CZECH UNIVERSITY OF LIFE SCIENCES IN PRAGUE

Faculty of Environmental Sciences

Department of Environmental Geosciences

DIPLOMA THESIS

Examining the efficacy of different biochars to reduce Zn and Pb uptake by ryegrass; a meta-analysis of diverse heavy metal contaminated soils

Supervisor: Manhattan Lebrun, Ph.D.

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DIPLOMA THESIS ASSIGNMENT

Bc. Sergei Zotin

Regional Environmental Administration

Thesis title

Examining the efficacy of different biochars to reduce Zn and Pb uptake by ryegrass; a meta-analysis of diverse heavy metal contaminated soils

Objectives of thesis

1. to evaluate heavy metals uptake into ryegrass on contracting soils amended by three alkaline biochars,
2. to elucidate the main mechanisms driving any observed differences in the impact of biochars on the contracting soils,
3. to discuss the findings in the context of the wider application of biochars to heavy metal contaminated soils, as part of remedial treatments.

Methodology

Evaluation of the biochar efficacy to regulate Zn and Pb mobility in contaminated soils was tested using a classical pot experiment. The collected soil from different locations after pretreatment was mixed with various biochars, distributed in pots in which ryegrass was subsequently grown. The studied metals were extracted from the surface of the biomass by microwave digestion, and pore water samples were taken directly from the substrate for analysis. Also, measurements of some physical and chemical properties of all samples of the studied soil were taken to establish the cause-effect relationship of the processes occurring in the soil. This study proposes the use of already collected data using the methodology described above. What follows is an analysis and discussion of the results.

The proposed extent of the thesis

40

Keywords

Heavy metals, contaminated soils, biochar, ryegrass, remedial treatments

Recommended information sources

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Author's Statement

I hereby declare that I have independently elaborated the diploma thesis with the topic of: «Examining the efficacy of different biochars to reduce Zn and Pb uptake by ryegrass; a meta-analysis of diverse heavy metal contaminated soils» and that I have cited all the information sources that I used in the thesis and that are also listed at the end of the thesis in the list of used information sources.

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Prague, 28.03.2023   Bc. Sergei Zotin

Acknowledgement

Examining the efficacy of different biochars to reduce Zn and Pb uptake by ryegrass; a meta-analysis of diverse heavy metal contaminated soils

Abstract

In this study the application of different doses (1 % and 5 %) of three biochar on contaminated soils from European countries (Czech Republic, Spain, France) was investigated. All soils have in common a rather high level of contamination in consequence of past mining activities or the automotive industry. In this work, attempts were made to assess the mobility and uptake of Zn and Pb by *Lolium perenne* ryegrass after biochar application. For this purpose, existing data from classical pot experiments that have been done before were analysed. The mobility of the investigated metals was assessed by collecting soil pore water. More effective results for increasing the dry plant biomass were recorded in almost all cases with a biochar dosage of 5 %. Similar results were obtained when observing Zn and Pb concentrations in soil pore water and in dry biomass of ryegrass. The main factors affecting the mobility and uptake of these metals were pH, SOM, and soil texture. Based on the results of this work, it is worth thinking about a wider application of biochar in different areas of human activity due to the availability of materials to produce these amendments and their production method that is not harmful to the environment.

Table of contents

List of Acronyms

ATSDR	Agency for toxic substances and disease
BQM	Biochar Quality Mandate
CEC	Cation exchange capacity
HM	Heavy metals
ICP-MS	Inductively coupled plasma mass spectrometry
LOI	Loss on ignition test
pH	Potential of hydrogen
PYREG	Systems provide a multi material registry
SOM	Soil organic matter
UN	United Nations
WHO	World Health Organization

1. Introduction

Living in an urban environment, many of us are faced with the term - environmental pollution. The types and sources of pollution in the urban environment and in the regions are very diverse. A special place is occupied by heavy metal pollution, in most cases of an anthropogenic nature (Джувеликян et al.). Heavy metals, as a special group of elements, stand out in soil chemistry because of the toxic effect they have on plants and other living organisms at high concentrations. There is no unambiguous definition of heavy metals. There is a definition rather generalizing properties of representatives of this group. Heavy metals are defined as those elements having an atomic number greater than 20 and atomic density above 5 or 6 g cm^{-3} and must exhibit the properties of metal (Basak et al., 2021).

Heavy metals spread by aerial route are dispersed over a long distance around metallurgical plants, coal-burning thermal stations, and other enterprises, and are deposited on nearby agricultural lands. Heavy metals enter agricultural lands from organic and mineral fertilizers, ameliorants, and plant protection products (Водяницкий et al.). These pollutants also enter the environment from quarries, and mines on extraction of polymetallic ores, transport, combustion of oil and various wastes, etc. (Байсеитова et Сартаева, 2014).

It is logical that among the three components of the biosphere, the soil is potentially more polluted than the atmosphere and water, because heavy metals enter this component both from the aquatic environment and from the air environment (Duruibe et al., 2007; Chibuike et Obiora, 2014). It also serves as a source of secondary pollution of surface air and waters that enter the World Ocean from it. In an urban environment, plants can actively uptake various kinds of pollution including heavy metals, which accumulate and have a negative effect either on the plant growth process or consequently on animals or humans that consume those plants (Воскресенский et Воскресенская, 2011). Thus, plants are a reliable indicator of the presence of heavy metals in the environment.

In Western Europe and the USA problems of heavy metal pollution date back to the technological revolution, although the extent of its further consequences was not recognized by the public for a long time (Копылов 2007). In the 1950s and 1960s, the parliaments of these countries passed stringent laws aimed at improving the quality of life, including restrictions on air, water, and soil pollution. In the United States, the Federal Law on State Environmental Policy was passed in 1970. After the adoption of this comprehensive law, others appeared in subsequent years: on clean water, air,

soil, and so on. With their adoption, the ecological situation in the country has changed dramatically for the better, the emission of new pollutants has sharply decreased (Водяницкий et al.).

The measures gradually taken in developed countries have had a positive effect on the planet's ecology. On a global scale, pollution of the biosphere with heavy metals has begun to decrease due, among other things, to the tightening of technical standards and requirements for enterprise technology and the evolution of environmentally friendly factories (Бабкина et al.). But there are still places heavily polluted because of previous unreasonable human activities.

Soil is an essential environmental object. Unlike other objects of the environment (air, water), in which there are processes of self-purification, the soil has these properties to a small extent. Moreover, for some substances, in particular for heavy metals, the soil is a caustic acceptor (Байсеитова et Сартаева, 2014). Heavy metals are firmly sorbed and interact with soil humus, forming a hard-soluble compounds. Thus, they accumulate in the soil. At the same time, under the influence of various factors in the soil there is a constant migration of the substances entering it and their transfer over long distances (Микшевич et Ковальчук, 1988).

Based on the already known facts of the behaviour of two heavy metals (lead and zinc) in the soil and our own observations, we will try to anticipate their behaviour when transferring in soil and when transferring in ryegrass under the condition of biochar application.

2. Literary research

2.1. Sources of soil contamination with heavy metals

The number of heavy metals is usually not specified. Often no more than 40 chemical elements are mentioned, although there is a list of the 19 most studied and frequently encountered elements: Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Ge, Mo, Cd, Sn, Sb, Te, W, Hg, Tl, Pb, Bi (Орлов et al., 2002). This list of metals does not include barium, latanides, or uranium, but rather antimony, which is a metalloid. Soil scientists usually include arsenic, which is also a metalloid, in this list (Ильин et Сысо, 2001). The group of natural heavy metals and metalloids will include all elements, starting with vanadium, i.e., all elements of the periodic table up to uranium, excluding halogens and noble gases, as well as metals that do not contain stable isotopes. Of the actinides, only thorium and uranium remain. A total of 57 elements belong to the group of heavy metals and metalloids (Greenwood et Ernsho, 2008).

2.1.1. Types of soil contamination with heavy metals

Having studied the three types of soil contamination by heavy metals (Водяницкий et al., 2012):

1) global,
2) regional,
3) local.

I would like to consider in detail only the local type of pollution, because it is directly related to the topic of the experimental part of this work. In any case, I will try to summarize the first two types of contamination.

Global pollution changes markedly over time. Isotope-dated snow or peat samples are often used to study the dynamics of global pollution.

After a vigorous scientific debate on the increasing pollution of the environment with heavy metals in Stockholm in 1972, the UN session developed the final materials that contributed to the overall reduction of pollution in the biosphere (©UN, 1972). After taking certain measures, we have already been able to observe the results after almost 20 years, published in 1991 in the journal "Nature". As the Greenland snow samples showed, the Pb content in the atmosphere and troposphere of the Northern Hemisphere decreased by 7.5 times, and the Cd and Zn content by 2.5 times (Boutron et al., 1991).

To analyze regional pollution, observe changes in the composition of river, lake, or sea sediments. They are formed in the process of washing away by rain and melt water of thin soil particles. The content of heavy metals is determined in the bottom sediments of rivers, lakes, and coastal parts of seas. According to an analysis of the metals in the silt of the Rhine, which flows through the industrial areas of 6 Western European countries, from the end of the 17th century to 1975 the concentration of chromium increased 9-fold, copper and lead 13-fold, zinc 19-fold, mercury 50-fold and cadmium 100-fold (Добровольский, 2003). The results of stricter national environmental laws have made themselves felt. Already in the early 1980s, high levels of metals in the bottom sediments of rivers and lakes decreased significantly (Fig. 1).
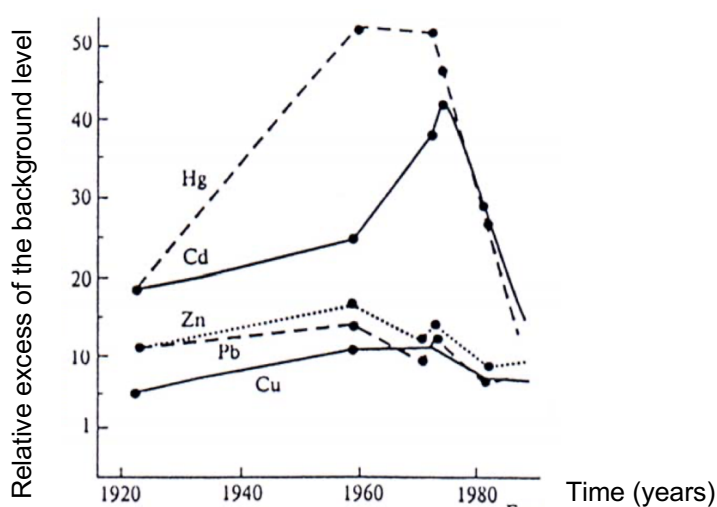
Figure 1. Changes in the content of heavy metals in floodplain sediments of the lower Rhine during the twentieth century (Добровольский, 2003).

And now directly to the local type of contamination. I suggest getting back to the main sources of soil contamination by hazardous heavy elements (Воробейчик et Козлов, 2012):

1) aerial deposition from stationary sources and vehicles,

2) hydrogenous pollution from industrial wastewater flowing into water bodies,

3) sewage sludge,

4) dumps of ash, slag, ores, sludge, etc.,

5) spills of oil and saline solutions at oil production sites.

Since the topic of this paper is related to the location of one of the largest mines of different polymetallic ores (Příbram) in the past, I would like to focus all attention now on this type of pollution sources (mine dumps).

An international team of scientists found elevated levels of Cu, Co, Ni, As, Pb and Zn at the tailings dump Kitwe (Zambia). Heavy metal concentrations were highest within 100 meters of the edge of the dump. Measurements at greater distances from the dump were correspondingly lower (Weissensteina et Sinkala, 2011).

