Effect of Two Different Biochars on Germination and Seedlings Growth of Salad, Cress and Barley

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Abstract**—**The application of biochar to soils is becoming more and more common. Its application which is generally reported to improve the physical, chemical, and biological properties of soils, has an indirect effect on soil health and increased crop yields. However, many of the previous results are highly variable and dependent mainly on the initial soil properties, biochar characteristics, and production conditions. In this study, two biochars which are biochar II (BC II) derived from a blend of paper sludge and wheat husks and biochar 005 (BC 005) derived from sewage sludge with a KCl additive, are used, and the physical and chemical properties of BC II are characterized. To determine the potential impact of salt stress and toxic and volatile substances, the second part of this study focused on the effect biochars have on germination of salad (*Lactuca sativa* L.), barley (*Hordeum vulgare*), and cress (*Lepidium sativum*) respectively. Our results indicate that Biochar II showed some unique properties compared to the soil, such as high EC, high content of K, Na, Mg, and low content of heavy metals. Concerning salad and barley germination test, no negative effect of BC II and BC 005 was observed. However, a negative effect of BC 005 at 8% level was revealed. The test of the effect of volatile substances on germination of cress revealed a positive effect of BC II, while a negative effect was observed for BC 005. Moreover, the water holding capacities of biochar-sand mixtures increased with increasing biochar application. Collectively, BC II could be safely used for agriculture and could provide the potential for a better plant growth.

Keywords**—**Biochar, phytotoxic tests, seedlings growth, water holding capacity.

I. Introduction

In recent years, biochar has received considerable interest for its potential use as a carbon sequestration agent and as a soil amendment for improved agricultural productivity [1]-[3]. A meta-analysis based on 371 studies showed that the addition of biochar to soils resulted in increased aboveground productivity, crop yield, soil microbial biomass, rhizobia nodulation, plant K tissue concentration, soil phosphorus (P), soil potassium (K), total soil nitrogen (N), and total soil carbon (C) compared with control conditions [4]. Hence, using the biochar as a soil amendment returns most of nutrients to the soils from which they came [5]. The physical and chemical properties of biochars derived from different sources of feedstock can vary tremendously leading to the high variability observed in terms of their effects on soil fertility. In addition to the many sources of feedstock, the thermal profile and the geographic variations in soil type and climate are some of the chief sources of variability when looking to the benefits of biochar as a soil amendment [6]. Thus, it is essential to characterize biochar properties prior to selecting a particular biochar for a specific application. However, the use of biochar is not without its critics. Research shows that biochar can contain dangerous inorganic contaminants and organic ones as well as dioxins and furans [7]-[10]. Furthermore, during the pyrolysis process that generates biochar, heating causes some nutrients to volatilize, especially at the surface of the material. These volatile organic compounds tend to form cyclic, aromatic molecules as pyrolysis temperature increases [11]. In the case of high levels of contaminants, there is a risk of their uptake by plants or migration down the soil profile to ground-waters. This may have negative effects on both the environment and living organisms. Therefore, biochar applied to soils should be free of toxic substances before any future large-scale application.

The aim of this study was to evaluate the effects of biochar II (BC II) and biochar 005 (BC 005) on (i) germination of salad (test for salt stress), (ii) barley (test for toxic substances), and (iii) cress seeds (test for gaseous phytotoxic emissions). Test procedures are adapted from Busch et al. [12].

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II. Materials and Methods

A. Cress Test

First, we calculated the needed amount of fresh weight (four replicates) for 100 g of dry weight per pot. Then, we set the water holding capacity (WHC) to 30% by adding tap water and split the mixture to four small Weck-glasses (volume of 200 ml). The small glasses were placed in 1L Weck-glasses that were also filled with 20 ml tap water. On top of the smaller glasses, we placed a wire with two pads of cotton that were moisten with tap water. On the pads, 0.5 g of seeds from cress was sowed. The 1L Weck-glasses were closed, and the system was placed in a greenhouse chamber by a light and dark rhythm of 16:8 with 15 Klux light intensity and a temperature of 20-22 °C during the day (14 °C by night), the humidity was around 60%. After seven days, the harvest takes place. We cut the seedlings near the cotton pads and we determine the fresh weight. The length of the hypocotyl from 10 plants per vessel is then measured.

B. Salad Test

For one replicate, an amount of fresh weight that equals 100 g of dry weight was taken out of the whole mixture (sand-biochar) and filled into a Petri dish. Tap water was added to set up the water content to 85% of the maximum WHC, taking into account that the mixture already contained water. 40 seeds of *Lactuca sativa L.* were evenly sowed, leaving a free space of around 1 cm to the border of the Petri dishes. They were softly pressed into the substrate. Afterwards, 90 g (dry weight) of coarse sand was distributed on top. The prepared Petri dishes were placed open in a plastic bag with zipper that was bloated and positioned in the green house. For the first 48h, a black plastic foil covered the dishes. After five days of incubation, the harvest started. We described the appearance of the seedlings in comparison to the control and determined the number of germinated seedlings, fresh and dry weight of above ground biomass. The Petri dishes which still contained the soil mixture were mixed, and 25 g of dry weight was taken. Again, around 100 ml of water was added, taking into account that there was already water in the substrate. After shaking for an hour by 150 rpm, we let the substrate settle down for 30 minutes and determined the pH and EC. All determinations were replicated three times.

