Czech University of Life Sciences Prague

Faculty of Agrobiology, Food and Natural Resources

Department of Agro-Environmental Chemistry and Plant Nutrition

The changes of risk element mobility and plant-availability in contaminated soil treated with dry olive residue

Diploma Thesis

Author of Thesis: Bc. Tereza Stejskalová

Thesis Supervisor: prof. Ing. Jiřina Száková, CSc.

Consultant: Mercedes García-Sánchez, Ph.D.

Declaration

I declare that this diploma thesis "The changes of risk element mobility and plant-availability in contaminated soil treated with dry olive residue" I prepared by myself under the professional leadership of my supervisor prof. Ing. Jiřina Száková, CSc. and my consultant Mercedes García-Sánchez, Ph.D. I worked with using scientific literature and other information sources which are listed in the attached bibliography at the end of this work. As the author of mentioned thesis, I also declare, I did not infringe the copyright of third parties in connection of creation this diploma thesis.

In Prague, 13th April 2017

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Tereza Stejskalová

Acknowledgement

Abstract

The importance of the olive oil agroindustrial activity in Mediterranean area is well known. The two-phase extraction system is the most widely used technology to obtain oil and generates a semi-solid organic waste called "alpeorujo" - olive mill dry residue (DOR). This residue due to its organic matter and mineral nutrient content might be used as a promising material for *in situ* remediation of the soil as a risk element stabilization agent and furthermore as fertilizer (Nogales et al. 1999). Unfortunately, like the majority of plant by-products, DOR can be phytotoxic. Approximately 4 – 5 million tons of "alpeorujo" per year are produced in Spain and uncontrolled disposal of the DOR might lead to serious environmental issue in the main olive growing regions. DOR cannot be directly applied to the soil but close relationship was found in saprophytic fungal transformation of DOR which can effectively stabilize the organic matter content, enhance C/N ratio and substantially reduce the phenolic fraction content in the waste (Sampedro et al., 2009).

In this experiment, the combined treatment of biotransformed DOR and arbuscular mycorrhizal fungi (AMF) was observed as suitable management option for the stabilization of the risk elements in extremely contaminated soil. Also, the impact on changing the nutrient status and bioavailability of risk elements in soil treated with biotransformed DOR by four species of fungi: *Penicillium chrysogenum, Funalia floccosa, Bjerkhandera adusta*, *Chondrostereum purpureum* and AM fungus *Funneliformis mosseae* was investigated as well as the effect of this treatment on the risk element uptake by wheat plant (*Triticum aestivum* L.) cultivated in a pot experiment with the Cd, Pb, and Zn contaminated soil.

The results showed substantial role of both biotransformation of DOR and AM inoculation in the plant growth and element uptake by wheat plants. Moreover, the mobile proportions of elements on the treated soils were related to increasing soil pH, where significantly decreasing Cd, Cu, Fe, Mn, P, Pb, and Zn mobility (r values between -0.36 and -0.46), and increasing Ca and Mg mobile proportions (r = 0.63, and r = 0.51, respectively) were determined. The biotransformed DOR application lowered the mobility of risk elements (Cd, Zn), and nutrients (Ca, Cu, Fe, Mg, Mn) in the aboveground biomass, where the elements retained in higher extent in roots. Thus, the biotransformed DOR application to the risk element contaminated soil can reduce the risk element accumulation in aboveground biomass of plants, although the reduction was insufficient for safe crop production in the extremely contaminated soil.

Keywords: biotransformed dry olive residue, risk elements, wheat, pot experiment, stabilization, immobilization

Abstrakt

Jak je dobře známo, nejdůležitější agroindustriální aktivitou v oblasti středomoří je výroba olivového oleje. Dvoufázová extrakce, která je nejvíce používanou technologií pro získání oleje, je též příčinou vznik polotuhého organického odpadu nazývaného "alpeorujo" – známého z literatury pod zkratkou DOR. Tato sušina, díky svému obsahu organické hmoty a množství minerálních živin by mohla být použita jako vhodný materiál pro stabilizaci rizikových prvků při *in situ* re-mediaci půdy a dále také jako hnojivo (Nogales et al. 1999). Avšak jako většina vedlejší produktů rostlin, DOR může působit fytotoxicky. Ve Španělsku se vyprodukuje přibližně 4–5 milionů tun "alpeoruja" za rok, přičemž nekontrolovaná likvidace takového množství odpadu by mohla vést až k vážným environmentálním problémům v této oblasti. DOR nemůže být aplikováno přímo do půdy, ale v případě transformace pomocí saprofytické houby je možné stabilizovat složení této organické hmoty, zvýšit poměr C/N a podstatně snížit obsah fenolických látek (Sampedro et al., 2009).

V tomto experimentu bylo zkoumáno, zda kombinace biotransformovaného DOR s ošetřením pomocí arbuskulární mykorhizní houby (AMF) má vliv na stabilizaci rizikových prvků v extrémně kontaminované půdě. Také byl sledován vliv na změnu obsahů živin a biologickou dostupnost rizikových prvků v půdě po aplikaci biotransformovaného DOR čtyřmi druhy hub: *Penicillium chrysogenum, Funalia floccosa, Bjerkhandera adusta*, *Chondrostereum purpureum* a AM houbou *Funneliformis mosseae.* Dále pak efekt tohoto ošetření na příjem rizikových prvků rostlinami pšenice (*Triticum aestivum* L.) pěstované v nádobovém pokusu na půdě kontaminované rizikovými prvky: Cd, Pb a Zn.

Výsledky potvrdily, že biotransformace a ošetření pomocí AMF hraje podstatnou roli v příjmu prvků rostlinou a jejím růstu. Dále se ukázalo, že mobilita prvků je závislá na zvyšující se hodnotě pH půdy, kde signifikantní poklesy mobility byly zřetelné u prvků: Cd, Cu, Fe, Mn, P, Pb a Zn (r hodnota v rozmezí <-0,36; -0,46>) a naopak nárůsty mobility u prvků Ca a Mg (r = 0,63, r = 0,51). Aplikace biotransformovaného DOR snížila mobilitu rizikových prvků (Cd, Zn) a živin (Ca, Fe, Mg, Mn) v nadzemních částech rostlin, zatím co ve větším rozsahu byly prvky akumulovány v kořenech. I presto, že v tomto experimentu bylo snížení obsahu rizikových prvků pro bezpečnou rostlinnou produkci nedostačující, pravděpodobně v důsledku použití extrémně kontaminované půdy, je aplikace biotransformovaného DOR možnou metodou pro snížení rizika hromadění těchto prvků v nadzemních částech rostlin.

Klíčová slova: biotransformovaná olivová sušina, rizikové prvky, pšenice, nádobový experiment, stabilizace, imobilizace.

Table of content

Introduction

German Advisory Council on Global Change (1994) in Bonn reported that 22 million hectares of global land is contaminated with heavy metals – nowadays, after further researches better to interpreted as a risk elements. For example, specifically in the European Union it was estimated that there could be up to 3 million potentially polluted soils. Fortunately, around 80,700 locations have been decontaminated over the last 30 years in the countries, where data about remediation was available. On the other hand, at least 250,000 sites contaminated by risk elements require our greater attention.

Remediation of hazardous soils by formal methods, including excavation and landfilling, is unpracticable on large scale because these techniques are environmentally disruptive and cost-prohibitive also. In contrast, phytoremediation, the use of vegetation for *in situ* resumption of contaminated soils, and other bioremediation measures are generally considered cost-effective and environmentally friendly approaches (Arthur et al., 2005).

The olive trees (*Olea europea* L.) planting and ensuing olive oil production represents one of the most relevant agroindustrial activities in the Mediterranean area and generates huge amount of a solid residue called as dry olive residue (DOR), meaning the dry olive mill waste „alpeorujo", a typical two-phase olive oil extraction solid by-product (Vlyssides et al., 1998). The uncontrolled disposal of such production might lead to serious environmental problems. However, there are several positive possibilities for re-use of DOR for example: combustion for production of energy, carbon source for production of biosurfactant surfactin, applicable for remediation of the soil contaminated with engine oil (Maass et al., 2016), production of DOR-based adsorbent for wastewater cleaning (Bhatnagar et al., 2014) or due to its content in organic matter and mineral nutrients, the material can be applied as organic fertilizer on agricultural soil (Sampedro et al., 2007b).

Before its application, the DOR needs to be stabilized, through its fungal transformation, because raw DOR is limited by their phytotoxic effect given by high contents of phenolic compounds (Sampedro et al., 2011). Various measures were tested to suppress the phytotoxicity of phenolic compounds. One of the suitable biotechnological treatment for detoxification of DOR is solid-state fermentation including composting (Diacono et al., 2012; Tortosa et al., 2012; Rodriguez et al., 2014). The chemical composition of the olive oil output wastes is suitable, where the phenolic compounds are degraded, and then the compost can be used for soil application (Balis et al., 2001; Chowdhury et al., 2013; Montemurro et al., 2015). The alternative is the fungal biotransformation of DOR which lead to polymerization of these

compounds, mediated by fungal laccases and peroxidases, resulting in their inaccessibility by plants (Sampedro et al., 2004; Bonanomi et al., 2006; Reina et al., 2013). For example: the DOR incubation with the fungus *Chondrostereum purpureum* reduced the phytotoxicity of DOR and indirectly stimulated the plant growth (Reina et al., 2017). Also, Sampedro et. al. (2007b) published that this residue´s detoxification and organic matter stabilization via incubation with saprobic fungi can solve the problem of its disposal to soil, enrich soils with limited organic matter and improve physical and chemical properties. Pardo et al. (2011) found the positive effect of composted DOR on the reduction of the mobile proportion of lead (Pb) and zinc (Zn) in contaminated soil. Similarly, Hovorka et al. (2016) evaluated the possible effect of biotransformed DOR on sorption of risk elements such as cadmium (Cd), Pb, and Zn in the soil.

Arbuscular mycorrhizal fungi (AMF) play an important role in the soil-plant interaction and it belongs to the most important microbial symbioses for the majority of plants influencing plant community development, nutrient uptake, water relations and above-ground productivity (Smith and Read, 2008). As reviewed by Muthukumar and Bagyaraj (2010) and Leung et al. (2013) AMF can facilitate the survival of their host plants growing on in risk element contaminated soils enhancing their nutrient acquisition, protecting them from the metal toxicity, or absorbing the risk elements. For instance, Carreon-Abud et al. (2013) reported protective effect of the mycorrhizal colonization of *Solanum lycorpersicum* L. against potential toxicity of chromium. Similarly, AMF strains isolated from old zinc wastes decreased risk element uptake by plants growing on risk element contaminated soil (Turnau et al., 2006). On the contrary, the inoculation of *Glomus spp.* resulted in enhanced plant uptake of Co, Cd, Zn, and Pb and increased translocation of these elements from roots to shoots in *Medicago sativa* L.,, *Eucalyptus globulus*, and *Helianthus annuus* plants (de Andrade et al., 2008; Arriagada et al., 2010; Zaefarian et al., 2011). However, the metal stabilization by AMF can be also improved through the application of organic amendments, as suggested (Alguacil et al., 2008; Kohler et al., 2015).

Therefore, this experiment was aimed to investigate if combining or not of biotransformed DOR and the inoculation with AM fungus may influence: the mobility of risk elements; the risk elements uptake by wheat plants (*Triticum aestivum*, L.) and the mobility and plant-availability of macro-and micronutrients in a contaminated soil with Cd, Pb, and Zn. In context, the results derived from experiment will allow to gain better understanding on the effectiveness of the fungal transformed DOR applications as immobilizing agent of risk elements along with AM fungus inoculation as profitable option for the remediation of risk element contaminated soils.

1 Work objective

In this experiment, the potential ability of the biotransformed DOR to decrease the mobility and plant-availability of the risk elements in contaminated soil will be investigated in combination with the AMF using the model pot experiment, where spring wheat will be cultivated.

The main hypothesis is:

- If the combined treatment involving biotransformed DOR and AMF, it can be considered as suitable management option for the stabilization of the risk elements in the contaminated soil.

Specific objectives are:

- Analyse the impact of the combined treatment, biotransformed DOR and AMF, in changing the nutrient status and bioavailability of risk elements in multi-element contaminated soil.
- Study if the impact of this combined treatment will reduce in the plant-available risk elements uptake, and thus it would allow the safe spring wheat cultivation.

Evaluate if the application of biotransformed DOR in combination with AMF will improve to wheat biomass production and nutritional status. The results were evaluated by using adequate statistical methods, interpreted and discussed in order to understand the potential capacity of this combined treatment reducing the soil mobility and plant-available uptake of risk elements.

Literature review

2 Soil contamination

Contamination of the soil belongs to processes that affect production and ecological functions of soil, along with water and wind erosion, land appropriation, land degradation, loss of organic matter and the acidification. As literature sources points, this is influenced by several inorganic and organic substances, whose source can be either natural processes or anthropogenic activity (Adriano, 2001; UNEP/GPA, 2004). Risk elements, originally based on anthropogenic activity, are the most common and belongs to the longest lasting environmental contaminants. In soil, risk elements can persist for thousands of years and it is very difficult to eliminate their effects on soil fertility and healthy plant growth (Tlustoš et al., 2007). As well as in soil, to small extent it can enter to animal or worse into human body system through food, water or even the air and bioaccumulate over period (UNEP/GPA, 2004).

2.1 Risk elements in soil

The term "Risk elements" (heavy metals – previously used) refers to any metallic element which density is relatively high and is poisonous or toxic even at low concentration. This is general name for metals and metalloids with atomic density greater than 4 g*cm^{-3}. Risk elements includes Pb, Cd, Zn, mercury (Hg), arsenic (As), silver (Ag) chromium (Cr), copper (Cu) iron (Fe), and the platinum group elements (Duruibe et al., 2007). According to Sáňka (2004), the group of risk elements includes: As, beryllium (Be), Cd, cobalt (Co), Cr, Cu, Hg, molybdenum (Mo), nickel (Ni), Pb, vanadium (V), Zn.

Essential elements such as Cu, Zn and Mo are necessary for plant growth. Elements such as Cu, Co, Mo, Zn, Cr, Ni and V are important for animal nutrition and except V, that also apply in human nutrition. As, Cd, Hg and Pb are toxic to all the organisms (Adriano et al., 2004).

Multi element contaminated soil can be considered as soil where amount of risk elements exceeded over the permitted limit of element content in soil. In the Czech Republic, the maximum permitted content of risk elements in farmland are defined in the Decree of the Ministry of the Environment No. 153/2016 Coll., setting forth the details protection of agricultural land. Acceptable and maximum limits of risk elements according to the Decree of the Ministry of the Environment are given in following Tables 1-2.

Table 1 - Preventive values of risk elements according to Decree Ministry of Environment No. 153/2016 Coll.

Precautionary value of risk elements (mg*kg^{-1} of Dry matter)											
Soil category	**As**	**Be**	**Cd**	**Co**	**Cr**	**Cu**	**Hg**	**Mn**	**Ni**	**Pb**	**V**	**Zn**
Common soil	20	2	0.5	30	90	60	0.3	1200	50	60	130	120
Light soil	15	1.5	0.4	20	55	45	0.3	1000	45	55	120	105

Table 2 - Indicating values beyond which may be compromised food safety, feed, animal and public health according to Decree Ministry of Environment No. 153/2016 Coll.

