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# The use of biochar compost to reduce toxic effects of copper in soil

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## Abstract

Decades of intensive use of copper-based fungicides in Germany led to a considerable accumulation of copper in hop gardens, orchards and vineyards resulting in negative effects on plants as well as soil micro- and mesofauna. Due to the high sorption capacity, the application of co-composted biochar was tested for soil amelioration in the present experiment. For this purpose, two biochar composts as well as a biochar-free compost were produced and mixed into a copper contaminated soil. Subsequently, plant response, soil respiration and earthworm avoidance behavior were investigated. The application of all composts significantly reduced the phytotoxic effects and also partially increased oxygen consumption of soil microorganisms. Moreover, copper-induced avoidance behavior of earthworms was reduced. However, a positive effect of biochar was only partly observed for plant growth. Thus, the application of compost, regardless of biochar co-composting, may be a suitable approach to reduce toxic effects of copper in soil.

## 1. Introduction

Copper-based fungicides have been used in Germany since more than 150 years (Kühne et al. 2009) as effective agents against fungal diseases such as downy mildew of grapes (*Plasmopara viticola*) and hops (*Pseudoperonospora humuli*), apple scab (*Venturia* spp.) and potato blight (*Phytophthora infestans*). In the absence of satisfying alternatives, annual application rates of 20 to 30 kg Cu $ha^{-1}$ and occasionally up to 80 kg Cu $ha^{-1}$ were common until well into the 20$^{th}$ century, resulting in a considerable copper accumulation in the topsoil (e.g. Strumpf et al. 2011). Although copper is an essential plant nutrient, excessive amounts can cause anatomical, morphological and physiological changes, and finally a decrease in total biomass of plants (e.g. Gong et al. 2019). Furthermore, the increased copper load can reduce the abundance of soil micro-, meso- and macrofauna and alter their community structure as well as their behavior (Naveed et al. 2014; Van Zwieten et al. 2004a). Due to its often-confirmed potential to adsorb heavy metals (Rizwan et al. 2016; Wang et al. 2018, 2021), the use of biochar could be a suitable approach to reduce the bioavailability of copper in polluted soils, and thus to decrease induced toxic stress to plants and soil organisms. Moreover, co-composting of biochar, especially if added directly at the beginning of the composting process, can further increase the affinity to copper, and thus optimize the sorption properties (Borchard et al. 2012). However, studies on the use of co-composted biochar composts for remediation of copper-contaminated soils are rather scare (Antonangelo et al. 2021). To fill this knowledge gap, the potential of biochar composts for reducing copper induced toxic effects was investigated by plant and earthworm response tests and measurements of soil respiration using an artificially copper-contaminated soil.

## 2. Data, Methods and Approach

*Biochars, composting process and test soil*

The two biochars (BC) used for the experiments were made from untreated wood from certified sustainable forestry and provided by a commercial producer (Carbuna AG, Memmingen, Germany). One of them (basic) was pyrolyzed at a temperature of 540 °C and had a pH ($CaCl_2$) of 8.6, an organic carbon content of 90%, a $H/C_{org}$ molar ratio of 0.3 and a specific surface (BET) area of 417 m² g$^{-1}$. The other biochar (premium) was pyrolyzed at a temperature of 850 °C and had a pH ($CaCl_2$) of 9.3, an organic carbon content of 85%, a $H/C_{org}$ molar ratio of 0.1 and a BET area of 282 m² g$^{-1}$. Both biochars fulfilled the requirement of the European Biochar Certificate (EBC) for use as soil improver. They were co-composted with grass clippings and woody scrap material from landscaping activities in composting boxes with a capacity of 2.0 m³. As control, a compost without biochar was produced in the same way. The proportion of each input material (Table 1) was in accordance with common practice, whereby the biochars were rated as structural components. For all experiments, an arable topsoil from the tertiary hilly country north of Freising was used. The soil was classified as sandy loam with a pH ($CaCl_2$) of 6.1 and calcium acetate lactate extractable amounts of phosphorus and potassium of 10 and 45 mg kg$^{-1}$, respectively (VDLUFA, 2014; method No. 6.2.1.1). The total copper content was 26 mg kg$^{-1}$ (VDLUFA, 2016; method No 2.1.1).

Table 1. Proportion of input materials in composting

| Compost | Type of Biochar | Input materials (% vol.) | | |
|---|---|---|---|---|
| | | Grass clippings | Woody scraps | Biochar |
| without BC | none | 60 | 40 | 0 |
| basic BC | basic | 60 | 15 | 25 |
| premium BC | premium | 60 | 15 | 25 |