C. Barley Test

This test is a modification of the original compost test procedure [12]. First, five biochar-peat mixtures have been prepared. We have used small amounts of biochar that equal 0%, 1%, 2.5%, 5%, and 10%, which means that 1% describes a mixture of 1 g dry biochar with 99 g dry peat. Then, we have determined the dry weight and WHC of the substrates. To prepare 1000 ml of dry weight for every mixture, we have calculated the amount of fresh weight of biochar and peat and the amount of water needed to set the mixture to 60% of WHC_{max}. Afterwards, we have put some textile/filter paper into the bottom of our plant pots (so smaller particles will not be lost) and split the mixtures into four replicates while leaving a bit that is needed to cover the seeds. After sowing in every pot 20 seeds of barley, we have distributed the rest of mixture over the seeds. We have noted the weight of the whole construction (pot + mixture with 60% of WHC_{max} + seeds) – this is our first weight. We placed the pots randomly in the greenhouse. Every day, we took the weight of pots and we calculated the difference to the first weight and we adjust it by adding tap water. We determined the consumption of water (by evapo-transpiration) and together with the produced biomass the water use efficiency (WUE). Nine days later, germination rate, biomass fresh weight, dry weight, water content, and WUE were determined.

D. Soil, Peat and Biochar Analysis

All physical and chemical analysis were performed in the Hassan II Agronomic and Veterinary Institute (IAV) soil science laboratory by using standard analytical methods. The pH and electrical conductivity (EC) were both measured in water extracts with standard electrodes. For chemical analysis, the total concentration of Na, K, Ca, and Mg were determined by flame emission spectrophotometer and Fe, Mn, Zn and Cu by atomic absorption spectrophotometer and calorimetrically for $NaNO_3$ and KH_2PO_4.

E. Statistics

For cress, barley, and salad germination tests, effects of different biochar additions on all replicated measurements were tested via one-way analysis of variance (ANOVA). Significance of differences among treatment groups was determined with the Tukey test. A result was considered significant at $p<0.05$. All statistical tests were performed with SigmaPlot (Systat Inc., Chicago, IL, USA).

III. Results and Discussion

A. Soil, Peat and Biochar Properties

TABLE I
PROPERTIES OF SANDY SOIL, PEAT AND BC II

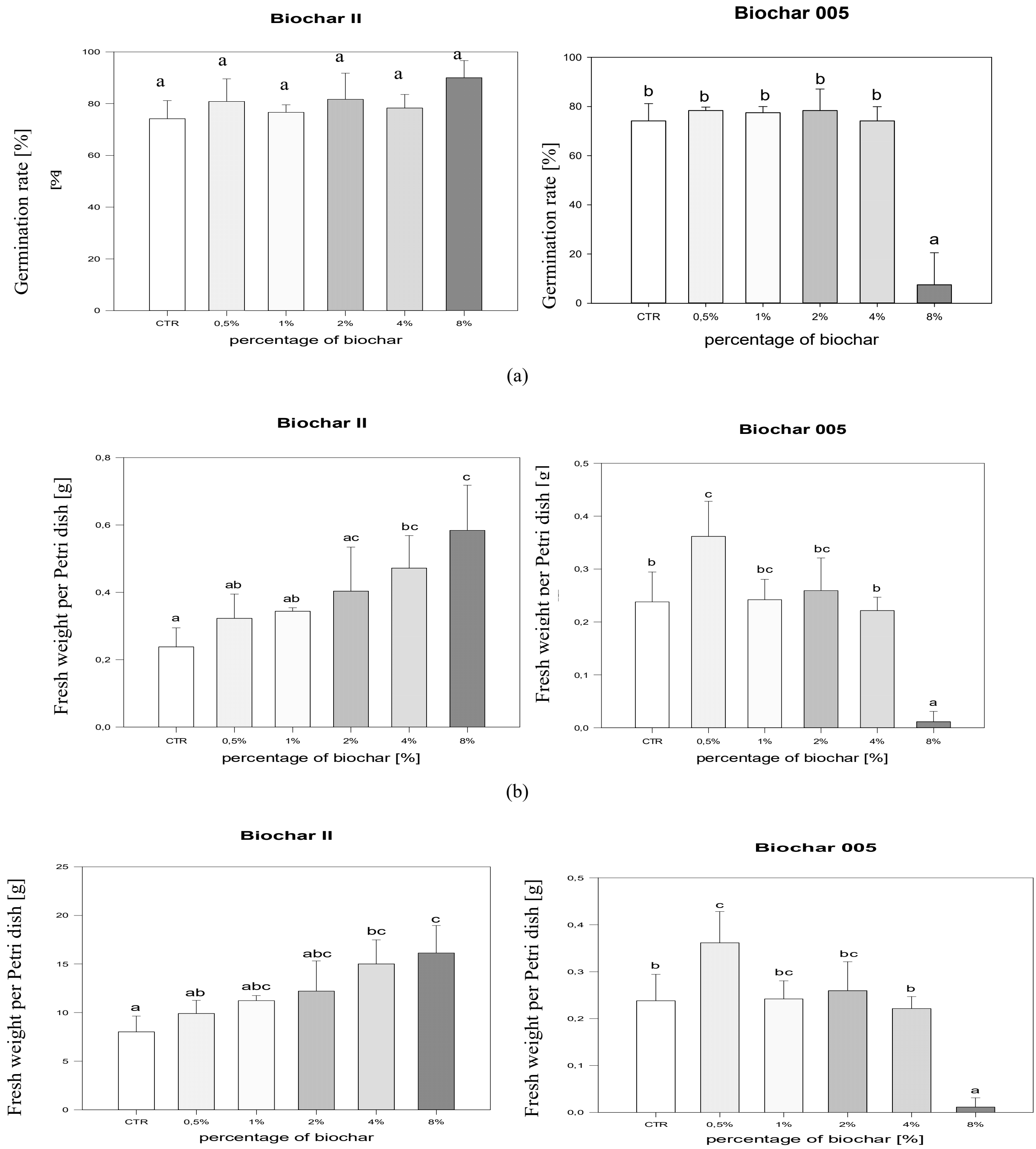
	Soil	Peat	Biochar
pH	7,93	5,81	8,25
EC (milliS cm^{-1})	0,48	0,94	1,33
K (ppm)	7,11	78,26	421,87
Na (ppm)	3,47	257,94	242,85
Ca (ppm)	34,09	190,9	0,6
Mg (ppm)	222,89	1191,58	1299,1
Fe (ppm)	9,07	0	0
Mn (ppm)	7,7	0,14	0,02
Cu (ppm)	1,3	0,06	0,062
Zn (ppm)	0,42	0,04	0,04
$NaNO_3$ (ppm)	100	133,33	2666,66
KH_2PO_4 (ppm)	1	2,33	1,93

