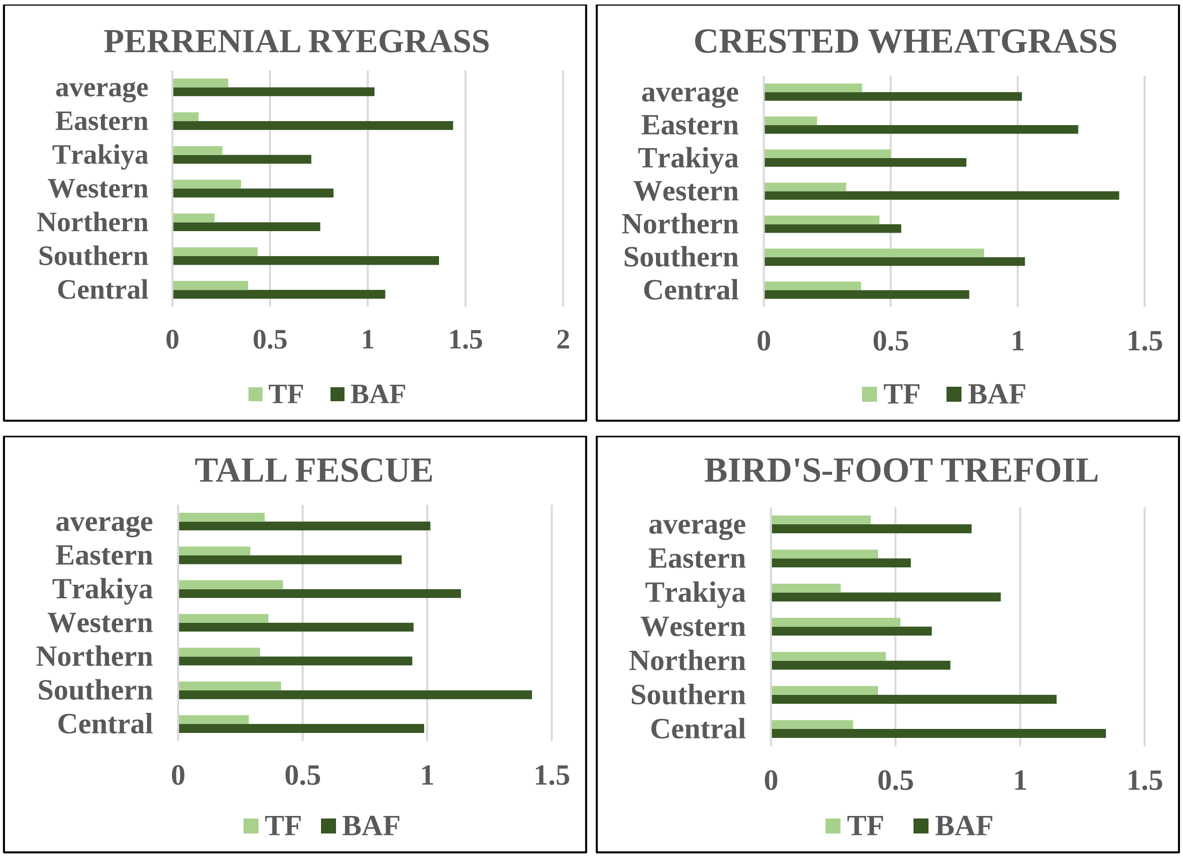

Fig. 17. Bioaccumulative potential for copper

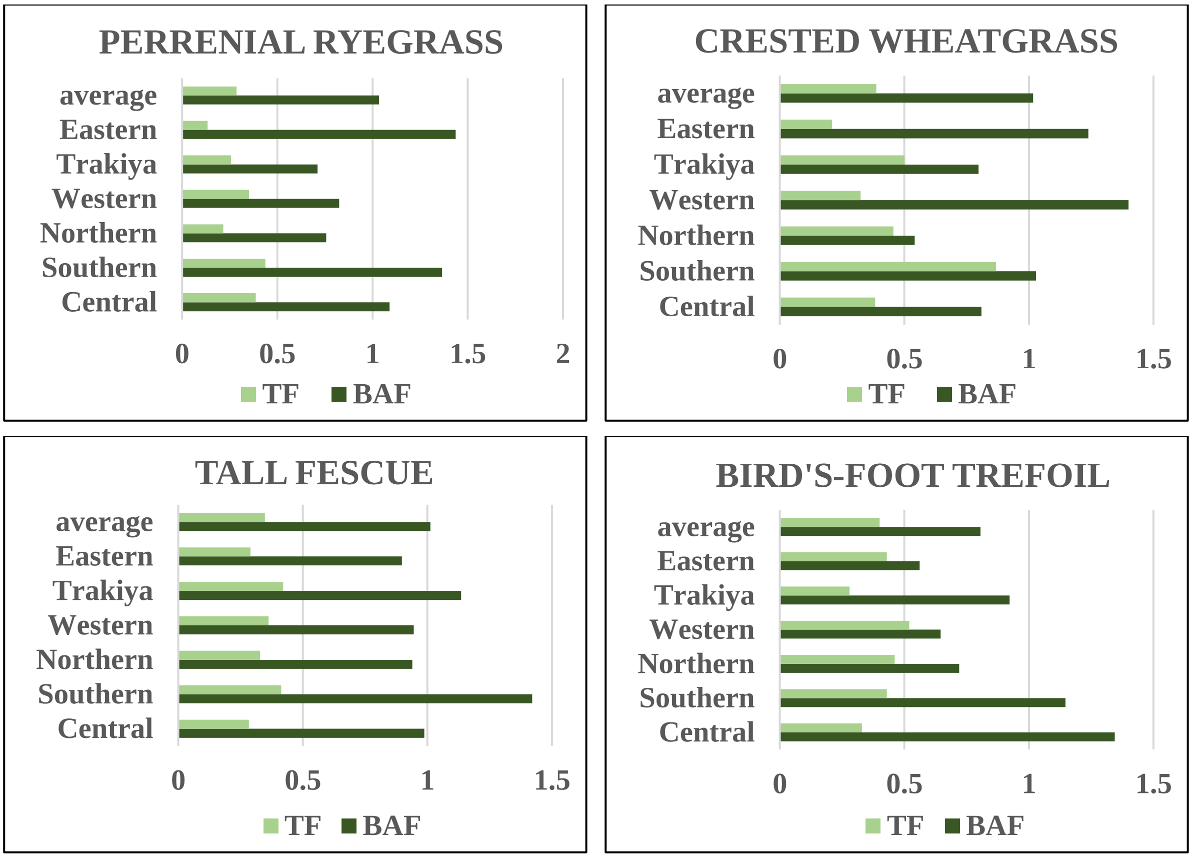

Fig. 18. Bioaccumulative potential for cadmium

According to the scale used to evaluate the bioaccumulation capacity of the studied plant species, the results show that Perennial ryegrass (*Lolium perenne* L.) variety "IFK Harmony" has a strong bioremediation potential for Mo (5.46), Zn (1, 81), Cd (1.03) and average potential for bioaccumulation for the remaining potentially toxic elements. Furthermore, the BAF valus for U (0.88), Cu (0.71) and Mn (0.70) are quite promising for its application in the construction of buffer green areas. Crested wheatgrass (*Agropyron cristatum* L.) variety "Svezhina" has a high bioaccumulative potential for Mo (2.16), Zn (1.56), U (1.05), Cd (1.02) and an average potential for the other PTEs studied, although the BAF values for Mn (0.82) and Cu (0.81) were quite elevated. Tall fescue (*Festuca arundinacea* Schreb) variety "Albena" demonstrated strong bioaccumulation of Mo (4.72), Zn (2.05), Cd (1.01) and promising potential for U (0.98), Cu (0.86) and Mn (0.74). Bird's-foot trefoil (*Lotus corniculatus* L.) variety "Leo" is the leader in accumulation of Mo (15.69) and U (1.05), but not as pronounced as a bioaccumulator of the other PTEs. The BAF values for Cd (0.81), Cu (0.75) and Zn (0.74) are promising.

Data from the mixed cropping of these four plant species indicate that co-planting can improve bioaccumulation potential compared to monoculture. The highest BAF values of Zn (2.32), Cd (1.19), Mn (0.89), Cu (0.92), Co (0.65) and Ni (0.63) were obtained at mixed cropping, being statistically significant for Zn, Cu, Co and Cd ($p < 0.05$). This fact clearly demonstrates that a higher phytocenosis biodiversity can not only increase the biodiversity of soil, but also has a positive influence on its properties.

