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BIOCHAR INDUCED CHANGES IN HEAVY METAL PLANT BIOAVAILABILITY IN ACIDIC SOILS AND TAILINGS AFFECTED BY COPPER METALLURGY

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ABSTRACT

Biochar (BC) has long been recognised as a soil ameliorant which immobilises contaminants and improves plant nutrition. The present study deals with acidic soils and tailings in the vicinity of a copper smelter and ore extraction plant. In a greenhouse experiment BC incorporation (20%) increased soil pH and cation exchange capacity, decreased electrical conductivity and caused Cu and other toxic heavy metal immobilization as analysed in soil solution (H2O), 0.01M CaCl2, and 1M NH4NO3. The 0,01M CaCl2 provides the most satisfactory relationship with the content of heavy metals and as in plants. Geochemical modelling (Visual Minteq) revealed that free M 2+ ions decreased following BC addition, while in the tailings the dominant M2+SO40(aq) complexes decreased at the expense of free more bioavailable M2+ ions. Nevertheless, there were measurable plant yields in the tailings for the BC and lime treated variants, due to improvement of general physico-chemical characteristics and full immobilisation of Cu. Amelioration with BC, caused statistically significant increases in plant biomass, often exceeding 4-13 times the original control yields, while for the tailings, no yields without BC were noted. Biochar amelioration led to lowering metal contents in plant biomass, as supported by Visual Minteq modelling, describing the increase of stable, high-molecular-weight organo-metallic complexes that can reduce metals' bioavailability. Although free M2+ ions of heavy metals in the tailings prevailed after BC addition, due to general improvement of the physico-chemical characteristics, plant yields and full Cu immobilization was observed. Biochar's long-term effects should be monitored if some plant-soil processes may transform its structure and properties.

KEYWORDS: Bioavailability, Biochar, Copper, Metal Speciation, Technogenic Soils.

1. INTRODUCTION

Numerous studies have dealt with biochar (BC) as effective amendment for immobilizing heavy metals in contaminated soils (Chen et al., 2018; Ibrahim et al., 2022; Sarraf et al., 2024; Lehmann and Joseph, 2015; Kong et al., 2014; Inyang et al., 2016). A meta-analysis (Chen et al., 2018) has shown that BC amelioration reduces heavy metal concentrations in solutions from contaminated soils and causes measurable decrease of Cd, Pb, Cu and Zn in plant tissues depending on BC and soil characteristics, plant species and the respective heavy metal.

The capacity of BC to adsorb heavy metals and metalloids in soil can be attributed to several mechanisms, such as physico-chemical adsorption (ion exchange, complexation), precipitation, redox reactions, with particular emphasis on the reduction of Cr^{6+} and the oxidation of As^{3+} (Wei et al., 2025).

The mechanisms by which BC adsorbs metals focus on the importance of specific surface area, porosity and water holding capacity, as well as the "BC aging" and transformation over time (Yuan et al., 2021; Nenova et al., 2025). Studies on predicting BC immobilization involving machine learning stress upon the importance of BC nitrogen content and application rate in heavy metals immobilization (Palansooriya et al., 2022).

BC incorporation in incubation and pot trials can cause significant decrease in the phytoavailable metal pool as assessed by 1M NH_4NO_3 extraction (Park et al., 2011; Kim et al., 2015) and by 0.005 M DTPA (Ibrahim et al., 2022), significant reduction of heavy metals in lettuce by over 80% (Kim et al., 2015) and by applying $KMnO_4$ modified water hyacinth BC (Yin et al., 2022), as well as improved nutrient availability alongside heavy metal immobilization in dill plants (Ghassemi-Golezani, et al., 2025). Co-pyrolysis of rice straw and swine manure produced effective BC capable of changing the shares of surface bound metals, e.g. the exchangeable heavy metals decreased in the BC-amended soils while the carbonate-bound metals increased (Meng et al., 2018).

Some key factors influence BC effectiveness, e.g. application rate, with higher application rates generally resulting in greater reductions in plant bioavailability; feedstock material (Lu et al., 2017), soil textural composition with coarse-textured soils showing larger decreases in plant heavy metal concentrations compared to fine-textured soils, pyrolysis temperature, with BCs produced at 450–500 °C being favored due to higher oxygen-containing functional groups and high pH (Tomczyk et al., 2020). Studies on the impact of BC on soils affected by copper metallurgy (Medyńska-Juraszek and Ćwieląg-Piasecka, 2020; Atanassova et al., 2024a, b; Nenova et al., 2025) have shown that BC application can reduce Cu, Pb, Zn or Cd mobility in acidic soils due to the liming effect. Bian et al., (2014) confirm that the reduction in metal availability persists over several years, while Wang et al., (2021) find that immobilization effect of BC can decrease over time due to abiotic (acid rain) or biotic factors. It has been demonstrated (Qin et al., 2022) that BC protonation in acid soils can weaken its effectiveness to reduce the bioavailability of heavy metals over time. These authors found out that BC significantly reduced the phytoavailability of some cationic heavy metals but not anionic, e.g. Cr.

Despite that many studies have dealt with BC effect on heavy metal decrease in soil extracts targeting various pools in contaminated soils and wastes, few (Atanassova et al., 2019; 2024a, b; Stoykova et al., 2024; Beesley et al., 2010) have examined heavy metal speciation in soil solution through geochemical modelling.

The aim of the present study is to assess the bioavailability of heavy metals, As and Cr, to plants in contaminated soils and tailings, and link it to metal speciation in soil solution, thus providing evidence of the "hidden" mechanisms of BC remediation and enabling a better understanding of how solution speciation predetermines plant uptake.

2. MATERIALS AND METHODS

2.1. Experimental Sites

The experimental sites of the study were selected in the vicinity of Aurubis-Pirdop copper smelter and refinery, and Asarel-Medet copper ore extraction and processing plant in Bulgaria (Nenova et al., 2025; Atanassova et al., 2024a, b).

The sites at *Aurubis-Pirdop* copper smelter and refinery (P) are agricultural lands (meadow grasses, birch, poplar and acacia), with shallow soil profile, low organic carbon content, low pH and heavy metals and metalloids contamination (Benkova and Atanassova 2015). Soil cores were sampled (0-20 cm) at two selected sites P1 and P6, 1- 2 km from the smelter, P1 (N 42° 42' 42.0" E 24° 09' 30.6") and P6 (N 42° 42' 29.2" E 24° 09' 20.2"), by taking composite samples from several points and pooling together (Atanassova et al. 2024b). Main physico-chemical properties and initial contents of heavy metals of the experimental soils are given in Table 1, i.e. (pH, electrical conductivity (EC), total organic carbon content (TOC) and cation exchange capacity (CEC).

The sites at the *Medet area* (M) of the Asarel-Medet mining and processing complex are the following: M1 (N42° 35' 19.3" E24° 11' 04.4") and M7 (N42° 39'

30.8" E24° 09' 27.5"), studied also by Atanassova et al., (2023). The Medet soil (M1) was taken from the "Great Southern Embankment", which was backfilled with materials from the open-pit mine "Medet" and was biologically reclaimed in the period 1998-2006 with pine and birch plantations. Soils from M7 were taken from the reclaimed tailings dam "Zlatishko Kale", which is situated a few kilometers from the Medet mine.

Waste materials (tailings) from the sites of the *Asarel-Medet* open pit copper mining and processing plant, site A1 (42˚32'13.70"N 24˚08'31.10"E) and A10 (42˚33'55.70"N 24˚05'52.20"E) were taken from 0-20 cm. The site at A1 was rock mass with Cu minerals from *Oxide embankment* filled with ballast ore with minimal copper content not subject to conventional enrichment. It is additionally processed with sulfuric acid (H_2SO_4), supplied to the embankment and the solution is taken away for extraction by electrolysis. The site A10 is a rocky mass from the *Western Embankment* from the mine overburden, not subject to subsequent processing.

2.2. Soils, Biochar and Waste Substrates Characteristics.

The following physico-chemical characteristics for the soils, substrates and BC were determined: total organic carbon (TOC) by oxidation with $K_2Cr_2O_7$ (Kononova, 1966) and through dry combustion on *Primacs SNC100 - SKALAR*; dissolved organic carbon (DOC) in soil solution (soil:water 1:5), and determination with Spectro quant assays, Merk Millipore, Darmstadt, Germany (Pharo 100), Katoh et al. (2012). Cation exchange capacity ($CEC_{8,2}$) as sum of titratable acidity ($pH_{8,2}$), CEC_{SA} with 1NKCl and $CEC_{WA} = CEC_{pH8,2} - CEC_{SA}$ (Ganev and Arsova 1980); electrical conductivity (EC) by ISO 11265:2002; soil pH/Eh in soil/water 1:2,5. Total (pseudo-total) forms of heavy metals, through aqua regia digestion (ISO 11466:1995). Extracts with 0,01 M $CaCl_2$ was prepared in a soil:solution ratio (1:5), shaking for 2 hours (Van Ranst et al. 1999). The use of 0,01 M $CaCl_2$ is recommended by Houba et al. (1996), due to the proximity of ionic strength to that of soil solution. Exchangeable heavy metals were determined by extraction with 1M NH_4NO_3 (Zeien and Brummer, 1989) referred to as the German national standard DIN 19730 (1995) (Prüess, 1997). Metals and metalloids were and analytically determined by ICP-OAS *Agilent 5800.*

2.3. Greenhouse Experiment

In 2024 a greenhouse experiment with alfalfa *(Medicago sativa* L.) was performed on the two acidic soils and waste substrates, based on preliminary incubation experiments in a *Climatic Chamber with Phytotron System KK 350 FIT DS–POL-EKO,* where the 20% w/w BC variants were chosen for assessing the effects of increasing (5-20%w/w) BC doses (Atanassova et al. 2024a, Nenova et al., 2025), because in these variants heavy metal immobilization was most pronounced. At the *Asarel* site, also lime ($CaCO_3$) was added due to the strongly acidic reaction. Preliminary soil preparation consisted of drying to air-dry state and sieving (< 2 mm). The pots of 3 kg of contaminated soil and BC (20%), homogenized and ground to 2 mm were mixed together (three replicates). After one month of composting at 75% field capacity (FC), *Rhizon soil moisture samplers, Rhizosphere Research Products, Wageningen, the Netherlands*) for collecting soil solution were installed in each pot at a depth of 10 cm from the soil surface. At the bottom of each pot, a layer of about 1-1,5 cm of perlite as filter material was placed, over which a fine mesh was installed, preventing the roots from passing through. Then the pots were filled with a layer of ~15 cm soil, two samplers were placed parallel to each other at a distance of about 10 cm, after which the pots were filled with soil a few centimetres below the edge. Before the planned collection of soil solution, all containers were watered at 100% of FC. Soil solution was collected at the beginning (before sowing) and at the end of experiment, and the following parameters were analysed: pH, electrical conductivity (EC), dissolved organic carbon (DOC) and anions (NO_3^-, SO_4^{2-}, PO_4^{3-} and Cl^-), K, Na, Ca, Mg, Cd, Cr, Cu, Zn, Pb, Fe, Mn and As. Before sowing the crop, mineral fertilisers were added in a ratio: 1N:1P:0.2S:0.5Ca:0,5K:0,2Mg. In each pot 0.5 g of seeds of *Medicago sativa* L. (alfalfa) were sown consistent with the planting rate of alfalfa, 45 kg/ha. Soil moisture was maintained at 75% of FC.