The physical and chemical properties of BC II compared to other media are shown in Table I. It showed some unique properties compared to the soil, such as high EC, high content of K, Na, Mg and low content of heavy metals. Depending on the pyrolysis conditions and the nature of the feedstock, different pH values ranging from 4 to 13 can be reached [6], [13]. In this sense, BC II resulted in the same pH range. The EC value was higher for biochar compared to soil. Previous research reported that added biochar with high EC value into soil with low EC value (indicating its low salinity) increased EC of soil [13], [14]. Thus, we would expect that the EC of the soil (0.48 mS/cm) would increase with biochar application. Given the high nutrient content of the BC II used in this study, a significant positive effect on plant growth especially in the sandy soil would be expected.

B. Salad Germination Test

Our results, as shown in Fig. 1 (a), revealed a positive effect of BC II on germination rate of salad in all of the biochar treatments (0.5, 1, 2, 4, and 8% biochar-sand mixture). In the other words, the biochar application increased the germination rate by 21% for BC II. A positive effect was also observed for the more sensitive parameter to biotoxic substances in biochar: fresh weight of seedlings (Figs. 1 (b) and (c)). Even when the biochar contributed with 8% volume of the germination mixture, no negative effect was observed. In the other words, BC II application increased the fresh weight per petri dish by

145% and the fresh weight per plant by 101%. However, a negative effect of BC 005 at 8% level was revealed on germination rate and fresh weight of lettuce seedlings which indicated high ash contents. In the other words, BC 005 at 8% level decreased significantly (P<0,001) the germination rate, fresh weigh per petri dish and fresh weight per plant of lettuce seedlings by 90%, 95%, and 84%, respectively, which is in line with the previous studies that confirm instances of decreasing yield due to a high biochar application rate [1], [4], [15], [16]. Collectively, our results indicate that biochar feedstock materials vary in their characteristics (e.g., pH, nutrient levels, ash content) which also influence application rate and germination results. Thus, before any large scale application, it is necessary to determine which biochar materials are best suited for application and at which rates to specific soils.

