CZECH UNIVERSITY OF LIFE SCIENCES

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PLANTS ADAPTATIONS TO CONTAMINATION WITH METALS AND METALLOIDS

BACHELOR THESIS

by

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Exchange programs

Thesis title

Plant adaptations to contamination with metals and metalloids

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Objectives of thesis

The aim of the review part of the thesis is to summarize basic information concerning toxic effects of metals and metalloids towards plants and connected plant adaptation mechanisms developed for reducing metal(loid) induced stress. Special focus will be given at processes of seed germination and plants growing at contaminated localities. Additionaly, use of nano zerovalent iron (nZVI) and biochar for remediation of contaminated soils will be reviewed with the special impact on plants and seeds.

In the experimental part, basic germination of seeds of two grass species (A. capillaris and F. rubra) from contaminated (Příbram, Czech Republic) and uncontaminated (bought from Czech company) sites will be firstly compared. Based on this preexperiment, parameters for the main experiment will be known. Here, germination of the same seeds planted in sand (uncontaminated control), Příbram soil (contaminated soil), and soil treated with nZVI, biochar, and nZVI+biochar (total of 5 treatments) will be compared to observe the influence of nZVI and biochar addition on seeds germination in metal contaminated soil.

Methodology

Review part is based on appropriate literature sources, eg., mainly scientific books and articles.

In the experimental part of the thesis, basic germination (germination percentage, root elongation) of seeds of two grass species (A. capillaris and F. rubra) from contaminated (Příbram, Czech Republic) and uncontaminated (bought from Czech company) sites will be firstly compared. Based on this preexperiment, parameters for the main experiment will be set. Here, germination of the same seeds planted in sand (uncontaminated control), Příbram soil (contaminated soil), and soil treated with nZVI, biochar, and nZVI+biochar (total of 5 treatments) will be compared to evaluate the effect of nZVI and biochar application on seeds germination in metal contaminated soil.

Obtained data will be processed, summarized and statistically evaluated. Student will discuss the results with existing literature and summarize main conclusions of the work.

The proposed extent of the thesis

around 30 pages as need

Keywords

phytotoxicity, germination, seeds, nano zerovalen iron, biochar

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Recommended information sources

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The Final Thesis Supervisor

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Prague on 14. 05. 2018

Declaration

I hereby declare that I wrote this diploma thesis, titled “Plants adaptations to contamination with metals and metalloids” independently, under the direction and supervision of Zuzana Michálková, PhD., and M.Sc. Manuel Teodoro Tenango. All literature and publications from which I have acquired information have been listed.

In Prague, 13 of May 2018

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Ermengol Ferrer Bustins

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Abstract

Soil metal contamination due to anthropogenic activities, such as mining or smelting, is an environmental concern that can cause drastic vegetation changes. Plants growing in these contaminated sites are highly affected by these pollutant concentrations, especially when metals are found in mobile or easily mobilizable forms because plants roots can uptake them more easily. In Příbram district (Czech Republic), Ag-Pb mining was held and therefore high concentrations of Pb and Zn are present in the nearby area and also along Litavka alluvium. Due to recent floods, these metals were leached and mobilized through the soils, and consequently, in the worst-case scenario metals could reach the groundwater system which would be an important hazardous health risk for living beings. To prevent such metal mobilization from happening, aided phytostabilization methods using native species could be carried out. *A. capillaris* and *F. rubra* grasses were found in Litavka alluvium growing naturally, which indicates that these species are tolerant to Příbram metal(loid) contamination. In this study, germination tests using BC and nZVI amendments applied to contaminated soils were conducted to determine the germination capability of the seeds specimens mentioned and to check if seedlings were positively or negatively affected by the amendments used. Around 90% of purchased *A. capillaris* seeds germinated under amended contaminated soils, whereas the seeds collected in Příbram presented not even a 10 % germination percentage. In *F. rubra* species, nearby a 35% germination percentage was observed under BC and nZVI amended soils, while not even a 30% was observed under BC+nZVI conditions. Regarding the results of the physiological experiment, Pnaturalis seedlings presented the longest shoot elongations when BC or nZVI amendment was used. Again, the longest root elongations in *F. rubra* seeds were recorded under one of these treatments conditions. Therefore, it was observed that BC and nZVI amendments when used separately and in low concentrations do not influence seed germinative capability and that these treatments can avoid seedlings from metal uptake provoking a seedlings physiologic enhancement regarding root and shoot elongations.

Keywords

Příbram; biochar; nano zero-valent iron (nZVI); *Agrostis capillaris*; *Festuca rubra*; metal contamination; Zn; Pb; seed germination; seedling physiology

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1 Introduction

Metal and metalloid contamination is a worldwide environmental affair because of its toxicological effects. Plentiful studies have been carried out concerning metal contamination regarding its harmful effects for living beings, e.g, humans, animals or plants, but also about its hazard with respect to soils.

In Czech Republic there are plenty of sites that were used for mining and smelting purposes. At those times, these anthropological activities were not properly environmentally attended but they were already endangering the adjacent environment. One of these cases occurred in Příbram district due to the smelter's chimney gas emissions. The emissions that were coming out of the chimney were highly metal concentrated, especially with Pb and Zn, and that developed into metallic particles displayed all over the soils and the Litavka alluvium.

This metallic soil contamination can be extremely problematic for the fauna and vegetation growing nearby because of the intake/uptake of these contaminants and consequently the health problems that entail, but also because of the difficulties and the stress that these contaminants induce to the growing of the plants species. The flora community can drastically change because of it, particularly, if it is not able to adapt to these new soil conditions. In the worst-case scenario vegetation can be completely wasted. In Příbram study field, just a few plants communities well-adapted to the metal contamination have been spotted growing naturally there. In view of grass exemplars, *Agrostis capillaris* and *Festuca rubra* have been noticed to be the dominant ones.

To overcome risks arising from metal pollution, different soil amendments have been studied to reduce the metal stress caused to the plants seeds and to improve the soil health conditions and so to make seed germination easier. For example, Jiang et al. (2018) made a revision on how to ameliorate the soil conditions using nano zero-valent iron particles (nZVI) to decrease the metallic contaminants mobility and consequently to provoke a metal stabilization in soils. Other researchers like Kookana (2010), also studied how soil amendments contribute to decreasing the metal contamination mobility and its bioaccessibility towards plants. In Kookana (2010) experiments biochar was the amendment investigated and like in the case of nZVI, it was also proved to be a useful amendment to stabilize metals and enhance the soil health.

So, nZVI and biochar amendments were found to be effective for stabilization of metallic contaminants in soil, but their effects on plants physiological functions (like e.g., germination) need to be investigated more.

2 Aims of the thesis

The main objectives of this study are to determine the germination capability of commercial and natural *A*. *capillaris* and *F*. *rubra* seeds collected from Příbram district in metal contaminated soil amended with biochar and nZVI, and to investigate how this amended metal stress is affecting early plant seedling development (e.g., effects on roots and shoots).

3 Literature review

3.1 General impact of metal(loid)s and metal contamination

Soil does not only serve as a medium upon which plants grow, but also provides habitat for animals and other micro-organisms. Fertile soil is vital to produce food, timber and fibre, which are all essential for human existence and economic prosperity. Soil therefore, constitutes part of vital ecological and agricultural resources that need to be protected.

The definition of soil varies widely, as it is dictated by its use and how we perceive it as a society for providing services, food, habitat, and enjoyment, where these functions are essential to soil health or quality. One well-established definition of soil is a medium that includes minerals, organic matter, countless organisms, liquid, and gases that together support life on earth through many services. Soil environment and functions are influenced by the parent materials and forming factors that contribute to the physical, chemical, and biological characteristics of soils (Lal and Olson, 2017).

The soil physical environment includes components of soil structure, aggregation, soil water potential and water movement, and soil thermal regime, along with governing forces. The soil biological environment includes all soil organisms (macro- and microorganisms), soil–plant relationships (plant root–soil interactions), plant growth and soil microorganisms, and plant root interface and nutrient cycling. In addition, the soil chemical environment discussion focuses on soil nutrient capacity and supply, nutrient cycling, and nutrient pathways and mechanisms (Lal and Olson, 2017).

Soil acts as both source and sink of metals for groundwater and plants (Khan et al., 2010). With the rapid development in industrialisation and excessive use of chemical based pesticides and fertilizers in agricultural fields, accumulation of these metallic elements in soil has emerged as a serious problem (Wuana and Okieimen, 2011).