The greenhouse experimental scheme was the following: *Pirdop* site: P1, control; P1 20% BC; P6, control; P6 20% BC; *Medet* site: M1, control; M1 20% BC; M7, control; M7 20% BC; *Asarel* site: A1, control; A1 BC 20% + 23,3 g $CaCO_3$/kg; A10, control; A10 BC 20% + 20 g $CaCO_3$/ kg. The amounts of liming material in the *Asarel* waste substrate were calculated based on a modified equation for determining the liming rate according to Ganev (1987). After harvesting the alfalfa, soil solution was collected from all the studied variants. All the above-mentioned parameters were analysed in the liquid phase. Plant analyses were performed by EPA – Method 3052 digestion and analysis by ICP-OES.

The BC used in this study is of mixed origin, (birch, sycamore, ash and maple, pyrolyzed at 400–420 °C, Nikimol, OOD, Asenovgrad, (Stoykova et al., 2024). Total organic carbon (TOC) was assessed by the methods mentioned above. In addition, solid-state

NMR spectra were measured on a Bruker Avance III HD 600 NMR spectrometer equipped with a 4 mm dual 1H/X broadband i-CPMAS probe with operating frequency for 1H 599.90 MHz (150.84 MHz for 13C). The BC treated samples were loaded in 4 mm zirconia rotors and spun at magic angle spinning (MAS) rate of 14 kHz for the acquisition of direct excitation ^{13}C spectra with high power ^{1}H decoupling, and at 10 kHz for the CPMAS and TOSS-CPMAS experiments.

3. RESULTS AND DISCUSSION

3.1. Soils Physico-chemical Characteristics and Total (Pseudo-Total) Contents of Metals

Soils near *Aurubis-Pirdop* smelter were classified as Alluvial-diluvial soil (P1) and Leached Cinnamonic forest soil (P6). Soils M1 and M7 were reclaimed soils at the *Medet* site. The cation exchange capacity (CEC) varied in the range of 17,5 – 23,1 cmol kg^{-1}, organic carbon content (TOC) was relatively low (1,02 and 2,9 %). The main pollutant in the area was Cu, with total contents of 2328 mg kg^{-1} (P1) site and 994 mg kg^{-1} (M1) site (Table 2), which was above the maximum permissible levels according to regulatory limits (Regulation No 3 (2008).

At the *Asarel* site a positive effect of liming and combined (BC + $CaCO_3$) effect is manifested, resulting in pH increase to pH ~5,6, rise of CEC, and especially of the strongly acidic exchange sites CEC_{sa}, as well as of the redox potential (Table 1). The BC ameliorant, decreases the salt content from 4,6 – 2,1 to 2,1-1,1 dS/m, respectively in the *Asarel* sites, but still the EC is in the range of slightly to moderately saline soils, (EC~2 dS/m, Schillaci et al., 2025). Some contents for Cu, Cd and Pb (P1) at the sampling sites (Table 2) are above the maximum permissible concentrations (MPC) according to the national regulatory standards (Regulation No 3, 2008). Manganese is also above the background levels for agricultural soils in Bulgaria, but is within the range observed worldwide (Atanassov et., al. 2001, Kabata-Pendias & Pendias, 2001). It is evident from Table 1 that soil environments become more oxidizing primarily due to BC's ability to act as an electron acceptor. Klupfel et al. (2014) find that biochars produced at temperatures (400–700 °C) show the highest capacities to accept and donate electrons, acting as key oxygen-containing functional groups that contribute to their redox properties. The quinone groups embedded within the aromatic network of biochar act as electron shuttle, which can reversibly obtain electrons from microorganisms and transfer them to specific electron acceptors (Jiang et al., 2025), thus enabling the electrons transfer between different microflora. The BC used in our study (solid state NMR, CPMAS and TOSS-CPMAS) indicate that it possesses aromatic C-C and C-H, guaiacyl C-2, C-6 and olefinic carbons in lignin acting as critical components in the electron-accepting capacity of BC. There is evidence that the quinones and poly-condensed aromatic functional groups are the components accepting electrons (oxidants), while the phenolic groups in BC are the main electron donating moieties i.e., reducers (Yuan et al., 2017). The biochar used possesses CEC 10,4 cmol/kg, $TOC_{K2Cr2O7}$ (19.8%) and $TOC_{dry\ combustion}$ (64,3%), (Table 3).

Table 1: Physico-Chemical Properties of the Experimental Soils and Wastes following Composting with Biochar (Before Sowing). Soil pH (SD 0.05-0.2), CEC (SD cmol kg^{-1} 0.03-0.08), TOC (SD % 0.07-0.2), EC electrical conductivity (SE 0,02-0,07 dS/m); oxidation-reduction potential (1-2 mV)

Site	pH/H_2O	TOC	EC	RedOx	$CEC_{8,2}$*	CEC_{SA}*	CEC_{WA}**	Al	Ca	Mg
		%	dS/m	mV	cmol kg^{-1}					
P1 C	4,6	1,5	0,146	110	22,3	16,7	5,6	2,2	12,1	2,1
P1 20%BC	5,4	5,1	0,132	132	23,1	18,3	4,8	1,3	14,2	2,2
P6 C	4,4	1,0	0,126	96	22,0	17,0	5,0	2,8	11,6	2,2
P6 20%BC	5,3	6,1	0,116	120	22,9	18,1	4,8	1,2	14,5	2,2
M1 C	4,4	1,3	0,142	88	17,5	12,5	5,0	1,8	7,8	2,5
M1 20%BC	5,7	5,2	0,118	128	18,5	15,0	3,5	06	11,8	2,5
M7 C	5,0	0,73	0,110	109	17,8	12,8	5,0	1,1	9,1	2,5
M7 20%BC	5,9	6,2	0,100	152	19,6	16,2	3,4	0,2	13,4	2,6
A1 C	2,5	0,42	4,60	-42	41,3	39,5	1,8	22,8	6,6	0,9
A1 20%BC+$CaCO_3$	5,6	5,2	2,13	198	42,2	39,9	2,3	0,5	38,2	0,9
A10 C	3,2	0,57	2,14	-12	33,6	30,2	3,4	14,0	9,2	1,8
A10 20%BC+$CaCO_3$	6,0	5,4	1,19	215	33,8	31,0	2,8	0,0	28,9	2,0

$CEC_{8,2}$ = total CEC at pH 8,2
CEC_{SA} = cation exchange at *strongly acidic sites* of soil/substrate exchanger
CEC_{WA}= cation exchange at *weakly acidic sites* of soil/substrate exchanger

Table 2: Total (pseudo-total) Contents (mg kg^{-1}, SD 2-7 %)

Site	Cu*	Zn	Mn	Fe	Cr	Pb	Cd*	As
	mg kg^{-1}			g kg^{-1}	mg kg^{-1}			
P 1	***2328***	188	3886	58,3	64,32	*281*	***6,80***	86,4
P 6	*425*	124	3568	52,3	69,49	49,7	***5,49***	117
M 1	***994***	89,1	794	38,1	25,18	53,5	***3,89***	**14,2**
M 7	***278***	91,9	614	43,3	49,57	21,1	***4,35***	**3,94**
A 1	***380***	17,9	89,1	33,5	2,21	27,5	***3,37***	**7,05**
A 10	***543***	126	707	45,3	18,73	65,0	***4,86***	**8,22**

*The values for Cu, Cd and Pb (P1) that exceed the MPC for cultivated land and grass land and the intervention concentrations for Cu (P1 and M1) under Regulation No 3 (2008) on the norms for permissible contents of harmful substances in soils, are marked in bold and italics,

Table 3: Biochar Physico-chemical Properties (Stoykova et al,, 2024)

pH /H_2O	CEC8,2	Al	Ca	Mg	TOC $K_2P_2O_7$	TOC ; TON C/N *SCALAR*	EC
	cmol kg^{-1}				%	%	dS/m
8,2	10,4	0,0	7,2	3,2	19,8+/-0,7	C 64,2; N 0,03	0,11

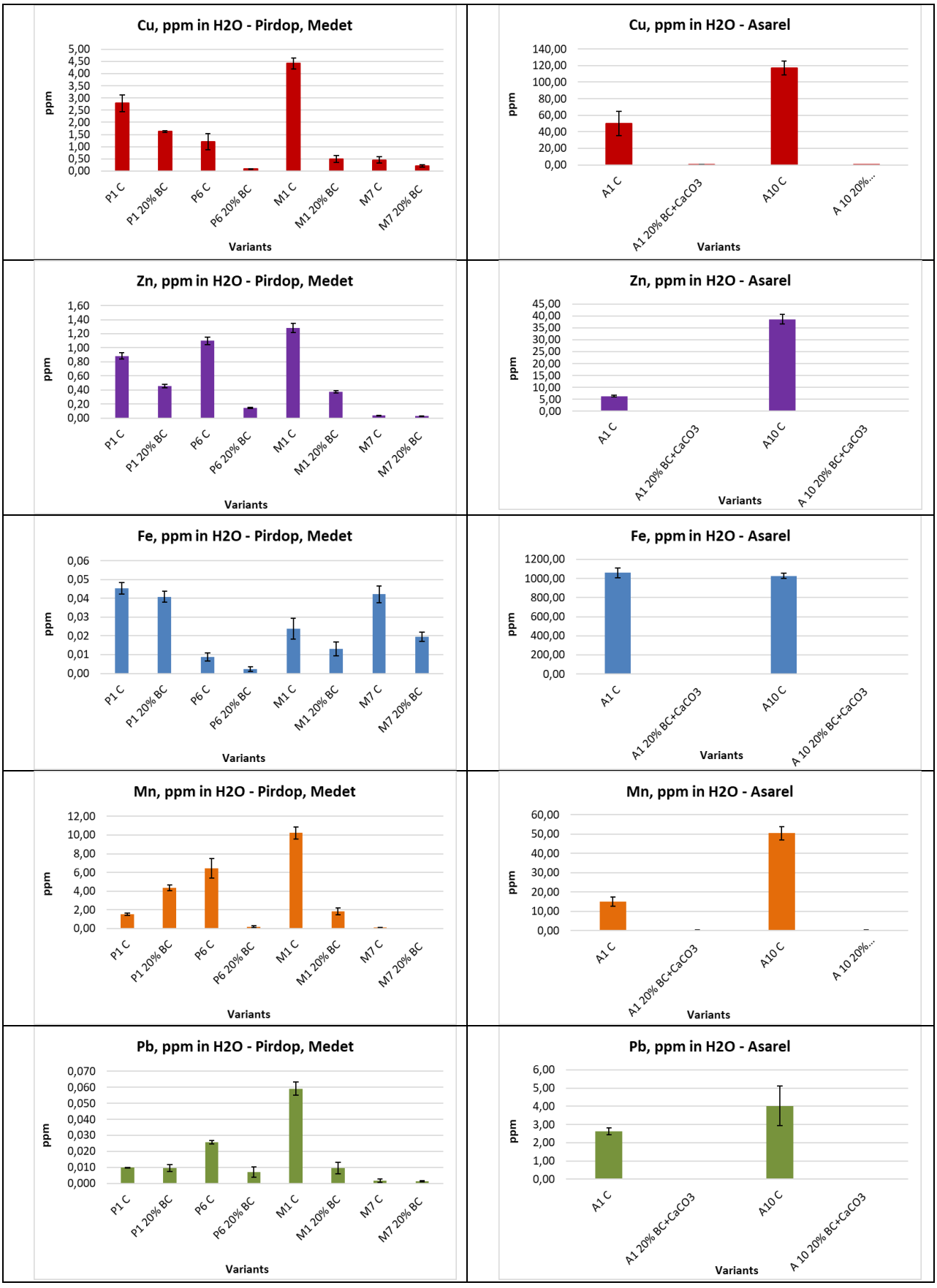
3.2. Predicting Mobility and Bioavailability of Heavy Metals

