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Application of olive mill waste-based biochars in agriculture: Impact on soil properties, enzymatic activities and tomato growth

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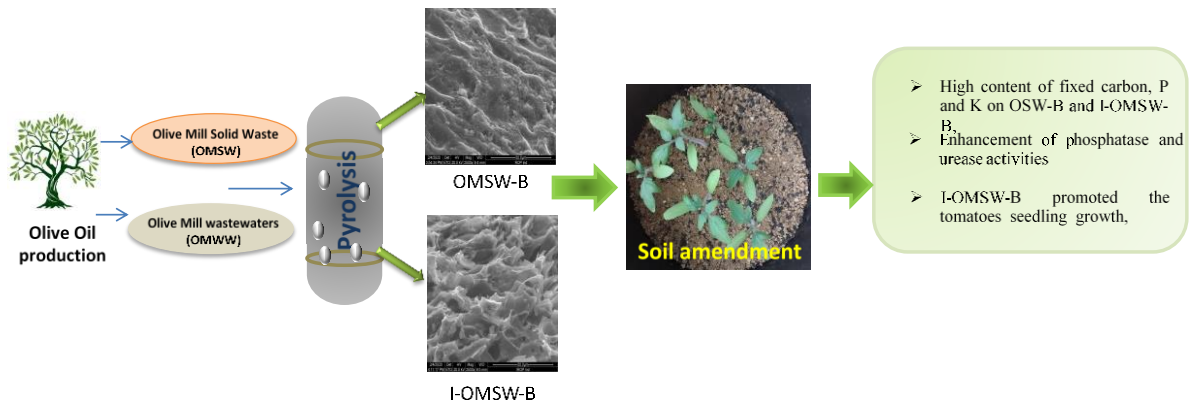
Abstract

The olive oil industry is an important economic sector in Mediterranean countries. However, oil production is unfortunately accompanied by the generation of huge amounts of olive mill solid wastes (OMSW) and olive mill wastewater (OMWW). In the present study, a strategy is proposed for converting these olive mill wastes into biochar through pyrolysis, for their later use as an organic amendment in agriculture. Specifically, two biochars were prepared from the pyrolysis of OMSW at 500°C, either alone or impregnated with OMWW (OMSW-B and I-OMSW-B).

The characterization of the OMSW and I-OMSW samples and their derived biochars showed that the fixed carbon and ash contents in the feedstocks increased by 38% and 11% respectively for OMSW-B, and by 37% and 12% respectively for I-OMSW-B. Interestingly, the impregnation process significantly increased Na, P, K, Ca and Fe contents in the produced biochars. The effect of OMSW-B and I-OMSW-B amendments at different application dose (1%, 2.5% and 5% wt/wt) on the enzymatic activity of an agricultural soil was performed at laboratory scale with a pot test. The experimental results showed that phosphatase and urease activity increased with biochar application rate; amendment with I-OMSW-B at 1%, 2.5% and 5% enhanced the phosphatase activity by 63%, 142% and 285% and urease activity by 50%, 116% and 149%, respectively. On the other hand, dehydrogenase and protease activities were higher for the application rate of 2.5 % biochar. Biochar amendment promoted tomatoes seedling growth after 10 weeks, which was highest in the application rates of 2.5% and 5% for both OMSW-B and I-OSMW-B. Thus, the produced biochars had great potential to be used as biofertilizers in agriculture.

Keywords: Olive mill wastes; Pyrolysis; Biochar; Biofertilization; Tomato growth.

Graphical Abstract



Highlights

- Slow pyrolysis performed to convert OMSW and its impregnated form onto biochars.
- Biochars characterized using proximate, mineral analysis and crystalline structure.
- I-OMSW-B exhibited a high contents of C, P and K comparing to initial feedstocks.
- I-OMSW-B amendment promoted tomatoes seedling growth and enzymatic activities.
- Studied biochars didn't have phytotoxic effect under tested doses.

1. Introduction

Olive oil production is the the main agricultural activity in Mediterranean basin countries, having a great economic importance (Asses et al., 2017). Tunisia, Spain and Greece annually produce a total of 1.7 million tones of olive oil, representing about 59% of the worldwide total production during the 2010/2016 period (IOOC, 2017). Depending on the extraction method, different types of olive mill wastes are generated during the oil extraction process. In countries such as Tunisia located in the the southern Mediterranean, the three-phase extraction system is the most common. Besides olive oil, this extraction system produces huge amounts of a liquid phase called olive mill wastewater (OMWW) and a solid cake called olive mill solid waste (OMSW). In Tunisia, the average annual production of olive oil in 2018 reached 210,000 tons and generated about 770,000 m³ of OMWW and about 550,000 tons of OMSW (Meftah et al., 2019). The management and disposal of these wastes represent a crucial issue in all olive oil-producing countries (Chouchene et al., 2010; Haddad et al., 2017).

The composition of OMWW is dependant on the variety of olive, pedoclimatic conditions and the oil extraction process (Achak et al., 2009). It is commonly characterized by high levels of chemical oxygen demand (COD) and biological oxygen demand (BOD) reaching values of more than 200 and 100 g L⁻¹ respectively (Lee et al., 2019). Direct disposal of OMWW in nearby aquatic systems, or spreading on arable lands (Mekki et al., 2007; Mechri et al., 2008; Magdich et al., 2016) have led to soil pollution, surface and ground waters contamination, odor nuisances, and inhibition of aquatic life and and vegetation (Karaouzas et al., 2011; Aggelis et al., 2003). For this reason, several treatment methods have been proposed such us ultrafiltration (Khemakhem et al., 2017), coagulation-flocculation (Sakira et al., 2004), advanced oxidation processes (Bargaoui et al., 2020), biological treatments (Tekerlekopoulou et al., 2017), composting (Chehab et al., 2019) and hydrothermal carbonization (Azzaz et al., 2020). However, despite their technical effectiveness, most of them are of low economic viability and

therefore other alternatives are required (Athanasios et al., 2017). It is important to underline that OMWW is very rich in nutrients and its recovery for subsequent use in agriculture is strategic as a circular economy strategy for materials and nutrient reuse (Haddad et al., 2017; Ditournie et al., 2019; Azzaz et al., 2020). In this context, the impregnation of OMWW onto lignocellulosic biomass and at adapted ratio, was proposed as an OMWW valorization with interesting agronomic impacts. The resulting biomass was then composted as a humidified raw material (Asses et al., 2017).

OMSW, also known as olive pomace, is a mixture of seeds, olive skins and pulp (Leon-Camacho et al., 2003). Its high content of polyphenols and heavy metals make it a waste of high phyto-toxicity which is recalcitrant to degradation (Leon-Camacho et al., 2003). Several treatment processes have been tested for the valorization of OMSW such as composting and co-composting with other green residues (Asses et al., 2017), anaerobic digestion with biogas, and liquid fertilizer production (Batuecas et al., 2019). However, OMSW toxicity and sensitivity of methanogenic bacteria to the contained phenolic compounds limits technical feasibility and economic viability of these methods (Orive et al., 2016).

Pyrolysis of olive mill wastes has been recently applied as an eco-friendly and effective method for their sustainable management (Atallah et al., 2019; Haddad et al., 2017). Indeed, the olive mill wastes are converted into three products: a bio-oil that can be used as a green biofuel, a gaseous fraction, rich in methane which can be valorized for energy production, and a solid fraction called biochar which can have various environmental and agronomic applications (Jeguirim et al., 2020; Dutournie et al., 2019; Haddad et al., 2017).

The use of biochars for soil restoration and biofertilization has received increasing interest as low-cost and eco-friendly amendements (Lone et al., 2015; Chen et al., 2018; Ibn Ferjani et al., 2020). Many studies have reported the role of biochars in carbon sequestration in soils, and therefore contributing to climate change mitigation (Soong et al., 2017; Han et al., 2020).

Moreover, biochars have been used as a remediation tool by limiting mobility of heavy metals and bioavailability of pesticides (Khorram et al., 2016; Oleszczuk et al., 2014). In addition, biochar has also been reported to have a positive impact on soil stability via, altering the size of aggregates and regulating water soil, which may promote plant growth (Obia et al., 2016; Chen et al., 2018). Amendment of agricultural soils with biochar is recommended as a promoting strategy to enhance ecosystem services because of its improvement of soil properties in world regions whose properties limit plant growth (Crane-Droesch et al., 2013), as well as other indirect positive effects on soil biological activities (Zhang et al., 2018). Among others, this practice has been reported to have significant effects on the distribution of soil microflora and invertebrate communities (Oleszczuk et al., 2014; Andrés et al., 2019), relative abundance of bacteria and fungi (Khadem and Raiesei, 2017), suppressive effect of soil born pathogenes (Jaiswal et al., 2017), and increases in enzymatic activity (Zhang et al., 2018; Tang et al., 2019). With such effects, biochar has often been reported to have positive effects on plant growth and improvement of crop yield (Kumar et al. 2020; Biederman et al., 2012) mainly due to carbon input increase and nutrient leaching reduction in soil (Xu et al., 2020; Hussain et al., 2017). In this context, Chen et al. (2018) demonstrated that after nine months of biochar amendment, soil bacterial and fungal structures were altered; which enhanced the plant productivity and species richness.