Metals from various sources may finally accumulate in the surface soil, and their fate depends on soil physical and chemical properties especially their speciation. This metal pollution of soil is believed to be a long-term threat to the environment. The accumulation of this group of elements in agricultural soil has attracted the attention of many investigators not only because metals can build up in the soil but also because they can be transferred to crops, where they constitute a significant potential risk to human health (Olubunmi and Olorunsola, 2010; Sabo et al., 2014).

Food crops such as cereals, vegetables, oil seeds and spices comprise the major part of daily human diet and are the main sources of essential nutrients, minerals and vitamins required by human beings for maintaining a good health (Grusak and DellaPenna, 1999; Welch and Graham, 1999). Cultivation of food crops in soils contaminated with metals, leads to accumulation of these metals in edible parts of the plants, which are consumed by human beings (Amin et al., 2013).

There are some metals which have a relatively high density and toxic at low quantity such as, e.g., arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd), chromium (Cr), thallium (Tl), etc. Some other 'trace elements' are, e.g., copper (Cu), selenium (Se) and zinc (Zn). They are essential to maintain the body metabolism, but they are toxic at higher concentrations. The pollutants can enter the bodies to a small extent via food, drinking water and air (Lenntech, 2004).

The toxic elements concerned with the environmental science chiefly include Pb, Hg, Cd, Cr, Cu, Zn, Mn, Ni, Ag, etc. Further, the metals are metallic elements which have a relatively high density, and they are poisonous at low quantity. The excess quantities of metallic elements are detrimental as these destabilize the ecosystems because of their bioaccumulation in organisms. All metals, in spite some of them are essential micronutrients, have their toxic effects on living organisms via metabolic interference and mutagenesis. Such toxic effects of these contaminants include reduction in fitness, interference in reproduction leading to carcinoma and finally death (Rajasthan, 2012).

3.1.1 Lead phytotoxic effects on plants

Lead is a major chemical pollutant of the environment; therefore, its concentration in vegetation in several countries has increased in recent decades owing to man's activities. Mainly, Pb is absorbed in soluble forms in nutrient solutions by roots hair and is stored to a considerable degree in cell walls. In physiologic terms, this cell walls metal accumulation can affect cells elasticity and plasticity resulting into a tissue wall rigidity. However, some plant leaves can also absorb this metalloid when its placed on them due to Pb particles emissions (Kabata-Pendias & Pendias, 2001).

Several plant species and genotypes can be adapted to grow in high Pb concentrations in the growth media. The highest bioaccumulation of Pb generally is reported for leafy vegetables (mainly lettuce) grown in surroundings of nonferrous metal smelters where plants are exposed to Pb sources of both soil and air. In these locations, highly contaminated lettuce may contain as much as 0.15% DW of Pb. Grass species tolerant to Pb metal contamination bioaccumulated around 2.1 ppm DW (Kabata-Pendias & Pendias, 2001).

Concerning Pb toxicity, findings have indicated mainly disturbance of fundamental biological processes such as photosynthesis, growth, mitosis, inhibition of water sorption etc (Kabata-Pendias & Pendias, 2001).

3.1.2 Zinc phytotoxic effects on plants

Zinc toxicity and tolerance is a growing matter of interest because of an enhanced Zn content in soil surfaces. Anthropogenic activities of the metal industry have been releasing this contaminant continuously leading to high Zn contamination. Plants are directly affected by this pollution because of the roots Zn uptake mostly in the hydrated Zn or Zn^{2+} forms (Kabata-Pendias & Pendias, 2001).

Although a lot of species have been greatly adapted to Zn metalloid, not all of them are capable to tolerate high concentrations. The sensitive ones can handle between 150 to 200 ppm whereas the tolerant ones are capable to tolerate between 100 to 500 ppm of Zn. Chlorosis, manly in new leaves and depressed plant growth are the common symptoms of this pollutant toxicity (Kabata-Pendias & Pendias, 2001).

3.2 Physiology of seed germination

A seed contains an embryonic plant in an inactive condition, and germination is its resumption of growth. The young plant consists of three components: embryo which contains the plant genetic material, endosperm which has reserves for metabolism, and seed-coat which protects the seed with layers of living and dead tissues. Both endosperm and embryo are the products of double fertilization, whereas the seed-coat develops from the maternal ovular tissues. Seed fertilization takes place within the protective tissues of the mother plant, and during its development the embryo is nourished by the mother plant (Boesewinkel and Bouman, 1984).

In some seeds development starts as soon as water is absorbed, but in others germination does not take place until additional requirements are fulfilled. Three distinct stages are evident in germinating seeds, namely: imbibition of water, cell elongation and increase in cell number. In a physiologic sense the start of germination depends upon coupling of respiration to growth. The established seedling results from resumption of development and its continuation through growth (Toole et al., 1956).

3.2.1 Impact of Pb and Zn contamination on seed germination

The effects of metals on germination of seeds from different plants depend on interspecies differences in seed structure, particularly seed coats, because seed coats have a wide range of anatomic forms that exists in no other plant organ or tissue. It is known that the most widely used acute phytotoxicity tests involving vascular plants are the seed germination test (a direct exposure method) and the root elongation test (Munzuroglu and Geckil, 2002).

Although the seed coat provides some protection from metal stress prior to germination, it will eventually crack or become more permeable upon germination. Some studies suggest that seed germination is affected by metals in two ways. Firstly, by their general toxicity, and secondly, by their inhibition of water uptake. Most papers show that metal treatment causes a concentration-dependent reduction in germination in many species (Kranner and Colville, 2011).

Though some metals, e.g., Mn, Cu, Zn, Mo, and Ni, are essential or beneficial micronutrients for plants at high concentrations all metals have strong toxic effects.

These pollutants can be especially toxic when seed germination takes place, so the seed becomes exposed to the soil characteristics. Some of these contaminants are not harmful to the seed in low concentrations and their inhibition rate is negligible (Lefèvre et al., 2009).

For example, lead case has been widely studied. It is considered a non-essential element for the seed germination and the seedling growth. This metal is well-known for its inherent toxicity even at low concentration rates. Seeds are hardly influenced when this metal is found in their growing soil. Actually, researchers agree that lead not only inhibits the seed from water uptake, but also delays greatly seed germination (Kranner and Colville, 2011).

Unlike lead, zinc is considered to be an essential micronutrient for the seedling development. In fact, some studies conclude that in absence of this element seeds were not able to germinate. Although this vital importance for the seed germination process, when zinc is present in high concentrations in soils then it becomes toxic and can inhibit the seed from water uptake in the imbibition stage, which is an essential metabolism activity for the seedling growth. So, this effect causes a decrease level in germination (Lefèvre et al., 2009).

3.3 Příbram, Czech Republic mining and smelting contaminated district

3.3.1 Localization inside Czech Republic

The mining and smelting district of Příbram is located approximately 60 km SW of Prague, the capital of the Czech Republic (Figure 3.1). The geology of the area is dominated by two Upper Proterozoic belts (volcano-sedimentary rocks), located within the Cambrian geological units (greywackes and conglomerates) and accompanied by the Variscan intrusions of Bohutín (diorite; SW of Příbram) and the Central Bohemian Pluton (granites and granodiorites; SE of Příbram) (Vlašímský, 1982).

The mineralization is related to the Variscan intrusions and occurs as two types of deposits: (i) polymetallic ore veins (Pb-Ag-Zn) located at Bohutín and Březové Hory, SW of Příbram (left Proterozoic belt dipping to the SE); and (ii) uranium deposits located in the contact aureole of the Upper Proterozoic rocks and the Central Bohemian Pluton, SE of Příbram (right Proterozoic belt dipping to the NW) (Vlašímský, 1982). The polymetallic ore deposits are mainly composed of Ag-bearing galena (PbS), sphalerite (ZnS), antimonite (Sb_2S_3) and various sulphosalts in the gangue formed especially by siderite ($FeCO_3$) (Vlašímský, 1982). The U deposits are mainly formed by uraninite (UO_2) within the mixed carbonate gangue minerals (Vlašímský, 1982).

The Příbram district has a long history of Pb-Ag mining, probably dating from the time of the Celtic civilisation, 6^{th}-1^{st} century B.C. (Ettler et al., 2001). The mining activity in the polymetallic ore district peaked between 1850 and 1950. About 3500 t Ag, 480 000 t Pb and 260 000 t Zn were mined in the polymetallic ore district at Bohutín and Březové Hory (Vlašímský, 1982). From 1786 to the 1970s, the smelter located 4 km NW of Příbram processed the Pb-Ag ores mined in the area (Ettler et al., 2001) and since 1972, secondary scrap, mainly car batteries, has been processed. The peak of mining activity in the U district occurred from the 1950s until it was terminated in 1989; the ore was processed outside the studied area.