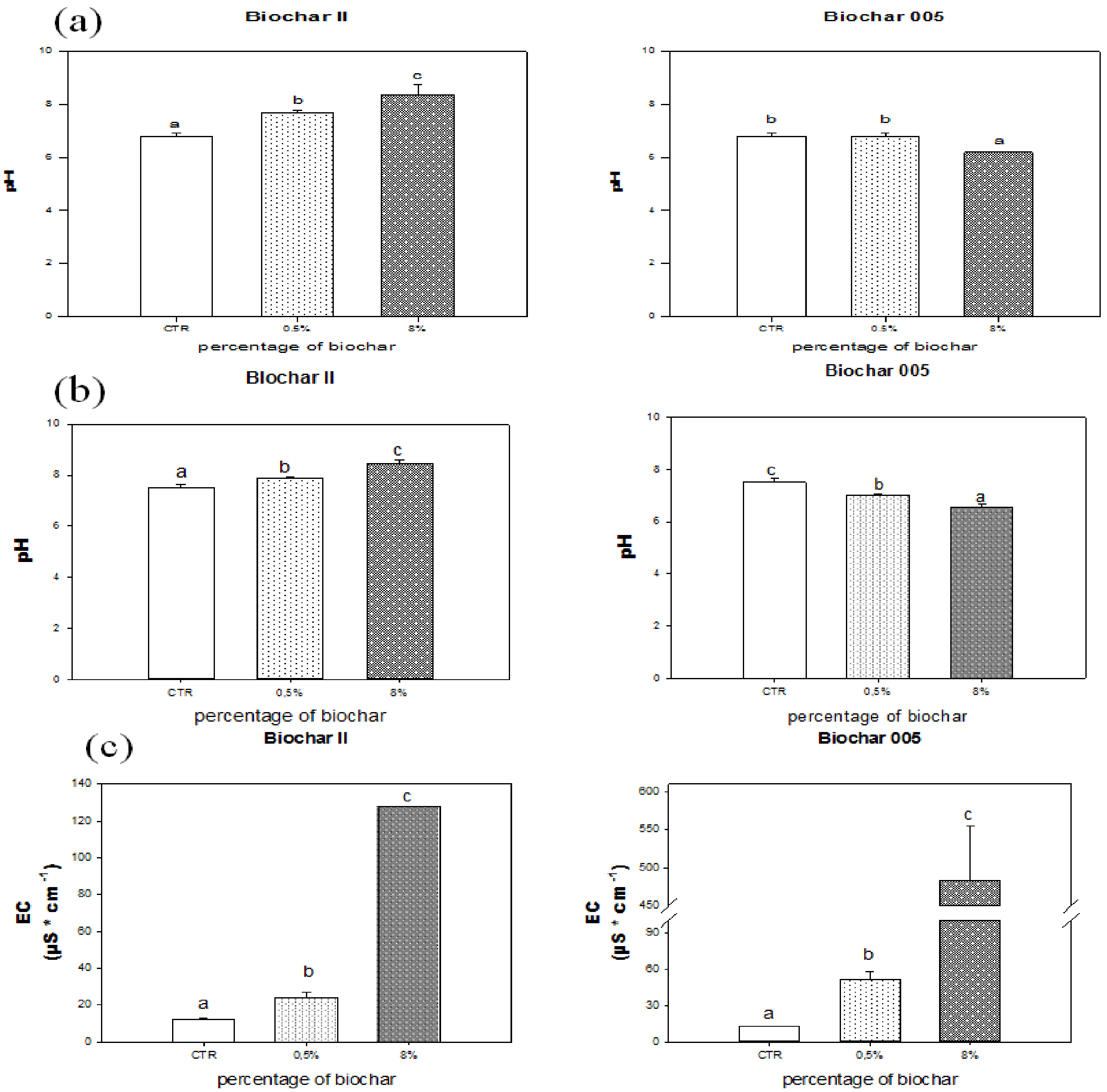


(c)

Fig. 1 Salad germination test results: (a): germination rate, (b): fresh weight per petri dish, (c): fresh weight per plant of three repeated test runs. The graphs present mean values and error bars present the standard deviation of the mean (n=3). Different letters indicate significant differences (one-way analysis of variance, ANOVA) between different mixtures with biochar and the control

The application of BC II at increasing rate significantly increased the pH before and after planting (Figs. 2 (a) and (b), respectively). The alkaline nature of biochar has been reported to be useful to increase the pH of acidic soils. The high pH of BC II revealed that a significant portion of the nutrient salts sequestered in biomass feedstock was concentrated during the pyrolysis process. The same results have previously been published [4], [17]. The application of BC 005 at 8% decreased significantly the pH before and after planting which can be explained by the acidic nature of biochar. In addition, the two biochars application at high level increased significantly the EC of mixture before and after planting (Figs. 2 (c) and (d)) which can be explained by the high content of more major nutrients in all biochars used in this study. Collectively, our results indicate that the two biochars used would be beneficial to soil except BC 005 at 8% volume.

The biochar application increased the WHC by 43% and 8% for BC II and BC 005, respectively, indicating that the most important value was observed for BC II. An increase in WHC has also been reported with the biochar application [6], [18].



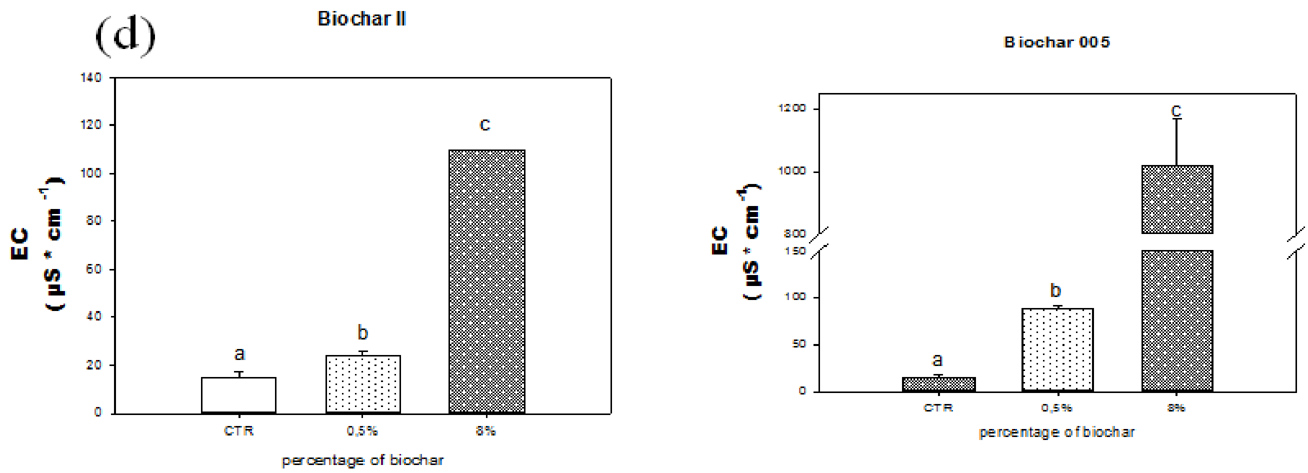
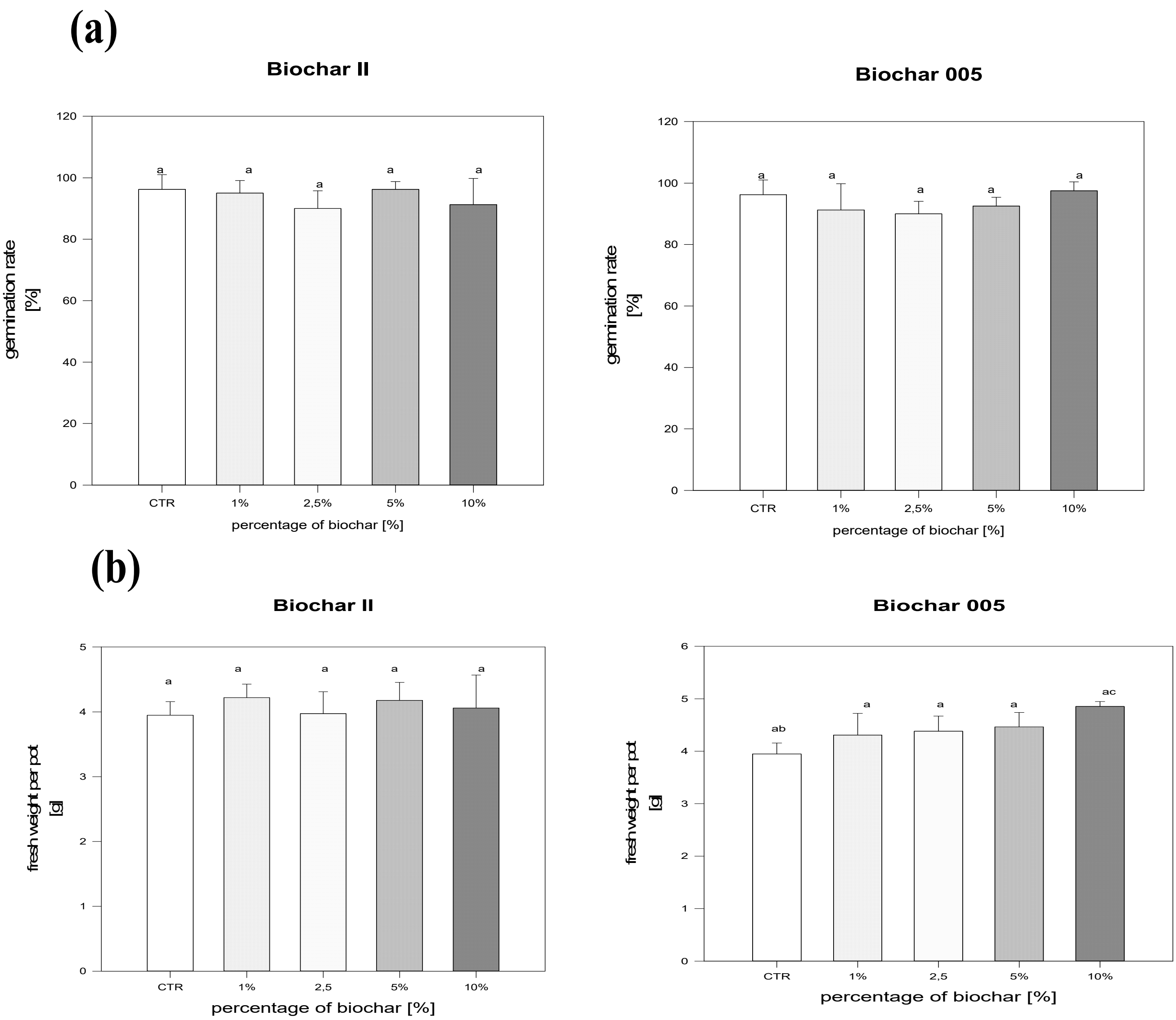


