Effect of the edaphic factors and metal content in soil on the diversity of *Trichoderma* spp.

Gordana Racić[a*], Péter Körmöczi[b], László Kredics[b], Vera Raičević[c], Beba Mutavdžić[d], Miroslav M. Vrvić[e] and Dejana Panković[a]

[a]Faculty of Environmental Protection, Educons University, Sremska Kamenica, Serbia

[b]Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

[c]Faculty of Agriculture, University of Belgrade, Belgrade, Serbia

[d]Faculty of Agriculture, University of Novi Sad, Novi Sad, Serbia

[e]Faculty of Chemistry, University of Belgrade, Belgrade, Serbia

[*]Correspondence to Gordana Racić, Faculty of Environmental Protection, Educons University, Vojvode Putnika 87, 21208 Sremska Kamenica, Serbia

Telephone: +381214893656

Fax number: +381214893618

E-mail: gordana.racic@educons.edu.rs

Acknowledgement

The research is co-financed by the European Union through the Hungary-Serbia IPA Cross-border Co-operation Program (PHANETRI, HUSRB/1002/214/068) and by the Ministry of Education and Science of the Republic of Serbia (Project No. III43010 and TR 31080).

Abstract. Influence of edaphic factors and metal content on diversity of *Trichoderma* species at 14 different soil sampling locations, on two depths, was examined. Fortyone *Trichoderma* isolates from 14 sampling sites were determined as nine species based on their ITS sequences. Our results indicate that weakly alkaline soils are rich sources of *Trichoderma* strains. Also, higher contents of available K and P are also connected with higher *Trichoderma* diversity. Increased metal content in soil was not inhibiting factor for *Trichoderma* species occurence. Relationship between these factors was confirmed by LOESS nonparametric smoothing analysis. *Trichoderma* strain (SZMC 22669) from soil with concentrations of Cr and Ni above remediation values should be tested for its potential for bioremediation of these metals in polluted soils.

Keywords: biodiversity, edaphic factors, metals, internal transcribed spacer, *Trichoderma*, LOESS

Introduction

The species composition of the genus *Trichoderma* is well described in diverse ecosystems (Kredics et al. 2014). Known as cosmopolitan soil fungi they are found to colonize soil niches from cool temperatures to tropical climates (Friedl and Druzhnina 2012; Yamazaki et al. 2011). *Trichoderma* biodiversity has been investigated by molecular methods in Europe, Asia, Africa and South America (Friedl and Druzhnina 2012, Körmöczi et al. 2013, Kredics et al. 2014). Although there are series of data reporting new species and genotypes of *Trichoderma*, there are just a few studies where *Trichoderma* biodiversity was examined in relation to well described locations/soil types as habitats (Friedl and Druzhinina 2012; Naár and Dobos 2006). Either the distribution of only one species in soil (Eastburn and Butler 1988a, 1988b, 1991), or the population density of a few species in one soil type (Muniappan and Muthukumar 2014) was examined. Although fungal species which belong to the genus *Trichoderma* are known as very effective soil colonizers with resistance to different organic and inorganic pollutants (Harman et al. 2004a, b; Lorito et al. 2010), there is a lack of information about the effect of metal presence in soil on *Trichoderma* species composition. Metal contamination of soil is a significant problem and presents threat to ecosystems with negative impact on life forms. It is impossible to completely eliminate metals from soil as they can only be modified in less toxic metal compounds. *Trichoderma* spp. developed several mechanisms that provide detoxification of metals and can be divided into four categories: biosorption, biovolatilazation, bioaccumulation and phitobial remediation (Tripathi et al. 2013). *Trichoderma* tolerance to a range of metal concentrations is mostly studied in *in vitro* tests, where Kredics et al. (2001) demonstrated that *Trichoderma* strains are able to tolerate more than one metal. Tripathi et al. (2013) reported

multiple tolerances against Ni, As and Zn in four *Trichoderma* isolates and suggested that indigenous metal-tolerant *Trichoderma* species isolated from contaminated sites might be promising bioremediation agents for metal removal.

Although, research on variability of *Trichoderma* spp. is well documented, effect of environmental parameters on their occurrence in soil has not been reported in depth. The aim of this work was examination of relationship between soil physico-chemical and microbiological characteristics, metal content and variability of *Trichoderma* species, determined by molecular methods. In addition, relationship between studied factors was confirmed by "flexible" nonparametric regression approach (Cleveland 1979). The final goal was to determine the richest soil habitat as source for *Trichoderma* species with high potential for metal remediation.

Materials and methods

Soil sampling and analysis

Based on the results of several authors (Škorić et al. 1985; Ćirić et al. 2012; Mrvić et al. 2013; Belić et al. 2013) 14 locations with most common soil types of the region were chosen (Figure 1, Table 1). Belić et al. (2013) analyzed 400 soil profiles in different locations of Vojvodina and determined 10 different soil types defined according to the WRB classification (IUSS Working group WRB, 2006). Recently, pedological map of Serbia was created on the basis of existing digitalized pedological maps (scale 1:50000) (Mrvić et al. 2013).

Soil samples were collected randomly from the top horizon (A) of soils at two depths 0-30cm and 30-60 cm (Figure 1). The only location where sample was taken from the 0-20cm depth was at Zmajevac, soil sample number 11; because dominant parental rock was

reached at 20 cm. Soil samples were placed in sterile polythene bags and transported to laboratory. For microbiological analysis, soil samples were immediately separated from roots and large particles, and the samples were air-dried and smashed in a sterile mortar to collect fine particles. Thereafter, each soil sample was spread into a sterile tray and divided into four equally sized fragments, two of which were discarded while the remaining two were thoroughly mixed and again spread on the same tray for subsequent subsampling. Finally 50 g of each soil was stored at -20 °C for further analysis.

For physico-chemical investigations, samples were air dried and sieved through a 0.2 mm sieve prior to analysis. Soil texture was determined by the pipette method. Sample preparation for analysis was done with Na-pyrophosphate (Gee and Bauder 1986; Karkanis et al.1991). Soil acidity in 1:2.5 soil-water and soil-KCl suspensions was determined using a Radiometer PHM 62 Standard pH Meter. Humus content was determined by the wet oxidation method with $K_2Cr_2O_7$ (Tyurin 1931) modified by Simakov (1957). The free $CaCO_3$ content was determined by the ISO 10693:1995 volumetric method. The available phosphorus (P_2O_5) and available potassium (K_2O) were determined by ammonium lactate extraction, followed by spectrophotometric and flame photometric detection, respectively (Egner et al. 1960). The total N was determined according to the AOAC 972.43:2000 method by elemental analysis on a Vario EL III CHNS Analyzer (Elementar, Germany). Soil water retention was measured at matrix potentials of -33 kPa using a porous plate apparatus (Soilmoisture Equipment Corp., Santa Barbara, CA) (Richards 1948).

