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The effect of dry olive residue-based biochar on the risk element mobility and nutrient status of the contaminated soil

Diploma Thesis

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Declaration

I declare that the Diploma Thesis ‘The effect of dry olive residue-based biochar on the risk element mobility and nutrient status of the contaminated soil’ is my own work and all the sources I cited in it are listed in the References.

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Abstract:

The Mediterranean areas depend on their production of olives and olive oil, with Spain taking the lead in olive oil production. A two-phase extraction system is used which creates a by-product known as “alpeorujo”, which undergoes a second centrifugation to extract any residual oil, creating dry olive residue (DOR) as a waste product. The DOR is rich in mineral and organic matter and phytotoxic compounds (phenolic compounds, organic acids, high molecular mass polyphenols and glycoproteins) that can inhibit growth of microorganisms and plants.

A possible way to decrease the phytotoxicity of the waste can be to transform it into biochar via pyrolysis and use it as an amendment for contaminated soils and to improve the nutrient status of soils. DOR from Spain was transformed into biochar at 350°C and 500°C in order to evaluate its effect on the mobility of As, Cd, Pb and Zn and Ca, Mg, P and K availability. A pot experiment was conducted over 3 months to observe the effect of each biochar on three levels of contaminated soil obtained from Přibram (low, medium and high levels of contamination). The biochar was applied at a 2% and 5% application rate. *Triticum aestivum* was grown in the soils to observe the availability and mobility of the risk elements. The different application rates and pyrolysis temperature were seen to influence the accessibility of the risk and essential elements in soils and *Triticum aestivum* plants.

Soil properties and especially pH was found to statistically affect the bioavailability of risk elements in the soils, however the treatments were less significant. A greater significance was found for the essential elements, where soil, biochar production temperature and plant parts were statistically significant in improving essential element concentrations in plants ($p<0.01$; $p<0.001$). The correlation analysis found a positive correlation between the risk elements in soils indicating the similar source of soil contamination. The negative correlations among the risk elements and nutrient levels in plants indicate that the increase in the risk elements has led to the decrease of some of the essential element concentrations in plants.

Key words: Dry olive residue, risk elements, mobility, nutrient availability, contaminated soil

Contents

1. Introduction

Soil is the basis of life; it is used to grow our food, filter our water and air, and is a platform to build our cities and houses. Soils are susceptible to chemical, biological and physical changes influenced by soil microorganisms, weathering, vegetation and climate. Different weather conditions such as precipitation or draught can influence and affect the conditions and fertility of soils, which in turn determines the occurrence of vegetation and microorganisms in the soils. However, one of the major influencers over soil properties is none other than humans. We have strived to achieved to grow our economies and cities to fit with the increasing population. As the population increases, the need to for more factories, food and energy has increased. Our economies are the influencers of the levels of anthropogenic soil contamination our soils face. Risk elements enter the soils through processes of aeolian deposition, colluvial deposition and dumping. Once in soils, the processes of flooding, leaching and runoff can further lead to contaminated water sources. According to the German Advisory Council on Global Change (1994), 22 million hectares of soils worldwide are degraded by chemical degradation from industries, including contamination by risk elements. The soils we used as a filter and source of life has now become a harbour for risk elements. The fate of the risk elements depends of soil chemical and physical properties and on their speciation. Risk elements in soils can be classified as bioavailable and unavailable to plants. The unavailable portion of the risk elements refers to the elements that are adsorbed into crystalline structures of clay minerals or on humus structures, thus, making them unavailable for plants to uptake them. However, any chemical or physical changes to soil properties can cause unavailable risk elements to become available for plants and *vice versa*. This has many consequences on soil, animal and human health. Vegetation, such as agricultural crops, grown on contaminated soils with high concentrations of bioavailable risk elements are prone to uptake these risk elements. This can have severe consequences on the plants health and ability to grow, and further on the health of humans who consume products with high levels of risk elements present. The human consumption of contaminated foods and water can cause many risks and side effects (McLaughlin et al., 2000, 2007).

Remediants and amendments are widely used and researched due to their ability to immobilise risk elements in soils. Clay minerals, humus, compost and biochar are examples of soil amendments that are being widely researched to observe their ability to amend contaminated soils. Biochar is distinguished as a “fine-grained, porous, carbon rich material”, and has had a rising interest in the scientific community thanks to its large surface area and cation exchange capacity (Beesley et al., 2011), making it an ideal amendment. According to the European Biochar Certificate (EBC), biochar is described as a “heterogeneous substance rich in aromatic carbon and minerals. It is produced by pyrolysis of sustainably obtained biomass under controlled conditions with clean technology and is used for any purpose that does not involve its rapid mineralisation to CO_2 and may eventually become a soil amendment” (EBC, 2012).

The economies of Mediterranean areas thrive on olive cultivation and olive oil production. According to Anania & Pupo D'Andrea (2011) the world production of olive oil in 2008/09 reached 2.8 million tons. Spain has been named the world’s major producer of olive oil, followed by Italy and Greece. Olive oil production for the year 2008/09 in 1000 tonnes for Spain was slightly over 1000 (Anania & Pupo D'Andrea, 2011). Spain uses a two-phase extraction system that produces olive oil and an organic sludge, also known as two-phase olive mill waste (TPOMW) (Aranda et al., 2007). Revolorization of this two-phase olive mill waste produces low quality olive oil and ‘dry olive residue’ or also known as DOR, as a by-product. DOR on its own is harmful to plants and microorganisms because of its high amount of phenolic compounds (Sampedro et al., 2009). However, the fungal transformation or composting of DOR, as well as the formation of DOR-based biochar can reduce the unfavourable properties of this material. The biotransformed DOR has been found to improve chemical characteristics of soil and bacterial and fungal communities (Sampedro et al., 2009).

2. Work Objectives

Two main objectives of the study were defined as follows:

1. To assess the effectivity of the dry olive residue (DOR)- produced biochar on the mobility and plant-availability of risk elements as well as on the nutrient status of the contaminated soil in a model pot experiment.

2. To verify the risk element immobilisation ability of DOR-based biochar in the contaminated soil and simultaneously the usefulness of this material as a source of available nutrients for plants growing in the contaminated soil.

Hypothesis: DOR-based biochar is able to improve growth parameters of plants via both risk element immobilisation and improvement of the soil nutrient status.

3. Literature Review

3.1. Occurrence and environmental cycle of risk elements

Elements within the ecosystem can be classified as occurring in four categories: atmospheric, organic, bioavailable and mineral. Within the atmosphere, all elements can be found in aerosol or gaseous forms. Organic phase of metals are elements that are incorporated in living and dead biomass of plants and animals. Bioavailable fractions are elements that are in free ionic or complexed forms which are either dissolved in soil solution or adsorbed on exchange sites of clays, humus or oxides of Fe, Mn and Al. Mineral fractions are primary and secondary minerals (Adriano, 2001). Many of the elements are important micro and macronutrients for plants, animals and humans. However, at elevated concentrations they can cause phytotoxicity and can affect human health, due to their non-biodegradable nature that allows them to accumulate in tissues of living organisms (FAO, 2018).

When focusing on the agroecosystem, two routes for the entrance of risk elements into an agroecosystem are present. These can be distinguished as aerial and land. Aerial referring to aeolian transport as aerosols, particulate matter or airborne dust. Land inputs are fertilisers, biosolids or various soil amendments. Mine tailings, heavy metal wastes, sewage sludge, waste water irrigation (Alloway, 2013) are further examples of entrance of risk elements into the agroecosystem. The biogeochemical cycle of elements is the continuous exchange of the elements between various sectors of the ecosystem and spheres (e.g. biosphere, lithosphere and atmosphere) (Adriano, 2001).

Soil systems constitute of mineral and organic fractions, allowing elements to change from one fraction to the other. Within soils, reactions of elements are within aqueous and solid phases of the soil. Solid phases are composed of clay minerals, hydrous oxides and organic matter. The aqueous phases, or also referred to as soil solution, is the water present in the soil that contains dissolved ions (Adriano, 2001).

Ion exchange, solubilization and adsorption are the main processes that determine biogeochemical speciation of elements. Speciation referring to the formation of new species of elements. In turn, this influences their solubility, mobility, bioavailability and toxicity. However, there are various other processes that ultimately affect the concentrations and biogeochemical dynamics of elements in soils; weathering of parent material, redox reactions, acid-based reactions and decompositions (Figure. 1) (Adriano, 2001).

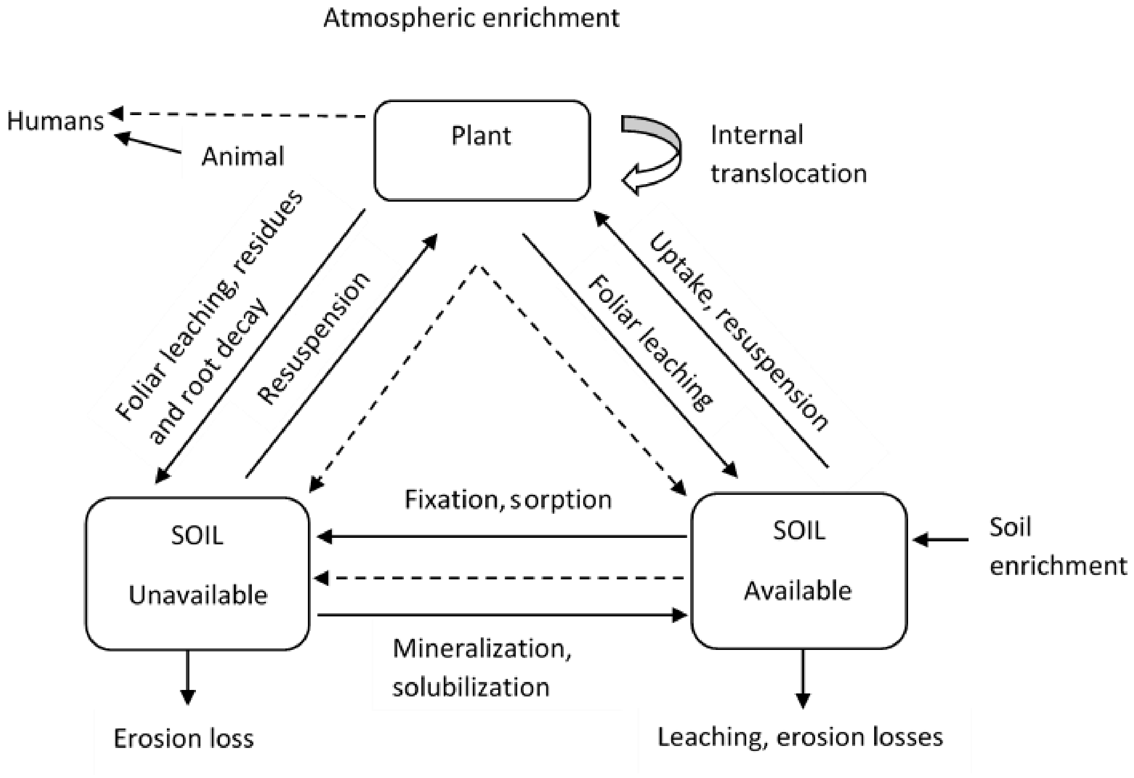

Figure 1: Biogeochemical cycle (Adriano, 2001).

3.2. Soil Contamination

The European commission defined a contaminated site in the Commission proposal (COM 2006) as “a site where there is a confirmed presence, caused by human activities, of hazardous substances to such a degree that they pose a significant risk to human health or the environment, taking into account land use” (Science Communication Unit, University of the West of England, 2013). According to FAO & ITPS (2015), soil contamination has been identified as the third most important threat to soil functions in Europe and Eurasia, fourth in North Africa and fifth in Asia. In the 1990s the United Nations Environment Programme (UNEP) attempted to conduct the unique global estimate of soil contamination and estimated that 22 million hectares had been affected by soil pollution (Oldeman, 1991). However, it is believed that the value presented by Oldeman (1991) underestimates the real degree of soil pollution. The Chinese Environmental Protection Ministry states that 16 percent of Chinese soils and 19 percent of agricultural soils are labelled as polluted (CCICED, 2015). According to the European Environment Agency, an estimate of around 3 million potentially polluted sites exist within the European Economic Area and countries in the West Balkans (EEA, 2014). Australia is believed to contain around 80 000 contaminated sites (DECA, 2010), while the USA have over 1300 polluted or contaminated sites in the Superfund National Priorities List (US EPA, 2013).

Soil contaminants from anthropogenic sources involves organic and inorganic contaminants. Organic contaminants can be oxidized to CO_2 by microbial activity, however many risk elements cannot be degraded by microbes or chemicals (Kirpichtchikova, 2006), thus allowing risk element concentrations in soils to persist for long periods of time (Adriano, 2001; Wuana & Okieimen, 2011). Once contaminants enter soils, they undergo physical, physiochemical, microbiological and biochemical processes that either retain, reduce or degrade them (FAO, 2018). Hence, the soil properties affect the level of soil contamination as they control the mobility, bioavailability and residence time of the contaminants (FAO & ITPS, 2015). Soil mineralogy, clay content, pH, organic content, temperature and moisture are all soil properties that play a role in the future of the contaminant in the soil. The risk elements that are the most common in contaminated soils are arsenic (As), lead (Pb), chromium (Cr), cadmium (Cd), zinc (Zn), copper (Cu), nickel (Ni) and mercury (Hg) (GWRTAC, 1997; Wuana & Okieimen, 2011).

The most persistent and complex kind of contaminants in nature are considered to be risk elements, especially metals and metalloids, as they degrade the quality of the atmosphere, water, food crops and threaten the health of animals and humans. Unlike majority of organic compounds, they are not subjected to metabolic breakdown, allowing them to accumulate in living organisms (McBride, 1994).

3.3. Sources

Risk elements entering the soil ecosystem can occur from lithogenic processes and sources as well as anthropogenic sources. Total concentrations of risk elements in soils is thus derived from the parent material the soil was developed on and inputs from anthropogenic sources brought on by humans. The total concentrations in soils are the total of all risk element inputs from both lithogenic and anthropogenic sources, allowing the following formula to summarise concentrations of total risk elements (Alloway, 1995):

$$M_{total} = (M_{pm} + M_{atm} + M_{sed} + M_{f} + M_{ac} + M_{tm} + M_{om} + M_{ic}) - (M_{cr} + M_{e} + M_{l} + M_{v})$$

M= metal(loid), pm= parent material, atm= atmospheric deposition, sed= deposited sediment, f= fertilizer, ac= agricultural chemicals, tm=technogenic materials, om= organic matter, ic= other inorganic contaminants, cr= crop removal, e= soil erosion, l= leaching, v= volatilisation.

3.3.1. Lithogenic sources

Lithogenic sources refers to the introduction of risk elements from parent materials through processes of weathering. However, the concentrations of risk elements introduced through the weathering of parent material is considered as trace, meaning they do not exceed 1000 mg/kg, and is seldomly toxic (Kabata-Pendias & Pendias, 2001; Pierzynski et al., 2000). The process of weathering evolves around the chemical decomposition of minerals, the release of part of their constituent elements in soluble forms and the formation of secondary minerals through chemical reactions between the products of weathering (Alloway, 2013). The part of the mineral not chemically decomposed remains as an insoluble residue and any risk elements retained in it are unlikely to become soluble and available to plants in a long time (Alloway, 2013). Some rock types that are lithogenic sources of risk elements in soils are: black shales, limestones, ultramafic rocks and iron oxide-rich sediments (concentrations shown in table 1). Black shales are high in organic matter and clay contents, enriched in a range of risk elements and trace elements. They can give rise to elevated concentrations of Ag, As, Au, Ba, Cd, Cr, Cu. Hg, Mo, Ni, Pb, Zn (Alloway, 2013). Limestones compromise of grains of calcite, derived from marine organisms which accumulate in marine basins. They can receive inputs of risk elements which in turn forms limestones with elevated risk element concentrations (Alloway, 2013). Ultramafic rocks contain relatively high concentrations of Ni, Cr and Co, which can lead to soils containing elevated concentrations of these elements that were developed on ultramafic rocks (Alloway, 2013). Iron oxide-rich sediments, such as ironstones, are found to accumulate high concentrations of As, Ba, Cu, Mn, Mo, Ni, Pb, V and Zn (Alloway, 2013).

Table 1: Concentrations of risk elements in certain rock types (Alloway, 2013).

	Black shales	Limestone	Ultramafic rocks	Sandstones
As	<500	1.5	0.7	0.5
Cd	<240	0.1	0.05	<0.04
Pb	<100	5	0.05	10
Zn	<2314	40	60	20

3.3.2. Anthropogenic sources

Anthropogenic sources refer to sources originating from human activities. Five major anthropogenic sources of risk elements in the environment are (Alloway, 1995):

i. Metalliferous mining and smelting
ii. Agricultural and horticultural materials
iii. Sewage sludges
iv. Fossil fuel combustion
v. Metallurgical industries

Contaminants from industrial activities are freed into the water, soil and atmosphere. Soil contamination occurring through such anthropogenic processes usually occur by aeolian or colluvial deposition, leaching and dumping. Aeolian deposition, or also considered atmospheric deposition, is the most extensive form of contamination due to long range transport (Alloway, 2013). Due to aeolian deposition, soils hundreds of kilometres away from the source can become contaminated. Sources of risk elements entering the atmosphere are from coal/oil fired electricity generating stations and industrial emissions (smelters, motor vehicle exhaust emissions, heating) and concentrations of risk elements in air in Europe can be seen in table 2.

Table 2: Median concentrations of several elements present in the air in Europe (ng/m3) (Bowen, 1979).

As	Cd	Cr	Cu	Mn	Pb	Zn
1.5-53	0.5-620	1-140	8-4900	9-210	55-340	13-16000

The processes of mining and smelting are pathways for contaminants to enter soils. Large quantities of risk elements are released to the environment from mining and smelting facilities and tend to persist for long periods of time (Ogundele et al., 2017). Mining wastes are formed by fine particles that contain different concentrations of risk elements, which can be dispersed by wind and water erosion (FAO, 2018). Thus, the final residence of risk elements can be kilometres from its source, entering agricultural or protected soils.

Landfills are also another source of environmental contamination. Municipal waste disposal and untreated waste water to the environment are sources of risk elements and other contaminants that can enter soils and waters (FAO, 2018). Households are also contributors to environmental contamination through the use of chemical detergents and personal care products which end up in sanitary sewage and in turn, can end up in soils and waters (FAO, 2018). A major source of lead contamination from households is for example the use of lead-based paint. When lead-based paint becomes pulverized into small particles from renovations or demolitions, it can enter the environment (Mielke & Reagan, 1998).

Agricultural sources of soil contaminants include agrochemical products such as fertilizers and pesticides. Such agrochemical products contain several trace metals which include Cu, Cd, Pb and Hg. Their elevated concentrations in soils can ultimately affect plant metabolism and crop productivity (FAO, 2018). Excess N and risk elements that enter the food chain are a threat to food security, water quality and human health (FAO & ITPS, 2015). Application of manure can on one hand be beneficial for soils, but on the other can be carriers of risk elements, antibiotics and pathogens. Risk elements in manure are introduced from animal feed and antibiotics are derived from growth promoters (FAO, 2018).

3.4. Risk elements

3.4.1. Cadmium

Cadmium (Cd) is a transitional metal that is usually divalent in all stable compounds, and most common compound found in nature being CdS. It is used as alloys, in electroplating, in pigments, as stabilisers for polyvinyl plastics, in batteries, and is a by-product of the Zn industry. Cd concentrations in natural soils is influenced by the concentrations of Cd in parent rocks (Adriano, 2001). In soils classified as non-contaminated, Cd levels are usually <1 mg/kg. However, due to anthropogenic sources and extreme weathering of high concentration bearing parent material, soils contain elevated concentrations of Cd. Cd contamination in the environment has been expeditiously rising as a result of the increasing consumption of Cd by various industries (Alloway, 1995) making it one of the most ecotoxic metals that have adverse effects on soil biological activity, plant metabolism and human health (Kabata-Pendias & Pendias, 2001). The toxicity of Cd is determined by the form it is present in, with Cd^{2+} most likely to be absorbed on the surfaces of soil solids. The speciation and concentration of Cd in soil solutions is dependent on the concentrations and stability of ligands in the soil solution (Alloway, 1995). Concentrations of Cd in topsoil in the vicinity of Pb and Zn mines and smelting operations are reported to be very high (Kabata-Pendias & Pendias, 2001).

Cadmium can be highly toxic even in low levels; causing renal dysfunction, lung disease, cadmium pneumonitis, and bone defects (Duruibe et al., 2007).

3.4.2. Lead

Known as the first metal to be extracted by man from its ore, lead (Pb) happens to be classified as a non-essential and non-beneficial element for both animals and plants. Pb is used in batteries, solders, cable covers, ammunition, pigments and plumbing (Wuana & Okieimen, 2011). Nevertheless, Pb is extremely toxic and can be extremely poisonous for mammals. Pb mining plays a leading role in soil contamination. In soils, lead tends to accumulate and remain bioavailable for extended periods. The greatest Pb concentrations are often found in organically rich top horizons of uncultivated soils (Kabata-Pendias & Pendias, 2001), making organic matter an important sink of Pb in contaminated soils. Surface soils worldwide usually have an average concentration of Pb of 32 mg/kg and generally ranges from 10-67 mg/kg (Kabata-Pendias & Pendias, 2001).

Pb is abundant in the earth's crust with 10-30 mg/kg (Wuana & Okieimen, 2011). It is abundant in shales and has an affinity for S. Pb is dangerous to humans and animals from two sources: food chain and soil dust inhalation (Wuana & Okieimen, 2011). Pb has the tendency to accumulate in the organs of organisms which can Pb to plumbism, affecting the kidneys, central nervous system and brain. Children that have been exposed to Pb have shown to experience lower IQ levels, hyperactivity, mental deterioration and impaired development (Wuana & Okieimen, 2011).

3.4.3. Zinc

Zinc (Zn) is a naturally occurring element in the earth's crust as constituents of rocks and Zn-rich ores, with concentrations ranging around 70 mg/kg in crustal rocks (Wuana & Okieimen, 2011; Adriano, 2001). Zn ores were formed by natural geological processes and are located worldwide, making it the 24th most abundant element on earth (Adriano, 2001). Zinc is abundantly used in the automobile and electrical industries. High concentrations of Zn in parent materials along with application of sewage sludge, mining and smelting have created an abundance of Zn in soils, beyond its micronutrient limit for biota. Concentrations of Zn in normal soils are quoted to range between 1-900 mg/kg, with a mean of 40-50 mg/kg of Zn was given for world soils (Adriano, 2001).

Zn is present in the +2-oxidation state in soils and the concentration of Zn in soil solution can be affected by precipitation-dissolution, sorption and solution complexation reactions. When

Zn is added to soils, an initial reaction takes places where Zn adsorbs onto the solid phases which is then followed by a process referred to as ageing; which is the removal of the metal from the labile pool (Alloway, 2013). Zn is easily adsorbed by mineral and organic components in most soils, causing an accumulation in surface horizons (Kabata-Pendias & Pendias, 2001). Soluble forms of Zn are readily available to plants and Zn adsorption differs among plant species and growth media. The nutrient solution and presence of calcium are important for Zn uptake (Kabata-Pendias & Pendias, 2001).

Zn is usually considered to be relatively non-toxic and is essential in certain concentrations. At elevated concentrations, health issues involving impairment of growth and reproduction, liver and kidney failure, anaemia, bloody urine and vomiting can be observed (Duruibe et al., 2007).

3.4.4. Arsenic

Arsenic (As) is a known toxic element that naturally occurs in the oxidation states between -III and +V. There are over 200 As containing minerals that have been identified which includes sixty percent being arsenates, twenty being sulphides and sulphosalts and the other twenty percent being arsenides, arsenites, oxides and elemental As (Alloway, 1995). As that has originated from parent material (Adriano, 2001) due to the weathering of materials containing shales, clay and phosphorites, contain higher levels of arsenic compared to that of sandstone and limestone. Areas close to mining operations are the main source of atmospheric deposition of arsenic to soils. As can originate from the processing of Cu, Pb, Zn, Ag and Au ores and from the ashes of coal combustion. Several As compounds can be strongly absorbed to soils preventing them from being transported long distances in ground and surface water. As concentrations in normal soils of surface soils in the U.S. was reported between <0.1 to 97 mg/kg. Concentrations of As in uncontaminated soils were found to seldom contain >10 mg/kg (Adriano, 2001).

As compounds are used in pesticides, which are now banned in the EU (Alloway, 1995). The use of pesticides, herbicides and silvicides in agriculture and forestry, can cause elevated concentrations of As in the soil (Adriano, 2001). Other uses of As are additions to animal feeds, paints, dyes, soaps and wood preservatives (Martin & Griswold, 2009). The main problems stemmed from being in contact with As are skin damage, increased risk of cancer and issues with the circulatory system (Wuana & Okieimen, 2011).

3.4.5. Risk elements in plants

There are several processes that can characterise the element metabolism of plants. These can be described as: uptake and transportation, enzymatic processes, concentration and forms, deficiency and toxicity, ion competition and interaction (Kabata-Pendias & Pendias, 2001). The ability of plants to uptake/accumulate risk elements also depends on plant species, different plants have different thresholds for element uptake. Plant species with the ability to grow in contaminated soil and accumulate high concentrations of risk elements in their above ground biomass are considered as hyperaccumulators (Rascio & Navari-Izzo, 2011), and can be used for phytoremediation. However, species that are grown for agricultural purposes such as mustard, radish, rape, amaranth and turnip were found to accumulate higher amounts of Cd, Cr, Cu, Ni and Zn (Kabata-Pendias & Pendias, 2001). This leads to issues of human consumption of contaminated crops that were grown on contaminated agricultural soils.

Determining the availability of risk elements to plants are their solubilities. For root uptake to occur, soluble forms of the risk elements must be present close to/around the root membrane (Cataldo & Wildung, 1978). The soluble form in which the risk element is present in the soil also determines its longevity in the soil solution, mobility and uptake rate (Cataldo & Wildung, 1978). Risk element solubility varies for different elements and its physiochemical properties, soil processes and properties (Cataldo & Wildung, 1978; Mortvedt, 1994). Soil properties that influence the availability and solubility of risk elements are soil pH, cation exchange capacity (CEC), soil texture, redox potential, clay content and organic matter content (Verloo & Eeckhout, 1990; Shah et al., 2010). Higher clay content, organic matter (OM), soil pH the more strongly bound the elements are to soil, rendering them unavailable for plant uptake (Shah et al., 2010). In comparison, the bioavailability of risk elements in soils is heightened by many factors, with focus on the release of phytosiderophores (class of chelate compounds) into the rhizosphere allowing for the solubilisation of risk elements bound in the soil (Kinnersely, 1993).