Fig. 2 Effect of biochar application on pH and EC before and after germination of salad. (a): pH values before planting, (b): pH values after planting, (c): EC values before planting, (d): EC values after planting of two repeated test runs. The graphs present mean values and error bars represent the standard deviation of the mean (n=3). Different letters indicate significant differences (one-way analysis of variance, ANOVA) between different mixtures with biochar and the control



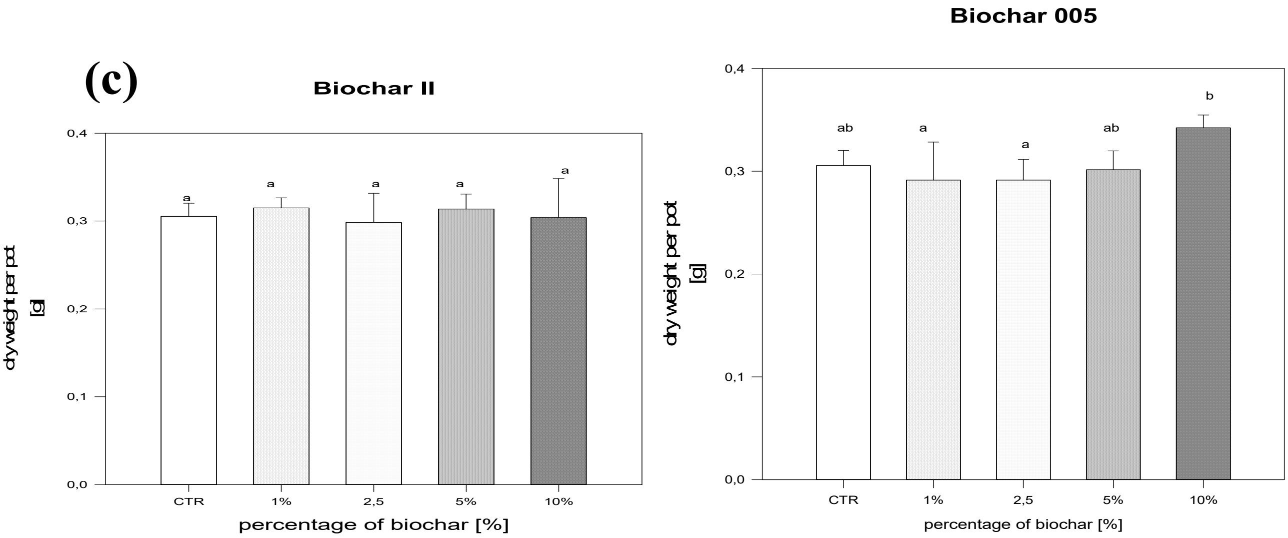


Fig. 3 Effect of two biochars on germination of barley. (a): germination rate, (b): fresh weight per plant and (c): dry weight per plant for four repeated test runs. The graphs present mean values and error bars present the standard deviation of the mean (n=4). Different letters indicate significant differences (one-way analysis of variance, ANOVA) between different mixtures with biochar and the control

C. Barley Germination Test

The germination results of different mixtures (peat-biochar) were compared with those of control (unfertilized peat, 0% biochar) where the fresh/dry weight was the most sensitive parameter to indicate negative effect of biochar on seedlings. In all biochar treatments (1, 2.5, 5, and 10% biochar-peat mixture), no negative effect of the two biochars on germination rate of barley was revealed (Fig. 3 (a)). The same result was observed for the more sensitive parameters to biotoxic substances in biochar: fresh and dry weight of seedlings (Figs. 3 (b) and (c)). More precisely, biochar application increased the fresh weight by 23% and 7% for BC 005 and BC II, respectively, indicating that the most important value was observed for BC 005. Furthermore, biochar application increased the dry weight by 12% and 3% for BC 005 and BC II, respectively, indicating that the most important value was observed for BC 005. Regarding the chemical properties of BC II and BC 005 (high value of EC), the observed increases in fresh and dry weight of biomass could be the results of high level of water soluble nutrients and low level of harmful substances in mixture with biochar application. Thus, for the two biochars, toxic effect by harmful substances was excluded as cause for the toxicity.

