Improving mining soil phytoremediation with *Sinapis alba* by addition of hydrochars and biochar from manure wastes

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Abstract

The use of phytoremediation to remove metals of contaminated soils is an interesting technique that is usually limited by adverse physical and chemical properties of this type of soils. The addition of biochar produced from manure waste could improve soil properties due to its nutrient content, high cation exchange capacity or water holding capacity. However, the high water content of manure wastes precludes its pyrolysis treatment use for biochar production, without a previous drying step. Indeed, hydrothermal carbonization (HTC) of manure wastes could be an adequate treatment method as it takes place in water solution. The product obtained after HTC of biomass, named hydrochar, exhibits different physiochemical properties than biochar that could affect their potential application. The objective of the present work is to study the effect of chars obtained by pyrolysis or HTC of manure wastes in mining soil phytoremediation with *Sinapis alba*. Two selected mining soils (PORT and GAM) were treated with two manure biochars prepared at 450ºC (BMW450) and 600ºC (BMW600), two hydrochars prepared by HTC of manure at 190ºC (HWM190) and 240ºC (HMW240) and raw manure waste (MW) at a rate of 10% in mass. Later, different soil samples were incubated with or without *Sinapis alba* growth. Experimental results shown that properties of chars have a great influence on the efficiency of the use of *Sinapis alba* in the phytoremediation of mining soils. The addition of BMW600 and raw material (MW) increased the production of aerial and root biomass for GAM soil. For PORT soil, HMW190, two biochars and MW increases root and aerial biomass whereas HMW240 only produces an increment on aerial biomass. Addition of two hydrochars and MW had a positive effect on the biochemical soil activities and the highest microbial biomass carbon of GAM and PORT soil samples was observed after addition of HMW190. Finally, the addition of biochars and hydrochars could improve the phytoremediation of mining soils by *Sinapis alba*. However, the heavy metal uptake greatly depends on the type of soil, the amendment and the target metal. The accumulation of As, Pb and Zn in the aerial part of *Sinapis alba* was very low. However, *Sinapis alba* acts as accumulator for As in the presence of BMW600 and HMW190, for Zn after amendment with HM190 and for Pb after BMW600 addition to PORT soil. For GAM soil, only the addition of BMW450 and BM600 improves the capacity of accumulation of As in roots.

***Keywords*:** Mining soil; Manure waste; Hydrothermal carbonization (HTC); Biochar; Hydrochar; Heavy metal.

Graphical abstract

Statement of novelty

Few researches have been performed about the use of hydrochar in the treatment of mining soils combined with phytoremediation. The addition of biochars and hydrochars from manure waste was compared.

Introduction

Over the years, mining and metallurgical activities have led to significant environmental damages. One of the more important has been the pollution of soils by a large variety of metal and metalloids. Unlike organic contaminants, metals and metalloids are non-degradable through natural processes and accumulate in the environment being a serious risk to human and animals due to direct exposure and food chain.

Traditionally, soil remediation techniques involved destructive and expensive practices such as soil excavation, metal immobilization or soil washing. However, in the last years, there is an increasing interest in the development and use of cleaner, low-cost and ecological technologies like phytoremediation which allows not only soil decontamination but also the recovery of some metals [1,2]. In addition, phytoremediation has positive perception by society and stakeholders and vegetation growth can prevent soil erosion or leaching of trace metals [1,2]. However, soil metal pollution has a pernicious effect on soil microbial properties and on the taxonomic and functional diversity of soils that can difficult the growth of plants and consequently, the process of phytoremediation [3]. For this reason, the addition of organic amendments as compost has been attempted for the improvement of soil properties as organic matter and nutrient content, cation exchange capacity, pH correction or water holding capacity could enhance the plant growth [4].

Recently, several authors have focused their research on phytoremediation of metal polluted soils combined with biochar amendment [1]. The use of biochar for heavy metal immobilization is a low cost technique and persistent for long periods compared to others organics amendments due to highest stability of biochar [5]. Also, the addition of biochar to metal polluted soil can reduce the bioavailability of heavy metals reducing the toxic effects in plants [6,7]. This fact can contribute to a higher biomass production and therefore, to a higher total heavy metal extraction by plants from contaminated soils [1]. The effect of biochar in polluted soils greatly depends on feedstock characteristics and pyrolysis conditions such as temperature, heating rate or time. Traditionally, biochar obtained from pyrolysis of biomass at higher temperatures show high stability and could be used as carbon sequestration [8]. On the other hand, biochars obtained at lower temperatures and from feedstocks with high nutrient contents like manure wastes or sewage sludges have good properties as amendments, with the potential to replace commercial fertilizers [9] and consequently, creating special interest in the restoration of metal polluted sites as mining areas after their exploitation [7]. The impacts of mining activities represents a threat to the ecosystem equilibrium, especially when is related to metal contamination. Mining soils are categorized as Technosols, which implies the modification of physical, chemical and biological soil properties due to industrial or artisanal human activities [10]. High amounts of metals in soils can produce losses of vegetation cover, alteration of soil biota (microorganisms) and even toxicity in plants and others levels of the trophic chain.

Despite of the interesting properties of manure wastes as raw material for the biochar preparation, their elevated water content can precluded its pyrolysis process being necessary an expensive and high-energetic drying process. For this reason, hydrothermal carbonization (HTC) of wet manure wastes could be an interesting alternative to traditional pyrolysis for the preparation of improved carbon rich amendments, named hydrochars [11]. Compared to pyrolysis, HTC is operated at low temperature, in the presence of water and under autogenous pressures [12]. Additionally, recirculation of process water can increase the overall system's efficiency and reduce both the operating costs and environmental impact of a commercial HTC plant [12]. Researches regarding soil amendment use of hydrochar are very limited in comparison to biochar. Hydrochars obtained from

HTC of organic wastes show different physical and chemical properties than biochars [5]. For example, hydrochars have acid pH, whereas biochars are in general more alkaline [13]. Hydrochars also tend to exhibit higher H/C and O/C ratios than biochar indicating that HTC leads to less aromatic structures than traditional pyrolysis [5,13]. Different works have described phytotoxic effect s of some hydrochar samples [14, 15, 16] while much less concerns have been reported about the phytotoxicity of biochar [14]. This negative effect was mainly reported at higher addition rates [14] and seems to be due to bio-degradable organic compounds. However, hydrochar characteristics could differ in terms of raw material properties and production parameters such as temperature, pressure and duration of HTC. For this reason, more researches are necessary in order to determine the potential use of hydrochars as soil amendments, specially, when hydrochars are prepared by HTC of biomass with high nutrient content like manure wastes or sewage sludges. The main objective of the present work is to study the effect of biochars and hydrochars prepared from manure waste, in the phytoremediation, using *Sinapis alba*, of two polluted soils from different Spanish mining areas. We suppose that manure wastes, rich in nutrients, are therefore interesting feedstock for the biochar and hydrochar production, especially when combined with phytoremediation techniques for metal pollution remediation since both amendments act as soil conditioner and slow release fertilizer.