Limit values of risk elements (mg*kg^{-1} of Aqua regia/Dry matter)							
Conditions	**As**	**Cd**	**Ni**	**Pb**	**Hg**	**Cu**	**Zn**
pH < 6.5	-/40	1.5/20	150/-	300/400	1.5/20	200/-	400/-
pH > 6.5	-/40	2/-	200/-	300/400	1.5/20	300/-	400/-

2.2   Mobility of risk elements in soil

Mobility and availability of risk elements is controlled by several soil processes. This is a particular deal of chemical and biochemical processes such as: precipitation, dissolution, adsorption, desorption, complexing, dissociation, oxidation and reduction (He et al., 2005). In connection with that, Kabata-Pendias (2011) mentions binding organic compounds, occlusion, diffusion in to the grid of minerals, uptake by organisms, volatilisation and transportation. Chemical reactions in the soil are very important for controlling the release elements in the soil and gives information about the deficiency or excess of the particular element (Violante et al., 2010).

Risk elements in soil can be divided into: soluble (H_2O), exchangeable, bound (O, C, organic matter) and residual forms (Adjia et al., 2008). The water-soluble and the exchangeable fractions are considered as bioavailable. Then, the oxygen fraction, the carbonate fraction and fraction bound into organic substances may be potentially bioavailable. Finally, the residual fraction is not available for any plants or soil microorganisms. Proportion of fractions differ depending on the soil type and determine mobility and availability of risk elements in soil (He et al., 2005). As described by Tlustoš et al. (2007), mobility of risk element decrease in the following order:

Cd> Zn> Ni> Mn> Cu> Pb> Hg

Tlustoš et al. (2007) are consistent with Kabata-Pendias and Pendias (2001) who reported Cd among other treace metals as the most mobile in the soil. Its mobility is most influenced by the

pH value of the soil while the pH 4.5 – 5.5 is the best condition for it (Kabata-Pendias and Pendias, 2001).

2.2.1 Cd in soil

The content of cadmium in soil vary from low values, characteristic for uncontaminated material, to the high values in material which historically received a large amount of this element in industrial and agricultural activities (Traina, 1999). However, generally it can be expected less than 1 mg*kg^{-1} of Cd in the most soil types (Alloway and Steinnes, 1999). Within the soil profile, usually the highest content of Cd is in surface horizons as a result of circulation through vegetation, application of a fertilizers containing Cd or soil adsorption of organic matter. As reported by Alloway (1995), the main source of this element in soil are: a parent material, atmospheric deposition, phosphate fertilizers, sewage sludge and manure from stables. According to Jansson (2002) Cd may occur in three forms in soil environment: as a **solid precipitate**, precipitate sorbet to the **solid soil particles** and particles **dissolved in the soil solution.**

Solid precipitate

Cadmium may form precipitates in soil with CO_3^{2-}, S_2^-, PO_4^{3-}, OH^-. However, in most arable lands the redox potential is too high and conversely pH value, Cd concentration and presence of anions too low for the formation of any precipitates (Christensen and Huang, 1999).

Solid soil particles

To the soil, Cadmium binds in two ways: by complexing or adsorption (Adriano, 2001). As **complexes**, it can be bound to organic matter or to OH^-, groups of surface ends oxides and clay minerals. These reactions are highly dependent on pH and in this case, need of pH is lower. On the other hand, **adsorption** of Cd to OH^- is stronger if pH value is higher (Jansson, 2002). Beyond pH, sorption on solid soil particles depends on other factors as for example, clay content, organic matter content, the amount of Al^-, Fe^-, Mn^- oxides or competition and cationic complexation with ligands in the soil solution (Cox, 2000). Because Cd is absorbed mainly to clay minerals with large share of permanent negative charges, Cd desorption is possible with presence of Ca_2^+, Zn_2^+ or H^+ in soil solution.

<u>Dissolved in the soil solution</u>

The main form of Cd in soil solution is Cd_2^+ but it can also occur in these complex ions: $CdCl^+$, $CdOH^+$, $CdHCO_3^+$, $CdCl_3^-$, $Cd(OH)_3^-$, $Cd(OH)_4^{2-}$ which are particularly dominant in the soil solutions of arable soils and soils treated with sludge (Alloway, 1995). It can form organic complexes as well although their share is relatively small (Alloway, 1995; Kabata-Pendias and Pendias, 2001). From the foregoing that the total concentration of Cd in the soil solution depends on both the sorption potential of soil and on the content of ligands (Jansson, 2002). While the content of soil solution is very important because plants absorbs Cd and other trace elements right there.

2.2.2 Pb in soil

Lead is among the elements that have been most extensively used by man over time. This has led to extensive pollution of surface soils on the local scale, mainly associated with mining and smelting of the metal and addition of organic Pb compounds to petrol. Other sources of soil Pb pollution are shooting ranges and sewage sludges. Release of Pb to the atmosphere from various high-temperature processes has led to surface contamination on the regional and even global scale. Lead is particularly strongly bound to humic matter in organic-rich soil and to iron oxides in mineral soil, and is rather immobile in the soil unless present at very high concentrations (Alloway, 1995). Transfer of Pb from the soil to green parts of plants is generally small, except in cases with extensive surface soil concentration (Kabata-Pendias and Pendias, 2001). Metallic lead is oxidized to more mobile forms in the environment. It is these subsequent oxidation products that determine the mobility of lead at shooting ranges and not the metallic lead itself. The rate of oxidation and the type of resultant oxidation products are highly variable and site-specific (Sherene, 2010).

<u>Solid soil particles</u>

Organic matter is important for the retention of metals by soil solids, thus decreasing mobility and bioavailability. However, because of the complexation of metals by soluble OM, the addition of OM can result in release of metals from solids to the soil solution (Sherene, 2010).

<u>Dissolved in the soil solution</u>

The dissolution and mobility of lead derived from lead bullets and shot are ultimately dictated by a number of geochemical processes including oxidation/reduction, precipitation/ dissolution, adsorption/desorption, and complexation/chelation (Sherene, 2010). The solubility of Pb in soil

solution is pH dependent, increasing as the pH is adjusted from 6 to 3. At near neutral pH, the activity of Pb^{2+} shows small but significant increase of solubility resulting from changing organic matter content. In the near neutral pH range, higher soil organic matter increases the dissolved organic matter, thereby promoting the formation of organo-Pb complexes and increasing Pb solubility (Sauve, 1998).

The dissolution of humic acid at higher pH is also responsible for dissolution of Pb from soil. The increase in the solubility of the Pb is related to the dissolution of the humic acid component of the organic matter. This indicates that dissolution reactions with organic matter dominate the partition of metals at higher pH because under high pH, the dissolved organic matter increases as a result of solubility of humic acid (Sherene, 2010).

2.2.3   Zn in soil

Zinc is naturally present in all soils in typical background concentrations $10 - 100$ mg*kg^{-1}. Human activities have enriched topsoil with Zn through atmospheric depositions, fertilization and sewage sludge application. Zinc contaminated soils with negative impact on the soil ecosystem are found around Zn smelters, near Zn mining sites and under galvanized structures (Alloway, 1995).

Zinc toxic soils are less widespread than deficient ones. Risk of Zn toxicity is manifested by effects on soil dwelling organisms, i.e. plants, invertebrates and soil microorganisms (Sherene, 2010). Toxic effects are identified at total Zn concentrations $100 >1,000$ mg*kg^{-1} and toxicity decreases with increasing soil cation exchange capacity (CEC). Risk assessments of Zn have proposed maximal additions as low as 26 mg*kg^{-1} in the EU to maintain soil ecosystem structure and function (Alloway, 1995).

Dissolved in the soil solution

The solubility of Zn in soils is almost invariably controlled by sorption reactions (Kabata-Pendias and Pendias, 2001). Pure Zn minerals (carbonates, silicates, hydroxides) have been detected at high total soil Zn concentrations ($>1,000$ mg*kg^{-1}) but are rarely controlling Zn solubility. Zinc is specifically sorbed as Zn^{2+} on pH-dependent binding sites of oxyhydroxides and organic matter and, at high concentrations, by ion exchange reactions on clay minerals. In general, soil solution Zn concentrations increase fivefold per unit pH decrease (Alloway, 1995; (Kabata-Pendias and Pendias, 2001). Zinc deficiency for agricultural crops is found in about 1/3 of worldwide soils due to low total Zn concentrations and high pH. Soils containing less

than 0.5 mg*kg^{-1} diethylenetriaminepentaacetic acid (DTPA) extractable Zn are potentially Zn deficient (Alloway, 1995).

2.3   Elements uptake by plants

The main source of risk elements for plants is their growth medium - rhizosphere, where through the root system risk elements penetrate into the plants (Kabata-Pendias and Pendias, 2001). Nevertheless, their uptake varies depending on the element species, growing species of plant, the growing stage and on soil and climate conditions also.

Plants usually take the form of elements that are dissolved in the soil solution, especially in the form of ions, chelates or complexes. The main principles of risk elements income have been summarized by Kabata-Pendias and Pendias (2001) as follows:

- Usually it takes a place at very low concentrations of metals in solution.
- It is dependent on the concentrations of elements in solution.
- It is highly dependent on the concentration of H$^+$ and other ions in solution.
- Its intensity varies depending on the plant species and stage of development.
- It is dependent on environmental factors such as temperature, aeration of the soil and the redox potential.
- Receipt may be selective for particular ions.
- Adoption of some metals can proceed against the concentration gradient.
- Mycorrhiza play an important role.

Uptake of elements through the rhizosphere can be both **passive** (non-metabolic) and **active** (metabolic) and its intensity positively correlates with the concentration of acceptable elements near the root surface. Passive income means diffusion of ions from an external solution to the root endodermis. Meanwhile, active income requires metabolic energy and is done against a concentration gradient.

A mechanism for receiving the individual elements differs: Pb and Ni are preferably accepted by non-metabolic way, whereas Cu, Mo, and Zn are actively taken up. If biological and structural properties of root cells are disturbed, all components are taken passively. This phenomenon occurs if the concentration of the elements is above the threshold value for a physiological barrier (Kabata-Pendias and Pendias, 2001). After reaction of risk elements with plant-excreted organic acids (leads to formation of chelates in the immediate proximity of the

roots), the diffusion gradient increase and element uptake speeds up. Elements penetrate to the plant roots by diffusion through the epidermis and further through cortex up to xylem. A part of the elements is bound by a non-metabolic fixation on the negative charges of the cell walls or is passing through the cell wall and is further transported into cells. The second part is directly transported by apoplast. Interaction of genotype and element itself determines if element is transported or bound (Procházka et al., 1998; Cibulka et al., 1991).

Generally, in plants, elements are transported by xylem, phloem, stored or immobilized. But mobility of the elements in plant tissues is controlled by factors such as pH, redox status, cations competition, hydrolysis, polymerization, the formation of insoluble salts and also it depends on element itself (Kabata-Pendias and Pendias, 2001). By mobility in plants Alloway (1995) divided risk elements into three groups: Easily movable – Mn, Zn, Cd, Mo, Se; Medium movable – Ni, Co, Cu; The least movable – Cr, Pb, Hg. Several years later, mobility has been interpreted by Kabata-Pendias and Pendias (2001) differently: Easily movable – Ag, B, Li, Mo, Se; Medium moveable – Mn, Ni, Cd, Zn; The least moveable – Co, Cu, Cr, Pb, Hg, Fe. Even the least mobile elements he defines as elements strongly bound in the root cells. Huang et al. (2009) states that the response of plants to soil contamination is directly dependent on its cultivar. For example: hard wheat, sunflower and flax are considered as crops highly accumulating Cd to compare with spring wheat, barley, corn or oats (Grant et al., 1999).

Israr et al. (2011) demonstrated that the uptake of the metals is influenced by soil content, if the soil is contaminated with one risk factor or by multiple risk elements. For instance: treatment Pb + Cu increased Pb uptake and vice versa uptake of Cu above-ground decreased compared to treatment only by individual metals. Similarly, it was with treatment Cu + Ni, where uptake of Ni increased and uptake of Cu decreased. Interesting point is, in combination of Cu + Zn treatment uptake of both metals increased above-ground but in roots were differences. When all metals were presented (Pb, Cu, Ni, Zn), the content of elements in above-ground parts and roots was significantly lower to compare with the presence of various metals or their combination in two (Stewart and Halley, 1999; Zheljazkov et al., 2006).

2.4 Soil biostabilization

Land pollution due to past mining and processing activities is a major environmental issue in many European countries. According to Alvarenga et al. (2009), for the remediation of soils affected by mining activities three different strategies can be adopted:

1) Digging out the polluted soil and replacing it by unpolluted soil.
2) *In situ* or *ex situ* treatment of the soil by means of chemical or physical techniques.
3) *In situ* immobilization of metals and subsequent revegetation of the area.

The first one is generally carried out *ex situ*, and simply soil structure deterioration and high costs, which limits their use on vast contaminated areas. Second and third remediation techniques of heavy metal-contaminated soils are based on the extraction or the stabilization of the contaminants (Pérez-de-Mora et. al, 2005).

Stabilization techniques are carried out *in situ* and are less expensive; as published by Barceló and Poschenrieder (2003), conventional procedures raise the average cost per contaminated hectare of soil from 0.27 to 1.6 million $, while phytoremediation costs from about 10 to 1,000 times less. Then, soils can naturally reduce mobility and bioavailability of heavy metals as they are retained in soil by sorption, precipitation and complexation reactions. This natural remediation of metals could be accelerated by the addition of a range of soil amendments (see Table 3) which contribute to the reduction of their bioavailability through a combination of mechanisms (Bolan et al., 2003). Phytostabilization also improves the chemical and biological characteristics of the contaminated soil by increasing the organic matter content, nutrient levels, cation exchange capacity and biological activity (Arienzo et al., 2004).

Although revegetation is desirable, mine contaminated soils or metalliferous wastes are very unfavourable environments for plants due to the presence of many growth limiting factors, in particular residual high levels of heavy metals, soil acidity, lack of organic matter and its associated nutrients, and poor substrate structure with only skeletal materials (Tordoff et al., 2000; Johnson, 2003; Wong, 2003). That is why, the use of organic bioremediated residues like sewage sludge, manure, compost or DOR is increasingly being considered in land rehabilitation (Walker et al., 2004; Brown et al., 2005; Pérez-de-Mora et al., 2006).

Organic residues play three major roles when applied to mine soils:

1) Improvement of the physical nature of the rooting medium, especially improving water and nutrient holding capacity.
2) Supply of plant nutrients in a slow release form, facilitating plant establishment.
3) *In situ,* chemical immobilization of metals, reducing metal leachability and phytotoxicity (Tordoff et al., 2000).

A vegetation cover further improves the chemical and biological characteristics of the contaminated soil by increasing the organic matter content, nutrient levels, cation exchange capacity and biological activity, allowing the creation of a self-sustaining ecosystem (Pérez-de-Mora et al., 2005, 2006). Plants cover also prevents wind-blow of contaminated particles and reduces water pollution by interception of a substantial proportion of the incident precipitation (Tordoff et al., 2000).

Soil enzymes, microbiological and biochemical status of a soil, have been reported to be highly sensitive to heavy metals, therefore, have been recommended as standard biochemical indicators to assess quality of heavy metal polluted soils (Tejada et al., 2006; Hinojosa et al., 2008). In fact, they may indicate the potential of a soil to sustain microbiological activity, which can be used to assess the effectiveness of a soil remediation process (Pérez-de-Mora et al., 2005, 2006; Tejada et al., 2006).