Assessment of photosynthetic efficiency and plant status in buffer green areas around transport arteries

In general, we found a higher content of total chlorophyll and carotenoids in Perennial ryegrass, followed by Crested wheatgrass and Tall fescue, while their values were significantly lower in the Bird's-foot trefoil. Regarding the influence of the region on these parameters, it can be noted that higher values are reported in the Central, Northern and Southern regions of the city of Plovdiv. The ratio chl a/chl b has similar values in the studied herbaceous species and into the different regions, with the exception of the values for Perennial ryegrass in the Northern region, Tall fescue and bird's-foot trefoil in the Central region.

Dynamics of enzyme activity in underground and aboveground plant biomass from the constructed buffer green areas

The results obtained show that the activity of catalase, in general, is the highest in the studied perennial grasses used (Tall fescue and Crested wheatgrass), followed by Perennial ryegrass, and in Bird's-foot trefoil (perennial legume) the activity of catalase is significantly lower – about 1.5–2 times. A probable reason for the inhibition of catalase activity observed in plants from individual experimental plots is the stronger anthropogenic load causing oxidative modification of proteins (Willekens et al., 2007).

With regard to the two studied enzymes of the glutathione cycle, it can be stated that, in general, the highest values for glutathione peroxidase activity were observed in Tall fescue and Bird's-foot trefoil, and with regard to glutathione reductase – in Perennial ryegrass and Crested wheatgrass. Glutathione is a widely used marker of oxidative stress in plants (Tauzs et al., 2004), and elevated levels have been measured under various stresses (Leipner et al., 1999; Koscy et al., 2001).

In the course of the present study, elevated levels of free proline were observed in plants from the constructed buffer green areas, which correlates with the literature data of a drastic increase in its level under various stress impacts (Saradhi et al., 1995; Alia et al., 2001). In the context of the experimental setup and the results of the complex analyzes of soils and biota, the high concentrations of free proline can be interpreted as a common, non-specific protective response of plants to the development of oxidative processes in their tissues due to the deteriorated ecological conditions in urban soils.

SUMMARY

The investigated perennial cereal grasses (Perennial ryegrass, Crested wheatgrass and Tall fescue) have a higher bioaccumulation coefficient compared to Bird's-foot trefoil (leguminous perrenial grass) in terms of lead, zinc and cadmium. Higher Translocation Factor of accumulated lead and zinc in the direction from the root system to the aerial organs is found fo Bird's-foot trefoil when compared to the other three species. Regarding their capacity for bioaccumulation and phytoremediation, the four studied herbaceous plant species can be arranged into the following descending order: Tall fescues > Crested wheatgrass > Perennial ryegrass > Bird's-foot trefoil.

The analysis of the adaptation processes in four herbaceous species, induced by their planting in experimental plots on the territory of the city of Plovdiv, characterized by a high degree of anthropogenic impact (along busy road arteries), is based on the changes in the enzymatic and non-enzymatic components of the antioxidant defense system in plants in relation to the content of potentially toxic elements in the soil.

The four studied species (Perennial ryegrass, Crested wheatgrass, Tall fescue, Bird's-foot trefoil) are in relatively good condition and show good adaptation reactions to the impact of the urban environment. The monitored parameters, directly related to the physiological status, show that, compared to the other studied species, the Tall fescue stands out as the most vital and physiologically active, which implies greater resistance and adaptability.

The higher levels of free proline found in Bird's-foot trefoil, in general, define it as more vulnerable to adverse environmental conditions, compared to the other studied species. However, in a mixed crop it developed successfully and did not show such high levels of oxidative stress.

We found that when the catalase activity is inhibited, the activities of glutathione peroxidase and glutathione reductase increase, probably as a compensatory mechanism in the process of neutralizing the highly increased concentration of hydrogen peroxide ($p < 0.05$).

The observed changes in the antioxidant defense system of the four herbaceous species are due to the combination of anthropogenic impacts in the urbanized environment and seasonal changes in the values of environmental factors. In order to optimally continue the vegetation in plants, a continuous process of adaptation is established by activating different signaling pathways in the cells, the exact mechanism of which remains still unclear. In general, experiments show that plants react in a specific way and have differentiated defense mechanisms according to the type of external stressor.

5.6. Analysis of the possibilities for sustainable management of urban soils through buffer green areas

To identify and rank the factors determining the behavior of the studied elements in the different soil samples and the relationships between them, a multifactorial statistical analysis was applied – Principal Component Analysis (PCA), containing six factors: 1) "Site type" with categories "background/control" and "experiment"; 2) "Physico-chemical parameters of soils" with "pH" and "EC" categories; 3) "Origin of elements" with categories "lithogenic (background level)" and "anthropogenic"; 4) "Location" with categories of the 6 administrative districts of the city of Plovdiv; 5) "Wind Rose"; 6) "Other anthropogenic activities".