Materials and methods

Mining soils

Two mining soils were selected from two different Spanish mining areas: Gamonedo soil (GAM) (Latitude: 43° 19´57.9573´´ N, Longitude: 4° 5´ 2.8573´´ W) and Portman soil (PORT) (Latitude: 37° 35´ 24.1593´´ N, Longitude: 0° 51´ 39.6248´´ W) both nearby to a lead and zinc ores exploitation areas.

Biochar and hydrochar preparation

Two biochars and two hydrochars were prepared using manure waste (MW) as feedstock using a thermal treatment equipment design by our research group and Demede enterprise (http://demede.es). MW was collected in a rabbit farm located in the practice field of the Universidad Politécnica de Madrid (UPM). The two biochars were prepared as follows: 500g of air dried MW were introduced in a 2 L reactor made of steel. Samples were heated at 3ºCmin^{-1} using N_2 flow of 0.5 L min^{-1} until 450 and 600ºC leading to BMW450 and BMW600, respectively. The final temperature was maintained during 1 hour. The reactor has two thermocouples, one is inserted into the recipient in contact with the sample and another is in contact with the external part of the steel wall. The temperature difference between the two thermocouples is 10ºC. Later, biochars were sieved below 2 mm.

The two hydrochars were prepared as follows: 1.0 L of wet MW solution with 30% of solid content was introduced in a Teflon recipient inside one Hastelloy reactor. The autoclave has two thermocouples, one is inserted into the Teflon recipient in contact with the sample and another one is connected with the Hastelloy wall. In this case, the difference between the final temperatures of two thermocouples was 20ºC. Samples were heated at 190 and 240ºC and the final temperature was maintained during 6 hours under autogenous pressures leading to HWM190 and HMW240, respectively. After cooling down to room temperature, the aqueous solutions were filtered and the solid fraction filtered and washed with 0.5 L of distilled water, in order to reduce potential phytotoxicity. Finally, the hydrochars were dried at 90ºC during 24 hours and sieved below 2 mm.

Characterization of soils, raw material, biochars and hydrochars

Selected soil samples (PORT and GAM) were air-dried, crushed and sieved through a 2 mm mesh prior to analysis. The pH and electrical conductivity (EC) were determined with a soil: water ratio of 1:2.5 (g mL^{-1}) using a Crison micro-pH 2000 and a Crison 222 conductivimeter, respectively. The effective cation exchange capacity (CEC) was determined using the standardized protocol ISO 23470 [17] that is based on the centrifuge extraction with 0.0166 M cobalt (III) hexamine chloride solution. The exchangeable K and Na were measured in the solution using a Perkin Elmer AAnalyst 400 Atomic Absorption Spectrophotometer. Easily oxidizable organic carbon (Coxi) was determined by the Walkley-Black method based on the oxidation of organic carbon with dichromate [18]. Total content in P, V, Cr, Fe, Co, Ni, Cu, Zn, As, Al and Pb were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) with an Optima 7300DV Perkin Elmer equipment for major elements and Nexion 300D equipment for minority elements after sample digestion with 3:1 (v/v) concentrated HCl/HNO_3 following USEPA 3051a method [19]. Manure waste, biochars and hydrochars were characterized in the same way that soils except for the pH and electrical conductivity (EC) that were measured in a 4 g L^{-1} relation and the available phosphorous (POlsen) that was determined by the Olsen method using a Shimadzu UV-1203 spectrophotometer at a wavelength of 430nm [20]. BET surface area (S_{BET}), V micro (cm^3g^{-1}), Vmeso (cm^3 g^{-1}) and Vmaco (cm^3g^{-1}) were determined from nitrogen isotherm and Hg porosimetry. Nitrogen isotherm was obtained using a Porosimetry System- ASAP 2020-Micromeritics and Hg porosimetry was carried out using a Micromeritics AutoPore IV 9500 equipment. The morphological study of the manure waste, biochars and hidrochars from manure waste was performed by scanning electron microscope (SEM), ZEISS Model DMS-942. The microscope is equipped with an energy-dispersive X-ray analysis system (Link-Isis II), SEM-EDX. Prior to SEM examination, the samples were covered with iridium to decrease the charging of the samples and to improve the SEM pictures examination.

Experimental design

The soils were amended at a rate of 10% (w/w) with the raw material and the four chars. After that, all treatments were initially watered to 60% of water holding capacity and afterwards watered daily to account for moisture losses. Mixtures were introduced in corresponding pots (volume of 500 mL). 10 seeds of *Sinapis alba* were sowed in the half of the mesocosm experiment while the remainder half were used as control without *Sinapis alba* growth. Then, pots were introduced in an incubator with light cycles every 12 h at a controlled temperature from 20 to 25°C for 60 days. Each treatment was replicated 3 times. After this time, leaves and stems (aerial part) and roots were collected and dry weights were determined after drying samples at 80ºC during 24 h. Later, the heavy metal content of plant tissues were extracted as follows: 0.4 g of dried shoots and roots were put in a digestion tube and 3 mL of HCl 37% and 1 mL of HNO_3 69%. Then, digestion tubes were heated up from 60ºC to 120°C for 1 hour. Following, heating was continued for another 2 hours at 160ºC, and allowed to cool down to room temperature. Soil heavy metal content was extracted following USEPA 3051a method [21]. Then, heavy metal content was determined using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Perking Elmer. With the heavy metal data, the bioconcentration factor (BCF) was calculated as the ratio of metal concentration in the plant (aerial part or roots) and metal concentration in the soil, indicating the efficiency of the plant regarding the accumulation process of metal. In this work, two BCF were calculated:

(i) BCF-1 = [metal concentration in aerial part (mg kg^{-1})]/ [metal concentration in the soil (mg kg^{-1})]

(ii) BCF-2 = [metal concentration in roots (mg kg^{-1})]/ [metal concentration in the soil (mg kg^{-1})]