The colony-forming units (CFUs) of total number of bacteria as well as numbers of ammonifiers, *Nitrobacter*, oligonitrofils, actinobacteria and fungi were determined by serial dilution and plating on selective media. The total number of microorganisms was

determined on soil agar, the number of azotobacters on nitrogen-free medium using the „fertile drops“ method (Anderson 1958), the number of ammonifiers on meat pepton agar – MPA (Pochon and Tardieux 1962), N-fixing bacteria on Fiodorov medium, actinobacteria on synthetic medium (Krasiljnikov 1965) and fungi on Czapek-Dox agar. Incubation temperature was 28°C, while the incubation time depended on the tested group of microorganisms. Soil was dried at 105°C for 2 h and the number of microorganisms was estimated as CFU g^{-1} dry soil (DW). Dehydrogenase activity (DHA) was measured spectrophotometrically by the modified method according to Thalmann and expressed as μg TPF g^{-1} soil (triphenylformazan g^{-1} soil) (Thalmann 1968). All measurements were performed in three replicates.

Metal content in soil samples

Determination of the total metal content in soil samples was performed according to EPA 6010C method using inductively coupled plasma-optima emission spectrometry (ISP-OES) as described previously (Stajic et al. 2016).

Isolation of *Trichoderma* strains from different soil types

Isolations were performed from soil on dichloran – Rose Bengal medium (5 g L^{-1} peptone, 1 g L^{-1} KH_2PO_4, 10 g L^{-1} glucose, 0.5 g L^{-1} $MgSO_4 \times 7H_2O$, 0.5 ml L^{-1} 0.2% dichloran-ethanol solution, 0.25 ml L^{-1} 5% Rose Bengal and 20 g L^{-1} agar supplemented with 0.1 g L^{-1} oxytetracyclin, 0.1 g L^{-1} streptomycin and 0.1 g L^{-1} chloramphenicol to inhibit bacteria) (King et al. 1979). The isolated strains were deposited at the Szeged Microbiology Collection (SZMC, szmc.hu).

Molecular identification of the isolated *Trichoderma* strains

DNA isolation and PCR amplification of the internal transcribed spacer (ITS: ITS1-5.8S rDNA-ITS2) region of the ribosomal RNA gene cluster were performed as described previously (Körmöczi et al. 2013). DNA sequencing of amplicons was performed at LGC Genomics, Germany. *Trichoderma* isolates were identified based on their ITS sequences with the aid of the barcoding program *TrichO*KEY 2.0 available online at the home page of the International Subcommission on *Trichoderma* and *Hypocrea* Taxonomy (www.isth.info) (Druzhinina et al. 2005). In the cases where *TrichO*key 2.0 was not able to identify the isolate at the species level, BLASTN homology searches were performed at the homepage of NCBI (National Center for Biotechnology Information) (Zhang et al. 2000). The validities of the BLASTN hits were checked with *TrichO*key 2.0 and literature searches. Sequences were deposited at the NCBI Genbank database (www.ncbi.nlm.nih.gov), accession numbers are listed in Table 5.

Data analysis

Robust locally weighted sequential smoothing (LOESS) of the curves was used to examine relations between environmental parameters including edaphic factors or metal presence and *Trichoderma* spp. isolated strain number (Cleveland 1979). LOESS is nonparametric simple strategy used to fit the smooth curves to empirical data. It is beneficial fitting technique as it doesn't require specification of the relationship between the dependent and independent variables. It is particularly valuable in case of the presence of outliers i.e. extreme parameter values. Mostly it is used as a scatterplot smoother, but also it can be generalized very easily to multivariate data (Jacoby 2000).

The relationship between each examined soil variable and number of strains was examined as individual model. The godness-of fit of the individual models was measured by estimating the Pearson linear correlation coefficient between the dependent variable and fitted values of the individual models. The relationship between pairs of variables is not expressed by equation whereas the value of the correlation coefficients reported as R^2 indicates the quality of the nonparametric regression models.

Results

Chemical and physical analysis of soil samples

The results from the chemical and physical analysis of the samples derived from different soil types are presented in Table 2. The total $CaCO_3$ content of the studied soil samples varied in the broad range from 0 to 20.62%, i.e. from non-calcareous to strongly calcareous. The lowest pH values were measured in sample 13 (5.24-H_2O; 3.78-KCl), while the highest ones were determined in sample 10 (8.48-H_2O; 7.9-KCl), however, most of the examined soil types were weakly alkaline. The humus content ranged from values characteristic for weakly humic soils (1.41%) to soils very rich in humus (7.35%). Total nitrogen content varied betwen 0.121% in sample 9 to 0.472% in sample11, which is considered as an optimal value in agricultural soils. Available phosphorus content was highly variable from very low, 0.30 mg P_2O_5/100 g DW in forest soil to very high, 79.3 mg P_2O_5/100 g DW in agricultural soil (sample number 26). Available potassium content in the examined soils ranged from <11 in forest soils to >50 mg K_2O/100 g DW in agricultural soils.

According to the soil mechanical composition analysis of the examined soil types, the following soil texture classes were represented: Clay Loam (sample numbers: 7, 8, 14, 15, 24-27), Clayish Loam (sample numbers: 1-6, 18-23), Fine Sandy Loam (sample numbers:

9, 10, 16, 17), Sandy Loam (sample numbers: 11, 12) and Sandy Clay Loam (sample number 13) (Food and Agriculture Organization of the United Nations 2006) (Table 2). Water retention capacity ranged from 17.3 kPa for sample 17 to 33.76 kPa for sample 26 (Table 2).

Metal content

Total content of metals in the examined soils are shown in Table 3. The highest concentration of Cd, Cu and Zn was found in sample number 1, from Sremski Karlovci, sampled from vineyard under conventional farming, whereas concentration of Cr, Co and Ni was the highest in sample number 11 from Zmajevac (forest soil). The highest concentrations of Pb and Mn were found in sample number 26, from Svilajnac.

Microbiological characteristics

Bacteria were the most abundant microbes in the examined soil samples (Table 4). Total number of bacteria ranged from 7.4×10^6 to 4.53×10^8 CFU g^{-1} DW soil in sample 10 and 20, respectively. Ammonifiers were the least abundant in sample 13, and the most abundant in sample 24. Oligonitrofils were present in the range of 1.5×10^5 (sample 13) to 3.81×10^7 CFU g^{-1} DW soil (sample 22). *Nitrobacter* species were absent in samples 12 and 13, while in the highest amount they occurred in sample 16. Samples 26 and 27 did not contain actinobacteria while samples from forest in Kac, sample 9, were the richest for them. Number of fungi was the highest in samples from Svilajnac (sample 24), and the lowest in sample 10. DHA in the examined soils ranged from 36 µg TPF g^{-1} soil in sample 27 to 823 µg TPF g^{-1} soil found in sample 5.

Species composition of the genus *Trichoderma* in the examined soil samples

A total of 41 strains were isolated from 11 out of 14 examined locations in Serbia (Table 5). According to identification based on their ITS sequences, the isolated strains could be divided into 9 taxa: *T. harzianum* species complex (THSC), *T. koningiopsis, T. koningii, T. atroviride, T. brevicompactum, T. gamsii, T. citrinoviride, T. virens* and *T. longibrachiatum*. The abundance of strains and species at examined sampling sites is presented in Figure 2. The diversity of the isolated *Trichoderma* species was the highest in agricultural soils. The 14 strains isolated from Svilajnac proved to belong to six different species. Six strains were isolated from Čenej and Crepaja and they were identified as members of three and two different species, respectively. Four strains, belonging to 3 species, were isolated from Sremski Karlovci, sampling site 1. Only one strain was isolated from the following sampling sites: Titelski Breg, Lok, Kaćka šuma, Zmajevac, put za Vrdnik, Ljutovo and Rimski Šančevi. No strains were isolated from the following sampling sites: Sremski Karlovci 2, Kisač and Svilajnac1 (Figure 2). Most of the strains were isolated from the depth 0-30cm. Out of 41 isolated strains only 3 were derived from the depth of 30-60 cm.