The relative chlorophyll content was measured with five replicated measurements on three leaves per plant by using a SPAD 502 device (Minolta, USA). Fig. 4 (a) shows the SPAD values in the two biochar tests compared to the control. The results indicate that BC II decreased the SPAD value even at a mixture with 1% biochar but none of the biochar additions resulted in a significant decrease while a small but non-significant increase was observed with BC 005 at 8% volume biochar addition. Furthermore, in all biochar treatments (1, 2.5, 5%, and 10% biochar-peat mixture), no negative effect of the two biochars on water content was revealed (Fig. 4 (b)). In the other words, biochar application increased the water content by 24% and 7% for BC 005 and BC II, respectively, indicating that the most important value was observed for BC 005. As found in the present study, the improvement of water retention capacities after biochar addition have previously been observed [18], [19] and reported to be due to its porous nature [20]. BC 005 had also a positive effect on water use efficiency (Fig. 4 (c)). They increased the WUE even in the lowest application rate; but the significant increase was observed at 10% level of BC005 by 17%. Our results are in line with the previous researches which have reported that the use of biochar as a soil amendment is anticipated to increase both nutrient and water use efficiency and thereby crop productivity where there is less drainage [19], [21].

D. Cress Germination Test

In this study, the cress fresh weight is >80% of the control for BC II, while it is <80% of the control for BC 005 (Fig. 5 (a)). The reduction of fresh weight compared to the control could be either attributed to direct negative effects of volatile substances on growth after germination, or to delayed germination that could have resulted in reduced time for growth and so led to reduced fresh weight. Delayed germination was also observed for cress and barley seeds exposed to volatiles from different biochars [22].