Ecotoxicity tests are able to measure the bioavailability of the contaminants and their effects on the soil community, and are recommended in a complete soil quality assessment (Conder et al., 2001; van Gestel et al., 2001; Leitgib et al., 2007). The tests using aqueous soil extracts can also be used to assess soil toxicity (van Gestel et al., 2001; Loureiro et al., 2005; Leitgib et al., 2007; Alvarenga et al., 2008a,b; Antunes et al., 2008). Because they are based on the assumption that chemical compounds present in the aqueous phase affect soil organisms.

Table 3 - Soil amendments in phytostabilization, suggested applicable to metal contaminants, and possible modes of contamination inactivation.

Amendment types		Possible target contaminants	Suggested mode of inactivation
Phosphate materials	H_3PO_4, apatite, calcium orthophosphates, Na_2HPO_4, KH_2PO_4, other phosphate fertilizers, high-phosphate by products (e.g. bone meal)	**Pb**	Formation of insoluble metal phosphate minerals, such as Pb pyromorphites
Hydrous Fe oxides	Iron rich by-products containing Fe oxides, isolated hydrous Fe oxides	**As, Cd, Cu, Ni, Pb, Zn**	Sorption of contaminants on oxide surface exchange sites, corprecipitation, or formation of contaminants – Fe compounds
Organic materials	Manure, composts, sludges and other biosolids	**As, Cd, Cu, Pb, Zn**	Sorption of contaminants on exchange sites, or incorporation into the organic materials
Inorganic clay materials	Synthetic zeolites, natural aluminosilicates, or aluminosilicates by-products from burning or coal refuse (e.g fly ash)	**As, Cd, Cu, Mn, Ni, Pb, Zn**	Sorption of contaminants on mineral surface exchange sites, or incorporation into the mineral structure

SOURCE: BOLAN ET AL., (2003)

2.5 DOR

In the Mediterranean region, olive oil production is one of the most important agro-industrial activities. Approximately 98% of global olive oil production is concentrated in this region. In 2013/2014, annual harvest yielding was 1,536,000 tons of olive oil (International Olive Council). In connection with, a large quantity of solid and liquid waste is produced, which needs to be handled properly because the accumulation or incorrect disposal of such waste may cause serious environmental problems (Sampedro et al., 2007b). One of the most widely used olive-mill extraction system is two-phase or Three-phase centrifugation (see Figure n.1). In addition to the oil, it generates a pomace waste "alpechín" (olive mill wastewater – OMW) and wet waste product commonly called "alpeorujo". A second centrifugation of alpeorujo results in an extra oil yield and a new by-product that is dried and extracted with solvents, producing a final solid waste called dry olive-mill residue (DOR). Due to its high organic and inorganic nutrient content, DOR has great potential as fertilizer (Sampedro et al., 2007a).

Unfortunately, DOR in a raw form contains phytotoxic compounds able of inhibiting the growth of microorganisms and thereby growth of plants (Sampedro et al., 2004; Saparrat et al., 2010). Consequently, DOR needs to be remediated before being applied to agricultural soils as an organic fertilizer. According to Sampedro et al. (2004; 2007a) promising approaches for DOR detoxification is bioremediation with saprobe fungi.

Figure 1 – Three-phase and two-phase olive oil extraction systems.
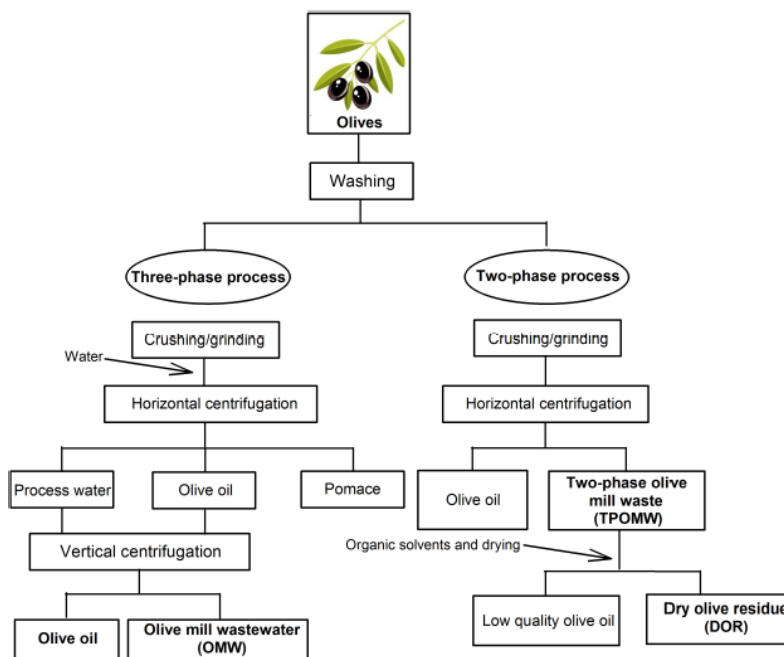
2.5.1 Biotransformation of DOR

Saprophytic fungi can be described as organisms that drives their nutrition from non-living or decaying organic matter. In general, they represent an effective enzymatic system for the degradation of a wide range of pollutants (Ballesteros, 2006), including phenolic compounds which are the main determinants of the phytotoxic effects of DOR (Sampedro et al., 2004, 2007b).

The ability of saprophytic fungi to bioremediate DOR is due to the presence of extracellular enzymes, more specifically laccase and peroxidase (Sampedro et al., 2004; Aranda et al., 2006, 2007). It has been demonstrated that a relationship is between the production of laccase by the saprobe fungus *Coriolopsis rigida* and degradation of phenolic compounds in aqueous extracts of DOR (ADOR) (Saparrat et al., 2010; Aranda et al., 2006, 2007). In addition, previous studies showed that the production of hydrolytic enzymes by the saprophytic fungus *Penicillium chrysogenum*-10 leads to decrease a phytotoxicity in ADOR (Aranda et al., 2004). Related to this, Reina et al. (2013) have demonstrated white-rot fungi as beneficial in the bioremediation of DOR due to the presence of extracellular enzymes, namely laccase and peroxidase.

Fundamentally, the inoculation of DOR with different species of fungi involves the transformation and stabilization of organic material accompanied by an increment in humifaction ratio, a decline in C:N rates, lower phenol content, alkalization and decrease the residue´s phytotoxicity (Sampedro et al., 2007b, 2009). Unfortunately, the application of saprobic fungi requires large amount of inoculum and long incubation time these days (Reina et al., 2013). On the other hand, there are some studies which demonstrate new inoculation media which can accelerate the process of phenol degradation and within 4 weeks shows significant results.

3 Methodology

3.1 Preparation of samples and their characteristics

As a soil for the incubation experiment was used contaminated Fluvisol from Trhové Dušníky village, approximately 60 km SW of Prague (Czech Republic), district of Příbram (see Figure n.2), where the smelter has been operating since 1786. Anthropogenic contamination of soil in surrounding area of the river Litavka originates from two main sources. Emission outputs from metallurgical industry is first source and then the more important one is represented by periodic floods of polluted water from metallurgical setting pits (Vaněk et al., 2005).

Figure 2 - Location of the soil sampling area.

This soil was sampled at a depth of 20 cm and homogenized immediately then sieved through 5 mm diameter mesh and stored at room temperature until the beginning of the experiment.

3.2 Biological transformation of DOR

The dry olive residue (DOR) sample used in this research was supplied by olive oil manufacturer Sierra Sur S.A. (Granada, Spain). According to characteristics described by Siles et al. (2014 a, b, c) the DOR was sieved and autoclaved in three cycles before use and then it was stored at 4°C.

Four species of fungi were used for the biotransformation of DOR: *Penicillium chrysogenum* (CAST-10 collection EEZ-10), *Funalia floccosa,* formerly known as *Coriolopsis floccosa* (Spanish Type Culture Collection, CECT 20449T), *Bjerkhandera adusta* (collection DSMZ, nº accession 23426), and *Chondrostereum purpureum* (collection DSMZ, nº accession DSM-4894). The fungi were pre-cultured on 2% MEA plates for 2 weeks at 24°C to obtain fresh inoculum.

The biological transformation of DOR was performed in solid state fermentation (SSF). Following the procedure described by Reina et al. (2013) the fungi were initially pre-cultured in barley–based media for 1 week. Then, fresh inoculum of each fungi was mixed into a flask with sterilized DOR in a 1:1 ratio (DOR: barley-based media, (w/w)) and were incubated at 28ºC for 4 weeks. Non-inoculated barley-based media and sterilized DOR samples were also incubated as well as inoculated one but those was used as controls later. The incubation was heat-inactivated by autoclaving. The biotransformed DOR samples were sieved to 5 mm mesh and the rest of the barley seeds were manually removed. After sieving, the *P. chrysogenum*-10-transformed DOR (DORPC), *F. floccosa*-transformed DOR (DORFF), *B. adusta*-transformed DOR (DORBA) and *C. purpureum*-transformed DOR (DORCP) were stored at 4ºC until its use.

3.3 Experimental design and set up

For whole experiment was prepared the set of samples with varying time of treatment. During The experiment consisted of three factor of variation:

1) Non-addition or addition of biotransformed DOR.
2) Non-inoculation or inoculation of (AM) fungus, *Funileformis mosseae*.
3) Time of soil treatment.

Model pot experiment was provided where biotransformed DOR was applied as a potential immobilizing agent. An extremely risk elements (Cd, Pb and Zn) contaminated soil from former mining and smelting area was used. Spring wheat (*Triticum aestivum*) was cultivated in the pots where used four biotransformed DOR:

1) *Penicillum chrysogenum* – 10 - transformed DOR.
2) *Funalia floccosa* – transformed DOR.
3) *Bjerkandera adusta* – transformed DOR.
4) *Chrondosterum purpureum* – transformed DOR.

These transformations were applied to reach a concentration of 50 g*kg^{-1} and mixed with 5 g of mycorrhizal inoculum of *Funeliformis mosseae*. Pots with and without biotransformed DOR and *F. mosseae* were established and used as control. Four replicates were set up per treatment. Soil samples were collected after 1, 3, 7, 14, 21, 30 and 60 days of incubation in order to evaluate the mobility of risk elements and nutrients. Wheat plant biomass (shoot, root, grain), plant-available risk elements and nutrients were monitored after 30 and 60 days of growing.

In this experiment *Funneliformis mosseae* (Nicol. and Ger.) Gerdemann and Trappe (BEG 12; Banque Européenne des Glomales) from Rothamsted Experimental Station (UK) was used as AM fungus which inoculum was collected from a pot culture of Alfalfa (*Medicago sativa* L.). Whole sample was contained of soil, spores and mycelia and colonized root fragments (10 sporocarps g^{-1}, with 1-5 spores per sporocarp).

3.4 Analytical methods

3.4.1 Total risk elements and nutrients determination

To determine a total content of the several elements in each sample decomposing of soil and DOR samples was needed. It was done by using of pressurized wet ashing in this way: ~0.5 g of air-dried matter was weighted into an extraction vessel. The reaction mixture was decomposed with 10 ml of *Aqua regia* (1:3 mixture of 65% nitric acid and 30% hydrochloric acid) and after that mixture was heated in an Ethos 1 (MLS, Germany) microwave-assisted wet digestion system (see Figure 3) for 33 min at 210 °C.

Similarly, to determine the element contents of plant biomass (shoot, root and grain): Firstly, an aliquot of the plant sample ~0.5 g of dry matter was weighted in extraction vessel. Secondly, dry matter was mixed with 8.0 ml of concentrated HNO_3 and 2.0 ml of 30% H_2O_2 (Analytika Ltd., Czech Republic). After that, the reaction mixture was heated in an Ethos 1 (MLS GmbH, Germany) microwave-assisted wet digestion system for 30 min at 220 °C.

In both cases, the digest was quantitatively transferred into a 20 ml glass tubes after cooling and filled up to the mark with deionized water. Each decomposition was carried out in triplicate. The quantity of Cd, Cu, Fe, Mn, Pb, Zn, and P elements was analysed by inductively coupled plasma-atomic emission spectrometry (ICP-OES, Agilent 720, Agilent Technologies Inc., USA) equipped with a two-channel peristaltic pump, a Struman-Masters spray chamber and a V-groove pneumatic nebulizer made of inert material (spectrometry parameters were: power: 1.2 kW; plasma flow: 15.0 l*min^{-1}; auxiliary flow: 0.75 l*min^{-1}: nebulizer flow: 0.9 l*min^{-1}). For Ca, Mg, and K contents of the extraction was used flame atomic absorption spectrometry Varian 280FS (F-AAS, Varian, Australia) (with parameters air flow: 13.5 l*min^{-1}, acetylene flow: 2.2 l*min^{-1}, burner height: 13.5 ml, nebulizer uptake rate: 5 ml*min^{-1}).

Figure 3 - Ethos 1 (MLS, Germany) - microwave-assisted wet digestion system: a) an appearance of apparatus, b) detail of inner rotor, c) detail of inner process inside of tube.

3.4.2 Available elements determination

To determine a ration of the bioavailable elements was weighted 0.5 g of each sample into a sealable plastic tube. Thereafter, 10 ml of 0.11 mol$*$l^{-1} solution of CH_3COOH was added, mixed and overnight shaken (Quevauviller et al., 1993). At the end of extraction, each of sample was centrifuged in a Hettich Universal 30 RF (Germany) at 3000 rpm (i.e. 460 g) for 10 min. With the greatest caution samples were taken out of centrifuge, by using a pipette supernatant was transferred into a glass tube and stored at 6°C before inductively coupled plasma-atomic emission spectrometry analysis (ICP-OES), same parameters as above (see Figure 4).

Figure 4 - ICP-OES, Agilent 720 used for plasma-atomic emission spektrometry.
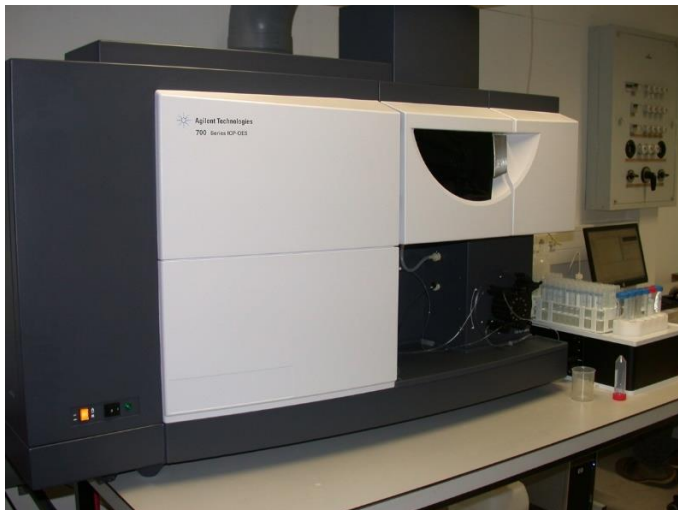
3.4.3 Determination of pH and CEC

Soil pH was determined in water extract. Tare weighted 1g of soil and 10 ml of deionized water (DI H_2O) was mixed in plastic tube, closed properly and left to shake for 1 hour. Next, quantification of pH by electrode probe was done.