The abundance of identified Trichoderma spp. at 11 locations is presented on Figure 3. In The most abundant taxon was the THSC with an overall abundance of 45 %. The frequences of *T. brevicompactum, T. koningiopsis, T. virens* and *T.longibrachiatum* were the second group of most abundant species detected at 3 locations. The abundance of the rest of the species was less than 20% (Figure 3).

Data analysis

Results shown in the Table 6 present correlation coefficients, reported as R^2 that indicates the quality of the nonparametric regression models, between environmental parameters (edaphic factors and metal content) and *Trichoderma* spp. isolated strain number. High R^2 values were obtained for models presenting relationship between isolated number of strains and available content of K and P, as well as pH, Cr, Co, Pb, Mn and Ni (R^2 equales 0.9147, 0.8058, 0.7678, 0.7111, 0.8833, 0.8221, 0.8425, 0.8006 respectively). The godness-of fit for the individual models describing relationships between number of isolated strains and other edafic factors such as content of $CaCO_3$, total N, humus, Cd, Cu and Zn was lower (R^2 equals 0.3128, 0.4228, 0.4555, 0.4747, 0.4895, 0.4356 respectively).

Discussion

The influence of environmental factors on the growth of different *Trichoderma* species was mostly examined under *in vitro* conditions (Antal et al. 2000; Kredics et al. 2003; Longa et al. 2009). In order to predict the behavior of *Trichoderma* species under changing natural conditions, mathematical models on combined effects of environmental parameters *in vitro* have also been developed (Begoude et al. 2007; Schubert et al. 2009). The most important factors influencing *Trichoderma* abundance *in vitro* are water availability, temperature, pH and the nutrient composition of the substrate (nutrient availability). Kredics et al. (2000) determined nearly linear correlation between water potential and *T. harzianum* colony growth rate. Schubert et al. (2009) showed that *T. atroviride* is more sensitive to the reduction of water activity than to temperature or nutrient status of the growth media. Similar results on sensitivity of another *T. atroviride* strain, isolated from decaying wood, to water availability in the substrate were

obtained by Longa et al. (2008). However, the information on the survivability and proliferation of *Trichoderma* species in relation to soil type and soil parameters is limited. The results of Muniappan and Muthukumar (2014) indicated that the fungal populations in the studied Alfisol soils were very stable and not easily influenced by edaphic factors at several locations. Our results indicate that water availability in the soil is an important factor for *Trichoderma* diversity. At the sampling site Svilajnac, soil type determined as Vertisol, with the highest measured water retention capacity (33.76 kPa) proved to be the location with the highest species diversity, six out of nine identified species as well as 14 out of 41 strains were isolated from Svilajnac2 (Table 5, Figure 2).

It was shown that the optimal pH for *in vitro* growth of different *Trichoderma* species varies between 4 and 6 (Kredics et al. 2004). According to our results, *Trichoderma* strains were present in all examined soil samples, the pH of which varied from 5.26 to 8.3. From soil samples with pH > 7.2, 37 strains belonging to 9 species were isolated. Our results indicate that weakly alkaline soils are rich sources of *Trichoderma* strains. Longa et al. (2008) have demonstrated that *in vitro* survivability of *T. atroviride* in three soil types was higher in soils with higher content of organic matter, nitrogen, P_2O_5 and K_2O. Anita and Ponmurugan (2011) have shown that the population density of *Trichoderma* spp. in soil samples from different locations was in significant positive correlation with the content of K (r=0.910), P (r= 0.686) and N (r=0.602). Our results indicate that the higher contents of available K and P and soil pH is connected with higher *Trichoderma* diversity (Tables 2 and 5), which is confirmed by LOESS data analysis (Table 6).

Soil contamination with increased metal concentrations in Serbia was rarely examined. Milenkovic et al. (2015) have investigated metal distribution in central Serbia around the city of

Kragujevac and their results indicate that mean concentrations of Cr (109.25 mg kg^{-1}) and Ni (120.12 mg kg^{-1}) exceed the Dutch standard target values for soil (100 and 35 mg kg^{-1}, respectively) (VROM 2000). Our results for the same two metals (Cr: 718 mg $kg^{-1}$and Ni: 1587 mg kg^{-1}) show that their concentration in the soil sample from Zmajevac significantly exceeds these standards. Sampling site Zmajevac, located on the mountain Fruška gora, is previously described as serpentinite soil lying on ultramafic rocks (Kostić et al. 1998). Indeed we have reached dominant parental rock at the depth of 20cm in soil sampling procedure. Soils from serpentinitic areas have high natural background of Ni and Cr (Bonifacio et al. 2010). Among serpentine soils Dystric Leptosol (Ranker, according to Serbian national classification) is standing out by significantly higher concentrations of Ni and Cr (Mrvić et al. 2013). It has been suggested that higher concentrations of Cr and Ni are the result of pedogenetic processes in the soil (Facchinelli et al. 2001; Lee and Kao 2004; Bonifacio et al. 2010). This sample was a source of only one *Trichoderma* species*, T. longibrachiatum*. The highest concentrations of Cd, Zn and Cu were determined in the soil sample from Sremski Karlovci, location 1, sampled from a vineyard which was under conventional farming, and thus it can be assumed that the metal content originates from the application of fungicides. However, 4 *Trichoderma* strains belonging to 3 species were isolated from this sample, indicating that it can be a good source of *Trichoderma* species.

Although most *Trichoderma* species are aggressive colonizers, they still have to compete with other soil microorganisms. The presence of other fast growing and opportunistic microbes in the vicinity of a *Trichoderma* fungus can reduce the substrate availability. According to microbiological characteristics of the examined soil samples in this investigation, fast growing bacterial populations which could compete with fungal populations were dominant (Table 4).

Among the examined soil types, bacterial populations were the most abundant in samples from Čenej, Crepaja and Svilajnac. In the same time the greatest diversity of *Trichoderma* species were found in these soil samples (Figure 2). According to our results, the investigated soil microbial characteristics did not affect *Trichoderma* diveristy in different soil samples (Table 4 and 5). The number of *Nitrobacter* in the sample from Zmajevac was significantly reduced probably due to high contentrations of nickel (Wyszkowska et al. 2007).