The Main hypothesis of this study is to effectively transform olive mill wastes (OMWW and OMSW) onto biochar with interesting biofertilizing impacts. The approach consists in the recovery of nutrients contained in OMWW and OMSW via impregnation with the latter, followed by a controlled pyrolysis step of the resulting solid matrix. The aims of this work were to: (i) To perform a deep characterization of produced biochar and underline the impregnation effect on its physico-chemical properties (ii) To assess the agronomic impact of the generated biochar (I-OMSW-B) as compared with raw OMSW (OMSW-B) at laboratory scale in tomato

growth pot tests with a Tunisian soil and (ii) To evaluate the adequacy of these biochars and potential effects on soil properties through phytotoxicity tests and measurement of soil enzymatic activities. To the best of our knowledge, this current investigation is the first research work dealing with the agricultural valorization of biochars prepared from OMSW and OMWW.

2. Materials and methods

2.1. Feedstock materials

Olive mill solid waste (OMSW) and the olive mill wastewater (OMWW) were collected from a three-phase olive mill press located in Grombalia, Nabeul city in the Northeast of Tunisia. For OMSW, particles were firstly sieved and only particles with sizes smaller than 2 mm were utilized in further assays. The sieved OMSW was air-dried and stored in hermetic bags for further use. Concerning the OMWW, it was first homogenized and then kept in plastic cans at 4°C until use. Before the impregnation experiment, the OMWW sample was filtered through a sieve with a mean mesh opening size of 100 µm in order to remove the relatively large particles contained in the raw OMWW.

2.2. OMSW impregnation with OMWW

For the impregnation phase, 400 g of OMSW were introduced into 2 L of OMWW and the solution was mixed using a magnetic stirrer (Yellow line OST basic) at a speed of 700 rpm during 4 hours (Jeguirim et al., 2020). This amount corresponds to 200 g of OMSW per liter of OMWW. The impregnated biomass was first dried at 60°C for 120 min to discard the excess water. The impregnation step was repeated several times to obtain a sufficient amount for the experimental agronomic assays.

2.3. Biochars synthesis

The obtained dried material (OMSW and I-OMSW) was subjected to slow pyrolysis in a tubular furnace reactor (Fig. A-1) as optimized in previous work (Ibn Ferjani et al., 2019). Briefly, 2

kg of these two samples were distributed along the pyrolyser's seven drawers. N₂ was constantly injected at a flow of 5 NL min⁻¹ for 30 min to discard the residual oxygen. The temperature increased at a range of 10 °C min⁻¹ under N₂ injection at flow rate of 5 L min⁻¹ until reaching 500°C. The pyrolysis reaction lasted 2 h at 500 °C followed by a natural cooling under N₂ flow. The obtained solid fractions after pyrolysis were designated as OMSW-B and I-OMSW-B for the non-impregnated and impregnated materials, respectively.

2.4. Characterization of feedstocks and biochars

Proximate, elemental and mineral compositions of the feedstocks and derived biochars were undertaken. The proximate analysis was performed according the TGA procedure as described previously (Azzaz et al., 2020).

The elemental analysis of initial feedstocks and generated biochars was performed using a CHONS analyzer (Flash 2000, Thermo Scientific, Cambridge, UK). The mineral composition was carried out using X-ray fluorescence spectrophotometer (Philips, Eindhoven, Netherlands - PW2540) for analysis) as described by Thomson and Leege (Leege, 1998).

The pH and the electrical conductivity (EC) of the produced biochars I-OMSW-B and OMSW-B were determined for the solutions resulting from the shaking of biochars in deionized water at a ratio of 1:10 (w/v). pH and EC values were measured by using a pH meter (HENNA instruments HI 2210) and a conductmeter (Consort C561).

Microscopic observations of the two produced biochars were performed using a scanning electron microscopy (SEM) (Philips model FEI model Quanta 400 apparatus, Amsterdam, The Netherlands). Before observation, samples were coated with a tinny gold layer using an Agar automatic sputter coater. Different magnification levels were assessed during the observations.

Specific surface area and porosity of the biochars were assessed through CO₂ adsorption using an ASAP 2020 gas adsorption apparatus (Micrometrics, Gerorgia, USA). Before analysis, 0.2

g of each biochar was degassed under vacuum ($> 10 \mu\text{mHg}$) for 24 hours at 200°C . Analyses were afterwards performed at 273K with progressive CO_2 gas doses. The specific surface area was determined using the BET model in the relative pressure range, P/P_0 0.01-0.03. Pore size distribution was determined using the CO_2 2D-NLDFT (Non-localized density functional theory) slit pores model. Moreover, X-Ray diffraction (XRD) was used to identify the crystalline phases in the biochars using a Panalytical X'Pert powder diffractometer (Malvern, UK) equipped with a copper anode. The crystalline composition was identified with the ICDD database on the Panalytical Highscore software as assessed previously (Graulis et al., 2009).

2.5. Phytotoxicity test

The phytotoxicity of the biochars was assessed through a germination test. Prior to use, Cress *Zea mays* (maize) seeds were soaked in dionized water for 4h. For each replicate, 30 seeds were sown in a Petri dish filled with 50 g of soil-biochar mixture (1%, 2.5% and 5%) after 5 days incubation. Then, all Petri dishes were incubated at 25°C , in dark conditions. After five days, seeds having 2 mm of emergent roots length were counted and considered as germinated seeds. The agricultural soil with no biochar was used as control treatment. The number of germinated seeds and the root elongation were measured to calculate the germination index (GI) as follows (Mohamed et al., 2017):

$$\text{GI (\%)} = (\text{Gbc} \times \text{Lbc}) / (\text{Gc} \times \text{Lc}) * 100 \quad (\text{Eq.1})$$

Where: Gbc and Lbc are the average number of germinated seeds and average root elongation in the biochars treated soils. Gc and Lc represent the same average measured parameters in the control assays (without biochars).

2.6. Tomato growth experiments

The effect of the biochars on properties of a local agricultural soil, enzymatic activities and phytotoxicity was carried out in pot experiments (height: 30 cm; inner diameter: 20 cm).

OMSW-B and I-OMSW-B were added to the agricultural soil at increasing application rates on a w/w basis: 1%; 2.5% and 5%. Each pot was filled with a total (soil and biochar) mass of 2 kg. The final treatments were: i) control test: agricultural soil without any amendment; ii) OMSW-B -1%, OMSW-B -2.5% and OMSW-B -5%: agricultural soil amended with 1%, 2.5% and 5% of OMSW-B, respectively, and iii) I-OMSW-B -1%, I-OMSW-B -2.5% and I-OMSW-B -5%: agricultural soil amended with 1%, 2.5% and 5% of I-OMSW-B, respectively. All these treatments were performed in triplicate and the reported results correspond to the mean values. All pots received a solution of nitrogen fertilizer (KNO_3) at a dose of 80 mg kg^{-1} at the beginning of the experiment.

In this study, tomato seeds (*Solanum lycopersicum*), were used as a model plant due to their high agricultural value and relevance. Tomato seeds were sown in all the pots with a seedling density of 1.5 g per pot. In total, 21 pots were prepared and 40 seeds were placed in each pot to ensure seed emergence (Asses et al., 2017). The soil used for experiment was composed in average of 35% silt, 22% clay, 14% coarse and 28% fine sands. It had a pH and a conductivity of 7.5 and 0.5 mS cm^{-1} respectively. It was a relatively poor soil with organic matter content of 0.71% wt and average contents in N, P, K about 0.03% wt, 0.052% wt, and 0.13% wt, respectively.

The experiment was carried out in ambient conditions in a local nursery irrigated with a volume of 200 mL water every 2 days. The total duration of the experiment was fixed at 10 weeks. The soil sampling was performed every week starting from May 1st 2019, and carried out till July 15th 2019.

On the same day, a sub-sample of 3 plants was randomly selected to measure growth parameters; stem length and root length. The plant growth reported in this work represents the sum of stem and root length expressed in cm per plant.

2.7. Enzymatic activity of the control and biochar amended soils

The pot test soils amended with OMSW-B and I-OMSW-B under different doses of biochar as described above was sampled after 10 weeks of incubation period, to assess the enzymatic activities of the following enzymes: dehydrogenase activity by the iodinitrotetrazolium chloride (INT)-formazan ml^{-1} assay method (Mersi and Sehinner, 1991); phosphatase activity using a calorimetric estimation of the p-nitrophenol released by phosphatase activity when soil is incubated with buffered (pH 6.5) sodium p-nitrophenyl phosphate solution and toluene at 37°C for 1 hr. (Tabatai and Bremner, 1969), urease activity which involves the determination of the ammonium released by incubation of the soil sample (5g) with tris(hydroxymethyl)aminomethane, urea solution and toluene (0-2 ml) at 37°C for 2 h, (Zantua and Bremner, 1975) and protease activity using proteins and dipeptide derivatives as substrates (Ladd and Butler, 1972).

2.8 Statistical analysis

The statistical analyses were undertaken with R v3.6.1 (R Development Core Team, 2019). For the study of plant growth, a mixed model approach was adopted in order to handle the different components of variance of the longitudinal dataset for, using the *nlme:lme* function. One model was tested for each biochar and considering observations of each plant from weeks 1-10. In the model, biochar concentration was defined as the explanatory variable, and plant and week as random variables. Global model statistical significance was tested using the *anova* function, and model adequacy was checked by visual inspection of a Q-Q and a Shapiro test for normality of residuals..

For enzymatic activities, due to the small number of observations, normality of the data cannot be ascertained. Therefore, a global non-parametric statistical test was used (Kruskal-Wallis, *stats:kruskal.test*) to test for differences within each biochar and enzyme assay group.