The effect of different treatments in soil biological properties was evaluated with the determination of microbial biomass carbon and dehydrogenase, phosphomonoesterase and β-Glucosidase activities. The microbial biomass carbon (C-biomass) was assessed with the variance in the carbon content of the fumigated and unfumigated samples (chloroform fumigation-extraction method) with a commonly used factor (Kc=0.45) for soils amended with organic materials [21, 22]. Dehydrogenase activity was determined according to Camiña et al. [22] using iodonitrotetrazolium formazan (INTF) as product (μmol INTF g^{-1} h^{-1}), while phosphomonoesterase and β-glucosidase activities were determined after incubating soils with a substrate containing a p-nitrophenyl moiety and then in the enzymatic hydrolysis the amount of released p-nitrophenol was measured (μmol p-nitrophenol) by a spectrophotometer at a wavelenght of 400 nm [23]. Finally, the geometric mean (GMea) index was calculated as suitable measure of soil biochemical activity that integrated the above mentioned enzymes [21] as follows:

$$GMea = (\text{phosphomonoesterase} \times \beta\text{-glucosidase} \times \text{dehydrogenase})^{1/3}$$

This index offers an integrative information from variables that possess different units and range of variation and it has been established as an adequate index for estimating soil biochemical quality after the addition of different amendments, including biochar [21].

The plant roots of *Sinapis alba* which grew in a soil contaminated by heavy metals, was studied by scanning electron microscope (SEM), ZEISS Model DMS-942. The microscope is equipped with an energy-dispersive X-ray analysis system (Link-Isis II), SEM-EDX. Prior to SEM examination, the samples were covered with iridium to decrease the charging of the samples and to improve the SEM pictures examination.

Statistical analysis

The statistical analysis of experimental results was performed using the Statgraphics Centurion XVI.I. software. Differences of means were tested using an analysis of variance (ANOVA) and means were considered to be different when $p < 0.05$ using Duncan's multiple range test.

Results and discussion

Table 1 shows main characteristics of GAM and PORT soils. Both soil pH was close to neutral value but other properties show important differences. Whereas GAM soil shows low CEC and a high P content the main characteristics of PORT soil are, high CEC and low EC and P content. According to the metal content, both soils had important amounts of Zn, Pb, As, V and Cd. The concentrations of these elements were above the critical soil total concentrations for the toxicity effects are likely [24] (Zn: 70-400 mg kg^{-1}; Pb: 100-400 mg kg^{-1}; As: 20-50 mg kg^{-1}; V: 50-100 mg kg^{-1}; Cd: 3-8 mg kg^{-1}). In addition, the content on Zn, Pb and As is above the guideline values established by the Finnish standard that are considered an approximation of the mean values of different national systems in Europe and have been used as reference values for agricultural soils [25]. With respect to the chars (Figure 1), a wide characterization was previously done by Cardenas et al., [5]. pH of chars is an important parameter when they were added to metal polluted soils as pH greatly influences on metal mobility. Figure 1.a shows that biochar pH increases with increasing pyrolysis temperature probably due to ash enrichment and polymerization/condensation reactions with the release of acidic groups, that take place during pyrolysis process [5]. However, hydrochars show lower pH than MW probably due to the adsorption on the hydrochar surface of organic acids produced during HTC. Instead of lower pH, hydrochars obtained from manure wastes show slightly basic pHs [5,11] that were favorable in the treatment of metal polluted soils. Electrical conductivity (EC) estimates the content of dissolved salts in samples. Figure 1.b shows that EC of two biochars was similar to that of MW, whereas hydrochars show lower EC that MW. This fact indicates that some soluble salts were probably removed during the washing stage after the hydrochars were prepared. Coxi was a measure of the labile fraction of carbon that was more available to soil microorganisms. Figure 1.c shows that Coxi decreased with the pyrolysis temperature of MW due to formation of more stable carbon structures. However, two hydrochars show highest Coxi values than MW indicating the presence of more labile structures than in MW. Finally, CEC related to the ability of materials to adsorb cations such as Ca^{2+}, Mg^{2+} or K^{+} (Figure 1.d), POlsen (Figure 1.e) and K (Figure 1.e) of MW significantly decreases with pyrolysis and especially HTC of MW. These properties could play great influence on heavy metals retention in the soil and plant growth and therefore, in the phytoremediation process. Porosity and porous size distribution play an important role on hydrophysical behavior of chars [11]. Also, porosity provides potential habitats for soil microorganisms. Figure 2 shows the specific surface area and the micro-, meso- and macro-porosity of MW and corresponding biochars and hydrochars. The study of the textural properties of the all materials indicates that the pyrolysis and HTC treatment develops the BET specific surface area and the porosity of the materials obtained, especially with the HTC treatment (Figure 2). Manure waste show a BET surface area value around of 1 m^2g^{-1}, value that increases to almost 5 m^2/g for manure waste biochars and to more than 14 m^2g^{-1} for manure waste hydrochars (Figure 2a). The development of the pore volume in the biochars and hydrochars from manure wastes follows a similar trend to the BET surface area and the values are higher in the materials obtained by the HTC process (Figure 2b). All samples show a macroporous structure with low development of micro- and meso- porosity due to the absence of activation processes during pyrolysis or after HTC (Figure 2b). However, it could be observed as pyrolysis slightly increases mesoporosity, whereas HTC increases the specific surface area, micro-, meso- and macroporosity with respect to the feedstock, being the increment related with HTC temperature. Manure wastes and biochars obtained from this residue are mainly macroporous, approximately the 95% of their porosity is due to the contribution of these pores and they do not have micropores (BMW450 show a macropore volume of 0.9056 cm^3g^{-1} *versus* a micropore volume of 0.002 cm^3g^{-1}). The manure waste hydrochars show macropore volume much higher than the other materials (up to 1.5375 cm^3g^{-1} for HMW240, which represents more than 86% of their porosity) and show also low development mesopores; these materials are the only ones that show micropore volume development, although it is relatively low (up to 0.1023 cm^3g^{-1} for HMW240, which is approximately 6% of their porosity).

Figure 3 shows selected SEM images at different magnifications of manure waste and biochars and hydrochars obtained from manure wastes. Figure 3.a) is a SEM picture of manure waste at 10000x magnification and their SEM-EDX analysis. Figures 3.b) and 3.c) corresponding at BMW450 at 2000x and BMW600 at 2500x. Figures 3.d) and 3.e) correspond to HMW190 and HMW 240 to 2500x and 5000x respectively. The SEM images of these materials show that the biochars and hydrochars conserved, to a large degree, the original structures of manure wastes. All the samples were studied by SEM-EDX and were identified the presence of alkaline, alkaline earth, silicon, phosphorus and sulfur compounds, as expected, largely due, to their vegetable origin.