For calculation of cation exchange capacity (CEC) was needed to weight 1g of soil and mixed it with 20 ml of 0.1 mol*l^{-1} $BaCl_2$ [1:20 (w/v)]. Mixture was shaken for 2 hours and finally centrifuged 3,000 rpm over 10′ (ISO, 1994). Obtained supernatant was collected in glass tubes and stored at 4°C until determination by titration (Schwertfeger et al., 2008). Total CEC was calculated as a quantitative sum of Ca, Mg, K, Na, Fe, Mn, Al contained in the extract.

3.5 Statistics

Analytical data were processed using a statistical software Statistica 12 (StatSoft, USA). One-way analysis of variance (ANOVA) was used with $\alpha = 0.05$ as the criterion for significance followed by the Tukey's test for:

- Assessment of the potential differences between the biotransformed DOR treatments.
- Definition of the differences between inoculated and non-inoculated variants.
- Analysis of the changes according to time of soil treatment.

Pearson's correlation coefficient was used to assess if changes in the mobility of risk element in soils might be associated with the changes in soil pH ($\alpha = 0.05$).

4 Results

4.1 Detailed description of the experimental soil

As described in procedure above, the determination of total physicochemical parameters of the soil was done. Results below, in the Table 4, see slightly acidic pH, relatively low CEC value and adequate nutrient status of the soil severely polluted by Pb, Cd and Zn due to mining and smelting activities. According to Decree Ministry of Environment No. 153/2016 Sb. characterizing the conditions for the protection of the agricultural soil quality in the Czech Republic, researched soil Cd and Zn values exceeds security border and represents the potential risk for crop contamination. In case of Pb, value exceeds even amount which can lead to damage of the animal and human health.

Table 4 - The main physicochemical characteristics of the experimental Fluvisol: data are presented as mean ± standard deviation, n=3.

pH	6.65±0.05	K (%)	0.30±0.10
CEC (mmol/kg)	149±3	Mg (%)	0.20±0.01
C (g/kg)	38.5±0.7	Ca (%)	0.23±0.01
N (g/kg)	3.13±0.04	Fe (%)	1.56±0.04
C/N	12.3±0.1	Mn (%)	0.17±0.01
TOC (g/kg)	17.1±0.4	Zn (%)	0.19±0.01
$N-NO_3^-$ (mg/kg)	117±10	Pb (%)	0.16±0.01
$N-NH_4^+$ (mg/kg)	0.70±0.08	Cu (mg/kg)	30.3±2.5
P (%)	0.03±0.01	Cd (mg/kg)	13.7±1.4

4.2 Detail description of biotransformed DOR

Table 5 shows the properties and nutrient status in the individual DOR samples. The results confirmed increasing pH of the biotransformed DOR samples against the non-transformed DOR. Biotransformed DOR samples represents significantly higher content of total N then non-transformed DOR. The total C contents remained unchanged as well as total organic carbon (TOC) levels which even decreased in DORPC sample. About levels of other nutrients like P, K, Mg, and Ca was not possible to predict any trend but generally both, non-transformed and biotransformed DOR, are good sources of the nutrients in soil.

*Table 5 - **The main physicochemical characteristics of the individual biotransformed DOR samples:** data are presented as mean ± standard deviation, n=3.*

	DOR	DORPC	DORFF	DORBA	DORCP
pH	4.69±0.01	6.50±0.02	5.60±0.02	5.28±0.01	6.93±0.01
CEC (mmol/kg)	647±20	518±16	512±11	499±11	611±13
C (g/kg)	463±5	472±3	472±2	470±2	462±3
N (g/kg)	14.0±0.9	22.1±0.5	22.8±1.1	27.7±0.1	28.2±1.4
C/N	33.3±2.1	21.4±0.4	20.8±0.9	17.0±0.1	16.4±0.8
TOC (g/kg)	150±3	131±7	152±3	155±3	160±4
P (mg/kg)	939±39	985±32	1033±36	1478±76	537±20
K (mg/kg)	7121±111	6728±613	6946±324	7928±613	8047±709
Mg (mg/kg)	47.6±3	35.60±0.7	45.13±1.8	57.6±2.5	32.0±1.7
Ca (mg/kg)	1247±91	1398±26	1553±48	1448±82	1676±71

4.3 Changes of pH value

Figures 5,6 represent the changes in soil pH after application of biotransformed DOR. The AM fungi inoculation seemed to be the most important factor affecting the soil pH. In comparison, regardless to biotransformed DOR application, soil pH significantly increased already during the first day of incubation compared to non-inoculated variants.

Comparing the individual treatments in Figure 5, and Figure 6, changes were more evident after biotransformed DOR application (see the letters in Table 5,6) where occurred immediately. Without inoculation, the biotransformed DOR variants tend to increase the soil pH compared to inoculated DOR from 7th till 30th days of cultivation. Predominantly, no changes were observed in the combined treatments (biotransformed DOR inoculated treatments plus AM fungi) during the time of soil incubation. However, in the end of experiment, all the pH changes were balanced and no significant differences among the treatments were observed between control and treated variants in both cases.

Figure 5 - The effect of the individual <u>not</u> inoculated treatments in soil pH during the experiment: data expressed as mean (bar) and standard deviation (line segment); The bars marked by the same letter did not significantly differ at P <0.05 within the DOR treatments.
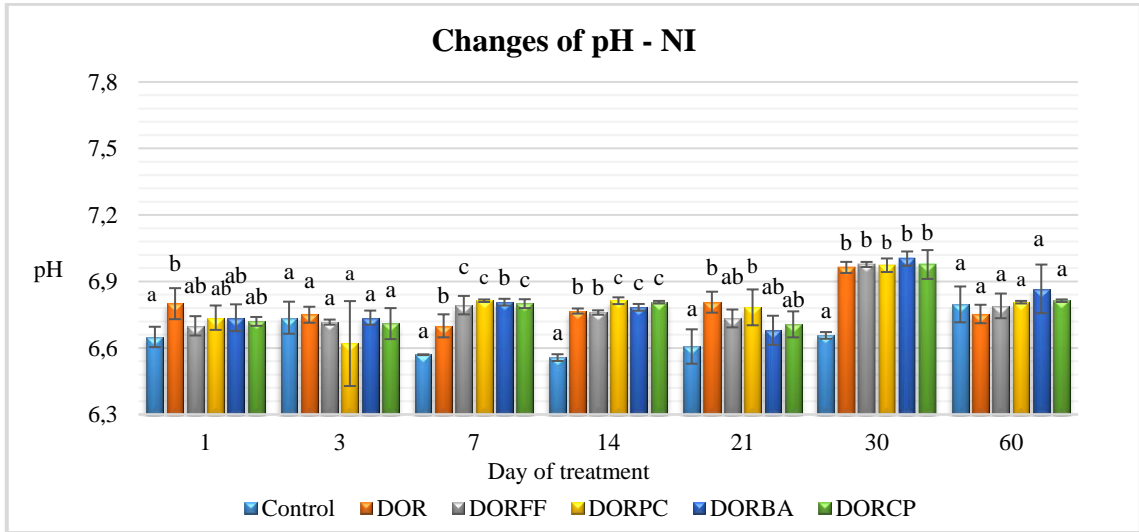
Figure 6 - The effect of the individual inoculated treatments in soil pH during the experiment: data expressed as mean (bar) and standard deviation (line segment); The bars marked by the same letter did not significantly differ at P <0.05 within the DOR treatments.
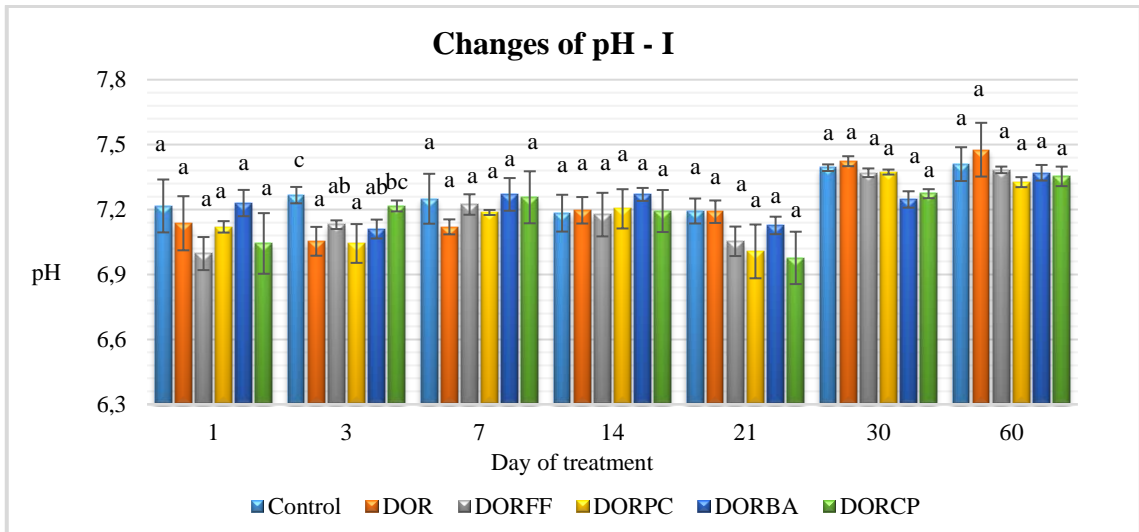
4.4 Changes in mobility of risk elements

The biotransformed DOR application into the soil resulted different alterations in mobility of risk elements documented by the Figures 7 – 12.

4.4.1 Mobility of Cd

In case of Cd, the mobility proportions increased after application of the not inoculated biotransformed DOR (Figure 7) compared to the inoculated biotransformed DOR (Figure 8), where mobility appeared to be unchanged.

When comparing the individual not inoculated treatments relative to control, significant increases were observed in: DOR – 7th and 30th day of treatment; DORPC – 1st and 30th day of treatment; DORBA – 3rd, 7th and 30th day of treatment; DORCP – 3rd, 7th and 30th day of treatment. Similarly, in the inoculated samples, the modest increases were demonstrated in: DORBA - 30th and 60th day of treatment and DORCP 30th day of treatment, compared to control. Difference between each combined treatment were not significant throughout the experiment except day 7, where not-inoculated samples shown slight increase in DORBA treatment against DORFF and DORPC.

Finally, after 60 days of incubation the mobility of Cd decreased below the primary observed level in all treatments.

Figure 7 - The cadmium concentrations in the treated soil extractable with 0.11 mol*l-1 acetic acid (mg*kg-1) during the experiment: <u>Not</u> inoculated; data expressed as mean (bar) and standard deviation (line segment); the bars marked by the same letter did not significantly differ at P <0.05 within the DOR treatments.
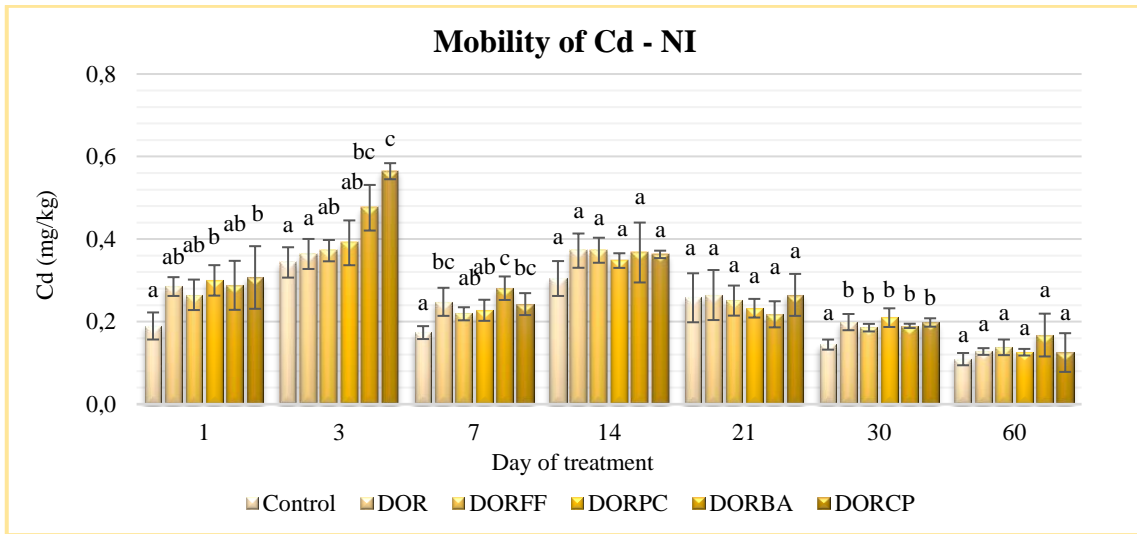
Figure 8 - The cadmium concentrations in the treated soil extractable with 0.11 mol*l-1 acetic acid (mg*kg-1) during the experiment: Inoculated; data expressed as mean (bar) and standard deviation (line segment); the bars marked by the same letter did not significantly differ at P <0.05 within the DOR treatments.
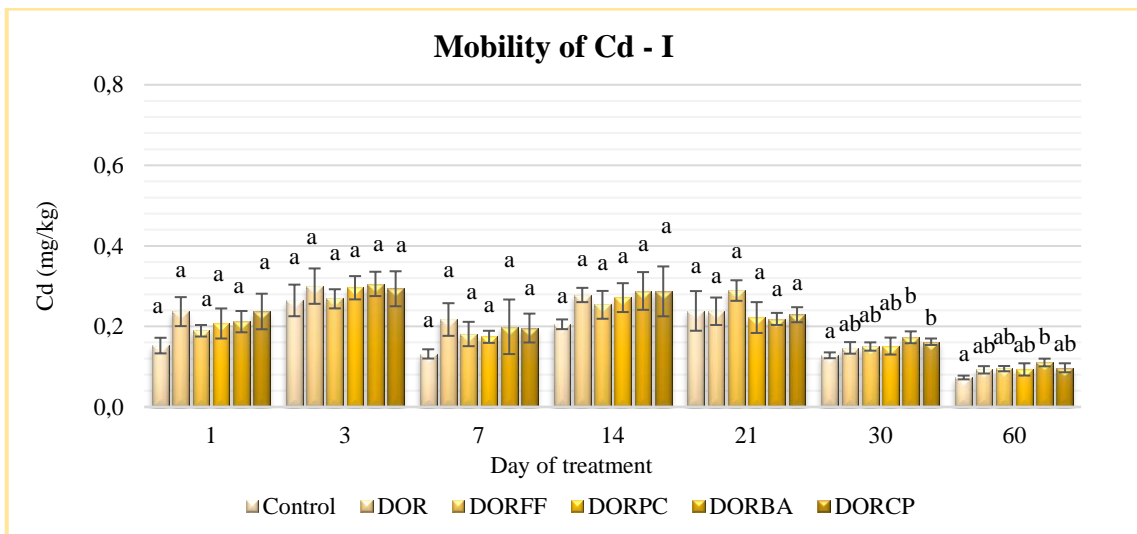
4.4.2 Mobility of Pb

In the case of Pb mobility, (Figures 9 and 10), the resulting effect was neutral, generally in both cases, not inoculated and inoculated biotransformed samples. Only not-inoculated samples shown slight difference between Control and DORPC at 30th days of incubation and between each combined treatment, increase of DORPC at 3rd day of incubation was observed.