3. Results and Discussions

3.1. Feedstocks and biochars characterization

. The carbonization yield of produced biochars was 40.5% and 36.0% for OMSW-B and I-OMSW-B, respectively. In fact, it was previously reported (Haddad et al., 2017) that the impregnation with OMW, allowed an efficient and rapid drying biomass process, since it contains a residual oil thin layer. An earlier degradation of feedstock could also be attributed to the mineral content such as K, Na and Mg brought by OMW impregnation. Such biochar yields are higher than those found in the literature for common lignocellulosic biomasses (Gai et al., 2014), which comprises an advantage for their economic viability when produced at large scale.

Proximate analyses were performed to determine the fixed carbon, volatile matter and ash contents for both feedstocks (OMSW and I-OMSW) and their derived biochars (OMSW-B and I-OMSW-B). Results, reported in Fig. 1, showed that raw feedstocks contain initially high percentages of volatile matter almost similar for the OMSW and I-OMSW (66% and 67% respectively). The percentage of fixed carbon was about 19.6% and 24% for OMSW and I-OMSW, respectively (Fig. 1). The incorporation of OMWW into the lignocellulosic matrix of OMSW enhanced its content in organic carbon and in fixed carbon percentages. After the pyrolysis at 500°C, the measured fixed carbon and ash contents (Fig. 1) in the feedstocks increased by about 65% and 45% for OMSW-B and by 63% and 48% for I-OMSW-B comparing to the raw feedstocks.

[Here Fig. 1]

After the pyrolysis at 500°C, carbon contents significantly increased as reported in Table 1.a. In fact, the C% was increased by about 19% and 25% for OMSW-B and I-OMSW-B compared to their initial feedstocks, respectively. The C% reached 63.83% and 71.08% for OMSW-B and I-OMSW-B. In contrast, a decrease in O, H, N and S percentages was observed after the thermal

treatment; the N(%) decreased by 53% and 63% and S(%) by 72% and 78% respectively for OMSW-B and I-OMSW-B. This fact is due to the degradation of biomass aromatic skeletal and a rearrangement of carbon structure mainly due to the release of oxygen and hydrogen content with volatile matter in the form of by-product gases (CH₄, CO₂, CO ...).

[Here Table 1 (a) and (b)]

The analysis of the mineral composition (Table 1.b) indicates that the initial feedstocks had relatively high contents of potassium (K), phosphorus (P) and calcium (Ca) compared to other biomasses (Ibn Ferjani et al., 2019). For instance, the K contents were 1.53% and 1.64% for OMSW and I-OMSW, respectively (Table 1.b). The impregnation process increased the contents of various elements such as Na, P, K and Fe contained in the I-OMSW. These elements which are found in at relatively high concentrations in OMWW (Haddad et al., 2017; Bargaoui et al., 2020) were thereby transferred to OMSW. This mass transfer could be ensured mainly through adsorption or intra-particle diffusion mechanisms from OMWW to OMSW (Bargaoui et al., 2020). However, an inverse mass transfer was observed for other elements such as Mg, Si and Ca, which can be attributed to the acidic pH of OMWW. Rajapaksha et al. (2016) reported that the surrounding acidity could cause the release of minerals from the solid to liquid fraction and it is considered as washing process for the release of minerals from solids.

Following pyrolysis, the mineral composition of the derived biochars (Table 1.b) showed that most of the measured mineral contents in OMSW-B and I-OMSW-B increased compared to their related feedstocks. The contents of Na, P, K, and Fe in the biochars reached 1.47%, 0.30%, 4.71% and 0.53%, for OMSW-B and 0.85%, 0.34%, 5.54% and 0.45% for I-OMSW-B, respectively. Calcium contents increased notably following pyrolysis, from 1.67% to 7.40% and from 1.58% to 5.80% for the OMSW and I-OMSW feedstocks, respectively (Table 1-b). It is particularly notable to mention that P and K contents in the I-OMSW-B were about 13% and 18% higher than in the non-impregnated biochar. Enrichment with these critical nutrients is an

important property when considering the use of this impregnated biochar as an amendment in agriculture. Also, both biochars obtained in this study had high contents of K compared to other biochars reported in other studies (Zhao et al., 2013). For instance, K contents of poplar and pine wood biochars reported by Marks et al. (2014) were between 0.4 and 0.9%, so based on the results of the present study, the contents of olive mill waste biochars may be between 5 and 10 times higher. The N contents of OMSW-B and I-OMSW-B were about 0.98% and 0.71% respectively and it is particularly similar to poultry manure derived biochar (Hadroug et al., 2019) produced at 400°C and 600°C. The P contents were about 0.3% and 0.34% which is notably higher than various biochars produced from lignocellulosic materials. For instance, the P contents for peanut shell, rice husk, wheat straw and hardwood were about 0.17 %, 0.12%, 0.02% and 0.03% (Zhao et al., 2013).

The measured pHs of the two produced biochars were alkaline, at pH 9.5 and 8.8 for OMSW-B and I-OMSW-B, respectively. This result is somehow expected considering the rich composition in alkaline metals. The pH of the I-OMSW-B is less alkaline due to the impregnation with OMWW, which has an acidic pH of 4.77 (Bargaoui et al., 2020).

3.2. Morphology, texture and crystalline structure of produced biochars

Figure 2 shows the SEM images of the two obtained biochars at three magnification levels of 500, 1000 and 2500 times.

[Here Fig. 2]

Observations of the micro-structure of the biochars showed that OMSW-B had a relatively smooth surface and hardly visible porosities without any sharp angles, such as those as observed for I-OMSW-B. The latter exhibited a discontinuous surface with well pronounced open porosities, clearly visible in images with the magnification of 2500x. The presence of sharp angles and elongated shapes made the particles rough and grainy. Therefore, the impregnation

of OMSW with OMWW affected the external morphology of the produced biochar, and in particular the macroporosity structure. To further emphasize the porosity modification of the materials CO₂ adsorption isotherms were measured as depicted in Figure 3a.

[Here Fig. 3]

Results showed a slight decrease in the CO₂ adsorbed volume which translates a decrease in the specific surface area of the produced biochars after the impregnation process. Indeed, these measured values were 166 m²/g and 115 m²/g for OMSW-B and I-OMSW-B, respectively. The decrease in CO₂ adsorbed volume in impregnated material could be explained by a partial blockage of the pores due to the minerals crystallization at the surface of the biochars. This tendency is further confirmed by the pore distribution profile (Figure 3b). For both cases, pores diameters varied between 0.40 nm and 0.63 nm with a maximum at around 0.47 nm suggesting that OMSW-B and I-OMSW-B are mainly composed of ultramicro-porous structures. In fact, the impregnation of OMWW onto OMSW led to a transfer reaction of variable concentrations of minerals (Mg²⁺, K⁺ and Na⁺) from liquid to solid fractions. Similar results were obtained by Haddad et al. (2020), who reported a micropores volume decrease of Cypress sawdust derived biochar after OMW impregnation (from 0.15 to 0.09 cm³g⁻¹). In fact, during pyrolysis, the presence of minerals on the surface of a lignocellulosic material usually enhances the dehydration of its carbohydrate polymers, causing a fast release of the volatile matter and eventually the formation of a ramified porous structure under the effect of heat (Shen et al., 2019). However, it has been reported that an excess in surface mineral concentrations, especially magnesium and calcium might lead to the formation of mineral oxides along with other intermediate thermal decomposition products blocking the pores and thus reducing the biochar's surface area (Zhang et al., 2020).

The crystalline structure of the biochars produced from OMSW and its impregnated derivatives at 500°C were assessed using the XRD technique (Fig.4).

[Here Fig. 4]

According to the XRD diagrams shown in Figure 4, OMSW and I-OMSW feedstocks presented relatively similar patterns in terms of crystalline composition. A number of common structures were detected, namely calcium carbonate (CaCO_3), potassium chloride (KCl) and silicon oxide (SiO_2). A specific broad peak was detected for both materials at 22.05° (2θ) corresponding to cellulose I amorphous structure with an increasing intensity for I-OMSW (Azzaz et al., 2016). After pyrolysis at 500°C , the peaks detected in both produced biochars are largely more intense compared to the feedstocks' peaks, confirming the immobilization of minerals inside the carbonaceous materials in the form of salts but also the increase in the size/crystallinity of minerals. The formation of these minerals is the result of reactions between the CO_2 , alkaline-earth metals and alkaline oxyhydroxides formed during biochar formation, whereas an increase in their mass concentration leads to higher crystalline affinity (Mohanty et al., 2013). Moreover, the intensity of some peaks decreased for I-OMSW-B compared to I-OMSW. These results are in concordance with the proximate and mineral analyses where a release of some minerals has occurred during the impregnation of OMWW onto OMSW. Also noted for both materials the presence of a large peak around 24.55° (2θ) with an increasing intensity for I-OMSW-B. This could be attributed to a rearrangement of the aromatic skeleton of lignocellulosic material into a graphitic-like carbon structure since the cellulose structure is known to be destroyed at temperatures beyond 400°C (Kim et al., 2012). The broadness of the peak indicates carbonaceous materials with disordered structure.