Figure 4 shows the dry weight of aerial part (leaves+stems) and roots of *Sinapis alba* growth on PORT and GAM soils after 60 days of incubation experiments. The effect of MW or char amendment on plant growth was more effective on GAM than in PORT soil. The addition of BMW600 and raw material (MW) increased the production of aerial and root biomass for GAM soil while the application of the two biochars (BMW450 and BMW600), two hydrochars (HMW190 and HMW240) and MW increased aerial biomass production for PORT soil. In addition, all amendments, except HMW240, increased root biomass in PORT soil. It is stablished that organic amendments could assist plant growth via enhanced chemical, biochemical and physical soil properties that depends on amendment characteristics and on soil properties. The highest total biomass production was obtained after addition of raw material (MW) for the both soils that could be related with the highest nutrient availability (N and P) of the raw material with respect to chars. These results show the great difficulty that still exists to generalize the use of biochar or hydrochar in the remediation of polluted soil. The results obtained will depend largely on the characteristics of the soil in addition to the properties of the biochar or hydrochar.

Organic amendments play an important influence of the soil biochemistry. The biochemical properties of two metal polluted soils were evaluated by Phosphomonoesterase, β-Glucosidase and Dehydrogenase activities (Figure 5). Soil dehydrogenase activity increased after the hydrochars addition to both soils probably due to the higher Coxi content of hydrochars (Figure 1.c). Indeed, dehydrogenase activity is involved in soil respiration [21] and it is normally improved by the addition of labile organic carbon [26]. β-Glucosidase activity diminished after biochar addition according to similar results obtained in previous works [27]; although, this activity also increased after hydrochar addition. The addition of two biochars also decreased the Phosphomonoesterase activity while the addition of MW increased it, probably due to the reduction of available P after biochar addition. The same trend was previously observed by Gascó et al. [8] after application of pig manure biochars to soil. Nevertheless, the Phosphomonoesterase activity increased after hydrochar addition instead their low POlsen content (Figure 1.e) implying that other soil properties apart from P content are involved in the process. Finally, it could be concluded that the GMea index, used as suitable measure of soil biochemical activity that integrated the above mentioned enzyme activities, increased after hydrochar addition. Therefore, experimental results obtained in this work show that hydrochars obtained by HTC at 190 or 240 ºC of manure waste had a positive effect on metal polluted soil quality. These results were contrary to other studies that show negative effects of hydrochar after addition to non-polluted soils [28]. However, Ren et al. [29] studied the effect of sewage sludge hydrochar on Cd polluted soil and reported an improvement on biochemical soil properties after hydrochar addition. These authors proposed that porosity of hydrochars takes an important role on the increment of soil microorganism's content. In addition, high porosity of hydrochars (Figure 2b) could promote the adsorption of metals on their surface.

Table 2 shows pH, EC and microbial biomass carbon (C-biomass) of GAM and PORT soil samples after growth of *Sinapis alba*. Addition of MW, biochars and hydrochar increases the soil pH. The highest increment was observed after addition of biochar BMW600 according to their high pH (Figure 1.a). With respect to EC their evolution was similar to pH, increasing with the addition of hydrochar, MW and specially biochar. Finally, addition of amendments combined with *Sinapis alba* significantly increases the microbial biomass carbon (C-biomass) of GAM and PORT soil samples. In both cases, the highest increment was obtained after the addition of hydrochar HMW190 in a similar way to results obtained by Ren et al. [29].

Finally, in order to study the effect of MW, biochars and hydrochars on metal behaviour on soil-plant system the bioconcentration factor were determined. Figure 6 shows the bioconcentration factors (BCF-1 and BCF-2) of As, Pb and Zn, for both soils. Values of BCF greater than one indicates a good accumulation efficiency and a potential accumulator plant [30, 31]. According to the data, the Zn, As and Pb were mainly accumulated in roots

(BCF-2) than in aerial part (BCF-1) as the accumulation of As, Pb and Zn in the aerial part was very low according to BCF-1 values. It could be observed that *Sinapis alba* acts as accumulator for As in GAM soil and in the presence of BMW600 and HMW190 in PORT soil. For GAM soil, the addition of BMW450 and BMW600 also improves the capacity of As accumulation in roots. *Sinapis alba* acts as accumulator for Pb in PORT soil after addition of BMW450, BMW600, HMW190 and HMW240 whereas for GAM soil the addition of amendments did not involve any improvement of the capacity of Pb accumulation in roots. Figure 7 shows the SEM-EDX of the *Sinapis alba* roots that grew in Portman soil with HMW190 amendment and where the Pb presence can be seen. Finally, *Sinapis alba* acts as bioaccumulator of Zn in PORT soil, increasing this value after amendment with HMW190. It could be concluded that the heavy metal uptake depends on plant ability, the type of soil, the amendment and the target metal. Experimental results show that the addition of amendments derived from manure waste changed the values of the bioconcentration factors of As and Pb (Figure 5).

Conclusions

Pyrolysis and HTC of manure waste leads to biochar and hydrochars with interesting benefits as amendments in the treatment of metal polluted soils. However, their effects will not depend only on the properties of the biochar or hydrochar but also on the previous characteristics of the soil being necessary future researches.

The addition of hydrochars obtained by hydrothermal carbonization of manure wastes had a positive effect on the biochemical soil properties of metal polluted soils selected in this work. GMea index significantly increased after hydrochars addition and HMW190 significantly increased microbial biomass carbon (C-biomass) of GAM and PORT soil samples after growth of *Sinapis alba* .

The addition of BMW600 and raw material (MW) increased the production of aerial and root biomass for GAM soil. For PORT soil, HMW190, the two biochars and MW increased root and aerial biomass whereas HMW240 only produced an increment on aerial biomass.

In general, the addition of biochars and hydrochars could improve the phytoremediation of mining soils by *Sinapis alba*. However, the heavy metal uptake by *Sinapis alba* greatly depends on the type of soil, the amendment and the target metal. Indeed, *Sinapis alba* acted as accumulator for As in GAM soil and in the presence of BMW600 and HMW190 in PORT soil. *Sinapis alba* acted as accumulator for Pb in PORT soil after addition of BMW450, BMW600, HMW190 and HMW240 whereas for GAM soil the addition of amendments did not involve any improvement of the capacity of Pb accumulation in roots. Finally, *Sinapis alba* acts as bioaccumulator of Zn in PORT soil, increasing significantly the bioaccumulation factor after amendment with HMW190.