Our iinvestigation on the diversity of *Trichoderma* species in Serbia showed that the most abundant taxon was THSC, the beneficial *T. harzianum* species complex (Table 5; Figure 3), the members of which are widely used for the biological control of plant pathogenic fungi. THSC strains were most frequently isolated from soils which are used for agricultural purposes. A second beneficial taxon, *T. virens* was identified at three soil sampling locations. It is reported that the most common BCAs of the genus *Trichoderma* are strains of *T. virens* and THSC (Benítez et al. 2004). THSC strains are also recommended for metal removal, as demonstrated *in vitro* (De Freitas Lima et al. 2011; Kredics et al. 2001). Furthermore, *T. atroviride* was isolated at 2 locations of agricultural soil samples. Lopez and Vazquez (2003) have demonstrated that *T. atroviride* isolate from sludge was able to tolerate higher concentration of Cd, Zn and Cu in different conditions. *T. koningiopsis* was mostly identified in samples from vineyards. *T. longibrachiatum* - a species of the genus known as rare opportunistic pathogen of immunocompromised humans (Hatvani et al. 2013) - was mostly isolated from forest soil samples in 3 out of 11 soil sampling locations. *T. brevicompactum* - a species known to produce a potent antifungal antibiotic trichodermin, which is also a protein synthesis inhibitor in mammalian cells (Degenkolb et al. 2008) - was present in 3 out of 11 soil sampling locations.

Increased metal content in soil was not inhibiting factor for *Trichoderma* species. For example *T. longibrachiatum* was isolated from the soil sample with the highest conentrations of Cr and Ni. Most of the *Trichoderma* strains (14 out of 41) were isolated from Svilajnac2 sampling location where the concentration of Ni (38.38 mg kg^{-1}) exceeded the Dutch stundard (35 mg kg^{-1}) and the Mn concentration was found to be very high compared to other examined samples (926.6 mg kg^{-1}). Application of *Trichoderma* spp. in metal removal was mostly examined *in vitro* (Kredics et al. 2001; Kacprzak and Malina 2005), only few studies have demonstrated results conducted in practice (Estudio y Gestión Ambiental, 2010). Tripathi et al. (2013) summarized the potential use of *Trichoderma* species in bioremediation and concluded that metal removal can be developed by reintroduction of mass-cultured metal-tolerant *Trichoderma* strains isolated from the contaminated sites. According to our results *Trichoderma* strain SZMC 22669 isolated from soil where determined concentrations of Cr and Ni were above remediation values should be tested for its potential for bioremediation of these metals in polluted soils.

Conclusions

The diversity of the isolated *Trichoderma* species was the highest in Svilajnac2, Čenej and Crepaja soil samples, which belong to agricultural soils. Weakly alkaline soil samples are rich sources of *Trichoderma* strains. The higher contents of available K and P seem to be an important edaphic factor connected with higher *Trichoderma* diversity. However, other examined chemical and microbial soil characteristics did not affect *Trichoderma* diversity. Our results suggest that *Trichoderma* strain (SZMC 22669) isolated from Cr and Ni contaminated soil sample could be tested for use in bioremediation of those site.

References

Anderson GR (1958) Ecology of Azotobacter in soils of the palouse region I. Occurrence. Soil Sci 86:57–62.

Anita S, Ponmurugan P (2011) In vitro evaluation of *Trichoderma* atroviride against Phomopsis theae a casual agent of collar canker disease in tea plants, Int J Agric Res 6:620–631.

Antal Z, Manczinger L, Szakács G, Tengerdy RP, Ferenczy L (2000) Colony growth, In vitro antagonism and secretion of extracellular enzymes in cold-tolerant strains of *Trichoderma* species. Mycol Res 104: 545–549.

Begoude BAD, Lahlali R, Friel D, Tondje PR, Jijakli MH (2007) Response surface methodology study of the combined effects of temperature, pH, and a_w on the growth rate of *Trichoderma asperellum*. J Appl Microbiol 103: 845–854.

Belić M, Manojlović M, Nešić Lj, Ćirić V, Vasin J, Benka P, Šeremešić S (2013) Pedoecological Significance of Soil Organic Carbon Stock in South-eastern Pannonian Basin. Carpath J Earth Env, 8, 171-178.

Benítez T, Rincón AM, Limón MC, Codón AC (2004) Biocontrol mechanisms of *Trichoderma* strains. Int Microbiol 7: 249–260.

Bonifacio E, Falsone G, Piazza S (2010). Linking Ni and Cr concentrations to soil mineralogy: does it help to assess metal contamination when the natural background is high?. J Soils Sediments 10(8):1475-1486.

Cleveland WS (1979) Robust locally weighted regression and smoothing scatterplots. Amer Statist Assoc, 74(368): 829–836.

Ćirić V, Manojlović M, Nesić Lj, Belić M (2012) Soil dry aggregate size distribution: effects of soil type and land use. J Soil Sci Plant Nutr 12(4): 689-703.

De Freitas Lima A, Ferreira De Moura G, Barbosa De Lima MA, Mendes De Souza P, Albero Alves Da Silva C, Maria De Campos Takaki G, Elsbao Do Nascimento (2011) Role of the morphology and polyphosphate in *Trichoderma harzianum* related to cadmium removal. Molecules 16:2486–2500.

Degenkolb T, Dieckmann R, Nielsen KF, Gräfenhan T, Theis C, Zafari D, Chaverri P, Ismaiel A, Brückner H, von Döhren H, Thrane U, Petrini O, Samuels GJ (2008) The *Trichoderma brevicompactum* clade: a separate lineage with new species, new peptaibiotics, and mycotoxins. Mycol Progr 7:177–219.

Druzhinina I, Kopchinskiy A, Komoñ M, Bissett J, Szakacs G, Kubicek CP (2005) An oligonucleotide barcode for species identification in *Trichoderma* and *Hypocrea*. Fungal Genet Biol 42:813–828.

Eastburn DM, Butler EE (1988a). Microhabitat characterization of *Trichoderma harzianum* in natural soil: evaluation of factors affecting distribution. Soil Biol Biochem 20:547–553.

Eastburn DM, Butler EE (1988b). Microhabitat characterization of *Trichoderma harzianum* in natural soil: evaluation of factors affecting population density. Soil Biol Biochem 20: 541–545.

Eastburn DM, Butler EE (1991) Effects of soil moisture and temperature on the saprophytic ability of *Trichoderma harzianum*. Mycologia 83:257–263.

Egner H, Riehm H, Domingo WR (1960) Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Böden. II. Chemische Extraktionsmethoden zur Phosphor- und Kaliumbestimmung. K Lantbrukshoegsk Ann 26:199–215.

Estudio y Gestión Ambiental (E&GA) (2010) *Trichoderma* spp. and its potential in soil bioremediation, Drynet Science & Technology Expertise, http://dry-net.org/wp-content/uploads/2015/09/100221_*Trichoderma*_spp_and_its_potential_in_soil_bioremedi.pdf

IUSS Working Group WRB, 2006 World reference base for soil resources 2006. World Soil Resources Reports No. 103. FAO, Rome

Facchinelli A, Sacchi E, Mallen L (2001) Multivariate statistical and GIS-based approach to identify heavy metal sources in soils. Environ Pol 114: 313–324.

Friedl MA, Druzhinina IS (2012) Taxon-specific metagenomics of *Trichoderma* reveals a narrow community of opportunistic species that regulate each other's development. Microbiology (UK) 158:69–83.

Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004a) *Trichoderma* species-opportunistic, avirulent plant symbionts. Nat Rev Microbiol 2, 43–56

Harman GE, Lorito M, Lynch JM (2004b). Uses of *Trichoderma* spp. to remediate soil and water pollution. Adv Appl Microbiol 56:313–330.

Hatvani L, Manczinger L, Vágvölgyi C, Kredics L, (2013) *Trichoderma* as a human pathogen, in: Mukherjee P.K., Horwitz B.A., Singh U.S., Mukherjee M and Schmoll M (Eds.), *Trichoderma* - Biology and Applications, CABI, Wallingford, pp. 292–313.

Jacoby WG (2000) Loess: a nonparametric, graphical tool for depicting relationships between variables. Electoral Studies, 19(4): 577-613.

Kacprzak M, Malina G (2005) The tolerance and Zn^{2+}, Ba^{2+} and Fe^{3+} accumulation by *Trichoderma* atroviride and Mortierella exigua isolated from contaminated soil. Can J Soil Sci 85:283–290.

Karkanis PG, Au K, Schaalje GB (1991) Comparison of four measurement schedules for determination of soil particle-size distribution by the hydrometer method. Can Agr Eng 33:211–215.

King ADJr, Hocking AD, Pitt JI (1979) Dichloran-rose bengal medium for enumeration and isolation of molds from foods. Appl Environ Microbiol 37: 959–964.

Körmöczi P, Danilović G, Manczinger L, Jovanović Lj, Panković D, Vágvölgyi C, Kredics L (2013) Species composition of *Trichoderma* isolates from the rhizosphere of vegetables grown in Hungarian soils. Fres Environ Bull 22:1736–1741.

Kostić N, Wilson J, Živkovic M, Bain D (1998) Mineralogy and geochemical speciation of heavy metals in some serpentine soils of Serbia. 16th World Congress of Soil Science. pp. 444- 455. Elsevier, Amsterdam.

Krasiljnikov NA (1965) Biology of some actinomycetes groups. Science, Moscow.

Kredics L, Antal Z, Manczinger L (2000) Influence of water potential on growth, enzyme secretion and in vitro enzyme activities of *Trichoderma harzianum* at different temperatures. Curr Microbiol 40:310–314.

Kredics L, Dóczi I, Antal Z, Manczinger L (2001) Effect of heavy metals on growth and extracellular enzyme activities of mycoparasitic *Trichoderma* strains. Bull Environ Contam Toxicol 66, 249–254.

Kredics L, Antal Z, Manczinger L, Szekeres A, Kevei F, Nagy E (2003) Influence of environmental parameters on *Trichoderma* strains with biocontrol potential. Food Technol Biotechnol 41:37–42.

Kredics L, Manczinger L, Antal Z, Pénzes Z, Szekeres A, Kevei F, Nagy E (2004) *In vitro* water activity and pH dependence of mycelial growth and extracellular enzyme activities of *Trichoderma* strains with biocontrol potential. J Appl Microbiol 96:491–498.

Kredics L, Hatvani L, Naeimi S, Körmöczi P, Manczinger L, Vágvölgyi C, Druzhinina I (2014). Biodiversity of the genus Hypocrea/*Trichoderma* in different habitats, in: Gupta V.K., Schmoll M., Herrera-Estrella A., Upadhyay R.S., Druzhinina I. and Tuohy M. (Eds.), Biotechnology and Biology of *Trichoderma*, Elsevier Science B.V., Amsterdam, pp. 3–24.

Lee CS, Kao MM (2004) Distribution of forms of heavy metals in soils contaminated by metallurgical smelter emissions. J Environ Sci Health A Tox Hazard Subst Environ Eng 39: 577–585.

Longa CM, Pertot I, Tosi S (2008) Ecophysiological requirements of a *Trichoderma* atroviride isolate with biocontrol potential. J Basic Microbiol 48, 269–277.

Longa CM, Savazzini F, Tosi S, Elad Y, Pertot I (2009) Evaluating the survival and environmental fate of the biocontrol agent *Trichoderma* atroviride SC1 in vineyards in northern Italy. J Appl Microbiol 106:1549–1557.

Lopez EE, Vazquez C (2003) Tolerance and uptake of heavy metals by *Trichoderma* atroviride isolated from sludge. Chemosphere 50: 137–143.

Lorito M, Woo SL, Harman GE, Monte E (2010) Translational research on *Trichoderma*: from 'Omics to the field. Annu Rev Phytopathol 48:395–417

Milenkovic B, Stajic JM, Gulan L, Zeremski T, Nikezic D (2015) Radioactivity levels and heavy metals in the urban soil of Central Serbia. Environ Sci Pollut Res, 22:16732–16741.

Muniappan V, Muthukumar T (2014) Influence of crop species and edaphic factors on the distribution and abundance of *Trichoderma* in Alfisol soils of southern India. Acta Bot Croat 73:37–50.

Naár Z, Dobos A (2006) Redundancy analysis of the influence of metal content and other edaphic parameters on the coexsistence of *Trichoderma* species. Appl Ecol Environ Res 4: 113-123.

Pochon J, Tardieux P (1962) Techniques d'analyse en microbiologie du sol. Editions de la Tourelle, Saint Mandé, 111p.

Richards LA (1948) Porous plate apparatus for measuring moisture retention and transmission by soil. Soil Sci 66:105–110.

Schubert M, Mourad S, Fink S, Schwarze FWMR (2009) Ecophysiological responses of the biocontrol agent *Trichoderma* atroviride (T-15603.1) to combined environmental parameters. Biol Control 49:84–90.

Simakov VN (1957) The use of phenylanthranilic acid in the determination of humus by Tyurin's method. Pochvovedenie 8:72–73.

Stajic JM, Milenkovic B, Pucarevic M, Stojic N, Vasiljevic I, Nikezic D (2016) Exposure of school children to polycyclic aromatic hydrocarbons, heavy metals and radionuclides in the urban soil of Kragujevac city, Central Serbia. Chemosphere, 146: 68–74.

Škorić, A, Filipovski G, Ćirić M (1985) Classification of Yugoslav soils. Academy of Sciencies

Thalmann A 1968. Zur Methodik der Bestimmung der Dehydrogenaseaktivität im Boden mittels Triphenyltetrazoliumchlorid (TTC). Landwirtschaftliche Forschung 21, 249–258.

Tripathi P, Singh PC, Mishra A, Chauhan PS, Dwivedi S, Bais RT, Tripathi RD (2013) *Trichoderma*: a potential bioremediator for environmental clean up. Clean Techn Envir, 15, 541–550.

Tyurin, I.V., 1931. A modification of a volumetric method of humus determination with chromic acid. Pochvovedenie. 36(5-6):36.

VROM (2000) Circular on Target values and intervention values for soil remediation annex A: target values, soil remediation intervention values and indicative levels for serious contamination. Dutch Ministry of Housing, Spatial Planning and Environment (VROM)http://www.esdat.net/Environmental%20Standards/Dutch/annexS_I2000Dutch%20Environmental%20Standards.pdf

Wyszkowska J, Boros E, Kucharski J (2007) Effect of interactions between nickel and other heavy metals on the soil microbiological properties. Plant Soil Environ 53, 544–552.