Uptake of risk elements by plant roots depend on concentrations of risk elements in the soil, without having a linear relationship with increasing concentrations (Shah et al., 2010). The adeptness of risk element uptake and accumulation by plants is found to be highest when they are present in low concentrations in soils (Shah et al., 2010). Certain elements such as Cd, Mn, Se, Zn and B are easily taken up and translocated within plant tissues, while on the other hand, Al, Cr, Fe, Hg and Pb are strongly bound to soils or root cell walls (Chaney, 1983a; Chaney, 1983b).

Zn in soluble forms are usually readily available to plant uptake. The ability of plants to absorb Zn varies with plant species and soil properties (Kabata-Pendias & Pendias, 2001). Zn was found bound to organic compounds in the xylem fluids and plant tissue extracts which indicate that Zn is relatively mobile in plants (Kabata-Pendias & Pendias, 2001). The roots of plants have found to accumulate more Zn than upper plant parts, but when grown in soils with higher concentrations of Zn, it can be translocated from the roots and accumulated in above ground plant biomass (Kabata-Pendias & Pendias, 2001). Zn is said to accumulate in chloroplasts, vacuole fluids and cell membranes (Tinker, 1981).

Cd is a nonessential element for plant metabolic processes, however it is absorbed by root and leaf systems, allowing it to accumulate in plants (Kabata-Pendias & Pendias, 2001). A linear relationship can be seen between Cd concentrations in plants and Cd concentrations in soils. The pH of soils is established as one of the dominant factors affecting the uptake of Cd by plants, nevertheless, soluble forms of Cd can easily be absorbed by plants (Kabata-Pendias & Pendias, 2001). Cd has been recognised to accumulate in the tissues of roots especially when concentrations of Cd increases in soils; leading to the concentrations in roots surpassing the concentration in above ground biomass by around 100 times (Kabata-Pendias & Wiacek, 1985). It was also reported by Cunningham (1977) that Cd was accumulated mostly in the roots, with lower concentrations in the stem nodes and leaves. Martin & Griswold (2009) also noted the accumulation of Cd in roots, and under high soil concentrations and plant stress, plants can accumulate high Cd levels in plant organs that are harmful to organisms.

Pb is not essential in plant metabolism and can be toxic to plants. The concentration of Pb in plants is determined by geochemical irregularities, contamination, seasonal changes and genotype ability to accumulate Pb (Kabata-Pendias & Pendias, 2001). Pb is not readily soluble in soils, but when it is, it is taken up by plants via root hairs and stored in cell walls. Hughes et al. (1980) found that Pb was absorbed by roots via passive uptake. Soluble Pb forms are easily available for plant uptake; with its uptake rate increasing with higher concentrations of Pb in soil solutions. It is found that Pb is accumulated in the roots as translocation of the element is limited and that around 3% of Pb in roots are able to translocate to other plant organs (Zimdahl, 1976). However, in the absence of phosphates, Pb can easily be translocated from root systems to above ground plant biomass (Martin & Griswold, 2009).

A linear relationship between arsenic concentration in plants and soils has been noticed for total and soluble As, indicating that arsenic uptake by plants is passive and with the water flow

(Kabata-Pendias & Pendias, 2001). As is also known to inhibit metabolic processes, decreasing yields when plants are grown on soils with high concentrations of bioavailable As. Interestingly, As can be found to be relatively less toxic to plants when the plants also have sufficient concentrations of P (Kabata-Pendias & Pendias, 2001). Translocation of As has been noted in grains of several plant species. Tlustoš et al. (1998) discovered that increasing concentrations of As in soils led to highest arsenic levels found in old leaves and root, alternatively, low concentrations of As in soils led to high As accumulation in leaves compared to roots.

3.5. Impacts of soil contamination on the food chain and ecosystem

Food security is a fundamental necessity for human health. It is defined as “the availability, access, utilization and stability of food supply” (FAO, 2018). With the pressing issue of soil pollution and contamination, food security is reduced. Crop yields are decreased due to toxic levels of contaminants and crop quality is decreased due to presence of contaminants, making them unsafe for human consumption (FAO & ITPS, 2015).

There are several pathways for contaminants to enter the food chain: wet or dry deposition of particles onto leaves, gaseous deposition on leaves, direct uptake from soil surface via roots and root uptake from soil solution (FAO, 2018).

Impairment of plant metabolisms and decrease of crop productivity occurs when soils contain high concentrations of risk elements such as As, Cd, Pb and Hg, putting pressure on arable land. Once they enter the food chain, food security, water resources and human health are all posed to risks (FAO, 2018). Each plant species has its own thresholds for uptake and translocation of elements into its tissues. These elements can interfere with metabolic processes and plant growth and can cause toxicity that can lead to plant death.

Two concepts that must be considered are the bioaccumulation of risk elements in soils and plants and its biomagnification. Bioaccumulation refers to accumulation of risk elements, substances and chemicals in an organism (Martin & Griswold, 2009). Biomagnification is the process whereby contaminants are transferred through the food chain, with concentrations increasing with increasing levels in the food chain. Uneasily degradable synthetic, lipophilic organic substances and risk elements are contaminants that are said to biomagnify throughout the food chain. The biomagnification occurs along the food chain due to successive trophic levels consuming larger quantities of biomass; therefore, consumption of large quantities of contaminated biomass also means the intake of large quantities of contaminants. The synthetic, lipophilic contaminants, such as persistent organic pollutants (POPs) are consumed from contaminated biomass and are absorbed and stored within the tissues and fats of consumers. The concentration of contaminants in fats and tissues increases with each trophic level. The biomagnification of risk elements differs depending on the element in question. Hg was found to biomagnify in the aquatic ecosystems (Gobas & Morrison, 2000), while Cd is one of the only metals found to biomagnify along the food chain. In order to quantify the extent of biomagnification in the food chain, the biomagnification factor (BMF) can determine any ecological risks of chemicals (Mackay & Fraser, 2000).

Oliver & Gregory (2015) summarised six soil-related human health risks, of which 3 are soil pollution related: risks from elemental contamination, organic chemical contamination and pharmaceutical contamination. The effect of soil contamination to human health depends on the levels of contamination, the contaminants and the pathway from soil to human. One of the ways in which risk elements enter the human metabolism is through consumption of contaminated plants or animals (Khan et al., 2015). Dermal exposure is another pathway in which humans are exposed to soil contamination from using parks and gardens (Chaparro Leal et al., 2018). Inhaling contaminants is also possible if soil contaminants have been vaporised or inhalation of dust particles (FAO, 2018).

3.6. Soil Contamination in the Czech Republic

Mining and Smelting in the Czech Republic had been going on for hundreds of years and slowly came to a halt in the 20th century. The subsurface mining of materials in the Czech Republic can be classified as one of the oldest in Europe, with over 800 of both ore deposits and metalliferous mineral occurrences. Exploitation of Ag ores, Sn and Au occurred around the Middle Age and Modern Period (Bufka et al., 2005). Ag ores were exploited in Kutná Hora, Příbram, Jáchymov, Rudolfov, Jihlava and Ratibořské Hory; tin mining occurred in Krupka, Cínovec, Horní Slavkov; gold mining took place in Jílové u Prahy, Nový Knín, Zlaté Hory and Kašperské Hory (Bufka et al., 2005). It had been one of the main contributors of soil contamination, especially in the surrounding areas of the cities such as Kutná Hora and Přibram. A precise number of contaminated sites within the Czech Republic is not known, however, an estimate of 10,000 contaminated sites exist (CENIA, 2014). However, the Evidence System of Contaminated Sites database in the year 2014 only contain 4829 contaminated sites (figure 2) (CENIA, 2014). Many contaminated sites in the Czech Republic have been or are undergoing remediation. According to CENIA (2014) the sites with the highest priority for investigation and remediation are located in South Moravia, Moravian-Silesian and Central Bohemian regions.

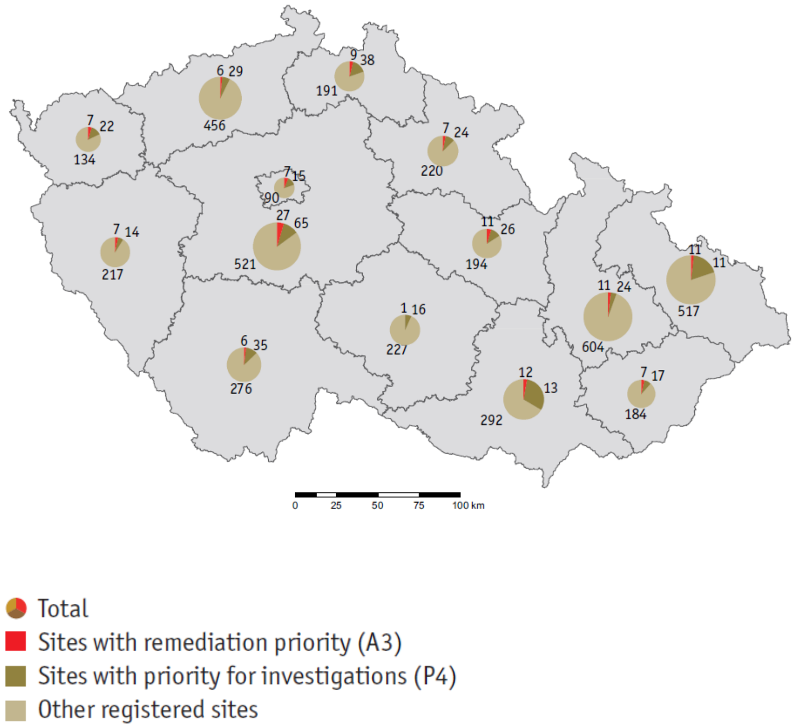

Figure 2: Number of contaminated sites registered in Evidence System of Contaminated Sites in the Czech Republic, 2014. Source: Ministry of Environment.

The processes of mining and smelting involved the processing of sulphidic ores that contained silver. These processes can be categorized into four stages (Vaněk & Velebil, 2007):

1. The sorting of ores followed by treatment to gain an initial sulphide concentrate.
2. The roasting of ores to oxidise the sulphides.
3. Reduction of metallic silver.
4. Silver separation and refining.

The sulphidic ores containing silver can theoretically be categorised into three groups: complex sulphide ores, galena ores and noble silver ores.

Complex sulphide ores are mixtures of sulphides of galena, sphalerite, chalcopyrite, pyrite and arsenopyrite with silver contents that range between 0.0X% to 0.X% as microscopic inclusions of silver-minerals or admixtures (Pauliš & Mikuš, 1998). Complex ores are processed by roasting, repeated smelting and matte roasting, production and liquidation of black copper and cupellation of lead-silver alloy. Galena ores are silver bearing galena with 0.X% of silver content as microscopic inclusions of silver sulpho-salts or admixtures. Processing includes ore roasting and subsequent reduction to lead which is then cupellated (Vaněk & Velebil, 2007). Noble silver ores contain macroscopic native silver, argentite, pyrargyrite and other containing high percentage of silver. As to prevent losses, they are smelted with lead which is cupellated (Vaněk & Velebil, 2007). The exploitation of gold mines can also result in the pollution of the environment with the chemicals used for gold mining; typically cyanide and mercury (Li et al., 2017; Lusilao-Makiese et al., 2014; Porgo & Gokyay, 2017; Stazi et al., 2017). The surroundings of both former and active gold mines are characterized by the presence of mercury-bearing (originating from amalgamation process), and arsenic-bearing (from arsenic minerals accompanying the gold-bearing veins and rocks) mine tailings. Arsenopyrite (FeAsS) is the main arsenic-bearing mineral in metallic deposits, such as mesothermal gold deposits in the Czech Republic (Morávek et al., 1992). Other primary arsenic minerals such as arsenic-bearing pyrite (FeS_2) or löllingite ($FeAs_2$) may be present in smaller amounts (Drahota et al., 2018).

The Czech Ministry of Environment have set preventive values for risk elements in agricultural soils as indicated in table 3. Soils exceeding the preventive values cannot be used for agricultural purposes and will in the future have to undergo remediation measures.

Table 3: Pseudo total preventive values for risk elements in agricultural soils set by the Ministry of Environment in the Czech Republic (Notice no. 153/2016 Coll.) (mg/kg).

As	Cd	Pb	Zn
20	0.5	60	120

3.7. Soil Amendments

The increasing soil contamination levels have brought forward a multitude of various amendment technologies and possibilities that help prevent further contamination and mitigation of contaminants. A soil amendment is the addition of a substance into the soil to improve its chemical and physical properties while immobilising contaminants via sorption or precipitation (Basta & McGowen, 2004). When referring to soil contamination, soil amendments are usually porous substances with a high cation exchange capacity that allow the risk elements to adsorb to the substance, preventing it from spreading. Soil amendments include clays, humus, oxides of manganese, aluminium and iron and biochar. The ability of soils and amendments to adsorb risk elements depend on several soil properties such as pH, redox potential, water content and salinity. Changes in soil properties can affect the adsorption abilities of soils, which can allow for unavailable concentrations of risk elements to become bioavailable and *vice versa* (Alloway, 2013).

Buekers et al., (2008) established that fractionation modelling predicted that a greater proportion of cadmium is bound to organic matter in soils with pH <6.5, whereas iron-oxides become most important adsorptive constituent at pH > 6.5. Phosphorous compounds have been proven to be the most effective amendments to immobilise lead (Geebelen et al., 2002) The content and nature of iron oxy/hydroxides has been proven to be the main controller of arsenic (arsenate and arsenite) sorption. Ion exchange onto clay mineral surfaces or on ionised groups of organic matters are all pathways of non-specific adsorption of zinc. The reactions are weakly selective, reversible and weakly pH dependent (Alloway, 2013).

3.8. Biochar

Biochar maintains a porous structure and high specific surface area which increases its popularity as a soil amendment for increasing soil fertility and adsorbing organic and inorganic contaminants in soil and water (Ahmad et al., 2014; Caporale et al., 2014). Different organic and inorganic fractions are involved in the composition of biochar and can react with contaminants (Beesley et al., 2011) through various mechanisms including adsorption, partitioning and aromatic-π and cation-π interaction (Ahmad et al., 2014; Inyang & Dickenson, 2015). Interactions between the biochar and contaminants have been recognised to change with pyrolysis temperature and nature of the parent material (Jindo et al., 2014). The temperature of pyrolysis greatly influences the partitioning of contaminants into non-carbonized and carbonised biochar fractions. Biochar is also known as a source of certain elements like calcium, magnesium, potassium and phosphorous, which are essential for plant growth (Amini et al., 2016). Application of biochar to agricultural soils have noted increase in plant growth (Chen et al., 2008), increase in soil water-holding capacity (Laird et al., 2010b), decrease in occurrence of crop diseases (Elad et al., 2010), immobilisation of risk elements (Park et al., 2011), reduction of N_2O emission (Kammann et al., 2011) and reduction in leaching of nutrients (Laird et al., 2010a).

Pyrolysis temperature of biochar influences the surface area and micropores development of biochar. Production of biochar is considered to be economical and eco-friendly as it involves the recycling of existing resources and waste by-products. Waste biomasses used for the production of biochar are, but not restricted to, crop residues, animal manure, paper mill waste, food processing waste, sewage sludge and municipal solid waste (Ahmad et al., 2014).

Temperature of pyrolysis and source material of biochar determine the degree of carbonisation which increases the surface area (Chen et al., 2008). Biochars pyrolyzed at higher temperatures (>550˚C) have higher aromatic content and are not as easily subjected to decomposition (Blasi et al., 2013). On the other hand, biochar pyrolyzed at lower temperatures (<550˚C) contain lower condensed C structure and aid in improving soil fertility (Abenavoli et al., 2016). Studies by Yu et al. (2006), Zhou, et al. (2009) and Kasozi et al. (2010) show that the increased pyrolysis temperature has shown to increase biochars' capability to absorb organic contaminants. A study by Chen et al. (2008) evaluated the surface area of biochars at different pyrolysis temperatures and established that biochars pyrolyzed at 700˚C had half the surface area of activated carbon, indicating the potential of biochar activation. Biochar produced at lower temperatures are found to absorb atrazine linearly into their non-carbonised organic matter (Cao et al., 2009) which suggests biochar is advantageous when remediating soils with organic and inorganic contaminants that compete for sorption sites. The thermo-chemical processes that are involved in the transformation of biochar are slow pyrolysis, fast pyrolysis, flash carbonisation and gasification (Abenavoli et al., 2016).

3.8.1. Mechanism of risk elemental immobilisation by biochars

Biochars have various mechanisms in which they immobilise contaminants in soils, however a greater understanding of these mechanisms on different contaminants is required. Figure 3 provides an illustrated overview of the mechanism that are used by biochar to adsorb and render risk elements immobile. One of the mechanisms is the electrostatic interactions between cationic risk elements and negatively charged biochar or soil surfaces (Tang et al., 2013).

The addition if biochar to soils increases the soils negative charges because of its zeta potential and increasing CEC (Tang et al., 2013). Increase CEC allows for the cation exchange of risk elements with Na^+, Ca^{2+}, Mg^{2+}, Al^{3+} (Bian et al., 2014). Biochars contain aromatic-π and cation-π which allow for the sorptive interactions with d-electrons of risk elements. Adsorption of risk elements is one of the most important factors in the immobilisation of risk elements (Wang et al., 2018). The greater the cation exchange, the greater the retention and immobilisation of risk elements (Lehmann et al., 2006).

Biochars contain a non-carbonised fraction, hydroxyl, carboxyl and phenolic functional groups, that allow for ligand complexation with contaminants (Chen et al., 2015).

Another mechanism is the (co)precipitation of risk elements by forming oxides, hydroxides, phosphates, carbonates, silicates and chlorates (Tang et al., 2013; Cao et al., 2011). Biochar increases dissolved organic carbon (DOC) and pH of soils (Beesley et al., 2010); increased soil pH leads to the decreased mobilisation of risk elements (Tang et al., 2013). The increase soil pH and the interactions of the risk elements with -OH, PO_4^{3-}, CO_3^{2-} can lead to the formation of carbonate, phosphate and hydroxide precipitates which immobilises risk elements (Wang et al., 2018).

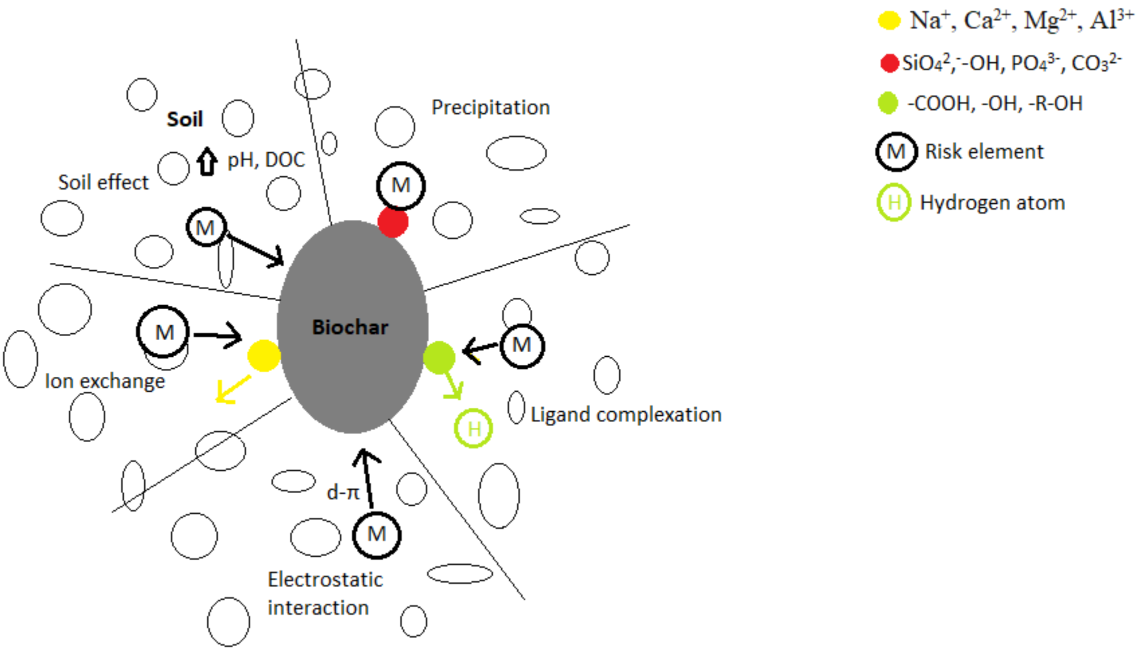

Figure 3: Mechanisms of remediation by biochar. Adapted from Wu et al. (2017) and Lu et al. (2012).

3.8.2. Physical and chemical changes of biochar in soils

The possibilities of biochar particle fragmentation can occur once the biochar is added to soils leading way to changes in biochar via chemical and biological processes. Processes that can affect the fragmentation of biochar ‘freeze-thaw cycle’, bioturbation, wind and rain, and penetration by plant roots and fungi hyphae (Lehmann & Joseph, 2009). The ‘freeze-thaw cycle refers to the process where water penetrates into soil pores and swells during freezing, breaking down large particles of biochar into smaller ones (Carcaillet, 2001). Soil microorganisms and animals aid in the incorporation of biochar into the soils. Microorganisms, such as earthworms, consume the biochar particles and distribute the broken-down particles into the soil profiles via excretion (Eckmeier et al., 2007). Pore sizes of biochar and its adsorption abilities can be affected by substances/elements that it is absorbing, blocking inner pores and disabling adsorption (Zackrisson et al., 1996). It was suggested by Zackrisson et al. (1996) that the deactivation of biochar occurs due to the clogging of pores and decrease of its adsorption ability.

Outer surface particles of biochar are subjected to oxidation and interactions with other soil constituents (clay/sand particles, organic matter, mineral matter) once it has been applied to soils. Oxidation of the oxygen- and hydrogen- functional groups that are present on the outer surfaces of biochar occur (Lehmann, 2007). Cheng et al. (2006) observed that at chemisorption of oxygen at higher temperatures resulted in spontaneous abiotic oxidation of particles of biochar, with acid functional groups increasing at these higher temperatures. An increase in these acid functional groups can result in the biochar to become further hydrophilic and increase weathering of the biochar to smaller particles which can be leached deeper into the soil profile or displaced from the soil profile (Cheng et al., 2006; Shindo, 1991). The formation of non-aromatic functions occurs on the surface of biochar that changes the elemental composition of the biochar particles, developing and expanding proportions of oxygen and hydrogen (Cheng et al., 2006; Hammes et al., 2006).

3.8.3. DOR based biochar

One of the Mediterranean’s most important agroindustrial activities is the production of olive oil. Olive oil extraction is done conducted a two-phase centrifugation system creating the by-product called “alpeorujo”, which is further treated by a second centrifugation to extract any residual oil (Aranda et al., 2007; Sampedro et al., 2005). Dry olive residue (DOR) is the by-product of the drying and chemical extraction with hexane from the second centrifugation (Aranda et al., 2007). DOR has high quantities mineral and organic matter such as sugars, tannins, phenolic compounds, polyphenols, pectins and lipids (Aranda et al., 2007). DOR contains phytotoxic compounds that can inhibit growth of microorganisms, which in turn can inhibit germination and growth of plants (Sampedro et al., 2004). Phytotoxic substances in DOR are phenolic compounds, organic acids, high molecular mass polyphenols and glycoproteins (Sampedro et al., 2005). Due to the abundance of DOR wastes and it's phytotoxicity, it must undergo transformations before its application to soils, which can involve biological transformations via bioremediation by microorganisms (such as fungi) (Siles et al., 2014) or pyrolysis processes.

Sampedro et al. (2005) conducted an experiment to investigate the ability of saprobe fungus *Fusarium lateritium* to degrade DOR into a less phytotoxic form and found it to be a promising technique to treat DOR before its application. Hovorka et al. (2016) conducted an experiment to compare the effect of various DOR that was transformed by four species of fungi: *Penicillium chrysogenum*, *Coriolopsis floccosa*, *Bjerkhandera adusta* and *Chondrostereum purpureum*, on its sorption ability of cadmium, lead, and zinc in soil. The results of the experiment proved DOR to have a good potential for the sorption of lead and less of cadmium and zinc. Biotransformed DOR showed to have better sorption characteristics, on the other hand, the desorption experiment showed certain instabilities of the elements bound to the DOR.

Hmid et al. (2015) conducted an experiment that evaluated the effect of slow pyrolyzed olive mill waste on the immobilisation of metals in the contaminated soils. The pyrolysis of the olive mill waste was conducted at temperatures between 400-450˚C and was applied at four application rates (0, 5, 10, 15% (w/w)) into the soils and left for 30, 90 and 120 days. In order to evaluate the phytotoxicity, Dwarf beans (*Phaseolus vulgaris*) were planted into the soils. The results of the experiment showed a considerable decrease in exchangeable fractions of risk elements and plant tissues contained lower levels of risk element concentrations, resulting in decreased phytotoxicity.

4. Methodology

4.1. Location and sampling

Přibram is a Czech city located approximately 60 km south west of the country’s capital, Prague. It has had a vast history with mining and smelting of lead, silver and zinc. The emissions from primary and secondary lead smelters have led to high concentrations of risk element contamination (lead, cadmium, zinc) in soils. The lead smelting activities also led rise to arsenic concentrations around the location. Vaněk et al. (2005) observed extremely high concentrations of lead, zinc and cadmium in soils, reaching up to 4500, 8700 and 68 mg/kg respectively. High spatial variability of the risk elements concentrations, especially lead, cadmium and zinc, in the soils was reported (Šichorová et al., 2004).

Three soils with differing risk element (arsenic, cadmium, lead, zinc) concentrations were chosen (figure 4). The areas chosen were: soil 1 (figure 5) with relatively low content of risk elements (49°43'15.730"N ; 13°58'33.126"E), soil 2 (figure 6) characterised by medium level of contamination (49°42'43.450"N ; 13°59'7.615"E), and soil 3 (figure 7) with extremely high risk element contents (49°43'9.353"N ; 14°0'49.828"E). According to the public notice characterising the conditions for the protection of agricultural soil quality in the Czech Republic (Anonymous, 2016), the values of arsenic, cadmium, lead, and zinc, exceeded the preventive values: 20 mg/kg for arsenic, 0.5 mg/kg for cadmium, 60 mg/kg for lead, and 120 mg/kg for zinc in all three soils. Soils were sampled at a depth of 20 cm and immediately homogenised, sieved through a 5-mm diameter mesh, and stored at room temperature prior to the experiment.