The presence of toxic elements was also found in samples of mine waste around Bokchegiano-Fontalchinaldo (South Tuscany). Mining activity in this place began as early as the 16th century, which makes this example similar to the Příbram location. However, the content of toxic elements in the samples (Cu, Pb, Zn, Bi, Cd, As) was extremely variable, with average values ranging from hundreds to thousands of ppm (with the exception of Bi and Cd). As in the previous example, the concentrations of the studied metals and acids were highest near the investigated wastes and rapidly decrease moving downstream some hundreds of meters or less (Benvenuti et al., 1997).

Zanetta-Colombo et al. (2022) found elevated concentrations of As, Sb, Cd, Cu, Mo, Ag, Pb, and S in several villages of Alto El Loa (Chilli) near the Talabre (Chilli) tailings dam. The researchers cited mining activities as one of the main causes of pollution, adding that wind direction and intensity played a major role in the transport of dust particles. Fig. 2 illustrates the process of contamination of adjacent areas in the aftermath of mining activities.
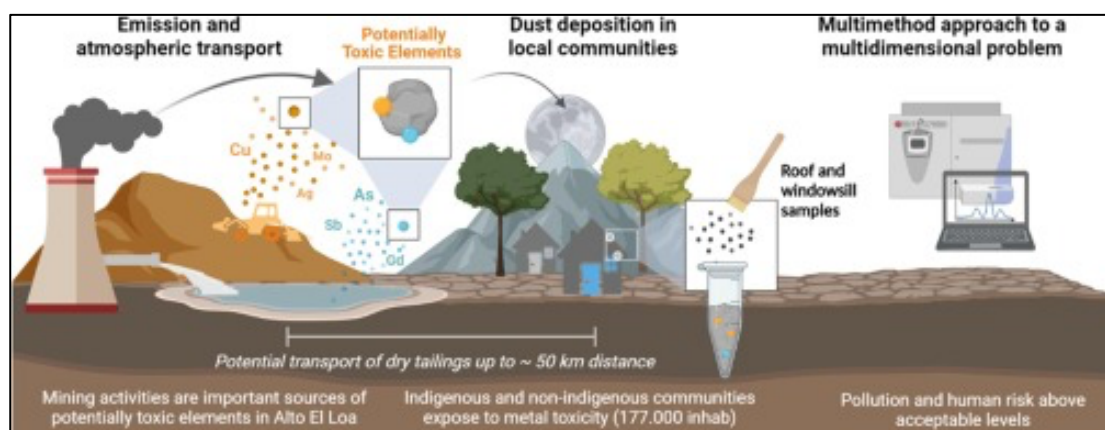

Figure 2. The process of contamination of adjacent areas in the villages of Alto El Los (Chile) in the aftermath of mining activities (Zanetta-Colombo et al., 2022)

The content of heavy metals/metalloids in dumps can be very high. For example, chromium enters soil and water from ore dumps, ferrochrome slag, metal scrap. In the sediments of wastewater treatment plants of electroplating plants, chromium content can reach 150000 mg/kg (Перельман et Касимов, 1999), and Ni up to 6000 mg/kg, Cu up to 11000 mg/kg and Cd up to 1600 mg/kg (Алтухова, 2010). Soils near such dumps are highly contaminated.

The environmental situation is acute in Altai (southern Siberia, Russia), where there are rich polymetallic deposits. Ore has been mined here since the 18th century. A huge number of heavy metals has accumulated in the abandoned workings and dumps. Over the years of the Altai Mining and Processing Combine, two large tailings dumps with a total area of 1 km^2 and a volume of 11 million m^3 were formed near the Gorniak (Russia) town. One of them is a tailing dump of Zmeinogorsk (Russia) gold refinery plant in the floodplain terrace of Korbolikha river. During floods this tailing dump is washed out and the generated wastes flow into the reservoir. The generated waste is hazardous: at the bottom of the tailings dump the Hg content reaches 1.7 mg/kg (at background 0.05 mg/kg), Cd - 8.9 mg/kg (allowed rate 0.2 mg/kg), Cu - 400 mg/kg (allowed rate 26 mg/kg) (Бабошкина et al., 2004).

Dumps of substandard ores on the site of the depleted Aktashskoye mercury deposit in Altai (Russia) are dangerous. The values measured near the dumps in soil averaged 65 mg Hg/kg (allowed rate 0.42 mg Hg/kg) and 152 mg Cr/kg (allowed rate 36 mg Cr/kg). In bottom sediments the allowed content of mercury exceeded by 10 thousand times (Архипов et al., 2004).

All of these examples confirm the presence of elevated concentrations of various heavy metals occurring in different forms. Concentrations of most of them are higher (sometimes several times higher) as they are closer to the local source. Scientific consensus is generally agreed that if nothing is done about this phenomenon, heavy metals and their various forms will eventually penetrate deeper into the soil. This can, in turn, lead to contamination of, for example, groundwater. Undoubtedly, this phenomenon can lead to a few other problems, such as entering the food chain of various animals through the plants.

It is also worth mentioning natural sources of soil contamination. In essence, it is the pollution of soils with lithogenic heavy metals and metalloids. We are talking primarily about direct enrichment of the soil with heavy metals. This phenomenon should be seen rather as a specific soil, formed over a long time. Also, imbalance of chemical elements in soil is dangerous. In other words when some elements are abundant or

in excess and other necessary elements are in deficit. And the third type of pollution is the activation of heavy metals in the soil. When, for example, a simple heavy rain can activate the mobility of some of the heavy metals with which a particular soil is enriched (Матвеев, Авдонькин, 2007).

2.1.2. Pathways of heavy metals entering the soil

A significant portion of heavy metals entering the environment from anthropogenic and natural sources subsequently enters the soil. Thanks to biogeochemical properties and the huge active surface area of the fine-dispersed part, the soil becomes a "warehouse" of toxic compounds such as mineral fertilizers, pesticides, heavy metals, and petroleum products (Попова et al., 2012). At the same time, it becomes one of the most important biogeochemical barriers for most of these pollutants on the way of their migration from the atmosphere to groundwater and river network (Попова et Наквасина, 2014).

Once in the soil environment, heavy metals enter into various chemical, physical, biochemical, and other interactions during which they can leach, accumulate, and enter plants. As a result of these interactions, the danger of metals to living organisms can change significantly (Парфенова, 2020). Heavy metals can be washed out by surface waters, accumulated in aquatic organisms, and bottom sediments, while elements accumulated in soils are slowly removed by leaching, consumption by plants, erosion, and deflation. The duration of contaminants in soils is much longer than in other parts of the biosphere, and soil contamination with toxic components is long-lived.

Heavy metals are concentrated in the near-surface soil layer (0-20 cm), where they are present in the form of exchange ions and are part of humus substances, carbonates, Al, Fe, and Mn oxides. The proportion of the water-soluble form is not high, but their absolute amount becomes an independent ecologically hazardous factor when heavily contaminated (Ильин, 1991). The composition and number of elements retained in the soil depend on the content and composition of humus, acid-base and redox conditions, sorption capacity and intensity of biological absorption.

2.1.3. Peculiarities of heavy metal transport in the soil-plant system

The soil cover is the main source of heavy metals entry in plants. Characteristics of soil cover largely determine the amount and nature of the consumed element and can also increase the toxicity of the metal to plants. The most important characteristics of

soils affecting the intake of heavy metals to plants are pH, cation exchange capacity and organic matter content (Лебедовский, 2007).

Maintaining acidity in soils with significant heavy metal content close to 7.0 prevents the phytotoxicity of many of them, but the same metal concentrations at pH equal to or lower than 5.5 can become lethal to plants. It is important to note that soil pH affects the mobility of metals and their assimilation by the root system of plants (Кравченко, 2016).

Soil organic matter retains metals in different ways. Some of them are fixed by it strongly, others - weakly. In addition, fertilization and liming can reduce or enhance the toxic effect of metals (Ильин, 1991).

Cation exchange capacity (CEC) is the total amount of cations of one kind that can be displaced from the soil. The higher the index, the more fertile the soil. In addition to the mineralogical and granulometric composition of the soil, the CEC is influenced by the content of organic matter, that is, the value depends mainly on the proportion of clay and humus in the soil. The value also increases with increasing site pH (Chibuike et Obiora, 2014).

There are phenomena of antagonism and synergism between heavy metals in soil and plants. The presence of certain elements can promote or hinder the entry of others into plants and mutually enhance or reduce the toxic effects of each other. For example, cadmium content in plants decreases when the concentration of zinc in soil increases (Зубкова, 2004). Cadmium at high concentrations in plants can act as zinc in many biochemical processes. All heavy metals differ in their toxic effects on plant organism by migration and accumulation peculiarities in soil, mechanisms of uptake by a plant community.

2.2. Migration of heavy metals in the soil profile

The danger of heavy metal migration lies in their final entry into receptors other than soil. Thus, heavy metals accumulated in the soil can be a secondary source of pollution of surface and groundwater and, later, the ocean, or they are absorbed by plants and subsequently enter the food chain. In these examples, soil acts as a pathway for pollutants to enter the above-mentioned receptors. Therefore, studying the migration processes of heavy metals can help us to anticipate possible contamination of, for example, groundwater. Fig. 3 illustrates the heavy metal migration pathways and their consequences from sources to the final receptor. We

can observe the complexity of this mechanism, the result of which depends on a huge number of variables.

This process is quite difficult to analyze only because it depends on many factors occurring in the soil itself for a long time, as well as factors occurring at the moment. So, the accumulation of the main part of pollutants is observed mainly in the humus-accumulative soil horizon, where they are bound by aluminosilicates, non-silicate minerals, organic matter due to various interaction reactions. The composition and number of elements retained in the soil depend on the content and composition of humus, acid-base and redox conditions, sorption capacity, intensity of biological absorption. A part of heavy metals is firmly retained by these components and not only does not participate in migration along the soil profile, but also does not pose a threat to living organisms. Negative ecological consequences of soil pollution are associated with mobile metal compounds (Горбунова et al., 2009).

Within the soil profile, the anthropogenic flux of substances meets a number of soil-geochemical barriers (Сарапулова, 2018). These include carbonate, gypsum, illuvial horizons (illuvial-iron-humus). Part of the highly toxic elements can pass into compounds that are difficult to uptake for plants, other elements that are mobile in this soil-geochemical environment, can migrate in the soil column, posing a potential danger to biota. The mobility of elements largely depends on acid-base and redox conditions in soils. In neutral soils, Zn, V, As, and Se compounds are mobile and can be leached during seasonal soil soaking (Соболева et Шишлова, 2018).
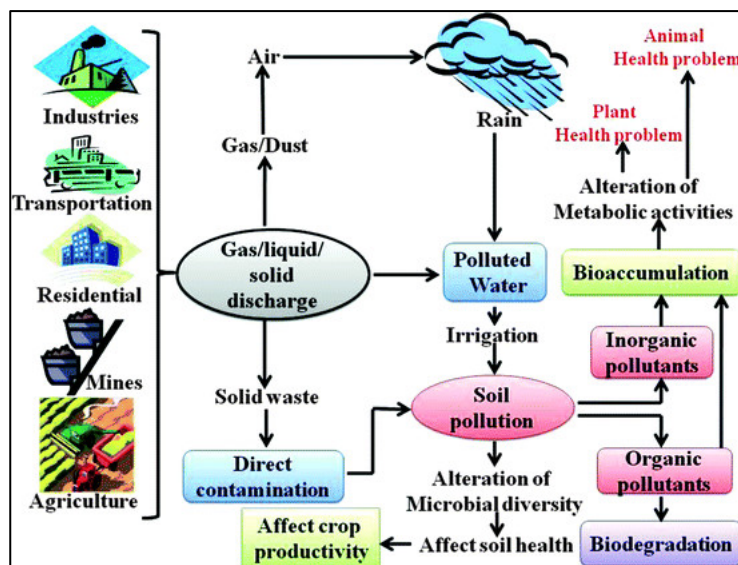

Figure 3. Migration pathways of heavy metals and their consequences (Oves et al., 2012)

However, these are not all factors on which the mobility and possible subsequent transformation of various elements, including heavy metals, depends.