3.3. *Effect of biochars on soil properties*

Biochar amendment of soils is generally followed by modification of soil pH and EC. The initial pH of OMSW-B and I-OMSW-B is estimated respectively to 9.5 and 8.8. As shown in Figure 5, the application of OMSW-B considerably increased the pH of the resulting soil-biochar mixture, an effect which increased with the biochars application rate. The pH values

were 6.72, 7.35, 7.54 and 7.87 respectively for control, 1%, 2.5% and 5% for OMSW-B amendments. The increase of pH for soil amended with I-OMSW-B was less pronounced than OMSW-B samples. It was previously reported that the alkalizing effect of biochar on soils was mainly due to the basic cations (K, Ca and Mg) in biochar which decreased the exchangeable ions of aluminium (Al) and hydrogen (H^+) by adsorption or chelation with organic compounds (Syuhada et al., 2016). Nevertheless, the pH increase on soils is related to biochar initial pH and its derived biomass (Tian et al., 2018). The pH increase could considerably improve nutrients availability in agricultural soils and thus facilitate their uptake by plants roots growth (Matsubara et al., 2002). The pH variation could also have an effect on the fungi-to-bacteria ratio and regulate the rate of organic carbon turnover (Kimetu and Lehmann, 2010). Chaturika et al., (2016) reported that a significant increase of pH in alkaline soils could limitate the nutrient bioavailability especially in which mineral fertilizer might be combined to biochar for a better efficiency.

[Here Fig. 5]

Biochar amendments also affected the EC of the tested treatments. Soil EC increased linearly with increasing dose of biochars (Fig. 5). Chaturika et al. (2016) suggested that the formation of carboxylic and phenolic functional groups increases biochar EC. The electrostatic attraction force was enhanced due to the cation retention by establishment of bonds with negatively charged sites of biochars, organic matter and organo-mineral complexes (Peng et al., 2011). Arif et al. (2018) showed that biochar amendment caused a reduction in soil EC, which is associated with microbial assimilation of NO_3^- and SO_4^{2-} . Meanwhile, increasing pH and EC on biochar amended soils were required for biofertilizing abilities of biochar.

3.4. *Phytotoxicity assay of biochar*

Prior to the agronomic tests, the possible phytotoxicity of the biochars produced from raw and impregnated OMSW was undertaken through specific assays using Cress *Zea mays* (maize) seeds according to the experimental protocol given in section 2-5. The estimated germination indexes (GI) are given in Table A.1. It is assumed that for seeds having GI above 60%, there is no significant phytotoxicity effect (Mohamed et al., 2017). Therefore, no phytotoxic effect was observed for the two used biochars. Furthermore, there were no significant differences in term of GI between OMSW-B and I-OMSW-B under the tested doses, compared to the control. The highest GI (72.3 ± 1.5 %) was observed when using OMSW-B at a dose of 2.5%. Similar trends were reported by Hmid et al. (2014), where the GI using raw OMSW derived biochars were assessed for washed and non-washed biochar, whereas the GI for biochar produced at the same temperature was considerably increased for washed biochar. It was recently reported that peanut straw derived biochars have an efficient impact on Al phytotoxicity of maize seeds due to its alkaline nature (Shi et al., 2020).

3.5. Effect of biochar on enzymatic activities

Enzymatic activities have been extensively used as fertility and productivity indicator for agronomic soils (Hussain et al., 2017). Estimating the enzymatic activities after 10 weeks of biochar amendment can lead to an understanding of the metabolic mechanisms of indigenous bacteria which influence nutrients bioavailability (Demisie et al., 2014; Tang et al., 2019). In general, high enzymatic activity values are used as indicator of a good soil quality (Hussain et al., 2017). As shown in Figure 6, amending the soil with biochar significantly improved the activity of alkaline phosphatase which can be partly explained by the increase in pH, deshydrogenase, urease and protease.

[Here Fig. 6]

As confirmed by the statistical analysis results in Table A.2, treatment led significant differences in enzyme activities in all of the groups tested. Also, each biochar concentration led

to different enzymatic activities in many cases. Since non-parametric post-hocs are highly insensitive for small sample numbers, differences between treatments should be only generally inferred from group means. On the basis of these results and Fig.6 (a, b, c, d), it is worth mentioning that enzymatic activities measured in the I-OMSW-B amended-soil are greater than activities measured for OMSW-B treatments, excepted for dehydrogenase activity under 5% amendment of biochar. This enhancement of enzymatic activities could be explained by the high percentage of fixed carbon (C%) (Fig.1) for I-OMSW-B (71.3%) compared to OMSW-B (63.8%). It was previously reported that soil enzymatic activities are positively correlated with organic C and/or total N (Gianfreda et al., 2005) and pH (Oleszczuk et al., 2014) as demonstrated in the above sections. Though not measured in the present study, there is a positive correlation between enzymatic activities and microbial biomass in the soil; an increase in enzymatic activities is an indicator of microbial biomass stimulation since most of enzymes are produced by soil bacteria (Demisie et al., 2014).

Phosphatase and urease activities (Fig. 6 a, b) increased with biochar application rate (an effect-dose relationship was observed). The urease enzyme is important for the hydrolysis of urea fertilizer into NH_3 (Makoi and Ndakidemi, 2008; Ibrahim et al., 2020) and an increase in urease activity has been reported to be caused by organic fertilization (Tu et al., 2020; Demisie et al., 2014). The phosphatase enzyme catalyses the hydrolysis of an anhydrous form of phosphoric acid in soil (Makoi and Ndakidemi, 2008). Soil amendment with I-OMSW-B increased the phosphatase activity by 63%, 142% and 285% and urease activity by 50%, 116% and 149%, respectively for doses of 1%, 2.5% and 5% of biochar. A similar trend was observed for OMSW-B added with the same doses, but with less marked tendency activity. Therefore, it is seen that an increasing dose of biochar promotes alkaline phosphatase and urease activities (Khadem and Raiesi, 2019). These data are consistent with Oleszczuk et al. (2014) results

showing that biochar amendment of 30 and 45T/ha could enhance the alkaline phosphatase activity by 198% and 120% and urease activity by 77% and 127%.

However, for dehydrogenase and protease activities, higher activities values were obtained for an amendment with biochars doses of 2.5 % rather than 1 and 5 %. After an amendment of 2.5% of biochar, the dehydrogenase activity increased by 192% and 186% respectively for I-OMSW-B and OMSW-B. However, at the application rate of 5% of biochar, the increase was about 77% and 101% respectively for I-OMSW-B and OMSW-B which is considerably lower. In the same sense, the protease activity increased about 134% and 107% with 2.5% of I-OMSW-B and OMSW-B, and about 70% and 64% fewer than 5% amendment with I-OMSW-B and OMSW-B, respectively.

Dehydrogenase activity is extensively used to evaluate the soil microbial activity (Brookes et al., 2008). Our results suggest that high doses of biochars inhibited the dehydrogenase and protease activities, which was previously reported by Oleszczuk et al. (2014) and Tang et al. (2019). Wang et al. (2016) demonstrated that biochar produced from three feedstocks (maize straw, pine wood dust and swine manure) at different pyrolysis temperatures and amended at a dose of 0.8 % could enhance the activities of invertase, neutral phosphatase, catalase, and polyphenol-oxidase, contrarily to urease and proteinase activities where the highest values were obtained for a biochar application rate of 0.2%. It was also reported that lower amounts of biochars enhanced the enzymes involved in C and S cycling, and that higher amendments decreased their activities (Wang et al., 2015). Therefore, considering the high C content (%) along with low S and N content (%) (Table 1.a), the produced biochars OMSW-B and I-OMSW-B could significantly decline the dehydrogenase and urease activities when applied at higher doses. Besides, the biochar composition could impact the micro-organisms abundance and community composition involved in enzymes synthesis. These effects could be explained by: (i) the adsorption of organic molecules and the inhibition of certain enzymes via adsorption

or blocking the active sites (Elzobair et al., 2016); (ii) depending on its origin, content and dose, biochar amendment might be toxic for indigenous microbial communities (Huang et al., 2017). Moreover, it was previously demonstrated that the effect of biochar on enzymatic activity is dependant on the temperature with which the biochar was produced (Zhang et al., 2018). Indeed, biochars produced at high temperatures generally didn't exhibit any effect on enzymatic activity and especially for dehydrogenase activity, due to its limited C and N content. Therefore, depending on its properties and its initial feedstock, the biochar can have different effect on soil characteristics (pH, EC, stability, soil aggregation), carbon content and mineral composition and thus determine its impact on diverse enzymatic activity.

3.6. *Effect of biochars amendment on growth parameters of tomato*

As explained in Section 2.6, we evaluated the effect of the biochars OMSW-B and I-OMSW-B on the growth of tomato seeds plants (*Solanum lycopersicum*) planted in a local agricultural soil, whereas the biometric parameters of stem height and root length was used as an indicator of vegetative growth over 10 weeks (Figure 7 a, b).

[Here Fig. 7]

The statistical analysis of plant growth is shown in Table A.3. The results of the models (one for each biochar), show that in both cases, biochar concentration had a significant, positive effect on plant growth (height) over the 10 weeks of the assay. Furthermore, the model estimates show that I-OMSW-B had a greater positive impact on plant growth than OMSW-B (0.44 as compared to 0.35). This is also reflected in the average values of Figure 7, where it is seen that the higher biochar application rates generally enhanced tomato growth. Under the OMSW-B treatment, during the first 4 weeks, growth was 4.56 and 3.99 cm/week for 2.5% and 5% of biochar amendements, respectively. Also, the highest growth rate compared to the control was obtained for a biochar application rate of 2.5%, measured to be 19.5 cm/plant at 10 weeks. For the I-OMSW-B amended soil, growth seems to have improved with increasing biochar

application rate (Figure 7). The growth rates during the first 4 weeks were 4.26 and 3.33 cm/week for I-OMSW-B 2.5 % and 5 %, respectively. Thus, at 10 weeks, the largest plants were found in the case of treatment with 5% I-OMSW-B, with an average size of 23 cm. Thus, based on above results, after 10 weeks, the I-OMSW-B led to better improvements in plant growth (Table A.3; Figure A.2).

