PHOTOSYNTHESIS OF VEGETABLES AND FIELD PLANTS IN RESPONSE TO BIOCHAR TREATMENT

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Abstract
The requirements of modern agriculture include besides obtaining maximum yields of crops also maintaining and improving soil fertility and environmental protection. In recent years, interest of usage solid phase (charcoal or biochar) obtained by pyrolysis as soil improver, increases. Biochar is a solid material obtained from the thermo chemical conversion of biomass in anoxygen-limited environment. Additions of biochar to soil have generally been shown to be beneficial for growing crops. From an agronomic perspective it is suggested that biochar could improve soil health by increasing nutrient retention. The present pot experiment was conducted to investigate the effect of biochar application on the plants photosynthetic activity. The experiment was carrying out on two soil types, treated with different BC ratio. The plants grown on cinnamic pseudopozolic soil show a high content of plastid pigments in comparison with these on alluvial meadow soil. The aim of study is to evaluate the biochar agronomic impact on the plants growth, development and quality indicators.

Keywords: chlorophyll content, Brix, plant development.

Introduction
The requirements of modern agriculture include, besides obtaining maximum yields of crops also maintaining and improving soil fertility and environmental protection. Traditionally, post-harvest residues of cereals crops in past were used for animals feed. Nowadays, under the conditions of greatly reduced livestock and lack of market, they have to be exported from the field or burned. This practice leads to deterioration of soil fertility, loss of fresh organic matter, disrupting soil structure and its properties, as well as release of large amounts of CO_2. Biomass is a sustainable source of organic carbon. There are many ways of its processing and use. Transformation of biomass into biochar (BC) as a result of pyrolysis has various everyday life applications - heating and barbecues, as well as a soil improver and composting element. In recent years, interest of usage solid phase (charcoal or biochar) obtained by pyrolysis as soil improver increases. The number research concerning biochar applications in order to mimic the effect of fertile soils in the Amazon Terra Petra (Glaser, 2002) were carried out in last ten years. The biochar structure, porosity, chemical composition, influence of external factors on the BC properties and its application, distribution and movement in the soil profile is necessary to be studied. In Bulgaria are made research only to determining the influence of systematic plowing of crop residues on the soil fertility and crop productivity on gray forest soil (Donkova, 2005). The studies of BC influence on the soil properties and plant growth are less. The aim of study is to evaluate the biochar agronomic impact on the plants growth, development and quality indicators.

Material and methods
The present pot experiment was conducted to investigate the effect of biochar application on the plants photosynthetic activity, soil agrochemical properties and plant development. Various methods for biochar preparation are described in the literature (Thomas B. Reed, 2004).Our team

has developed a small pyrolysis stove according to the "Lucia Stove" methodology. The wooden residues used in the process of pyrolysis are from cherry, apple, plum, quince and oak. The process of pyrolysis runs at 300-500°C, the obtained BC is without impurities from petroleum products. The obtained biochar was crushed to 1 mm particles. The experiment was carried out on two soil types- cinnamic pseudopozolic from Sekirovo village and alluvial meadow soil from Tsalapitsa village. The tested crops were maize (*Zea mays*), after harvesting to determine the effect of biochar, was planted lettuce (*Lactuca sativa*) as a second crop. The biochar used for conducting the path experiment was in rate of 200 kg / ha and 400 kg / ha.

The following variants are tested:
1. Control 1 / clean soil /
2. Control + Biochar 1 / 6 g per pot/
3. Control + Biochar 2 / 12 g per pot /
4. Control 2 - (soil + NPK)
5. NPK + Biochar 1 / 6 g per pot /
6. NPK + Biochar 2 /12 g per pot/