2.2.1. Properties of soil based on water content

Also, not a less important factors are water permeability, capillarity, moisture capacity and evaporation capacity of soil.

Perhaps the most important property of the above is water permeability. The ability of the soil to conduct water from its upper to lower horizons consists of three phenomena: water absorption by the soil, soil soaking, and filtration. Water absorption into the soil is carried out by sorption and capillary forces, soaking by capillary forces, and filtration by gravitational forces. Water permeability depends on granulometric composition of soil, presence of humus substances and structure (Гаргуша, 1962).

In soils of heavy granulometric composition with high content of clay particles and with small pores water permeability is weaker and vice versa. Sandy soils have the best filtration capacity, while clayey soils have the worst filtration capacity. In clayey soils, characterized by very fine porosity, water infiltration is so difficult that the filtration rate is sometimes reduced to zero, as the soil is almost impermeable to water. Soil structure has a great influence on filtration rate: water permeability of structural soils is better than that of unstructured soils (Лившиц et Цыганов, 1999). Essential role in this respect is played by the presence of humus in soil, and its value is different in soils with different texture. Finally, the degree of soil compaction has a great influence on water permeability. Looser soils are more permeable. Conversely, more compacted soils have less water permeability (Гаргуша, 1962).

Accumulation of mobile forms of metals depends on water and air regime of soils. Thus, the least HM accumulation is observed in water permeable soils with abundant precipitation exceeding evaporation. It increases in soils with uniform regime when the amount of precipitation corresponds to the evaporation value. And maximum value in soils with prevailing evaporation and low amount of rainfall (Авдощенко et Климова, 2021).

2.2.2. Redox potential

The redox potential plays a significant role in internal processes in the soil. During oxidation, the oxidizing element loses an electron; during reduction, the restoring element gains an electron. Oxidation reactions are always accompanied by reduction reactions and run concurrently. This factor regulates the solubility and availability of hazardous elements in the soil-plant system (Kabata-Pendias et Pendias, 2001).

The main oxidizing agent in soils is oxygen of soil air and dissolved in soil moisture, while reducing agents are organic matter, hydrogen, and hydrogen sulfide. Conditions that determine intensity and direction of redox processes are soil moistening and aeration, content of organic matter and activity of microflora. Redox potential of soils is heterogeneous within the soil profile and dynamic over time (Иванова et Синицын, 2017).

Elements are more tightly bound in heavy neutral and alkaline soils and can be taken up slowly by plants. Light soils are a source of readily available metals. Losses can occur more easily in these soils (Wenzel et al., 1999).

2.2.3. Soil pH

This soil factor is one of the most important characteristics of soil and is therefore a common analysis. The medium reaction (pH) depends on the content of hydrogen ions (H^+) and serves as an indicator of the acidity or alkalinity of the soil. This indicator depends mainly on ionic exchange with mineral and organic colloids and the presence of calcium, sodium, potassium, and other cations. Soil pH varies from 4.0 and less (strongly acidic) to 7.0 (neutral) to 11.0 (strongly alkaline). As the pH increases, the possibility of formation of insoluble hydroxides and carbonates increases (Соболева et Шишлова, 2018).

Calcium, magnesium, potassium, and sodium can displace hydrogen ions, reducing the acidity. Therefore, not only soils with a lot of lime (calcium), but also saline soils with excessive sodium can be alkaline. Acidity in most soils is due to the concentration of cations on the surface of small clay particles. It is on the surface of these small soil particles that nutrients are held and the size of the total surface of these particles determines the properties of the soil and its fertility (Иванова et Синицын, 2017).

The acidity of soils is caused by many factors, one of which is the dissociation of functional groups of humus and the other is the microbiological decomposition of organic matter. Other sources of soil acidity are clay-silicate minerals and iron and aluminum hydroxides. The intensity of soil acidification depends to some extent on the equilibrium between hydrogen and aluminum ions (Kabata-Pendias a Pendias, 2001).

A huge number of experiments have been conducted confirming the influence of soil pH on the mobility and concentration of various heavy metals. For example, in the pH range from 4 to 7.7, the sorption of Cd increases up to three times with an increase in pH by one unit (Christensen, 1989). Black soils and rendzinas contain a lower

proportion of the mobile fraction of Pb and Zn than podzols and Cambisols with low pH (Makovníková, 2000). Another example shows that changing the pH from 5 to 7 significantly affected the available Cd and Zn content of the soil. A decrease from 75 % to 15 % was found for Cd and from 50 % to 5 % of the available amount for Zn (Tiller et al., 1984). In the following experiment, it became known that when the pH of the contaminated soil was changed from 5.7 to 7.3, the mobile fraction of zinc, cadmium and lead in the soil decreased by 70 %, 50 % and 20 % respectively (Tlustoš et al., 2006). Studies with arsenic showed higher plant uptake in parallel with increasing pH. Thus, under oxidizing conditions (when pH changed from 5 to 8), 3 times more soluble As was found in the soil solution, and almost all of it was in the form of As^{5+}. Under reducing and alkaline conditions, it was mainly in the form of As^{3+}, and its concentration was lower than under acidic conditions (Masscheleyn et al., 1991).

The research team noticed that as the pH increased, the surface potential and proton competition decreased, resulting in higher uptake of copper (Yujun Yin et al., 2002).

The adsorption of cations of hazardous elements depends on the density of negative charges on the surface of soil colloids, which in turn is affected by the pH of the medium. However, the distribution of the displacement force between cations is uneven, so some of them may have a greater displacement force than others and may be selectively bound to sorption sites. In addition, these elements compete with more common ions such as Ca and Mg to occupy these sorption sites (Tlustoš, 1999). It has been found that the amount of adsorbed Cd increases almost linearly with increasing pH, and this process is reversible. In general, it can be assumed that this principle is applicable for most cations. The cation exchange capacity (CEC) affects the absorption of the required elements both directly, through the number and strength of the binding sites of elements in the soil, and indirectly, through the influence on other soil properties (Adriano, 2001).

The interrelation of several factors that ultimately influenced soil adsorption of heavy metals was observed by a group of researchers Wang et al. (2023) from China. They reported that higher soil pH generally promotes soil adsorption of heavy metals. The higher the soil pH was, the more negatively charged surface areas were present. These conditions favor complexation and ionic exchange between soil and heavy metals. Competition between H^+ and metal ions was observed for active adsorption sites in soil at low pH and increasing amounts of H^+ (Sauve et al., 1997). A correlation was found between soil CEC and heavy metal adsorption (McBride et al., 1997; Hooda et Alloway, 1998). The content of clay minerals in the soil, which tend to be

small in particle size with a large specific surface area, and therefore can bind heavy metals through complexation and electrostatic attraction reactions, was also considered (Ugwu et Igbokwe, 2019). These results showed that increasing soil pH, CEC, and clay content can promote soil adsorption of heavy metals and prevent metal migration (Wang et al., 2023).

2.2.4. Soil organic matter

We should not forget about no less important factor as soil organic matter. This characteristic is responsible for the fertility of the soil. Substances representing the complex of organic compounds are of two types: humus and non-humus substances. Significant part (85-90 %) of organic matter are fulvic, humic and humin acids, which are typical representatives of humus substances. Almost all representatives of heavy metals exhibit coordination properties, contributing to the formation of soluble complexes when interacting with certain substances in the soil. A special role in this process is played by trace elements with humic and fulvic acids, which in interaction with heavy metal ions can form the very complexes. The formation of strong compounds of metals with organic matter largely determines the conditions of migration or accumulation of metals and humic substances in the soil profile (©AGROTEST 2021).

A group of researchers noticed a pattern in the distribution of heavy metals by groups of organic matter: in almost all genetic horizons, fulvic acids accumulate large amounts of HM compared to humic acids (their share ranges from 52 to 98 %). They also noticed the predisposition of the higher molecular weight components of fulvic acids to contain much higher amounts of HM. A fraction with a molecular weight of more than 700 a.u.m. accumulates 30-35 times more copper and cobalt, 40 times more cadmium, and 60 times more zinc than a fraction with a molecular weight of 258 a.u.m. (Галактионов et Карпухин, 2007).

The content of non-humified organic matter in the soil is much less, but their importance is important. This includes animal and plant residues of varying degrees of decomposition, as well as intermediate products of decomposition such as proteins, resins, fats, fiber, organic acids, etc. Their role rather focuses on plant and animal nutrition. Some of them influence the transformation of nutrients into available forms for absorption. They are the source of all macro- and microelements (©AGROTEST 2021).

The solubility of soil organic matter increases with increasing pH, which affects the stability of complexes consisting of humus and metal. Humic acids are insoluble in

neutral and acidic soil reactions and thus can contribute to the retention of harmful elements in the soil and reduce the amount of metals in the soil solution. On the other hand, fulvic acids and their complexes are soluble even in acidic reaction and therefore can increase the mobility of elements bound to them (Borůvka and Drábek, 2002).

2.2.5. Microorganisms' activity

Increasing the content of humus in the soil creates favorable conditions for the development and activity of microorganisms. Microorganisms activate many biochemical processes in the soil, participate in the mineralization of organic matter, and increase the availability of soil nutrients to plants. Therefore, soils rich in microorganisms are more fertile and provide higher crop yields (©AGROTEST 2021).

Heavy metals in soil affect cell division of microorganisms and change the variety of soil microflora, which is represented mainly by trophic groups. Microorganisms have a role in the migration of heavy metals in the soil. They can act as producers, consumers and transporting agents in the soil ecosystem. Many soil fungi can exhibit the ability to immobilize heavy metals by accumulating them in mycelium, temporarily excluding them from the cycle of substances (Барабанщиков et Сердюкова, 2017).

2.2.6. Coefficient of biological absorption

It is also worth paying attention to the coefficient of biological absorption. Vegetation plays an important role in the biogeochemical cycle of substances in nature. In addition to the elements necessary for their vital activity, plants absorb and accumulate heavy metals in different parts of their bodies. It is the intensity of absorption that is the key indicator. Concentration of trace elements in plants depends on many independent factors: on the content of elements in soil-forming rocks, their mineralogical composition, soil type, topography and location of groundwater level, morphological features of plants, peculiarities of their vegetation, etc. Therefore, the distribution of concentrations in vegetation samples is determined by statistical laws (Добровольский, 1983). Plants in this context resist the vertical migration of HM into the soil depth, being at the same time indicators of pollutants.

However, this factor influencing the migration of HM in the soil can be considered as a separate type of receptor in the system "source-pathway-receptor".

I would also like to mention such a factor as soil air. The amount and composition of soil air have a great influence on the development and vital activity of plants and

microorganisms, the solubility of chemical compounds and their migration in the profile, on the intensity of soil processes (Марчик et Ефремов, 2006).

2.3. Biochar production and application

Biochar is charcoal of plant origin, i.e., it is not obtained only from wood. In particular, to produce this type of biochar it is allowed to use forestry and agricultural waste: bark, shredded wood, including secondary wood in the form of pallets and others. The product has a high carbon content (93-99 %) and is free from harmful and toxic impurities (Лелеков, 2020). Biochar is produced by pyrolysis, in which biomass is burned in the absence of oxygen (400-650°C and above) (Huang et al., 2017).