3.2.1. Heavy Metals in Soil Solution (H2O)

The "*in situ*" monitoring of the soil solution in the present study was performed by the use of *Rhison soil moisture samplers*. The advantages of this technique include its easy installation, high temporal and spatial resolution of monitoring solute movement and quality. Data obtained for the concentrations of the main contaminants in the H_2O solution are presented in Fig. 1.

The Cu content (mg/L) in soil solution from the control soils of the *Pirdop, Medet* and *Asarel* sites varies in a wide range from 0,46 mg/L in *Medet* (M7) to 117,03 ppm in *Asarel* (A10), (Fig 1.) The application of BC significantly reduces the water-soluble copper concentrations in all the studied soils, especially in the substrates from *Asarel* with 20% BC+$CaCO_3$. In (A1) there is a Cu decrease from 50,06 mg/L to 0,10 mg/L in A1-20BC+$CaCO_3$ variant, and even stronger is the immobilization of water-soluble Cu in A10, a decrease from 117,03 mg/L to 0,03 mg/L in A10 with 20%BC+$CaCO_3$.

Water-soluble concentrations of Zn, Pb and Cd also decrease markedly after BC application in all three sites (Fig. 1). Water-soluble forms of Fe in the sites of *Pirdop* and *Medet* are very low, below 0,05 mg/L, only in the control substrates from *Asarel* are the concentrations very high, 1060 mg/L in A1 and 1026 mg/L in A10. For Mn and As in *Pirdop* (P1), mobilization of water-soluble forms was observed after adding 20% BC. The same trend was found for As in the *Medet* site, (M7), an increase in mobility after adding BC. The reasons are owing to a couple of factors: 1) BC induces desorption of hydroxyl ions which compete with arsenate for sorption sites on iron and aluminum oxides in acidic soils; 2) release of dissolved organic carbon (DOC) which can form soluble complexes with arsenic, preventing it from adsorbtion onto soil surfaces and facilitating it's mobility; 3) dissolution of iron oxides in acidic soils through reduction, e.g. BC can serve as an electron donor, stimulating biological or chemical reduction of As bound to F(III) oxides, which also undergo reduction to Fe(II); 4) being a cation exchanger, BC has a net negative charge, which leads to anion repulsion. As pH increases, the surface becomes more negatively charged, therefore repels anionic arsenate ions and prevents their adsorption. In a study by Lomaglio et al., (2017), similarly to the present study, an increase in the concentration of water-soluble forms of As was also found after adding BC due to reactions of desorption from the surfaces of Fe, Mn, and Al oxides and hydroxides most likely due to increase of phosphorus with BC addition, resulting in desorption of As (Moreno-Jimenez et al., 2012).


Cu, ppm in H2O - Pirdop, Medet
ppm
5,00
4,50
4,00
3,50
3,00
2,50
2,00
1,50
1,00
0,50
0,00
P1 C
P1 20% BC
P6 C
P6 20% BC
M1 C
M1 20% BC
M7 C
M7 20% BC
Variants
Cu, ppm in H2O - Asarel
ppm
140,00
120,00
100,00
80,00
60,00
40,00
20,00
0,00
A1 C
A1 20% BC+CaCO3
A10 C
A 10 20%...
Variants
Zn, ppm in H2O - Pirdop, Medet
ppm
1,60
1,40
1,20
1,00
0,80
0,60
0,40
0,20
0,00
P1 C
P1 20% BC
P6 C
P6 20% BC
M1 C
M1 20% BC
M7 C
M7 20% BC
Variants
Zn, ppm in H2O - Asarel
ppm
45,00
40,00
35,00
30,00
25,00
20,00
15,00
10,00
5,00
0,00
A1 C
A1 20% BC+CaCO3
A10 C
A 10 20% BC+CaCO3
Variants
Fe, ppm in H2O - Pirdop, Medet
ppm
0,06
0,05
0,04
0,03
0,02
0,01
0,00
P1 C
P1 20% BC
P6 C
P6 20% BC
M1 C
M1 20% BC
M7 C
M7 20% BC
Variants
Fe, ppm in H2O - Asarel
ppm
1200,00
1000,00
800,00
600,00
400,00
200,00
0,00
A1 C
A1 20% BC+CaCO3
A10 C
A 10 20% BC+CaCO3
Variants
Mn, ppm in H2O - Pirdop, Medet
ppm
12,00
10,00
8,00
6,00
4,00
2,00
0,00
P1 C
P1 20% BC
P6 C
P6 20% BC
M1 C
M1 20% BC
M7 C
M7 20% BC
Variants
Mn, ppm in H2O - Asarel
ppm
60,00
50,00
40,00
30,00
20,00
10,00
0,00
A1 C
A1 20% BC+CaCO3
A10 C
A 10 20%...
Variants
Pb, ppm in H2O - Pirdop, Medet
ppm
0,070
0,060
0,050
0,040
0,030
0,020
0,010
0,000
P1 C
P1 20% BC
P6 C
P6 20% BC
M1 C
M1 20% BC
M7 C
M7 20% BC
Variants
Pb, ppm in H2O - Asarel
ppm
6,00
5,00
4,00
3,00
2,00
1,00
0,00
A1 C
A1 20% BC+CaCO3
A10 C
A 10 20% BC+CaCO3
Variants

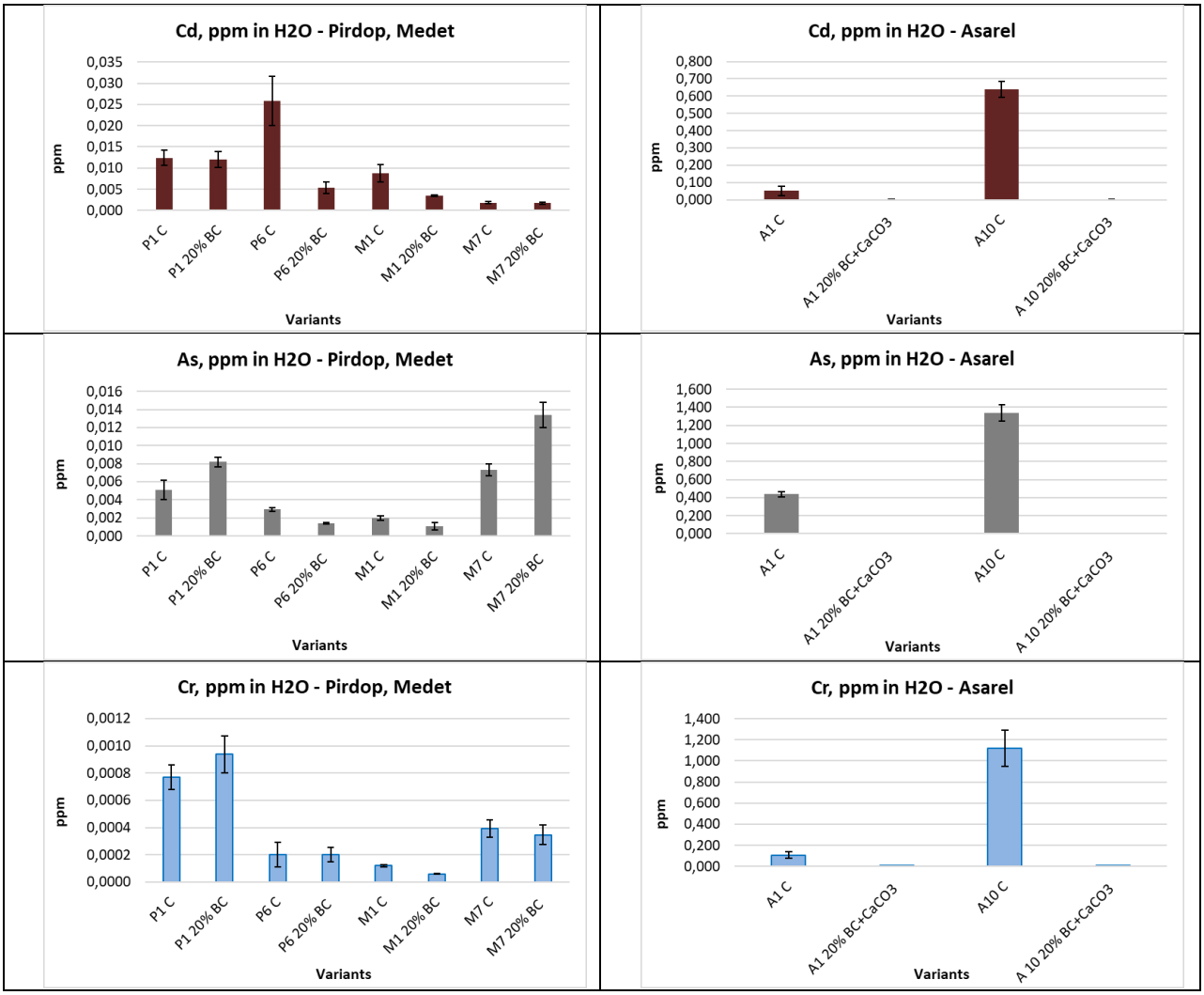


Figure 1: Heavy metals in soil solution (mg/l (+/-SE)