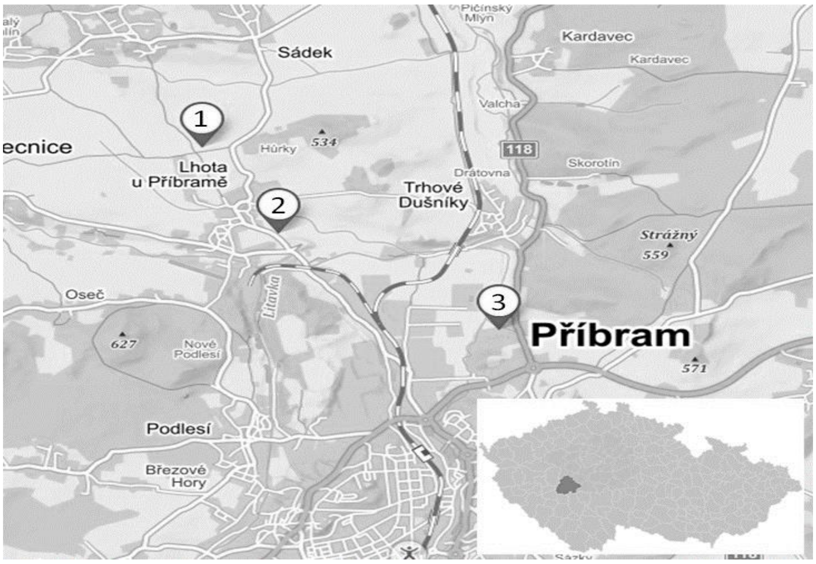

Figure 4: Location of sampling

Figure 5: Location of Hluboš (low) soil sample. Source: Ing. Jana Najmanová

Figure 6: Location of Halda (medium) soil sample. Source: Ing. Jana Najmanová

Figure 7: Location of Litavka (high) soil sample. Source: Ing. Jana Najmanová

4.2. Experimental design

The model pot experiment was set up to evaluate dry olive residue (DOR)-based biochar as a potential immobilising agent for arsenic, lead, cadmium, zinc and improving the nutrient status of the soil. The experiment was set up in a series of identical 0.3 litre polypropylene pots containing 300g of the studied contaminated soils and was conducted by Estación Experimental Del Zaidín, Granada-Spain.

The experimental design consisted in a randomised factorial system with three factors of variation. The first factor was the addition of DOR biochar and comprised five levels: control without biochar, biochar from 350°C and 500°C with a 2% application rate, biochar from 350°C and 500°C with a 5% application rate. The second factor was the time of soil treatment, which were 30, 60 and 90 days. Thirdly, the soils used varied in levels of contamination; Hluboš with low contamination, Halda with medium contamination and Litavka with high contamination. The DOR biochars were applied and mixed manually with the soil to reach concentrations of 2% and 5%. The moisture of the soil was brought to 60% of water holding capacity of the soil. Four replicates were established per each treatment. One 15-day-old wheat plant (*Triticum aestivum*) was planted in each pot (shown in figure 8). The experiment was run in greenhouse conditions (supplementary light 25/19°C and 50% relative humidity) and plants were regularly watered in order to maintain the same initial moisture conditions. After 30, 60 and 90 days of treatment, soil samples from each pot were homogenised, sieved (2 mm mesh), and air-dried at room temperature for chemical analysis. The plant samples were separated to roots, shoots, and grain. The roots were washed after harvest in order to eliminate the soil and all the plant parts were dried at 60°C for 48 hours and homogenised before analysis.

Figure 8: Images of the experimental set up. **Source: Dr. Inmaculada García Romera**

4.3. Soil characterisation

The three soil samples were homogenised and sieved, followed by several tests to characterise the soil before the experiment. The pH, CEC, bioaccessible essential elements and risk elements, and pseudototal levels of risk elements were analysed prior to the experiment.

4.4. Biochar Preparation

The DOR used for the experiment was obtained from the manufacturing company Sierra Sur S.L. (Granada, Spain). Before pyrolysis the DOR was sterilised by autoclave three times (121°C for 20 min) and was frozen at -20°C until use. For biochar production in the laboratory conditions, the pyrolytic furnace Carbolite 301 (Carbolite Gero, Great Britain) (figure 9) was applied. The pyrolysis was provided in the electrically heated quartz tube for 25 min at the target temperature of 350°C and 500°C in the presence of nitrogen (nitrogen flow 4.5 L per minute).

Figure 9: Pyrolytic furnace Carbolite 301 (Carbolite Gero, Great Britain). **Source: Kateřina Vejvodová**

4.5. Analytical Methods

4.5.1. Determination of pH and CEC

For soil characterisation the soil pH was determined using $CaCl_2$. The soil $pH_{(CaCl2)}$ was measured with 0.01 M $CaCl_2$ solution. The pH was obtained using a 1:5 ratio of soil to solution. Thus, 5g of soil was weighed and 25ml of 0.01 M $CaCl_2$ was added and left to shake for one hour and then stand for one hour. The pH of the samples was measured using a pH meter (pH 315i/SET, WTW Wissenschaftlich- Technische Werkstätten, Germany).

4.5.2. Determination of pseudo-total element contents in soils and total element contents in plants

The pseudo-total concentration of risk elements was determined by the digestion of 0.5g of air-dried soil samples that was decomposed in digestion vessels with 10ml of aqua regia, a mixture of 65% nitric and 30% hydrochloric acid in the ratio of 1:3. The mixture was heated in an Ethos 1 (MLS GmbH, Germany) (figure 10) microwave-assisted wet digestion system for 35 minutes at 210°C (Fröhlichová et al., 2018).

Similarly, for the determination of total element concentrations in plant biomass (root, shoot and grain), 0.5g of plant biomass was weighed into digestion vessels. 8ml of 65% nitric acid and 2ml of 30% hydrogen peroxide were added into the digestion vessels. The mixture was heated in an Ethos 1 (MLS GmbH, Germany) microwave-assisted wet digestion system for 30 minutes at 220°C (Fröhlichová et al., 2018).

Figure 10: Ethos 1 (MLS GmbH, Germany) microwave-assisted wet digestion system. Source: Kateřina Vejvodová

After digestion of both soil and plant samples, the samples were left to cool. After cooling, the digests were transferred into 20ml glass tubes and filled to the mark using deionized water. Each sample was done in triplicates.

The total concentrations of elements in the DOR formed biochar were also determined following the same procedure as the digestion for plant biomass.

4.5.3. Determination of bioaccessible elements

The bioaccessible fraction of elements was determined by using acetic acid. A soil sample of 1g was weighed and 20ml of 0.11 M acetic acid was added to it. The samples were left to shake overnight and were then centrifuged for 10 minutes and the supernatant was transferred into clean test tubes (Quevauviller et al., 1993).

4.5.4. Measurements

Inductively coupled plasma-atomic emission spectrometry (ICP-OES, Agilent 720, Agilent Technologies Inc., USA), equipped with a two-channel peristaltic pump, a Struman-Masters spray chamber, and a V-groove pneumatic nebulizer made of inert material (figure 12), was used to determine As, Cd, Pb, Zn and P concentrations of the soil and plant digests as well as soil extracts. Flame atomic absorption spectrometry using a Varian 280FS (F-AAS, Varian, Australia) was used to determine the Ca, Mg and K contents of the solutions (figure 11). The low concentrations of As, Cd and Pb in the plant digests were measured by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x, Agilent Technologies Inc., USA) using a collision cell to reduce potential interferences.

Figure 11: Flame atomic absorption spectrometry Varian 280 FS (F-AAS, Varian, Australia).
Source Kateřina Vejvodová

Figure 12: Inductively coupled plasma-atomic emission spectrometry (ICP-OES Agilent 720, Agilent Technologies Inc., USA). **Source Kateřina Vejvodová**

4.5.5. Analyses of results

All statistical analyses were performed using Statistica 12.0 software (www.StatSoft.com). A one-way analysis of variance (ANOVA) at significance level of α=0.05 followed by the Tukey's test were applied to assess the effect of the individual treatments. The interactions of the treatment and other variables (e.g. biochar rate, pyrolysis temperature, and part of the plant analysed) were analysed by factorial analysis of variance, ANOVA. Correlation analysis was used for the assessment of relationships between variables, where Pearson's correlation was used with α=0.05 as the criterion for significance.

5. Results

The results that are labelled ‘Hluboš’, ‘Halda’ and ‘Litavka’ respectively refer to the results from low, medium and high contaminated soils.

5.1. Element concentrations in DOR and biochar

From table 4 it can be seen that As, Cd and Pb were below the detection limits (DL), whereas Zn concentrations can be seen to increase in concentration when transformed to biochar and with pyrolysis at higher temperatures. As for the essential elements, it can be seen to follow the same pattern as for Zn. Ca, K, P and Mg concentrations indicate the effect of pyrolysis temperature on the nutrient status of DOR-based biochar. However, the results that stand out are of K concentrations, proving DOR and biochar (containing double/triple the concentrations) to be the great source of K. The percentage of C increased after the pyrolysis of biochar, however, the temperature of pyrolysis did not affect its concentration. Percentage of N were found to increase when pyrolyzed at 350°C and were higher than when pyrolyzed at 500°C, while the percentage of H decreased with pyrolysis.

Table 4: Element contents in DOR before pyrolysis and in the biochars.

	Unit	DOR	Biochar 350°C	Biochar 500°C
As	mg/kg	<0.3	<0.3	<0.3
Ca	mg/kg	4540	7460	12700
Cd	mg/kg	<0.03	<0.03	<0.03
Co	mg/kg	<0.05	0.37	0.63
Cr	mg/kg	2.28	4.13	6.96
Cu	mg/kg	16	25.9	39.1
Fe	mg/kg	672	1010	1940
K	mg/kg	196000	380000	573000
Mg	mg/kg	134	2110	3530
Mn	mg/kg	16.9	27.1	44.4
P	mg/kg	1370	2450	3910
Pb	mg/kg	<0.2	<0.2	0.92
S	mg/kg	1250	937	1150
Zn	mg/kg	39.8	55.5	103
C	%	47.3	63.6	66.7
H	%	6.55	5.83	3.88
N	%	1.60	1.98	1.68
C/N ratio		29.6	32.2	39.8
C/H ratio		7.22	10.9	17.2

< data under detection limit

5.2. Soil characterisation results

Table 5: pH and CEC values.

	pH_{CaCl2}	CEC (mmol/kg)
Hluboš	5.88	102
Halda	5.84	136
Litavka	5.73	77.8

Table 6: The pseudototal element contents in soil extractable with Aqua regia (mg/kg) at the start of the pot experiment; data are presented as mean ± standard deviation; n=3.

	Hluboš	**Halda**	**Litavka**
As	21.3±0.68	34±1.16	344±20
Ca	3930±47.4	3430±211	2880±52
Cd	1.29±0.11	2.48±0.18	23.8±2.71
Cu	15±0.6	29.2±1.14	58.7±6.1
Fe	23900±939	27400±325	31500±724
K	9230±54.7	6010±61.3	5180±622
Mg	4350±36	4170±181	4330±34
Mn	1080±98	1130±211	3660±296
P	993±38	572±19	617±54
Pb	332±21.4	643±21	2110±299
Zn	145±1.65	372±44	3340±112

Table 7: The element proportions extractable with 0.11M CH3COOH (mg/kg) at the start of the pot experiment; data are presented as mean ± standard deviation; n=3.

	Hluboš	**Halda**	**Litavka**
As	0.71±0.01	0.60±0.00	1.59±0.03
Ca	1510±32.5	1690±22.6	1580±75.3
Cd	0.40±0.00	0.77±0.08	13.2±0.27
Cu	0.10±0.01	0.37±0.03	2.52±0.03
Fe	2.90±0.48	3.14±0.88	4.81±0.64
K	320±13.1	176±3.36	165±0.29
Mg	111±2.22	176±5.94	179±0.00
Mn	64.9±0.84	69.6±5.43	152±6.28
P	111±5.39	15.9±2.91	5.90±0.17
Pb	2.63±0.11	6.83±0.38	39.6±1.41
Zn	16.1±0.05	37.8±5.94	1530±4.99

The three soils, Hluboš (low), Halda (medium) and Litavka (high), used for the experiment were all slightly acidic, with the pH ranging from 5.7-5.9. The CEC was found to be the highest for Halda followed by Hluboš and then by Litavka and can be seen in table 5.

The pseudototal concentrations can be seen in table 6; for all risk elements, it increases with the level of soil contamination (low, medium and high) and essential elements can be noted to decrease with level of soil contamination. Litavka soil has ten times higher concentration of As, Cd and Zn than Halda soil. Halda soil had double the concentration of Cd, Pb and Zn compared to Hluboš. Pb concentrations in Litavka soil is triple the concentration than of Halda. Pseudototal concentration for nutrients such as Ca, K and P can be seen to decrease with increasing level of contaminated soil. The differences in Mg concentrations are relatively insignificant, with concentrations of Mg in Hluboš and Litavka being approximately the same, and Halda containing a little under 200 mg/kg less.

The bioaccessible fractions of the risk elements shown in table 7 can be observed to increase with the level of soil contamination and vice versa with nutrients; with Litavka having the highest concentration of bioaccessible As, Cd, Pb and Zn, and lower concentrations of K and P. However, Halda had slightly lower concentration of As than Hluboš. Bioaccessible Pb concentrations significantly increase with level of soil contamination 39.6>6.83>2.63 mg/kg (Litavka>Halda>Hluboš). Litavka contained significantly high concentrations of bioaccessible Zn at 1530 mg/kg compared to Halda and Hluboš, which had 37.8 mg/kg and 16.1 mg.kg, respectively.

5.3. Experimental results

The results presented below, depict the changes in concentrations of bioaccessible risk elements and essential elements in the Hluboš, Halda and Litavka contaminated soil samples as well as in the grains, shoots and roots of *Triticum aestivum* after 90 days of being in contact with DOR biochar. Results are presented as means with standard error (SE) where n=4, and statistics were α=0.05. In certain cases, the absence of results of standard error bars can be noticed due to results being lower than the detection limit.

5.3.1. Bioaccessible risk element and essential element concentrations in soils

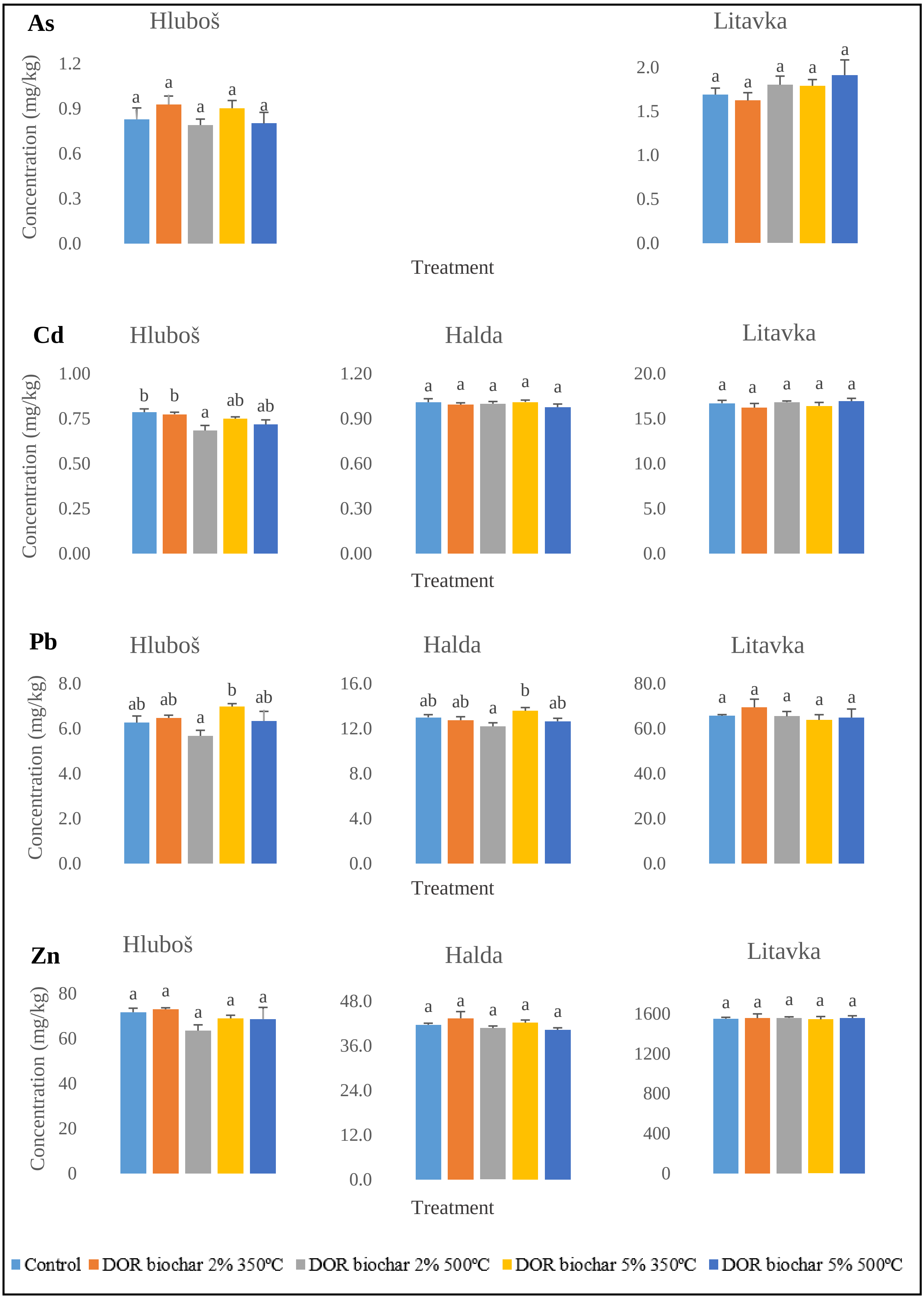

Figure 13: Concentration of bioaccessible risk elements in the soils after 90 days: n=4, α=0.05. Absence of As concentration for Halda soil due to results below DL.

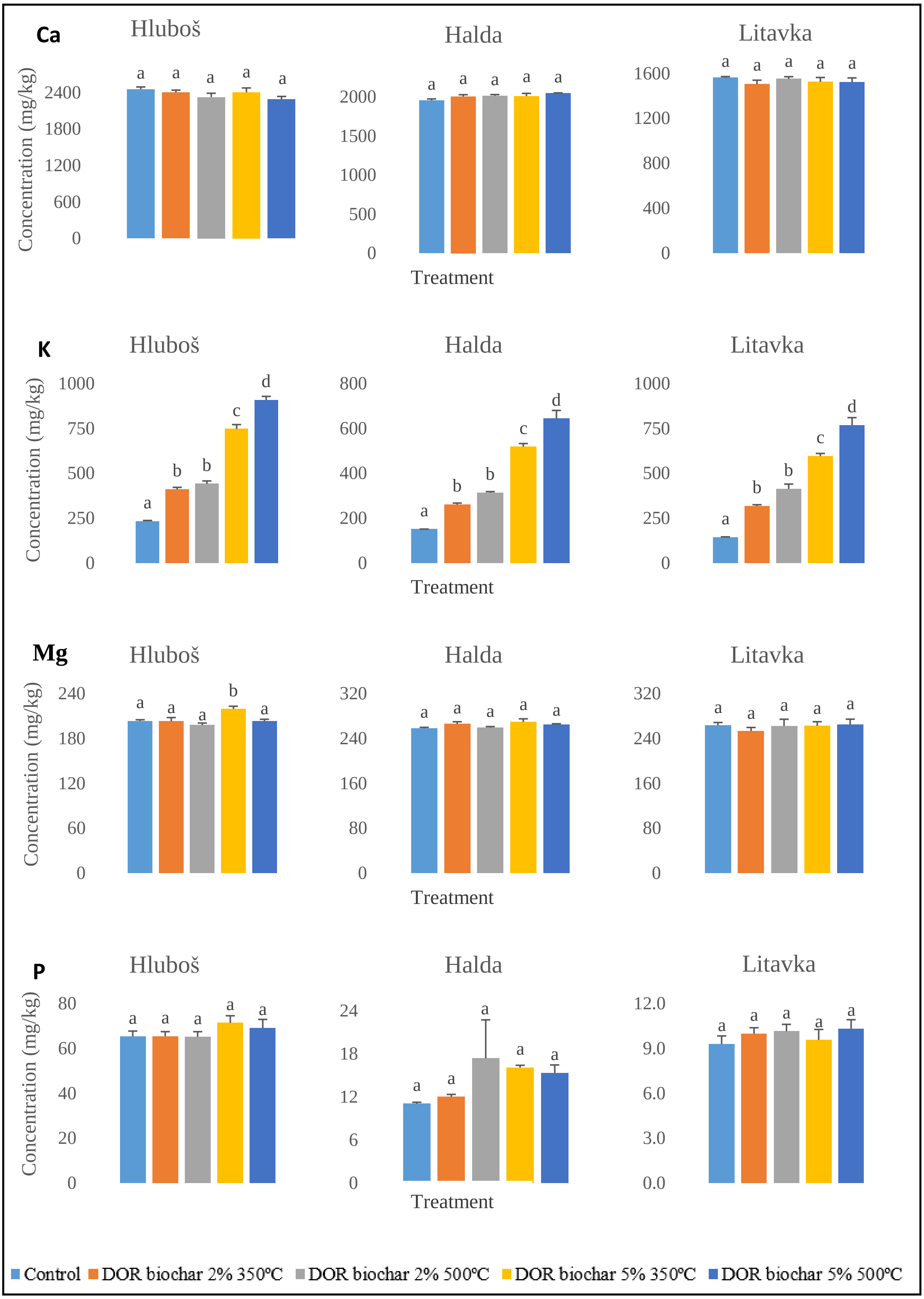

Figure 14: Concentration of bioaccessible essential elements in the soils after 90 days: n=4, α=0.05.

5.3.2. Element concentration in Hluboš, Halda and Litavka soils

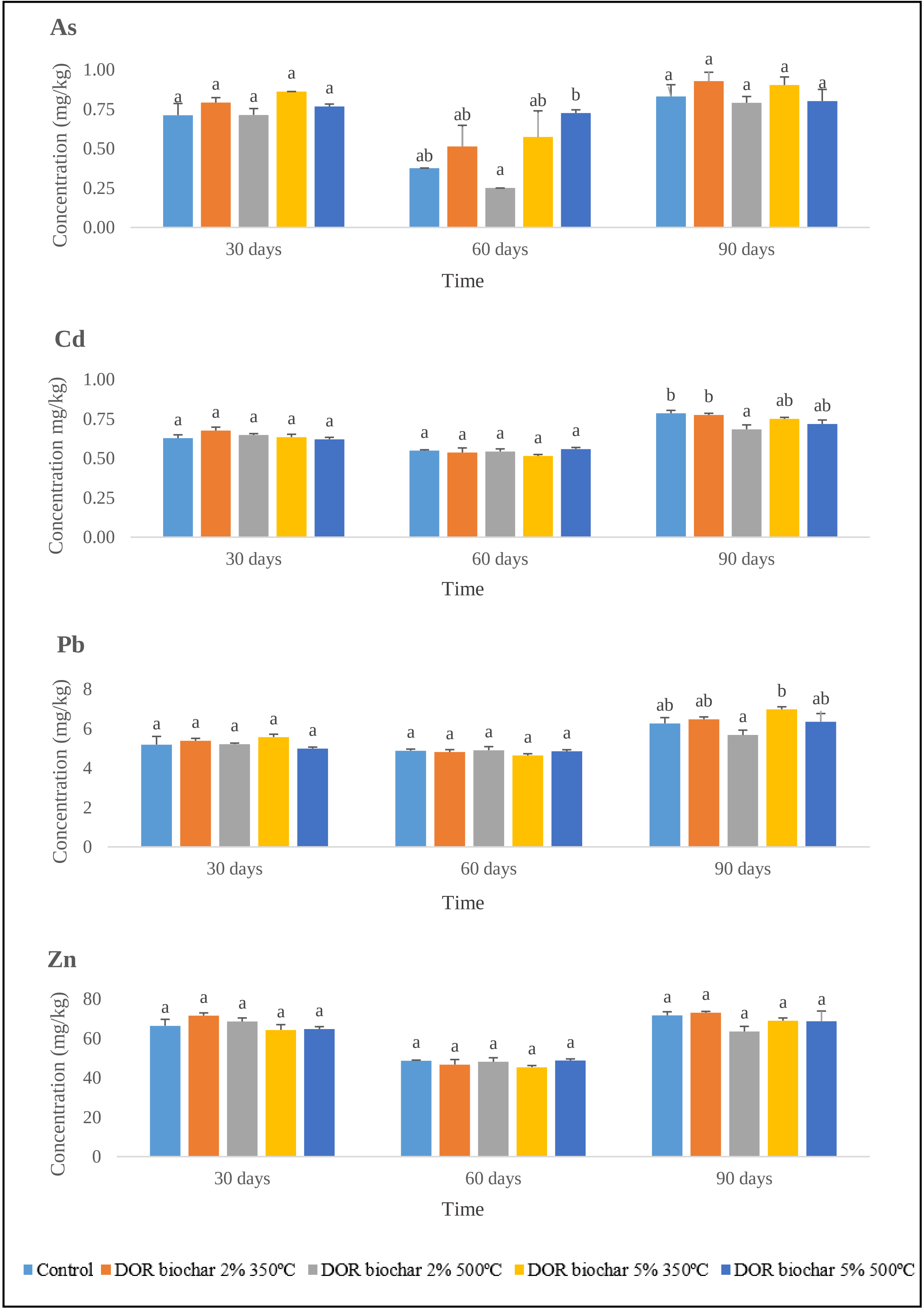

Figure 15: Concentration of risk elements in Hluboš soil: n=4, α=0.05. Note: As results SE=0 for Control, DOR biochar 5% 350˚C and 2% 500 ˚C due majority of results below DL.