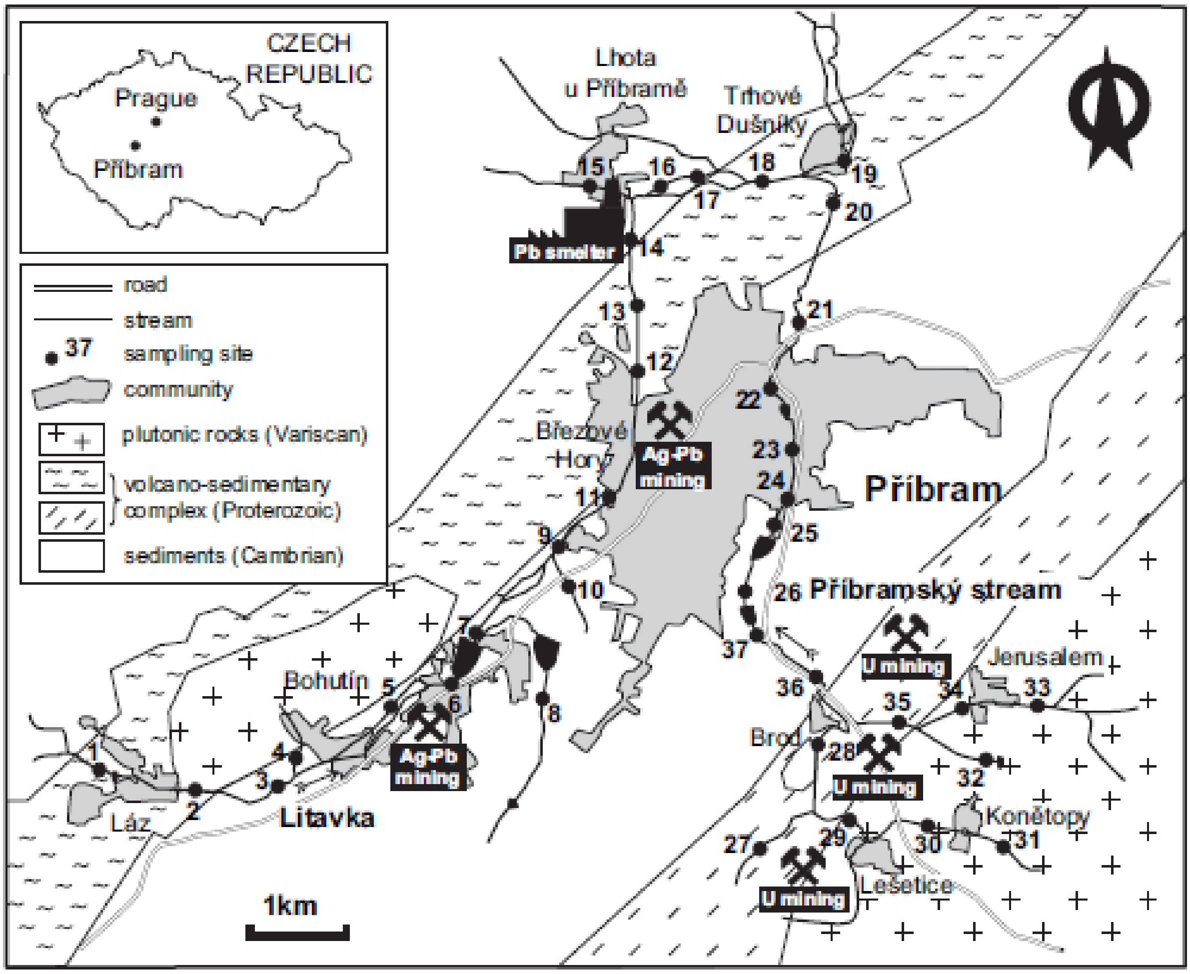

Figure 3.1: Historic mining and smelting areas and geology of the Příbram district (Ettler et al., 2006)

The Příbram district is drained by two principal perennial streams: the Litavka and the Příbramský streams (Figure 3.1). The Litavka springs out W of Láz and flows through the area of the historical mining sites at Bohutín and Březové Hory and the smelter area at Lhota (total length 56 km, total drainage basin approximately 200 km^2, channel and alluvium widths at Trhové Dušníky 4 m and 20 m, respectively) (Vlašímský, 1982).

3.3.2 Mining and smelting, soil main source of metal contamination

Mining and smelting constitute one of the principal sources of metals in the environment at a large number of sites as some articles verify (Hillier et al., 2001; Hudson-Edwards et al., 1996; MacKenzie and Pulford, 2002; Monna et al., 2000).

In the mining district of Příbram, Czech Republic, extensive Ag–Pb mining has occurred since Middle Ages up to the 1970s and U mining was practiced mainly in the second half of the 20th century. Furthermore, the Pb smelter, which is in operation for over 200 years, constituted another significant source of pollution in the area.

Specifically, high concentrations of Pb, Zn and Cd elements have been found around this area, mostly deposited in the surface soil layer because of the pollutants emission caused by the Pb smelter chimney. These contaminants have got direct impact on the environment and plants development on site (Ettler et al., 2000; Šichorová et al., 2004). In the last few decades, a large number of studies have focused on the distribution of metallic pollution in soils, alluvial soils and plants (Ettler et al., 2006).

Soils represent direct sinks for contaminants emitted to the atmosphere by smelters. A representation of this event is shown in Figure 3.2. Contaminants associated with particulates emitted from mining operations are usually concentrated in the fine fraction (<2 μm), while those from smelting concentrates predominantly in the ultrafine particle fraction (<0.5 μm), which may travel greater distances into the environment (Csavina et al., 2011; Csavina et al., 2012; Ettler et al., 2005).

Focusing again on Příbram soil pollution concentration, Šichorová et al. (2004) studies examine the most emission-damaged arable area highly concentrated of Pb, Zn and Cd metal pollutants. Three layers are differentiated depending on the depth of the soil sampling. The first layer including samples between 0-20cm deep turned out to be the most metal contaminated containing the highest Pb and Zn concentrations. In the second and the third soil layers samples between 20-40cm and 40-60cm respectively were studied and its Pb and Zn concentration registered values significantly lower than the ones obtained from the first layer.

Metal and metalloids bearing particles derived from mining-related activities also enter fluvial and alluvial systems primarily through mine or processing waste discharging (tailings from crushing, milling or dressing operations), or remobilization of mining-contaminated alluvium and mine drainage (Hudson-Edwards, 2003). It is not uncommon for more than 90% of the total metal load in rivers to be transported in the solid phase, either adsorbed onto particle surfaces and coatings, or incorporated into mineral grains (Miller, 1997). Fluvial processes exert the greatest influence on deposition and redistribution of these particles in the ecosystems surrounding the stream.

Figure 3.2: Non-ferrous smelter's metal emission scheme (Ettler, 2016)

So, the mobilization of these pollutants must be taken into consideration. Depending on the metal contaminants fractionation in soils, these can easily be leached into the groundwater system. Consequently, this fact enhances the possibility for the plants to uptake these pollutants by root absorption. Příbram metal contaminated soil has been analysed to determine this metal mobility and bioaccessibility by Michálková et al. (2017). Fractionation of the metals in soil was determined using the modified BCR extraction and the high fractionation concentration in Příbram soil for Zn and Pb was found. The exchangeable fraction for Zinc reached up values around 1822 mg/kg, the reducible one arrived at 816 mg/kg value, around 300 mg/kg for the fraction oxidizable one and the residual got as far as 1066 mg/kg. Following the same order, Pb concentration values achieved 281 mg/kg, 2165 mg/kg, 705 mg/kg and 388 mg/kg respectively (Michálková et al., 2017).

These great concentration values demonstrated that Zn and Pb metal pollutants are easily reachable and thereby must be stabilized to avoid plants from its uptake.

3.3.3 Plants species growing in Příbram contaminated soil

Mine spoil heaps are habitats with specific ecological conditions that originate from the mining of ores and subsequent pollution of soil with extreme quantities of metals. Due to the toxicity of most metalloids, only a few plant species can grow there. They have developed different mechanisms to cope with the hostile environment and form distinctive plant communities (Banásová et al., 2006).