Yamazaki Y, Tojo M, Hoshino T, Kida K, Sakamoto T, Ihara H, Yumoto I, Tronsmo AM Kanda H (2011) Characterization of *Trichoderma* polysporum from Spitsbergen, Svalbard archipelago, Norway, with species identity, pathogenicity to moss, and polygalacturonase activity. Fungal Ecol, 4, 15–21.

Zhang Z, Schwartz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. J Comput Biol 7:203-214.

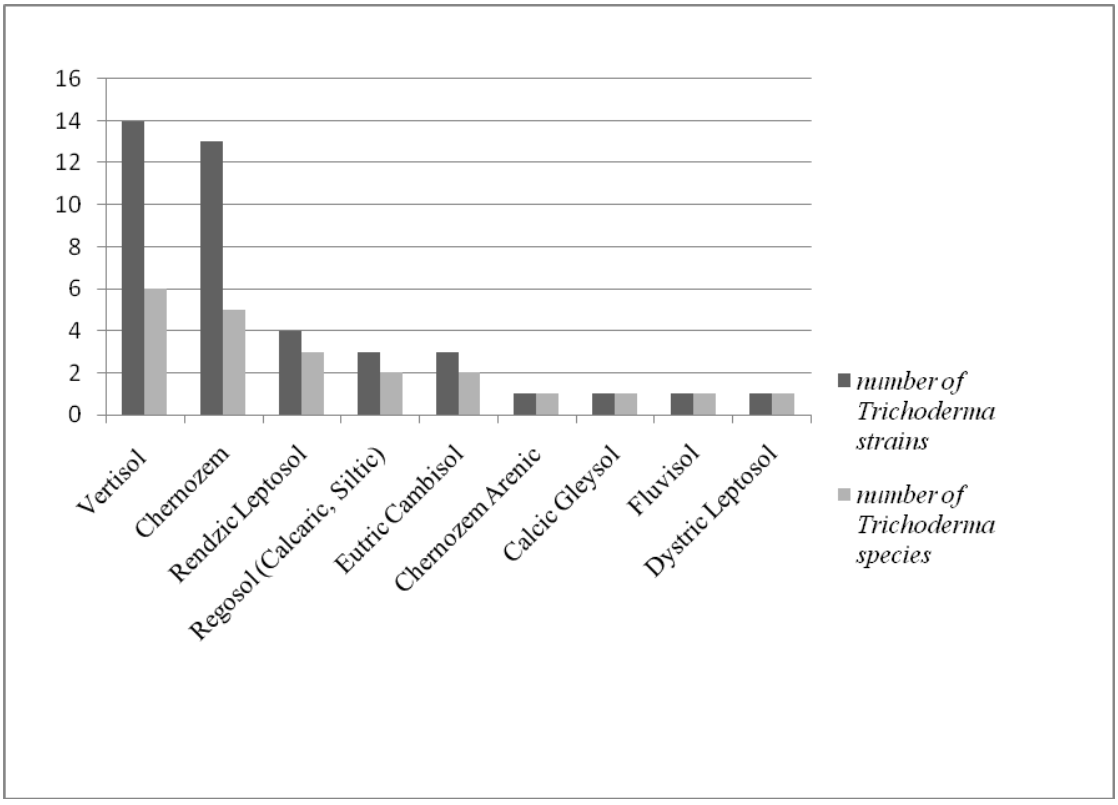

Figure 1. Abundance of *Trichoderma* species and strains in different soil types

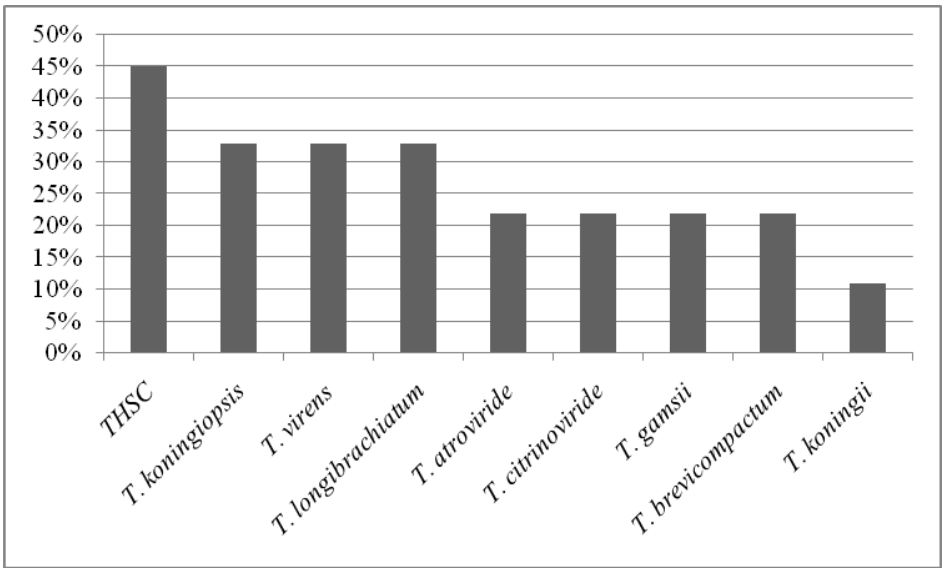

Figure 2. Frequency of *Trichoderma* species isolated from Serbian soil samples during the study.

THSC: *Trichoderma harzianum* species complex.