Figure 9 - The lead concentrations in the treated soil extractable with 0.11 mol*l⁻¹ acetic acid (mg*kg⁻¹) during the experiment: Not inoculated; data expressed as mean (bar) and standard deviation (line segment); the bars marked by the same letter did not significantly differ at P <0.05 within the DOR treatments.
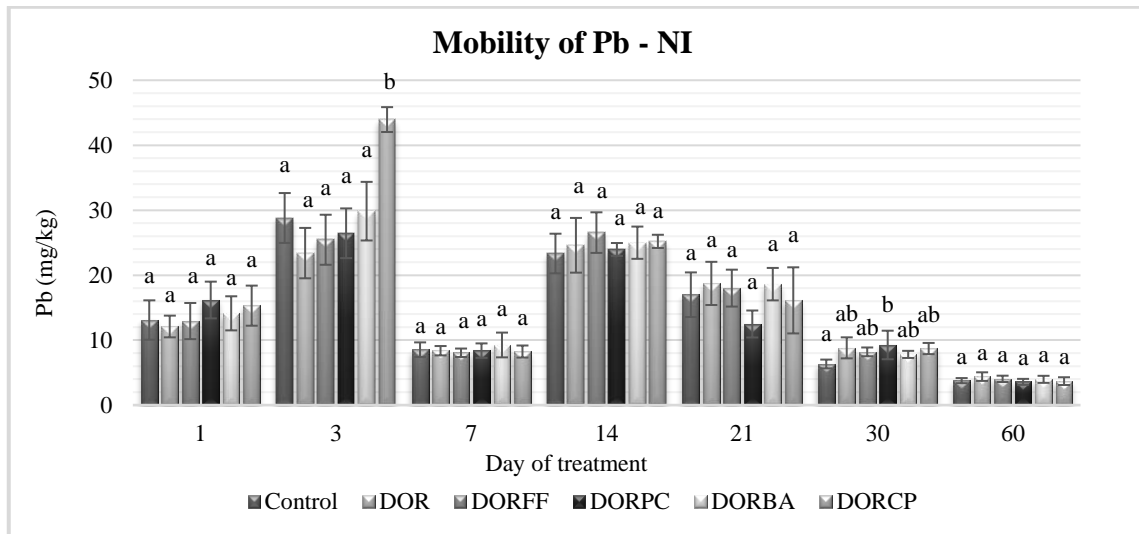

Figure 10 - The lead concentrations in the treated soil extractable with 0.11 mol*l⁻¹ acetic acid (mg*kg⁻¹) during the experiment: Inoculated; data expressed as mean (bar) and standard deviation (line segment); the bars marked by the same letter did not significantly differ at P <0.05 within the DOR treatments.
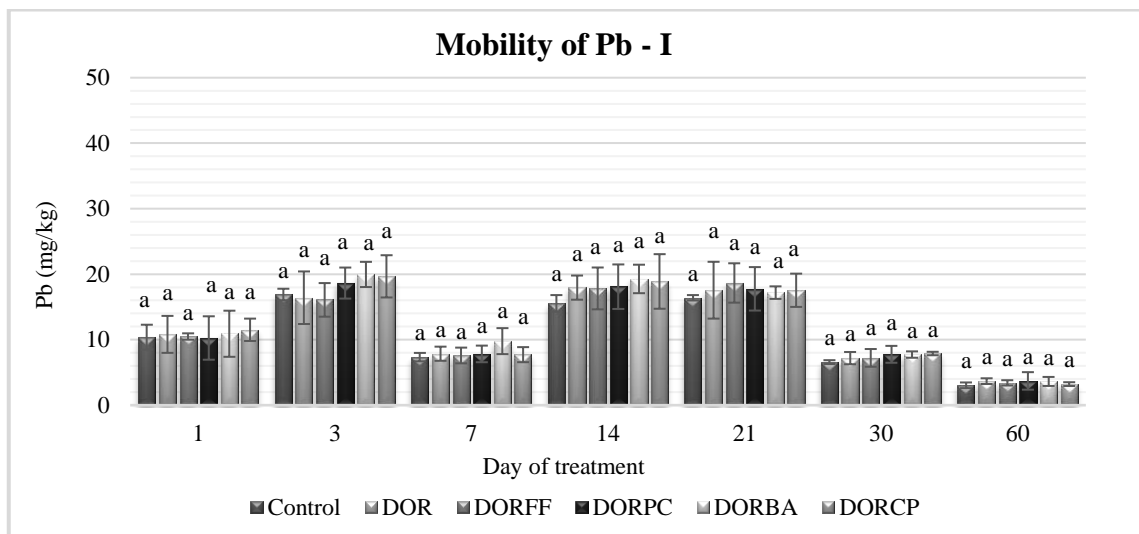
4.4.3 Mobility of Zn

In this experiment, application of biotransformed DOR, not inoculated and inoculated, had no effect on mobility of Zn (Figures 11 and 12).

Figure 11 - The zinc concentrations in the treated soil extractable with 0.11 mol*l⁻¹ acetic acid (mg*kg⁻¹) during the experiment: Not inoculated; data expressed as mean (bar) and standard deviation (line segment); the bars marked by the same letter did not significantly differ at P <0.05 within the DOR treatments.
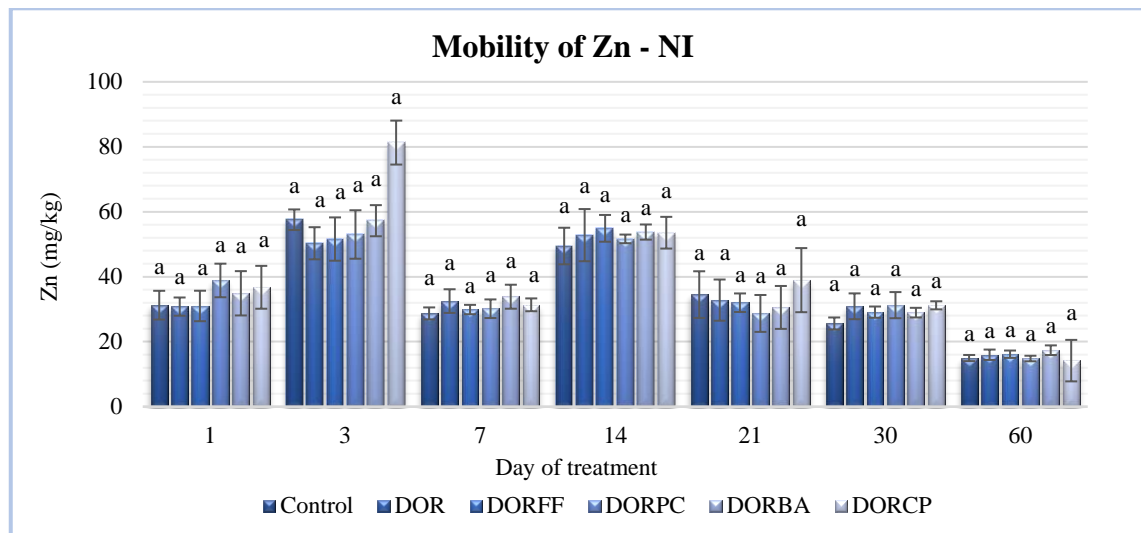
Figure 12 - The zinc concentrations in the treated soil extractable with 0.11 mol*l⁻¹ acetic acid (mg*kg⁻¹) during the experiment: Inoculated; data expressed as mean (bar) and standard deviation (line segment); the bars marked by the same letter did not significantly differ at P <0.05 within the DOR treatments.
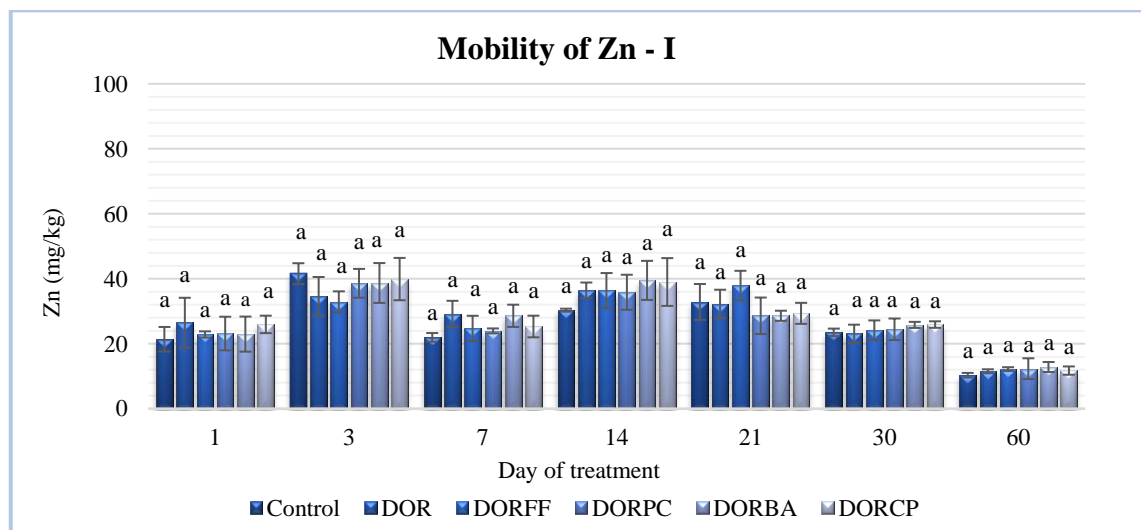
4.5 Bioavailable nutrients

Changes in the mobility of essential macro- and micronutrients after application of biotransformed DOR into the soil were seen immediately (Tables 6 - 12). Whereas the mobility of Fe, Mg and P remained unchanged, the application of biotransformed DOR tended to increase the K mobility in the biotransformed samples.

The AM fungi application decreased the mobility of most of the elements, except Ca and K compared to the not-inoculated variants. Alterations were observed for AM fungi inoculated and not inoculated variants regardless to DOR application. The AM fungi inoculated variants showed lower mobility of Cu and P, no difference was observed in the case of Fe, K and Mg, compared to not-inoculated ones. On the contrary, higher mobility was observed in the case of Ca and Mn, compared to not-inoculated variants.

Within the individual DOR treatments no difference occurred for Fe, Mg and Mn. Increasing mobility was determined for Cu, mostly due to DORBA and DORCP application, then for K, where all treatments responded almost without distinction, and for P, where increases were caused due to DORBA and DORCP as in case of Cu.

Generally, as documented by the following Tables (6 – 12), the mobility of all investigated elements varied during the cultivation regardless of the DOR application. Merely significant increase in mobile proportions of available soil K was observed during the whole experiment.

Finally, after 60 days of treatment, the element mobility significantly decreased for almost all the elements compared to day 1, except Ca and Mg where no distinctive changes in mobility of this elements were observed (Table 12). Moreover, the mobile proportions of the elements were stabilized and no significant differences were recorded for the variants not inoculated with AM fungi except Ca, where the only significant difference was found in the case of the Ca mobility compared to the control.

Table 6 - The element concentrations in the treated soil extractable with 0.11 mol*l⁻¹ acetic acid (mg*kg⁻¹) in the beginning of the experiment (1ˢᵗ day of incubation): *The averages marked by the same letter did not significantly differ at p <0.05 within individua columns, data are presented as mean ± standard deviation, n=4.*

Treatment	Ca mg/kg	Cu mg/kg	Fe mg/kg	K mg/kg	Mg mg/kg	Mn mg/kg	P mg/kg
				Not inoculated			
Control	186±18^a	0.699±0.043^a	155±26^a	101±10^a	28.7±3.4^a	7.66±2.32^a	6.33±0.71^a
DOR	279±37^b	0.940±0.102^{ab}	122±22^a	483±132^b	33.0±1.4^a	21.0±2.7^b	8.55±0.95^a
DORFF	224±11^a	1.11±0.26^{ab}	134±28^a	319±114^{ab}	30.6±3.3^a	8.72±1.72^a	8.68±2.20^a
DORPC	209±16^a	1.09±0.15^{ab}	175±31^a	363±101^{ab}	34.6±1.8^a	10.1±1.6^a	10.3±1.9^a
DORBA	202±9^a	1.35±0.44^b	151±29^a	306±114^{ab}	30.2±4.1^a	8.90±1.53^a	9.76±3.08^a
DORCP	225±31^a	1.30±0.45^{ab}	159±29^a	361±189^{ab}	32.6±3.1^a	10.3±2.4^a	8.49±2.20^a
				Inoculated			
Control	387±56^a	0.568±0.051^a	119±21^a	102±2^a	46.1±6.8^a	6.10±0.91^a	5.12±0.64^a
DOR	434±39^a	0.803±0.085^{ab}	119±30^a	370±70^b	57.4±9.3^a	9.78±1.30^b	7.14±1.40^a
DORFF	331±51^a	0.924±0.138^{bc}	119±4^a	294±10^b	43.1±5.8^a	6.49±0.20^{ab}	7.38±1.14^a
DORPC	381±96^a	0.905±0.099^{bc}	113±31^a	401±49^b	46.8±6.3^a	6.41±2.26^{ab}	7.66±1.18^a
DORBA	420±16^a	1.00±0.09^{bc}	111±32^a	281±44^b	50.1±4.3^a	7.58±2.46^{ab}	6.94±1.24^a
DORCP	414±45^a	1.10±0.24^c	127±8^a	356±82^b	49.2±4.3^a	7.51±1.36^{ab}	7.32±1.20^a

Table 7 -The nutrient concentrations in the treated soil extractable with 0.11 mol*l^{-1} acetic acid (mg*kg^{-1}) in the course of the experiment (3rd day of incubation): *The averages marked by the same letter did not significantly differ at p <0.05 within individua columns, data are presented as mean ± standard deviation, n=4.*

Treatment	Ca	Cu	Fe	K	Mg	Mn	P
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Not inoculated							
Control	217±5[a]	1.14±0.08[a]	308±24[a]	146±6[a]	43.4±1.6[a]	16.4±3.0[a]	12.1±0.7[a]
DOR	261±29[a]	1.22±0.10[a]	241±34[a]	406±72[b]	40.2±4.8[a]	16.1±2.6[a]	12.4±1.0[a]
DORFF	231±37[a]	1.38±0.23[a]	252±43[a]	317±52[b]	40.3±5.9[a]	14.4±2.3[a]	14.2±3.3[a]
DORPC	235±21[a]	1.44±0.22[a]	265±41[a]	356±85[b]	40.7±5.5[a]	14.8±2.1[a]	14.0±2.3[a]
DORBA	284±49[a]	1.98±0.23[b]	276±29[a]	386±75[b]	45.2±4.5[a]	19.1±4.1[a]	15.8±2.2[ab]
DORCP	251±53[a]	1.94±0.18[b]	430±37[b]	435±55[b]	57.2±1.4[b]	26.1±1.2[b]	19.3±1.8[b]
Inoculated							
Control	532±43[a]	0.871±0.203[a]	231±88[a]	151±31[a]	65.1±8.9[a]	12.3±6.6[a]	10.0±3.3[a]
DOR	500±49[a]	0.953±0.066[a]	165±36[a]	455±62[b]	58.7±7.0[a]	17.1±8.4[a]	9.79±1.57[a]
DORFF	465±69[a]	1.12±0.20[ab]	166±21[a]	376±64[b]	56.5±7.0[a]	9.06±1.37[a]	10.4±2.0[a]
DORPC	461±71[a]	1.14±0.09[ab]	200±25a	422±66[b]	61.4±6.6[a]	10.0±1.2[a]	11.7±1.5[a]
DORBA	518±33[a]	1.29±0.15[b]	197±31a	316±46[ab]	62.2±6.5[a]	11.6±1.0[a]	10.7±1.4[a]
DORCP	536±43[a]	1.11±0.13[ab]	207±33a	360±64[b]	65.7±4.3a	11.0±1.8[a]	10.4±1.5[a]