Depending on the type of metal the mine is extracting, the soil around the mining and smelting place will be contaminated by some metals or some others. For example, Slovakian region Banská Štiavnica is characteristic by Pb-Zn ore excavation and some studies have concluded that the soils in mine heaps contain high concentrations of Zn, Pb, Cd and a slightly increased content of Cu. In this contamination profile grass species such as *Agrostis capillaris, Avenella flexuosa, Festuca rubra* and, dicotyledons like *Arabidopsis arenosa, Dianthus carthusianorum,* and *Acetosella vulgaris* have been found naturally growing there (Banásová et al., 2006).

In Příbram was carried out Ag-Pb mining and there was also a Pb smelter. So, both activities concluded in a high Pb, Zn and Cd contamination of the soil. Due to these environmental stress conditions, the vegetation that can be found in this habitat is specific to these conditions. Some researchers have studied this area and concretely the vegetation and flora that is well-adapted to the pollutants that are spread there. Just a few tree communities are naturally growing there; particularly spruces (*Picea abies*), alder (*Alnus*), pine (*Pinus*) and birch (*Betula*) mostly. Other climatically more demanding types such as linden (*Tilia*), beech (*Fagus*), maple (*Acer*) or hazel (*Corylus*) can also be found in Příbram vicinities (Brizova, 2008; Ettler et al., 2005).

Regarding the grasslands plants, similarly as in Banská Štiavnica, the greatest population of herbs found belonged to the plant family of *Cyperaceae* and *Poaceae.* The most common grass species spotted there growing naturally are *Molinia caerulea*, *Calamagrostis epigeios*, *Agrostis capillaris* and *Festuca rubra* (Brisova, 2008; Suchara et al., 2011).

3.4 Phytoremediation as a soil decontamination solution

It is possible to safeguard the public health through the recovery of contaminated sites, which can also be reused for various future activities. Furthermore, the ecologically sustainable remediation techniques involve a substantial reduction of waste volumes produced using physicochemical methods such as soil incineration or excavation and transfer to the landfill. These techniques have other disadvantages: high cost of remediation, possible pollution caused by the release of substances used in remediation processes. Then, eco-friendly biological methods are taken into consideration for soil remediation (Ali et al., 2013).

Bioremediation is defined as the process whereby the pollutants are biologically removed or degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities (Kumar et al., 2011).

Phytoremediation is an applicable technique to several reclaiming treatments because it does not interfere with the ecosystem, it requires little manpower and therefore is not very expensive compared to traditional physicochemical methods. Advances in this sector have been significant in recent years thanks to the use of modern biotechnology as phytoextraction and phytodegradation (Rajakaruna et al., 2006; Souza et al., 2014).

Phytoremediation techniques could be applied for the recovery of the heavily contaminated industrial sites using natives species which are adapted to the soil conditions (Fernández et al., 2017). It is an emerging technology based on the use of green plants to remove, contain, inactivate or destroy harmful environmental pollutants. Like any other new approach, phytoremediation will only be accepted if its success is demonstrated. The key factors are low cost (compared to classical remediation techniques) and aesthetic aspects, making it suitable to remediate large contaminated sites in populated areas (Lelie et al., 2001).

Several types of phytoremediation can be defined:

• Phytoextraction, the use of pollutant-accumulating plants to remove metals or organics from soil by concentrating them in harvestable plant parts;

• Phytotransformation, the degradation of complex organic molecules to simple molecules or the incorporation of these molecules into plant tissues;

• Phytostimulation, or plant-assisted bioremediation, the stimulation of microbial and fungal degradation by release of exudates/enzymes into the root zone (rhizosphere);

• Phytovolatilization, the use of plants to volatilize pollutants or metabolites;

• Rhizofiltration, the use of plant roots to ab/adsorb pollutants, mainly metals, but also organic pollutants, from water and aqueous waste streams;

• Pump and tree, the use of trees to evaporate water and thus to extract pollutants from the soil;

• Phytostabilization, the use of plants to reduce the mobility and bioavailability of pollutants in the environment, thus preventing their migration to groundwater or their entry into the food chain;

• Hydraulic control, the control of the water table and the soil field capacity by plant canopies.

Besides metabolization, one of the most striking features of all phytoremediation techniques is the extensive evaporation of water from plant-covered sites. This high consumption of water that may almost equal the amount of water added to an area via precipitation prevents pollutants wash out and slows down the possible migration in the soil and into the ground water. Furthermore, upward movement of water will also transport soluble pollutants into the plants (Schwitzguébel, 2004).

Whereas the removal of contaminating metals seems to be governed by the processes of ion transport and hyperaccumulation in tolerant plants after mobilizing the metals in the rhizosphere, phytodegradation and phytovolatilization of organic xenobiotics have both to rely on the metabolism of foreign compounds in the plant. Even more complicated, organic pollutants under consideration are often lipophilic or covalently bound to soil components which causes a severe problem to the uptake of the compound into the plant. Once in the plant rhizosphere, the pollutants have to migrate into the root, then become translocated into other tissues and organs of the plant, where detoxification and metabolization will take place (Schwitzguébel, 2004).

3.4.1 Suitable plant species for phytostabilization purposes

Revegetation of metal contaminated soils based on aided phytostabilization is recognized as a practical and environmentally sustainable remediation practice. This technique requires both incorporation of adequate amendments and revegetation with suitable plant species, basically those which are able to germinate and adapt to metal contamination but also to survive in highly acidified soils (Yang et al., 2016).

Several studies proved successful aided phytostabilization cases. Most of them were carried out in acidified soils but also in mining metal contaminated soils. For example, Gray et al. (2006) successfully established a good *Festuca rubra* cover on a moderately acidic (pH = 4.7) mine site by the addition of lime and red mud (at 3% or 5%). Bleeker et al. (2003) revegetated a highly acidic (pH = 3.6) As-contaminated mine spoil tips in Portugal using a combination of three additives (beringite, steel shots and organic matter) and two native grass species (*Agrostis castellana* and *Holcus lanatus*) (Yang et al., 2016).

3.4.2 *Festuca rubra* and *Agrostis capillaris* study case

Festuca rubra L. is a perennial grass widespread along the northern hemisphere from temperate regions to arctic wastelands. Frequently grows on dry to wet sites in open habitats from sea level to high elevations in clay, loam and sandy soils. It mostly grows on sand dunes, dry beaches, coastal headlands, freshwater shores, bogs, marshes but also it often colonizes abandoned mines and it can easily be found close to the road and along the railway tracks. In Europe and North America it has been intensively used in the formation of man-made meadows, pasture and lawns, in the most diverse kinds of sites and climatic conditions (Brizova, 2008; Ettler et al., 2005).

Several studies prove that this grass belonging to *Poaceae* family have the potential to colonize areas under metal contamination stress conditions. For instance, *F. rubra* can germinate under metal contaminated soil because of its mechanisms of antioxidant protections. It has also the potential to retain metal contaminants in its densely tufted root system. These specific characteristics make *F. rubra* an excellent plant for phytostabilization purposes (Gajić et al., 2016).

Agrostis capillaris L. properties are also similar to *F.* r*ubra.* It is a perennial grass that inhabits various environments ranging from urban to coastal wetland, including grassland as well as near arctic regions of the world. Disturbed areas and roadsides are frequently invaded with this grass. It impacts native biodiversity in its known introduced range by out competing and replacing native species (Johnston and Pickering, 2001).

This perennial grass tolerates contaminated soils and it physiologically adapts to metallicolous ecotypes (Austruy et al., 2013) and presents high As tolerance (Meharg and Macnair, 1991). Some studies verified that *A. capillaris* in support of biochar amendment and exposed to Pb-Zn contaminated soils was capable to develop genetic adaptations to cope with metal contamination. This high metal contamination stress toleration makes it successful and suitable for phytostabilization purposes (Austruy et al., 2013; Doubková and Sudová, 2016; Houben and Sonnet, 2015).

3.4.3 Nano zero-valent iron and biochar as amendments for polluted soils

Unlined waste grounds containing elevated concentrations of non-ferrous metals can be a continuous source of metals spreading to the surroundings. Due to high amounts of non-ferrous-metals, the top layer of such contaminated soils can be strongly phytotoxic, thus preventing the establishment of vegetation. The absence of a vegetative cover facilitates lateral wind erosion of metal contaminated particles. It also drastically changes the soil water balance and enhances the volume of water percolating through the dump and eventually, reaching the underlying ground water (Stern et al., 1995).