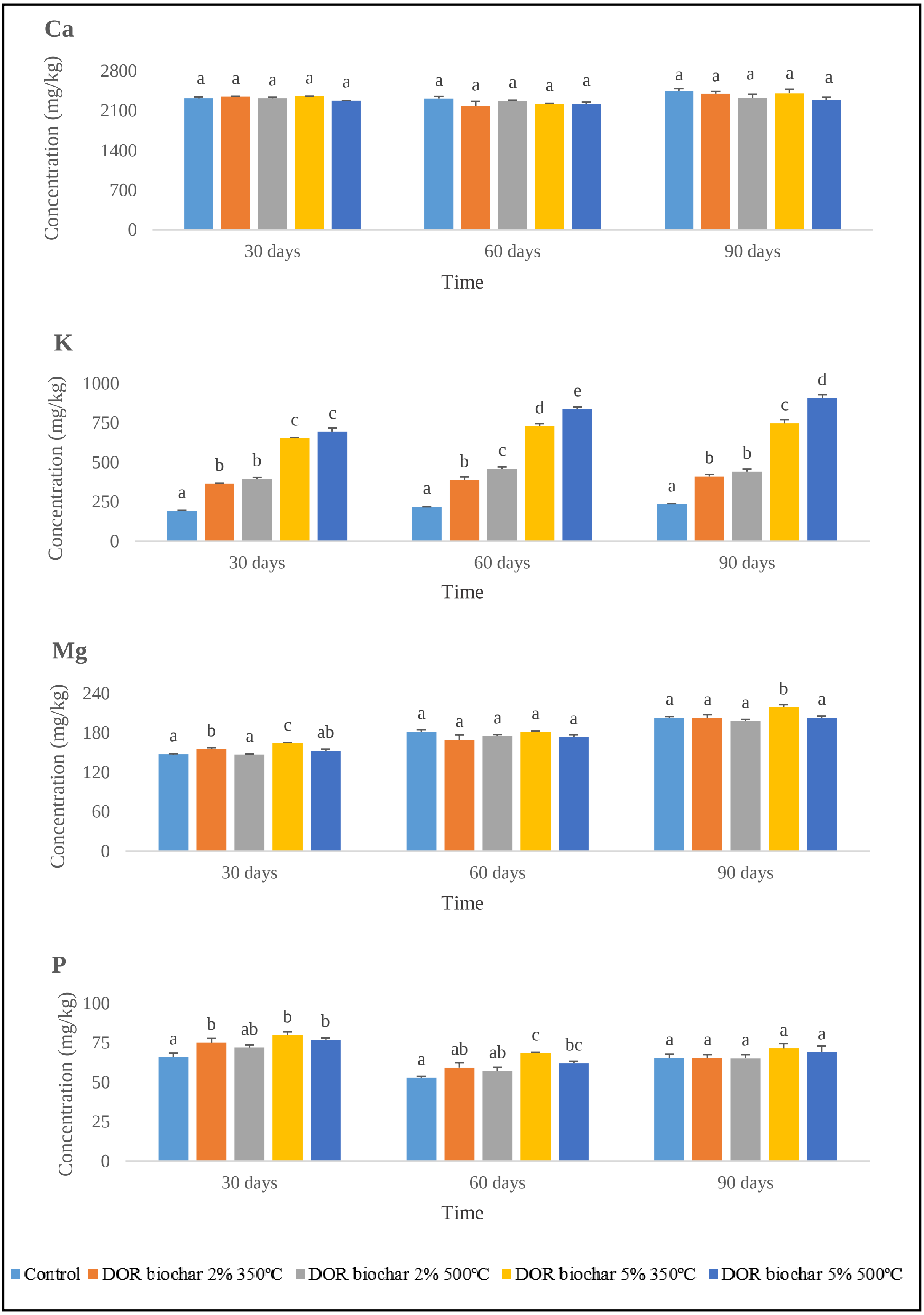

Figure 16: Concentration of essential elements in Hluboš Soil: n=4, α=0.05.

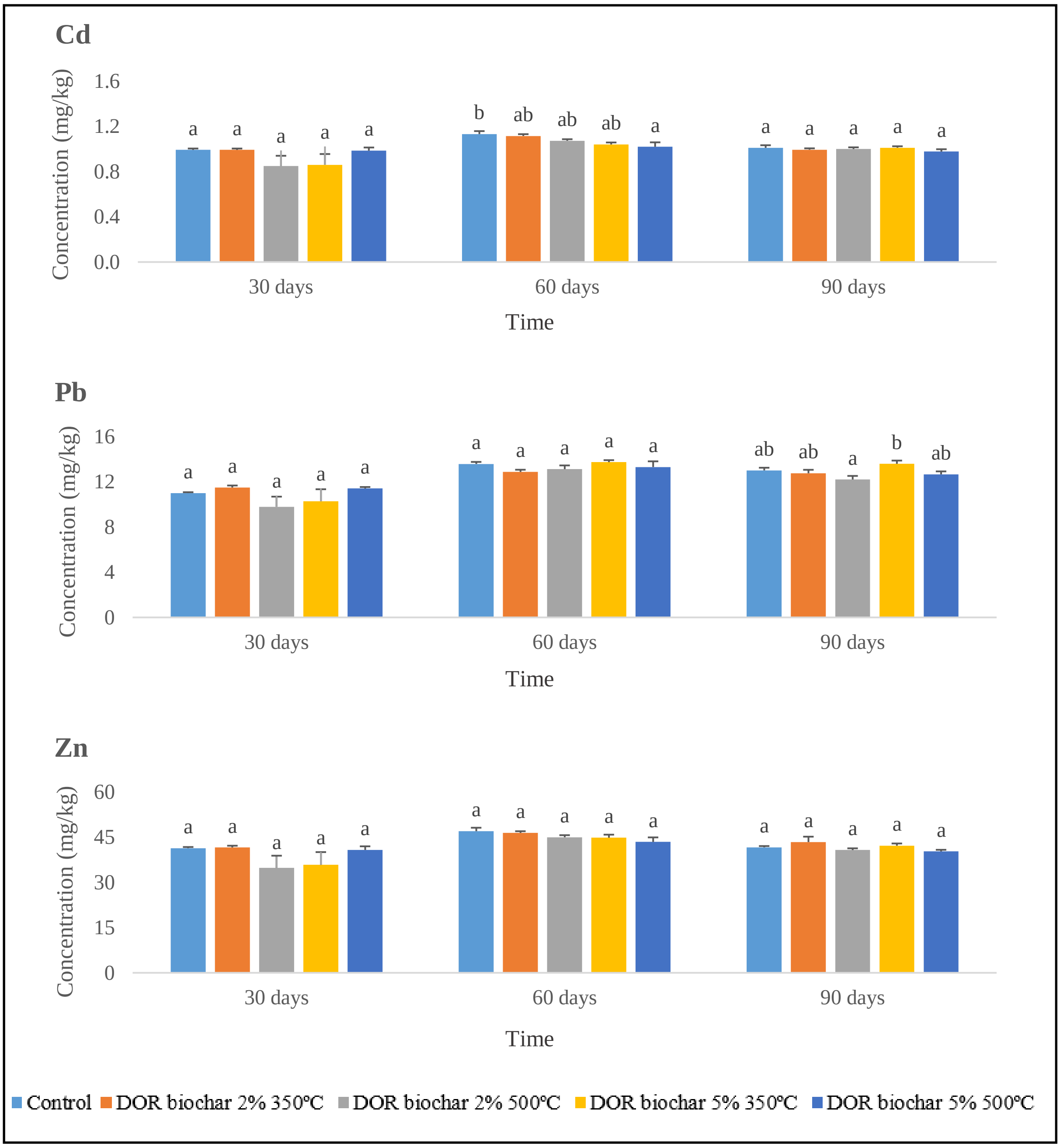

Figure 17: Concentration of risk elements in Halda soil: n=4, α=0.05. Note: the absence of the As concentration is due to results being below the DL.

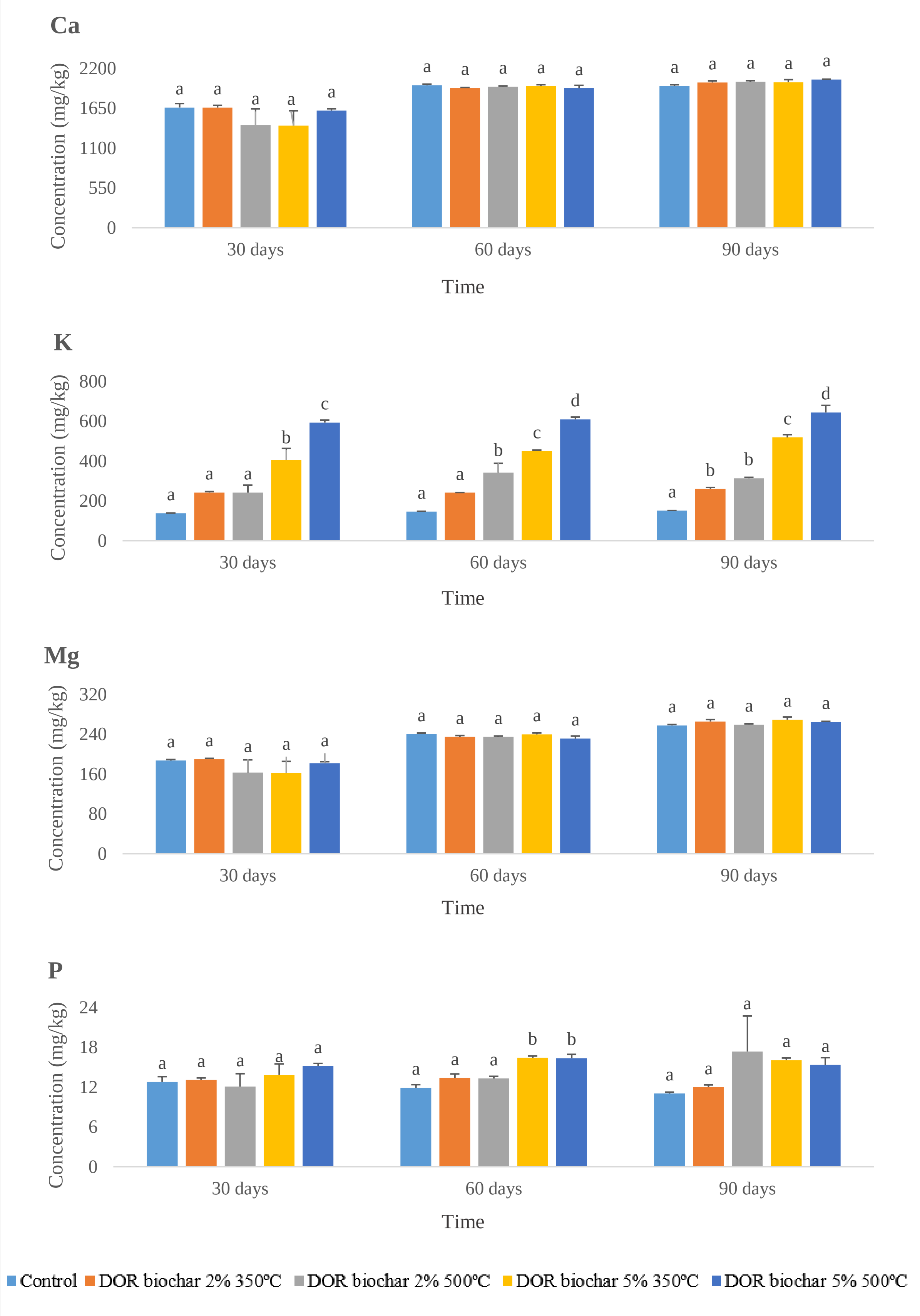

Figure 18: Concentration of essential elements in Halda soil: n=4, α=0.05.

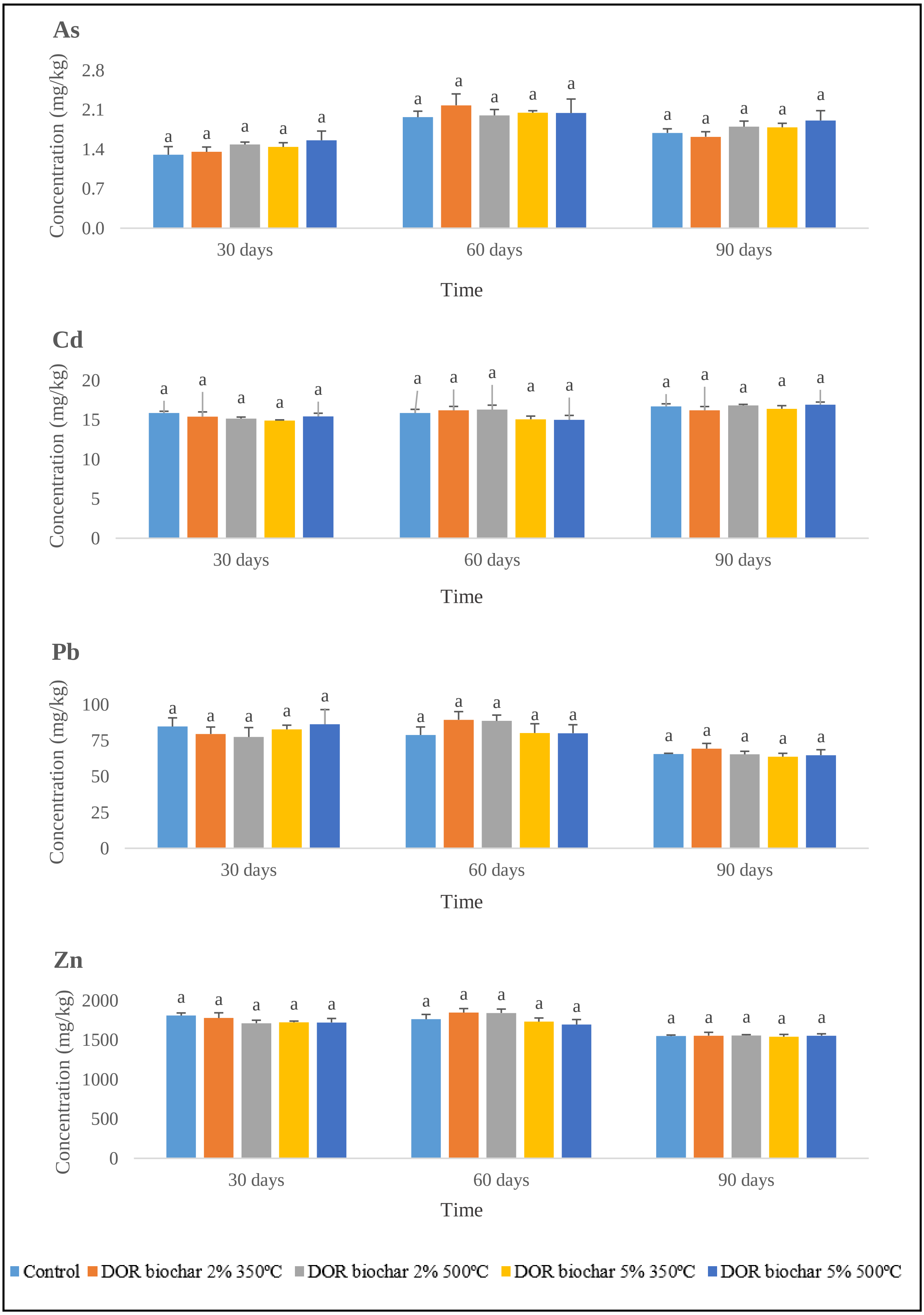

Figure 19: Concentration of risk elements in Litavka soil: n=4, α=0.05.

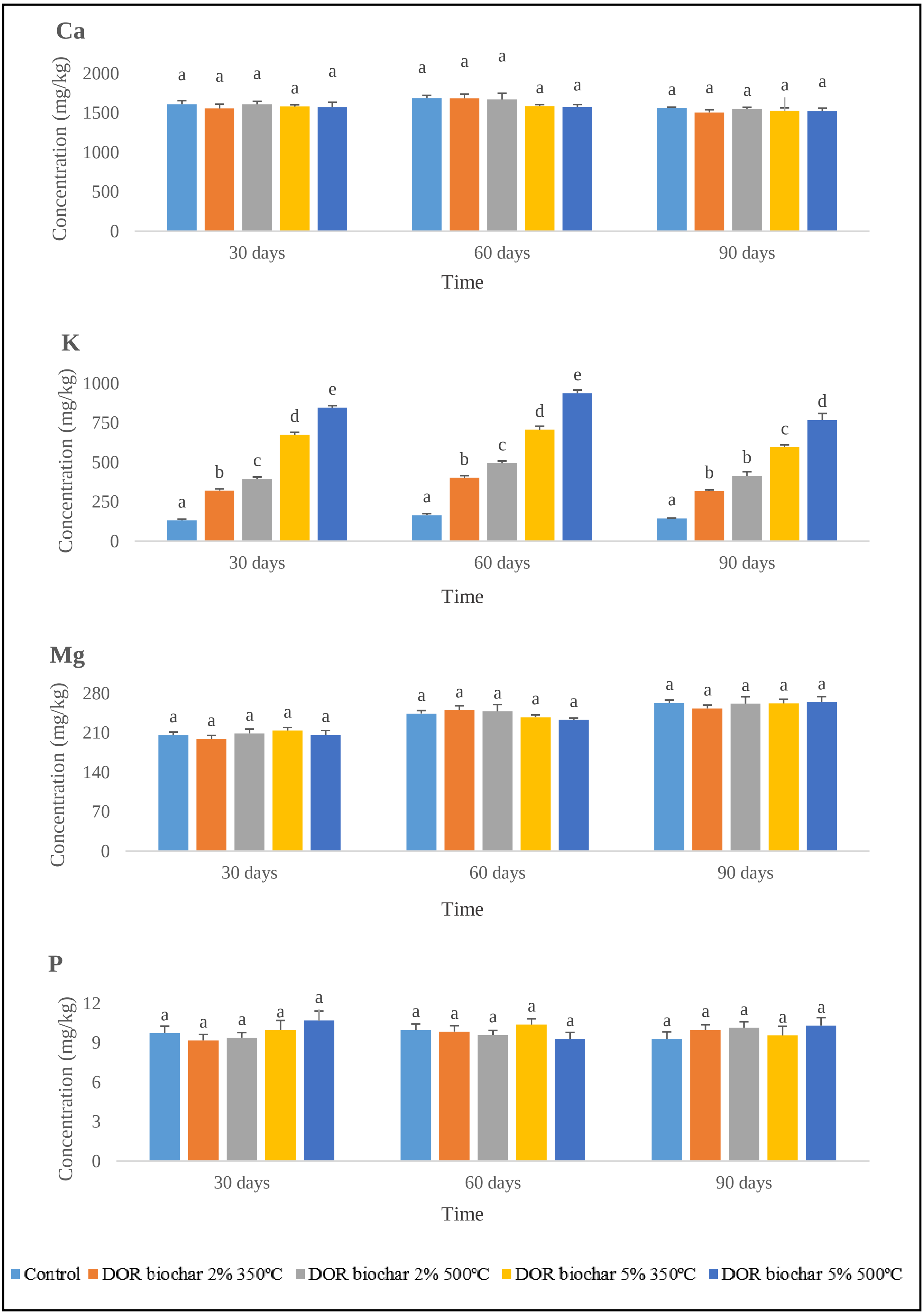

Figure 20: Concentration of essential elements in Litavka soil: n=4, α=0.05.

In figure 13 it can be observed that there are relatively insignificant changes in concentrations of the bioaccessible risk elements. The graphs in figures 15, 17 and 19 can be described to be a homogenous group with no significance between treatments. However, for Hluboš a decrease in concentration of the risk elements can be seen when amended with DOR biochar 2% 500°C. It can be observed for Cd concentrations in Hluboš that DOR biochar 2% 500°C is significant compared to the control and DOR biochar 2% 350°C. A similar significance can be seen in Zn concentrations between Hluboš and Halda, where a significance between biochar 2% 500°C and biochar 5% 350°C can be seen.

The bioaccessible essential element concentrations in the soils after 90 days can be observed in figure 14. More detailed graphs depicting concentrations of essential elements over time can be seen in figures 16, 18 and 20. Comparatively, low changes can be noticed for Ca, P and Mg with no significance in the different treatments. Exceptionally, the results for K can be seen to be more pronounced. There is a gradual increase in K concentrations increasing in said order: DOR biochar 5% 500°C > DOR biochar 5% 350°C > DOR biochar 2% 500°C > DOR biochar 2% 350°C > Control. Concentrations of K in the soils amended with DOR biochar 5% 500 ˚C can be seen to increase by 4-5 times compared to the control. This could be due to the high concentration of K that was present in the DOR

There is a low number of significant differences, indicating that the bioaccessible concentrations of risk elements and essential elements in soils were not significantly changed by using the different treatments. There is one exception to this, and that is the K concentrations, as DOR and DOR biochar present itself as a source of K for soils.

5.3.3. Risk element and essential element concentrations in *Triticum aestivum* grown on Hluboš, Halda and Litavka soils after 90 days

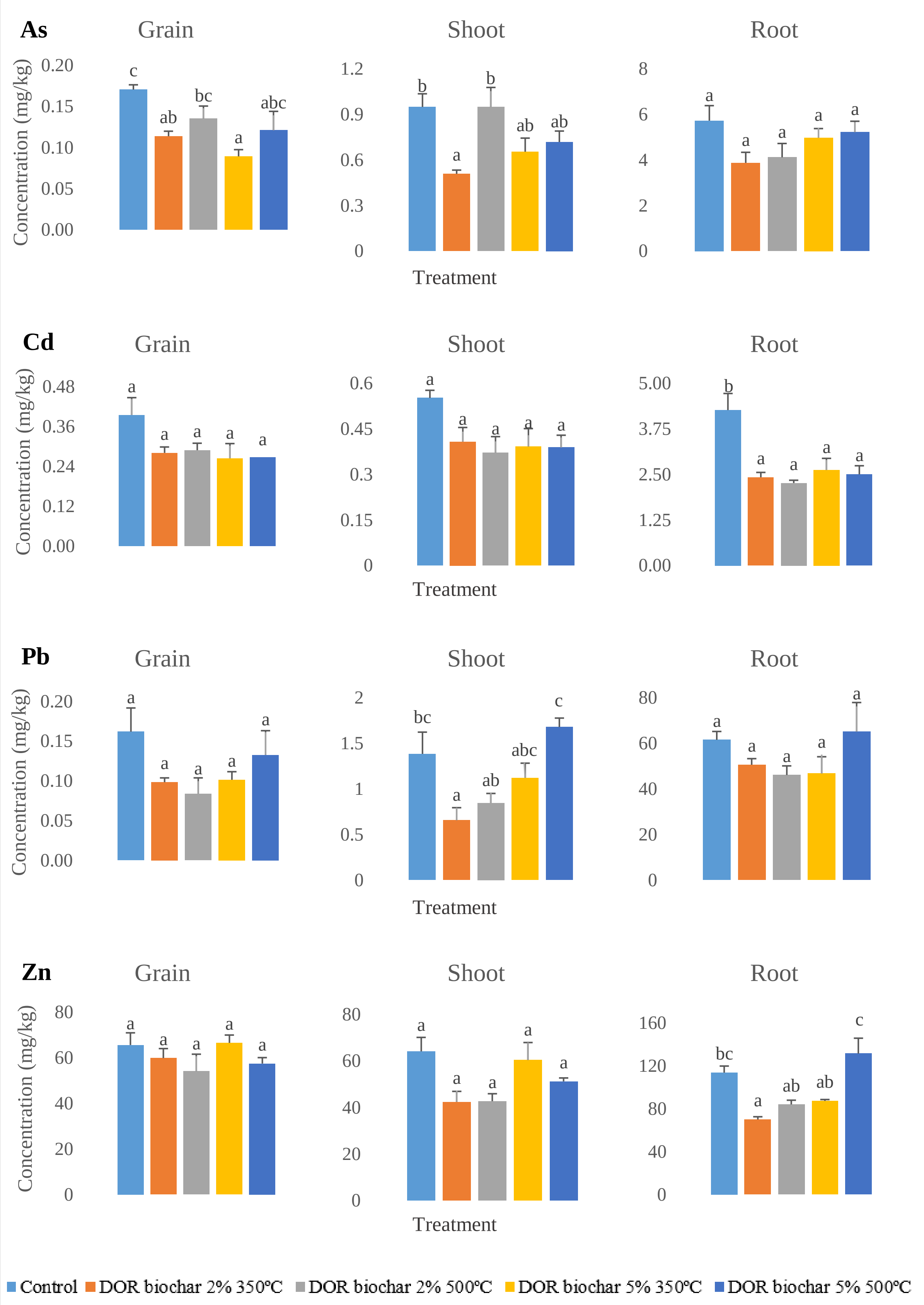

Figure 21: Concentration of risk elements in *Triticum aestivum* grown on Hluboš soil after 90 days: n=4, α=0.05.

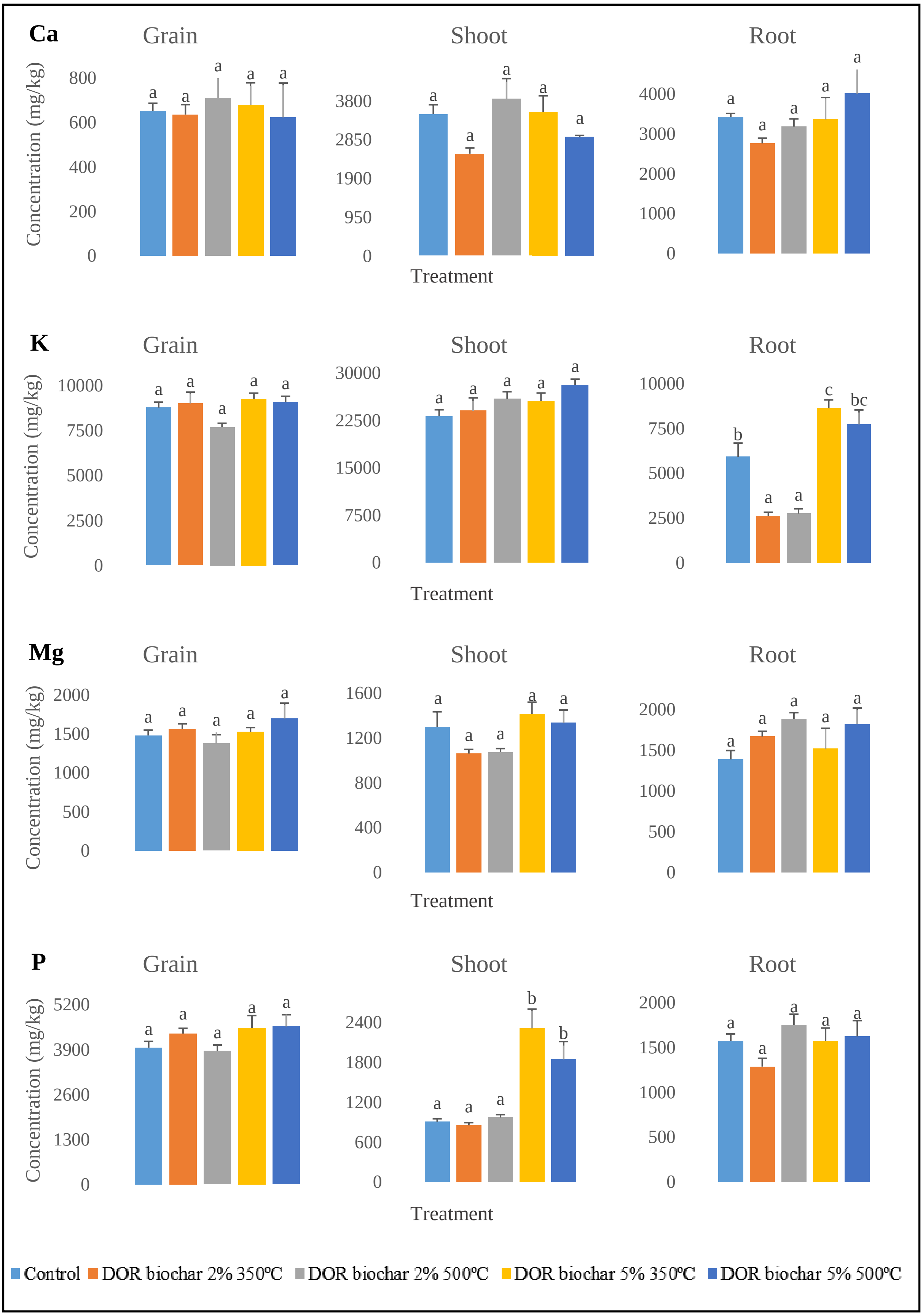

Figure 22: Concentration of essential elements in *Triticum aestivum* grown on Hluboš soil after 90 days: n=4, α=0.05.