Table 1. Physico-chemical characteristics of the examined soil samples																		
	Soil type	Sampling site	Vegetation	Depth(cm)	***$CaCO_3$ (%)***	***pH (H_2O)***	***pH (KCl)***	***Humus (%)***	***Total N (%)***	***P_2O_5 (mg /100 g DW)***	***K_2O (mg /100 g DW)***	***grit 2.0–0.2 mm***	***fine sand 0.2-0.02 mm***	***powder 0.02 – 0.002 mm***	***clay <0.002 mm***	***total sand >0.02 mm***	***powder + clay <0.02 mm***	***water retention -33 kPa***
1	Rendzic Leptosol	Sremski Karlovci	vineyard	0-30	17.61	8.17	7.64	1.74	0.150	32.7	24.10	4.300	38.580	36.92	20.20	42.88	57.12	24.60
2				30-60	10.12	8.17	7.62	1.84	0.158	25.8	19.10	4.100	36.260	39.20	20.44	40.36	59.64	25.30
3		Sremski Karlovci	vineyard (ecological table)	0-30	10.90	7.94	7.31	1.49	0.128	43.8	28.60	2.100	40.660	35.76	21.48.	42.76	57.24	23.40
4				30-60	11.32	8.13	7.43	1.48	0.127	41.2	25.00	2.300	42.860	31.80	23.04	45.16	54.84	23.20
5	Regosol (Calcaric, Arenic)	Titelski Breg	agricultural soil	0-30	7.96	8.08	7.51	3.26	0.223	42.5	35.50	0.700	39.740	35.56	24.00	40.44	59.56	26.60
6				30-60	9.22	8.20	7.33	3.21	0.220	39.6	28.20	0.500	38.540	34.60	26.36	39.04	60.96	26.00
7	Calcic Gleysol	Lok	agricultural soil	0-30	3.35	8.03	7.35	4.05	0.260	69.4	16.40	1.300	48.340	21.92	28.44	49.64	50.36	25.00
8				30-60	2.43	7.88	7.26	4.65	0.298	60.9	14.10	1.100	48.300	21.80	28.80	49.40	50.60	26.40
9	Fluvisol	Kaćka šuma	forest soil	0-30	19.70	8.31	7.73	1.41	0.121	5.8	7.30	0.900	79.180	12.56	8.36	80.08	19.92	17.40
10				30-60	20.54	8.48	7.90	1.52	0.131	1.9	5.50	1.400	83.600	9.20	5.80	85.00	15.00	22.90
11	Dystric Leptosol	Zmajevac	forest soil	0-20	0.34	7.63	6.77	7.35	0.472	3.1	24.50	36.500	35.420	21.04	7.04	71.92	28.08	29.80
12	Eutric Cambisol	put za Vrdnik	forest soil	0-30	0.00	5.51	4.26	5.07	0.325	0.9	10.90	2.500	37.700	45.08	14.72	40.20	59.80	29.30
13				30 -60	0.00	5.24	3.78	3.59	0.246	0.3	10.00	1.500	35.460	47.52	15.52	36.96	63.04	25.80
14	Chernozem on loess terrace	Kisač	agricultural soil	0-30	0.70	8.30	7.47	3.68	0.252	30.8	41.80	0.100	30.180	37.84	31.88	30.28	69.72	25.40
15				30-60	1.82	8.18	7.51	3.46	0.237	15.5	35.00	0.100	31.180	35.08	33.64	31.28	68.72	25.40
16	Chernozem (Arenic)	Ljutovo	agricultural soil	0-30	7.13	8.27	7.65	2.65	0.197	36.1	30.00	8.800	65.880	13.48	11.84	74.68	25.32	18.10
17				30-60	6.71	8.21	7.69	2.57	0.191	33.0	17.30	12.000	62.240	13.72	12.04	74.24	25.76	17.30
18	Chernozem	Rimski Šančevi	agricultural soil	0-30	1.96	8.19	7.54	2.74	0.204	9.8	21.80	0.400	37.480	32.80	29.32	37.88	62.12	24.60
19				30-60	1.82	8.12	7.46	2.20	0.163	10.5	22.30	1.000	36.760	31.68	30.56	37.76	62.24	25.30
20		Čenej	rhizosphere	0-30	1.13	7.75	7.10	3.99	0.273	38.5	23.20	0.440	44.840	30.44	24.28	45.28	54.72	30.61
21		Crepaja	rhizosphere	0-30	20.62	8.00	7.39	3.25	0.220	34.8	52.73	1.320	40.600	39.06	19.01	41.92	58.08	31.10
22	Vertisol	Svilajnac	rhizosphere	0-30	0.00	5.90	4.70	2.87	0.204	5.7	13.65	3.615	34.045	34.88	27.46	37.66	68.04	28.90
23		Svilajnac	rhizosphere	0-30	0.51	7.30	6.69	4.04	0.259	79.3	118	3.97	32.390	37.08	26.56	36.36	63.64	33.76
MIN					0.00	5.24	3.78	1.41	0.121	0.3	5.50	0.100	30.180	9.20	5.80	30.28	15.00	17.40
MAX					20.62	8.48	7.90	7.35	0.472	79.3	118	36.500	83.600	47.52	33.64	85.00	69.72	33.76

*MIN=minimum value; MAX=maximum value; DW: dry weight.

Table 2. Total heavy metal content of the examined soil samples

Sample	Soil type	Depth(cm)	Cd (mg/kg)	Cr (mg/kg)	Co (mg/kg)	Cu (mg/kg)	Fe (g/kg)	Pb (mg/kg)	Mn (mg/kg)	Ni (mg/kg)	Zn (mg/kg)
1	Rendzic Leptosol	0-30	0.5659	37.47	7.345	72.820	26.87	11.830	402.2	22.11	80.50
2		30-60	0.4974	35.10	6.867	58.610	28.58	11.550	363.7	23.89	73.13
3		0-30	0.3616	44.91	8.773	58.840	31.08	12.660	484.9	25.26	76.92
4		30-60	0.3619	43.42	8.808	61.600	29.83	11.730	477.2	23.70	71.23
5	Regosol (Calcaric. Arenic)	0-30	0.3005	39.64	7.781	20.110	28.82	9.936	486.1	21.97	56.57
6		30-60	0.3105	39.52	7.646	20.170	29.54	9.894	484.8	21.57	55.61
7	Calcic Gleysol	0-30	0.2481	41.22	6.892	26.740	22.21	11.430	216.8	24.27	58.18
8		30-60	0.2915	39.96	6.983	27.570	20.28	11.890	217.3	24.18	61.32
9	Fluvisol	0-30	0.2076	22.61	5.528	11.540	14.54	6.984	302.4	15.61	47.77
10		30-60	0.1630	20.29	5.983	10.330	14.10	5.965	290.1	15.88	42.51
11	Dystric Leptosol	0-20	< 0.1	718.10	57.180	10.630	50.25	15.920	595.9	1587.00	66.49
12	Eutric Cambisol	0-30	0.1327	102.80	9.319	8.148	28.00	14.800	220.4	57.58	51.10
13		30-60	0.1642	97.38	9.923	9.119	27.14	13.290	220.6	57.03	52.12
14	Chernozem on loess terrace	0-30	0.2764	51.89	9.036	26.290	32.35	11.970	457.3	26.32	68.69
15		30-60	0.2580	45.70	8.853	25.260	29.50	11.090	423.7	24.92	65.33
16	Chernozem (Arenic)	0-30	0.2202	23.26	3.878	35.530	15.00	6.236	329.3	10.67	43.92
17		30-60	0.2046	23.29	3.973	28.610	14.87	6.029	326.8	10.57	44.79
18	Chernozem	0-30	0.2839	50.04	8.941	23.480	33.92	12.710	523.2	26.79	64.41
19		30-60	0.2667	49.13	8.700	23.220	32.94	12.610	520.9	24.90	65.11
20		0-30	0.2662	48.49	8.957	23.400	30.48	12.200	598.7	24.66	56.96
21		0-30	0.4181	36.99	6.886	22.320	26.10	9.662	566.3	19.64	50.31
22	Vertisol	0-30	0.1733	60.72	11.090	21.650	31.83	20.560	748.7	30.35	56.69
23		0-30	0.2708	71.76	16.590	33.940	35.59	24.480	926.6	38.48	73.81
MIN			< 0.1	20.29	3.878	8.148	14.10	5.965	216.8	10.57	42.51
MAX			0.5659	718.10	57.180	72.820	50.25	24.480	926.6	1587.00	80.50
LIM			0.8	100	9	36	/	85	/	35	140
REM			12	380	240	190	/	530	/	210	720