Restoration of a vegetation cover can significantly limit both routes of continuous metal dispersion. Such a vegetation cover fixes and stabilises the top layer and blocks wind erosion. The revegetation of such waste sites is, however, often problematical due to the phytotoxic potential and the dryness of the substrate. Under such conditions, a solution to the problem can be the use of metal-tolerant plant ecotypes, in combination with an enhanced immobilization of the metals on the soil complex, through the addition of metal-immobilizing soil additives (Vangronsveld et al., 1995).

However, when the metal availability to the plants is very high, even metal-tolerant plants are not always able to survive. Some factors that increase the metal availability to the plants are the pH, the cation exchange capacity, the organic matter concentration, the speciation of the element or the type of soil. Under these conditions, mixing of metal-immobilizing additives in the soil to reduce its phytotoxic potential should be considered (Vangronsveld et al., 1995).

Incompletely combusted or charred materials from agriculture, such as biochar, have been suggested as a potential amendment to trap pollutants in contaminated soils. Due to its high aromaticity and high surface area, biochar is considered as a strong and effective sorbent for both organic and inorganic pollutants (Ahmad et al., 2014; Kookana, 2010).

When applied in contaminated soils, biochar can potentially reduce pollutant bioavailability (Chai et al., 2012) and pollutant reactivity (Xu et al., 2011). Besides, with the high porosity, cation exchange capacity and sorption capacity, biochar provides a suitable habitat for microorganisms and might affect different microbial processes (Jindo et al., 2012; Thies and Rillig, 2009).

In the last 15 years, the use of nanoscale zero-valent iron (nZVI) to remediate polluted sites has gained increasing attention due to its metal phytostabilization potential (Mar Gil-Díaz et al., 2014). Because of its reduced size, nZVI has a higher reactivity towards a broad range of contaminants, including halogenated compounds, nitrates, phosphates, polycyclic aromatic hydro-carbons, and metal(loid)s, and a higher mobility compared to its microscale counterpart.

By virtue of its sorption properties, nanoiron particles are supposed to decrease mobile, bioavailable and bioaccessible fractions of the metal(loid)s and minimize thus possible risks of environmental contamination, leaching and uptake by soil organisms, plants, crops and humans (Komárek et al., 2013).

In addition, its application does not require excavation as highly concentrated nZVI slurries are injected directly underground, at or near the source of contamination (Lefevre et al., 2016).

4 Materials and methods

4.1 *Agrostis capillaris* and *Festuca rubra* germination test in absence of contaminants

In this first experiment, *Agrostis capillaris* and *Festuca rubra* seeds germination tests were carried out under non-contaminated conditions. Seeds collected from Litavka alluvium, in Příbram polluted area were collected and preserved inside glass pots, and pure seeds from the same plant species were bought from a company named *Planta Naturalis*.

Five hundred seeds of each specie were selected to run the experiment. The set up consisted of placing two filter papers inside each culture cell. In total 10 repetitions per plant species was set and 50 seeds were sowed per culture cell.

Disinfection of the seeds was conducted to ensure that none hazardous microorganisms were attached on the seed surface or seed-coat. In order to do that, closely 100mL of a peroxide 30% solution was poured into four different 250mL capacity beakers.

Afterwards, in each of these beakers seeds were soaked into the decontamination solution. The seeds were soaked inside the beakers for 15 minutes under a gas extractor hood. Past this period, the seeds were filtrated in a small hole strainer, due to the small seed size, and they were also rinsed with abundant distilled water.

The disinfection and drying procedures were done thus the seeds were ready for the germination test.

As shown in Figure 4.1, seeds were placed carefully in each petri dish using stainless tweezers and they were all moistened with distilled water to break the seed dormancy and initiate the seedling germination.

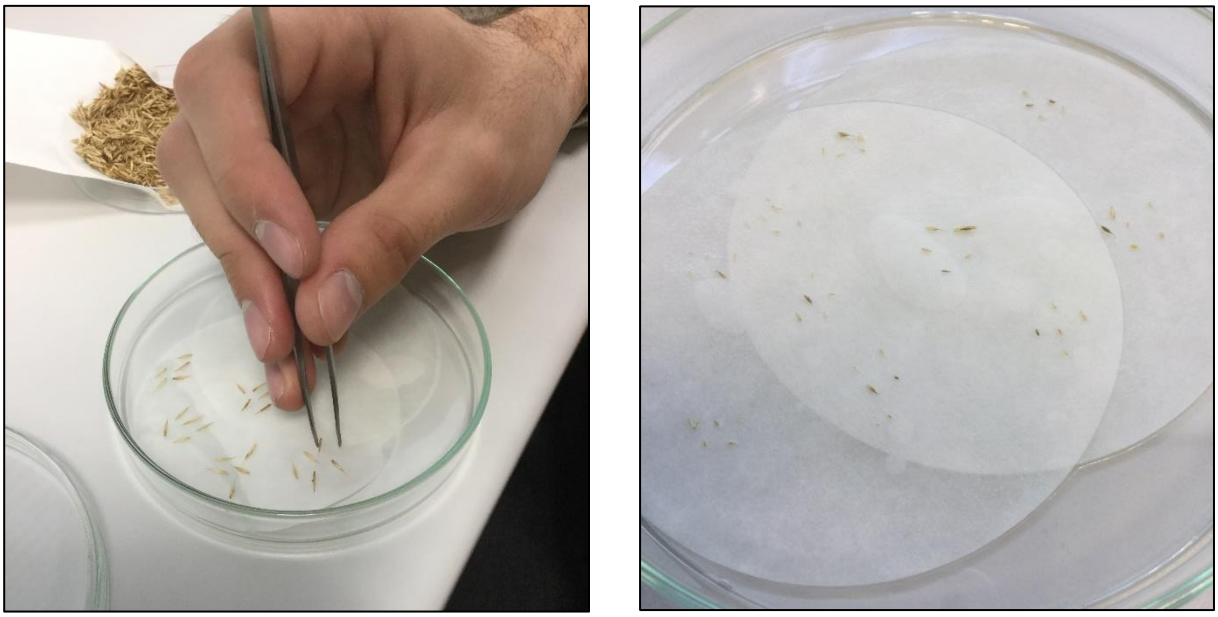

Figure 4.1: *Festuca rubra* and *Agrostis capillaris* placement set-up inside the already prepared culture cells

Then, the entire set of culture cells were placed inside a germinative chamber under optimal germination conditions according to these specifications: 20ºC concerning the temperature, 65% of relative humidity regarding the humidification conditions and 16h of light and 8h of darkness in respect to the light frequency (Figure 4.2).

Germination controls started to take place by the sixth day after the placement of the culture cells inside the germinative chamber and each day seedling germinations were counted and recorded. In addition, when the filter papers were not wet enough, some distilled water was added to achieve the optimal germination conditions. The germination test concluded when there were no more seedling germinations along three days.

Figure 4.2: Entire set of petri dishes distributed on the top shelf of the Binder KBWF growth chamber

4.2 *Agrostis capillaris* and *Festuca rubra* germination test using Příbram contaminated soil in presence of biochar and nZVI amendments

In this second experiment, metal contaminated soils were collected from Příbram area and germination tests of *Agrostis capillaris* and *Festuca rubra* coming from Litavka riverside and from Planta Naturalis enterprise were carried out in four different amendment treatments: 1% nanoiron (NANOFER STAR air-stabilized nanopowder, NANOIRON, Czech Republic), 1% biochar and 1% nanoiron plus 1% biochar and one non-treated contaminated soil. Each treatment was conducted in six repetitions and each repetition contained 20 seeds.

Firstly, as shown in Figure 4.3, metal polluted soil from Příbram area, which basic chemical properties were analysed by Michálková et al.(2017) and the results are displayed in Table 4.1. As visible, the soil is contaminated with Cd, Pb, Zn and As, with especially high concentrations of Pb and Zn. The soil was collected using a previously cleaned plastic flowerpot and poured into a plastic bucket. Exactly, a dozen fully charged flowerpots were poured into a plastic bucket and the total content of soil was weighted in a laboratory scale.

Figure 4.3: Contaminated soil from Příbram being collected with a plastic flowerpot

Once the measurement was done, 1% of the total mass was calculated and the value of that 1% weight was the amount of amendment added to the soil. Inside the first plastic bucket approximately 757g of contaminated soil and 8g of nZVI were poured. Into the second bucket 894g of polluted soil and 9g of biochar were added and in the third one 1065g of Litavka soil, 11g of nZVI and 11g of biochar were poured.