The Biochar Quality Mandate - BQM - has been developed at the University of Edinburgh. The BQM specifies requirements for biochar production technology (e.g. complete combustion of pyrolysis gases or capturing them and limiting atmospheric emissions), raw material preparation, and most importantly, it regulates product analyses and sets maximum allowable concentrations of individual groups of substances (Shackley, 2014). Biochar of standard and high quality is produced. Classification into the second category is determined primarily by low content of mineral and toxic compounds. This means that biochar meeting the requirements of BQM can be used in agriculture and animal farming as absolutely safe and is not harmful to the environment in its production.

A large consumer of biochar is agriculture. Above all, it is an effective soil substrate: it increases soil fertility by reducing soil hardness and density. In this way, the roots penetrate the soil more easily and grow better. In addition, a special habitat is created for soil micro-organisms and fungi, which ensures plant health (Fortaine et al., 2007). In addition, biochar retains water in the soil, increases fertilizer efficiency and absorbs nutrients and beneficial minerals, thus contributing to high agricultural activity and higher yields (Huang et al., 2017). Due to its adsorption capacity, this type of charcoal can be used to remove pollutants and disinfect soil, drinking water and wastewater, and serve as a barrier to prevent pesticides and herbicides from entering surface water (Lelekov, 2020).

Biochar reduces the necessity for fertilizer and prevents the deposition of nutrients and nitrates in groundwater, increases the microbial mass, water absorption and water retention of the soil, while reducing methane emissions, and enhances plant growth (Huang et al., 2017).

2.4. Lead in soils

Pollution of soils with lead is a frequent global phenomenon, leading to an excess of this element and its compounds, mostly in the upper soil horizon due to its sorption by soluble organic matter. Its presence in deeper soil layers was observed in podzolic soils (Kabata-Pendias et Barbara Szteke, 2015).

Lead compounds are fixed by clay minerals, hydroxides, and soluble organic matter (SOM). Their content in soil increases at higher pH values. This element exhibits similar properties and behavior as SOM and tends to form complexes when reacting with certain soil components. Pb^{2+} tends to replace elements such as Sr, Ca, K, and Ba in minerals (Kothe et Ajit, 2012). The peculiarities of lead distribution in the soil profile find their relationship with hydroxides. As with iron and manganese hydroxides, it is uneven. Kabata-Pendias (2010) found in her studies with lead-contaminated soil the formation of hydrocerussite and pyromorphite. By nature, Pb mobility in soils is quite low, but it increases under more acidic conditions. Some lead complexes are much more mobile.

In soil solution, lead occurs in cationic (Pb^{2+}, $PbCl^+$ and $PbOH^+$) and anionic ($PbCl_3$, $Pb(CO)_2$) forms (Kabata-Pendias et Sadurski, 2004). Its solubility on the surface of iron minerals can be affected by microbial activities. Also, microorganisms such as *Rhizopus Arrhizus* can accumulate up to 33 mg Pb/g at a fairly wide pH range of 3-7 (Perelomov et al., 2013). These examples prove the important role of microorganisms in transformation and migration of heavy metals in soil.

Baron et al. (2006) argue that the first anthropogenic traces of lead contamination of soils go back to the Middle Ages, when active mining for construction began, and subsequently to the first industry. Thus, a study was carried out which revealed that the source of 40 % as much as 100 % of the total lead in contaminated soils in France was the activity of medieval workshops.

One source of lead contamination is its mining and processing activities. As a result of this activity, Pb can disperse over long distances as a result of erosion and weathering of tailings materials. Elevated concentrations of Pb have been recorded near metal processing plants (Kabata-Pendias et Barbara Szteke, 2015).

The low mobility of lead is a favorable factor for further actions to clean the soil from this representative of heavy metals. However, rather active ability of Pb uptake by plant roots and its subsequent transfer to shoots was recorded (Kabata-Pendias et Barbara Szteke, 2015).

In modern science, there are several methods for remediating lead-contaminated soil. Plant-based lead remediation methods are relatively ineffective due to the nature of this metal, but there are some hyperaccumulators for this pollutant, such as corn, sunflower or broom (*Thlaspi*). These species are used for phytostabilization or phytoextraction purposes. Phytoremediation is adopted in this case. It has been recorded that this method becomes more effective after the addition of chelators such as ethylenediaminetetraacetic acid or diethylenetriaminepentaacetic acid. These chelators affect lead mobility by increasing it. Fixation of Pb in the rhizosphere zones of the roots of some plants makes it possible to stabilize this metal for a certain time. Stabilization is also possible by other methods, such as using iron-rich lime, using P-fertilizers, and using minerals such as barite (Kabata-Pendias, 2010). It is also worth mentioning the possibility of adding a lignite preparation, which was used to reduce the phytoavailability of lead (Kwiatkowska-Malina et Maciejewska, 2013). Usually this preparation consists of coal, peat and ash.

2.4.1. Toxicity of lead in living organisms

Lead has potential carcinogenic properties. Most of its compounds and lead itself are toxic, especially water-soluble compounds (such as lead acetate $Pb(CH_3COO)_2$). Lead affects the photosynthesis of plants, namely: disrupts the anatomical structure of leaves and the structure of chloroplasts, reduces the number of pigments, disrupts light reactions in photosynthesis, and inhibits the activity of Calvin cycle enzymes. This heavy metal has its negative effect on plant respiration: it disrupts the structural organization of mitochondria, inhibits the activity of glycolysis and Krebs cycle enzymes. Do not forget about its effects on water metabolism, such as: reducing the water content in cells, slowing transpiration. Also, Pb reduces the flow of macro- and microelements in the roots and inhibits the transport of elements into the above-ground organs. After all this, cell division and hormonal balance of the plant is impaired, and it also leads to a decrease in productivity. We can also note the competitive displacement of some metal ions that are involved in the mineral metabolism of the plant (Fe, Mn, Zn). The content of phosphorus, calcium and potassium also drops (Доршакова et al., 2020).

Lead poisoning has also been found in many animals. This phenomenon is common in cattle and other ruminants. As in many cases, the degree of exposure is affected by the type of lead compounds, the acidic environment of the gut, the type of animal and other factors. As for symptoms or effects: central nervous system, gastrointestinal tract, muscle coordination and red blood cell synthesis disorders. Also, excessive

concentrations of this metal can cause blindness, muscle stress, teeth grinding, and seizures in cattle. Symptoms such as anorexia, abdominal pain, depression, diarrhea, and anemia are typical in sheep. Osteoporosis in young lambs and abortions in sheep grazing in Pb mining areas have also been reported. Long-term exposure to Pb negatively affects the kidneys, liver, cardiovascular, immune, reproductive and nervous systems (Kabata-Pendias et Szteke, 2015).

Stupor, depression, anemia, laryngeal paralysis, and resultant breathing severity have been found in horses with lead intoxication. A reduction in the manifestation of many of the symptoms was observed with a diet enriched with zinc. A correlation with Ca in the diet was also observed. An increased accumulation of Pb in the liver of young horses was seen at low calcium levels. Lead can contribute to the inactivation of some enzymes. Nuclei, mitochondria, and microsomes at the cellular level are particularly susceptible to lead. This heavy metal can also accumulate in the bones, liver, kidneys, and intestines of the fetus (Kabata-Pendias et Szteke, 2015).

Lead can enter the human body from paint, food, water, dust, soil, leaded gasoline, etc. However, the most common way of ingesting lead is by ingestion (absorption through the intestines). Again, this process is influenced by a huge number of factors: age of the person, iron and calcium content, pregnancy, etc. Of the physical and chemical factors: mineralogy, solubility, particle size, type of Pb compound (©WHO 2011c). Lead is then transported through the body and accumulates in the soft tissues and bone tissue. The transfer process is accomplished with the help of the blood red blood cells. A very small amount of Pb binds to the plasma and subsequently enters the brain, lungs, kidney cortex, spleen, teeth, and bones with the plasma. This metal can remain in our bones for up to 30 years (Kabata-Pendias et Szteke, 2015).

Lead toxicity shows itself in the inactivation of enzymes and disruption of Mg, Ca and Zn homeostasis. Further, Pb disrupts the balance between pro-oxidants and antioxidants. These effects can be regulated by adding vitamins E, C, B6, β-carotene, Zn, and Se to the diet (Hsu et Guo, 2002). The most affected organ in adults is the central nervous system. In children, intoxication appears in decreased intelligence, impaired motor function, and affects neurobehavioral development. Disorders such as brain damage, anemia, gastrointestinal symptoms, kidney, immune and reproductive system damage, cardiovascular effects, hematological disorders, headaches, cramps, and many others are typical in lead intoxication (Kabata-Pendias et Szteke, 2015).

2.5. Zinc in soils

Soil texture plays the main role in soil zinc content. For example, the lowest Zn concentrations are observed in light sandy soils. However, the opposite situation can be observed for calcareous and organic soils (Kabata-Pendias et Szteke, 2015). Zinc penetration into soil and its further accumulation, transformation or migration depend on parent materials, content of soluble organic matter, soil formation processes, and content of clay fractions (a particularly pronounced reaction was observed when the fractions included vermiculite or gibbsite). Two of the above factors, namely SOM and clay fraction, show increased sorption ability with zinc, thereby influencing its surface accumulation. Soil pH and added metals are responsible for the fixation of this heavy metal. It is worth mentioning that the process of fixation of this pollutant itself is relatively slow, but over time, accumulation of fixed zinc occurs. It is worth noting the tendency of Zn to become more mobile with increasing temperature (Li et al., 2005).

$ZnHCO_3$ and $Zn(OH)_3$ are among the most common mobile zinc compounds formed in soil solution, but a number of more complex compounds ($ZnFe_2O_4$, $Zn(PO_4)_2$, etc.) can also be formed. These substances form simple and complex ions (Kabata-Pendias et Sadurski, 2004).

pH strongly affects the concentration of this metal in the soil. Thus, at pH < 4 (very acidic environment), the Zn concentration averaged 7137 µg/l, but in contaminated soils its content reached 17,000 mg/l (Kabata-Pendias, 2010). The high mobility of this heavy metal is still not an obstacle for its retention in the soil under certain conditions. The main conditions are a neutral or alkaline environment and are also influenced by SOM, clay fractions and P compounds (Kumpiene et al., 2005). One explanation for the accumulation of zinc in the upper soil layers is reactions with SOM, which results in relatively stable forms.

The presence of some Al, Fe and Mn oxides and hydroxides affects Zn fixation in some soils (for example goethite (αFeOOH), which exhibits strong sorption properties to Zn). Not to forget the ability of some microorganisms to accumulate zinc in their living cells, thereby reducing its accumulation in the soil (Perelomov et Kandeler, 2006). Zn mobility is affected by dissolved organic matter at higher pH values (7-7.5) (Kabata-Pendias, 2010). As pH increases, organic complexes of zinc affect its solubility. And under more acidic conditions with a sandy structure, SOM plays a major role in binding Zn because it is under these conditions that this heavy metal is easily leached down the soil profile. Several studies have revealed two mechanisms of zinc sorption: at low pH values this metal is bound to cation exchange sites; at high

(alkaline) pH values it is chemisorption, which is strongly dependent on organic ligands (complex compounds with organic molecules). The presence of clay minerals as well as Ca, P and S compounds in the soil induce zinc immobilization (Kabata-Pendias et Szteke, 2015).

Zinc also has several similar behavioral traits as lead. The effect of rhizosphere on the surface of grass roots accompanies the formation of Zn pyromorphite $[Zn_5(PO_4)_3OH]$ when soil contamination with this heavy metal is strongly pronounced. The microorganism *Rhizopus Arrhizus* described above can accumulate up to 28,000 mg/kg of zinc, but at a slightly lower pH range of 4-7.5 than that of lead (Perelomov et al., 2013).