Individual pots have a capacity of 2 kg and the total number of experimental pots is 36. Each variant is set in three replications. The mineral fertilizer rate was calculated based on our previous studies with both crops (Stoimenov, et al 2009). The fertilization norms are: nitrogen fertilizer - ammonium nitrate - 150 mg nitrogen per 1 kg of soil; Triple superphosphate - 120 mg P_2O_5 per 1 kg of soil and Potassium sulphate - 100 mg of K_2O per 1 kg of soil. In the first stage of experiments 5 maize seeds were planted per pot from variety Kn 209. Three plants are left after germination. Maize plants are harvested in the 6-7 leaf stage. After maize harvesting, lettuce from pre-produced seedlings was planted. Five plants per pot were planted leaving only one till the end of vegetation. The average duration of vegetation is 40 days. Throughout the growing season, irrigation is maintained close to the optimal. Phenological observations and biometric measurements were made during vegetation. The content of the soils macro- and microelements is determined by standard methodologies (Arinushkin, 1970). Ammonium and nitrate nitrogen were determined calorimetrically, mobile forms of phosphorus and potassium by method of P. Ivanov (1984), Movable forms of microelements with EDTA-extract, pH-potentiometrically, in aqueous extract and potassium chloride solution. Total nitrogen content in the plants was determined by the Keldahl method by decomposition with concentrated H_2SO_4 and 30% H_2O_2. The remaining macro- and microelements were determined by "dry" burning in muffle furnaces and subsequent dissolution in 20% HCl, followed by measuring on Atomic Absorption Spectrophotometer (Mincheva M., A. Brashnarova, 1975). In the study, the chlorophyll was determined in fresh mass (mg %), spectrophotometrically in 80% acetone leaching by the Vernon method, 1960. The total sugars content was determined refractometrically (%) (Digital Refractometer – 32145).

Results and discussion

The two soil types- cinnamic pseudopozolic from Sekirovo village and alluvial-meadow soil from Tsalapitsa village were used to carry out the vegetation pot experiments.The soil of the experimental field of Tsalapitsa village is with a light mechanical composition, which resulted in faster development of the processes. According to Stoycheva, (2007) soil chemical analysis of the Tsalapitsa village is characterized by a low content of total N (0.052%) and humus (0.70%), slightly acidic reaction throughout the profile and a sorption capacity of 22.5 meq / 100g soil. The soil from the Sekirovo village is characterized by a low humus content which gradually decreases in depth. The soil has a medium acidity in the surface horizon and low acidity in the ´By´ horizon. The availability of total nitrogen is very low. There is a very low sorption capacity. The distribution of cation exchanges is diffuse. There is an exchangeable acidity. The chemical composition -pH in KHL is a 5.5 for the surface horizon (tab.1).

Table 1. Chemical analysis of alluvial-meadow soil from the village of Tsalapitsa and cinnamic pseudopozolic from the of Sekirovo village used in pot experiments

	pH KCl	Sum N NH_4+NO_3 mg/kg	P_2O_3 mg/100g	K_2O mg/100g	Humus %
Tsalapica	7,3	23,6	25,2	43.7	2,98
Sekirovo	5,5	19	22,7	33,3	2,15

Soil analysis after harvesting of the experimental plants reported a slight increase in pH values due to the BC use, the highest value was recorded in variants with- 12 g/BC on both soil types (Tsalapica pH-7.5; Sekirovo pH-6.00). Similar results have also been obtained from many other authors (Nigussie et al. 2012; Arocena, J.M. and C. Opio, 2003; Khanna, et al. 1994). Nigussie et al. show a statistically proven (P <0.01) increase in pH after BC use in ratio- 10 t / ha. The residual NH4 + NO3 content in the test treatments ranges between 10.6-77.7 mg / kg of soil for Sekirovo and between 5.00-75.00 mg / kg of soil for Tsalapitsa. The content of mobile K and P on both soil types and both forms of fertilization increases as the fertilizer rate increases. In the maize growing experiment, results were obtained for the amount of plastid pigments in the leaves of each treatment. The content of plastid pigments in plants depends on the species, variety, development stage, temperature, lighting, feeding conditions, etc. Therefore, the chlorophyll content can serve as an indirect indicator of plant growth, as chlorophyll "a" is more sensitive to external influences in compeers with chlorophyll "b" (Petrova, 2010). Figure 1 shows the chlorophyll content of maize leaves in the 6-7 leaf stage. There is a trend of chlorophyll “a” growth from the control to the treatment with imported 12 g/ BC per kg/ soil. The chlorophyll "a" is ranges from 0.39 to 1.35 mg / g in fresh mass. The highest values on both soil types in both fertilization variants are reported for the variant with imported 12 g / BC per kg/ soil. Chlorophyll ´b” ranges from 0.19 to 0.65 mg / g in fresh mass. The ratio of Ch "a" to Ch "b" is 2 (3): 1, which is within the normal range.