Table 8 - The nutrient concentrations in the treated soil extractable with 0.11 mol*l⁻¹ acetic acid (mg*kg⁻¹) in the course of the experiment (7ᵗʰ day of incubation): *The averages marked by the same letter did not significantly differ at $p < 0.05$ within individua columns, data are presented as mean ± standard deviation, n=4.*

Treatment	Ca	Cu	Fe	K	Mg	Mn	P
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
				Not inoculated			
Control	195±19^a	0.943±0.112^a	131±12^a	84±1^a	28.3±0.7^a	3.86±0.57^{ab}	6.77±1.03^a
DOR	240±50^a	1.18±0.13^{ab}	120±8^a	334±68^b	32.6±5.7^a	7.18±3.08^b	8.63±1.07^{ab}
DORFF	199±28^a	1.25±0.07^{ab}	119±7^a	266±63^b	30.1±3.5^a	4.04±0.31^{ab}	9.05±1.94^{ab}
DORPC	188±44^a	1.28±0.13^{ab}	122±13^a	272±70^b	27.8±4.4^a	3.70±0.64^a	9.08±1.23^{ab}
DORBA	241±23^a	1.70±0.17^c	128±19^a	267±28^b	33.8±1.4^a	5.01±1.43^{ab}	11.1±2.1^b
DORCP	198±29^a	1.51±0.27^{bc}	124±8^a	332±57^b	29.1±2.9^a	4.12±0.90^{ab}	8.76±1.04^{ab}
				Inoculated			
Control	468±47^a	0.676±0.026^a	115±6^a	118±7^a	57.2±5.3^a	3.36±0.41^a	6.55±0.66^a
DOR	409±77^a	0.995±0.162^{ab}	126±14^a	331±68^b	54.4±9.4^a	6.59±1.95^a	7.99±1.01^a
DORFF	413±66^a	1.04±0.18^{ab}	117±18^a	259±42^b	55.3±7.8^a	3.48±0.53^a	7.69±0.93^a
DORPC	339±29^a	1.12±0.12^b	116±7^a	289±31^b	45.8±3.2^a	3.75±0.73^a	7.94±0.73^a
DORBA	332±45^a	1.29±0.26^b	140±55^a	246±33^b	48.1±8.6^a	4.81±0.73^a	9.95±3.33^a
DORCP	381±89^a	1.20±0.28^b	118±11^a	337±66^b	50.4±9.7^a	3.83±0.75^a	8.13±1.50^a

Table 9 - The nutrient concentrations in the treated soil extractable with 0.11 mol*l⁻¹ acetic acid (mg*kg⁻¹) in the course of the experiment (14th day of incubation): *The averages marked by the same letter did not significantly differ at $p < 0.05$ within individual columns, data are presented as mean ± standard deviation, n=4.*

Treatment	Ca mg/kg	Cu mg/kg	Fe mg/kg	K mg/kg	Mg mg/kg	Mn mg/kg	P mg/kg
			Not inoculated				
Control	223±21[a]	0.987±0.081[a]	252±30[a]	113±9a	41.0±4.0a	14.5±1.9a	10.4±1.1a
DOR	219±32[a]	1.24±0.09[ab]	253±49[a]	319±62b	41.2±3.3a	17.7±1.6a	12.6±2.0a
DORFF	200±4[a]	1.34±0.20[b]	277±28[a]	276±59b	43.6±2.9a	16.4±2.9a	14.1±2.5a
DORPC	206±9[a]	1.21±0.12[ab]	256±16[a]	262±82b	40.9±1.5a	14.4±1.2a	12.5±1.0a
DORBA	190±29[a]	1.31±0.15[b]	265±68[a]	233±24ab	41.3±9.4a	15.9±4.6a	12.7±2.2a
DORCP	193±28[a]	1.34±0.08b	264±23[a]	256±18ab	40.6±4.1a	15.7±0.7a	12.6±1.1a
			Inoculated				
Control	450±76[a]	0.703±0.011[a]	166±4[a]	114±8[a]	59.5±10.0[a]	9.46±1.60[a]	7.53±0.38[a]
DOR	396±40[a]	0.878±0.018[ab]	185±15[a]	286±42[b]	54.9±5.7[a]	12.7±1.1[a]	9.25±0.54[a]
DORFF	433±54[a]	0.922±0.121[ab]	191±31[a]	245±50[b]	62.5±7.0[a]	10.8±2.3[a]	10.2±1.0[a]
DORPC	376±76[a]	1.03±0.13[b]	185±31[a]	262±42[b]	54.8±7.7[a]	11.1±1.7[a]	9.70±1.00[a]
DORBA	419±33[a]	1.05±0.19[b]	202±27[a]	242±44[b]	61.1±2.2[a]	11.9±1.0[a]	10.2±1.9[a]
DORCP	415±70[a]	1.09±0.18[b]	198±35[a]	276±67[b]	58.8±10.6[a]	11.7±3.3[a]	10.2±1.8[a]

Table 10 - The nutrient concentrations in the treated soil extractable with 0.11 mol*l^{-1} acetic acid (mg*kg^{-1}) in the course of the experiment (21st day of incubation): The averages marked by the same letter did not significantly differ at p <0.05 within individual columns, data are presented as mean ± standard deviation, n=4.

Treatment	Ca mg/kg	Cu mg/kg	Fe mg/kg	K mg/kg	Mg mg/kg	Mn mg/kg	P mg/kg
Not inoculated							
Control	222±63^{ab}	0.928±0.150^a	170±38^a	174±92^a	32.5±8.8^{ab}	10.6±3.0^a	8.87±1.92^a
DOR	313±41^{ab}	0.900±0.222^a	171±29^a	180±50^a	41.0±12.6^{ab}	12.2±2.5^a	9.35±1.69^a
DORFF	337±92^{ab}	0.870±0.111^a	167±22^a	196±82^a	42.5±11.1^{ab}	11.2±1.4^a	8.62±1.38^a
DORPC	187±16^a	0.975±0.044^a	126±20^a	187±29^a	25.7±3.9^a	7.61±1.42^a	8.57±2.33^a
DORBA	379±77^b	0.759±0.119^a	174±36^a	207±70^a	50.8±8.6^b	11.3±1.1^a	8.18±1.10^a
DORCP	207±30^{ab}	1.02±0.29^a	159±47^a	181±87^a	29.8±4.8^a	10.0±3.3^a	9.36±2.29^a
Inoculated							
Control	304±57^{ab}	0.963±0.406^a	164±11^a	189±63^a	40.2±13.7^{ab}	10.0±0.2^a	8.67±1.76^a
DOR	293±90^{ab}	0.818±0.195^a	168±30^a	180±78^a	38.5±7.2^{ab}	11.0±3.1^a	8.14±1.54^a
DORFF	169±14^a	1.07±0.09^a	186±27^a	230±16^a	29.5±2.9^a	11.4±2.2^a	10.4±1.0^a
DORPC	447±82^b	0.726±0.109^a	164±34^a	252±64^a	58.8±9.3^b	11.3±2.7^a	8.65±0.15^a
DORBA	377±31^b	0.760±0.016^a	158±10^a	185±31^a	46.2±2.7^{ab}	10.3±0.8^a	7.68±0.50^a
DORCP	360±80^{ab}	0.797±0.091^a	159±15^a	252±72^a	44.7±8.6^{ab}	10.6±1.8^a	8.17±1.02^a

Table 11 - The nutrient concentrations in the treated soil extractable with 0.11 mol*l⁻¹ acetic acid (mg*kg⁻¹) in the course of the experiment (30ᵗʰ day of incubation): *The averages marked by the same letter did not significantly differ at p <0.05 within individual columns, data are presented as mean ± standard deviation, n=4.*

Treatment	Ca mg/kg	Cu mg/kg	Fe mg/kg	K mg/kg	Mg mg/kg	Mn mg/kg	P mg/kg
			Not inoculated				
Control	206±14^a	0.790±0.099^a	109±9^a	62±3^a	28.2±1.2^a	2.85±0.36^a	5.77±0.44^a
DOR	203±38^a	1.06±0.14^b	132±20^a	216±24^{bcd}	31.2±0.9^a	5.03±0.98^b	7.66±1.24^{ab}
DORFF	176±11^a	1.21±0.07^{bc}	125±8^a	184±14^c	29.4±0.9^a	4.11±0.76^{ab}	8.12±1.05^b
DORPC	198±10^a	1.22±0.10^{bc}	137±24^a	245±23^d	32.0±2.9^a	4.49±1.07^{ab}	8.42±1.04^b
DORBA	183±20^a	1.33±0.08^c	120±7^a	170±3^{bc}	28.9±2.6^a	3.81±0.37^{ab}	8.24±1.29^b
DORCP	210±26^a	1.02±0.12^{ab}	134±8^a	156±45^b	31.8±2.0^a	4.23±0.58^{ab}	7.53±0.61^{ab}
			Inoculated				
Control	401±36^{bc}	0.730±0.084^a	118±6^a	93±6^a	51.1±4.8^{ab}	2.94±0.12^a	6.61±0.93^a
DOR	434±27^c	0.890±0.100^{ab}	111±12^a	218±8^{cd}	54.4±3.4^b	3.54±0.57^a	6.82±1.24^a
DORFF	376±31^{ab}	0.831±0.069^{ab}	115±18^a	183±19^{bc}	49.8±5.1^{ab}	3.53±0.69^a	7.52±1.04^a
DORPC	378±41^{ab}	0.886±0.075^{ab}	115±16^a	223±11^d	49.4±3.1^{ab}	3.90±0.99^a	7.06±0.86^a
DORBA	339±28^{ab}	1.07±0.20^b	119±3^a	184±20^{bc}	45.5±3.0^a	3.66±0.33^a	7.89±1.22^a
DORCP	315±35^a	0.934±0.044^{ab}	120±5^a	179±28^b	43.3±3.0^a	3.72±0.38^a	7.50±0.91^a

Table 12 - The nutrient concentrations in the treated soil extractable with 0.11 mol*l^{-1} acetic acid (mg*kg^{-1}) in the end of the experiment (60th day of incubation): The averages marked by the same letter did not significantly differ at p <0.05 within individual columns, data are presented as mean ± standard deviation, n=4.

Treatment	Ca mg/kg	Cu mg/kg	Fe mg/kg	K mg/kg	Mg mg/kg	Mn mg/kg	P mg/kg
			Not inoculated				
Control	241±18[b]	0.673±0.197[a]	62.7±3.3[a]	64.1±3.57[a]	30.1±2.0[a]	1.58±0.27[a]	3.78±0.42[a]
DOR	190±17[ab]	0.716±0.066[a]	66.2±7.6[a]	229±14.3[b]	25.8±2.2[a]	2.00±0.56[a]	4.58±0.44[a]
DORFF	223±35[ab]	0.737±0.064[a]	62.6±5.8[a]	201±14.0[b]	31.3±4.4[a]	1.72±0.29[a]	4.79±0.28[a]
DORPC	221±47[ab]	0.714±0.064[a]	55.6±5.3[a]	244±24.5[b]	28.6±4.3[a]	1.36±0.22[a]	4.50±0.28[a]
DORBA	213±22[ab]	0.932±0.280[a]	59.1±6.1[a]	211±27.5[b]	32.1±6.2[a]	1.66±0.30[a]	4.67±0.52[a]
DORCP	166±36[a]	0.693±0.171[a]	54.3±3.9[a]	204±55.4[b]	25.2±1.2[a]	1.54±0.31[a]	3.83±1.55[a]
			Inoculated				
Control	418±24[b]	0.484±0.033[a]	54.0±4.6[a]	92.2±2[a]	49.2±1.8[a]	1.29±0.29[a]	3.21±0.30[a]
DOR	419±19[b]	0.611±0.057[ab]	58.2±4.4[a]	251±6[c]	48.8±2.3[a]	1.67±0.18[a]	3.87±0.26[ab]
DORFF	297±51[a]	0.686±0.046[b]	55.4±3.6[a]	188±8[b]	41.1±3.5[a]	1.42±0.31[a]	4.18±0.13[b]
DORPC	323±62[ab]	0.650±0.049[b]	58.2±6.3[a]	228±22[c]	42.6±9.5[a]	1.70±0.73[a]	4.10±0.69[b]
DORBA	338±33[ab]	0.752±0.116[b]	57.7±7.2[a]	191±17[b]	42.0±2.0[a]	1.63±0.37[a]	4.17±0.32[b]
DORCP	326±67[ab]	0.626±0.053[ab]	53.6±6.2[a]	187±23[b]	38.6±6.6[a]	1.39±0.15[a]	3.60±0.32[ab]

4.6 Element contents in plant biomass

The effect of the individual treatments on the yield of wheat plants is presented in Figures 13 - 15. The results showed increasing yield of both straw and grain dry biomass in the AM fungi inoculated pots. For roots, the dry biomass yield tended to increase in DOR, DORFF, and DORPC variants but the differences were not significant. Straw and grain biomass yields significantly decreased after non-transformed DOR application. On the contrary, the application of the biotransformed DOR samples led to increase of the wheat straw and grain yield compared to control, where the most apparent yield increase was observed after DORFF and DORBA application.