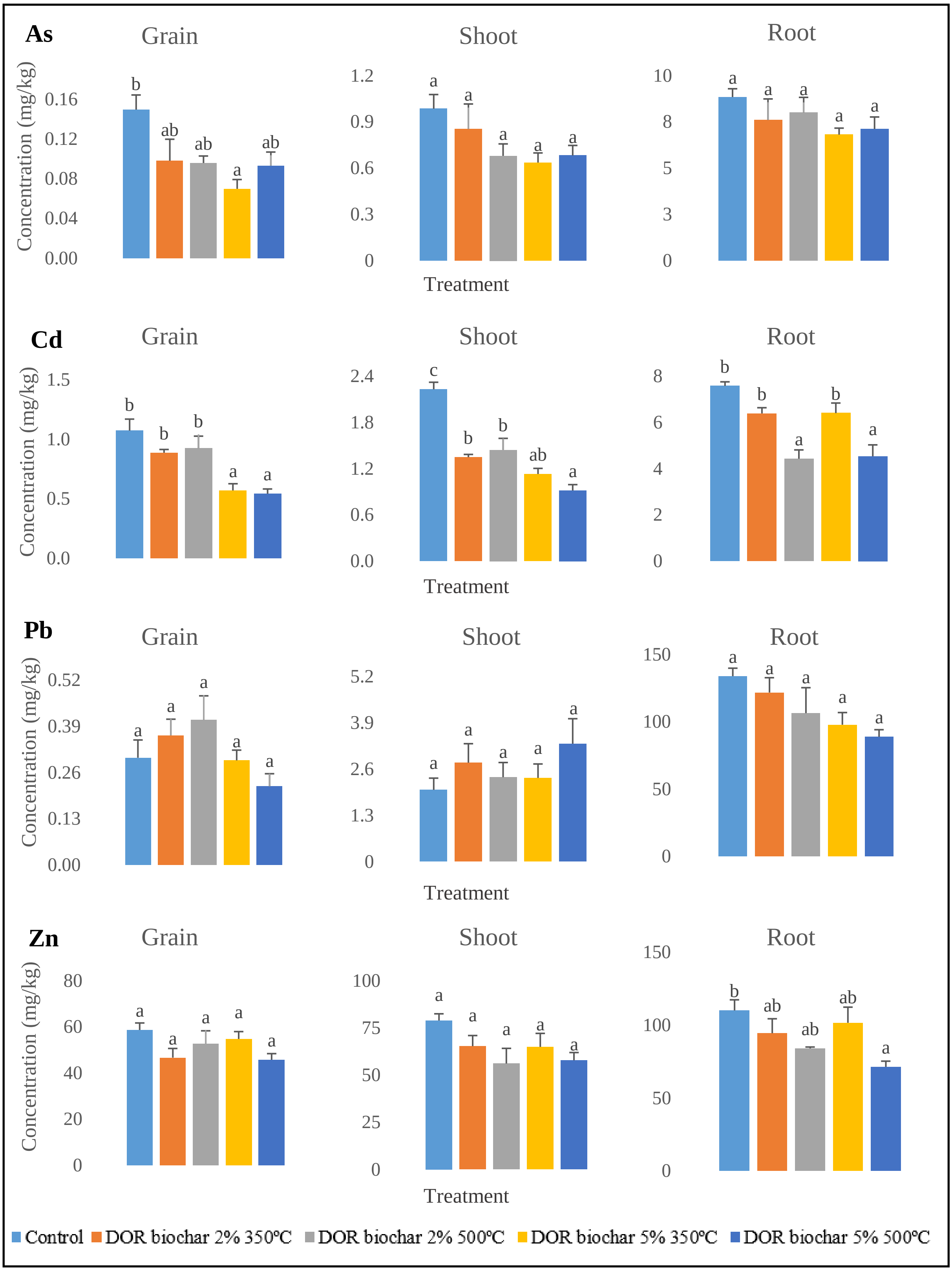

Figure 23: Concentration of risk elements in *Triticum aestivum* grown on Halda soil after 90 days: n=4, α=0.05.

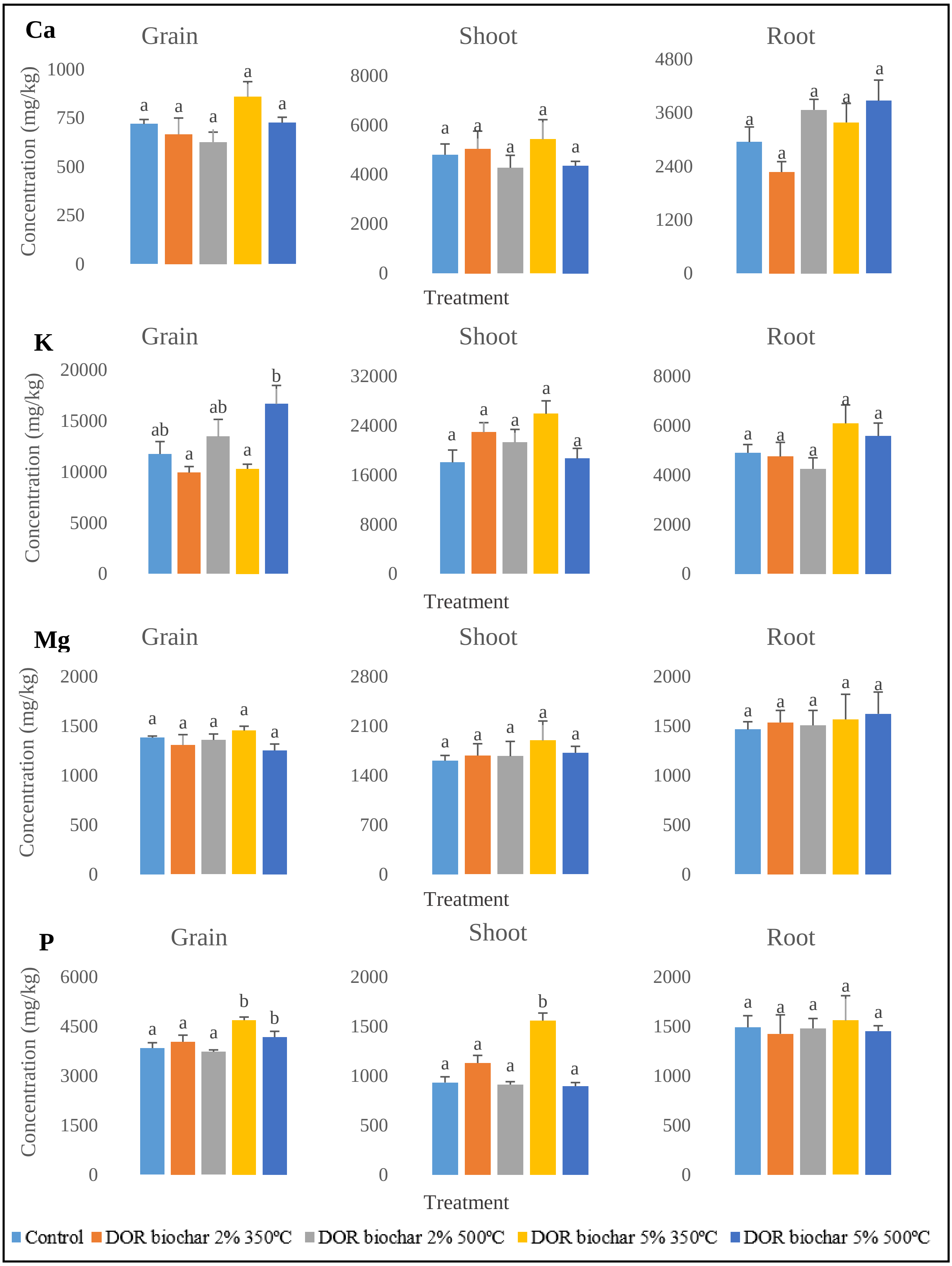

Figure 24: Concentration of essential elements in *Triticum aestivum* grown on Halda soil after 90 days:: n=4, α=0.05.

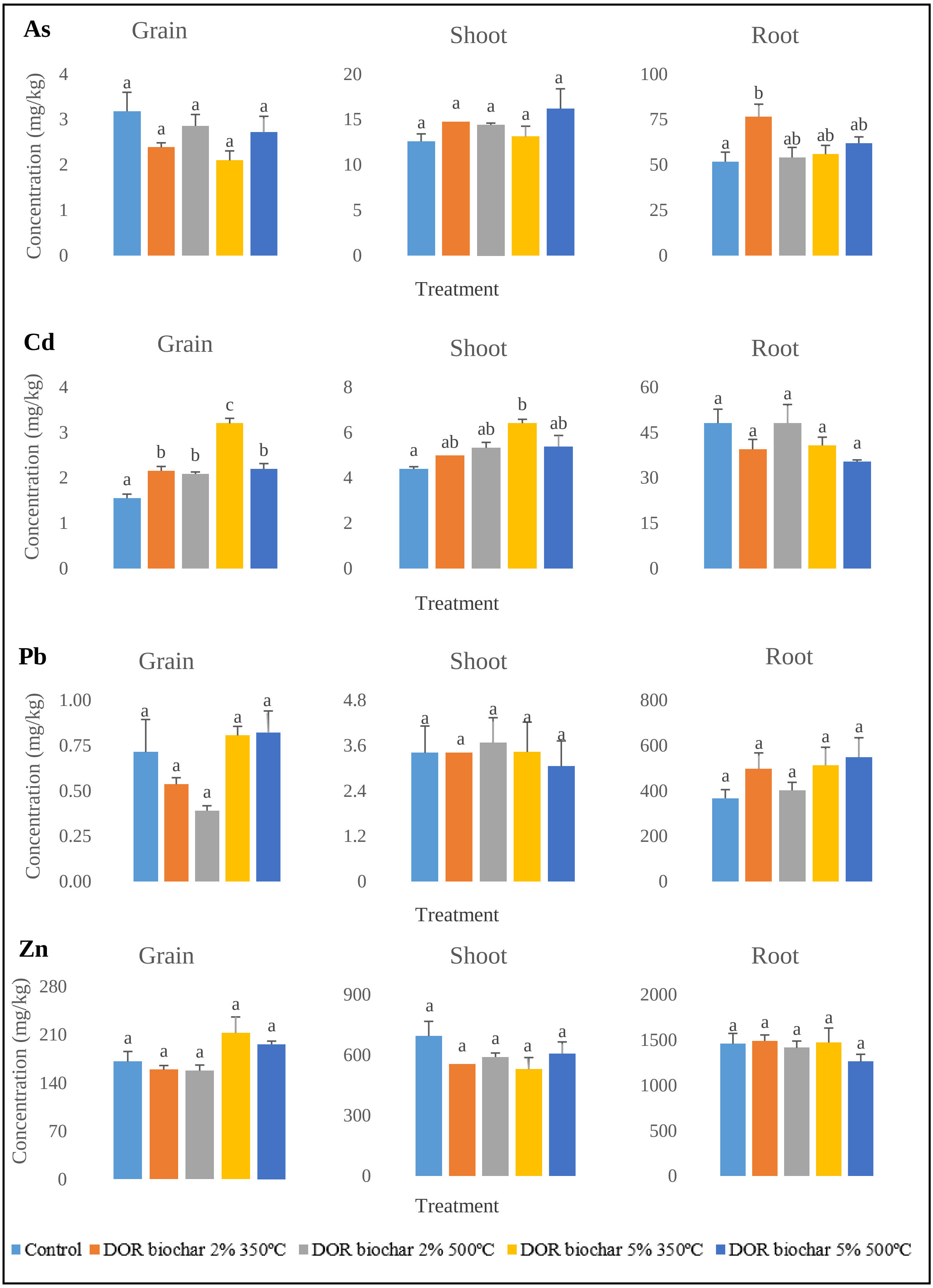

Figure 25: Concentration of risk elements in *Triticum aestivum* grown on Litavka soil after 90 days: n=4, α=0.05.

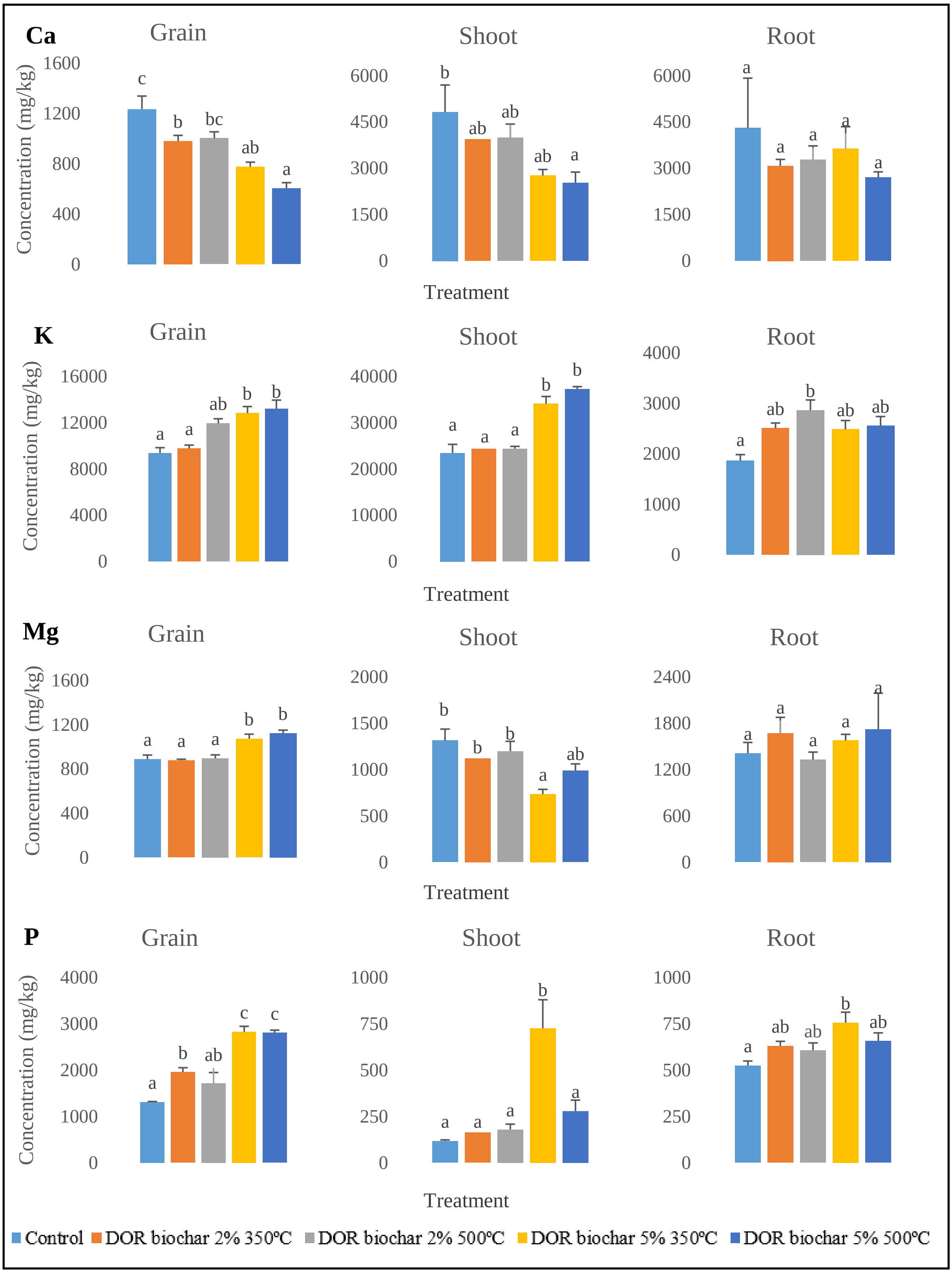

Figure 26: Concentration of essential elements in *Triticum aestivum* grown on Litavka soil after 90 days: n=4, α=0.05.

The effect of dry olive residue (DOR) biochar, specifically its application rate and pyrolysis temperature can be noticed in *Triticum aestivum* shown in figures 21-26. Biochar, however, was not as successful in decreasing risk element and increasing essential element concentrations in soil. One notable essential element that was seen to increase in soils was the bioaccessible concentration of K, due to the high concentrations of K in the DOR biochar. The pyrolysis temperature and application rate appear to affect the risk element concentrations differently in the different soils and different parts of *Triticum aestivum*. Concentrations of As appear to be highly influenced by the pyrolysis temperature of 350°C with a 5% application rate decreasing As concentrations by approximately 50% in the grain grown in Hluboš and Halda soils. In the shoot a 50% decrease can be seen with the treatment of DOR biochar 2% 350°C, with significant decreases when also treated with DOR biochar 5%. In Litavka soils, an increase of As concentrations can be noted. Cd concentrations were approximately 50% lower with all treatments in the root grown on Hluboš soil and displayed that DOR biochar 2% and 5% pyrolyzed at 500°C were statistically successful in decreasing concentrations in the root grown on Halda soil. In Hluboš and Halda soils, the concentrations of Pb were decreasing with treatment, but are not statistically significant. The shoot grown on Hluboš soil proved 2% and 5% biochar application rate pyrolyzed at 350°C significant in decreasing Pb concentrations, also by relatively 50%. The root grown on Hluboš soil shows significant decrease of Zn concentrations by DOR biochar 5% 350°C and especially by 2% 350°C, while in Halda soil the Zn concentrations were lowered by all treatments, significantly 5% 500°C.

Concentrations of Mg and Ca in the plant parts were not statistically significant in Hluboš and Halda soil. No statistical significance can be seen for P concentrations in grain and roots in Hluboš soil and roots in Litavka soil. P concentrations in shoot in Halda soil increased with the application of biochar 5% 500°C. K concentrations in grain and shoot grown in Hluboš soil were not significant. K concentrations was significantly decreased with biochar 2% application and increased with biochar 5% application. P concentrations in the shoot can be seen to increase with the application of biochar 5%. In Halda soil K concentrations increased with biochar 2% and 5% pyrolyzed at 500°C in the grain and decreases with biochar 2% and 5% pyrolyzed at 350°C. The treatments managed to decrease concentrations of Ca in grain, shoot and root of *Triticum aestivum.*

Additional results can be found in appendix 1, where concentrations of risk elements and essential elements can be observed over 30, 60 and 90 days. In Hluboš soil, the concentration of As decreases in grain with treatment, significantly with biochar 5% 350°C and 500°C after 60 days and can be seen in supplement figure 1. In the shoot grown on Hluboš soil, As concentrations were significantly decreased by biochar 2% 500°C, 5% 350°C and 5% 500°C after 60 days. Supplement figure 2 shows the decrease of Cd in grain grown on Halda soil, with high significance of biochar 2% 500°C, 5% 350°C and 5% 500°C. In supplement figures 3, 6 and 9, the concentration of As decreases with the application of biochar, significantly the biochar applied at 5% pyrolyzed at 350°C and 500°C. In supplement figure 6, changes in Pb and Zn concentrations in shoots can also be noted, with significant decreases after 60 days. While Zn concentrations were also decreased in roots after 30 and 60 days.

Supplement figures 12, 13, 14 and 15 all present results that show increased P concentrations with high significance of the biochar treatment. Furthermore, figure 12 and 15 depict decreased concentration of Ca when in contact with the biochar treatment.

The results confirm a homogeneous group with no significance of treatment. The application rate of the biochar and the pyrolysis temperature showed to have relatively no influence in the immobilisation of risk elements or the release of nutrients into the soil. This is quite interesting due the increasing concentrations in Ca, P and Mg when in DOR when pyrolyzed, which cannot be noticed in the soils.

5.3.4. Results of 3-way ANOVA

Table 8: Summary of the effects of soil, biochar temperature and biochar rate on the mobile contents of risk elements in soil, and soil pH after 30 days.

	Soil	Biochar temp.	Biochar rate	Soil* Biochar temp.	Soil* Biochar rate	Biochar temp.* Biochar rate	Soil* Biochar temp.* Biochar rate
As	215***	ns	ns	ns	ns	ns	ns
Cd	11508***	ns	ns	ns	ns	ns	ns
Pb	789***	ns	ns	ns	ns	ns	ns
Zn	9660***	ns	ns	ns	ns	ns	ns
pH	25.6***	43.7***	20.9***	2.74*	ns	6.42**	ns

Values shown represent F-value of 3-way ANOVA; ns not significant; * p<0.05; ** p<0.01; *** p<0.001

Table 9: Summary of the effects of soil, biochar temperature and biochar rate on the mobile contents of risk elements in soil, and soil pH after 60 days.

	Soil	Biochar temp.	Biochar rate	Soil* Biochar temp.	Soil* Biochar rate	Biochar temp.* Biochar rate	Soil* Biochar temp.* Biochar rate
As	413***	ns	ns	ns	ns	ns	ns
Cd	5518***	ns	4.49*	ns	3.82*	ns	ns
Pb	1050***	ns	ns	ns	ns	ns	ns
Zn	5689***	ns	ns	ns	3.60*	ns	ns
pH	40.0***	54.2***	36.4***	12.3***	5.75**	16.2***	3.62*

Values shown represent F-value of 3-way ANOVA; ns not significant; * p<0.05; ** p<0.01; *** p<0.001

Table 10: Summary of the effects of soil, biochar temperature and biochar rate on the mobile contents of risk elements in soil, and soil pH after 90 days.

	Soil	Biochar temp.	Biochar rate	Soil* Biochar temp.	Soil* Biochar rate	Biochar temp.* Biochar rate	Soil* Biochar temp.* Biochar rate
As	593***	ns	ns	ns	ns	ns	ns
Cd	12827***	ns	ns	ns	ns	ns	ns
Pb	2963***	ns	ns	ns	ns	ns	ns
Zn	21624***	ns	ns	ns	ns	ns	ns
pH	877***	124***	59.3***	10.4***	ns	14.9***	ns

Values shown represent F-value of 3-way ANOVA; ns not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$

The results of the 3- way ANOVA shown in table 8, 9 and 10 indicate that the soil and pH were the main factors of influence in the concentrations of the risk elements in the soil, with high significance. The biochar application rate and the temperature of pyrolysis seems to not be significant in the concentrations of risk elements in the soil.

5.3.5. Results of 4-way ANOVA

Table 11: Summary of the effects of soil, biochar temperature, biochar rate, and part of the plant on the total contents of elements in plants after 30 days.

	As	Cd	Pb	Zn	Ca	K	Mg	P
S	1047***	2697***	347***	1349***	19.8***	132***	3.65*	148***
BT	11.8***	4.88**	n.s.	19.2***	4.18*	15.5***	7.07**	40.9***
BR	6.33*	n.s.	n.s.	4.62*	n.s.	15.5***	n.s.	15.4***
PP	844***	2609***	1407***	979***	n.s.	1341***	64.9***	382***
S*BT	9.66***	6.42***	n.s.	18.1***	n.s.	n.s.	2.49*	3.54**
S*BR	4.47*	n.s.	5.34**	n.s.	n.s.	4.44*	n.s.	n.s.
BT* BR	5.08**	n.s.	n.s.	n.s.	n.s.	3.87*	n.s.	5.22**
Soil*PP	543***	2078***	327***	721***	12.0***	3.60*	n.s.	25.5***
BT* PP	9.22***	4.56*	n.s.	9.44***	5.21**	3.67*	7.63***	27.9***
BR* PP	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	4.51*
S*BT*BR	6.91***	n.s.	4.35**	n.s.	n.s.	n.s.	n.s.	n.s.
S*BT*PP	7.40***	5.50***	n.s.	9.56***	5.32***	n.s.	3.71**	n.s.
S*BR*PP	n.s.	n.s.	5.15**	n.s.	n.s.	n.s.	n.s.	n.s.
BT*BR*PP	3.60*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
S*BT*BR*PP	5.29***	n.s.	4.29**	n.s.	n.s.	n.s.	n.s.	n.s.

S=Soil, BT=Biochar temp, BR=Biochar rate, PP=Plant part

Values shown represent F-value of 4-way ANOVA; ns not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$

Table 12: Summary of the effects of soil, biochar temperature, biochar rate, and part of the plant on the total contents of elements in plants after 60 days.

	As	Cd	Pb	Zn	Ca	K	Mg	P
S	2486***	1034***	532***	1388***	n.s.	n.s.	4.47*	157***
BT	7.99***	8.76***	4.79**	12.9***	3.47*	23.6***	10.4***	20.8***
BR	28.1***	4.63*	n.s.	n.s.	7.72**	8.81**	8.74**	12.4***
PP	2123***	1362***	1984***	961***	464***	616***	199***	229***
S*BT	4.24**	9.99***	8.51***	9.48***	10.15***	5.15***	n.s.	2.57*
S*BR	23.6***	9.12***	n.s.	n.s.	7.24***	n.s.	3.59*	n.s.
BT* BR	9.99***	8.79***	n.s.	n.s.	3.34*	n.s.	9.04***	4.18*
Soil*PP	1235***	894***	531***	729***	3.29*	21.1***	21.9***	10.4***
BT* PP	4.43**	7.81***	5.29***	7.45***	13.1***	4.61**	2.84*	5.20***
BR* PP	5.61**	4.55*	n.s.	n.s.	n.s.	n.s.	9.12***	n.s.
S*BT*BR	7.19***	8.16***	n.s.	n.s.	n.s.	n.s.	2.70*	n.s.
S*BT*PP	2.38*	9.28***	9.25***	4.98***	4.37***	5.76***	3.97***	2.32*
S*BR*PP	4.74**	7.97***	3.16*	n.s.	n.s.	n.s.	2.87*	n.s.
BT*BR*PP	3.36*	8.87***	n.s.	n.s.	n.s.	n.s.	9.45***	n.s.
S*BT*BR*PP	n.s.	8.06***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

S=Soil, BT=Biochar temp, BR=Biochar rate, PP=Plant part

Values shown represent F-value of 4-way ANOVA; ns not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$

Table 13: Summary of the effects of soil, biochar temperature, biochar rate, and part of the plant on the total contents of elements in plants after 90 days.