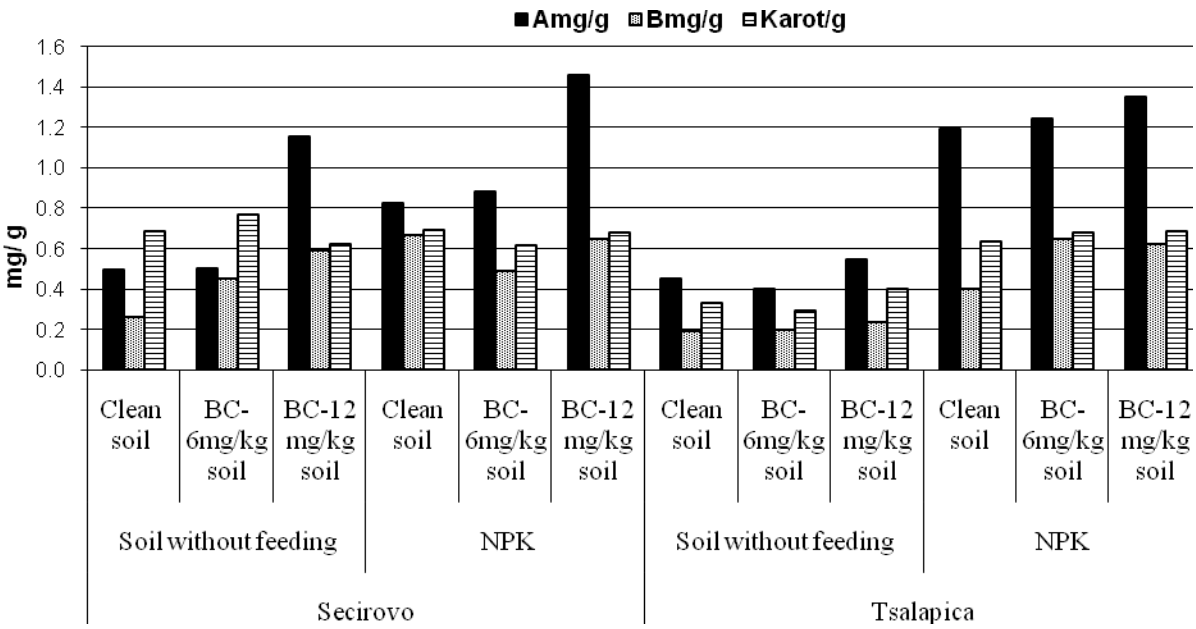

Figure. 1 Content of plastid pigments in pot experiment with maize 6-7 leaf phase

The content of plastid pigments is an important factor determining the growth and development of plants (Dinev, 1996; Stancheva, 2002; Stancheva, 2004). As an indirect indicator, it can be associated with the absorption of nutrients, especially nitrogen. The lower content of N in variant with BC on alluvial meadow soil from Tsalapitsa, compared to cinnamic pseudopozolic from Sekirovo leads to a lower content of plastid pigments. This is probably due to the fact that the nitrogen content of these soils has not ensured the optimal nitrogen nutrition regime. In both soil types the content of plastid pigments increases with increasing of fertilizer rate. The Brix percentage is a very important as it is a direct measurement of how the plant is performing, as all plants use 6 molecules of water and 6 molecules of carbon dioxide with the radiation from the sun to make 1 molecule of basic sugar and 6 molecules of oxygen. The one molecule of sugar produced in the photosynthesis process is the foundational building block for everything we see growing above and below ground in the form of leaves, stems, tree trunks, branches, fruit, bulbs, grain, roots and even algae. So, when we measure the sugar levels in plants it directly corresponds to how much sugar production has taken place in the plant. The reported total sugars content on both soil types ranges between 3.15 and 7.2 Brix % (fig.2). In the treatments with added BC and organic fertilizer, it was observed increasing of sugar content on both soil types. For NPK-added variants, the highest values were reported for the 6 g/ BC + NPK.