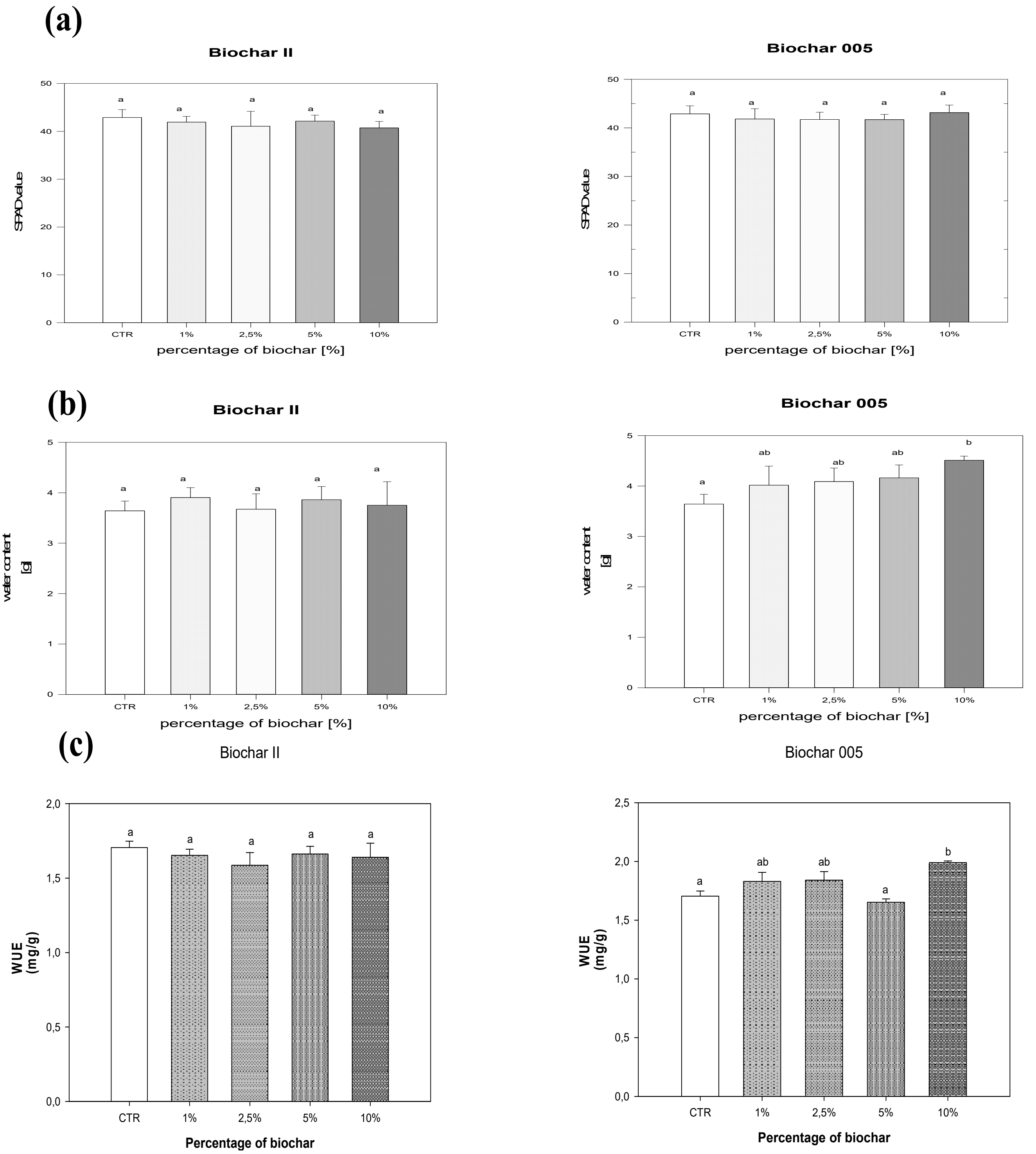


Fig. 4 Effect of biochars on SPAD values, water content and water use efficiency after nine days of germination of barley. (a): SPAD values, (b): water content, (c): water use efficiency for four repeated test runs. The graphs present mean values and error bars present the standard deviation of the mean (n=4). Different letters indicate significant differences (one-way analysis of variance, ANOVA) between different mixtures with biochar and the control

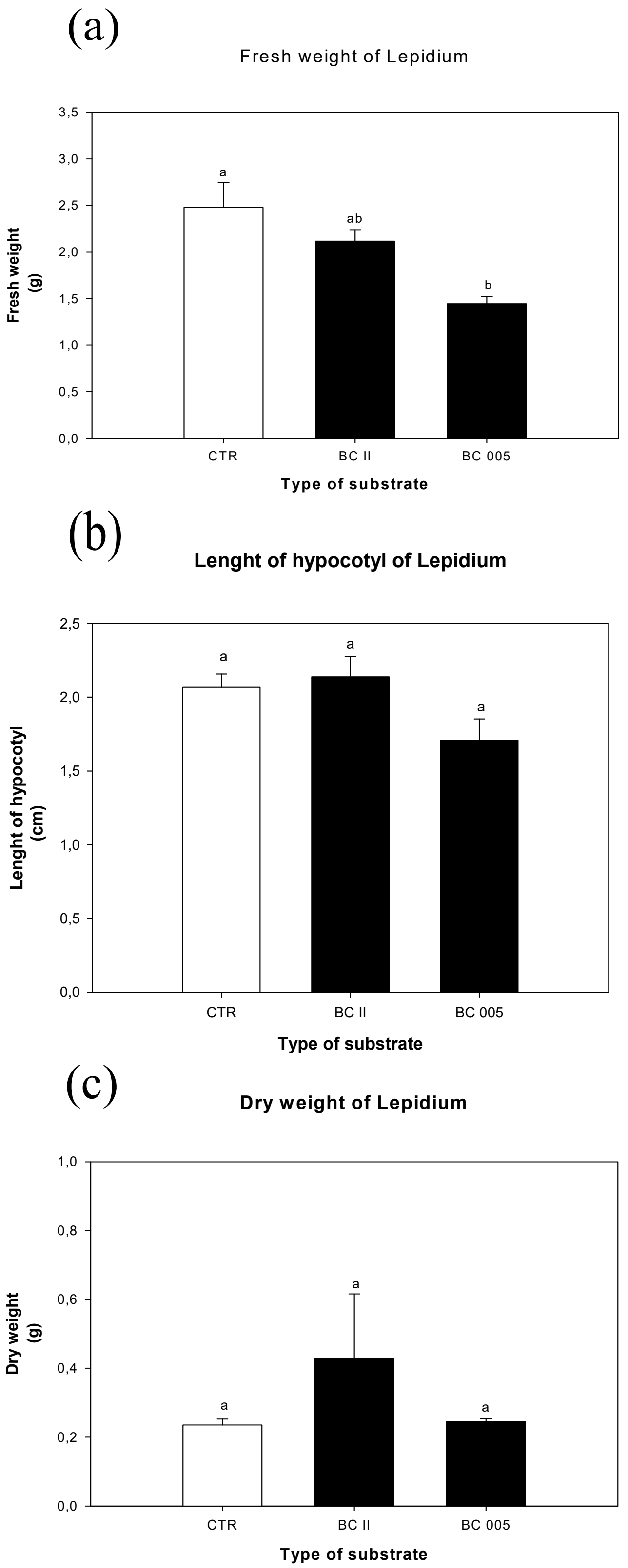


Fig. 5 Effect of biochars on germination of cress. (a): fresh weight of biomass, (b): length of hypocotyl, (c): dry weight of biomass. The graphs present mean values and error bars present the standard deviation of the mean (n=4). Different letters indicate significant differences (one-way analysis of variance, ANOVA) between different biochars and the control

IV. Conclusion

Biochar is the residual material after pyrolysis of organic feedstock. The physical and chemical properties of the resultant biochar depend on source material and production conditions. In this study, biochars obtained from different biomass showed some unique properties compared to the soil, such as high EC, high content of K, Na, Mg and low content of heavy metals. However, BC 005 showed some unique properties compared to the other media such as acidic pH and high EC. When used in mixture with sandy soil, they increased the WHCs but the most important value was observed for BC II. In addition, the results obtained from the two phytotoxicity test (test for salt stress and test for toxic substances) revealed no negative effect of BC II. However, a negative effect of BC 005 at 8% level was revealed on salad germination results indicating a salt stress effect of this biochar at this level but no toxic effect by harmful substances was observed. The phytotoxic test of the biochar samples caused by volatile substances revealed a positive effect of BC II, while a negative effect was observed for BC 005 on fresh weight of cress seedlings. Thus, before any large scale application, it is necessary to determine which biochar materials are best suited for application.

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References

[1] J. Lehmann, J. Gaunt, and M. Rondon, “Bio-char sequestration in terrestrial ecosystems- a review,” Mitigation and adaptation strategies for global change vol. 11, pp. 403-427, 2006.