Figure 13 - The effects of the individual treatments on yield of straw biomass: data expressed as mean (bar) and standard deviation (line segment); the bars marked by the same letter did not significantly differ at P <0.05 within the DOR treatments.
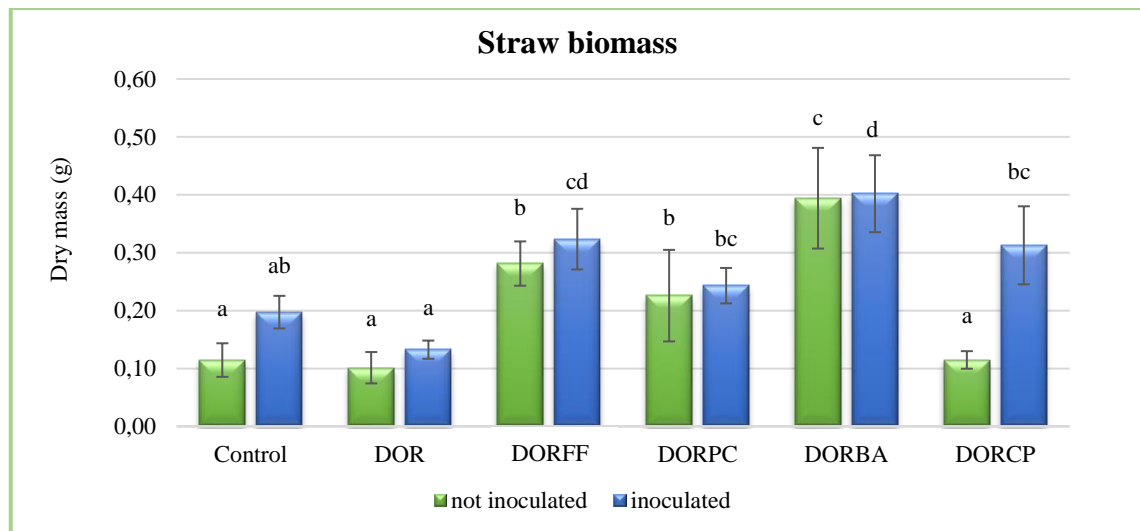
Figure 14 - The effects of the individual treatments on yield of grain biomass: data expressed as mean (bar) and standard deviation (line segment); the bars marked by the same letter did not significantly differ at P <0.05 within the DOR treatments.
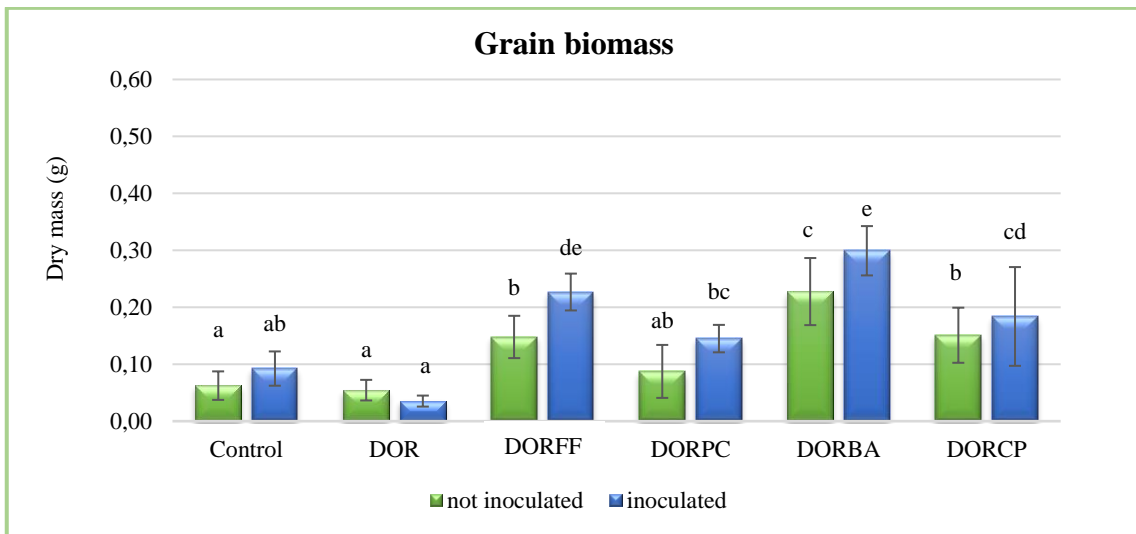
Figure 15 - The effects of the individual treatments on yield of roots biomass: data expressed as mean (bar) and standard deviation (line segment); the bars marked by the same letter did not significantly differ at P <0.05 within the DOR treatments.
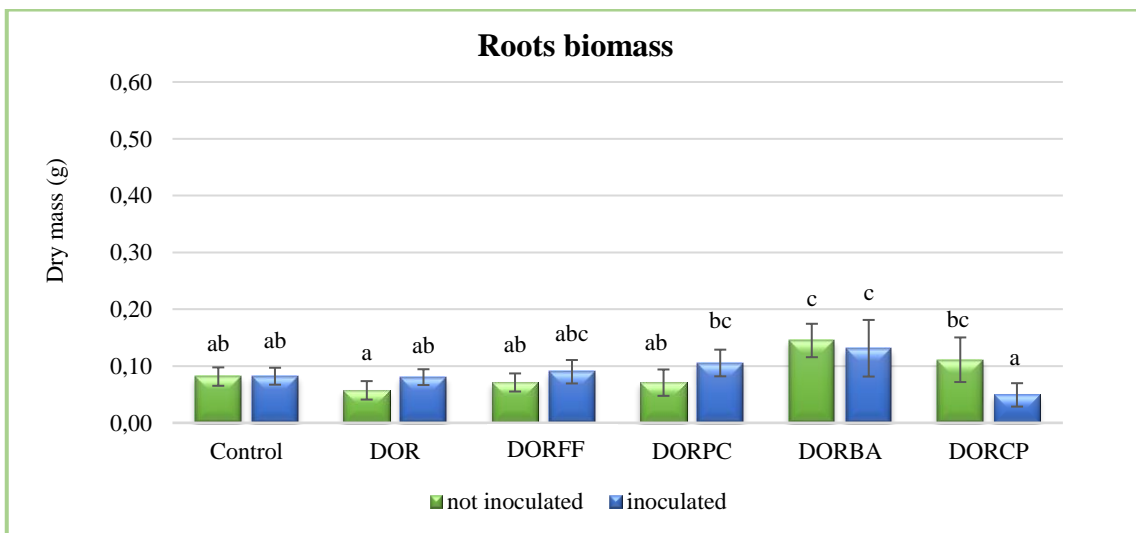
The contents of investigated elements in the wheat plants are summarized in Supplementary Tables 1 - 3. Irrespective of the high Pb contents in the experimental soil, the Pb contents in the aboveground biomass were low, not exceeding the detection limit of the analytical procedure. The substantial proportion of Pb was retained in roots. In the roots, the DOR application resulted in higher Pb accumulation, especially for DORPC and DORCP treatments.

In the AM inoculated variants, similar trends were observed among the treatments, with lower variability of the experimental data indicating the stabilization effect of the AM fungi. For Cd and Zn, their translocation within the plants was similar. In the roots without AM inoculation, the Cd and Zn contents increased at the DOR treated variants regardless of the biotransformation, where higher element contents were recorded for DORPC and DORCP treated soils. The AM inoculation increased Cd and Zn accumulation in the control sample, exceeding in this case the samples treated by non-transformed DOR. However, no significant effect of the AM inoculation was observed for the whole dataset. The shoot Cd and Zn accumulation showed opposite pattern with the highest element contents in the control samples. In this case, the application of DORFF and DORPC tended to be more effective in the reduction of the element uptake than DORBA and DORCP. Again, the AM inoculation did not lead to the change in the Cd and Zn contents in shoots. However, lower grain Cd and Zn was found in the AM fungi inoculated variants when compared with the non-inoculated ones.

The decrease in straw and grain contents of nutrient elements (Supplementary Tables 3-4) showed similar pattern as for Cd and Zn, with some exceptions. Whereas K was predominantly accumulated in shoots and P in grain, other nutrients were retained in roots. In wheat shoots, the AM fungi inoculation did not affect the element contents in the biomass except K and P, where the element contents significantly increased in the inoculated variants. In wheat grain the AM fungi inoculation resulted in higher contents of most of the investigated elements except Cu, Mn, and P. In the roots, the AM fungi inoculation lead to increase of Ca and Mg contents. Whereas the P contents in the wheat shoots remained unchanged regardless of the treatment, other elements decreased after addition of both non-transformed and biotransformed DOR. Similar response on the DOR application was observed in wheat grain and opposite pattern was recorded for roots.

The shifts in the translocation of the elements within the plants as demonstrated by changing shoot/root ratio calculated for the individual treatments (Table 13). Among the DOR samples application, the lowest element contents in the wheat roots after non-transformed DOR application associated with the lowest root biomass yield indicate better translocation of elements into the aboveground biomass compared to the application of the biotransformed DOR. The biotransformed DOR application suppressed the translocation of both nutrients and risk elements from roots to shoots. This pattern was apparent in higher extent in non-inoculated variants when compared to the AM fungi inoculated ones.

Table 13 - Shoots/roots ratio of the individual elements according to the treatments.

Treatments	Ca	Cd	Cu	Fe	K	Mg	Mn	P	Pb	Zn
					Not inoculated					
Control	8.82	0.15	0.41	0.17	8.55	3.39	0.14	2.97	#	0.98
DOR	1.57	0.04	0.13	0.03	4.20	0.85	0.04	1.23	#	0.38
DORFF	0.80	0.01	0.05	0.03	1.56	0.28	0.00	0.46	#	0.14
DORPC	0.49	0.01	0.04	0.01	1.25	0.28	0.01	0.48	#	0.11
DORBA	0.54	0.02	0.05	0.01	2.95	0.22	0.00	0.52	#	0.14
DORCP	0.57	0.02	0.04	0.01	2.00	0.30	0.01	0.50	#	0.13
					Inoculated					
Control	1.40	0.04	0.11	0.04	2.51	0.91	0.03	0.96	#	0.37
DOR	0.78	0.02	0.06	0.03	2.21	0.56	0.07	0.50	#	0.16
DORFF	0.78	0.02	0.07	0.04	2.05	0.53	0.03	0.80	#	0.20
DORPC	0.45	0.01	0.04	0.01	1.98	0.20	0.01	0.67	#	0.13
DORBA	0.51	0.02	0.05	0.01	3.07	0.44	0.01	0.84	#	0.24
DORCP	0.55	0.02	0.05	0.01	2.57	0.38	0.01	1.02	#	0.19

4.7   Correlation analysis of elements

The correlation analysis suggested important role of pH in the element mobility, where the Pearson's correlation coefficients (r) indicated significantly decreasing Cd, Cu, Fe, Mn, P, Pb, and Zn mobility (r values varied between -0.36 and -0.46), and increasing Ca and Mg mobile proportions (r = 0.63, and r = 0.51, respectively) according to increasing soil pH, if the whole set of data was concerned. However, weaker correlations (r values between -0.19 and -0.31) were found for non-inoculated variants, whereas the inoculated variants showed the correlation coefficients between r= -0.31 and r= -0.54. These findings indicate that the significant change in soil pH due to the AM fungi inoculation lead to the change of the element mobility regardless of the DOR application. The mobility of K was not related to the soil pH.

5 Discussion

Száková et al. (2016b) published, that the biological transformation of DOR showed good potential for sorption of risk elements in contaminated soil especially for Pb. Thus, fungal transformation of DOR could be considered as a potential material suitable for the immobilization and stabilization of risk elements in soil.

5.1 Parameters of the experimental soil

The determination of total physicochemical parameters of the soil shown slightly acidic pH, relatively low CEC value and adequate nutrient status of the soil severely polluted by Pb, Cd and Zn due to mining and smelting activities. On the contrary, the previous experiments done by Hovorka et al. (2016) showed negligible contents of Cd (under detection limit), Pb (not exceeding 2 mg*kg^{-1}), and Zn (varying between 15 and 30 mg*kg^{-1}) in both non-transformed and biotransformed DOR samples, as confirmed also in this experiment. The properties and nutrient contents in the individual DOR samples confirmed increasing pH of the biotransformed samples compared to the non-transformed DOR as in the previous investigations, but the alterations in CEC levels did not correspond to the trends in the previous experiment (Hovorka et al., 2016).

5.2 Impact of biotransformed DOR and AMF inoculation on changing of soil pH value

As is known, metal polluted soils are characterized by low pH which determines the mobility of metals in the soil. In that case, suitable opinion was to stabilise them, since they cannot be removed from the soil. The best option how to do it is by changing soil pH, which can be achieved also by organic amendments (García-Sánchez et al., 2015). It corresponds to study documented by Zahedifar et al. (2012) for Zn, and by Adhikari, and Singh (2008) for Cd, where the role of soil pH was highlighted. In this study, the correlation analysis suggested important role of pH in the element mobility as identically as was reported by Hovorka et al. (2016), where the main limitation of tested material was its pH. However, if compare of additional organic material such as compost, ashes, sludge or other liming materials pH of biotransformed DOR is relatively low. On the other hand, Smith and Read (2008) examined the remediation potential of several bio-wastes and showed that sewage sludge has a positive effect in remediation of metal contaminated soils, however, together with background elements in fact

it can raise the risk element content in the soil and the ability of crops plants to uptake these elements.

5.2.1 Biotransformed DOR

Without inoculation, the biotransformed DOR treated variants tended to increase the soil pH compared to control sample from 7th till the 30th day of cultivation. The changes in soil pH occurred immediately after biotransformed DOR application whereas the alterations in the soil pH in biotransformed DOR treated variants were recorded after three-days delay.

5.2.2 Combined treatment of biotransformed DOR and AMF inoculation

In this study, the AM fungi inoculation seemed to be the most important factor affecting the soil pH, where the soil pH increased significantly compared to the not-inoculated variants regardless to the biotransformed DOR application. Siles et al. (2015) observed a slight decrease in soil pH after soil application of DOR, and biotransformed DOR (using *F. floccosa*, and *F. oxysporum*) after 60 days of incubation. If comparing to individual treatments, the changes were more apparent after non-transformed DOR application (Siles et al., 2015), which does not correspond to this research.

5.3 Impact of biotransformed DOR and AMF inoculation on stabilization of the risk elements in contaminated soil

In the previous study, Hovorka et al. (2016) observed abilities of biotransformed DOR material for reducing the mobility of Cd, Pb and Zn, which supposed to be connected with the stabilized organic matter supplied into the soil, which is provide by the fungal treated biotransformed DOR. The effect of biotransformed DOR application on the element mobility in the Pb and Zn contaminated soil was investigated also by de la Fuente et al. (2008). Consistent with this statement was the mobility of Cd, which increased after application of non-transformed DOR, then with not inoculated treatments like DORPC, DORBA, DORCP and DORBA, DORCP inoculated ones. In case of Zn application of biotransformed DOR, not inoculated and inoculated, had no significant effect on mobility of this risk elements. On the contrary, increased mobility of elements (Cd, Pb, Zn) for various organic treatments and soils compared to controls has been published by Hernandez-Soriano and Jimenez-Lopez (2012), where the element mobility was significantly correlated with the soluble organic matter content. Slight increase in the mobile proportions of risk elements after application of composted DOR were reported by

other authors like Burgos et al. (2002) or Maqueda et al. (2015). On the other hand, Díaz-Barrientos et al. (1999) suggested that the increase of Zn mobility after compost application was caused by the contribution of the metals originally present in the composts. The increased solubility of Cd and Zn in soils can be related to the increasing concentrations of dissolved organic acids in the soils (Güngör and Bekbölet, 2010). These authors documented that humic and fluvic acid treatments can increase the release of Zn from soils. Thus, the potential immobilization efficiency of the risk elements depends on the composition of the applied organic matter. In accordance with the literature, higher pH, CEC, and Mn oxide content are responsible for greater absorption and retention of Cd, Pb, and Zn (Vega, 2008). Responsibility of pH in mobility was represented by Pearson's correlation coefficient in this study as well. However, organic matter has been noted as an important factor that influences the adsorption of elements in biotransformed DOR due to the high specific surface area, and the presence of carboxyl groups, amine, or phenolic hydroxyl can chelate metals to form stable complexes (Paradelo and Barral, 2012).

5.4   Impact of biotransformed DOR and AMF inoculation on changing soil nutrient status in metal polluted soils

During the experiment biotransformed DOR treated with the AMF inoculation resulted an enhancement in some contents of micro- and macronutrients due to partial decomposition of the organic matter but generally, both, non-transformed and biotransformed DOR, are good sources of the nutrients in soil.

5.4.1   Biotransformed DOR

Burgos et al. (2002) and Maqueda et al. (2015) reported increased mobility of Fe, Cu and Mn after application of composted DOR. Rodriguez-Rubio et al. (2003) investigated the effect of the composted DOR on the Cu sorption in calcareous soil, where the Cu retention tended to be enhanced by organic amendments. The sorption enhancement was apparent especially in the silt fraction, as a consequence of the organic matter accumulation in this fraction. Similarly, Gondar and Bernal (2009) observed increasing Cu binding ability of the DOR-amended soil and decreasing Cu concentrations in the soil solution. In this study, biotransformed DOR application shows different pattern, whereas the mobility of Fe, Mg and P remained unchanged, the mobility of Ca and Mn was slightly increased and the mobility of K was highly increased. Similarly, significant increase in available soil K was observed by Siles et al. (2015) after soil

application of DOR after 60 days of incubation. As documented by Zahedifar et al. (2012), the application of biotransformed organic matter into the soil can result in lower mobility of elements in soils.