On one hand, growth improvements compared to the control could be attributed to the minerals contained in the biochars. This is likely to be the reason for improved growth in the impregnated biochar treatment as opposed to the non-impregnated biochar (a direct fertilization effect).

Improvements crop yield observed after biochar amendment could be a direct contribution of high fixed carbon (58.8% and 61.2%) in amended biochars (Yang et al., 2020), as it was demonstrated to be effective in enzyme synthesis, seed germination and nutrition promotion (Verma et al., 2019). The yield increase is also the result of P and K content which highly influenced the seedling growth. On the other hand, indirect effects due to biochar addition may explain enhanced plant growth: the enhancement of phosphatase, urease, dehydrogenase and urease enzyme activities are directly correlated with the microbial community, which is responsible for nutrient bioavailability. It has been previously shown that biochar can enhance the microbial community responsible for phosphate solubilization and nitrogen cycling (Ducey et al., 2013) as well as suppression of phytopathogenes (Jaiswal et al., 2017). Chen et al. (2018) demonstrated that biochar amendment enhanced plant productivity, diversity and ecological performance which is attributed to bacterial function enhancement and nutrient cycling.

Finally, regarding physical effects, the morphology structure of the biochars, with pronounced porosities (especially in I-OMSW-B) could improve the specific surface area and nutrients bioavailability. Consequently, significant modifications occurred in relation with physical (aggregates stability, porosity) and chemical properties (pH, EC, moisture) of the soil

which lead to conclude that the I-OMSW-B have a great potential to be used as biofertilizer (Figure A.2).

It is worthy to mention that the difference on plant growth impact between I-OMSW-B and OMSW-B treatments could be mainly due to difference in carbon input, nutrients adsorption and slow release (Tan et al., 2017). Finally, since a fixed amount of water was applied during the study period, changes in soil structure and increased availabilities or water use efficiencies may also be responsible (Zao et al., 2020). It has also been reported that biochar can strongly affect the roots length density and mass density more than above-ground parts (Brennan et al., 2014; Wang et al., 2019).

The increased the height of seedlings with biochar application is consistent with previous reports (Wang et al., 2019; Bruun et al., 2014). Agbna et al. (2017) reported that biochar amendment of 25 t ha⁻¹ under irrigation deficit system increased water retention in soils and enhanced the yield of tomato crops whereas an amendment of 50 t ha⁻¹ could decrease the water use by 50% without a significant effect on yield. It was also noticed that biochar could affect strongly the roots length density and mass density more than above-ground parts (Brennan et al., 2014; Wang et al., 2019). Chen et al. (2018) demonstrated that biochar amendment enhanced plant productivity, diversity and ecological performance which is attributed to bacterial functions enhancement and nutrient cycling.

Conclusions

In this study, the slow pyrolysis of raw and impregnated OMSW with OMWW permits to get biochars with relatively high contents of fixed carbon, P, K and Ca contents. The significant enhancement of phosphatase and urease activity under OMSW-B and I-OMSW-B amendments made organic carbon as well as P and K available to the tomato cultivated crops. The tested biochars didn't present any phytotoxic effects for maize. Based on the positive effects for the

growth of tomato seedlings, it could be considered as a promising and eco-friendly organic fertilizer for the growth of various high added-values crops. The recycling of these materials could thus serve both as a treatment system for problematic wastes, and constitute a useful material for the agricultural sector. Thus, based on its physical and chemical properties (surface area, microporosity, light weight), it might be also be attractive for environmental technologies such as wastewater treatment. Therefore, regarding above properties, the generated biochars could be used as a promising biofertilizer for agronomic soils.

Credit Authorship Contribution Statement

Leila El-Bassi: Conceptualisation, Methodology, Investigation, Funding acquisition, Formal analysis, Resources, Writing - original draft. **Ahmed Amine Azzaz:** Methodology, Investigation, Formal analysis, Data curation. **Salah Jellali:** Conceptualisation, Methodology, Investigation, Writing – review & editing. **Hanene Akrouf:** Conceptualisation, Formal analysis, Resources, Writing – review & editing. **Evan A.N. Marks:** Formal analysis, Data curation, Writing – review & editing. **Camélia Matei Ghimbeu:** Investigation, Formal analysis, Funding acquisition, Writing – review & editing. **Mejdi Jeguirim:** Methodology, Conceptualization, Supervision, Funding acquisition, Writing - review& editing

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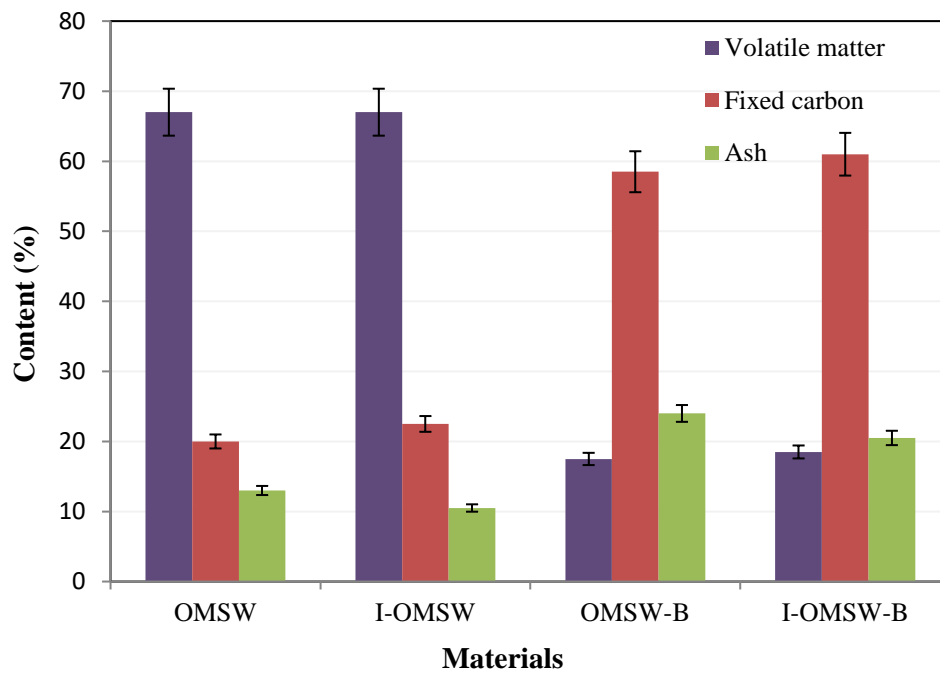


Figure 1. Proximate analysis of OMSW, I-OMSW, OMSW-B and I-OMSW-B materials.