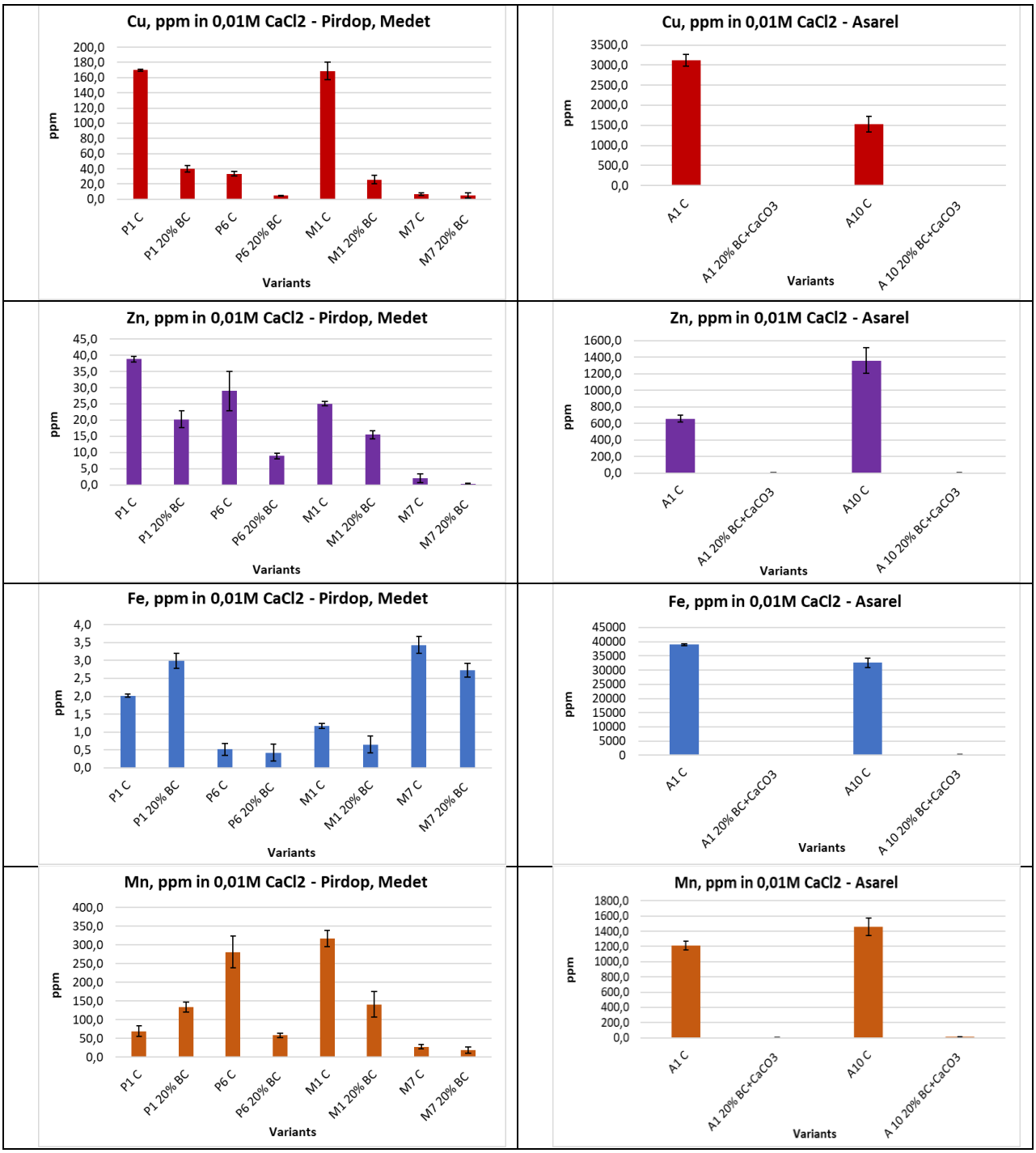
Adding BC leads to increasing Mn in soil solution in *Pirdop* P1 site and simultaneously immobilizing copper Cu due to their different chemical behaviour and mainly differences in bonding mechanisms to soil components and biochar. Manganese in soil solution usually exists as Mn^{2+}, it is electrostatically attracted to the negatively charged surfaces of soil colloids and is exchangeably adsorbed, therefore it can be replaced by other cations like Ca^{2+} and Mg^{2+}. Manganese is the least susceptible to hydrolysis due to its lowest hydrolysis constant (pK_h = 10,6, Baes and Mesmer, 1976). In addition, BC application can host microbes and stimulate microbial processes that lead to reduction of insoluble Mn(IV) oxides to soluble Mn, thus increasing Mn mobility (Bolan et al., 2023). In the remaining soils and substrates studied, the addition of BC significantly reduced the concentrations of water-soluble forms of Mn and As. The trends are identical to those found in the extracts with 0,01M $CaCl_2$, with the latter extracting more than one order of magnitude higher concentrations of the studied metals compared to the soil solution.

3.2.2. Heavy Metals in 0,01M CaCl2 Extracts

Data on the concentrations of heavy metals and metalloids in the soils of the *Pirdop, Medet* and *Asarel* sites, determined in the extracts with 0,01M $CaCl_2$ are given in Fig. 2.

In accordance with the high concentrations of Cu in the control soils *Pirdop* P1 (Cu 2328 mg/kg) and *Medet* M1 (Cu 994 mg/kg), the contents of mobile forms of Cu in the extracts with 0,01M $CaCl_2$ in the control samples from these two soils are high, 170 mg/L and 169 mg/L, respectively. The addition of 20% BC w/w leads to a significant reduction in mobile Cu in variant P1-20% BC, therefore decreasing more than 4 times to 39,8 mg/L. In the *Medet* soil (variant M1, 20% BC), the concentration decreases to 25,8 mg/L or 6,5 times lower, compared to the untreated control. In the *Asarel* site, mobile Cu in the extract with 0,01M $CaCl_2$ in the controls is very high, ~ 3122 mg/L in A1 and 1525 mg/L in A10 (Fig. 2). The addition of BC+$CaCO_3$ lead to fixation of copper (2,0 – 2,2 mg/L Cu were detected in the extract with 0,01M $CaCl_2$), i.e. Cu decreased 1560 and 693 times, respectively, compared to the control variants.

The behaviour of Zn and Pb after the addition of BC is very similar to that of copper. The introduction of 20% BC led to a significant reduction and fixation of mobile forms of the studied heavy metals. Considering that contents of Zn and Pb in the studied soils are not as high as of copper, the mobile concentrations recorded in the extract with 0,01M $CaCl_2$, are lower. In the controls from the *Pirdop* and *Medet* sites, Zn in 0,01M $CaCl_2$ ranges from 38,8 ppm (P1) to 2,1 ppm (M7). The addition of BC caused a decrease of 34% and 134% of mobile Zn, respectively. In the *Asarel* site, again the Zn values are much higher than in *Pirdop* and *Medet* sites (Fig. 2), 655 mg/L in A1 and 1357 mg/L in A10, respectively. However, the addition of BC+$CaCO_3$ reduced the mobile forms of Zn to 0,5 and 0,7 mg/L. The concentrations of Pb in the extract with 0,01M $CaCl_2$ in the soils of *Pirdop and Medet* are between 0,28 (P1) and 0,06 (M7) ppm, while in *Asarel* they are an order of magnitude higher, e.g. in A1 (Pb 3,85 mg/L) and A10 (Pb 1,97mg/L). The addition of BC+$CaCO_3$ in *Asarel* significantly reduced their bioavailable concentrations (Fig. 2).



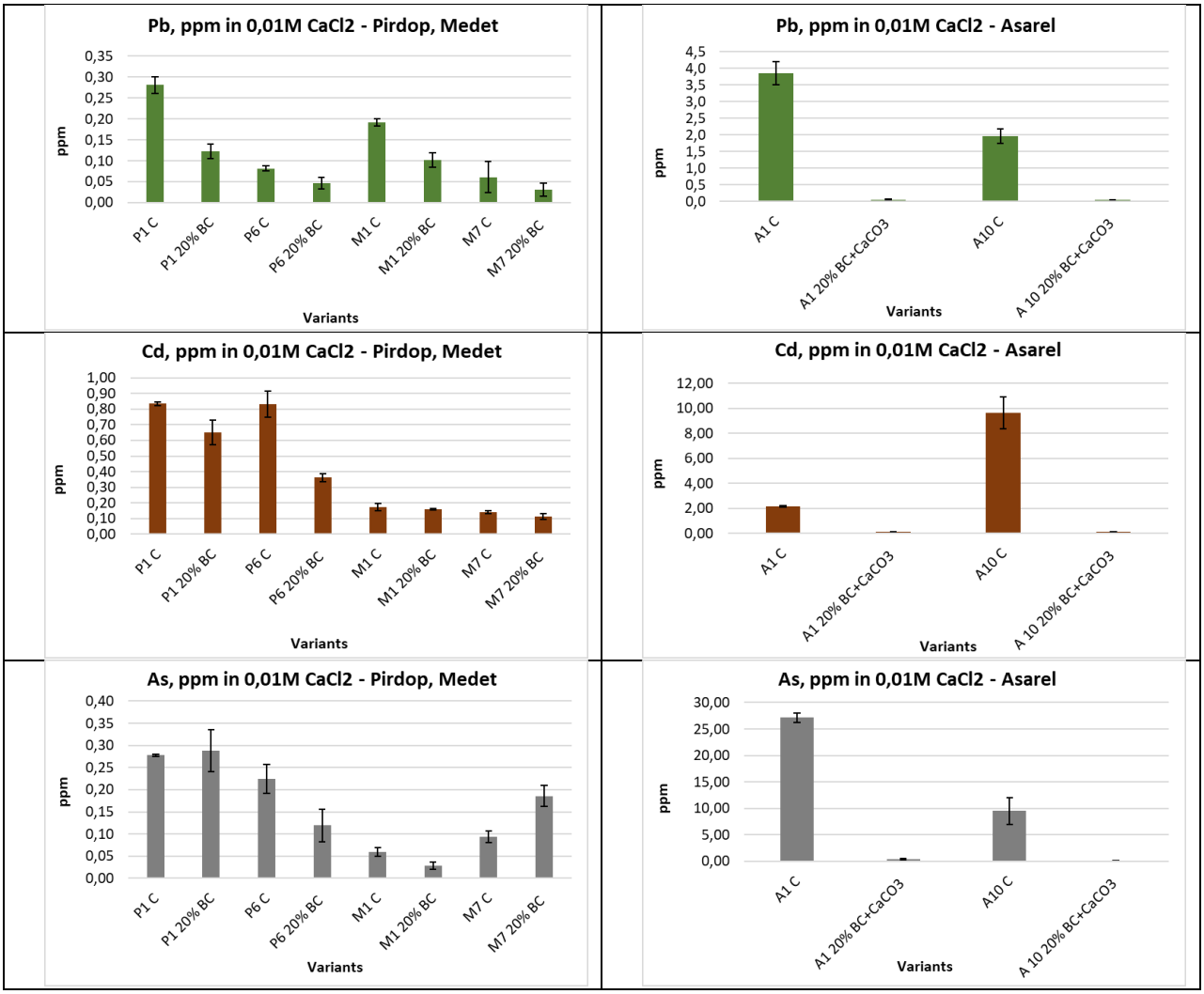


Figure 2: Heavy Metals in 0,01MCaCl$_2$ solution (+/-SE)