At certain concentrations, zinc is an important component in the life of many organisms, but its elevated levels are toxic to most microorganisms (toxicity is especially pronounced in acidic soils). Another type of soil organisms that show good Zn uptake capacity are earthworms. Studies on earthworm uptake of Zn in soil showed concentrations in the range of 114-369 mg/kg. The concentration was dependent on changes in the physical quality of the soil through the addition of different soil amendments (Siebielec et al., 2013). The maximum concentration of this metal was recorded by Kabata-Pendias (2010) in her studies, where earthworms absorbed as much as 2000 mg/kg. This fact once again emphasizes the great importance of individual microorganisms in the processes to which heavy metals are subjected after entering the soil profile.

Once in the soil, zinc can stay there for a long time. The estimated half-life of this metal is 70-80 years (Kabata-Pendias, 2010). There are several methods to reduce Zn concentrations in contaminated soils, which are based on the principles of controlling the bioavailability of this heavy metal. This method is widely applied using lime or organic matter, or a combination of the two. In order to reduce the phytoavailability of zinc, brown coal, which usually consists of coal, peat and fly ash, is added to contaminated soil (Kwiatkowska-Malina et Maciejewska, 2013). Various organic zinc complexes can influence the phytoavailability of Zn (the uptake of this element and its compounds by plant roots), thereby increasing it (Degryse et al., 2006).

2.5.1. Toxicity of zinc in living organisms

The toxicity of this heavy metal refers to the consequences of its increased levels in plants, animals, and humans.

Excess zinc inhibits the growth and development of plants, reduces their yield and seed quality, and causes necrosis. Like many heavy metals, Zn has the ability to accumulate in different parts of plants. The degree of zinc influence on quality of seed progeny depends on soil type and agrochemical properties. Moreover, its bioavailability increased when Zn was applied in the form of soluble compounds. These reactions were revealed in a study by a group of scientists from my hometown in Russia, Obninsk. Plants were stressed on sod-podzolic soils already at increased metal content of 100 mg/kg of soil, on peaty soils and chernozem at average (250 mg/kg) and high (500 mg/kg) soil contamination, respectively. Zinc stress affected the morphological indices of barley, reducing straw weight or weight of 1000 seeds. Soil zinc contamination was able to negatively affect barley seed progeny by increasing the frequency of cytogenetic disorders in the root meristem of seedlings, reducing mitotic activity of root meristem cells and seed germination (Anisimov et al., 2019).

Increased zinc concentrations in animals lead to displacement of Cu and Fe. Toxic effects in rodents contribute to weakness, weight loss, anemia, reduced growth, hair loss, changes in liver and serum enzyme levels, enzymatic and morphological changes in the brain, and histological and functional changes in the kidneys (©WHO 2001d).

Zinc in Foodstuffs (mg/kg FW)

Product	Content
Food of Plant Origin	
Bread	9.9
Miscellaneous cereals	9.4
Rice, brown[a]	44.2
Rice, different species[b]	15.0
Green vegetables	3.26
Other vegetables	2.62
Fresh fruits	0.61
Nuts	31.0
Food of Animal Origin	
Carcass meat	64.8
Meat products	46.5
Offal	23.0
Poultry	16.3
Fish	7.67
Eggs	11.4
Milk	3.71
Dairy products	9.66

Table 1. Zn concentrations in plant/animal-based food (Rose et al., 2010)

Intake of disproportionate amounts of zinc leads to displacement of copper, which can cause leukopenia and/or hypochromic microcytic anemia. High levels of Zn lead to a

decrease in the number of white blood cells in the human body and a deterioration of their function. This metal also displaces iron stores (©ATSDR 2005d). Gastrointestinal disorders, nausea, and diarrhea are considered typical symptoms of poisoning (©WHO 2001d). The bioavailability of zinc is high in eggs, meat, and seafood, and it is present in all food groups (Tab. 1).

3. Hypothesis and objectives

This thesis examines the effectiveness of three different biochars to reduce the mobility and uptake of lead and zinc by ryegrass. Following the proposed method, I propose three hypotheses:

1. I expect a decrease in the concentration of the above-mentioned metals in the dry biomass of ryegrass and in the soil pore water.
2. The combination of pH and organic matter are the main factors influencing the mobility of metals in the amended soil. Biochar is therefore expected to be more effective in reducing Zn concentrations in soil leachate and dry ryegrass biomass than Pb.
3. Increasing the biochar dose will show more effective results.

To test above hypothesis, this study has three primary objectives:

1. to evaluate heavy metals uptake into ryegrass on contracting soils amended by three alkaline biochars,
2. to elucidate the main mechanisms driving any observed differences in the impact of biochars on the contracting soils,
3. to discuss the findings in the context of the wider application of biochars to heavy metal contaminated soils, as part of remedial treatments.

4. Materials and methods

4.1. Characteristics of the study area

For this study soils of contrasting pH, organic matter content and heavy metal total concentrations were selected to, as best as possible, represent a wide range of soils of European post-industrial areas. The first soil, (denoted as Soil CZ) Czech Republic near the town of Příbram, soil from Murcia in Spain (denoted as Soil S) and soil from France (denoted as soil F).

Příbram region is known as the mining region of silver, tin, zinc, uranium, and many other polymetallic ores in the past (Velfl, 2003). Active mining of certain ores took place until the 80s of the 20th centuries. The duration of mining lasted a relatively long time, so the inevitable consequences such as the emission into the air of certain elements and substances, dust particles, road traffic, noise and pollution of groundwater, destruction of local ecosystems, contamination, and removal of huge amounts of fertile soil were unavoidable (Lepka, 2003). Therefore, it is reasonable to assume that the samples of this soil will contain above the allowable number of heavy metals under study.

Soil sample F was created by a mixture of industrial sludge and industrial soil. The industrial soil was collected from the top layer of a Cambisol covered with natural forest in the Vosges region of north-eastern France, which was contaminated with organic compounds and various metals (this soil was alkaline with a pH of 8.45). These samples were mixed with industrial sludge from the automotive industry. This sludge had an acidic pH (3.9) and contained large amounts of metals. The soils were mixed at a dry weight ratio of industrial sludge to treated industrial soil material of 1:9 (Rue et al., 2019).

S soil samples were taken from the mining area of La Unión Cartagena, in the south-eastern region of Murcia (Spain). Mining of minerals and metals has been carried out here since ancient Roman times, with a peak of activity in 1980 (Bes et al., 2014).

4.2. Soil and biochar samples

The required amount of surface soil (up to 10 cm) was collected from each of the locations from the plots allocated to the experimental activity. These plots did not have any vegetation on the surface. The soil was then brought to a homogeneous state (large pieces of soil were scattered). 40 kg of soil from each sample was air dried for 1 week at 22°C. The dried soil was then sieved (<11.2 mm) to remove debris and

unwanted material. The soil was then distributed in equal proportions into four parts: a control part, three parts followed by the addition of three different biochars.

Three biochars were selected for this study, being sourced from waste wood and woody residues. Biochar 1 was produced from wood sieving residues (50 % hardwood, 50 % softwood) and pyrolyzed at 650°C using PYREG process. Biochar 2 was produced from *Miscanthus x giganteus* biomass and pyrolyzed at 500-550°C. Biochar 3 was produced from softwood and pyrolyzed at 480°C. Each biochar was air-dried and sieved to 2 mm (Beesley et al., 2011).

Next, the samples with the additives were stirred to obtain a homogeneous consistency. The next goal was to achieve 70 % water-holding capacity of the soil by pouring it with distilled water. Then the samples were placed in plastic bags for 4 weeks at 15°C for equilibration purposes. After small sub-samples were taken from each soil sample for further drying in the above-described manner. Sub-samples were then sieved to fractions <2 mm for subsequent analyses (pH, organic matter, total element concentration and granulometric composition) (Beesley et al., 2011).

4.3. Soil physicochemical analysis

The pH analysis of the amended soils was performed as follows: 10 g of each soil sample was taken and subsequently stirred with 25 ml of deionized water for equilibration. The samples were left for 30 min at room temperature afterwards. The samples prepared as described above were measured with a pH meter. The organic matter content was analyzed by loss on ignition at 500°C (LOI). To analyze the total element concentration, the prepared samples were first dissolved in aqua regia and subsequently extracted in 0.01 M $CaCl_2$. The granulometric composition was carried out by laser granulometry (Beesley et Dickinson, 2010; Beesley et al., 2011).

4.4. Planting ryegrass in prepared soil samples

Prepared soils with three different biochars (as well as control samples without additives) were distributed in 2 l pots. Each pot was placed on a saucer so that in no case the leachate was lost from the prepared system. Afterwards, 2 g of ryegrass *Lolium perenne* was sown into each of the pots. Three replicates were prepared for each soil sample. The ryegrass cultivation experiment was conducted in greenhouses at 18°C with 150 ml of distilled water every day. The crop was harvested at two consecutive intervals (in this study, biomass was used only after the first harvest - after 6 weeks). Ryegrass biomass was harvested as follows: leaves of the plant were cut off, leaving 1 cm above the soil surface. After the collected shoots were carefully

washed with deionized water, collected residual water with a tissue, weighed and then placed in dryers for 24 hours at a temperature of 45°C. Already dried biomass was re-weighed, then it was subjected to grinding in an electric mill. After that, 10 ml of HNO_3 was added to 0.2 g of the milled sample. Then, the obtained solution was subjected to extraction by microwave digestion. The resulting substrate was analyzed for metal concentration by ICP-MS (Beesley et al., 2011).

After 6 weeks, pore water samples were also taken from each experimental pot using a sampler hermetically integrated into each pot. Pore water samples were analyzed for the metal concentration using ICP-MS (Beesley et al., 2011).

Data was collated and appropriately statistically treated. First, general tests were done for all data (Shapiro test and Bartlett test), which revealed that our data have no normal distribution and no parametric distribution (only in the case of DW). So, a two-way ANOVA statistical analysis was made, the results of which are presented in Table 2.

5. Results

5.1. Statistical analysis of the results

The effect of biochars and soil, and their interaction on zinc and lead concentrations in pore water (SPW [Pb], SPW [Zn]), dried shoot weight (DW) and zinc and lead concentrations in ryegrass shoots (Plant [Pb], Plant [Zn]) were evaluated.

Based on the data from this analysis, only the soil factor significantly influenced all the parameters investigated (except SPW [Zn]). Perhaps these results are due to the rather strong contrasts between each soil type. Biochar also has different composition and properties, but they are not significantly different from each other.

Table 2. Effect of biochars, soils and biochars*soil on the Pb and Zn soil pore water concentrations, plant dry weight and concentrations of Pb and Zn in shoots of ryegrass

Parameter	Biochar	Soil	Biochar*Soil
SPW [Pb]	ns	***	ns
SPW [Zn]	ns	ns	ns
DW	ns	***	ns
Plant [Pb]	ns	***	ns
Plant [Zn]	ns	**	ns

ns = non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

5.2. Soils and biochars characteristics

Some physical properties of each type of investigated soil were measured on the soils and the biochars. The results of this measurement can be observed in tab. 3, 4, and 5 for the soils. Relatively high sand content is observed in S and CZ soils. On the contrary high silt fraction is observed in F soil. Highest clay content is found in F soil. In the discussion chapter these data will be taken into consideration.

Using the texture triangle plot and data from table 3 I was able to classify each of the soils described above: soil S - loamy-sandy, soil F - silty-loamy, soil CZ - loamy-sandy.

Table 3. Soil texture parameters of soil from Spain (S), from France (F), and from Czech Republic (CZ)

Parameter	Soil S	Soil F	Soil CZ
Clay (<2 µm) (%)	0.9	2.8	0.3
Silt (2-50 µm) (%)	14.3	70.1	28.1
Total sand (50-2000 µm) (%)	84.9	27.0	71.6
Texture	Loamy-sandy	Silty-loamy	Loamy-sandy

The key soil properties are its pH and organic matter content. These measurements are presented in tab. 4. From this table it can be observed that soils are neutral to strongly acidic, so the addition of further alkaline biochar may be a justified solution. The organic material values are the highest for the CZ soil but cannot be called optimal. The addition of biochar with a higher organic matter content could improve the quality of the final soil composition, which again justifies its use in this case.