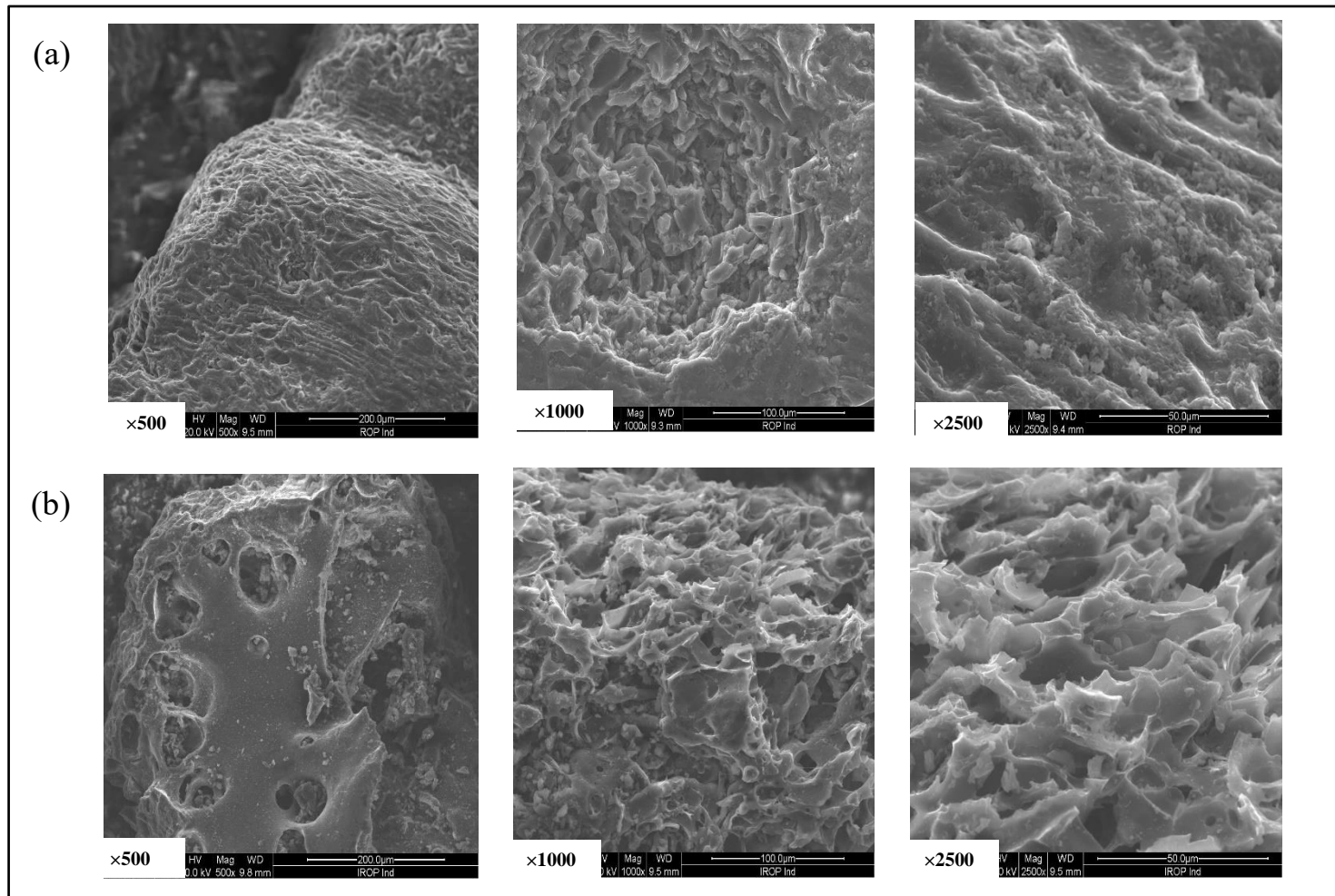
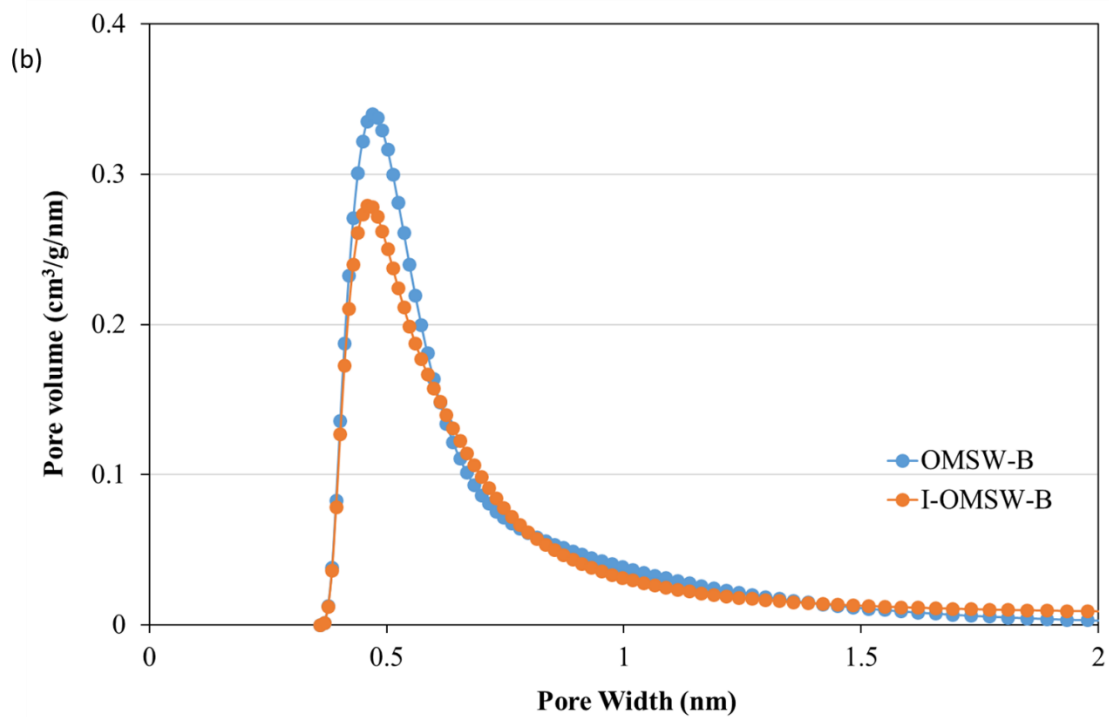
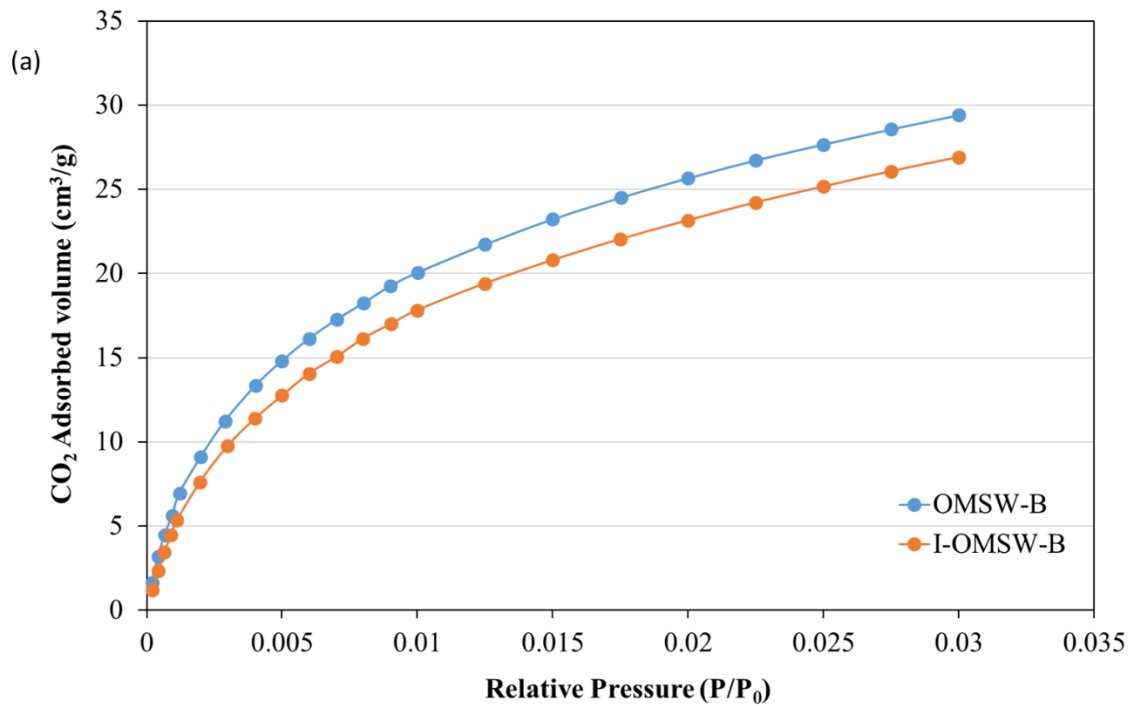


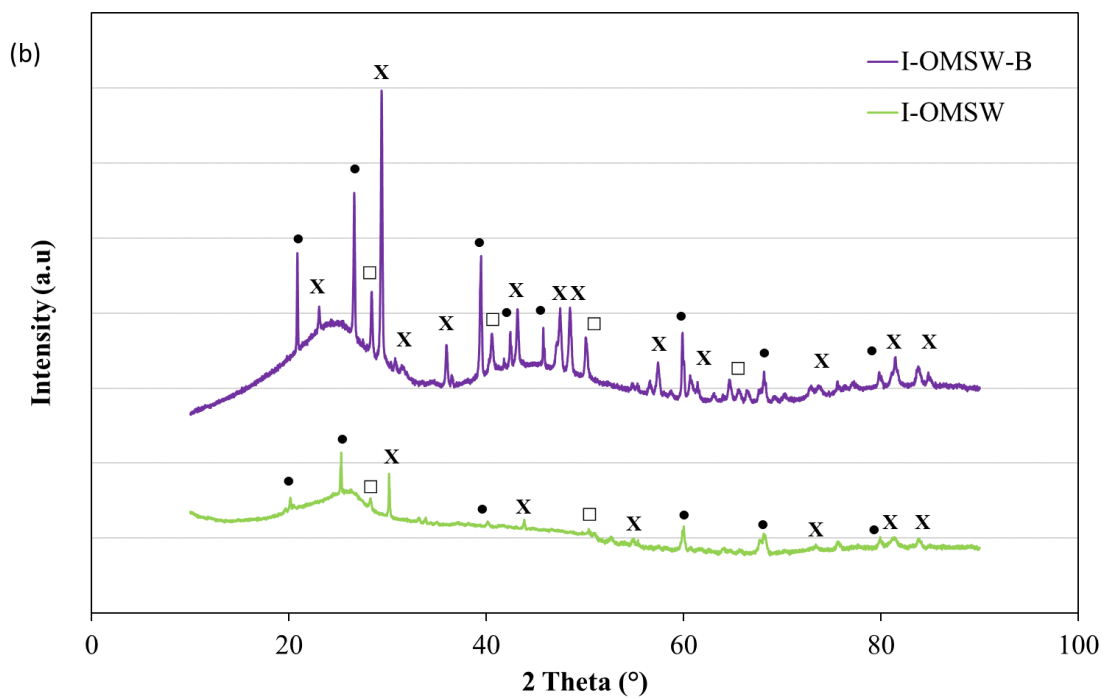
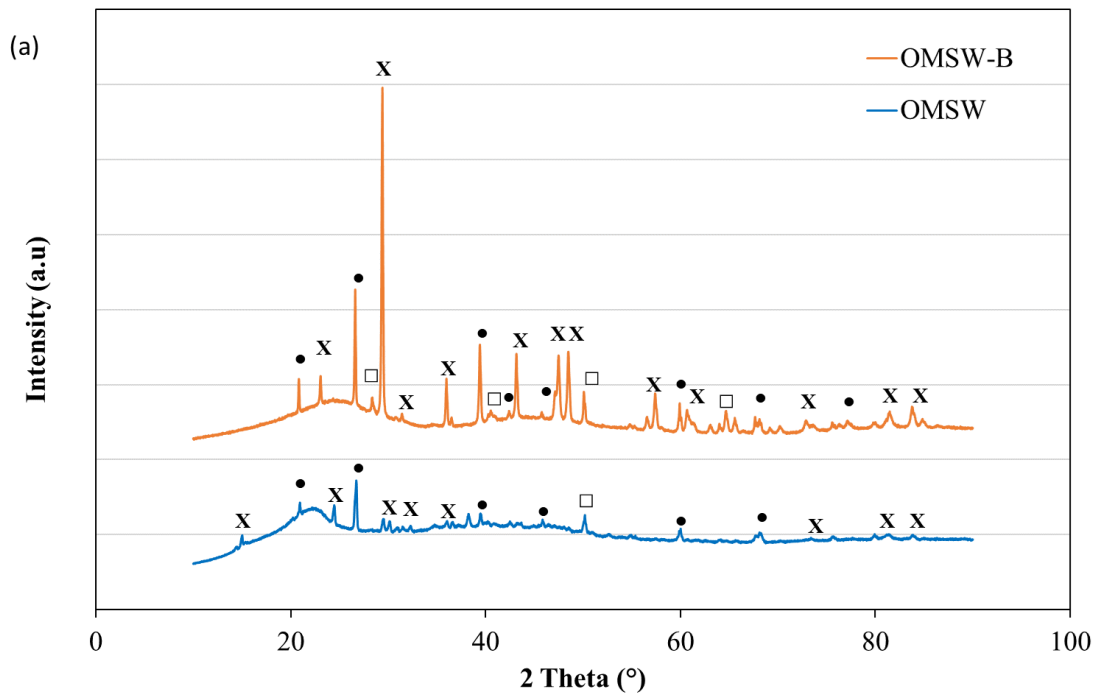
Figure 2. SEM images of (a) OMSW-B and (b) I-OMSW-B