5.4.2 Combined treatment of biotransformed DOR and AMF inoculation

Application of AM fungi treated samples led to decreases in mobility of most of the elements. However, this study showed increasing mobility of Cu, in the combined treatment, involving biotransformed DOR and AMF inoculation, indicating the dominant role of pH in the alteration of the element mobility. This result was also supported by the positive correlation between the pH and the Cu mobility as indicated the Pearson correlation coefficient. Moreover, the mobile proportions of the elements were stabilized after 60 days of incubation and no significant differences were recorded for the variants not inoculated with AM fungi except Ca, where both non-transformed and biotransformed DOR samples tended to decrease, compared to control.

5.5 Impact of biotransformed DOR and AMF inoculation on element uptake by wheat plants

5.5.1 Plant biomass

Application of the biotransformed DOR samples like DORFF and DORBA increased the yield of the straw and grain dry biomass the most apparently. Similarly, to these results of Reina et al., (2017). In the AM fungi inoculated pots, the results showed increasing yield of straw and grain dry biomass. On the contrary, after non-transformed DOR application yield of straw and grain significantly decreased. According to Sampedro et al. (2008), presence of AM fungi to facilitate the translocation of phenolic compounds from soil to plants, and non-transformed DOR decreased the percentage of AM root length colonization of the plants. Sampedro et al. (2008) showed that the application of DOR incubated with *P. farinosus*, *C. rigida* and *P. subvermispora* during 20 weeks increased the shoot and root dry weight of AM colonized *Solanum lycopersicum* and *M. sativa* in comparison to AM colonized plants cultivated in absence of DOR. Thus, the *F. mossae* inoculation together with the biotransformed DOR improved the wheat growth in the highly-contaminated soil. Corresponding result was investigated also by Curaqueo et al. (2014); the higher doses of composted olive waste (COW) in combination with AM fungi increased shoot and root biomass production of *T. articulata* by 96 and 60 %, respectively.

5.5.2   Risk elements uptake

Irrespective of the high Pb contents in the experimental soil, the Pb contents in the aboveground biomass was not detected. The substantial proportion of Pb was retained in roots as observed also for other plants growing in Pb-contaminated soil (Száková et al., 2016a). In the roots, the boitransformed DOR application resulted in higher Pb accumulation, especially for DORPC and DORCP treatments. In the AM inoculated variants, similar trends were observed among the treatments, with lower variability of the experimental data indicating the stabilization effect of the AM fungi. However, no significant effect of the AM inoculation was observed for the whole dataset in straws and in shoots. Only lower content of Cd and Zn in grain was found in the AM fungi inoculated variants. For example, Liu et al. (2016) observed lower Cd contents in wheat plants growing in soil treated by municipal sludge compost. Thus, the final decrease in Cd and Zn plant-availability in the treated soils is the result of both the enrichment of soil by organic matter and an increase in soil pH (Ociepa et al., 2013).

5.5.3   Nutrients plant-availability

After biotransformed DOR application, K was predominantly accumulated in shoots and P in grain, other nutrients were retained in roots. AM fungi inoculated variants increased content of K and P in the wheat biomass, other nutrients mostly in wheat grain and in roots was increased content of Ca and Mg. Grant et al. (2005) observed that under low-P conditions, encouragement of AM associations may enhance P uptake by crops early in the growing season. Curaqueo et al. (2014) reported that the dilution effect of increased plant biomass due to the AM fungi inoculation resulted in significant decrease of the Cr, Ni, and Pb contents in shoot tissues, as well as Cr and as in root tissues. The improvement of plant nutrient uptake (mainly P) was also reported. Lower plant-availability of Cu in composted DOR-treated soil compared to the control was observed by Shaheen et al. (2015) in the Cu polluted soil. Fresh olive husk favoured bioavailability and solubility of Mn in soil, but did not increase Mn uptake by plants (de la Fuente et al., 2011). The shifts in the translocation of the elements within the plants show that the lowest element contents in the wheat roots, after non-transformed DOR application, associated with the lowest root biomass yield indicate better translocation of elements into the aboveground biomass than application of the biotransformed DOR samples. The lower plant-availability could be related to their complexation with the humic substances where the stability of these complexes increases with increasing soil pH (Loganathan and Hedley, 1997; Kabata-Pendias, 2011).

6 Conclusion

Summarizing the results, the DOR application to the risk element (Cd, Pb, Zn) contaminated soil confirmed to be a promising material for reduction of the uptake of these elements uptake by plants, where biotransformation of DOR is reasonable measure for minimizing the potential phytotoxic effect of DOR and improvement of the plant growth. The AM fungi inoculation supported the plant growth, as well. The decrease of the Cd and Zn contents in the wheat biomass seems to be the result of various factors, such as the dilution factor due to the enhanced biomass production, and lower translocation of elements into the shoots. The potential decrease of the plant-available proportion of the elements in soil was not confirmed by the single extraction procedure (0.11 mol*l^{-1} acetic acid extractable). However, the shifts in the element distribution into the soil fractions can be speculated in this case and the element bounds especially into DOR-derived organic matter, and should be investigated in more details in further research. The important aspect of the bioremediation techniques is the further use of the remediated soil. Unfortunately, despite of the Cd and Zn contents in the wheat shoots and grain decreased in DOR treated soil, the levels exceeded the limits for Cd and reached the phytotoxicity level for Zn in most of cases.

The efficiency of the remediation methods based on the biological principles is usually better for moderately contaminated soils when compared with the extreme contamination as used in this experiment (Tlustoš et al., 2007). Thus there are two possible ways for further research, 1) to verify the potential effectivity of the biotransformed DOR application as the risk element stabilization agent in the moderately contaminated soils, and 2) to combine DOR application with other element stabilizing materials such as ash from the biomass combustion or the waste from biogas production (Ochecová et al., 2014, García-Sánchez et al., 2015), or to investigate alternative methods of DOR transformations, for instance pyrolysis to produce biochar (Hmid et al., 2014).

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Appendices

Supplement table 1 - *The element contents in wheat shoots (mg*kg^{-1}):* *The averages marked by the same letter did not significantly differ at p <0.05 within individual columns, n=3.*

Treatments	Ca mg/kg	Cd mg/kg	Cu mg/kg	Fe mg/kg	K mg/kg	Mg mg/kg	Mn mg/kg	P mg/kg	Pb mg/kg	Zn mg/kg
					Not inoculated					
Control	8298±614^b	3.97±0.54^b	6.44±0.17^b	165±15^b	16080±2628^b	1563±210^b	10.1±0.4^c	802±109^a	*	732±89^b
DOR	2754±145^a	2.03±0.20^a	3.78±0.34^a	82.2±34.8^{ab}	12168±1720^{ab}	753±84^a	5.05±1.13^{ab}	765±87^a	*	419±46^a
DORFF	2259±951^a	1.60±0.71^a	2.85±0.92^a	65.6±32.9^a	11178±986^{ab}	564±199^a	3.59±0.64^a	655±294^a	*	354±153^a
DORPC	2118±923^a	1.58±0.70^a	2.60±1.12^a	80.4±56.9^{ab}	7596±3444^a	572±255^a	4.51±2.26^a	689±317^a	*	333±131^a
DORBA	2427±599^a	2.24±0.33^a	3.08±0.86^a	63.4±7.4^a	15189±4127^b	580±108^a	5.22±1.24^{ab}	581±74^a	*	415±68^a
DORCP	2799±385^a	2.35±0.13^a	3.40±0.29^a	102±16^{ab}	13737±2176^{ab}	698±99^a	8.86±0.52^{bc}	739±45^a	*	468±6^a
					Inoculated					
Control	6849±599^b	3.63±0.30^c	7.34±1.06^b	131±31^a	18378±5636^a	1788±336^b	8.57±1.40^b	1203±215^a	*	773±83^c
DOR	2580±235^a	1.68±0.10^{ab}	2.94±0.25^a	86.3±22.7^a	11265±1157^a	936±79^a	21.7±2.6^c	755±64^a	*	208±9^a
DORFF	2829±45^a	1.97±0.21^{ab}	3.29±0.13^a	95.1±24.9^a	15363±1426^a	831±68^a	6.37±1.03^{ab}	1101±104^a	*	367±44^{ab}
DORPC	1896±790^a	1.14±0.36^a	2.55±0.89^a	63.4±14.1^a	14553±3418^a	579±85^a	3.90±1.10^a	878±380^a	*	280±58^{ab}
DORBA	2961±70^a	2.43±0.19^b	3.59±0.14^a	83.9±17.4^a	17799±1716^a	963±48^a	6.84±0.82^{ab}	1044±206^a	*	530±195^{bc}
DORCP	3000±529^a	2.36±0.58^b	4.16±0.86^a	101±27^a	17556±3443^a	966±167^a	7.78±1.22^{ab}	1329±264^a	*	510±96^{bc}

*under detection limit

Supplement table 2 - The element contents in wheat grain (mg*kg^{-1}): The averages marked by the same letter did not significantly differ at p <0.05 within individual columns, n=3.

Treatments	Ca	Cd	Cu	Fe	K	Mg	Mn	P	Pb	Zn
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Not inoculated										
Control	2553±242[b]	1.43±0.09[b]	5.96±0.27[b]	98.4±5.1[c]	8884±503[bc]	1131±140[b]	5.95±0.36[a]	1455±216[ab]	*	202±39[a]
DOR	1701±178[a]	1.08±0.26[ab]	5.57±0.80[b]	51.9±7.5[a]	7775±531[a]	1094±121[b]	11.9±2.2[b]	1886±235[b]	*	159±26[a]
DORFF	1464±58[a]	0.915±0.199[a]	5.13±0.25[b]	65.9±2.0[ab]	7829±204[ab]	931±72[ab]	6.05±0.83[a]	1803±181[b]	*	139±23[a]
DORPC	1661±134[a]	1.06±0.10[ab]	5.87±0.06[b]	93.9±11.1[bc]	8336±244[abc]	1107±51[b]	8.29±0.38[a]	1908±76[b]	*	183±17[a]
DORBA	1518±129[a]	0.897±0.094[a]	4.11±0.39[a]	77.0±5.1[ab]	9040±487[c]	761±73[a]	6.24±0.41[a]	1149±149[a]	*	143±15[a]
DORCP	1602±130[a]	0.718±0.038[a]	5.39±0.14[b]	77.3±8.6[ab]	8628±138[abc]	924±10[ab]	8.46±0.89[a]	1662±78[b]	*	137±3[a]
Inoculated										
Control	1935±151[c]	1.27±0.20[b]	7.65±0.74[b]	61.4±2.8[b]	9344±352[c]	1191±77[c]	7.19±0.56[a]	1785±262[a]	*	230±20[b]
DOR	980±154[b]	0.646±0.152[a]	4.86±1.02[ab]	96.3±6.4[c]	7453±543[bc]	1022±223[bc]	18.1±5.0[b]	1791±407[a]	*	116±24[a]
DORFF	750±89[ab]	0.562±0.227[a]	4.59±1.75[ab]	50.2±4.2[b]	4041±492[a]	712±260[a]	5.89±1.33[a]	1584±639[a]	*	91.8±19.0[a]
DORPC	499±103[ab]	0.341±0.069[a]	2.93±0.65[a]	23.1±5.6[a]	4862±691[ab]	493±96[ab]	3.74±1.08[a]	1098±253[a]	*	60.5±10.5[a]
DORBA	522±112[ab]	0.480±0.056[a]	3.54±0.62[a]	28.1±2.4[a]	5918±626[ab]	577±264[ab]	5.14±1.87[a]	1296±608[a]	*	73.0±15.0[a]
DORCP	345±46[a]	0.227±0.030[a]	2.37±0.22[a]	18.7±4.3[a]	3437±576[a]	355±33[a]	2.67±0.28[a]	793±81[a]	*	44.0±3.9[a]

*under detection limit

Supplement table 3 - The element contents in wheat roots (mg*kg⁻¹): *The averages marked by the same letter did not significantly differ at p <0.05 within individual columns, n=3.*

Treatments	Ca mg/kg	Cd mg/kg	Cu mg/kg	Fe mg/kg	K mg/kg	Mg mg/kg	Mn mg/kg	P mg/kg	Pb mg/kg	Zn mg/kg
					Not inoculated					
Control	932 ± 146^a	27.2 ± 6.5^a	16.0 ± 2.7^a	968 ± 95^a	1903 ± 201^a	456 ± 93^a	72.8 ± 10^a	273 ± 59^a	79.4 ± 11.4^a	750 ± 193^a
DOR	1728**	51.3**	29.4**	2007**	2907**	865**	204**	541**	166**	990**
DORFF	2448 ± 115^a	99.3 ± 11.9^b	56.8 ± 6.7^b	2606 ± 643^a	7457 ± 759^b	1800 ± 649^{abc}	989 ± 14^b	1197 ± 51^b	207 ± 39^a	2093 ± 185^b
DORPC	4395 ± 347^b	137 ± 10^c	68.6 ± 6.5^b	7665 ± 747^b	6023 ± 250^b	2064 ± 105^{bc}	775 ± 112^b	1470 ± 120^c	887 ± 119^b	3195 ± 206^c
DORBA	4497 ± 149^b	106 ± 8^b	68.3 ± 1.4^b	9558 ± 890^b	5120 ± 869^{ab}	2604 ± 552^c	1065 ± 45^b	1128 ± 68^b	1158 ± 187^b	3006 ± 94^c
DORCP	4980 ± 997^b	136 ± 7^c	87.7 ± 8.2^c	10251 ± 637^c	6893 ± 39^b	2364 ± 358^{bc}	1086 ± 209^b	1476 ± 39^c	1244 ± 178^b	3534 ± 257^c
					Inoculated					
Control	4974 ± 736^{abc}	95.6 ± 8.2^b	64.4 ± 3.4^b	3297 ± 159^a	7350 ± 409^a	1953 ± 68^{ab}	310 ± 27^a	1249 ± 62^a	350 ± 36^a	2100 ± 292^b
DOR	3281 ± 121^a	70.1 ± 2.9^a	45.8 ± 1.8^a	2921 ± 197^a	5297 ± 708^a	1701 ± 369^{ab}	332 ± 74^{ab}	1454 ± 6^a	264 ± 5^a	1341 ± 38^a
DORFF	3650 ± 121^{ab}	87.6 ± 1.3^{ab}	44.7 ± 1.0^a	3024 ± 115^a	7177 ± 43^a	1643 ± 6^a	238 ± 29^a	1368 ± 64^a	272 ± 20^a	1908 ± 51^{ab}
DORPC	4488 ± 752^{abc}	95.3 ± 9.6^b	68.4 ± 7.4^{bc}	6744 ± 854^b	7448 ± 599^a	2961 ± 402^c	644 ± 95^{bc}	1359 ± 118^a	683 ± 85^b	2247 ± 185^{bc}
DORBA	5904 ± 852^c	101 ± 7^b	67.4 ± 4.9^{bc}	8091 ± 406^b	5952 ± 838^a	2190 ± 213^{abc}	844 ± 129^{cd}	1236 ± 53^a	909 ± 118^c	2229 ± 162^{bc}
DORCP	5394 ± 405^{bc}	107 ± 8^b	79.0 ± 2.8^c	9105 ± 624^c	6861 ± 259^a	2556 ± 305^{bc}	1050 ± 137^d	1323 ± 86^a	981 ± 63^c	2688 ± 131^c

**single sample only