Regarding Fe, Mn in *Pirdop* (P1), the mobility of the two elements increased after the addition of BC. For as, the increase is insignificant from 0,28 ppm in *Pirdop* P1 to 0,29 mg/L (P1+20%BC), which is within the SD, but in M7 of *Medet,* especially for as, there was an increase in from 0,09 mg/L (M7) of 0,19 mg/L (M7-20%BC). Iron in *Pirdop* P1 increased from 2,0 mg/L (P1) to 3,0 mg/L (P1+20%BC), and Mn from 69,1 mg/L (P1) to 135 mg/L (P1+20%BC). In fact, only in this particular site of *Pirdop* P1 there was an increase in mobile forms of Fe, Mn after adding BC. The reasons must be again, release of DOC, which can facilitate the reduction and dissolution of ferric oxides, especially in acidic environments with high iron oxide content. In the other studied soils, as well as in the substrates from *Asarel*, the introduction of BC reduced the concentrations of mobile forms of Fe, Mn and as,

3.2.3. Heavy Metals in 1M NH4NO3 Extracts

In all the variants (P1, P6, M1, M7, A1, A10) of the three sites *Pirdop, Medet and Asarel*, a large decrease in the concentrations of the studied metals in the extracts of 1M NH_4NO_3 was observed with the addition of 20% BC or a combination of BC and $CaCO_3$ in *Asarel* (Fig. 3). The largest decrease was observed for Cu. In the *Pirdop* site, Cu decreased by 74% from 81,4 to 21,2 mg/kg (P1), and in the *Medet* by 74% from 40,8 to 10,5 mg/kg (M1). A drastic decrease in the mobility of Cu and Zn was observed in the *Asarel* sites (A1 and A10) and the efficiency of heavy metal immobilisation reached 99,8% (e.g. Zn decreased from 30,9 to 0,05 mg/kg in variant A1). High reductions in Pb concentrations (over 60% - 90%) were also reported, through fixation by adsorption on BC's porous surface (Beesley et al., 2011). The addition of $CaCO_3$ increased the pH, which additionally lead to specific sorption of heavy metals (Atanassova & Okazaki, 1997; Atanassova, 1999), making them unavailable to plants.

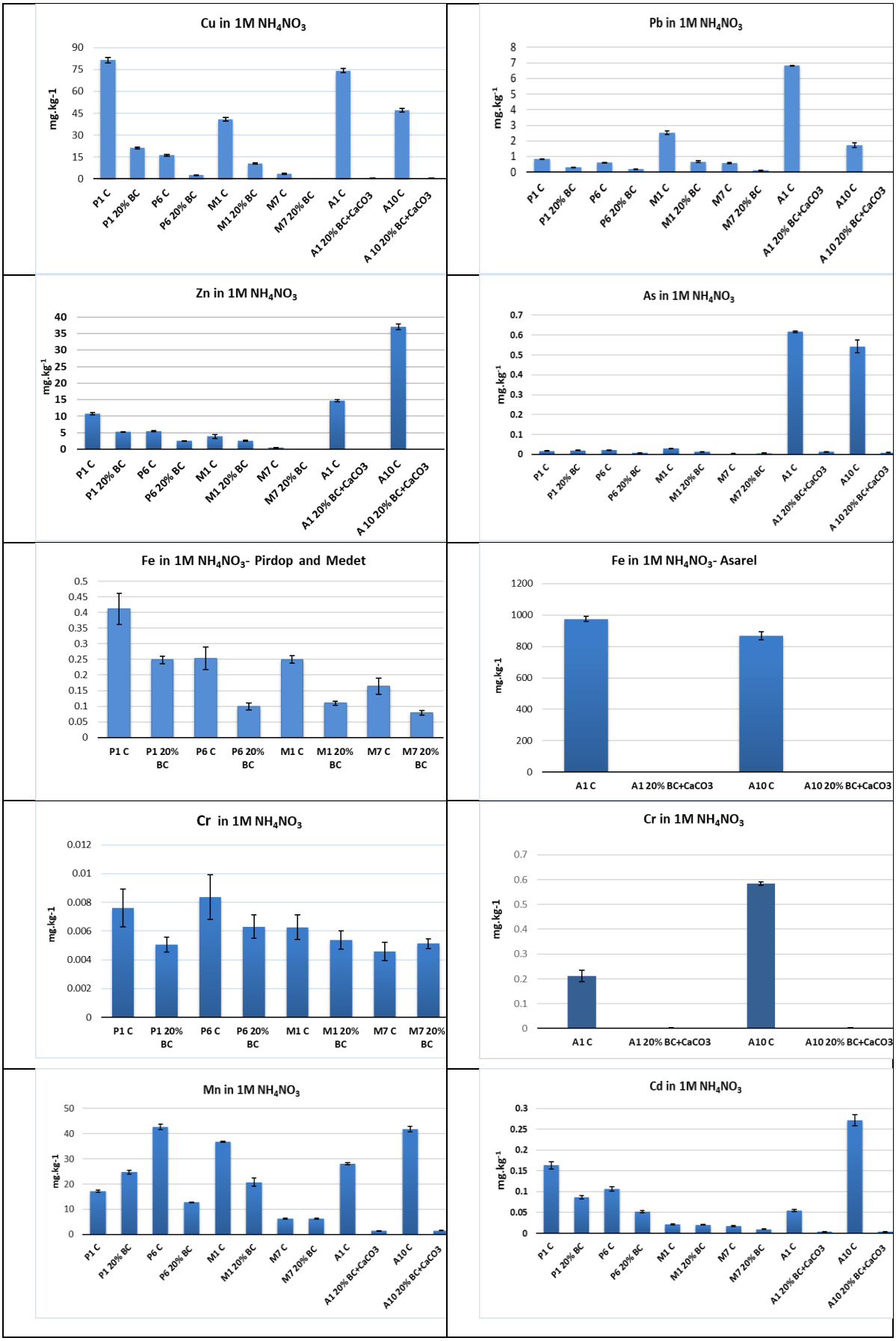


Figure 3: Heavy Metals in 1M NH4NO3 Extracts

In the variants P1 and M7, the concentration of Mn increases or remains stable when BC is added. This again, can be explained by the higher pH, introduction of Ca^{2+} and Mg^{2+} from BC, which stimulates the release of Mn^{2+} before fixing them (Xu et al., 2022), However, when $CaCO_3$ is also added (*Asarel*), Mn is almost completely fixed (decline from 41,7 to 1,5 mg/kg), This confirms the fact that alkalization is the leading factor for its fixation. In the variants A1 and A10, the Fe levels in the initial samples were extremely high (869 – 974 mg/kg), but after BC and $CaCO_3$ addition, Fe decreased below 0,2 mg/kg, indicating almost complete adsorption and fixation of Fe. In the variants P1 and M7, a slight increase in as was observed, following BC application. This increase in as mobility (15% and 65%, respectively in P1 and M7) was confirmed also by studies by Namgay et al. (2010), which conclude that arsenate competition with hydroxyl and phosphate ions for sorption sites can lead to as mobilisation.

The results show that the combined application of BC and $CaCO_3$ in waste mine tailings (variants A1 and A10, *Asarel*) leads to almost complete immobilization of mobile forms of metals.

3.2.4. Plant Biomass Yields and Metal and Metalloids' Contents

Statistically significant increases in above-ground biomass, often exceeding 4-13 times (M1) the original control yields were achieved, while at the *Asarel* tailings lack of biomass yields without amelioration was noted (Fig. 4). Biochar incorporation with additional liming (*Asarel*) led to increase of soil pH and cation exchange capacity (CEC), decrease of electrical conductivity and achieved toxic heavy metal immobilization (Table 1., Fig.1, 2 and 3), which may also be due to enhanced soil nutrient retention as described in our previous studies (Benkova et al., 2023).

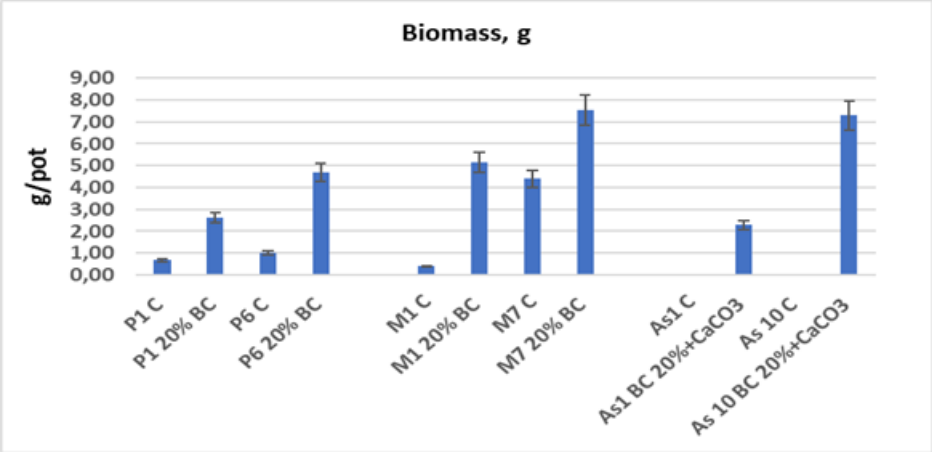
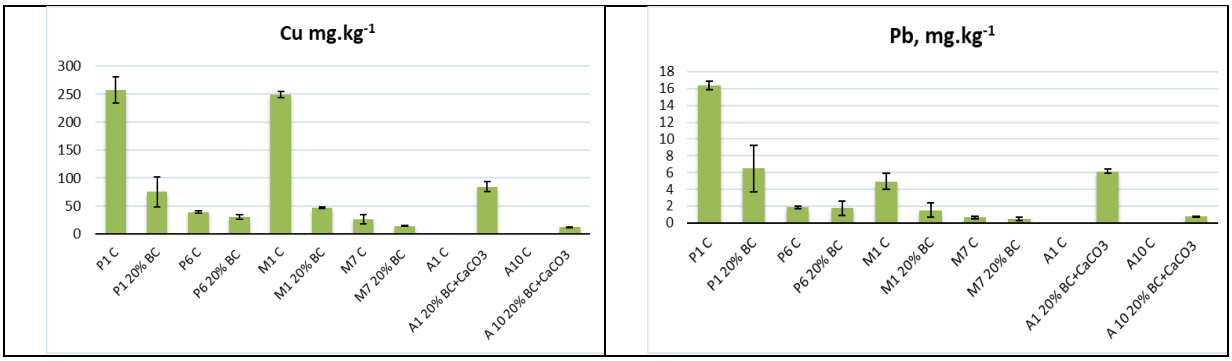


Figure 4: Biomass Yields (+/- SE)

The largest decreases in heavy metals in plant tissue concentrations upon treatment with 20% BC occurred for Cu, Pb, Zn, Mn and Fe in the acidic Pirdop and Medet soils (except Cu and Pb in P6, and Mn and Zn in P1), while As increased upon treatment in P6 (Pirdop) and M7 and M1 (Medet + 20% BC) (Fig. 5) similarly to the results of Chen et al., (2018). According to Kabata-Pendias (2010), normal heavy metal concentrations in mg/kg dry weight generally fall within these ranges: Pb (5–10), Cu (5–30), Zn (25–150), Mn (30–300), Fe (20–300) and As (0,01 – 1,0), which in this study indicates toxicity of Cu in the sites P1, (Pirdop) and M1(Medet) and of As in P1, M1 and A1 (Asarel), toxic levels of Fe and Mn in the control variants in all the samples analysed, except in A10 (20% BC+CaCO3), and of Pb in P1(Fig. 5).