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Figure 3. CO₂ adsorption isotherms (a) and the related pore size distribution of OMSW-B and I-OMSW-B (b) determined using the slit pores CO₂ NLDFT model)

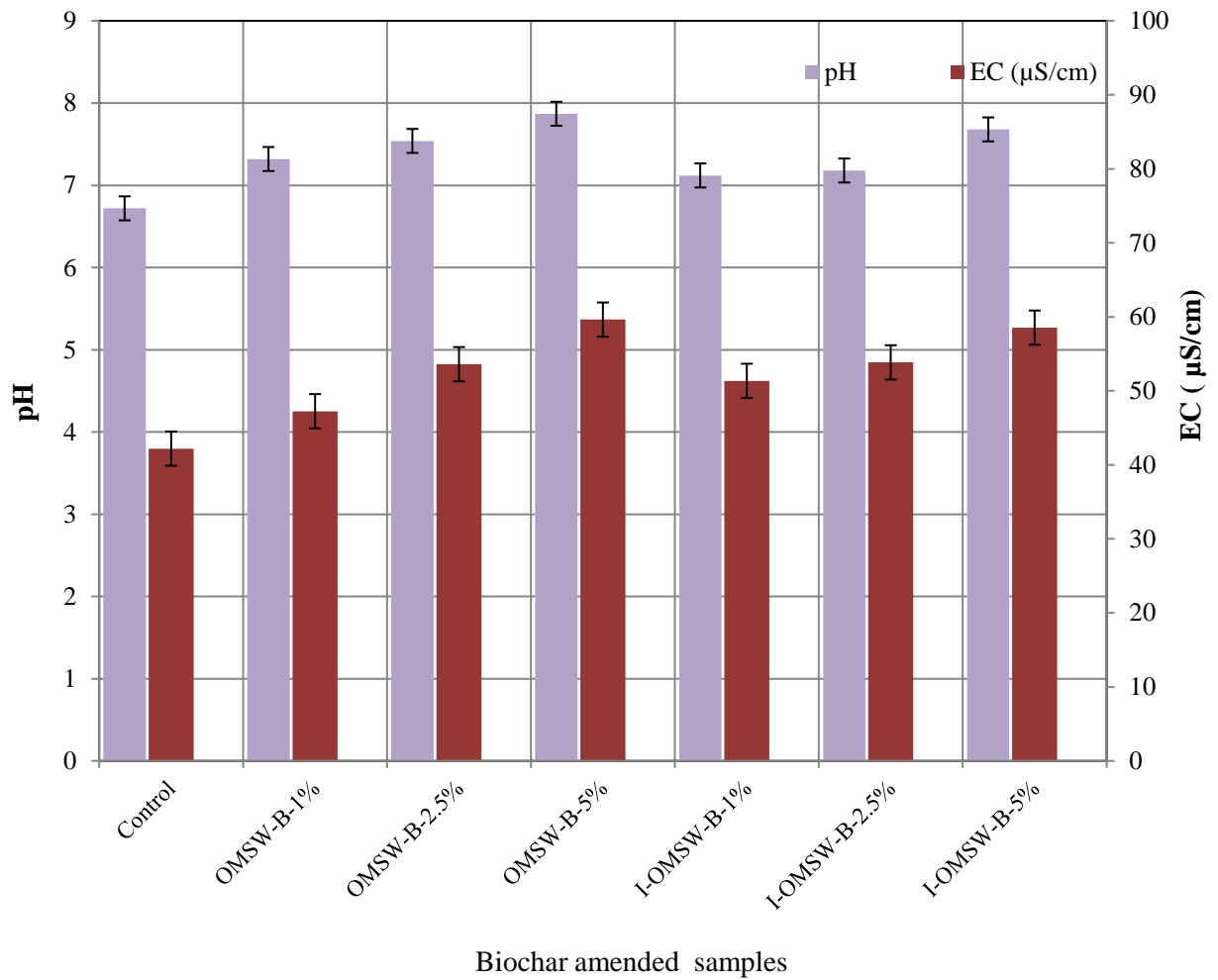
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9 **Figure 4.** XRD diagrams of OMSW and its derived biochar (a) and I-OMSW and its biochar
 10 (b) produced at 500°C (●: SiO₂, □: KCl, X: CaCO₃)

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12

13 **Figure 5.** pH and EC of biochar amended soil under different treatment OMSW-B (OMSW-B
 14 -1%, OMSW-B-2.5%, OMSW-B-5%); I-OMSW-B (I-OMSW-B-1%, I-OMSW-B-2.5%, I-
 15 OMSW-B-5%) and control without biochar amendment.

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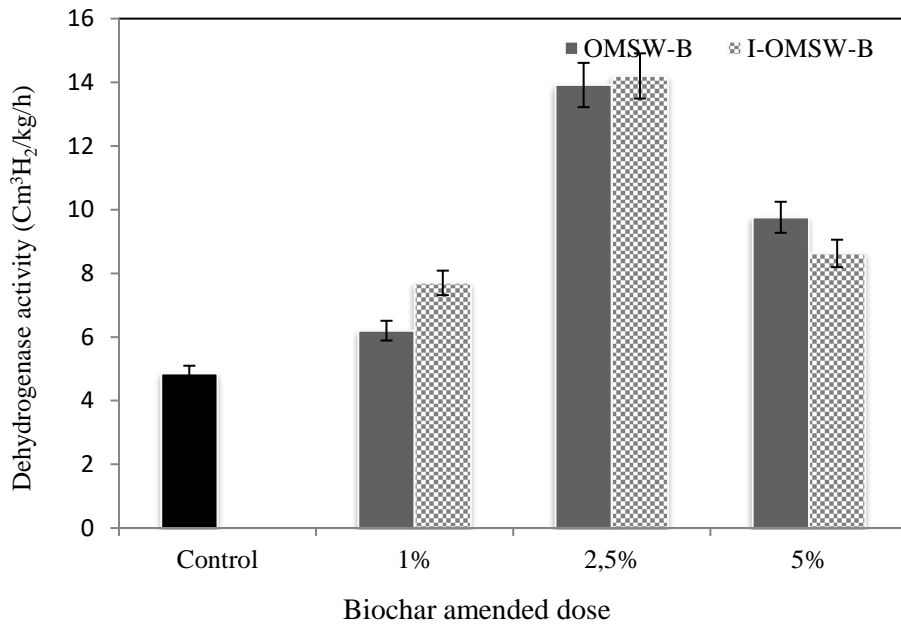
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(a)