Table 4.1: Basic physico-chemical characteristics of the investigated contaminated soil. Limit concentrations of metals/metalloids in agricultural soils according to the Ministry of the Environment of the Czech Republic (Act No. 13/1994) (Michálková et al., 2017).

		Fluvisol
pH_{H2O}		5.95
pH_{KCl}		5.14
CEC (cmol kg^{-1})		9.08 ± 0.52
TOC (%)		2.15
Particle size distribution (%)		
Clay (%)		7
Silt (%)		31
Sand (%)		62
Texture		sandy loam
Total metal concentrations (mg kg^{-1}) (n = 3)		Limit concentrations (mg kg^{-1})
As	296 ± 6	30
Pb	3539 ± 375	140
Cd	39 ± 1	1
Zn	4002 ± 68	200
Cu	68 ± 3	100
Fe	$37\,408 \pm 195$	no limit
Mn	4276 ± 34	no limit

The materials shown in Figure 4.4, plus a plastic spoon for the nanoiron, were previously cleaned and used to add up the required quantity of amendment.

Figure 4.4: Laboratory instruments used to measure and add up the amendments to the soil. In addition, a plastic spoon was also used for nanoiron amendment processing

Once all the buckets were filled with the soil and the correct amount of amendment, then all the containers were closed using its corresponding cover and they were completely agitated to homogenize the mixture.

Subsequently, an amount of distilled water according to 60% of water holding capacity of the soil was poured and stirred to moisten the soil mixture and to activate the amendments. At that point, humidity controls were conducted twice per week for a month to prevent the soil dryness.

Passed that month, the activation of the amendments was completed so the different treated soils were ready to be used for the germination tests of the four different species.

As shown in the Figure 4.5, a metallic spoon was used to place the soils from the container inside the petri dishes and afterwards the 20 seeds sowing per culture cell was executed using metallic tweezers and latex gloves.

Figure 4.5: Treated soil placement inside the culture cells before the seed sowing

As it was also done in the first experiment, the total amount of culture cells was placed inside the germinative chamber under the same light, temperature and humidity conditions that were selected in the first experiments. As shown in Figure 4.6, germination controls started to take place by the sixth day after the placement of the culture cells inside the germinative chamber and each day seedling germinations were counted and recorded.

Once again, the experiment concluded when no more seedlings were growing for three consecutives days

Figure 4.6: Example of a daily seedling control taken along the 20 experiment days

4.3 Statistical analysis

For the germination tests, an excel table to record new daily seedling germinations and graphics of germinated seeds during the experiment were made. This germination computation started the 6^{th} day after the seed sowing day and concluded the 20^{th} day, when no more seeds were germinating for 3 consecutive days.

Maximum germination time and Relative Germination Rates (RGR) were calculated. These RGR values were obtained dividing the germination percentage of each treatment and specimen by the germination percentage of each specimen obtained in the first experiment.

Analysis of variance and Tukey tests at p=0,01, using R+ 3.3 software, were performed to compare the results obtained from these excel charts and conclude whether substantial differences between experimental results could be confirmed or not.

5 Results

In Figure 5.1 can be seen that *A. capillaris* seeds from Pnaturalis were the ones germinating the most with a total of 380 out of 500 seeds. They mostly germinated between the 6th and the 11th day. During this time interval 265 new seedlings germinated reaching the accumulative number of 337 seedling.

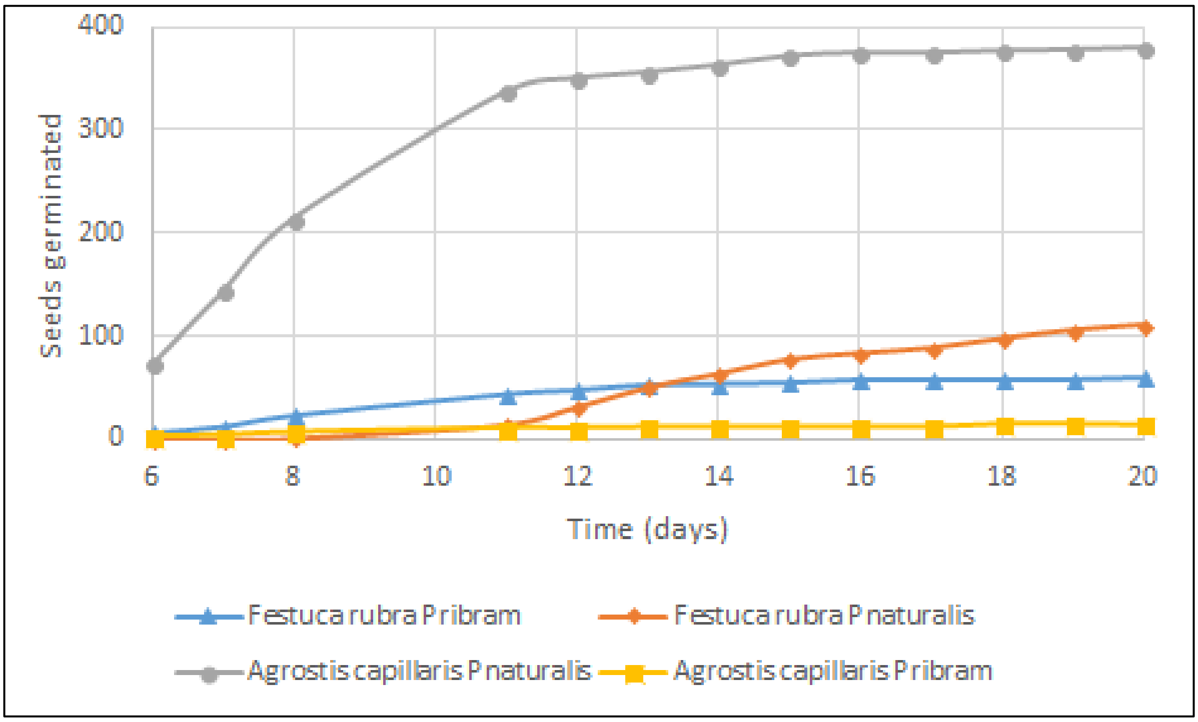

Figure 5.1: Number of seeds germinated on filter paper culture cells along the first experiment, which lasted 20 days, classified by grass specimen and provenance

F. rubra from Pnaturalis was the following specie which germinated more with a total amount of 110 seedlings. Unlike *A. capillaris* from Pnaturalis, the records from the results table showed that *F. rubra* started mostly to germinate the 11th day and then regular daily germinations were noted until the 18th day when the seed germination was decreasing.

Seeds from *F. rubra* and *A. capillaris* from Příbram registered the lowest values of seedling germinations, when just 60 and 14 seeds of them germinated on the filter paper culture cells.

It should be mentioned that despite the disinfection processes the seeds were subjected, both of *F. rubra* specimens presented growth of fungi on the filter paper culture cells.

For the second experiment, *A. capillaris* seeds from Pnaturalis were the most succeeding ones in germination terms, similarly to the first experimental set. No substantial differences were observed between the different treatments for this specie (Figure 5.2). In addition, a high percentage of the seeds germinated because in all the cases around 110 out of 120 seedlings were counted.