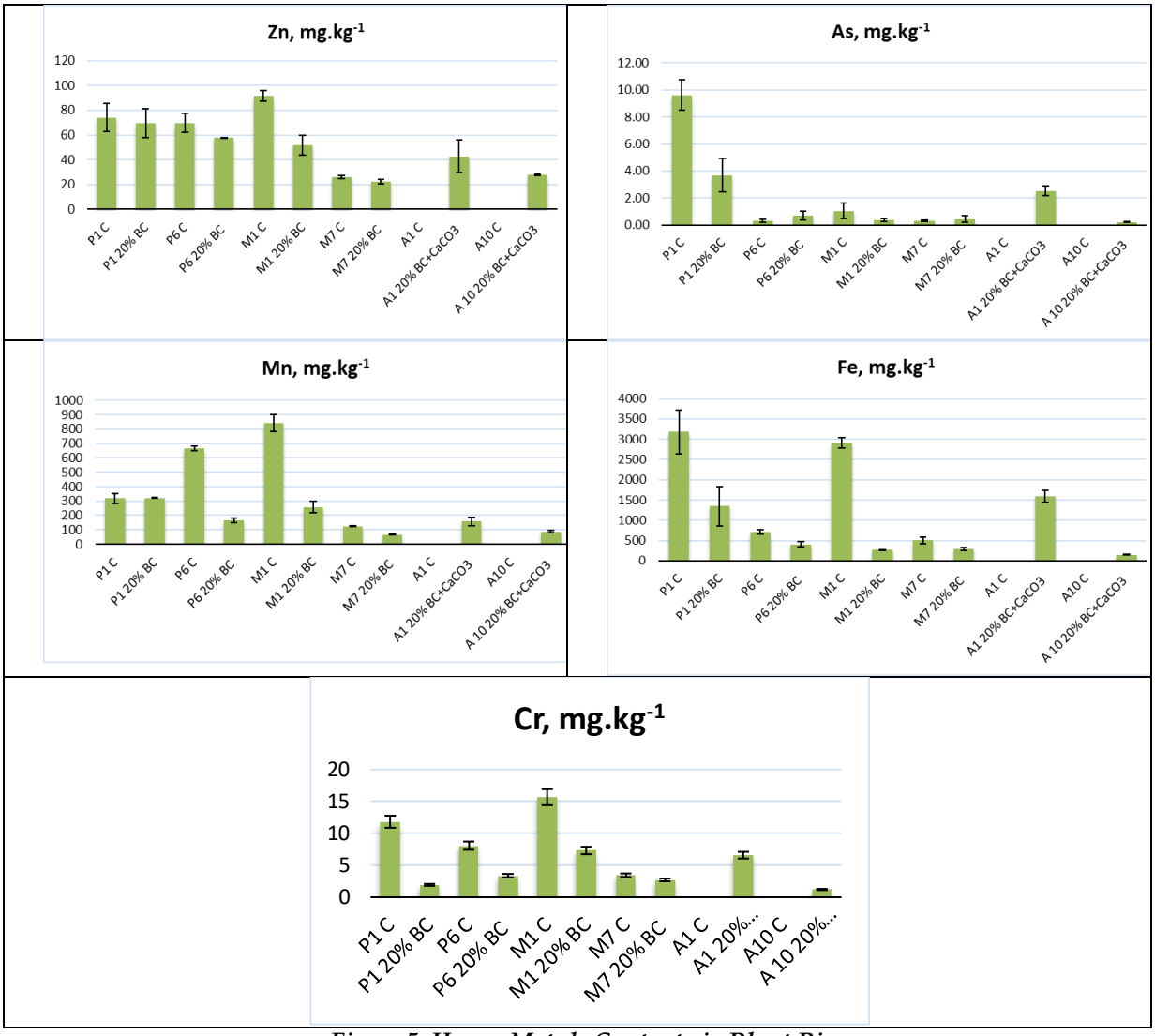


Figure 5: Heavy Metals Contents in Plant Biomass

Despite the significant decrease in soil bioavailable metal pools, alfalfa growth declined with time in the BC treatments, as well. This might be attributed to adsorption of available nitrogen on to the BC resulting in nitrogen deficiency. Therefore, it should be considered that when BC is used for metal immobilization in agricultural soils, maintaining soil macro-nutrient optimal status is of crucial importance to ensure normal growth of plants.

3.2.5. Relationships between Plant Biomass Contents and Bioavailable Heavy Metals

Data processing was carried out for metals behaviour at the three experimental areas of investigation. Simple and multiple regression models were presented on Table 4. assessing relationships between heavy metals concentrations in plant biomass at the end of the experiment and the concentration of Cu, Zn and Mn in the soil solution. For the multiple regression model total organic C % in soil was additionally used as an independent variable. The simple linear regression model was selected because regarding copper which is the main pollutant in the investigated areas, the linear model yields the highest R^2 of 71,44%. Simple regression was performed also for the elements Fe, Pb and as, but no significant relationship between the soil solution and plants' concentrations for those HMs was found.

Table 4: Simple Regression (SR) and Multiple Regression Models (MR) of Selected Heavy Metals in Plants and Soil Solution Concentrations

Analysis	Dependent_independent variable	P-value	R^2 (%)	Equation of the fitted model
SR	Cu-plant_Cu-H_2O	0,0000	71,44	Cu-Plant = 16,94 + 0,05*Cu-H_2O
MR	Cu-plant_Cu-H_2O_C %	0,0003	71,44	Cu-Plant = 15,9 + 0,05*Cu-H_2O + 0,1724* C
SR	Zn-plant_Zn-H_2O	0,0001	68,79	Zn-Plant = 33,43 + 0,05*Zn-H_2O
MR	Zn-plant_Zn-H_2O_C %	0,0000	78,25	Zn-Plant = 17,25 + 0,05*Zn-H_2O + 2,62*C
SR	Mn-plant_Mn-H_2O	0,0000	88,04	Mn-Plant = 123,9+ 0,06*Mn-H_2O
MR	Mn-plant_Mn-H_2O_C %	0,0000	90,04	Mn-Plant = 185,5 + 0,06*Mn-H_2O - 10,96*C

Table 5: Simple Regression (SR) and Multiple Regression Models (MR) of Selected Heavy Metals in Plants and 0,01M $CaCl_2$

Analysis	Dependent/independent variable	P-value	R^2 (%)	Equation of the fitted model
SR	Cu-plant_Cu-$CaCl_2$	0,0000	91,69	Cu-plant = 22,48 + 0,001*Cu-$CaCl_2$
MR	Cu-plant_Cu-$CaCl_2$ and OC %	0,0000	91,89	Cu-Plant = 15,65 + 0,001*Cu-$CaCl_2$ + 1,17*C
SR	Zn-plant_Zn- $CaCl_2$	0,0000	71,35	Zn-plant = 30,7576 + 0,0016*Zn-$CaCl_2$
MR	Zn-plant_Zn- $CaCl_2$ and OC %	0,0002	73,09	Zn-plant = 24,65 + 0,002*Zn-$CaCl_2$ + 1,05*C
SR	Mn-plant_Mn- $CaCl_2$	0,0000	88,00	Mn-Plant = 83,3 + 0,002*Mn-$CaCl_2$
MR	Mn-plant_Mn-$CaCl_2$ and OC%	0,0000	89,77	Mn-plant = 143 + 0,0017*Mn-$CaCl_2$ - 10,31*C
SR	Pb-plant_Pb-$CaCl_2$	0,0003	61,95	Pb-plant = -0,116617 + 0,0441*Pb-$CaCl_2$
MR	Pb-plant_Pb-$CaCl_2$ and OC%	0,0017	62,47	Pb-plant = -0,77 + 0,05*Pb-$CaCl_2$ + 0,11*C
SR	As-plant_As-$CaCl_2$	0,0163	34,70	As-plant = -0,39 + 0,01*As-$CaCl_2$
MR	As-plant_As-$CaCl_2$ and OC%	0,0444	38,06	As-plant = 0,3358 + 0,01*As-$CaCl_2$ - 0,16*C

The simple linear regression between the Cu content in plants and 0,01M $CaCl_2$ extracts shows a strong significant relationship R^2 = 91,69 % (p≤0,001, Table 5). The comparison between the relationship in 0,01M $CaCl_2$ and in soil solution shows a stronger relationship in 0,01M $CaCl_2$. This finding indicates that 0,01M $CaCl_2$ can serve as a more reliable predictor of the Cu availability in plants than water. Adding total organic carbon (TOC%) as independent variable in the multiple regression model slightly improves the relationship (R^2 = 91,86%). The regression relationships between the content of Zn and Mn in plants and in the extracts of 0,01 M $CaCl_2$ are strong and significant (R^2 = 71,35% and 88,00%, p≤0,001). Adding the values for % TOC in the multiple regression slightly improves the degree to which the approximated model explains the variability of Zn and Mn in plants. Unlike the soil solution, the regression analysis of Pb and As in 0,01M $CaCl_2$ in relation to their contents in plants shows a significant relationship. For Pb, the simple regression relationship shows that 61,95% of the variability of the Pb concentration in plants is determined by the content of this element in the extracts, while for As this percentage is only 34,7%. No relationship was found between the concentration of iron (Fe) in the extracts and those reported in plants.

The simple regression analysis for Cu and Zn, in relation to their concentration in 1M NH_4NO_3, shows a significant relationship (p≤0,05), but the R^2 = 78,95% for Cu and 53,05% for Zn, are lower than those obtained in the extracts of 0,01M $CaCl_2$. The multiple regression after adding the total organic carbon (TOC %) in the equation does not noticeably improve the relationship between the studied parameters (Table 6). Similarly, to the results obtained for water and 0,01M $CaCl_2$ extracts, no significant relationship is established between the content of iron Fe in plant biomass and its concentrations in 1M NH_4NO_3. For Mn, a significant regression relationship R^2 = 81,43% (p≤0,01) was observed, and for the studied heavy metals the relationship between the concentrations in 1M NH_4NO_3 and those in the plant tissue, is the strongest. For Pb and as insignificant relationships were obtained in 1M NH_4NO_3 extracts.