In Fig. 19, results are presented regarding the factors influencing the content of potentially toxic elements in the studied soils from the 12 plots in the city of Plovdiv in 2019. The most significant of these we found to be "Site type" – control or experimental (Factor 1 = 46.36%), followed by "Physico-chemical parameters of the soils" (Factor 2 = 35.16%).

"Origin of the elements" (Factor 3 = 7.92%) and "Location" (Factor 4 = 4.56%) have an extremely weak impact on the distribution of the investigated potentially toxic elements in the soils.

The remaining two factors – "Wind rose" (Factor 5 = 2.61%) and "Other anthropogenic activities" (Factor 6 = 1.90%) have negligible low values.

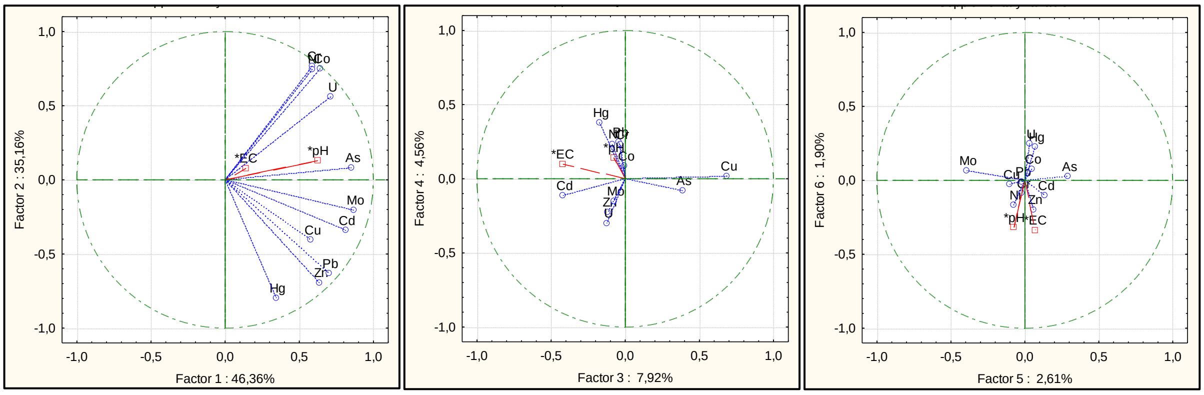

Fig. 19. Principal component analysis (MCA) of the factors affecting the distribution of potentially toxic elements in the studied soils in 2019.

Fig. 20 presents the results regarding the factors affecting the content of potentially toxic elements in the studied soils from the 12 plots in the city of Plovdiv in 2022. The most significant of these we found to be "Site Type" – control or experimental (Factor 1 = 54.54%), followed by "Physico-chemical parameters of the soils" (Factor 2 = 7.90%), but with much more little weight. This result clearly indicates that the constructed buffer green areas in the experimental plots effectively reduce the content of potentially toxic elements in the soils.

When comparing the weight of these two factors in 2019 and in 2022 there is an increase in the importance of the first factor, which shows that in the long term the effect of the buffer green areas is increasing.