	As	Cd	Pb	Zn	Ca	K	Mg	P
S	807***	617***	184***	1477***	4.23*	3.96*	28.2***	573***
BT	n.s.	4.19*	n.s.	n.s.	n.s.	19.2***	n.s.	39.7***
BR	n.s.	n.s.	n.s.	n.s.	n.s.	37.6***	n.s.	62.2***
PP	721***	730***	531***	521***	208***	1625***	18.9***	1840***
S*BT	4.13**	n.s.	3.21*	n.s.	3.25*	2.98*	n.s.	3.66**
S*BR	n.s.	n.s.	n.s.	n.s.	n.s.	4.63*	n.s.	n.s.
BT* BR	3.98*	n.s.	n.s.	n.s.	n.s.	9.48***	n.s.	17.8***
S*PP	367***	416***	187***	420***	6.00***	44.8***	14.0***	53.8****
BT* PP	n.s.	4.67**	n.s.	n.s.	n.s.	10.4***	n.s.	10.9***
BR* PP	n.s.	n.s.	n.s.	n.s.	n.s.	3.73*	n.s.	11.1***
S*BT*BR	4.02**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
S*BT*PP	3.79***	n.s.	3.33**	n.s.	n.s.	4.70***	n.s.	2.83**
S*BR*PP	n.s.	n.s.	n.s.	n.s.	n.s.	10.2***	n.s.	5.25***
BT*BR*PP	2.91*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	5.04***
S*BT*BR*PP	2.96**	n.s.	n.s.	n.s.	n.s.	3.84***	n.s.	n.s.

S=Soil, BT=Biochar temp, BR=Biochar rate, PP=Plant part

Values shown represent F-value of 4-way ANOVA; ns not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$

The 4-way ANOVA shown in table 11, 12 and 13 proves that biochar has a significance in the concentrations of risk and essential elements in the plants. In table 11 and 13 it can be seen that soil influenced the concentrations of the elements, however in table 12, soil was not a factor in the concentrations of Ca and K. Plant part and biochar temperature*plant part were factors in concentrations of elements and was of high significance in plants harvested after 60 days, the results after 30 days can see the same pattern with an exception for Pb with regards to biochar temperature and Ca with regards to plant part. Plant part was also of high significance in concentration of all elements after 90 days. Soil*plant part was a significant factor for concentrations of elements after 30 days and 90 days. From table 12, it can be noted that majority of the factors were significant in the concentrations of elements in the plants.

5.3.6. Results of correlation analysis

Correlation analysis results can be found in appendix 2. Supplement tables 1-3 depict the correlation coefficient results of pH and mobile risk elements in soils after 30, 60 and 90 days. A negative correlation can be seen between pH and the risk elements. Correlations between the risk elements are all positive, indicating an increase in one element leads to increased concentrations of another element.

Supplement tables 4-6 show the correlation coefficient values of pH and elements in plants after 30, 60 and 90 days. The tables indicate that the risk elements, As, Cd, Pb, and Zn, along with Ca have a negative correlation with pH, the same can be seen for Mg after 30 and 60 days. Another pattern that can be observed is K and P concentrations are negatively correlated with As, Cd, Pb and Zn after 30, 60 and 90 days. In the case of P concentrations, after 60 days there was also a negative correlation with Ca, K and Mg, while after 90 days there was also a negative correlation with K. After 30 days a negative correlation can be noticed between Ca and As, Cd, Pb and Zn. The negative correlations indicate that the increase in the risk elements has led the decrease of some of the essential element concentrations in plants.

6. Discussion

Contaminated soils have dire and adverse effects on the environment and its inhabitants. With possibilities of further contamination of rivers and groundwater systems. The presence of contaminants in plants decrease crop productivity and interfere with the metabolic processes and growth of plants (FAO, 2018). Once consumed, the elements enter the animals' body where they can biomagnify or bioaccumulate (Gobas & Morrison, 2000; Mackay & Fraser, 2000). The Czech Republic experienced years of mining and smelting which influenced the levels of soil contamination throughout the country. CENIA (2014) estimated around 10,000 contaminated sites in Czech Republic, with many highest priority sites for remediation located in Central Bohemia and South Moravia. In order to prevent plant contamination, the Ministry of Environment in the Czech Republic has set preventive values for concentration of risk elements allowed in agricultural soils; the pseudototal values for As, Cd, Pb and Zn were 20 mg/kg, 0.5 mg/kg, 60 mg.kg and 120 mg/kg, respectively. In table 6 it can be observed that all three soils (low, medium and highly contaminated) had breached these preventive values and cannot be used for agricultural purposes.

The use of amendments has been used widely due to their ability to improve chemical and physical properties of soils, while using processes of sorption or precipitation to immobilise risk elements. Such amendments can include clays, Mn/Al/Fe oxides, humus and biochar (Alloway, 2013). Biochar have been found to immobilise risk elements, reduce leaching of essential elements, increase soil water-holding capacity and serves as a source of essential elements (Laird et al., 2010a, 2010b; Park et al., 2011; Amini et al., 2016). Biochar produced from dry olive residue (DOR) enables to convert the phytotoxic compound into a functional soil amendment, while decreasing DOR wastes. The transformation of DOR can occur via pyrolysis or through the transformation by microorganisms.

Hmid et al. (2015) produced biochar from olive mill waste with slow pyrolysis in a downdraft gasifier at a temperature range of 400-450°C with application rates of 0, 5, 10 and 15% (w/w) and were equilibrated for 30 and 90 days. The effect of biochar on soil toxicity was determined using plants (*Phaseolus vulgaris*), bacteria and earthworms. The increase of biochar application rate and equilibration period led to decreased $Ca(NO_3)_2$ exchangeable risk elements, improved growth of *Phaseolus vulgaris* with reduced metal concentrations in the leaves. The results obtained by Hmid et al. (2015) can be comparable to that of this experiment, where accessible risk elements were decreased in *Triticum aestivum* grains and shoots.

Hmid et al. (2015) also observed the effect of pH and noted an increase in $pH_{(KCl)}$ of the soil after the addition of biochar especially with higher application rates. The pH of the soil was initially 6.2 and after 30 days of equilibrium with 15% application of olive mill waste- derived biochar the pH of the soil rose to 7.3, while after 90 days of equilibrium the soil pH rose to 7.5. An increase in soil pH can also be seen in the experiment conducted in this paper. The average soil pH of the low, medium and high contaminated soils were 6.4, 6.3 and 6.0, respectively. The pH of soils after the addition of the DOR biochar rose in all samples, with emphasis on the soils amended with DOR biochar pyrolyzed at 500°C and applied at a 5% application rate. Low, medium and high contaminated soils had a pH between 6.4 and 7; 6.6 and 6.8; and 6.1 and 6.8, respectively, after the addition of DOR biochar pyrolyzed at 500°C and applied at a 5% application rate, proving the liming effect of biochar on slightly acidic soils (Hmid et al., 2015).

The ability of biochar to adsorb risk elements onto its surface due to its carbonaceous properties can be said to be the reason for the decrease of risk elements in the rhizosphere therefore decreasing the availability to plants (Uchimiya et al., 2010; Zhou et al., 2015).

Kargar et al. (2015) found a decrease in Cu, Zn, Cd and Pb in sandy loam after biochar (maple wood, 450°C) application. A decrease by 99, 61 and 42% of bioavailable Cr, Ni and Mn respectively was reported by (Herath et al., 2015) after 5% application of biochar. Immobilisation of risk elements in soils changes with soil type and biochar application (Yang et al., 2015; Jiang et al., 2014). Kim et al. (2015) found paper mill sludge derived biochar more effective in decreasing Cd and Zn phytoavailability compared to that of distillery sludge and pruned branches. Yang et al. (2015) found rice straw derived biochar to immobilise risk elements greater than bamboo derived biochar in sandy loam. However, bamboo derived biochar immobilised Cd to greater extent than coconut shell, sugarcane bagasse or pine wood shaving derived biochars in Ultisol. Indicating the effect of soil type on the behaviour of different biochars in immobilising risk elements. The temperature of pyrolysis also influences the immobilisation of risk elements in soils (Uchimiya et al., 2010). Pellera & Gidarakos (2015) found dried olive pomace derived biochar prepared at 700°C to immobilise Cd and Ni compared to when it is prepared at 400°C. However, otherwise was indicated by Rajapaksha et al. (2015), where buffalo weed derived biochar produced at 300°C was able to decrease bioavailability of Pb and Cu than that of the biochar produced at 700°C. In this experiment it can be noted that the treatments varied for different elements in different soils. As and Pb were highly influenced by the pyrolysis temperature of 350°C with a 2% and 5% application rate. On the other hand, Cd concentrations decreased with all treatments, especially, DOR biochar 2% and 5% pyrolyzed at 500°C.

A met-analysis conducted by Biederman & Harpole (2013) studying the effects of biochar on plant productivity and nutrient cycling found that the addition of biochar to soils increased in aboveground productivity, crop yield, rhizobia nodulation and plant K tissue concentrations. The ability of risk elements to be taken up by plants depends on the type of biochar (Fellet et al., 2014), the temperature of biochar production, application rate, the soil type and the plant species (Rizwan et al., 2016).

Miscanthus derived biochar produced at 600°C for 30 mins and applied to Cd, Zn and Pb contaminated sandy loam at 0, 1, 5 and 10% w/w application rates with rapeseed grown in it. Biochar was found to increase plant dry mass and concentrations of Ca, Mg, K and P, with decreasing concentrations of Cd, Pb and Zn in shoots during 1st and 2nd harvest (Houben et al., 2013). Stinging nettles was pyrolyzed at 450°C for 6 hours to transform it into biochar and applied at a 0, 1 and 5% w/w ratio to sandy soil with sunflower grown on it. There was decreased concentrations of As and Cu in the plants and increase in the plant biomass (Sneath et al., 2013).

Bian et al. (2013) used biochar made from wheat straw which was pyrolyzed at 350°C and 550°C and applied at 0, 20 and 40 t/ha. The risk element in concern was Cd and the plant species studied was rice. The results of the experiment showed decrease bioavailable Cd in soils and decrease of Cd concentration in the rice grains. Rice residues pyrolyzed at 500°C for 4 hours and applied at 5% w/w to clay loam contaminated with Cd, Pb, Zn and As with wheat grown on it showed that the biochar managed to decrease available Cd, Pb and Zn, but increased As concentrations. An increased plant growth and biomass was noted by Zhang et al. (2013).

In this experiment there can be seen a notable effect of biochar on the concentrations of risk elements in the different plant parts of *Triticum aestivum* and the effect of pyrolysis temperature and application rate. As concentrations were influenced by the pyrolysis temperature of 350°C with a 5% application rate decreasing As concentrations by approximately 50% in the grain and by 50% in the shoot with the treatment of DOR biochar 2% 350°C. The concentrations of Pb decreased with treatment, specifically with 2% and 5% biochar application rate pyrolyzed at 350°C in shoots. Zn concentrations in roots were decreased by DOR biochar 5% 350°C and especially by 2% 350°C.

Different studies by Nigussie et al. (2012), Rees et al. (2015) and Yang et al. (2015) indicate that the effect of biochar on nutrient uptake by plants varies with time and also the type of biochar. Nigussie et al. (2012) found that the application of maize stalk biochar in Cr contaminated soils increased the N, P and K concentrations in lettuce. Rees et al found that biochar decreased concentrations and availability of N, P and Ca in Cd and Zn contaminated soils. Whereas, Yang et al. (2015) reported that P concentrations in soils increased with biochar application in a dose-dependent manner. DOR derived biochar increased in essential element concentration with increased temperature of pyrolysis. With a notable concentration of K in the DOR and DOR derived biochars. Concentrations of Ca, Mg, K and P were all statistically significant in increasing concentrations in different plant parts under different treatments.

7. Conclusion

The focus of the thesis was to evaluate the effectivity of biochar produced from dry olive residue (DOR) on the bio-accessibility of risk elements as well as the nutrient status of contaminated soils via a model pot experiment. The main objective of the study was to verify DOR-based biochar on the immobilisation of risk elements in contaminated soils and to assess the usefulness of the biochar as a source of nutrients for plants grown on contaminated soils.

Summarising the results, over the three-month period, different application rates and pyrolysis temperatures can be seen to influence the accessibility of the different risk and essential elements, both in soils and *Triticum aestivum* plants. The results indicate greater importance of the DOR-based biochar for increasing the nutrient status of soils, notably K concentrations, which were exceptionally high in DOR and DOR-based biochar. The essential element concentrations in DOR and DOR-based biochar increased with increasing pyrolysis temperatures. Signifying the value of DOR-based biochar on improving nutrient status in soils. The bioaccessible concentrations of As, Pb and Zn can be observed to be highly influenced by the pyrolysis temperature of 350°C, while for Cd concentrations DOR pyrolyzed at 500°C, proved to be more significant, for both application rates. Expressing the influence of temperature of pyrolysis and application rate on the immobilisation of risk elements and increase of essential elements. The results from the 4-way ANOVA provides an insight to the effect of the different factors that significantly affected the concentrations of bioaccessible risk essential elements in plants. The most significant factors were the soil, pyrolysis temperature, plant part, soil and plant part and biochar temperature and plant part. It can be concluded that DOR-based biochar was able to improve growth parameters of plants by decreasing risk element concentrations and increasing essential elements, and thus nutrient status of the soil.

Further studies in this field could examine wider range of the temperature of pyrolysis and application rate. In order to maintain economic stability of the use of biochar (as high application rates can be expensive) the use of biochar with other amendments can be a cost-effective solution.

8. References

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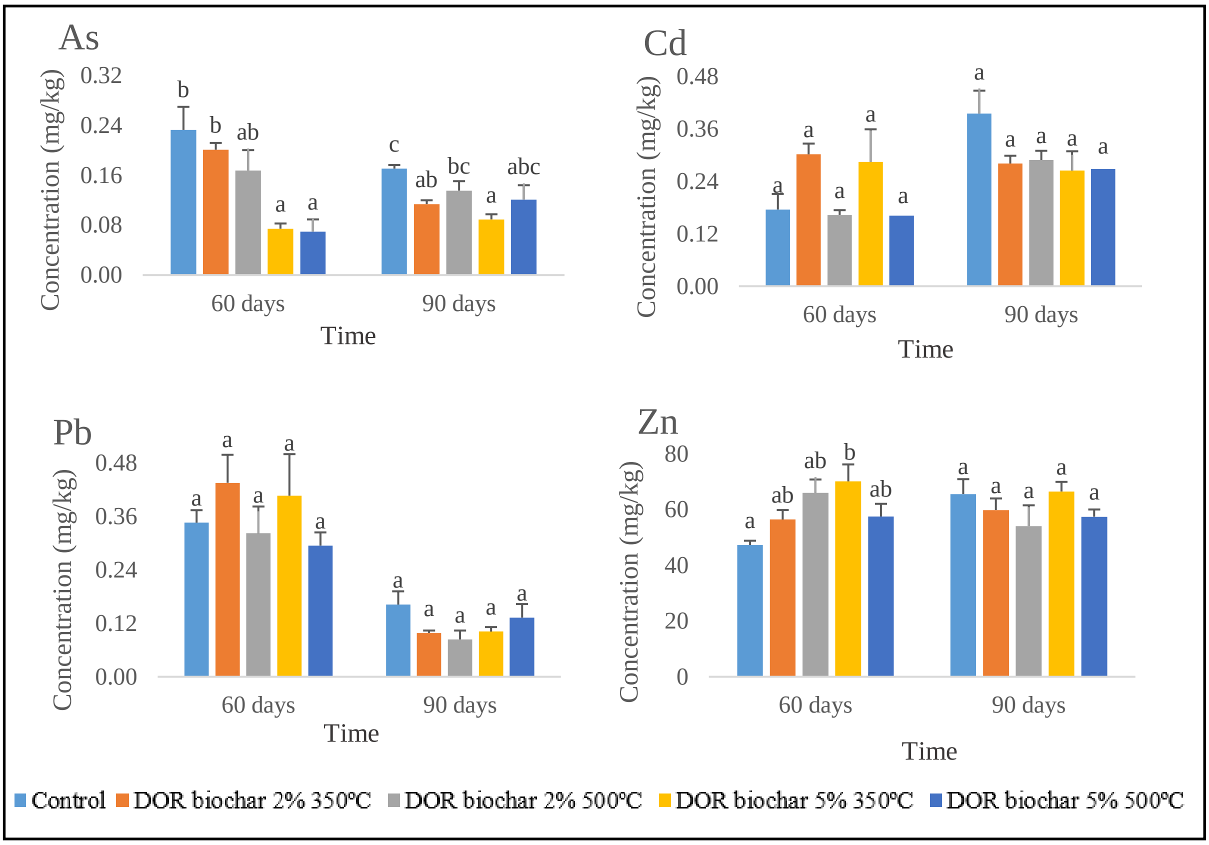
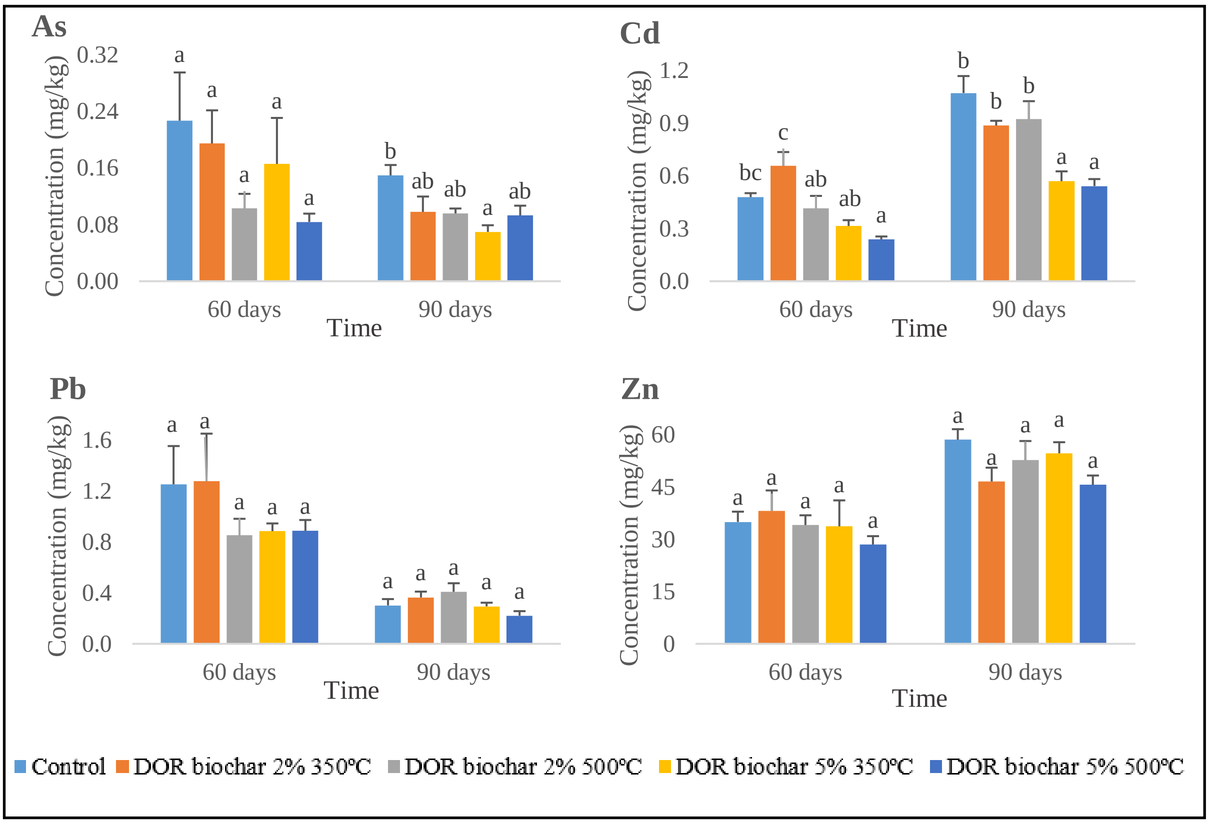
9. Appendices

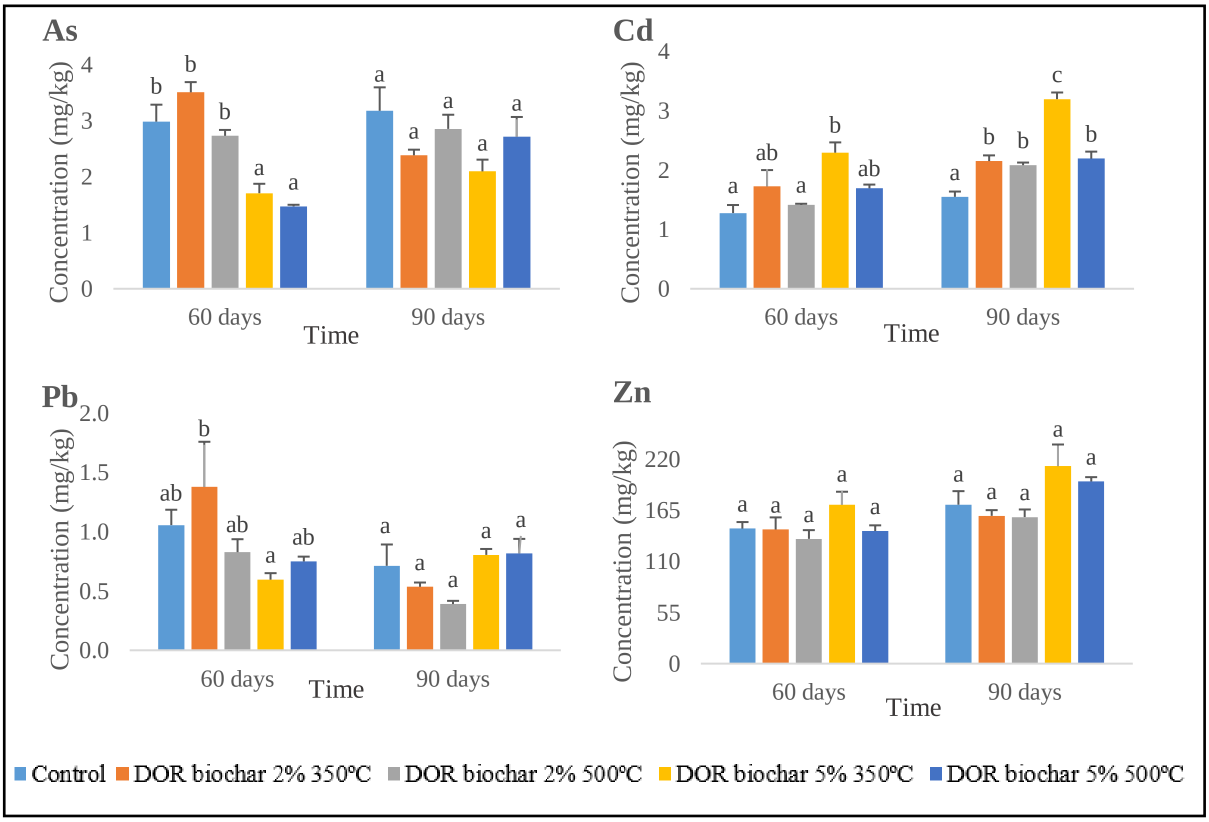
9.1. Appendix 1



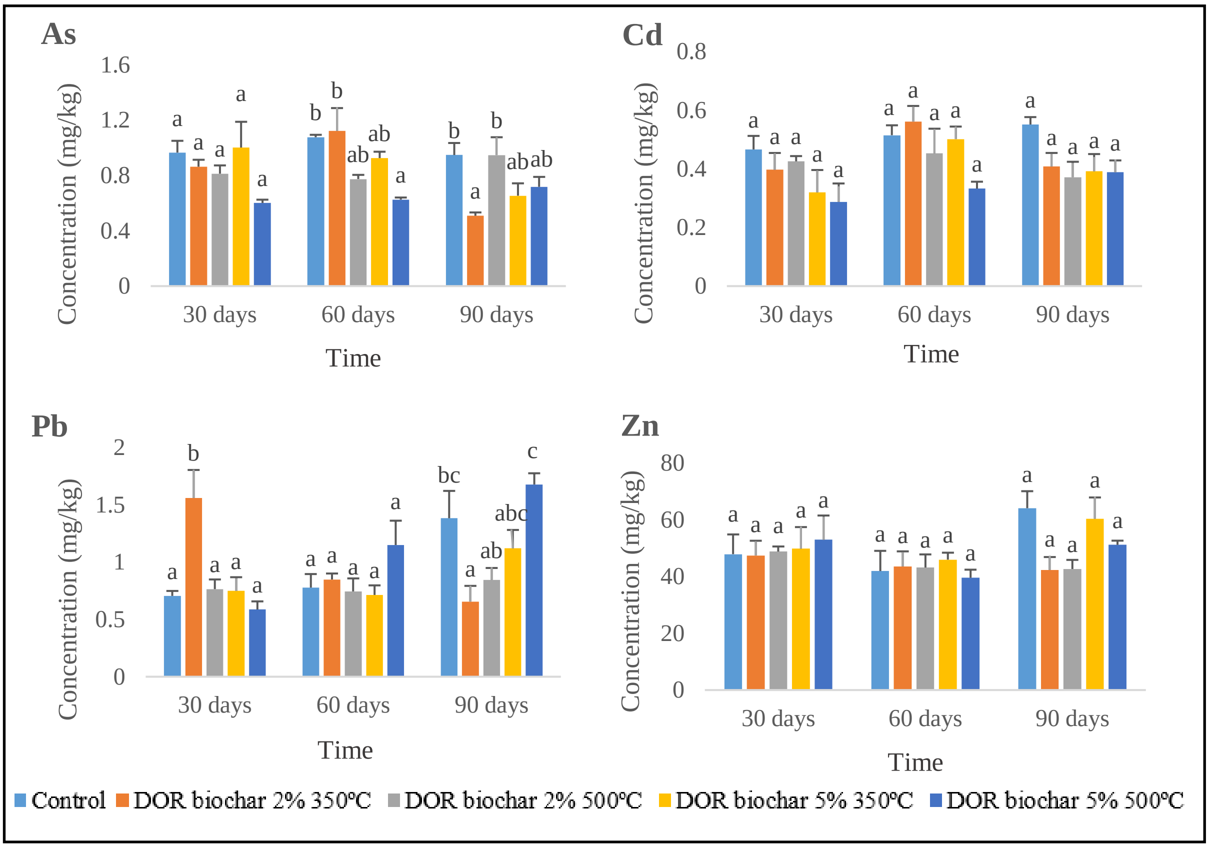
Supplement figure 1: Concentration of risk elements in grain of *Triticum aestivum* grown on Hluboš soil: n=4, α=0.05.