Table 6: Simple Regression (SR) and Multiple Regression Models (MR) of Selected Heavy Metals in Plants and 1M NH_4NO_3

Analysis	Dependent_independent variable	P-value	R^2 (%)	Equation of the fitted model
SR	Cu-plant_Cu-NH_4NO_3	0,0000	78,95	Cu-plant = 20,1 + 0,01*Cu-NH_4NO_3
MR	Cu-plant_Cu-NH_4NO_3 and OC %	0,0000	78,96	Cu-plant = 18,6+0,01*Cu-NH_4NO_3+0,2696*C %
SR	Zn-plant_Zn- NH_4NO_3	0,0014	53,05	Zn-plant = 34,7 + 0,01*Zn-NH_4NO_3
MR	Zn-plant_Zn-NH_4NO_3 and OC%	0,0068	53,55	Zn-plant = 31,6+0,02*Zn-NH_4NO_3+ 0,5542*C %
SR	Mn-plant_Mn- NH_4NO_3	0,0000	81,43	Mn-plant = 22,1 + 0,04*Mn-NH_4NO_3
MR	Mn-plant_Mn- NH_4NO_3 and OC%	0,0000	82,91	Mn-plant = 80,38+0,04*Mn-NH_4NO_3 - 9,51*C %

In general, the extraction with 0,01M $CaCl_2$ provides the most satisfactory relationship with the content of HM and as in plants in the studied contaminated soils and substrates. Unlike the other two extractants, H_2O and 1M NH_4NO_3, in which the simple regression is significant only for the elements Cu, Zn and Mn, in the extraction with 0,01M $CaCl_2$ there is also a significant relationship for Pb and As. The highest R^2 (CD) in all three extracts were obtained for the elements Cu and Mn in the simple linear regression models.

Adding TOC % as an independent variable in a multiple regression model improves the model predictability for the heavy metal(oid)s content especially for Zn, in the water extracts, which better explains the variability of Zn in plants, compared to the simple regression model.

3.3. Geochemical Modelling for Predicting Metal Species' Distribution in Soil Solution

The concentrations of major ions in soil solution were used for geochemical modelling of metal speciation. Metal species distributions for the soils *Pirdop 1 (P1), Medet (1)* and the waste *Asarel* 10 (A10) generated by *Visual Minteq* are represented in Tables 7, 8 and 9.

The Stockholm humic model (SHM) was used for modelling DOC properties (Stoykova et al., 2024). It is evident that in the acidic *Pirdop* and *Medet* soils, BC incorporation except increasing pH leads to an increase of metal organic complexes of Cu (Table 7) from 57% in the control variant P1 to 84% (P1+20% BC), and for Pb from 47% to 73,6%, while free Cu^{2+} ions decreased from 38% to 12,8%, while free Pb^{2+} decreased from 39,3% to 15,8%. Organic complex formation for Zn (an increase from 10% to 21,3%) was lower, and least for Mn, as free Mn^{2+} ions decreased only by 8,9% due to BC addition. Even higher was the Cu^{2+} ions decrease in the *Medet* soil, from 65,2% to 3,9% and the fulvic acid complexation increased from 28,7% to 95% (Table 8). For Zn, predominance of organic complex formation in the *Medet* (M1) soil+20% BC is noted, as well. Fulvic acid as a major, highly reactive ligand controls the solubility, mobility, and bioavailability of heavy metal ions in aquatic environments and soils by forming stable complexes with them, reducing their toxicity and limiting the concentration of free, uncomplexed cations. In numerous studies (Wu et al., 2000); Ren et al., 2015), the free metal species M^{n+} are most mobile in soil solutions and ground water and are also the major bioavailable species to plants and soil biota, thus being the major bioavailability indicator rather than the total metal concentration (sum of free ion species and complexes).

The fractions of various species of metals differed markedly among the control variants and the 20% BC-amended soils (Tables 7-9). It can be noted that the metal species complexed with soluble organic ligands (represented as /FA-Cu^{+2}G(aq); /FA_2Cu(aq); /FACu^{+}(aq)) prevailed (esp, for Cu and Pb in the solutions of BC-amended soils, with concomitant decrease of M^{2+} ions. Manganese (Mn) showed the slightest decrease of the free Mn^{2+} species and the lowest increase of organic ligand complexation due to the lower stability of Mn complexes with FA ligands and the lowest hydrolysis constant at pH 5,6-5,7 (pKh_1 = 10,6 compared to the other heavy metals (Baes and Mesmer, 1976). For iron Fe(III), there was no significant effect of BC on the presence of organic species, since Fe principally forms strong complexes with organic ligands in soil solution. The chemical equilibrium modelling in *Visual MINTEQ (SHM*) for iron, indicates limited or no complexation in the "gel" fraction of fulvic acid at low pH, favouring more aqueous, hydroxy-fulvic species FA_2FeOH(aq).

The Stockholm Humic Model (SHM) in *Visual MINTEQ* is used to describe the complexation of heavy metals with fulvic acids (FA), (Gustafsson, 2001). It allows for prediction of free heavy metal ion concentrations (e,g,, Cd, Cu, Pb, Zn, etc,) by accounting for mono- and bidentate binding to carboxylic and phenolic sites, while considering electrostatic effects that change with ionic strength and pH. The model assumes that fulvic and humic acids represent a "gel-like" molecule, and the binding is calculated for metal-organic complexation, including monodentate and bidentate coordination. For Fe complexation we interpret the obtained results to the extreme stability of aqueous organic species and the specific assumptions of the thermodynamic model, rather than a lack of affinity for the "gel" like fraction of humic substances.

In studies of Smebye et al. (2016) it is speculated that BC micropores can act as size exclusion sieve resulting in exclusion of larger DOM of aromatic properties, therefore increasing DOM leached from soil.

Comparing data from metal speciation through geochemical modelling, soluble and bioavailable metals in soil solution and the extracts, as well as biomass contents (Fig. 5) it can be concluded that metal contents in plant biomass decrease, because the formation of stable, high-molecular-weight organometallic complexes can reduce their bioavailability (Walker et al., 2003) due to the following mechanisms: 1) increased organic complexation shifts the equilibrium away from free ions towards complexed forms, lowering the "bioavailable" fraction, as plant roots primarily take up free hydrated metal ions; 2) humic substances (humic and fulvic acids) form inner-sphere complexes. These complexes, particularly with humic acids, are often large and stable, immobilizing the metals and preventing them from being absorbed by root membranes (Wang et al., 2020; Maqbool et al., 2024); 3) increased soil pH (5,3-5,9) promotes the formation of specifically sorbed species of metals (Atanassova, 1999; Atanassova & Okazaki, 1997) provoking tighter binding to soil components, thus reducing metal availability.

Gu et al., (2020) found that different BCs may decrease the mobility of metals and increase that of metalloids in tailings through decreasing phytotoxicity < 55,8% or increasing it < 20,7%, increase microbial activities < 28,3% or decrease it <

24,0%. These authors also found that release of metalloids may compromise BC treatments on mine tailings.

In this study soil solutions from the BC-untreated materials from the mine tailings at *Asarel* site were dominated by the sulphate MSO_4^0(aq) complexes followed by M^{2+} free ions, with a reversal of dominance in the BC-treated variants (Table 9). The concentration of SO_4^{2-} in the control variants reached 20-30 g/L, which decreased 10-13 times following BC and lime incorporation, but still exceeds by a factor of 20-100 the sulphate concentrations in soil solutions of normal soils (15-40 mg/L) for optimal plant growth, and EC < 2 dS/m (Calvo et al., 2009). In spite of the increase of free more bioavailable M^{2+} species, the general improvement of the physico-chemical soil characteristics (Table 1), pH, CEC, EC, TOC and RedOx potential led to seed germination and yield registration (Fig. 4). Arsenic (As) is present as neutral species H_3AsO_3 (As^{3+}) at pH 2,3 and reducing conditions (Table 9), it is less strongly sorbed to sesquioxides than $H_2AsO_4^-$, and is therefore highly mobile.

Table 7: Heavy metals species distribution in soil solution of the Pirdop 1 Soil (SE 2-7%)

P1 control pH 4,9	**% of total**	**Species**	**P 1 20% BC pH 5,6**	**% of total**	**Species**
Cu^{+2}	38,006	Cu^{+2}	Cu^{+2}	12,793	Cu^{+2}
	0,067	CuOH+		0,104	CuOH+
	0,094	$CuCl^+$		0,049	$CuCl^+$
	4,131	$CuSO_4$ (aq)		3,036	$CuSO_4$ (aq)
	0,5	$CuNO_3$+		0,177	$CuNO_3$+
	0,234	/FA-Cu^{+2}G(aq)		0,13	/FA-Cu^{+2}G(aq)
	21,994	/FA_2Cu(aq)		46,289	/FA_2Cu(aq)
	34,945	/FACu^+(aq)		37,096	/FACu^+(aq)
	0,028	/FA_2CuOH(aq)		0,326	/FA_2CuOH(aq)
Fe^{+3}	0,024	$FeOH^{+2}$	Fe^{+3}	0,318	$Fe(OH)_2^+$
	0,245	$Fe(OH)_2^+$		99,36	/FA_2FeOH(aq)
	98,029	/FA_2FeOH(aq)		0,315	/FA_2Fe^+(aq)
	1,702	/FA_2Fe^+(aq)			
Mn^{+2}	90,299	Mn^{+2}	Mn^{+2}	81,358	Mn^{+2}
	0,112	$MnCl^+$		0,156	$MnCl^+$
	7,618	$MnSO_4$ (aq)		14,989	$MnSO_4$ (aq)
	0,596	$MnNO_3^+$		0,565	$MnNO_3^+$
	0,556	/FA-Mn^{+2}G(aq)		0,83	/FA-Mn^{+2}G(aq)
	0,811	/FAMn^+(aq)		2,092	/FAMn^+(aq)
Pb^{+2}	39,306	Pb^{+2}	Pb^{+2}	15,794	Pb^{+2}
	0,242	/FA-Pb^{+2}G(aq)		0,161	/FA-Pb^{+2}G(aq)
	0,055	$PbOH^+$		0,102	PbOH+
	1,766	$PbCl^+$		1,099	PbCl+
	9,133	$PbSO_4$ (aq)		8,014	$PbSO_4$ (aq)
	0,068	$Pb(SO_4)_2^{-2}$		0,167	$Pb(SO_4)_2^{-2}$
	2,419	$PbNO_3^+$		1,024	$PbNO_3^+$
	0,022	$Pb(NO_3)_2$ (aq)		0,01	$Pb(NO_3)_2$ (aq)
	39,589	/FAPb+(aq)		45,181	/FAPb^+(aq)
	7,394	/FA_2Pb(aq)		28,443	/FA_2Pb(aq)
Zn^{+2}	80,463	Zn^{+2}	Zn^{+2}	62,835	Zn^{+2}
	0,496	/FA-Zn^{+2}G(aq)		0,641	/FA-Zn^{+2}G(aq)
	0,287	$ZnCl^+$		0,016	$ZnOH^+$
	8,352	$ZnSO_4$ (aq)		0,347	$ZnCl^+$
	0,09	$Zn(SO_4)_2^{-2}$		14,242	$ZnSO_4$ (aq)
	0,841	$ZnNO_3^+$		0,429	$Zn(SO_4)_2^{-2}$
	9,446	/FAZn^+(aq)		0,692	$ZnNO_3^+$
	0,02	/FA_2Zn(aq)		20,686	/FAZn^+(aq)
AsO_4^{-3}	0,219	H_3AsO_4	AsO_4^{-3}	0,04	H_3AsO_4
	1,145	$HAsO_4^{-2}$		5,991	$HAsO_4^{-2}$
	98,636	H_2AsO_4-		93,969	H_2AsO_4-