Table 4. pH and organic matter of soil S, F, CZ

Parameter	Soil S	Soil F	Soil CZ
Organic matter (%)	4.1	3.5	5.7
pH in water	4.0	6.5	5.5

Table No. 5 shows the total concentration of the two metals investigated, measured as an arithmetic mean as well as their standard deviation. Extremely high levels of lead were exhibited by soils S and CZ, with the soil from Spain with the highest values measured in. High zinc values were detected in all soils, but the highest value was measured for soil F. The table also shows that the concentrations of the pollutants of interest exceed the maximum permissible concentrations in agricultural and forest soils, described in the last right-hand column of the table No. 4 (issued by the Ministry of Nature Protection of the CR in 2004).

Light soils are defined as sandy and loamy-sandy soils with a percentage of clay particles (< 0,002 mm) ranging from 0 to a maximum of 20 %. Other soils are medium soils (sandy-loamy to loamy soils) and heavy soils (clay-loamy, clay soils, clay) with a percentage of clay particles from 20 to a maximum of 75 % according to Novak (Annex 8 to Decree No. 275/1998 Coll., as amended) (MŽP ©2004). However, Pb did not exceed permissible limits in the F soil. In the remaining cases, the data exceed the permissible values extremely high, especially samples from Spain in the context of lead concentration and samples from France in the context of zinc concentration.

Table 5. Total (aqua regia) concentrations of Pb and Zn in soils S, F, CZ (Mean ± sd, n=4)

Parameter	Soil S	Soil F	Soil CZ	Permissible levels*	
				Light soils	Other soils
Total Pb (mg/kg)	18346.9 ±10748.6[a]	79.5 ±56.4	3736.2 ±56.3	100	140
Total Zn (mg/kg)	2684.9 ±1482.8	5999.4 ±520.6	3646.7 ±15.2	130	200

* Maximum permissible levels (aqua regia) of hazardous elements in soil according to Decree No. 13/94 Coll (MŽP ©2004).

Table No. 6 provides data on the measured properties of three different biochars (A, B, C). Based on the pH of all three additives, we can say that their pH is very high; accordingly, they will all exhibit alkaline properties in the soil profile. Based on the information above, it can be assumed that the use of alkaline biochar is a logical solution to increase pH of strongly acidic soil samples to a neutral level (close to normal). It is also worth mentioning the rather high content of organic material in the composition of all three additives, which is typical for this type of biochar.

CEC values were in some specific range that could lead to positive effects. This factor tells us about a good exchange ability of the additives to exchange cations interacting with soil cations. In other words, a good ability to accept heavy metal cations from contaminated soil.

Table 6. Physicochemical properties of the three biochars A, B, C

Parameter	Biochar A	Biochar B	Biochar C
pH in water	9.62	10.10	8.90
Electrical conductivity (mS/cm)	0.23	0.79	0.08
Organic matter (%)	79.30	89.90	82.30
Organic C (g/kg)	685	784	700
Olsen P_2O_5 (g/kg)	0.09	1.69	0.15
CEC (cmol+/kg)	3.20	3.63	5.24
Total con. of Pb (mg/kg)	4	4.20	4.30
Total con. of Zn (mg/kg)	94	259	73
CaCl2 extr. Pb (µg/kg)	<3	<3	<3
CaCl2 extr. Zn (µg/kg)	<10	16.8	<10

However, it is worth paying attention to Biochar B, which has a slightly high zinc content. This phenomenon can be explained either by the high zinc content of the material from which the biochar was made, or by the fact that the amendment was

contaminated during the production process. In either case, the biochar may be a source of transferring unwanted heavy metals and their compounds into the soil.

5.3. Effect of biochars application on the S soil

This chapter will deal directly with the results of applying three different biochar A, B, C in different proportions (1 % and 5 %) on soil from Spain. In Figure No. 4, we can observe the weight of cuttings of ryegrass shoots for each of the attempts (viz. weight of shoots grown in contaminated soil without adding any biochar; weight of shoots grown with the addition of 1 % or 5 % biochar A, B, C). In this context, it can be observed that when 1 % of each biochar was added, the mass of ryegrasses increased in relation to the mass of the plant without addition. The highest result was shown by biochar B (2679 mg), increasing the mass of ryegrass shoots by around 33 % compared to shoots in control pots (2009 mg). When the biochar dose was increased to 5 %, a significant increase in shoot weight was observed in all cases. Biochar A was the leader (3065 mg) within this dose of additives, increasing the shoot mass by around 49 %. Biochar B, however, had almost similar results as biochar A (2988 mg), increasing the mass of the ryegrass by 48 %.
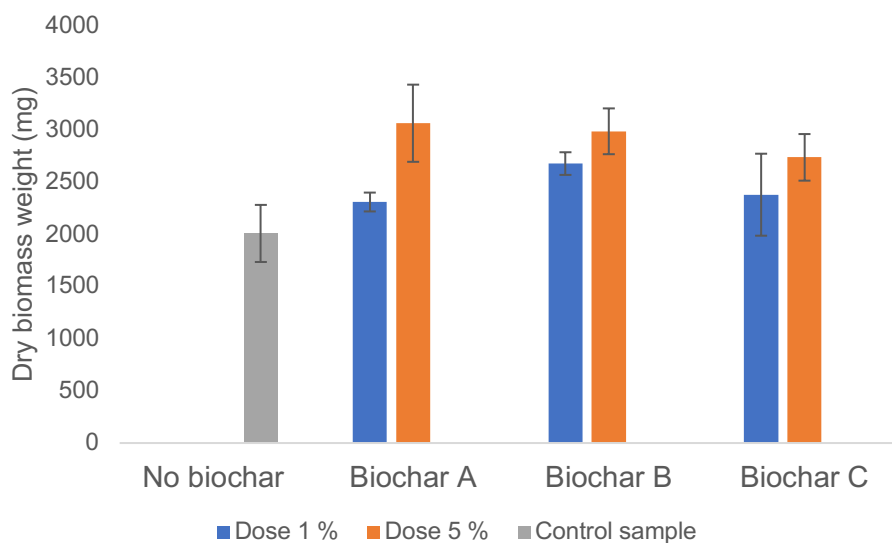

Figure 4. Effect of the addition of three biochars (A, B and C) at 0 % (grey bar), 1 % (blue bar) and 5 % (orange bar) on the dry biomass production of ryegrass grown on the Spanish soil.

The next two indicators that were measured are lead and zinc concentrations in ryegrass shoots (Fig. 5 and 6). In the case of 1 % addition of biochar A and C, a slight decrease in Pb concentrations in the shoots was observed. Zn concentrations in shoots, on the contrary, decreased markedly (in some of the attempts, a decrease of more than twofold was observed). In the case of a 5 % dose of each of the additives,

we observe significant results of reducing both Pb and Zn concentrations to acceptable norms from tab. 5 (except for biochar C, which also performed well, but it failed to reduce both metal concentrations to the required minimum).
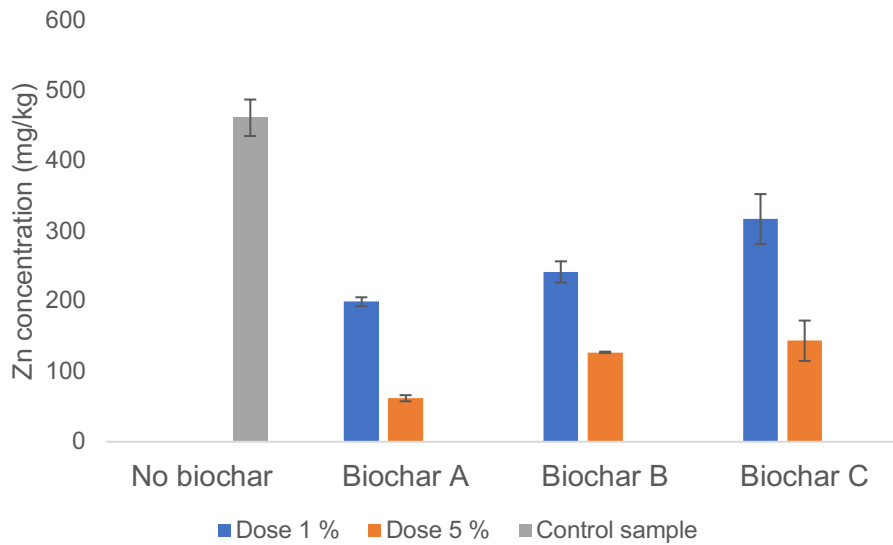

Figure 5. Effect of the addition of three biochars (A, B and C) at 0 % (grey bar), 1 % (blue bar) and 5 % (orange bar) on the Zn concentration in ryegrass shoots grown on the Spanish soil.
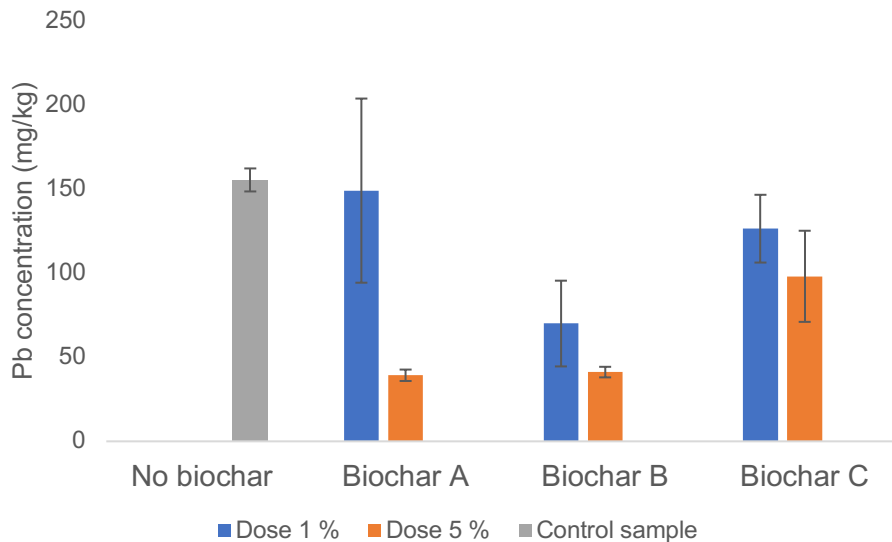

Figure 6. Effect of the addition of three biochars (A, B and C) at 0 % (grey bar), 1 % (blue bar) and 5 % (orange bar) on the Pb concentration in ryegrass shoots grown on the Spanish soil.

The last indicator is the concentration of the two heavy metals investigated in the pore water (tab. 7) of the soil S. Samples with 1 % additive already show quite good results, reducing the concentrations of lead by 76 % (A), 75 % (B), and 52 % (C); zinc by 65 % (A), 54 % (B), and 45 % (C). The use of 5 % additives was more efficient than 1%, reducing concentration of lead by 99 % (A), 98 % (B), and 84 % (C); zinc by 99 % (A),

99 % (B) (again except for biochar C, which again performed well (reducing by 75 %), but nevertheless its use did not lead to identical results for the other two additives).