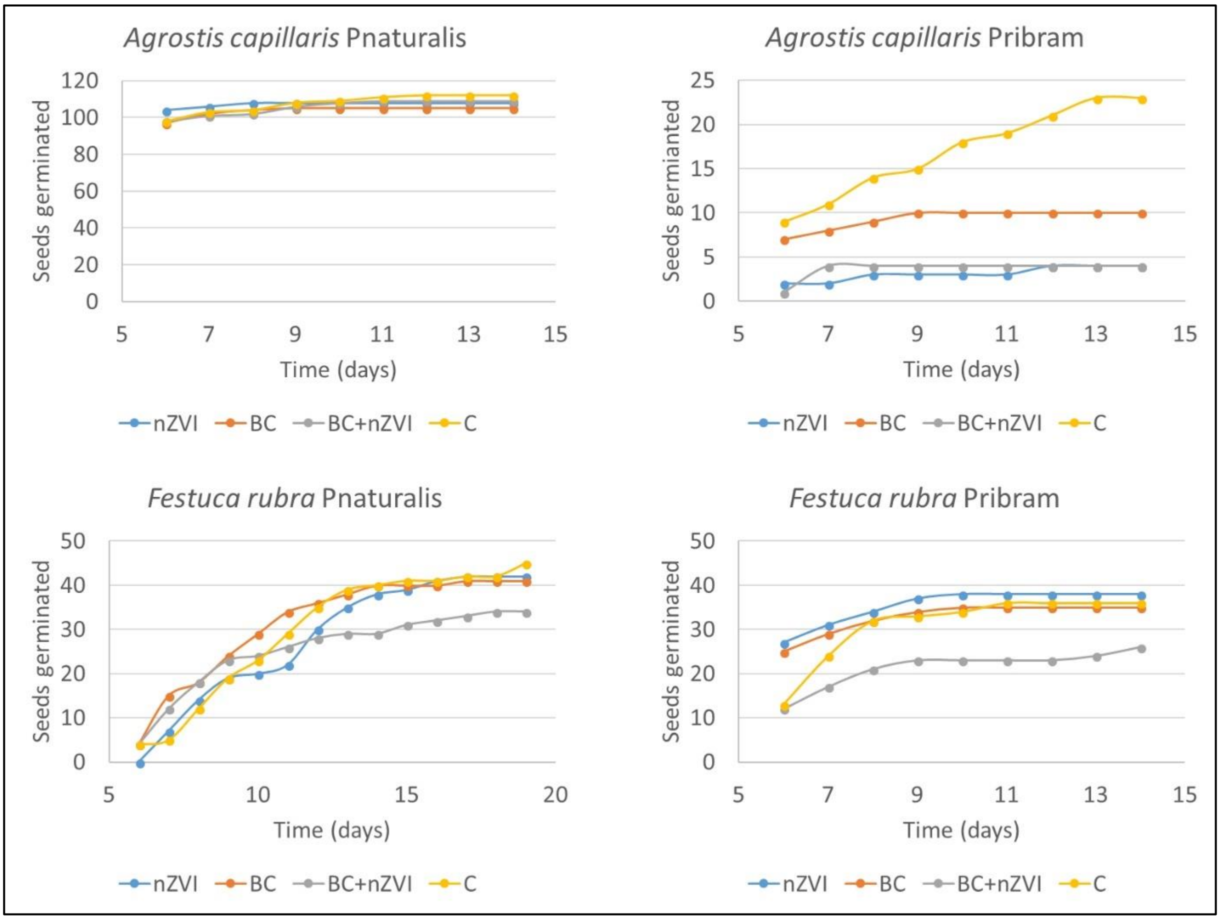

Figure 5.2: Number of germinated seeds corresponding to the second experiment, which lasted 19 days, according the plant specimen, provenance and submitted amendment treatments. nZVI corresponds to the treated soil with 1% of nanoiron, BC to the one treated with 1% biochar, BC+nZVI to the one treated with 1% biochar plus 1% nanoiron and C to the non-treated soil

Results in *A. capillaris* from Příbram showed difference between amendments conditions as observed in Figure 5.2, where the control soil presented higher number of germinated seeds than the other treatments where there were no differences between the three of them.

As it can be seen in the last two graphics*, F. rubra* seeds germination processes did not differ a lot in terms of seed origin. A similar number of seedlings germinations were found in both cases, achieving a total of nearby 40 out of 120 germinations. However, it was observed a germination difference under BC+nZVI amendment. In contrast with the other amendment conditions, BC+nZVI lessen the germinative process in *F. rubra* Příbram seeds.

One of the only significative differences is seen in Figure 5.2 between *F. rubra* from Pnaturalis and *F. rubra* from Příbram, is concerning the time it takes for most of the seeds to germinate, also known as maximum germination time (observed in Table 5.1). In this case, Příbram seeds just needed about 8-9 days to completely germinate while Pnaturalis seeds required more days to reach the same amount of germinated seeds.

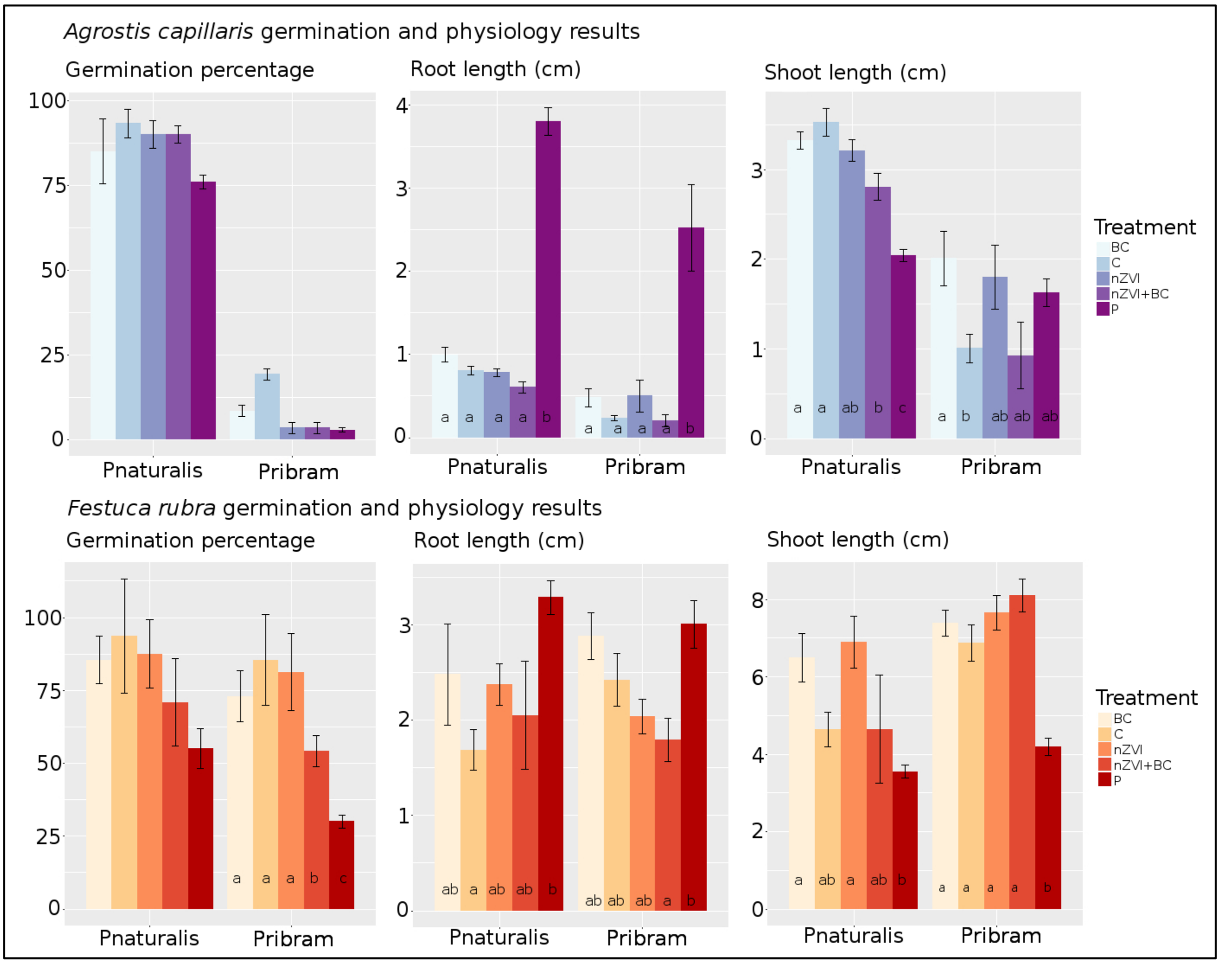

Figure 5.3: A. capillaris and F. rubra mean values and standard error of germination percentage, root length and shoot length. Lowercase letters in the bars (a, b, c, ab) represent significant differences by Tukey test at p=0.01

In all the cases, a highest percentage of seeds germinating in amended or non-amended soils rather than in filter paper was registered (Figure 5.3). These differences are clearly shown in Table 5.1, where all the values for Relative Germination Rate are above 1.

Moreover, the analysis of variance showed significative differences between germination percentage according to seeds species. Regarding provenance characteristics, in *F*. *rubra* instance no substantial germination percentage was found, whereas *A*. *capillaris* case study presented notable divergence in terms of germination percentage results. *A. capillaris* Příbram seeds were notoriously unsuccessfully germinating, compared to the Pnaturalis ones, no matter in which soil or conditions they were growing on.