Table 8: Metal species distribution in soil solution of the Medet 1 (M1) soil

M1 Control pH 4,6	% of total concentration	Species name	M 1 20% BC pH 5,7	% of total concentration	Species name
Cu^{+2}	65,185	Cu^{+2}	Cu^{+2}	3,9	Cu^{+2}
	0,047	$CuOH^{+}$		0,041	$CuOH^{+}$
	0,074	$CuCl^{+}$		0,012	$CuCl^{+}$
	5,812	$CuSO_4$ (aq)		0,403	$CuSO_4$ (aq)
	0,177	$CuNO_3^{+}$		0,099	$CuNO_3^{+}$
	0,177	/FA-Cu^{+2}G(aq)		0,027	/FA-Cu^{+2}G(aq)
	7,834	/FA_2Cu(aq)		81,24	/FA_2Cu(aq)
	20,69	/FACu^{+}(aq)		13,575	/FACu^{+}(aq)
Fe^{+3}	0,105	$FeOH^{+2}$		0,704	/FA_2CuOH(aq)
	0,44	$Fe(OH)_2^{+}$	Fe^{+3}	0,025	$Fe(OH)_2^{+}$
	95,46	/FA_2FeOH(aq)		99,718	/FA_2FeOH(aq)
	3,992	/FA_2Fe^{+}(aq)		0,256	/FA_2Fe^{+}(aq)
Mn^{+2}	92,831	Mn^{+2}	Mn^{+2}	89,219	Mn^{+2}
	0,053	$MnCl^{+}$		0,138	MnCl+
	6,425	$MnSO_4$ (aq)		7,153	$MnSO_4$ (aq)
	0,126	$MnNO_3^{+}$		1,13	$MnNO_3^{+}$
	0,252	/FA-Mn^{+2}G(aq)		0,03	$Mn(NO_3)_2$ (aq)
	0,313	/FAMn^{+}(aq)		0,612	/FA-Mn^{+2}G(aq)
Pb^{+2}	60,895	Pb^{+2}		1,716	/FAMn^{+}(aq)
	0,165	/FA-Pb^{+2}G(aq)	Pb^{+2}	13,783	Pb^{+2}
	0,035	$PbOH^{+}$		0,095	/FA-Pb^{+2}G(aq)
	1,261	$PbCl^{+}$		0,114	$PbOH^{+}$
	11,607	$PbSO_4$ (aq)		0,775	$PbCl^{+}$
	0,068	$Pb(SO_4)_2^{-2}$		3,043	$PbSO_4$ (aq)
	0,772	$PbNO_3^{+}$		0,026	$Pb(SO_4)_2^{-2}$
	23,043	/FAPb^{+}(aq)		1,63	$PbNO_3$+
	2,152	/FA_2Pb(aq)		0,029	$Pb(NO_3)_2$ (aq)
Zn^{+2}	87,986	Zn^{+2}		34,664	/FAPb^{+}(aq)
	0,239	/FA-Zn^{+2}G(aq)		45,839	/FA_2Pb(aq)
	0,145	ZnCl+	Zn^{+2}	67,29	Zn^{+2}
	7,491	$ZnSO_4$ (aq)		0,462	/FA-Zn^{+2}G(aq)
	0,063	$Zn(SO_4)_2^{-2}$		0,022	ZnOH+
	0,189	$ZnNO_3^{+}$		0,3	ZnCl+
	3,88	/FAZn^{+}(aq)		6,637	ZnSO4 (aq)
AsO_4^{-3}	0,556	H_3AsO_4		0,083	$Zn(SO_4)_2^{-2}$
	0,449	$HAsO_4^{-2}$		1,351	$ZnNO_3^{+}$
	98,994	$H_2AsO_4^{-}$		23,368	/FAZn^{+}(aq)
				0,484	/FA_2Zn(aq)
			AsO_4^{-3}	0,032	H_3AsO_4
				7,299	$HAsO_4^{-2}$
				92,669	$H_2AsO_4^{-}$

Table 9: Metals and as species distribution in soil solution of the Asarel A10 soil

A10 Control pH 2,3	% of total concentration	Species name	A10 20%BC+Ca pH 5,5	% of total concentration	Species name
Cu^{+2}	24,03	Cu^{+2}	Cu^{+2}	52,04	Cu^{+2}
	0,408	$CuCl^{+}$		0,294	CuOH+
	74,49	$CuSO_4$ (aq)		0,034	$Cu_2(OH)_2^{+2}$
	0,541	$CuHSO_4^{+}$		0,035	CuCl+
	0,482	$CuNO_3$+		37,946	$CuSO_4$ (aq)
	0,042	/FACu^{+}(aq)		0,643	$CuCO_3$ (aq)
Fe^{+2}	23,112	Fe^{+2}		0,569	$CuHCO_3^{+}$
	0,124	$FeCl^{+}$		0,034	/FA-Cu^{+2}G(aq)
	76,762	$FeSO_4$ (aq)		3,061	/FA_2Cu(aq)
Mn^{+2}	29,195	Mn^{+2}		5,312	/FACu+(aq)
	0,248	MnCl+		0,02	/FA_2CuOH(aq)
	70,246	$MnSO_4$ (aq)	Fe^{+3}	0,449	$FeOH^{+2}$
	0,293	$MnNO_3^{+}$		14,858	$Fe(OH)_2^{+}$
Pb^{+2}	6,172	Pb^{+2}		0,01	$FeSO_4^{+}$

	1,905	$PbCl^{+}$		84,385	$/FA_2FeOH(aq)$
	0,06	$PbCl_2$ (aq)		0,294	$/FA_2Fe^{+}(aq)$
	40,903	$PbSO_4$ (aq)	Mn^{+2}	63,618	Mn^{+2}
	50,359	$Pb(SO_4)_2^{-2}$		0,021	$MnCl^{+}$
	0,579	$PbNO_3$+		36,01	$MnSO_4$ (aq)
	0,011	$Pb(NO_3)_2$ (aq)		0,22	$MnHCO_3^{+}$
	0,01	$/FAPb^{+}(aq)$		0,042	$/FA\text{-}Mn^{+2}G(aq)$
Zn^{+2}	10,788	Zn^{+2}		0,076	/FAMn+(aq)
	0,264	$ZnCl^{+}$	Pb^{+2}	33,055	Pb^{+2}
	31,935	$ZnSO_4$ (aq)		0,149	$PbOH^{+}$
	56,832	$Zn(SO_4)_2^{-2}$		0,401	$PbCl^{+}$
	0,172	$ZnNO_3^{+}$		51,532	$PbSO_4$ (aq)
H_3AsO_3	100	H_3AsO_3		4,687	$Pb(SO_4)_2^{-2}$
$Cr(OH)_2^{+1}$	15,638	Cr^{+3}		0,029	$PbNO_3^{+}$
	0,174	$CrOH^{+2}$		0,235	$PbCO_3$ (aq)
	0,02	$CrCl^{+2}$		4,561	$PbHCO_3^{+}$
	83,728	$CrSO_4^{+}$		0,022	$/FA\text{-}Pb^{+2}G(aq)$
	0,245	$CrOHSO_4$(aq)		4,202	$/FAPb^{+}(aq)$
				1,126	$/FA_2Pb(aq)$
			Zn^{+2}	55,27	Zn^{+2}
				0,036	$/FA\text{-}Zn^{+}{}_2G(aq)$
				0,053	$ZnCl^{+}$
				38,489	$ZnSO_4$ (aq)
				5,06	$Zn(SO_4)_2^{-2}$
				0,303	$ZnHCO_3^{+}$
				0,762	$/FAZn^{+}(aq)$
			AsO_4^{-3}	0,049	H_3AsO_4
				5,439	$HAsO_4^{-2}$
				94,512	H_2AsO_4-
			$Cr(OH)_2^{+1}$	1,238	$Cr(OH)_2^{+1}$
				0,398	Cr+3
				12,977	$CrOH^{+2}$
				0,143	$Cr(OH)_3$ (aq)
				0,744	$CrSO_4^{+}$
				4,297	$CrOHSO_4$ (aq)
				36,015	$/FA_2Cr+(aq)$
				44,187	$/FA_3Cr_2(OH)_2^{+}(aq)$

4. CONCLUSIONS

The experimental sites of the present study were selected in the vicinity of Aurubis-Pirdop copper smelter and refinery, and Asarel-Medet copper ore extraction and processing plant in Bulgaria. In a greenhouse experiment BC incorporation (20%) increased soil pH and cation exchange capacity, decreased electrical conductivity and caused toxic heavy metal immobilization, as proved by decline of heavy metal concentration in soil solution (H_2O), and 0,01M $CaCl_2$, and 1M NH_4NO_3 extracts of Cu, and other heavy metals. Multiple regression analysis revealed that 0,01M $CaCl_2$ provides the most satisfactory relationship with the content of heavy metals and as in plants in the studied contaminated soils and wastes. The highest R^2 in all the three extracts were obtained for the elements Cu and Mn in simple linear regression models. Geochemical modelling for predicting metal species distribution in soil solution revealed that free M^{2+} ions decreased following BC addition in the acidic soils, while in the acidic tailings the dominant $M^{2+}SO_4^{0}(aq)$ complexes decreased at the expense of increase of free more bioavailable M^{2+} ions. Nevertheless, there was a measurable yield of alfalfa in the tailings for the BC and $CaCO_3$ treated variants, due to improvement of general waste substrate characteristics, i.e. pH, CEC, EC, TOC and full immobilisation of Cu (from 117 to 0,03 mg/L).

Amelioration with 20% BC, caused statistically significant increases in above-ground biomass, often exceeding 4-13 times the original control yields, while at the tailings site, no yield without BC amelioration was noted. Biochar amelioration led to lowering metal contents in plant biomass, as supported by the results from *Visual Minteq* modelling, describing the increase of stable, high-molecular-weight organo-metallic complexes that can reduce metals'

bioavailability.

Biochar proved to be efficient ameliorant for immobilizing Cu and other heavy metals and reducing their bioavailability in acidic technogenic polluted soils through decreasing the free metal ion species in the soil solution. In acidic tailings from Cu ore processing metallurgy when added simultaneously with lime, it improved the physico-chemical characteristics of the tailings and achieved plant biomass yields. However, BC's long-term reliability and sustainability (over 2–5 years) should be monitored if some plant-soil interactions could transform its structure and properties.

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