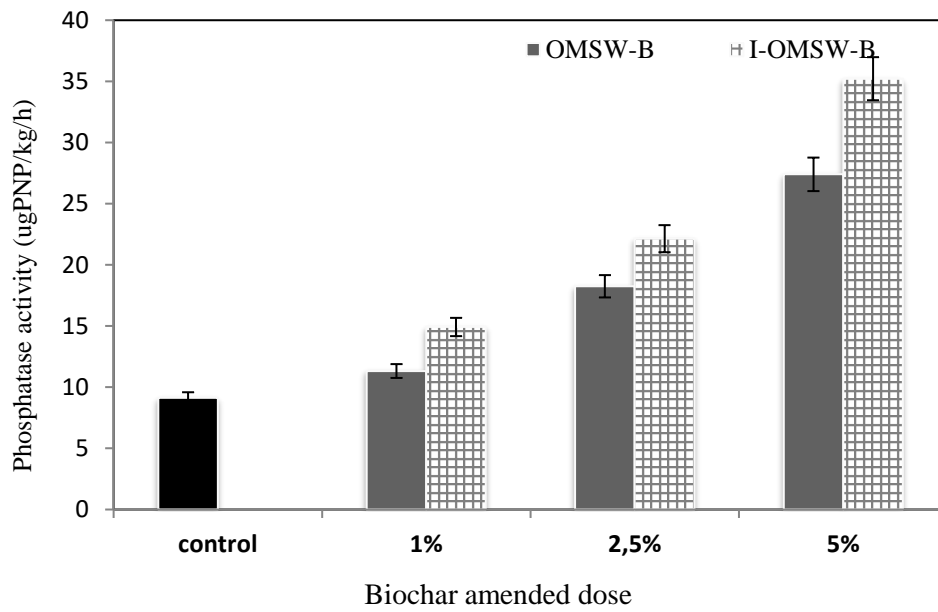


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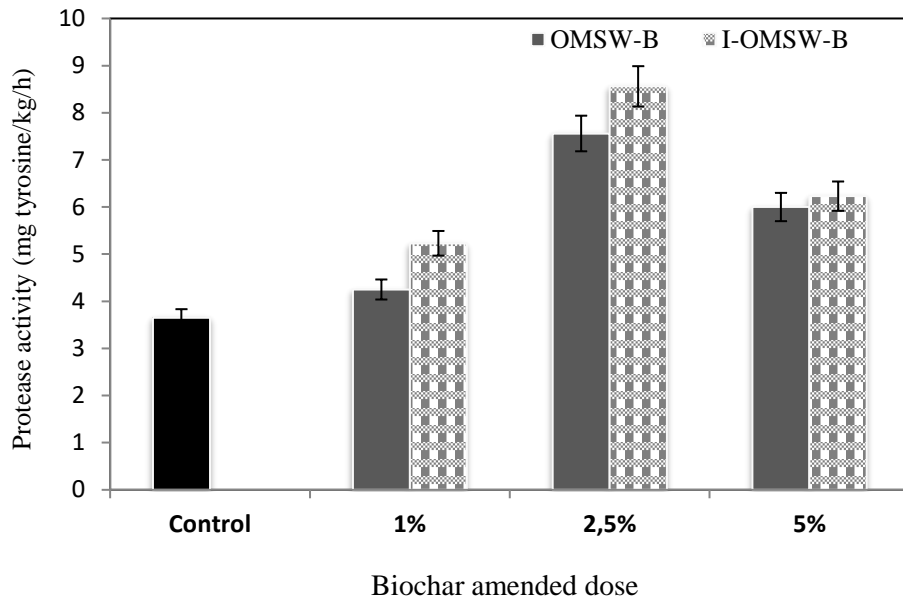
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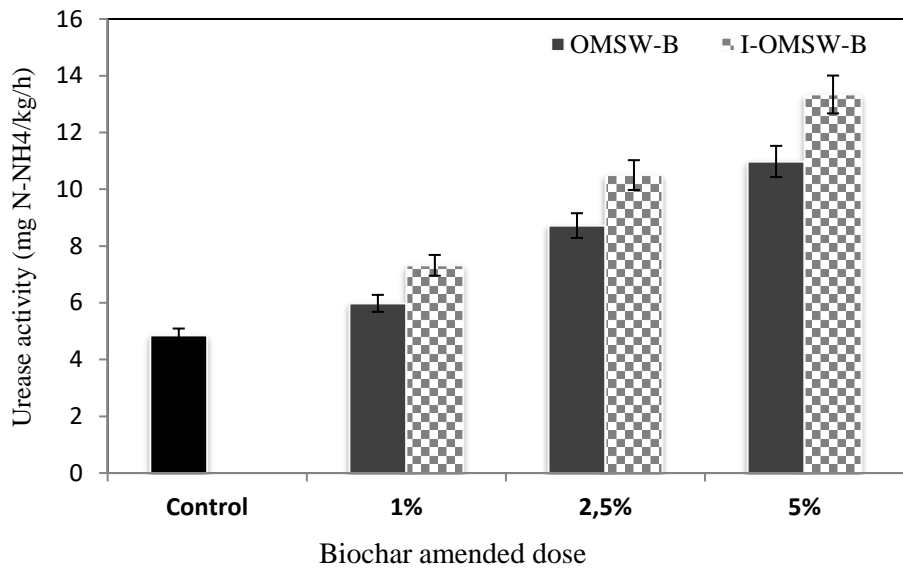
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34 (c)



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36 (d)



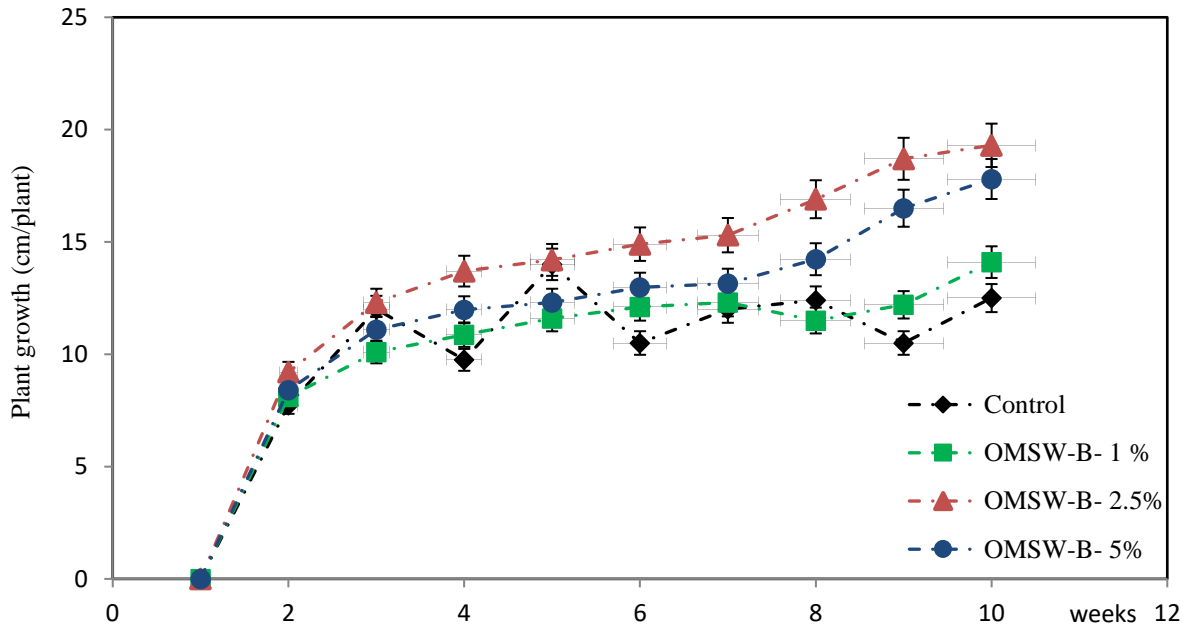
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39 **Figure 6.** Effect of OMSW-B and I-OMSW-B amendments on the enzymatic activities of the
40 amended soil under different doses, (a) : dehydrogenase activity, (b) : phosphatase activity, (c) :
41 protease activity, (d) : urease activity ; Errors bars represent the standard deviation of the mean
42 (n=3).

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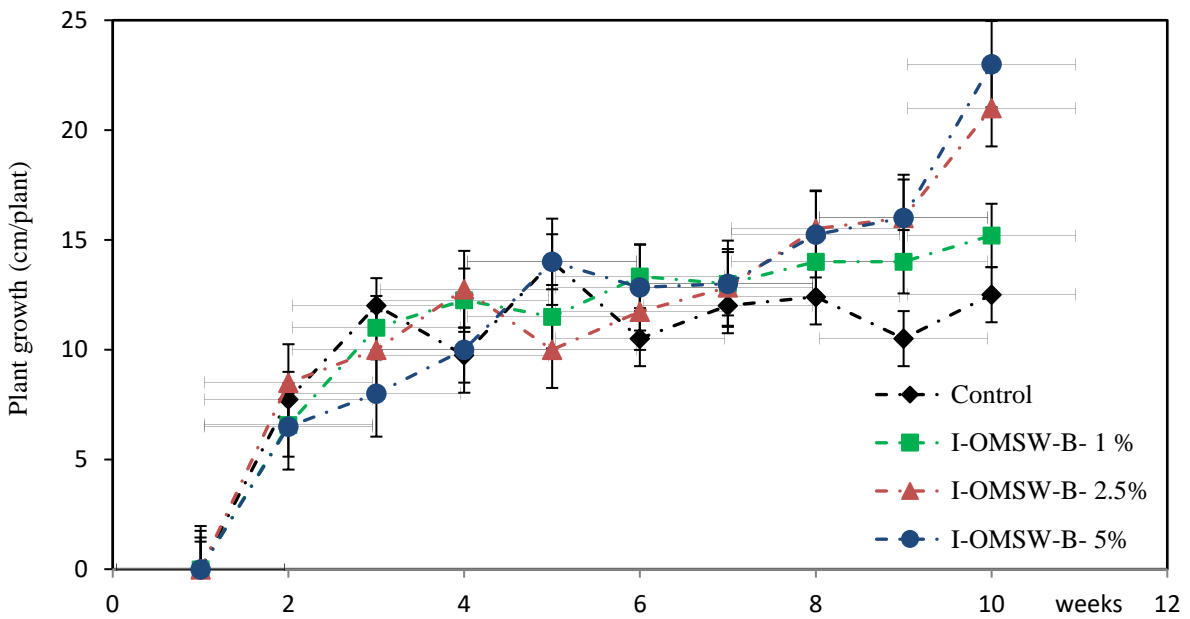
44 (a)



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47 (b)



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49 **Figure 7.** Plant growth of *Solanum lycopersicum* seeds under biochar amendment OMSW-B (a)
 50 and I-OMSW-B (b) amendment under different doses

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