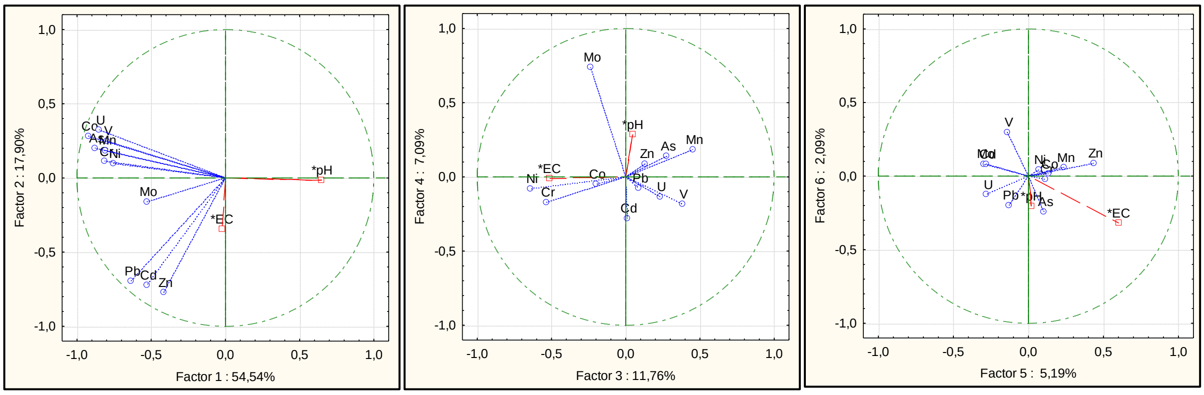

Fig. 20. Principal component analysis (MCA) of the factors affecting the distribution of potentially toxic elements in the studied soils in 2022.

The established differences in the bioaccumulation abilities of mobile forms of heavy metals of the perennial cereal grasses included in the study, Crested wheatgrass (*Agropyron cristatum* L.) variety "Svezhina", Perennial ryegrass (*Lolium perenne* L.) variety "IFK Harmony", Tall fescue (*Festuca arundinacea* Schreb) variety "Albena" and the perennial leguminous grass – Bird's-foot trefoil (*Lotus corniculatus* L.) variety "Leo", grown both independently and in a mixed grass stand, determine a differentiated approach when choosing them for grassing urban areas, depending on the type and share in pollution with various heavy metals in the soil. In case of main accumulation of Pb, Zn and Cd in the surface soil layer, it is recommended for their bioaccumulation to use the monocultural cultivation of Perennial ryegrass (*Lolium perenne* L.) variety "IFK Harmony" and Tall fescue (*Festuca arundinacea* Schreb) variety "Albena" in the construction of urban lawns. In case of pollution of the surface soil layer with mobile forms of the heavy metals Mo, Zn, U and Cu, promising crops with the ability to bioaccumulate them are Crested wheatgrass (*Agropyron cristatum* L.) variety "Svezhina" and Bird's-foot trefoil (*Lotus corniculatus* L.) variety "Leo", grown as monoculture.

Model validation for sustainable urban soil management

Based on the results of all studies, a model for sustainable management of urban soils by construction of buffer green areas around transport arteries has been developed and validated. It includes 5 steps, visualized in Fig. 21.