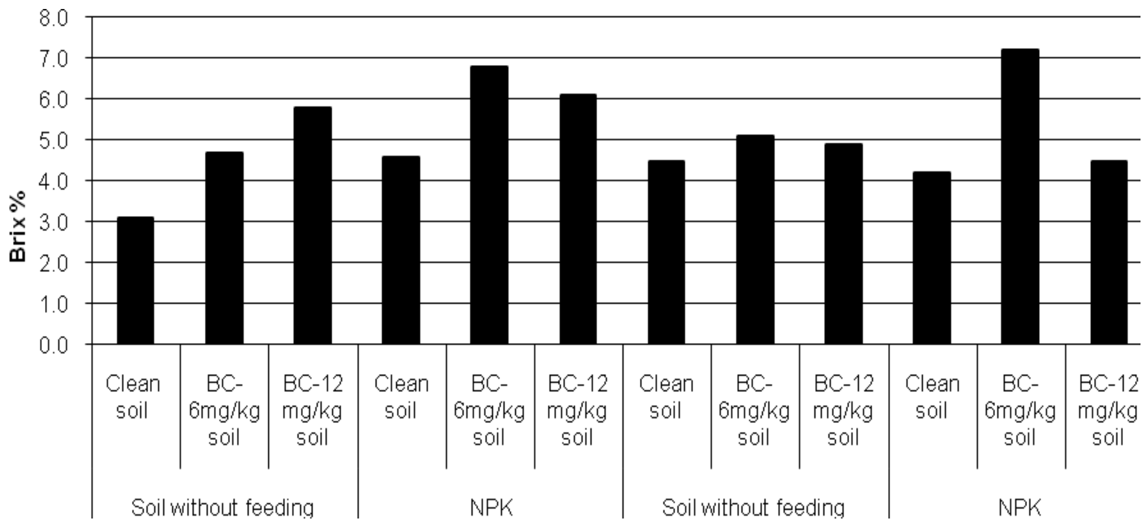

Figure 2. Total sugars content in maize 6-7 leaf phase

The yield of fresh biomass from the maize growing experiment varies between 12.08 g and 48.4 g (fig.3).

The results from fresh biomass measurements in the organic fertilizer variants shows, that the differences between the control and the two variants with BC are minimal. This proves that the chosen BC norm, as well as the step between them, is small. The highest yield on cinnamic pseudopozolic soil was observed in variant with 6 g/BC + NPK - 48, 4 g. While in the alluvial meadow soil, which has lower baseline nitrogen content, the yield increases from the control to the variation by12 g/ BC + NPK (from 26.9 to 42.13 g). The mass of the maize roots in variants without mineral fertilization increases with increasing of the BC norm. This relationship is reversed in the version with the addition of NPK. It is known that leafy vegetable crops are particularly suitable as indicators for testing certain factors (Dinev and Mitova, 2011, Dinev and Mitova, 2013, Mengel and Kirkby, 1982). That's why after harvesting maize, lettuce (*Lactuca sativa*) was planted as a second crop to determine the effect of biochar. The similar trends in yields obtained from maize are seen in the second crop. Fig. 4 shows the yields of lettuce fresh biomass.