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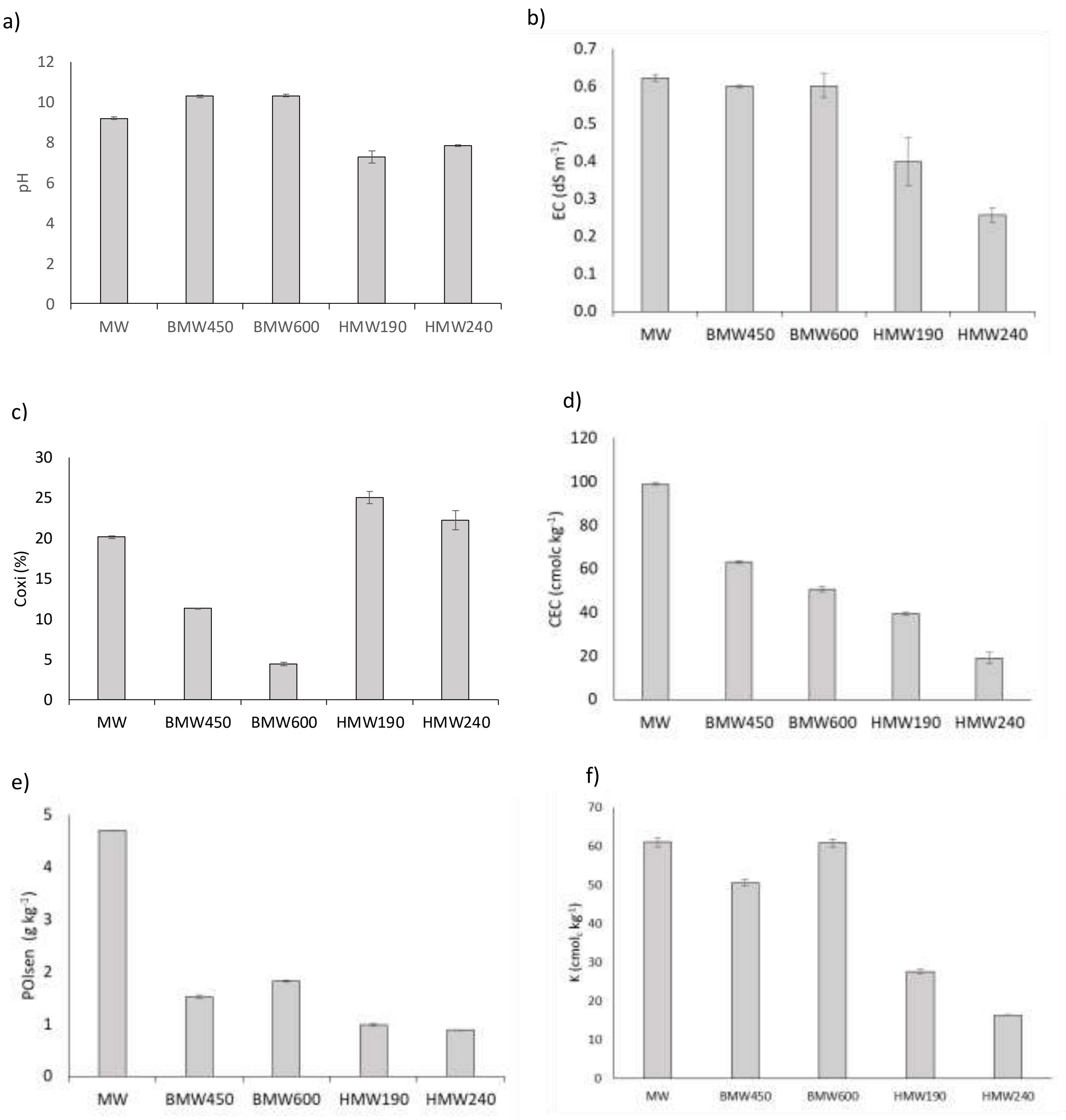


Figure 1. Properties of MW, of biochars (BMW450 and BMW600) and hydrochars (HMW190 and HMW240). X axis in all figures (a-f) correspond to the MW, biochars and hydrochars. Y axis differ between each graphic: a: pH values, b: electrical conductivity (EC), c: Easily oxidizable organic carbon (Coxi), d: cation exchange capacity (CEC), e: available phosphorous content (Polsen), f: exchangeable potassium content (K).

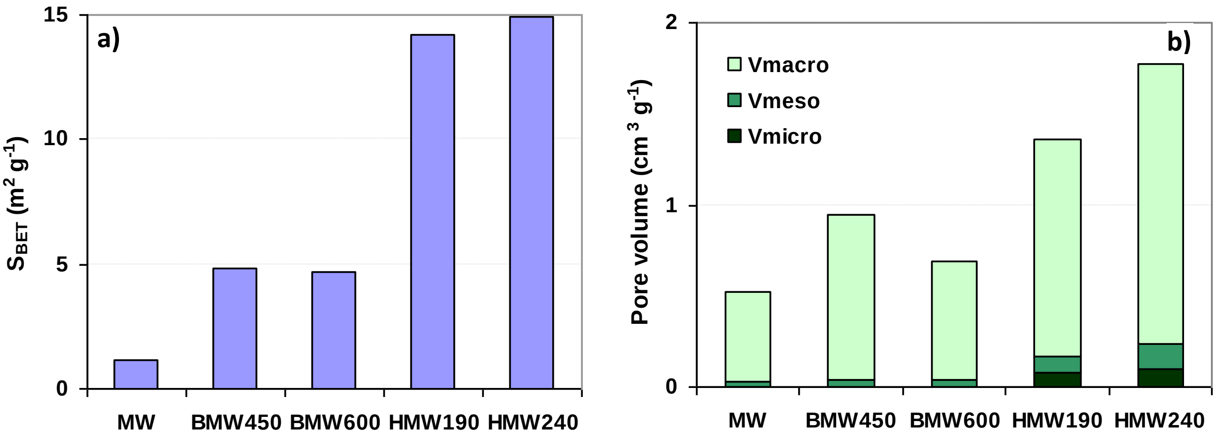
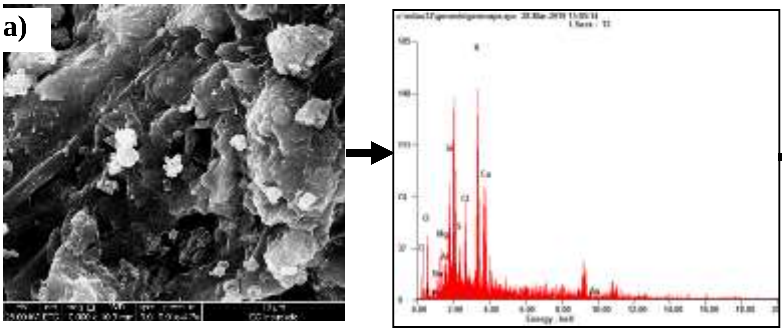


Figure 2. BET specific surface area (a) and micro-, meso- and macro-pore volume (b) of manure waste (MW), biochars (BMW450 and BMW600) and hydrochars (HMW190 and HMW240).