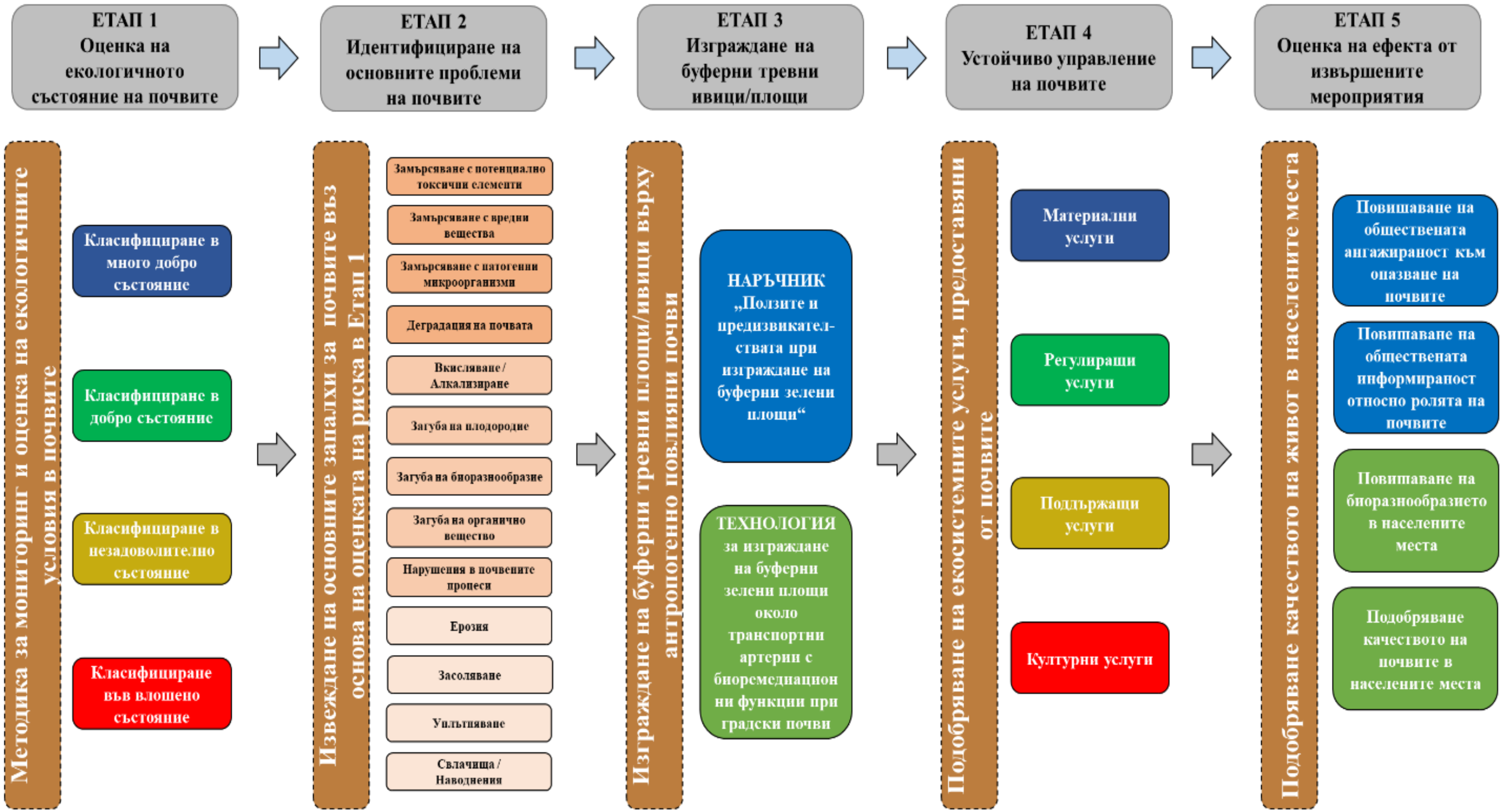

Fig. 21. Model for sustainable management of urban soils through buffer green areas

SUMMARY

The factor analysis proved that the type of plot (control or experimental), followed by its location (administrative region) plays a determining role for the content and distribution of elements in urban soils. This highlights the applicability of buffer green areas with an appropriately selected species composition for sustainable management of soils in urban areas.

Due to the complex contamination of the surface soil layer with various mobile forms of heavy metals (Zn, Cd, Mn, Cu, Co, Ni), it is recommended to create mixed grass stands with the participation of perennial cereal and leguminous grasses. On the one hand, this will improve their bioaccumulation potential compared to the monoculture cultivation, and on the other hand, the increased biodiversity in the phytocenosis will increase the biodiversity of animals and microorganisms. The mixed cropping could enhance the water and physical properties of the soil, as well as to protect it from wind and water erosion during the construction of the buffer green areas on sloping terrains.

6. CONCLUSIONS

1. The four studied species (Crested wheatgrass (*Agropyron cristatum* L.) variety “Svezhina”, Perennial ryegrass (*Lolium perenne* L.) variety “IFK Harmony”, Tall fescue (*Festuca arundinacea* Schreb) variety “Albena” and Bird’s-foot trefoil (*Lotus corniculatus* L .) variety “Leo”) show good adaptive responses to the impact of the urban environment. The Tall fescue stands out as the most vital and physiologically active, which implies greater resistance and adaptability.

2. The observed changes in the antioxidant defense system of the four herbaceous species are due to the combination between anthropogenic impacts in the urbanized environment and seasonal changes in the values of environmental factors. In general, experiments show that plants react in a specific way and have differentiated defense mechanisms according to the type of external stressor.

3. The constructed buffer green areas in the experimental plots have the ability to extract potentially toxic elements from the soils, and in the long-term perspective their effect increases.

4. It has been proven that the constructed buffer green areas near the main transport arteries in the city of Plovdiv effectively influence the content and migration of potentially toxic elements in soils ($p<0.05$).

5. The studied perennial cereal grasses (Perennial ryegrass, Crested wheatgrass and Tall fescue) have a higher bioaccumulation coefficient compared to Bird’s-foot trefoil (leguminous grass) in terms of lead, zinc and cadmium. The combined crop of the four species – Perennial ryegrass (*Lolium perenne* L.), Crested wheatgrass (*Agropyron cristatum* L.), Tall fescue (*Festuca arundinacea* Schreb.) and Bird’s-foot trefoil (*Lotus corniculatus* L.) managed to create suitable conditions for increasing microbial diversity and the bioremediation effect.

6. The data showed that the number of major groups of soil microorganisms was higher in all experimental variants compared to the background plots. In this regard, we recommend the combined planting of different herbaceous species in the construction of buffer green areas, as together they will form a better rhizosphere microbiome compared to single-seeded species (monocultures).