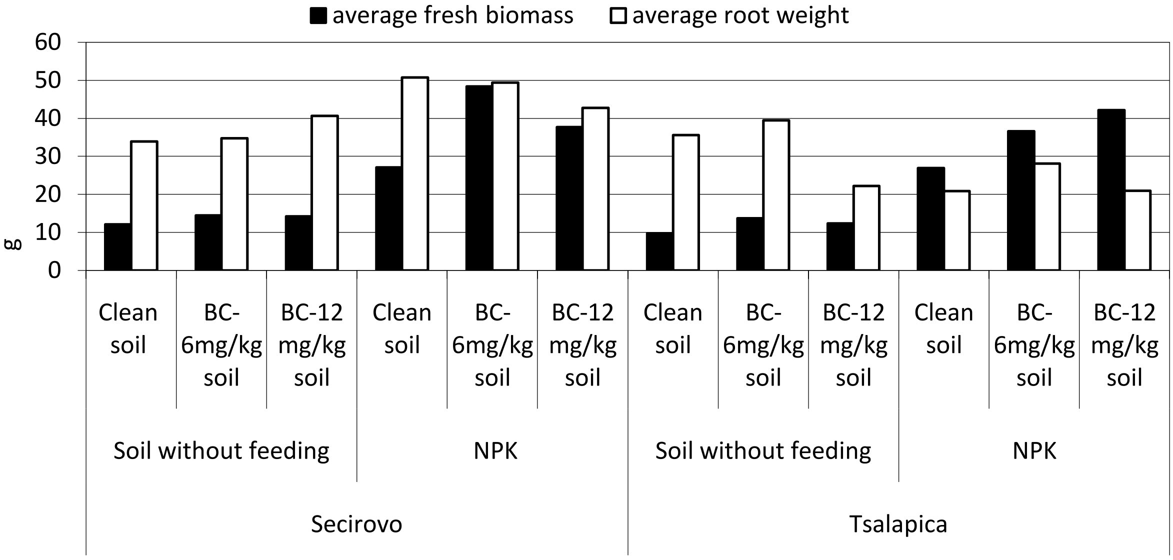

Figure 3. Average weights of fresh biomass and roots from pot experiment with maize on two soil types

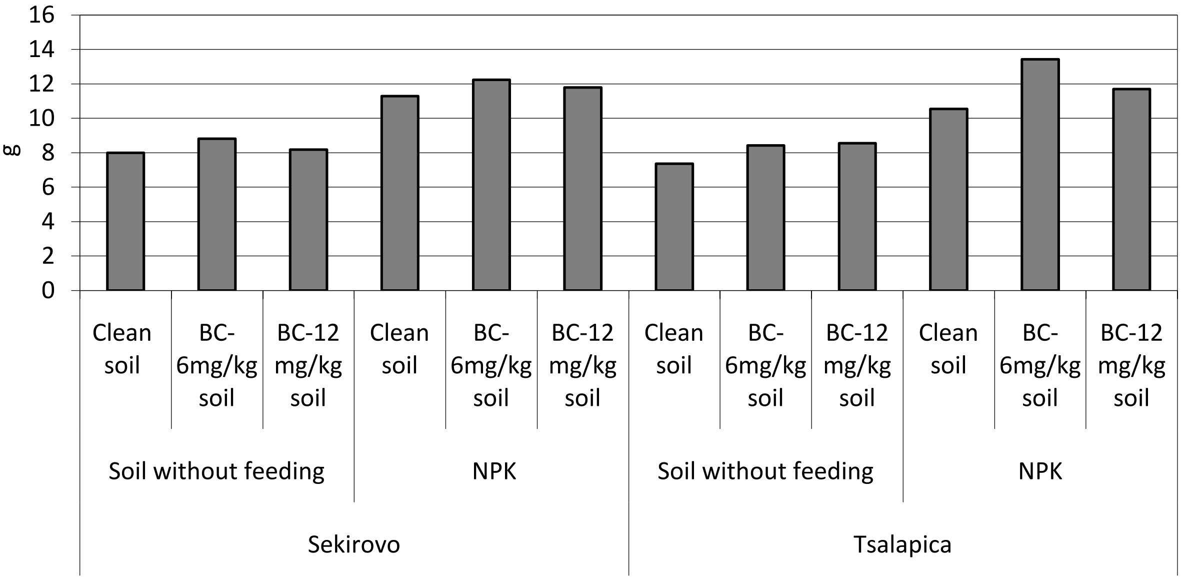

Figure 4. Average weight of fresh biomass from pot experiment with lettuce on two soil types

The largest yield was reported for variants with 6g/ BC on both soil types, which appears to be optimal for lettuce development. In variants with imported 12 g/ BC, the reported yield is slightly lower from 8.2 g to 11.8 g. It has been reported that leaf colour can indicate the amount and proportion of chlorophyll in leaves which are, in turn, closely related to plant nutrient status. Figure 5 presents the results obtained for the content of plastid pigments.