Soil amendments were also considered while doing the Tukey and variance tests. In this study case, *p* statistical value determined that *A*. *capillaris* seedling germinations were not affected despite of the amendment used, but it affected indeed regarding *F. rubra* seedling germinations, particularly in Příbram ones. A decreased germination percentage was founded when BC+nZVI amendment treatment was applied.

Variance and Tukey results concerning the seedlings physiology, basically onto roots and shoots lengths, displayed a great variability within amended treatments, species and provenance (see Figure 5.3).

For *F*. *rubra* species, BC and nZVI amendment were the ones which helped root growing the most. Respecting *A. capillaris*, no matter what amendment was used that seedling roots length were not significantly different. There were only differences between seed provenance and filter paper or soil environment. Additionally, in all cases filter paper conditions recorded the longest root elongations.

In both *A*. *capillaris* seeds, BC and nZVI amendment were the environmental optimal conditions concerning the shoot elongation length, whereas nZVI+BC and filter paper settings recorded the lowest measured values. A similar case occurred with the Pnaturalis *F. rubra* seeds, but not in the Příbram ones which no significative length registers where examined between treatments.

Table 5.1: Maximum seed germination time expressed in days and Relative Germination Rate (RGR) of each different seed specimen referred to the seed germination test conducted on the filter paper culture cells

Specie	Source	Treatment	Max. Germination Time (days)	Relative Germination Rate (RGR)
A. capillaris	Pnaturalis	P	6	1,00
A. capillaris	Pnaturalis	nZVI	6	1,18
A. capillaris	Pnaturalis	BC	6	1,15
A. capillaris	Pnaturalis	BC+nZVI	6	1,20
A. capillaris	Pnaturalis	C	6	1,23
A. capillaris	Pribram	P	6	1,00
A. capillaris	Pribram	nZVI	6	1,19
A. capillaris	Pribram	BC	6	2,98
A. capillaris	Pribram	BC+nZVI	7	1,19
A. capillaris	Pribram	C	6	6,85
F. rubra	Pnaturalis	P	13	1,00
F. rubra	Pnaturalis	nZVI	12	1,59
F. rubra	Pnaturalis	BC	7	1,55
F. rubra	Pnaturalis	BC+nZVI	7	1,29
F. rubra	Pnaturalis	C	8	1,70
F. rubra	Pribram	P	11	1,00
F. rubra	Pribram	nZVI	6	2,71
F. rubra	Pribram	BC	6	2,43
F. rubra	Pribram	BC+nZVI	6	1,81
F. rubra	Pribram	C	6	2,85

6 Discussion

Seed germination and seedling growth processes are highly influenced under the conditions in which take place. Li et al. (2005) investigations pointed out that metal contaminants (e.g. Pb and Zn) were more hazardous on seedling growth stages rather than in seed germination process concerning *Arabidopsis thaliana*. To overcome this seedling growth and consequently seedling physiology phytotoxic effects that metals produce, biochar and nZVI are commonly used to immobilize and reduce the bioavailability of these pollutants as several studies from many researchers like Michálková et al. (2017), Soudek et al. (2017) Trakal et al. (2016) or Vítková et al. (2017) confirmed.

In this study case, *A. capillaris* and *F. rubra* seedlings physiology showed a significative enhancement regarding root and shoot elongation when BC or nZVI amendments were used. This proves that these two amendment treatments were more successful decreasing the Pb and Zn mobility and consequently the metal uptake by the seedling roots, rather than the combination of both amendments together. However, it should be mentioned that the amendments were added to the soil in low concentration rates. Libralato et al. (2016) studies focused particularly on different sized iron particles addition to plant roots growing inside controlled solutions and their phytotoxic effects in three different species, concretely two dicotyledonous *(Lepidium sativum* and *Sinapis alba*) and one monocotyledon (*Sorghum saccharatum*). In these studies, it was concluded that micro- and nano-iron sized particles produced positive moderate biostimulation concerning seedlings length at 10.78 mg/L and 33.56 mg/L respectively. At this high concentration exposure, metal uptake by the plants roots was also reported as black spots corresponding to nZVI where found attached to the roots.

Particularly, these two amendments were chosen because of their negligible impact concerning seed germination. Martínez-Fernández et al. (2016) studies concluded that no germination differences could be appreciated when nZVI was present in low concentrations in the soil compared to when it was not present. Although, it was also mentioned that the effects of nZVI on germination were concentration dependant. Also Yehia & Joner (2012) studies were focused on this amendment toxicity regarding seed germination inhibition of ryegrass, barley and flax specimens. Their studies observed complete inhibitory effects on seed germination when nZVI concentration was higher than 1000 mg/kg in soils. Libralato et al. (2016) investigations were also focused on seed germination inhibition. Angiosperms species, concretely two dicotyledonous *(Lepidium sativum* and *Sinapis alba*) and one monocotyledon (*Sorghum saccharatum*) were again the subject of his studies and, as in the physiology case, no significative germination changes were recorded in micro- and nano-sized zerovalent irons.

Solaiman et al. (2012) observed the biochar effect on seed germination processes and concluded that seed germination was not being affected or it could even be improved when in biochar amendment presence depending on biochar origins.

As seen in the results, *A. capillaris* and *F*. *rubra* seed germinations were not affected by 1% biochar or 1% nZVI amendments. That could mean that the concentrations used were appropriated in the BC and nZVI cases, whereas phytotoxic effects could have been produced when both amendments were used together in *F. rubra* Příbram seeds. Remarkably, these seeds that presented seed germination inhibition when both amendments were used, presented no physiologic differences in root and shoot elongation characteristics under any treatment. In fact, these Příbram seeds presented the longest shoot and root lengths. These factor could be bounded to the positive biostimulation effect that was observed under high nZVI concentrations recorded in Libralato et al. (2016)

Another point of discussion that could be interesting to mention is concerning the seeds provenance. *A. capillaris* seed germination case, the germination percentage drastically improved in comparison to the ones from Příbram. The ones from Pnaturalis reached seed germination percentages nearby 90% were achieved whereas the ones collected in Příbram where hardly germinating. In their case just the 10% of the seeds germinated, which was the lowest germination rate recorded. This fact shows a huge difference between seeds genetics. Seeds that are being formatted or created in the contaminated plants from Příbram area could present some physiologic damage during its formation process, because they are not even able to germinate under non-stressful conditions, whereas the ones from Pnaturalis, which supposedly have not genetically modified and have not been formed under metal contaminated soils, showed an outstanding performance regarding seed germination process.

Surprisingly this provenance fact was not a decisive factor in *F. rubra* species. In point of fact, seed germination percentage between *F. rubra* from Pnaturalis and Příbram barely changed, though only *F. rubra* seeds from Příbram showed germinative significative differences under BC+nZVI amendment.

Germination time was also a studied value. *A. capillaris* seeds presented a faster germination time in comparison to *F. rubra* seeds. This timing did not significantly differ despite the treatments used regarding the same seeds species. So, the specimens proved not being affected by the different amendment conditions in seed germination timing terms.

7 Conclusion

After the completion of all germination tests and the revision of the results, these information points were reached:

- *A. capillaris* seeds from Pnaturalis were found out to be the seed germinating the most, compared to the ones collected in Příbram and *F. rubra* species
- All *A. capillaris* and *F. rubra* seeds from Pnaturalis showed no germination differences despite the treatment applied
- *A. capillaris* seeds germination time is shorter than in *F*. *rubra* seeds
- *F. rubra* seeds from Příbram germinated less under BC+nZVI amendment rather than the other ones
- All *A. capillaris* seedling root length was not affected by any different amendment treatment
- Biochar and nZVI amendment, when applied separately, improved Pnaturalis and Příbram *A. capillaris* shoot elongation and Pnaturalis *F. rubra* root and shoot elongation
- *F. rubra* seedlings from Příbram presented no physiological differences under any amendment used

Therefore, it was proved that almost all the amendment conditions did not affect in terms of seed germination, in exception from *F. rubra* from Příbram that germination percentage significantly decreased under BC+nZVI amendments. It could be concluded then, that *A. capillaris* and *F. rubra* germination rates are not affected by 1% biochar and 1% nZVI amendment treatments, when applied separately.

So, 1% biochar amendment and 1% nZVI amendment showed no negative influence concerning seed germination and, in both cases, seedlings presented some enhanced physiologic conditions whether in root or shoot elongation. Thus, based on the obtained data, 1% nZVI or 1% biochar amendments demonstrated an enhancement on soils conditions regarding metal particles immobilization and consequently roots metal uptake avoidance regarding *A. capillaris* and *F. rubra* species.

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