7. Urbanization has a significant impact on the ecological state and biological processes in the soils of the city of Plovdiv. Quantitative indices of microbial biomass and their ratios are important biomarkers of urbanized soils and can be used as criteria for their ecological assessment. As a result of the complex studies, urban areas with more strongly influenced soil characteristics and altered properties of the microbial communities were highlighted.

8. The developed and validated in the conditions of the city of Plovdiv model for sustainable management of urban soils by buffer green areas can be successfully applied to improve the quality of soils in other populated areas.

7. CONTRIBUTIONS

Contributions of an original scientific nature

1. Original scientific data were obtained on the elemental composition of the studied urban soil samples as well as of the underground and aboveground plant biomass from the constructed buffer green areas around transport arteries in order to evaluate the effect of bioremediation.

2. Buffer green areas around the transport arteries with selected perennial leguminous and cereals herbaceous species with proven tolerance to the conditions of the urban environment and high bioaccumulation capabilities have been constructed for the first time in the city of Plovdiv.

Contributions of a scientific and applied nature

1. The technology for construction of buffer green areas around transport arteries with bioremediation functions for urban soils has been validated in the city of Plovdiv by studying stress responses to assess their physiological state.

2. A model for sustainable management of urban soils through buffer green areas around transport arteries has been approved, leading to long-term improvement of soil quality and living conditions in the city.

The developed model for sustainable management of urban soils will be provided free of charge to the interested state and municipal structures to serve as a scientific basis for the development of regulatory documents at the regional and national level and for undertaking effective management decisions.

8. PUBLICATIONS AND PARTICIPATION IN SCIENTIFIC FORUMS RELATED TO THE PHD THESIS

List of publications

1) Petrova S., Nikolov B., Velcheva I., Angelov N., Valcheva E., Katova A., Golubinova I., Marinov-Serafimov P. (2022). Buffer Green Patches around Urban Road Network as a Tool for Sustainable Soil Management. Land, 11, 343, 1-23. https://doi.org/10.3390/land11030343

2) Petrova S., Velcheva I., Nikolov B., Angelov N., Hristozova G., Zaprjanova P., Valcheva E., Golubinova I., Marinov-Serafimov P., Petrov P., Stefanova V., Varbanova E., Georgieva D., Stefanova V., Marhova M., Tsankova M., Iliev I. (2022). Nature-Based Solutions for the Sustainable Management of Urban Soils and Quality of Life Improvements. Land, 11, 569, 1-22. https://doi.org/10.3390/land11040569

List of conferences

1) Angelov, A., Nikolov, B., Petrova, S., Velcheva, I., Zheleva, E., Petrov, P., Stefanova, V., Zapryanova, P., Hristeva, G., Valcheva, E. (2020). Geochemical spectra of some chemical elements in urban soils. Twelfth scientific conference for students and young scientists "Ecology – a way of thinking" 12, November 1, 2020, Plovdiv

2) Angelov, A., Valcheva, E., Velcheva, I., Petrova, S., Nikolov, B. (2021). Bioaccumulative potential of some herbaceous species. Thirteenth scientific conference for students and young scientists "Ecology – a way of thinking" 13, October 9, 2021, Plovdiv.

3) Nikolov, B., Petrova, S., Velcheva, I., Angelov, N., Valcheva, E., Zapryanova, P., Hristozova, G., Varbanova, E., Georgieva, D., Stefanova, V. (2020). Accumulation and mobile forms of some heavy metals in roadside urban soils. CBU International Conference on Innovations in Science and Education, 23-25 March 2020, Prague, Czech Republic.

4) Nikolov, B., Petrova, S., Velcheva, I., Angelov, N., Valcheva, E., Zapryanova, P., Hristozova, G., Varbanova, E., Georgieva, D., Stefanova, V. (2020). Macro- and microelements content of urban soils from Plovdiv (Bulgaria). Agriculture for Life, Life for Agriculture, 4-6 June 2020, Bucharest, Romania.

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7) Azimi, R., Heshmati, G., Farzam, M., Goldani, M. (2019). Effects of mycorrhiza, zeolite and superabsorbent on growth and primary establishment of *Agropyron desertorum* in mining field (Case Study: Mashhad′s Shargh Cement Factory, Iran. J. Rangel. Sci., 9, 172–183.

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