In the variants with imported BC and of both soil types, those treated with 6 g /BC +NPK appear to be optimal. The lowest value was recorded for the non-added BC variants on both soil types.

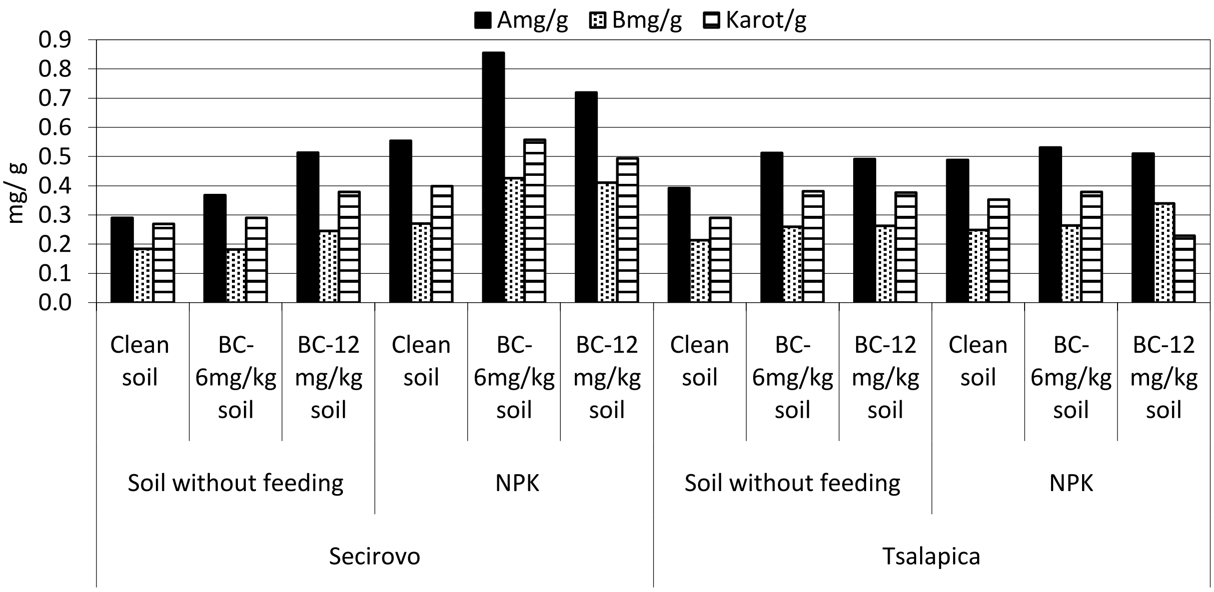

Figure 5. The content of plastid pigments in pot experiment with lettuce on two soil types

Conclusions

The biomass yield in both tested crops follow common pattern. While in the variants with different ratio BC, the differences in the obtained vegetative mass are lower, in the BC+ NPK treatments there was increase in yield in parallel with the increase of the quantity of BC applied. The variants with a higher content of total N in the plants tissues (maize and lettuce) on cinnamic pseudopozolic soil from Sekirovo village, a higher content of plastid pigments was measured as compared to the plants grown on alluvial meadow soil from Tsalapitsa village. In both tested cultures an increase in the BC norm also increases the chlorophyll content, which is particularly noticeable on the cinnamon pseudopozolic soil. The highest total sugars content in maize, for both soil types, was found in the variant with 12g/ BC. In the case of NPK + BC fertilization, the maximum is reached in variant with 6 g/BC + NPK. As a result of studies, the combined use of BC with mineral fertilization in the cultivation of trenches and vegetable crops can be recommended.

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