Element	Wt%	At%
C	19.80	36.89
O	20.16	28.19
Fe	03.96	01.59
Na	01.52	01.48
Mg	03.26	03.00
Al	01.98	01.64
Si	06.93	05.52
S	02.71	01.89
Cl	05.44	03.44
K	16.05	09.18
Ca	11.48	06.41

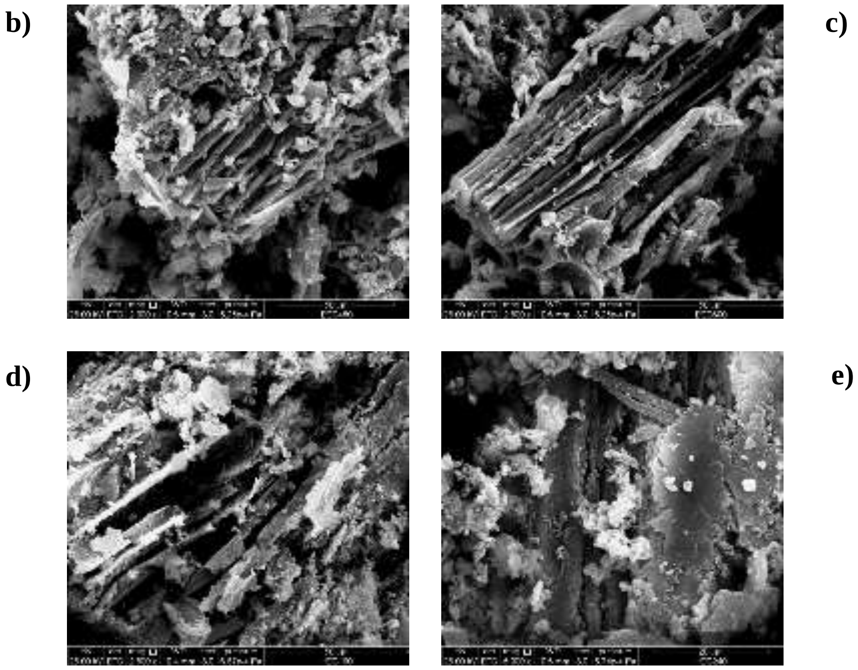
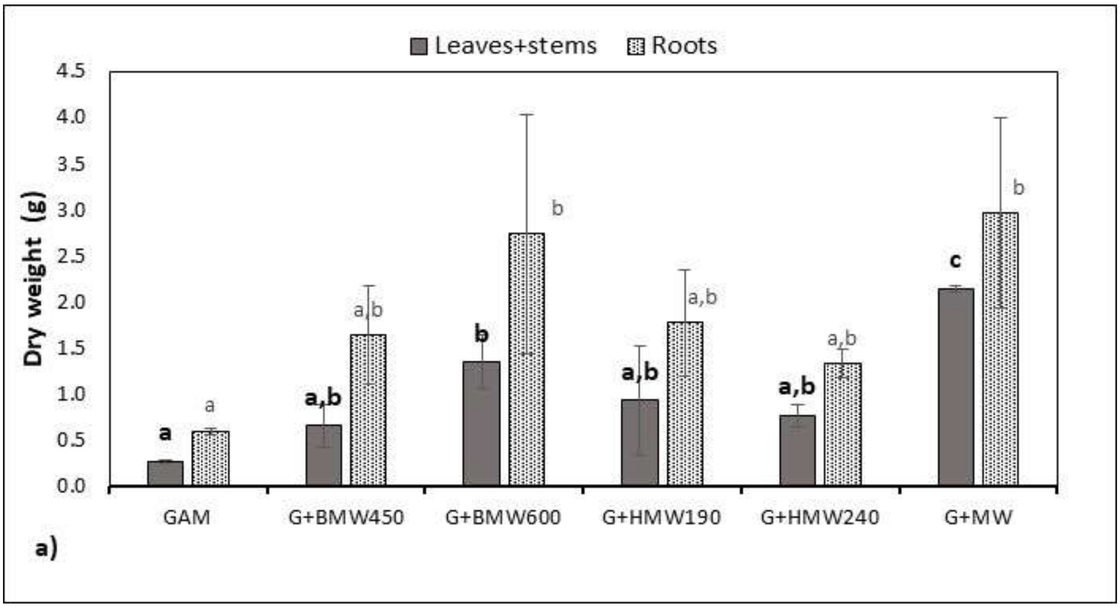


Figure 3. SEM-EDX of manure waste (a) and SEM images of biochars (b, c) and hidrochars (d, e) from this waste.



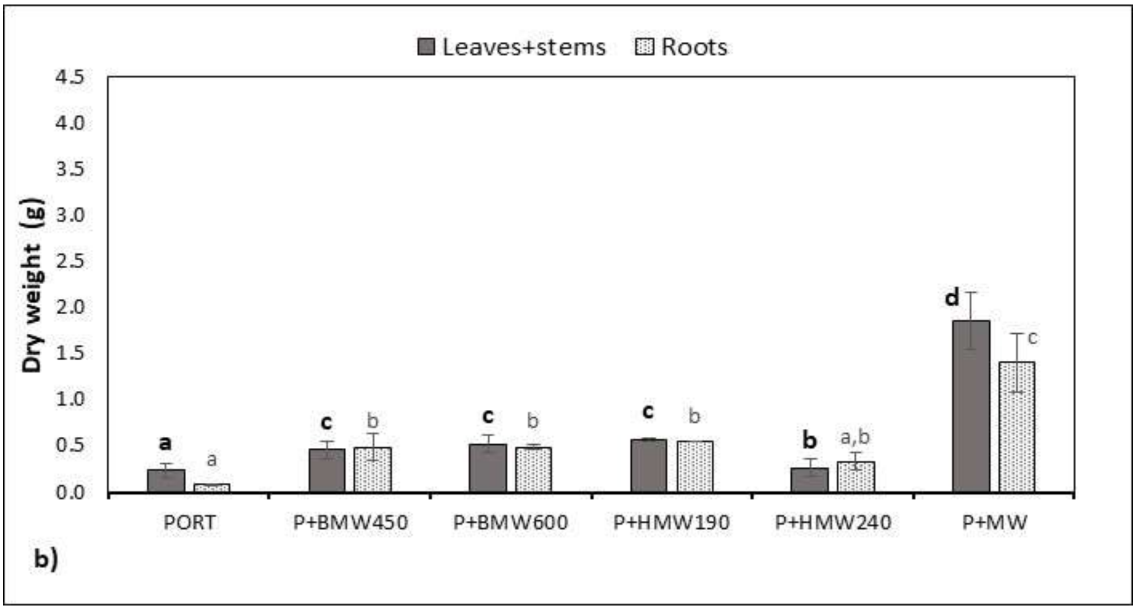


Figure 4. Dry weight (g) of aerial part and roots in different soils (GAM and PORT). Values are reported as means ± standard deviation. Means with common letter are not significantly different ($p > 0.05$) using the Duncan test.