*MIN=minimum value; MAX=maximum value; LIM=limit values; REM=remediation values

Table 3. Microbiological characteristics of the examined soil samples

	Soil type	Sampling site	Vegetation	Depth(cm)	Number of microorganisms (CFU g^{-1} absolutely dry soil))						DHA µg TPF g^{-1} of soil
					Total No. of bacteria $\times 10^6$	ammonifiers $\times 10^6$	*Nitrobacter* $\times 10^1$	oligonitrofils $\times 10^5$	actinobacteria $\times 10^2$	fungi $\times 10^3$	
1	Rendzic Leptosol	Sremski Karlovci	vineyard	0-30	19.6	21.1	121	24.6	194	14.0	290
2				30-60	8.0	9.5	111	12.9	167	12.4	307
3		Sremski Karlovci	vineyard (ecological table)	0-30	23.2	23.1	109	23.3	64	10.8	446
4				30-60	21.7	16.8	105	15.6	52	8.3	281
5	Regosol (Calcaric. Arenic)	Titelski Breg	agricultural soil	0-30	21.7	21.7	79	23.0	48	5.8	823
6				30-60	13.1	13.7	32	21.0	44	5.6	436
7	Calcic Gleysol	Lok	agricultural soil	0-30	14.9	20.0	65	21.3	52	5.8	125
8				30-60	19.1	22.9	105	21.7	31	5.2	134
9	Fluvisol	Kaćka šuma	forest soil	0-30	13.2	13.6	24	13.4	377	7.7	148
10				30-60	7.4	8.2	6	9.6	274	3.3	108
11	Dystric Leptosol	Zmajevac	forest soil	0-20	27.9	19.3	4	30.3	27	8.6	709
12	Eutric Cambisol	put za Vrdnik	forest soil	0-30	19.2	9.5	0	1.7	88	22.2	357
13				30 -60	8.2	6.7	0	1.5	19	9.4	109
14	Chernozem on loess terrace	Kisač	agricultural soil	0-30	8.4	17.1	81	26.1	10	10.2	195
15				30-60	8.2	21.4	65	16.2	5	6.7	358
16	Chernozem (Arenic)	Ljutovo	agricultural soil	0-30	23.0	19.1	139	22.3	65	7.8	782
17				30-60	12.0	13.1	83	16.3	30	8.6	314
18	Chernozem	Rimski Šančevi	agricultural soil	0-30	14.5	14.6	36	18.4	80	14.2	215
19				30-60	13.7	13.6	32	17.4	53	12.9	244
20		Čenej	rhizosphere	0-30	453	108	93	329	50	40	149
21		Crepaja	rhizosphere	0-30	443	118	56	381	110	32	390
22	Vertisol	Svilajnac	rhizosphere	0-30	316	143	27	273	90	66	203
23		Svilajnac		0-30	206	52	14	157	0	43	40
MIN					7.4	6.7	0	1.5	0	3.3	40
MAX					453	143	139	381	377	66	823

*MIN=minimum value; MAX=maximum value DHA: dehydrogenase activity; TPF: triphenylformazan

Table 4. - Isolation data and identification details of the examined *Trichoderma* strains

	Soil type	Sampling site	Vegetation	Depth (cm)	SZMC Number	GenBank Accession number (ITS)	*TrichO*key 2.0 or NCBI diagnosis
1	Rendzic Leptosol	Sremski Karlovci	vineyard	0-30	SZMC 20966 SZMC 20969 SZMC 20976 SZMC 20978	**KP316411** **KP316414** **KP316421** **KP316423**	*T. koningiopsis* *T. harzianum* *T. atroviride* *T. koningiopsis*
2				30-60	-	-	-
3		Sremski Karlovci	vineyard (organic farming)	0-30	-	-	-
4				30-60	-	-	-
5	Regosol (Calcaric. Arenic)	Titelski Breg	agricultural soil	0-30	SZMC 22661 SZMC 22662	**KP316440** **KP316450**	*T.brevicompactum* *T. gamsii*
6				30-60	SZMC 22663	**KP316441**	*T. brevicompactum*
7	Calcic Gleysol	Lok	agricultural soil	0-30	SZMC 22664	**KP316444**	*T. longibrachiatum*
8				30-60	-	-	-
9	Fluvisol	Kaćka šuma	forest soil	0-30	SZMC 22668	**KP316445**	*T. citrinoviride*
10				30-60	-	-	-
11	Dystric Leptosol	Zmajevac	forest soil	0-20	SZMC 22669	**KP316442**	*T. longibrachiatum*
12	Eutric Cambisol	put za Vrdnik	forest soil	0-30	SZMC 22665 SZMC 22666	**KP316443** **KP316446**	*T. longibrachiatum* *T. virens*
13		put za Vrdnik		30 -60	SZMC 22667	**KP316447**	*T. virens*
14	Chernozem on loess terrace	Kisač	agricultural soil	0-30	-	-	-
15				30-60	-	-	-
16	Chernozem (Arenic)	Ljutovo	agricultural soil	0-30	-	-	-
17				30-60	SZMC 22660	**KP316448**	*T. harzianum*
18	Chernozem	Rimski Šančevi	agricultural soil	0-30	SZMC 22659	**KP316449**	*T. virens*
19		Rimski Šančevi		30-60	-	-	-
20		Čenej	rhizosphere	0-30	SZMC 20974 SZMC 20977 SZMC 20979 SZMC 20985 SZMC 20986 SZMC 20988	**KP316419** **KP316422** **KP316424** **KP316430** **KP316431** **KP316433**	*T. brevicompactum* *T. brevicompactum* *T. koningiopsis* *T. gamsii* *T. brevicompactum* *T. harzianum*
21		Crepaja	rhizosphere	0-30	SZMC 20971 SZMC 20973 SZMC 20975 SZMC 20982 SZMC 20990 SZMC 20994	**KP316416** **KP316418** **KP316420** **KP316427** **KP316435** **KP316439**	*T. brevicompactum* *T. brevicompactum* *T. harzianum* *T. harzianum* *T. brevicompactum* *T. harzianum*
22	Vertisol	Svilajnac	rhizosphere	0-30			
23		Svilajnac		0-30	SZMC 20965 SZMC 20967 SZMC 20968 SZMC 20970 SZMC 20972 SZMC 20980 SZMC 20981 SZMC 20983 SZMC 20984 SZMC 20987 SZMC 20991 SZMC 20992 SZMC 20993 SZMC 20989	**KP316410** **KP316412** **KP316413** **KP316415** **KP316417** **KP316425** **KP316426** **KP316428** **KP316429** **KP316432** **KP316436** **KP316437** **KP316438** **KP316410**	*T. harzianum* *T. harzianum* *T. atroviride* *T. harzianum* *T. koningii* *T. harzianum* *T. harzianum* *T. koningiopsis* *T. koningiopsis* *T. citrinoviride* *T. virens* *T. harzianum* *T. koningiopsis* *T. harzianum*

1 Table 5. Relationship between isolated number of *Trichoderma* strains and the environmental
2 variables used in localy weighted regression scatterplot smoothing analysis (LOESS)

Environmental variables	$CaCO_3$	pH (H_2O)	pH (KCl)	Humus	Total N	P_2O_5	K_2O	Cd	Cr	Co	Cu	Fe	Pb	Mn	Ni	Zn
r^2	0.3147	0.6355	0.6361	0.4318	0.387	0.7996	0.8961	0.3281	0.7111	0.8508	0.479	0.7525	0.8221	0.8309	0.8766	0.3827

16