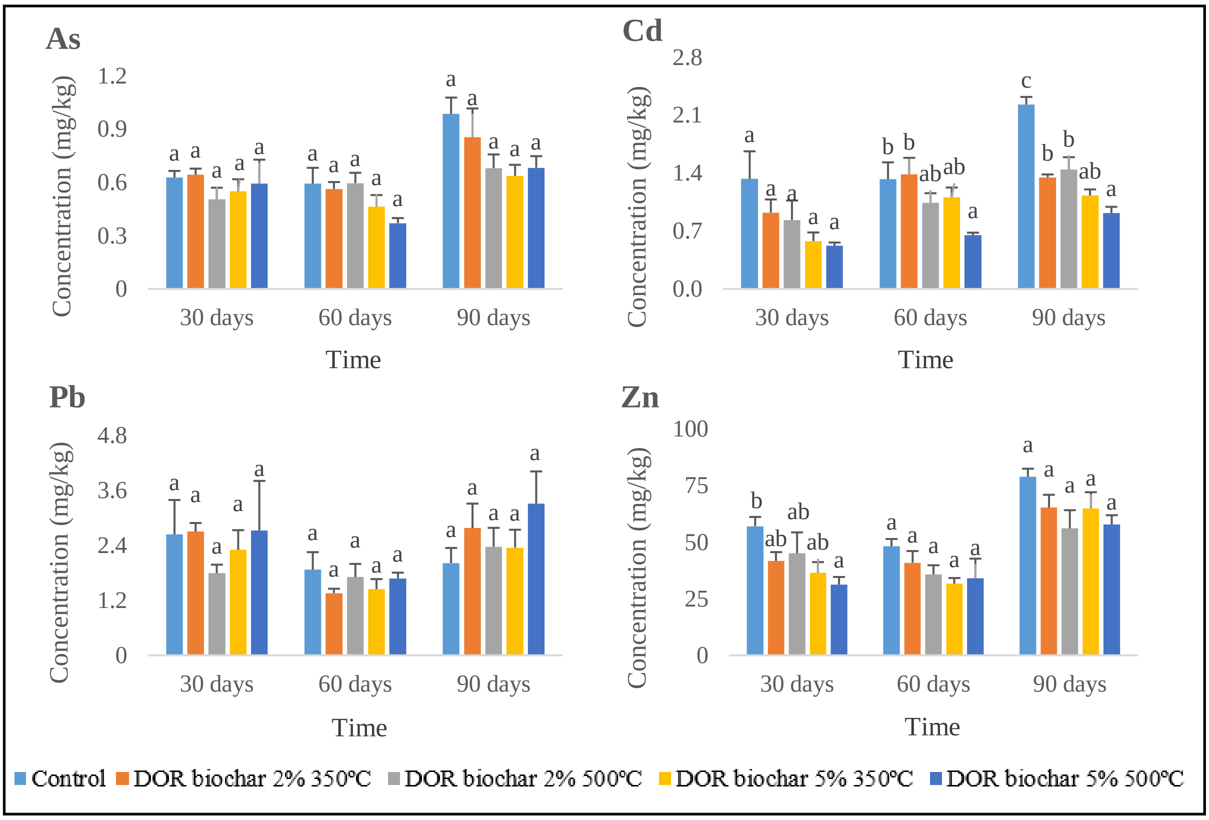
Supplement figure 2: Concentration is risk elements in grain of *Triticum aestivum* grown on Halda soil: n=4, α=0.05.



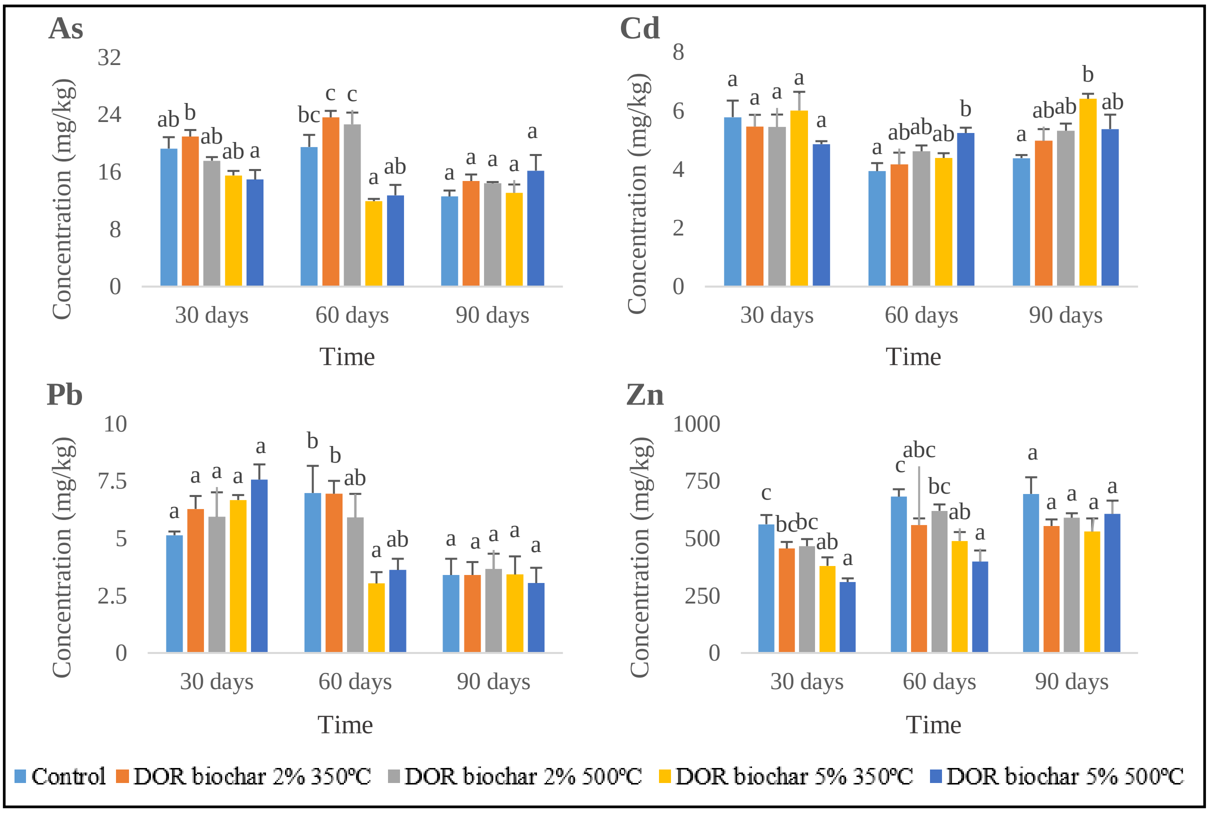
Supplement figure 3: Concentration of risk elements in grain of *Triticum aestivum* grown on Litavka soil: n=4, α=0.05.



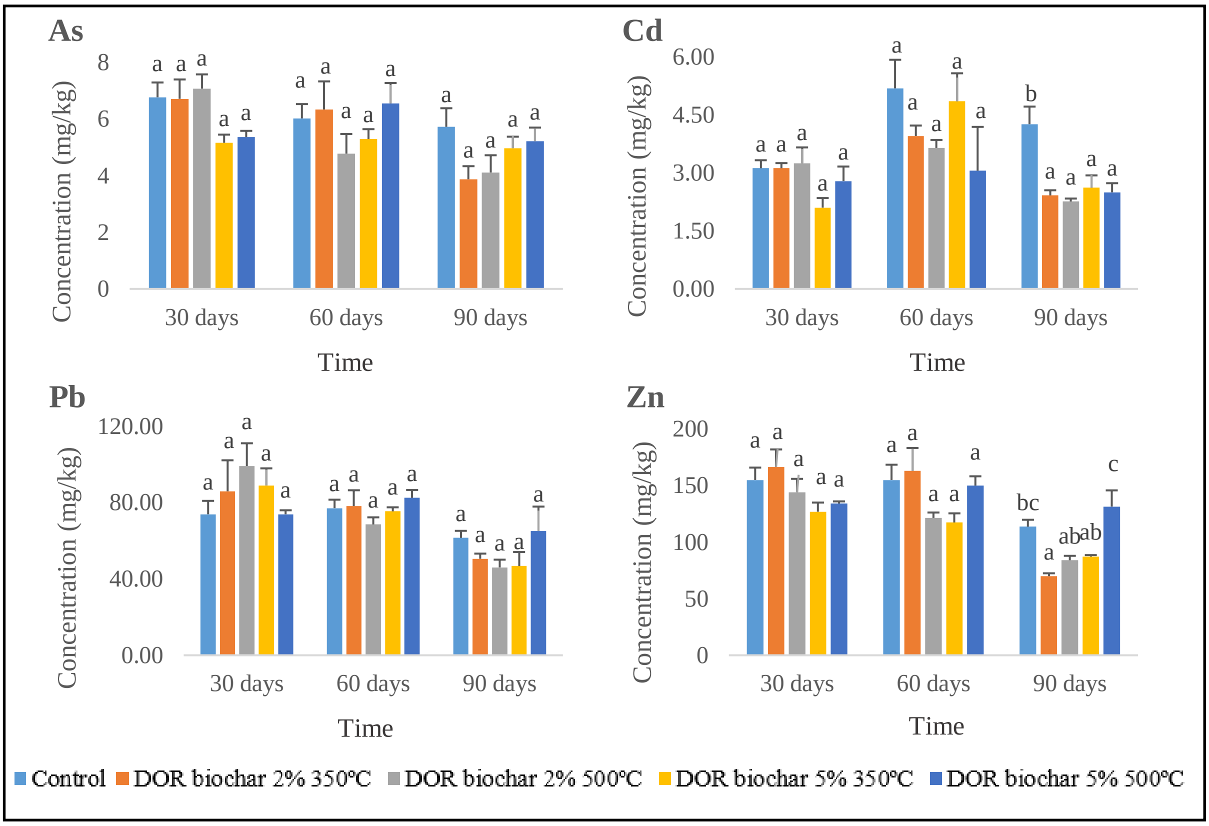
Supplement figure 4: Concentration of risk elements in shoot of *Triticum aestivum* grown on Hluboš soil: n=4, α=0.05.



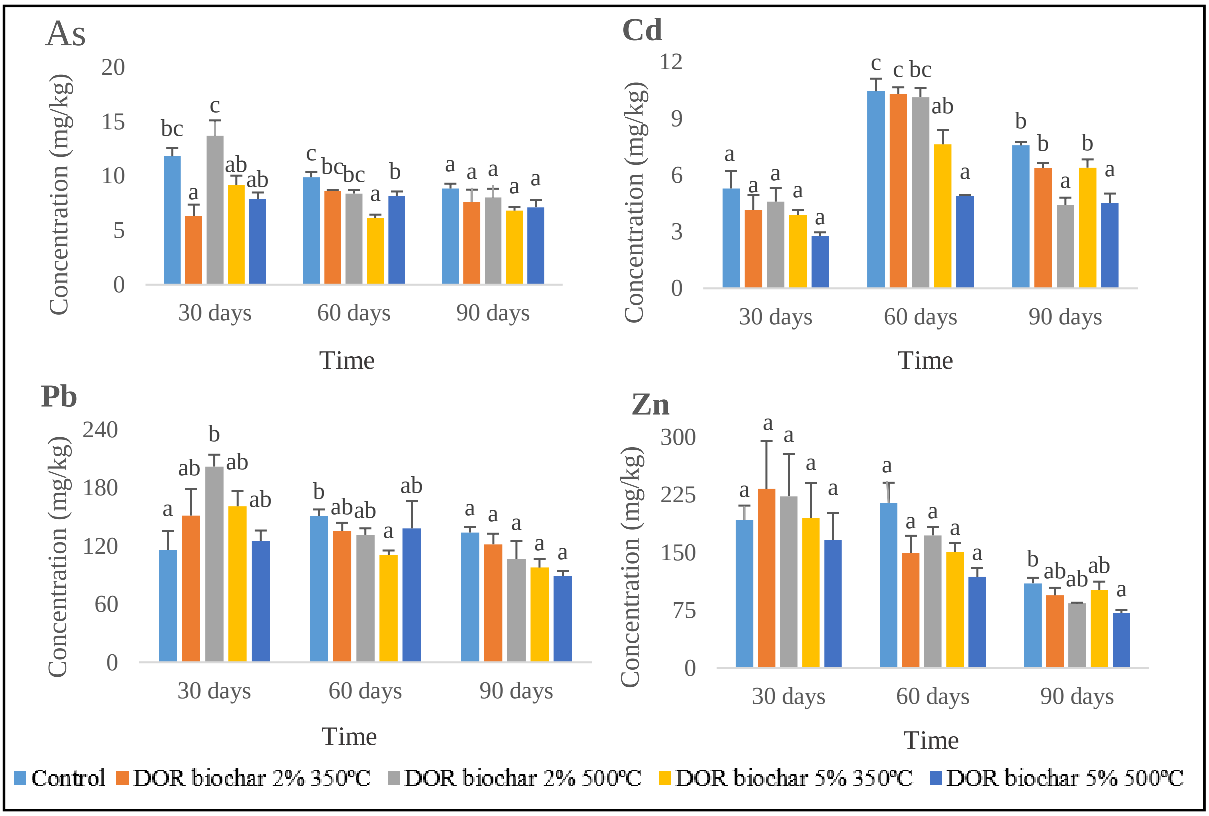
Supplement figure 5: Concentration of risk elements in shoot of *Triticum aestivum* grown on Halda soil: n=4, α=0.05.



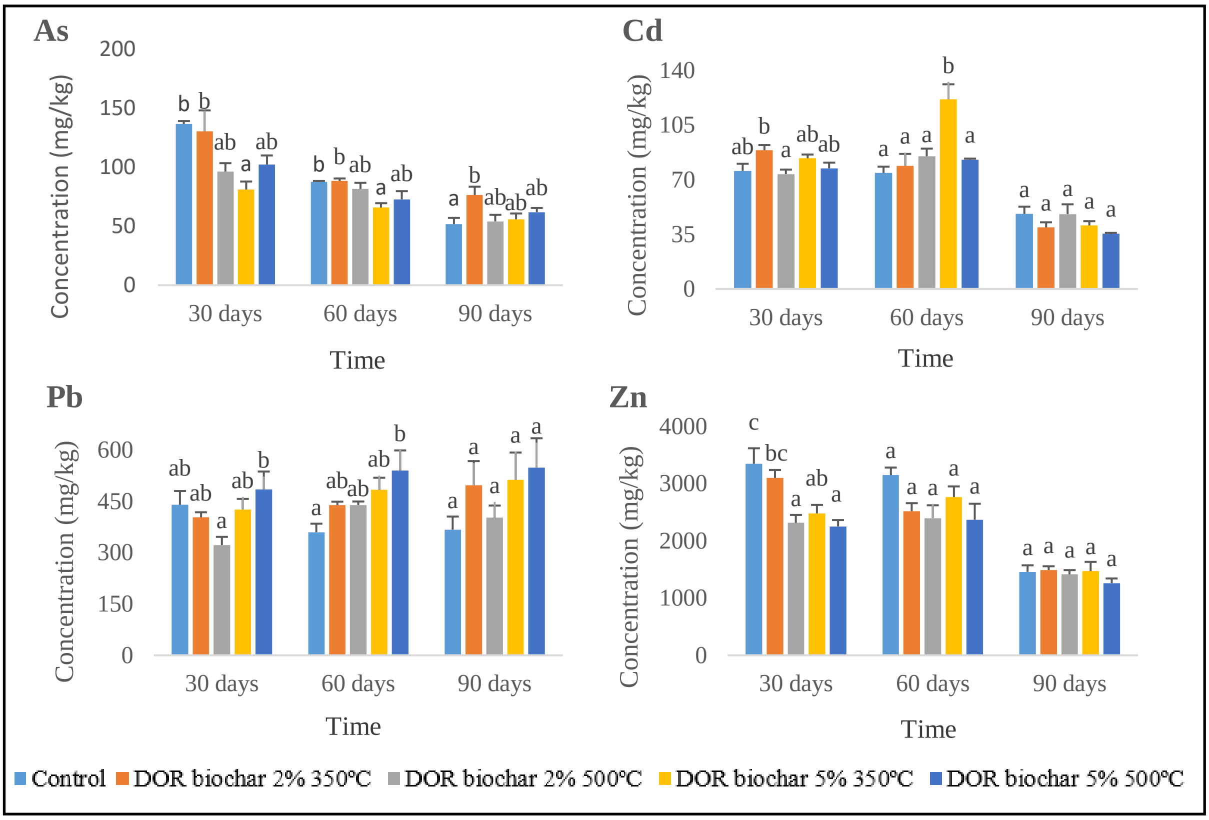
Supplement figure 6: Concentration of risk elements in shoot of *Triticum aestivum* grown on Litavka soil: n=4, α=0.05.



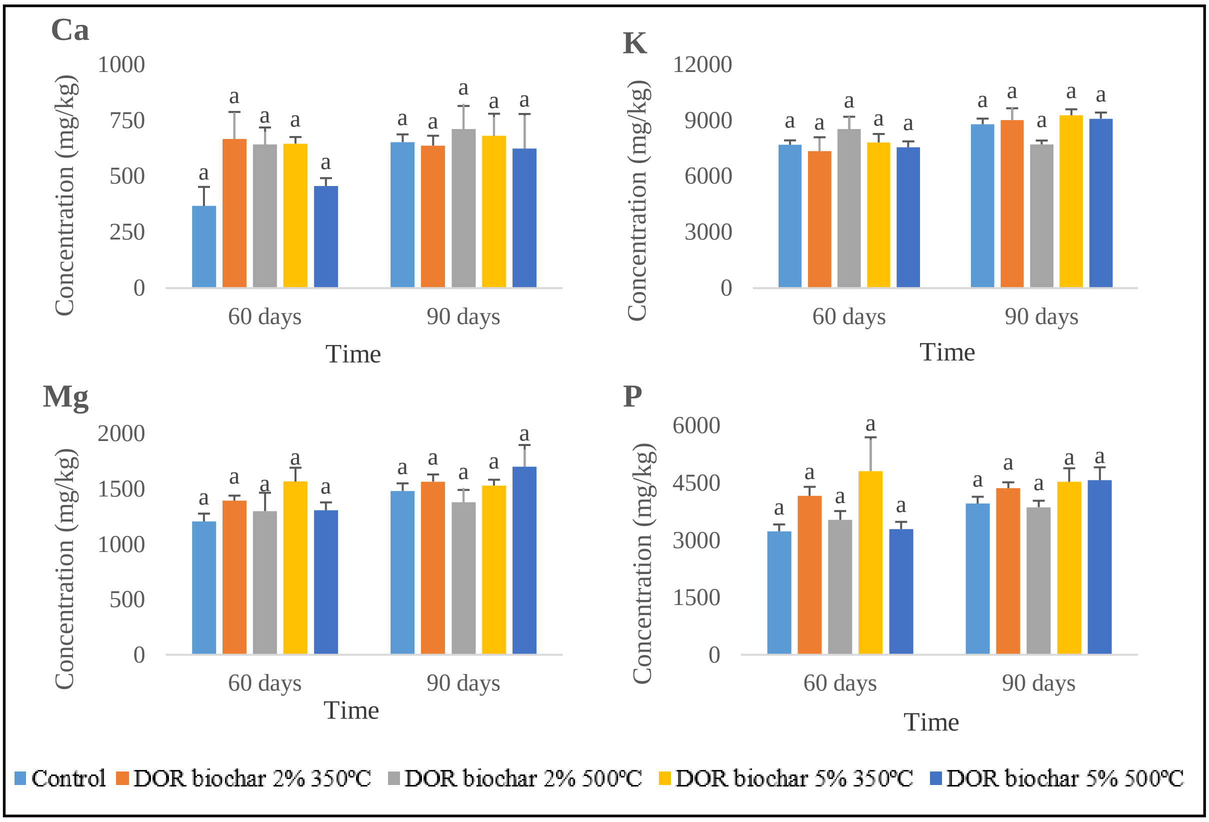
Supplement figure 7: Concentration of risk elements in root of *Triticum aestivum* grown on Hluboš soil: n=4, α=0.05.



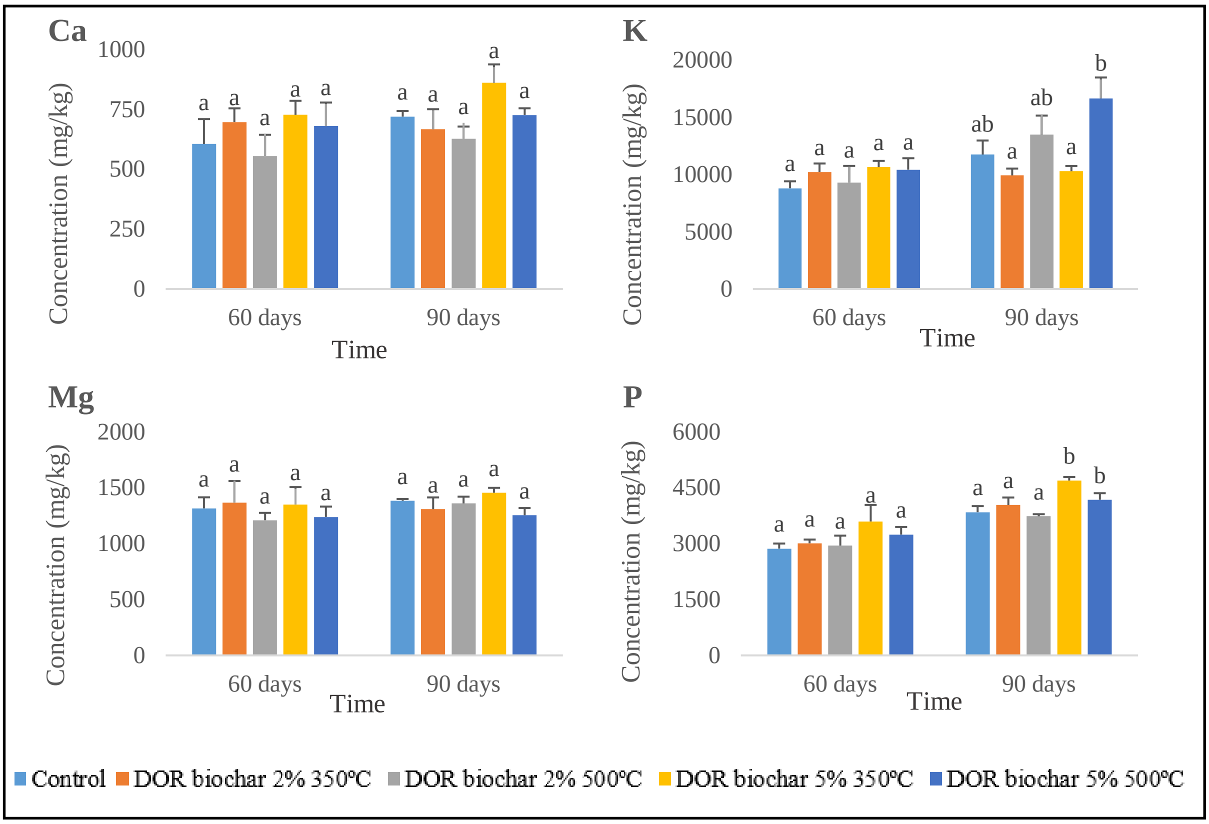
Supplement figure 8: Concentration of risk elements in root of *Triticum aestivum* grown on Halda soil: n=4, α=0.05.



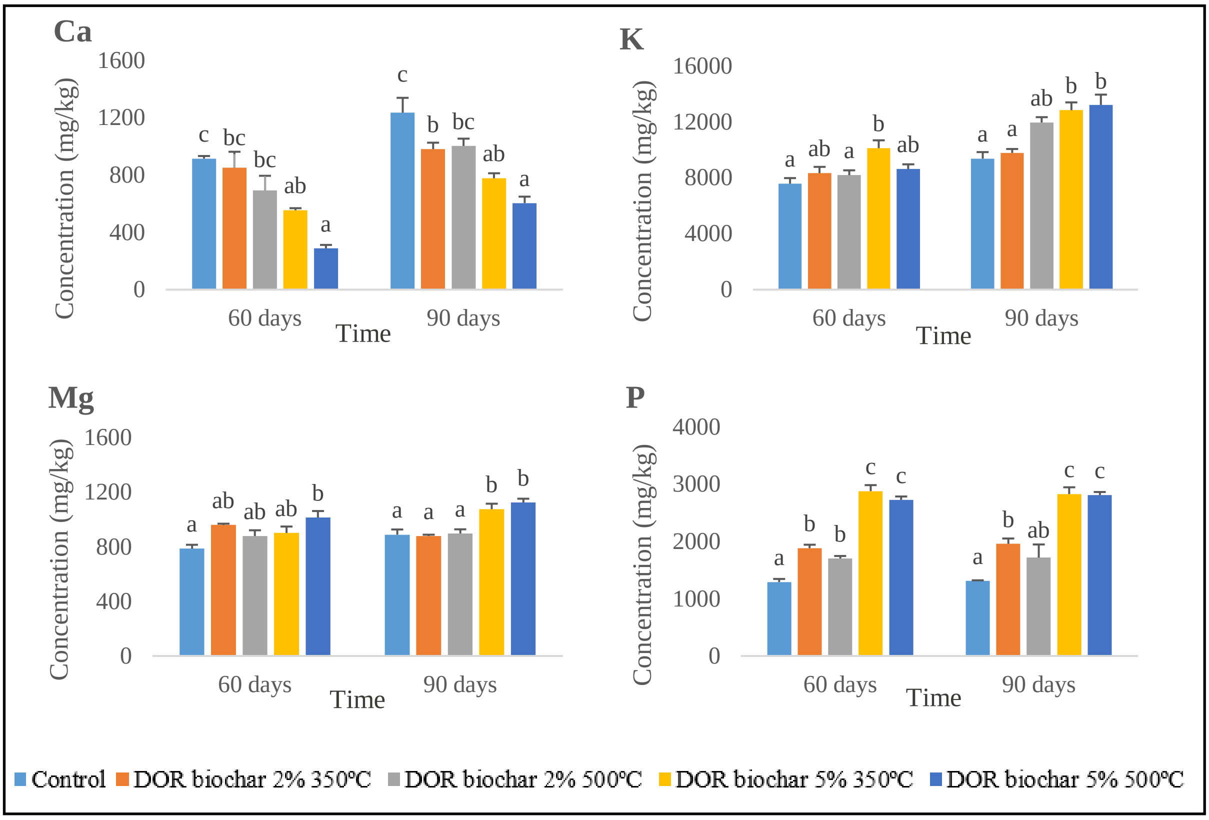
Supplement figure 9: Concentration of risk elements in root of Triticum aestivum grown on Litavka soil: n=4, α=0.05.