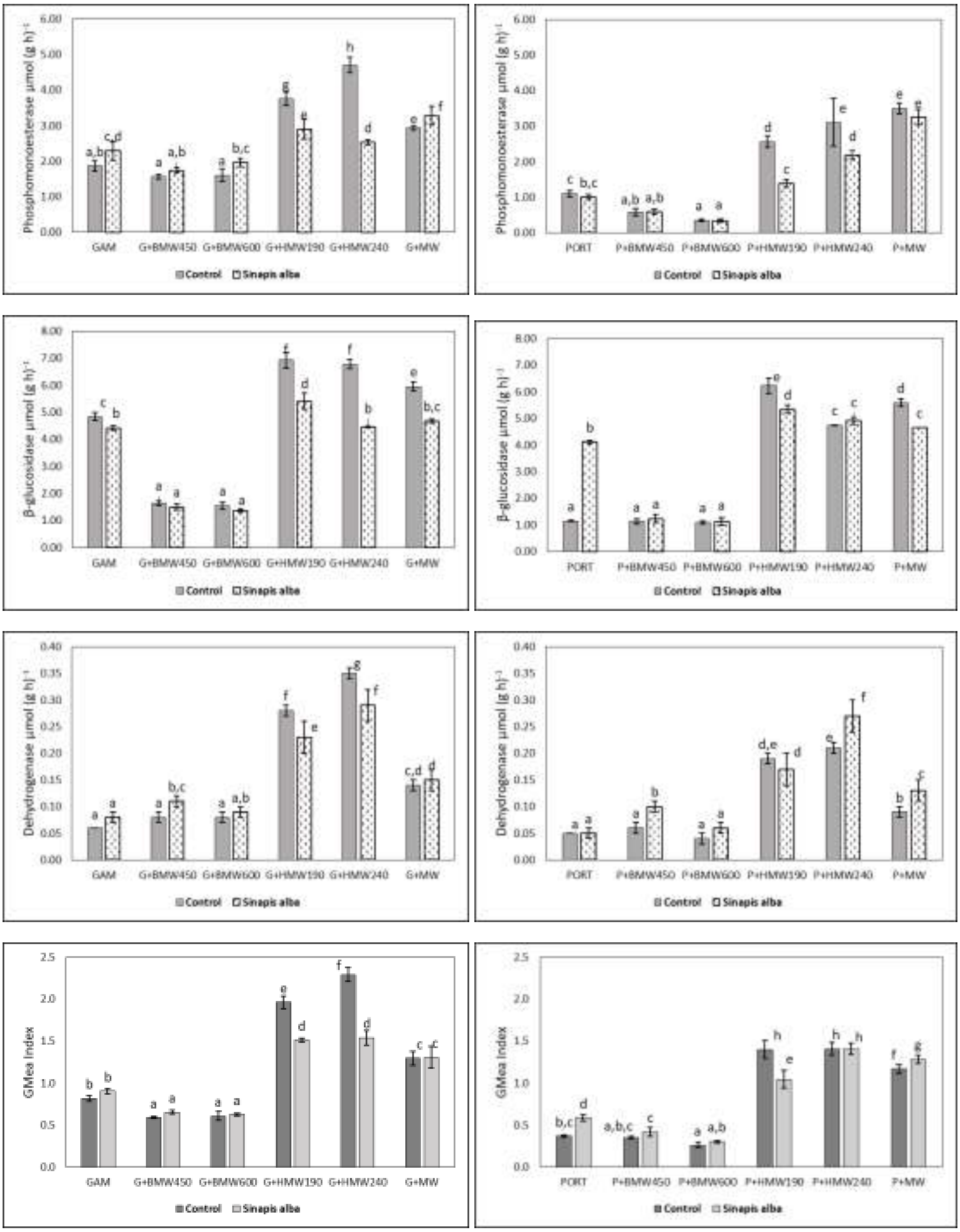


Figure 5. Phosphomonoesterase, β-Glucosidase, Dehydrogenase activities and GMea index for different treatments after two months of *Sinapis alba* growth. Values are reported as means ± standard deviation. Means with common letter are not significantly different ($p > 0.05$) using the Duncan test.