[2] S. Shackley, S. Sohi, R. Ibarrola, J. Hammond, O. Mašek, P. Brownsort, A. Cross, M. Prendergast-Miller, and S. Haszeldine, “Biochar, tool for climate change mitigation and soil management,” In: Geoengineering Responses to Climate Change, Lenton, T., Vaughan, N. (Eds.), Springer, New York, 2013, pp. 73–140.

[3] P. Smith, “Soil carbon sequestration and biochar as negative emission technologies, ”Journal of Global change biology, vol. 22, issue 3, pp. 1315–1324, 2016.

[4] L. A. Biederman, and W.S. Harpole, “Biochar and its effects on plant productivity and nutrient cycling: A meta-analysis,” GCB Bioenergy vol. 5, pp. 202–214, 2013.

[5] D. A. Laird, R. C. Brown, J. E. Amonette, and J. Lehmann, “Review of the pyrolysis platform for coproducing bio-oil and biochar,” Biofuels, Bioprod. Bioref., vol. 3, pp. 547–562, 2009.

[6] Y. Wang, L. Zhang, H. Yang, G. Yan, Z. Xu, Ch. Chen, and D. Zhang, “Biochar nutrient availability rather than its water holding capacity governs the growth of both C3 and C4 plants, ”Journal of Soils and Sediments, Vol. 16, issue 3, pp. 801-810, 2016.

[7] S.E. Hale, J. Lehmann, D. Rutherford, A.R. Zimmerman, R.T. Bachmann, V. Shitumbanuma, A. O’Toole, K.L. Sundqvist, H.P.H. Arp, and G. Cornelissen, “Quantifying the total and bioavailable polycyclic aromatic hydrocarbons and dioxins in biochars,” Environ. Sci. Technol, vol. 46, pp. 2830-2838, 2012.

[8] P. Oleszczuk, I. Jo_sko, and M. Ku_smierz, “Biochar properties regarding to contaminants content and ecotoxicological assessment,” J. Hazard. Mater, vol. 260, pp. 375-382, 2013.

[9] W. Buss, and O. Masek, “Mobile organic compounds in biochar: A potential source of contamination-Phytotoxic effects on cress seed (Lepidium sativum) germination” Journal of Environmental Management, vol. 137, pp. 111-119, 2014.

[10] M. Kołtowski, and P. Oleszczuk, "Toxicity of biochars after polycyclic aromatic hydrocarbons removal by thermal treatment," Ecological Engineering, vol. 75, pp. 79–85, 2015.
[11] E. N. Yargicoglu, B. Y. Sadasivam, K. R. Reddy, and K. Spokas, "Physical and chemical characterization of waste wood derived biochars," Waste Management vol. 36, pp. 256–268, 2015.
[12] D. Busch, C. Kammann, L. Grünhage, and Ch. Müller, "Simple Biotoxicity Tests for Evaluation of Carbonaceous Soil Additives: Establishment and Reproducibility of Four Test Procedures," Journal of Environmental Quality, vol. 41, pp. 1023-1032, 2012.
[13] K.Y. Chan, L. Van Zwieten, I. Meszaros, A. Downie, and S. Joseph, "Using poultry litter biochars as soil amendments" Australian Journal of Soil Research, vol. 46, pp. 437–444, 2008.
[14] G.C. Sigua, J.M. Novak, D.W. Watts, M.G. Johnson, and K. Spokas, "Efficacies of designer biochars in improving biomass and nutrient uptake of winter wheat grown in a hard setting subsoil layer" Chemosphere, vol. 142, pp. 176–183, 2016.
[15] K. -W. Jung, K. Kim, T. –U. Jeong, and K. –H. Ahn, "Influence of pyrolysis temperature on characteristics and phosphate adsorption capability of biochar derived from waste-marine macroalgae (Undaria pinnatifida roots)," Bioresource Technology, vol. 200, pp. 1024–1028, 2016.
[16] C. I. Kammann, S. Linsel, J. W. Gößling, and H. –W. Koyro, "Influence of biochar on drought tolerance of Chenopodium quinoa Willd and on soil–plant relations" Plant Soil, vol. 345, pp. 195–210, 2011.
[17] D. Zhang, G. Pan, G. Wu, G. W. Kibue, L. Li, X. Zhang, J. Zheng, and J. Zheng, "Biochar helps enhance maize productivity and reduce greenhouse gas emissions under balanced fertilization in a rainfed low fertility inceptisol" Chemosphere, vol. 142, pp. 106–113, 2016.
[18] C. T. N. Cao, C. Farrella, P. E. Kristiansenc, and J. P. Rayner, "Biochar makes green roof substrates lighter and improves water supply to plants" Ecological Engineering, vol. 71, pp. 368–374. 2014.
[19] A. Obia, J. Mulder, V. Martinsen, G. Cornelissen, and T. Børresen, "In situ effects of biochar on aggregation, water retention and porosity in light-textured tropical soils" Soil and Tillage Research, vol. 155, pp. 35–44, 2016.
[20] X. Yu, C. Wu, Y. Fu, P. C. Brookes, and S. Lu, "Three-dimensional pore structure and carbon distribution of macroaggregates in biochar-amended soil," European Journal of Soil Science, vol. 67, issue 1, pp. 109–120, 2016.
[21] F. Sun, and Sh. Lu, "Biochars improve aggregate stability, water retention, and porespace properties of clayey soil" Journal of Plant Nutrition and Soil Science, vol. 177, Issue 1, pp. 26–33, 2014.
[22] I. Bargmann, M.C. Rillig, W. Buss, A. Kruse, and M. Kuecke, "Hydrochar and biochar effects on germination of spring barley," J. Agron. Crop Sci., vol. 199: 360-373, 2013.