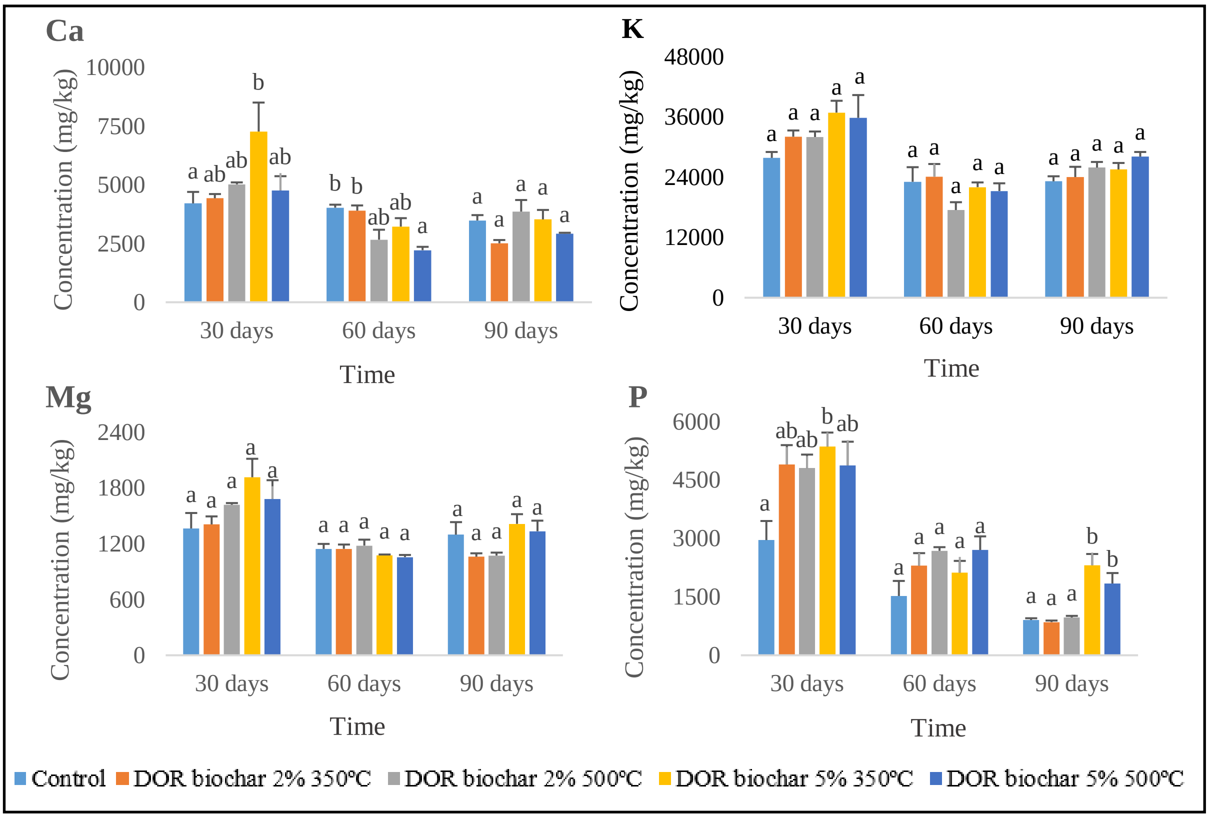
Supplement figure 10: Concentration of nutrients in grain of *Triticum aestivum* grown on Hluboš soil: n=4, α=0.05.



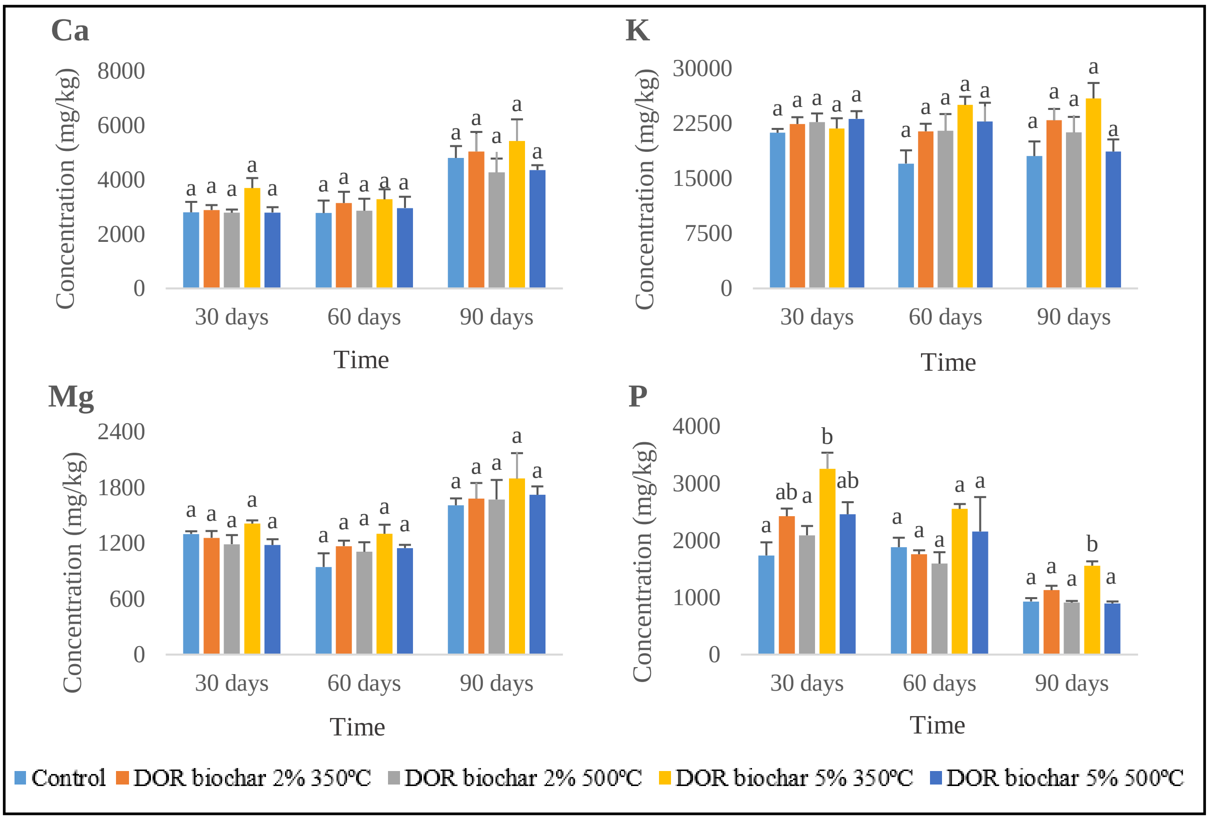
Supplement figure 11: Concentration of nutrients in grain of *Triticum aestivum* grown on Halda soil: n=4, α=0.05.



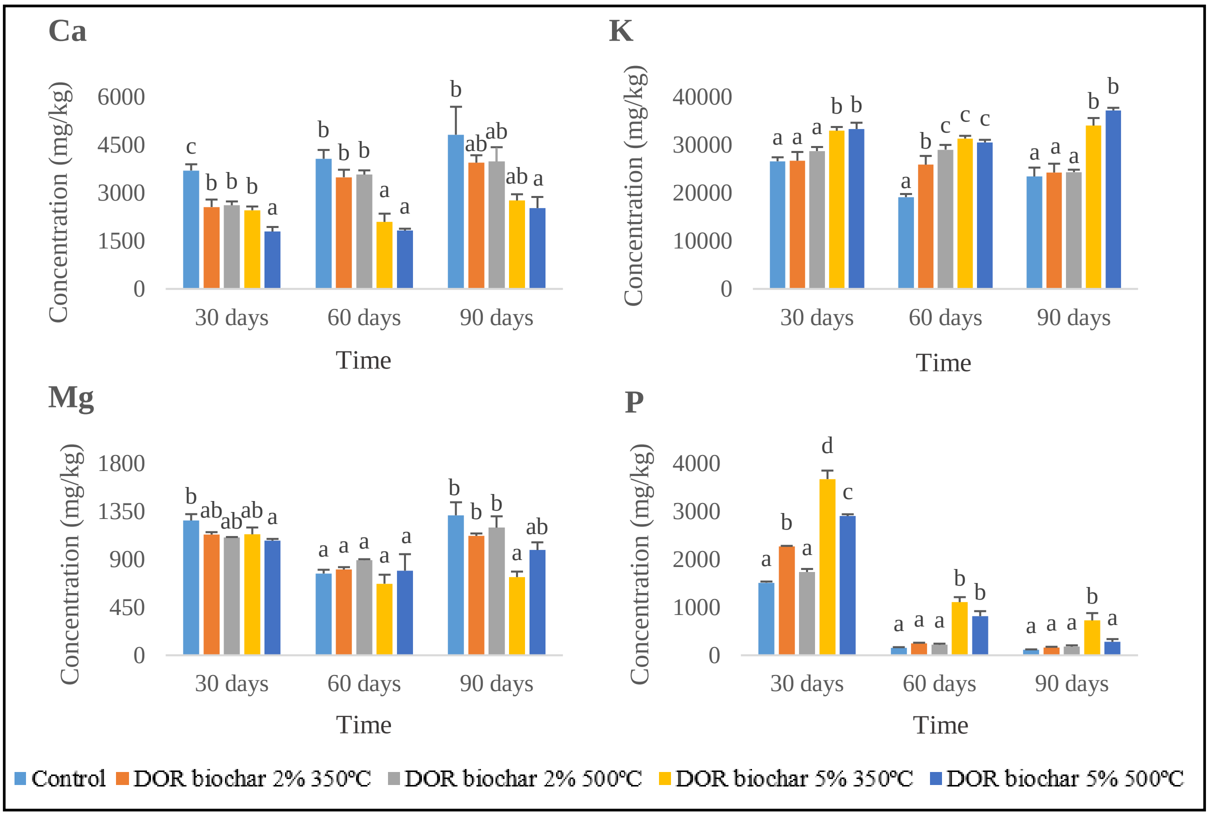
Supplement figure 12: Concentration of nutrients in grain of *Triticum aestivum* grown on Litavka soil: n=4, α=0.05.



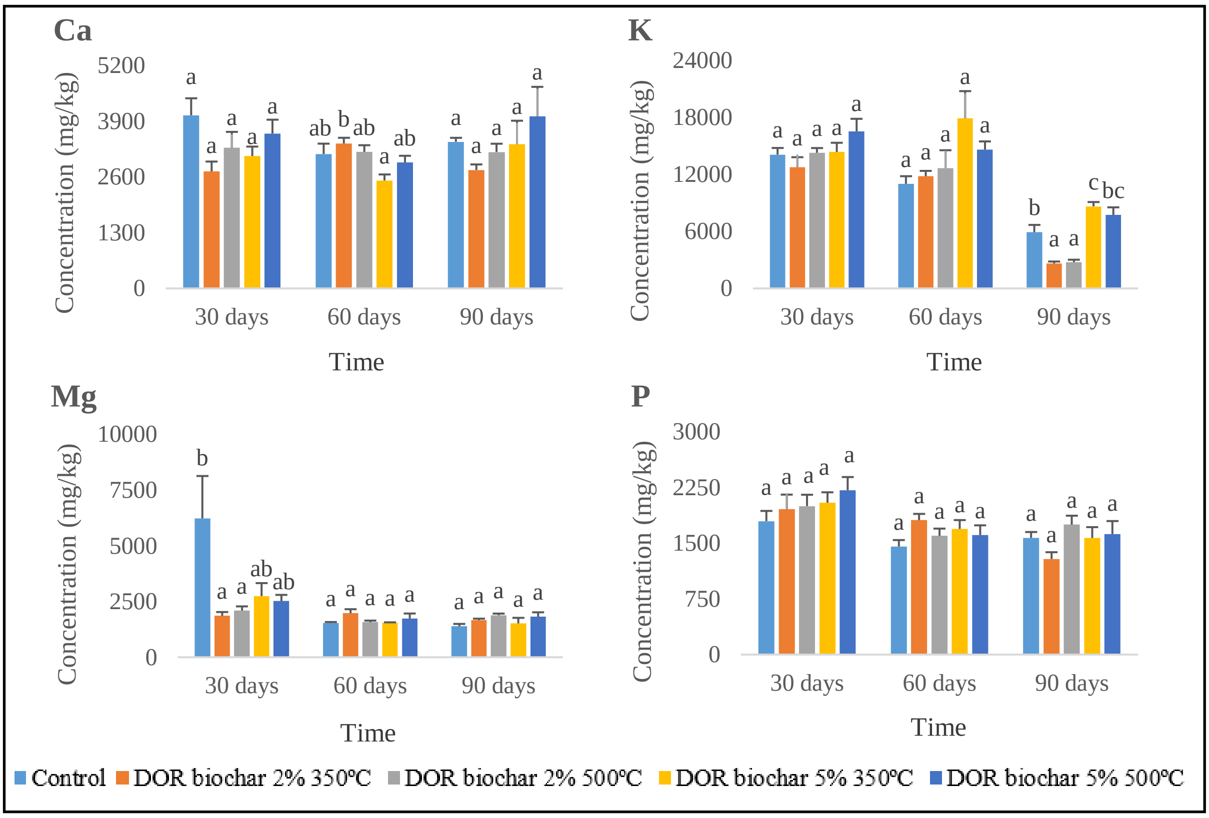
Supplement figure 13: Concentration of nutrients in shoot of *Triticum aestivum* grown on Hluboš soil: n=4, α=0.05.



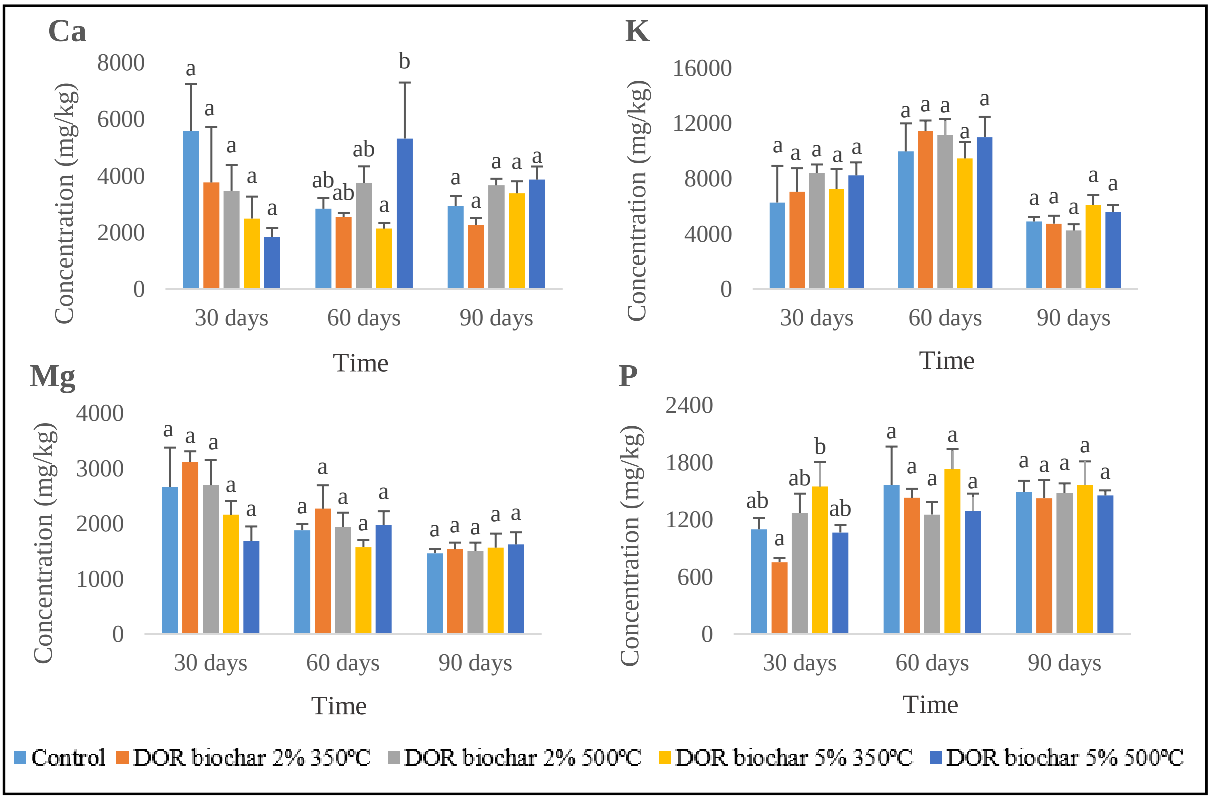
Supplement figure 14: Concentration of nutrients in shoot of *Triticum aestivum* grown on Halda soil: n=4, α=0.05.



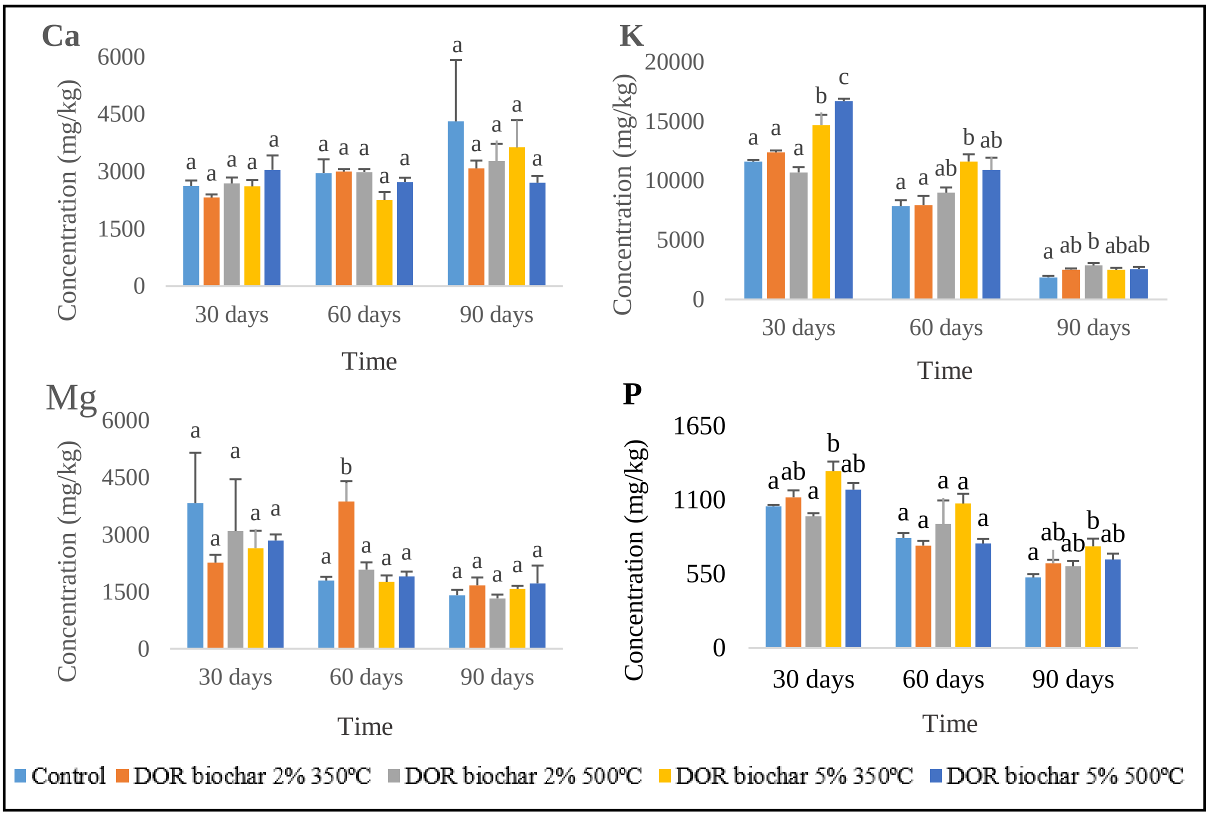
Supplement figure 15: Concentration of nutrients in shoot of *Triticum aestivum* grown on Litavka soil: n=4, α=0.05.



Supplement figure 16: Concentration of nutrients in root of *Triticum aestivum* grown on Hluboš soil: n=4, α=0.05.



Supplement figure 17: Concentration of nutrients in root of *Triticum aestivum* grown on Halda soil: n=4, α=0.05.



Supplement figure 18: Concentration of nutrients in root of Triticum aestivum grown on Litavka soil: n=4, α=0.05.

9.2. Appendix 2

Supplement table 1: Correlation coefficients of pH and mobile risk elements in soil after 30 days.

	pH	As	Cd	Pb	Zn
pH	1.000	-0.343	-0.461	-0.435	-0.466
As	-0.343	1.000	0.853	0.801	0.860
Cd	-0.461	0.853	1.000	0.984	0.999
Pb	-0.435	0.801	0.984	1.000	0.985
Zn	-0.466	0.860	0.999	0.985	1.000

Red values indicate correlation coefficient significant at $p<0.05$

Supplement table 2: Correlation coefficients of pH and mobile risk elements in soil after 60 days.

	pH	As	Cd	Pb	Zn
pH	1.000	0.008	-0.111	-0.119	-0.091
As	0.008	1.000	0.945	0.912	0.947
Cd	-0.111	0.945	1.000	0.988	0.999
Pb	-0.119	0.912	0.988	1.000	0.988
Zn	-0.091	0.947	0.999	0.988	1.000

Red values indicate correlation coefficient significant at $p<0.05$

Supplement table 3: Correlation coefficients of pH and mobile risk elements in soil after 90 days.

	pH	As	Cd	Pb	Zn
pH	1.000	-0.642	-0.872	-0.887	-0.868
As	-0.642	1.000	0.859	0.801	0.870
Cd	-0.872	0.859	1.000	0.991	0.999
Pb	-0.887	0.801	0.991	1.000	0.989
Zn	-0.868	0.870	0.999	0.989	1.000

Red values indicate correlation coefficient significant at $p<0.05$

Supplement table 4: Correlation coefficients of pH and elements in plants after 30 days.

	As	Cd	Pb	Zn	Ca	K	Mg	P	pH
As	1.000	0.955	0.893	0.978	-0.241	-0.334	0.255	-0.418	-0.406
Cd	0.955	1.000	0.915	0.970	-0.236	-0.342	0.244	-0.407	-0.313
Pb	0.893	0.915	1.000	0.891	-0.202	-0.567	0.368	-0.520	-0.190
Zn	0.978	0.970	0.891	1.000	-0.243	-0.317	0.270	-0.411	-0.422
Ca	-0.241	-0.236	-0.202	-0.243	1.000	0.270	0.196	0.441	-0.001
K	-0.334	-0.342	-0.567	-0.317	0.270	1.000	-0.386	0.801	0.024
Mg	0.255	0.244	0.368	0.270	0.196	-0.386	1.000	-0.233	-0.103
P	-0.418	-0.407	-0.520	-0.411	0.441	0.801	-0.233	1.000	0.243
pH	-0.406	-0.313	-0.190	-0.422	-0.001	0.024	-0.103	0.243	1.000

Red values indicate correlation coefficient significant at $p<0.05$

Supplement table 5: Correlation coefficients of pH and elements in plants after 60 days.

	As	Cd	Pb	Zn	Ca	K	Mg	P	pH
As	1.000	0.922	0.904	0.975	0.262	-0.166	0.505	-0.495	-0.100
Cd	0.922	1.000	0.933	0.941	0.171	-0.226	0.502	-0.380	-0.031
Pb	0.904	0.933	1.000	0.878	0.251	-0.291	0.645	-0.409	-0.013
Zn	0.975	0.941	0.878	1.000	0.211	-0.169	0.438	-0.455	-0.099
Ca	0.262	0.171	0.251	0.211	1.000	0.533	0.201	-0.588	-0.068
K	-0.166	-0.226	-0.291	-0.169	0.533	1.000	-0.337	-0.291	0.097
Mg	0.505	0.502	0.645	0.438	0.201	-0.337	1.000	-0.083	-0.005
P	-0.495	-0.380	-0.409	-0.455	-0.588	-0.291	-0.083	1.000	0.235
pH	-0.100	-0.031	-0.013	-0.099	-0.068	0.097	-0.005	0.235	1.000

Red values indicate correlation coefficient significant at $p<0.05$

Supplement table 6: Correlation coefficients of pH and elements in plants after 90 days.

	As	Cd	Pb	Zn	Ca	K	Mg	P	pH
As	1.000	0.935	0.935	0.955	0.236	-0.357	0.103	-0.431	-0.529
Cd	0.935	1.000	0.898	0.924	0.206	-0.423	0.075	-0.379	-0.500
Pb	0.935	0.898	1.000	0.843	0.205	-0.512	0.263	-0.339	-0.366
Zn	0.955	0.924	0.843	1.000	0.263	-0.229	0.011	-0.463	-0.643
Ca	0.236	0.206	0.205	0.263	1.000	0.223	0.375	-0.685	-0.056
K	-0.357	-0.423	-0.512	-0.229	0.223	1.000	-0.247	-0.223	0.036
Mg	0.103	0.075	0.263	0.011	0.375	-0.247	1.000	0.071	0.347
P	-0.431	-0.379	-0.339	-0.463	-0.685	-0.223	0.071	1.000	0.431
pH	-0.529	-0.500	-0.366	-0.643	-0.056	0.036	0.347	0.431	1.000

Red values indicate correlation coefficient significant at $p<0.05$