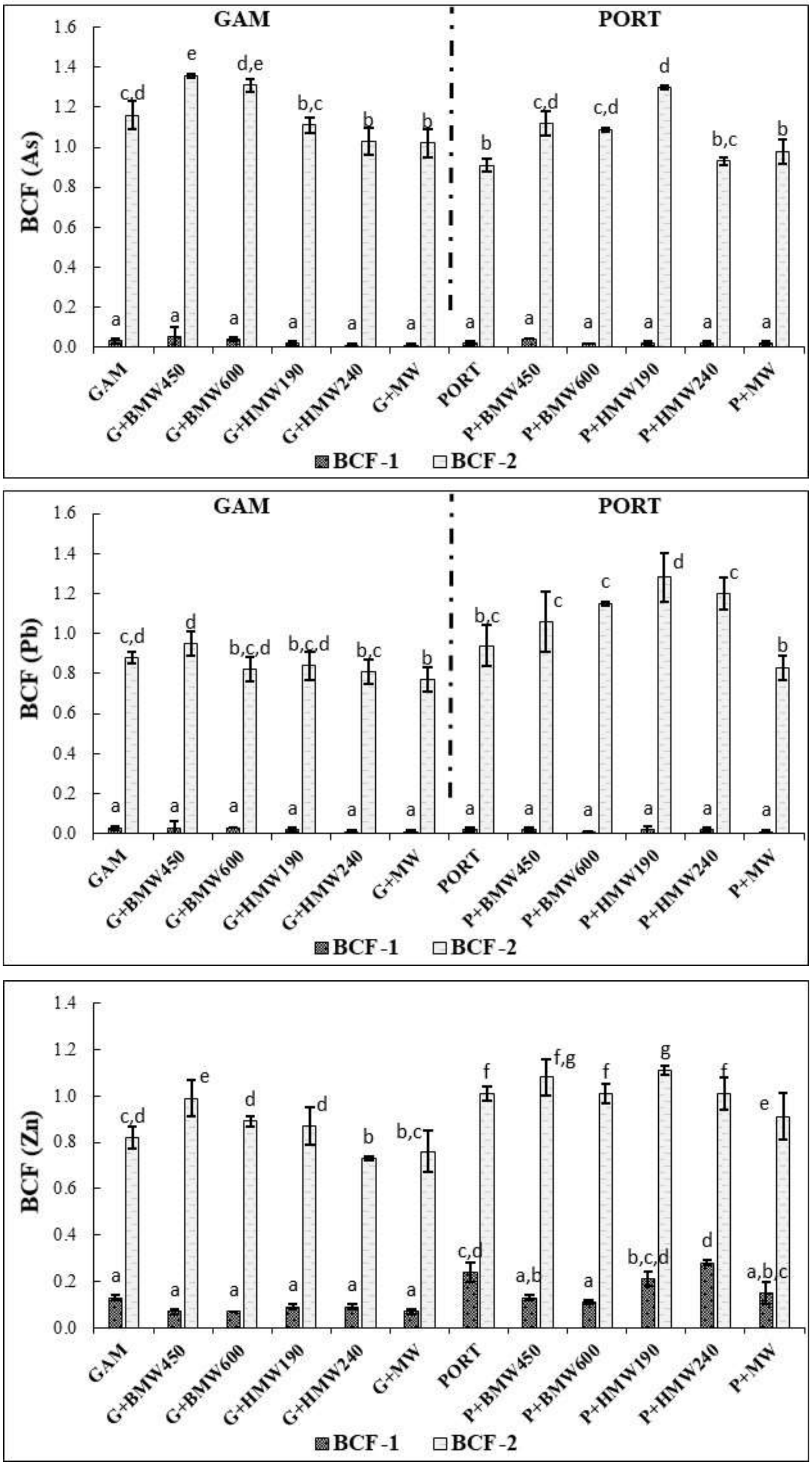
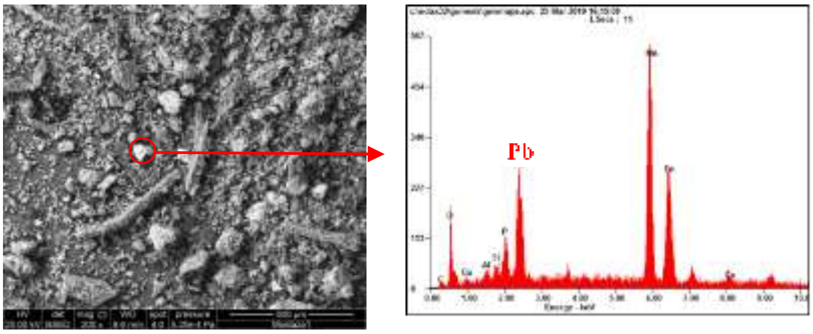


Figure 6. Bioconcentration factor (BCF) of As, Pb and Zn throught *Sinapis alba* grown in different treatments. Values are reported as means ± standard deviation. Means with common letter are not significantly different ($p > 0.05$) using the Duncan test.



Element	Wt%	At%
C	02.06	08.83
O	09.07	29.20
Al	01.08	02.05
Si	01.19	02.17
P	02.70	04.49
Mn	30.40	28.51
Fe	14.62	13.49
Cu	02.82	02.28
Pb	36.08	08.97

Figure 7. SEM-EDX of *Sinapis alba* roots in PORT soil.

Table 1. Main characteristics of GAM and PORT soils

	GAM	**PORT**
Soil texture	Sandy clay loam	Sandy loam
pH	7.13 ± 0.08	7.7 ± 0.10
EC (dS m^{-1})	0.91 ± 0.08	0.19 ± 0.02
C_{oxi} (%)	1.73 ± 0.09	0.78 ± 0.05
CEC ($cmol_c$ kg^{-1})	10.23 ± 0.20	23.89 ± 0.78
Na ($cmol_c$ kg^{-1})	17.0 ± 0.8	13.0 ± 0.3
K ($cmol_c$ kg^{-1})	22 ± 1	55 ± 3
P (mg kg^{-1})	862 ± 33	337± 9
Cd (mg kg^{-1})	10.5 ± 0.3	3.0± 0.2
V (mg kg^{-1})	62.34 ± 3.39	70.99 ± 1.52
Cr (mg kg^{-1})	48.48 ± 0.81	44.32 ± 1.61
Fe (g kg^{-1})	170 ± 5	90 ± 4
Co (mg kg^{-1})	20.5 ± 2.5	14.9 ± 0.4
Ni (mg kg^{-1})	38.1 ± 5.1	42.1 ± 3.9
Cu (mg kg^{-1})	16.5 ± 0.4	35.1 ± 1.12
Zn (g kg^{-1})	10.0 ± 0.3	2.2 ± 0.06
As (mg kg^{-1})	61 ± 3	151 ± 6
Al (g kg^{-1})	16 ± 0.6	24 ± 2.3
Pb (g kg^{-1})	2.3 ± 0.1	2.7 ± 0.2

Table 2. pH, EC and microbial biomass carbon (C-biomass) of GAM and PORT soil samples after growth of *Sinapis alba*

	pH	EC (dSm^{-1})	C-Biomass (µg C/g)
GAM	7.54±0.12[a]	0.75±0.03[c]	174.39±79.07[a,b]
G+BMW450	8.09±0.07[d]	2.15±0.15[h]	286.16±9.31[a,b,c]
G+BMW600	8.25±0.03[e]	2.10±0.10[h]	466.76±40.47[d,e]
G+HMW190	7.64±0.12[b]	1.15±0.01[e]	845.29±57.92[f]
G+HMW240	7.49±0.02[a]	0.87±0.02[d]	586.94±39.45[e]
G+MW	7.68±0.00[b]	1.59±0.04[g]	571.88±49.15[e]
PORT	8.25±0.01[e]	0.14±0.00[a]	165.55±51.57[a]
P+BMW450	8.47±0.02[f]	1.17±0.01[e]	302.97±46.10[b,c]
P+BMW600	8.54±0.04[f]	1.27±0.04[f]	291.43±82.99[a,b,c]
P+HMW190	8.01±0.01[c,d]	0.42±0.00[b]	415.88±24.34[c,d]
P+HMW240	7.95±0.03[c]	0.44±0.03[b]	306.30±73.78[c]
P+MW	8.06±0.01[d]	0.75±0.01[c]	321.39±83.11[c]