Table 7. Pore water concentration of Zn and Pb from the soil S, non-amended or amended with 1 % or 5 % of the different biochars (Mean ± sd, n=4)

Biochar ID	Biochar dose	Zn (mg/l)	Pb (mg/l)
no biochar	0 %	26.52 ±3.08	14.38 ±1.30
A	1 %	9.23 ±1.83	3.43 ±0.44
A	5 %	0.15 ±0.01	0.10 ±0.03
B	1 %	12.10 ±5.26	3.42 ±1.58
B	5 %	0.80 ±0.03	0.29 ±0.06
C	1 %	14.70 ±3.05	6.95 ±1.47
C	5 %	6.56 ±1.15	2.35 ±0.16

5.4. Effect of biochars application on the F soil

It is worth mentioning here that the concentration of lead initially does not exceed the norms (tab. 5), so observations of Pb values in both biomass and pore water are omitted.
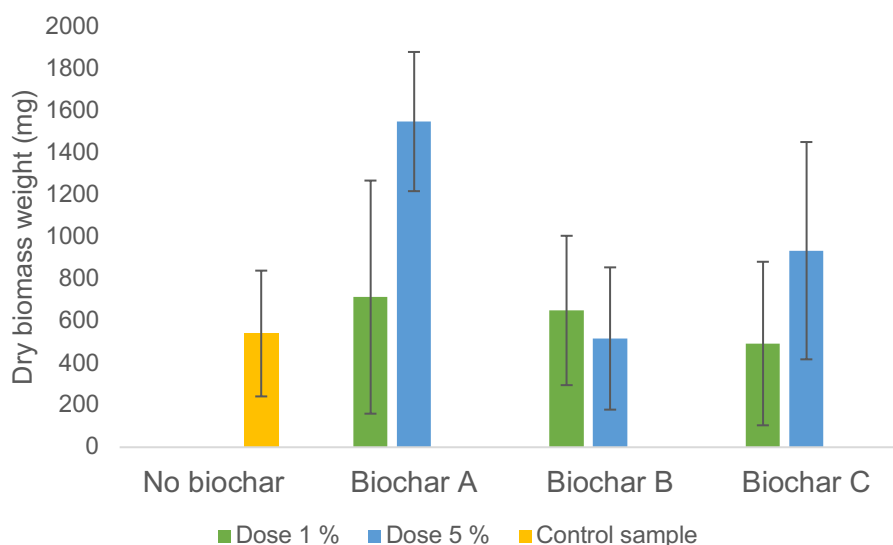

Figure 7. Effect of the addition of three biochars (A, B and C) at 0 % (yellow bar), 1 % (green bar) and 5 % (blue bar) on the dry biomass production of ryegrass grown on the French soil.

Let's pay attention to the shoot mass (fig. 7): 1 % of biochar is not efficient in all cases. When a 5 % dose of biochar B is added, there is no significant increase in biomass as with the other two (A and C). On the contrary, the shoot mass does not even reach the values of a 1 % dose of biochar A and B. It is also worth noting that shoot growth when 1 % biochar C is added does not reach the results of control pots, the average

of which is 541 mg (but the difference is not that big, only nearly 4 %). The best result was obtained with biochar A at the dosage of 5 % (286 % increase).

From Figure No. 8, we observe a decrease in zinc concentration in ryegrass shoots after the addition of 1 % of each biochar to the soil. However, adding more dosage of biochar (5 %) did not lead to further improvements. Biochar C showed the best result, reducing the Zn concentration from 145, 21 mg/kg to 105.18 mg/kg (28 % decrease).
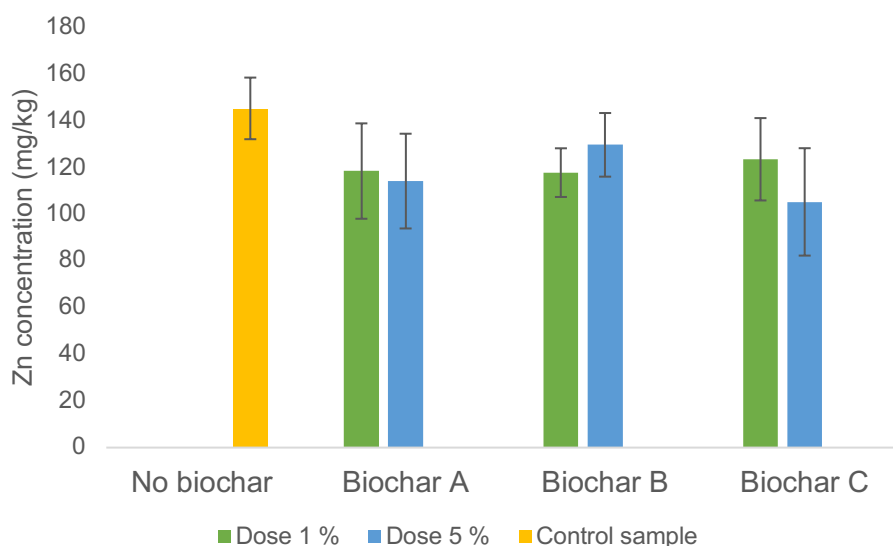

Figure 8. Effect of the addition of three biochars (A, B and C) at 0 % (yellow bar), 1 % (green bar) and 5 % (blue bar) on the Zn concentration in ryegrass shoots grown on the French soil.

As for the zinc concentration in pore water of soil F, it decreased in all cases and in all ratios (both at 1 % and 5 %). The best result was achieved with 5 % biochar A (reduce by 48 %). The tab. 8 also shows the concentration results for lead, but the values are so small that these data will not be used in the discussion.

Table 8. Pore water concentration of Zn and Pb from the soil F, non-amended or amended with 1 % or 5 % of the different biochars (Mean ± sd, n=4)

Biochar ID	Biochar dose	Zn (mg/l)	Pb (mg/l)
no biochar	0 %	8.31 ±0.74	0.13 ±0.01
A	1 %	6.82 ±0.93	0.13 ±0.01
A	5 %	4.29 ±0.63	0.12 ±0.01
B	1 %	7.14 ±0.71	0.12 ±0.01
B	5 %	6.72 ±0.93	0.12 ±0.01
C	1 %	7.08 ±1.54	0.13 ±0.01
C	5 %	5.57 ±0.43	0.12 ±0.01

5.5. Effect of biochars application on the CZ soil

In this chapter we will look at the results of zinc content in ryegrass shoots and in pore water of soil from the Czech Republic.
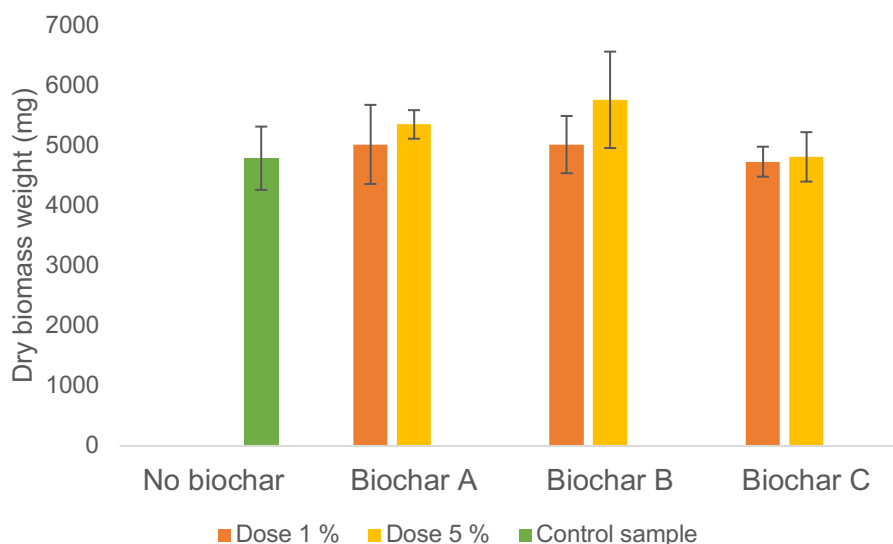

Figure 9. Effect of the addition of three biochars (A, B and C) at 0 % (green bar), 1 % (orange bar) and 5 % (yellow bar) on the dry biomass production of ryegrass grown on the Czech soil.

From the figure 9, we observe the same result in growth as non-amended sample in all cases with the addition of 1 % of each biochar, except biochar C. With the addition of 5 % amendments, more intensive growth is observed as a result with higher shoot weights. All the values are higher than those of the control pots. However, considering the deviation values only biochar B showed the real high result of 5762 mg (20 % increase).

Looking at the fig. 10, we observe that already after the addition of 1 % of each of the biochars, there is a significant decrease in the zinc concentration in the samples. In this case, all additives performed well, but biochar C was the leader (32 % reduction) compared to biochar A (30 % reduction) and biochar B (29 % reduction). A significant reduction of heavy metal concentration was also achieved after the addition of 5 % dose of each of the biochar. The best result was applying biochar B (82 % reduction).
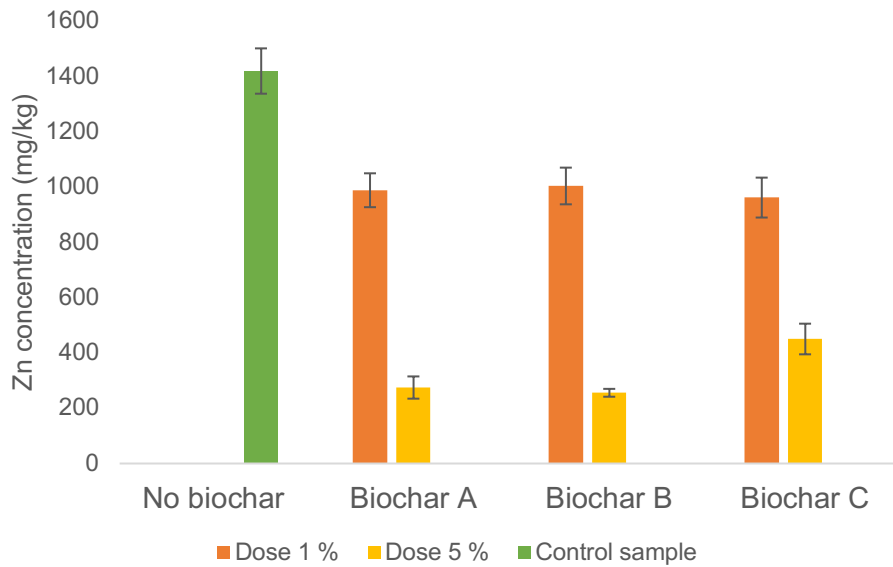
Figure 10. Effect of the addition of three biochars (A, B and C) at 0 % (green bar), 1 % (orange bar) and 5 % (yellow bar) on the Zn concentration in ryegrass shoots grown on the Czech soil.
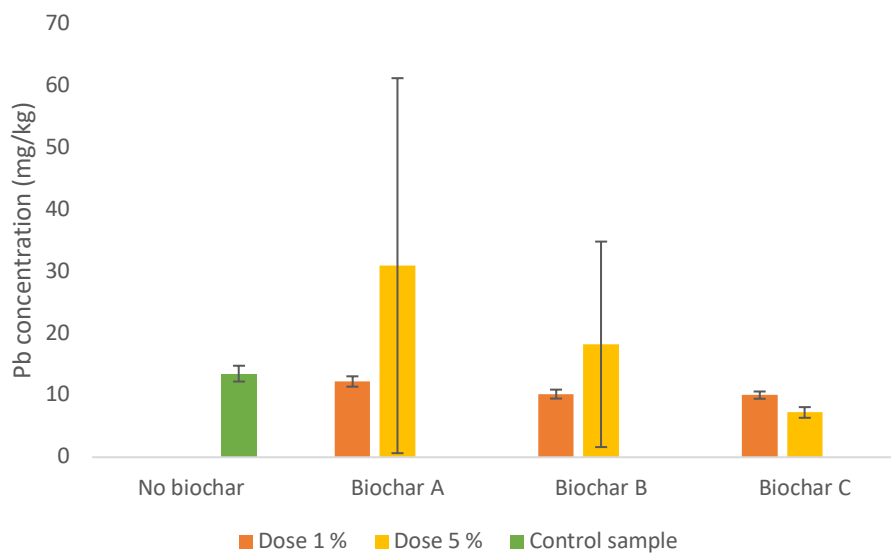
Figure 11. Effect of the addition of three biochars (A, B and C) at 0 % (green bar), 1 % (orange bar) and 5 % (yellow bar) on the Pb concentration in ryegrass shoots grown on the Czech soil.

Biochar B proved to be more effective, reducing initially quite high Zn concentration to 256 mg/kg (over 81 % reduction). The results for lead concentrations (fig. 11) are initially below the tab. 5, so this will not be emphasised in the discussion chapter. After addition of 5 % of A and B biochars no effect was observed, probably due to elevated variability.

Table 9. Pore water concentration of Zn and Pb from the soil CZ, non-amended or amended with 1 % or 5 % of the different biochars (Mean ± sd, n=4)

Biochar ID	Biochar dose	Zn (mg/l)	Pb (mg/l)
no biochar	0 %	4.26 ±0.94	0.01 ±0.01
A	1 %	3.86 ±0.69	0.05 ±0.02
A	5 %	1.39 ±2.18	0.04 ±0.04
B	1 %	6.19 ±0.78	0.03 ±0.01
B	5 %	2.77 ±0.92	0.02 ±0.02
C	1 %	7.63 ±1.79	0.02 ±0.01
C	5 %	3.94 ±1.38	0.02 ±0.02

As for zinc concentration in pore water of CZ soil, it increased after addition of 1 % of biochar B and C. In all other cases, the concentration decreased below that of the control pots. The best result was achieved when 5 % biochar A was used. The tab. 9 also shows the results of lead concentration in the pore water, which had very low values, not affected by the biochar additions.

6. Discussion

6.1. Soil Properties

At the beginning of the discussion, I would like to discuss the role of texture in each of the soils studied. The particle size distribution influences infiltration, the ability to bind nutrients and water and aeration (Strahler, 1999). Soil S and soil CZ are similar based on this classification, meaning that these soils have good infiltration and aeration because they are dominated by sand fractions. However, the low accumulation capacity, the inability to retain water, again due to the predominance of coarse fractions, also contributes to the low nutrient content (Máčka, 2017). Soil CZ contains about 28.1 % silt, so we can assume that it makes it more fertile, as this soil will have more nutrients and better water retention capacity. Soil F is dominated by silt, which means acceptable infiltration and water retention for plant growth as well as sufficient nutrients.

Further details on the pH and organic matter of each of the soils follow. Soil S has the lowest pH of 4.0, which is certainly considered acidic soil and unsuitable for the growth of most plants. Elements that are more toxic to plants may become more available (heavy metals) in an acidic soil, while many important elements (essential for plant growth) become less available. Willsher et al. (2017) in their pot experiments came to the result that at pH 4 and below, the growth of H. *tuberosus* was retarded compared to pots with pH from 5-6. When a combination of low pH and an elevated heavy metal content (Zn and Pb were included in this list) were present, plant growth was severely stunted. Soils with this kind of acidity lead to a reduction of organic degrading bacteria, which leads to accumulation of organic matter and reduces the availability of nutrients, especially nitrogen. Zhao et al. (2011) found that at low pH values (4-5) the availability of nutrients ($NH4^+$, available P, etc.) decreases. In the other two soils we can observe a higher pH of 5.5 for CZ soil (which is certainly also an acid soil) and 6.5 for F soil, which is very close to neutral. The usage of alkaline biochar implies higher pH values for soils. However, if we turn our attention to the biomass results, the French soil had the lowest results, which in this context cannot be explained by the low pH. A significant role was probably contributed by the lowest SOM of the soil F.

The organic matter content in the soils studied varies from 3.5 to 5.7 %. There is an example information on the average content of organic matter in soils: e.g., in podzolic soils and grey soils the SOM varies from 1-3 % and from 8-10 % or more in thick chernozems (Муравин et Смирнов, 1984). The investigated soils cannot be called completely poor in OM, on the contrary, CZ soil has quite a good index. Biochar added

to each soil is rich in OM, so its use implies an improvement of soil properties. In their studies with columns Pontoni et al. (2016) were able to demonstrate that the accumulation of heavy metals in the topsoil was inversely proportional to the SOM content. The 10 % SOM content of the supplements showed the best results than the 2.5 %, 5 % and 7.5 % supplements.

6.2. The effect of biochars on ryegrass growth

Let's pay attention to the graphs of biomass weight (Fig. 4, 7, 9): there is a clear dependence of the amount of biomass in control pots on the organic matter content in the soil. The higher the percentage of organic matter in the soil, the greater the mass of ryegrass in the control pots. Accordingly, plant growth in CZ soil (OM 5.7 %) was the most fertile compared to the others. The addition of a 1 % dose of each of biochar A, B, C resulted mostly in a slight increase in ryegrass biomass in all cases except the results of biochar C in the F and CZ soil. The low biomass weight results in the case of soil F can be explained by the insufficient dosage of biochar C to change the OM content of the soil for the better. Also, the high concentration of zinc in the soil should not be forgotten, which certainly affects the growth and development of ryegrass in combination with the particle size distribution of this soil in question (described above) can create less suitable conditions for soil aeration, which can subsequently disrupt plant respiration. Puga at al. (2015) also observed an increase in Jack beans weight after biochar application at different doses. Maximum increase in plant weight was observed when 5 % dose of biochar was added. In the case of CZ soil, this experiment did not result in a significant reduction in biomass compared to control shoots. The factor of pH increases of soils after addition of this dosage of each biochar was reflected insignificantly.

Increasing the dosage of each biochar applied to 5 % has obviously led to better results than applying a dosage of 1 %. However, it is worth examining in more detail two cases that do not fit the above formulation: 1) the mass of ryegrass grown in soil F using 5 % biochar B and 2) the mass of shoots grown in soil CZ with the addition of 5 % biochar C. In the first case, a decrease in shoot weight was observed compared to the control samples, which may be due to the higher zinc content of biochar B (tab. 6). Soil F contains high concentrations of Zn as mentioned above, so this combination began to increase the zinc content of the shoots (Fig. 8), which eventually had a negative effect on plant growth (however, adding some available Zn with biochar it is not necessarily accompanied by a subsequent negative effect). Biochar B has the highest pH (10.1), which could eventually affect the pH of every soil, especially soil F

(6.5), raising it more into an alkaline condition. Strongly alkaline soils are known to have disturbed water balance and promote worse nutrient uptake by plants, which affects plant growth and development. Alvarenga et al. (2016) found in their experiments that high pH hinders nutrient adsorption and reduces metal bioavailability. The second case raises the question of the cost-effectiveness of biochar C application at the suggested dosages in CZ land. The use of the highest dosage (5 %) is characterised by only a slight increase in weight in comparison with the control pots (Fig. 9). The concentration of zinc in the shoots decreased markedly, but this had no significant effect on plant growth. Perhaps the application of a bit higher dosage of biochar C probably can help to achieve better results. We should remember that there is also a risk of nutrient immobilisation when doses are increased. However, the soil pH factor must be considered, which with increased doses of biochar C (and the other two as well) can increase more than pH 7 and the soil will start to show alkaline properties.

6.3. The effect of biochars on reducing metals concentrations in dry biomass

Based on the results of the reduction of zinc concentration in shoots after using three biochars, it is quite difficult to answer the question of which one is more effective and why. Each of them has its own unique composition, but we do not observe any significant differences in composition. Therefore, the main factor in this question is peculiarities of each soil. They fulfilled their main purpose by proving their adsorption capacity in contaminated soil, thereby reducing the bioavailability of this heavy metal for ryegrass. Zhou et al. (2023) also succeeded in reducing the bioavailability of lead by more than 50 % and zinc by almost 90 % after adding biochar compost to the soil. Since zinc shows increased mobility under low soil pH conditions and increased plant uptake, the application of alkaline biochar to increase soil pH and SOM can create conditions for stabilization and immobilization of this metal. Moreover, unlike Pb, Zn is an essential nutrient and its uptake by plants is subject to physiological regulation (Kabata-Pendias, 2010).

Regarding the results of lead concentration in the shoots, only the S soil samples showed striking results. Increasing the dosage of biochar applied reduced the content of this metal in the dry matter to a minimum. The F soil data did not contain increased concentrations of Pb. The CZ soil samples contained more lead in themselves. In general, lead was not absorbed by ryegrass in the same amount as zinc, which may explain its unlike zinc behaviour in soil. Lead is less mobile, and plants have features in their physiology that inhibit Pb uptake. Lead often exhibits strong adsorptive

properties in soils, so its phytoavailability is low, especially at higher soil pH (Yoon et al., 2006).

6.4. The effect of biochars on reducing metals concentrations in pore water

From the results of the lead content in pore water in soil S we observed a decrease in concentration with increasing biochar dosage. It is important to mention the low pH of this soil, which may have provoked its movement through the soil profile, resulting in this metal being immobilized in the soil. In the case of CZ, where the Pb content of the soil was elevated, no concentrations of this metal were observed in the pore water. Possibly due to the low mobility of this heavy metal in the soil profile at pH 5.5 (after the addition of biochar the pH of the soil most likely increased as well). We can assume that Pb was immobilised by the added biochar. Beesley et al. (2014) observed a decrease of Cd, Pb and Zn concentration in pore water after application of 10 % biochar (orchard waste) in contaminated soils.

The results of zinc content in pore water in each of the investigated soils showed similar results: the metal concentration decreased when the biochar dose was increased. This trend appeared in all cases except after the addition of 1 % biochar B and C in CZ soil. It can be assumed that this result is due to dominant sand in CZ soil structure (71.6 %), which increases zinc mobility with water down the soil profile. In the case of biochar B, we should not forget the increased Zn content in its composition, which could also have contributed to this result. However, after increasing the dose of both biochar to 5 %, the Zn concentration in the pore water decreased significantly.

7. Conclusions

In this study the effect of three different biochars on the ability to reduce Zn and Pb uptake by ryegrass and its mobility down the soil profile was examined. A series of identical experiments were performed using three European contrasting soils. Some Some key findings of the study:

- In each individual experiment, the expected results of reducing Zn and Pb concentrations in both the dry mass of ryegrass shoots and in pore water were achieved, confirming the first hypothesis.

- One of the key factors influencing the reduction of investigated metal concentrations after biochar addition is soil pH and organic matter, which confirms the second main hypothesis of the thesis. However, during the discussion, as an explanation of some of the results, it became clear that in addition to soil pH and SOM, soil texture is also an important factor, which affects infiltration, nutrient and water binding capacity, and aeration.

- The effect of reduced zinc uptake by ryegrass is more visible than that of lead. During data work, it was discovered that zinc shows increased mobility in the soil and is an essential nutrient for plants. These facts suggest a more intensive uptake of zinc by ryegrass, and consequently more visible reduction results after amendment application. The behaviour of lead is reversed, which can be seen in the results with ryegrass as well as in the soil leachate.

- The biochars used demonstrated alkaline properties and contained a high amount of organic matter, which also helped to improve the physicochemical properties of the soils (it was assumed that the pH and organic matter would increase). The decision to use these alkaline amendments in acidic soils was certainly a justified decision.

- Biochar clearly showed its adsorption abilities in each of the contaminated soils, thus reducing the bioavailability of Zn and Pb for ryegrass and reducing their content in the soil leachate. During data work, more effective results were recorded in almost all cases when the biochar dosage was increased from 1 to 5 %.

- The decisive factors for the effective result of biochar application are the combination of soil characteristics and biochar properties.

Based on the results of this work, it is worth thinking about a wider application of biochar in different areas of human activity. In addition to their effective ability to

adsorb pollutants, these amendments can improve the properties of soils, making them more fertile and resistant to various abiotic factors.

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