*Plant response, soil respiration and avoidance behavior of earthworms*

For the plant response test, Chinese cabbage (*Brassica rapa* ssp. *pekinensis)* was used as a model plant for hop, vine and fruit cultivation since seedlings are sensitive to high copper levels (Gossow et al. 1995). The two biochar composts as well as the compost without biochar were mixed into the test soil at an application rate of 23 g per kg soil on dry matter (DM) basis. In practice, i.e. application of biochar compost closely around the planting hole of hop plants, this is equivalent to 60 t DM ha$^{-1}$ in the application area. However, related to the entire hop garden this gives only an application rate of 5 to 6 t DM ha$^{-1}$. The prepared mixtures were either left unpolluted or spiked with copper at rates of 250 and 500 mg kg$^{-1}$ using copper sulfate, moistened to 60% of maximum water capacity and filled into styrofoam trays (20 • 16 • 4 cm, approx. 1.3 L volume) according to bulk density. As control, the soil without compost was treated in the same way. Three trays per treatment were prepared and 40 seeds of Chinese cabbage were sown per tray. Subsequently, the trays were arranged in a randomized block design in a glass-sheltered greenhouse. The climate settings during the 21-days cultivation period were adjusted to 22/20 °C (day/night) for heating and 25 °C for ventilation. To meet nutrient demand of plants, a complete water-soluble fertilizer (15% N + 9% $P_2O_5$ + 16% $K_2O$ + 2% Mg + trace elements except copper; custom blend provided by Planta, Regenstauf, Germany) was applied by fertigation according to VDLUFA (2016; method No 10.2.1). Soil moisture was maintained between 60% and 80% of maximum water capacity by applying deionized water evenly to the soil surface. At the end of the experiment, plants were cut off just above the soil surface and the above-ground fresh weight was recorded. Furthermore, the copper concentration of the harvested biomass was analysed by ICP-OES after microwave-assisted digestion in $HNO_3/H_2O_2$ (VDLUFA 2014; method No 2.1.1).

In addition to the cabbage growth test, the oxygen consumption as an indicator of microbial activity was measured in all compost and copper treatments using the respirometry OxiTop® system (WTW, Germany), which consists of 2.7 L glass jars with sodium hydroxide (1M) as carbon dioxide trap in the headspace and a pressure measuring head (Platen and Wirtz 1999). Oxygen consumption was measured in triplicate over a period of 5 days at 25 °C in a climate cabinet and expressed as respiration rate in mg $O_2$ per kg dry matter and day.

The earthworm response test was performed according to ISO 17512-1 (DIN 2020). The two biochar composts as well as the biochar-free compost were mixed into the test soil at application rates equivalent to 30, 60 and 120 t DM $ha^{-1}$, respectively. The mixtures as well as the soil without compost were either left unpolluted or spiked with copper at rates of 50 and 100 mg $kg^{-1}$, respectively, using copper sulfate, moistened to 60% of maximum water capacity and filled into plastic vessels (18 • 12 • 6 cm, approx. 1.3 L volume) according to bulk density. Thereby, the vessels were divided into two equal sections. One was filled with the uncontaminated soil or soil-compost mixtures, while the other was filled with the Cu-polluted ones. Afterwards, 10 worms of the species *Eisenia fetida* were placed in the middle of each vessel. To prevent the worms from getting off the vessels, vessels were covered with a perforated lid. After 48 h at 20 °C ambient temperature in the greenhouse, the number of worms was determined in both sections of the vessels. The test was performed in four replicates.

*Statistical Analysis*

The above-ground fresh weight of the Chinese cabbage plants, the copper content of the above-ground biomass as well as the respiration rate of all treatments were first subjected to a two-way ANOVA with type of amendment (control, without BC, basic BC and premium BC) and copper loading (0, 250 and 500 mg Cu $kg^{-1}$) as factors. If there were no significant interactions between the factors, the means of each factor averaged over the other one were tested against each other for significant differences (Tukey test, $p \leq 0.05$). Otherwise, the dataset was splitted along the type of amendment and one-way ANOVAs were conducted for each data subset and significance of means was tested pairwise (Dunnett test, $p \leq 0.05$) to the soil without copper as control. The same procedure was performed for the percentage differences in fresh weight between treatments spiked with Cu and the corresponding treatments without Cu addition, using the respective spiked soil without compost as control (Dunnett test, $p \leq 0.05$). For the earthworm response test, the percentage of worms in the contaminated section was calculated for each treatment and the associated 95% confidence interval (Student-t-distribution) was computed. Data pre-processing and visualization were done with Microsoft Excel 2016, whereas the Minitab software package (V 18) was used for further statistical calculation.

## 3. Results and Discussion

*Effects of amendments on plant performance*

Both the 250 and 500 mg Cu addition resulted in a significant reduction of above-ground fresh weight in the soil without compost addition (control; Figure 1). If compost was applied, this was only true for 500 mg Cu. At the lower Cu level of 250 mg, the compost application generally negated the toxic effect observed in the control. Considering the percentage difference in above-ground fresh weight due to Cu contamination, the application of compost – regardless of biochar co-composting – significantly reduced the fresh weight loss at 250 mg Cu addition compared to the control soil without compost (data not shown). In contrast, if copper addition was doubled, the fresh weight loss in treatments with biochar-free compost as well as with basic biochar compost was comparable to that of the control. Only the premium biochar compost was able to achieve a significantly lower fresh weight loss than the soil without compost.

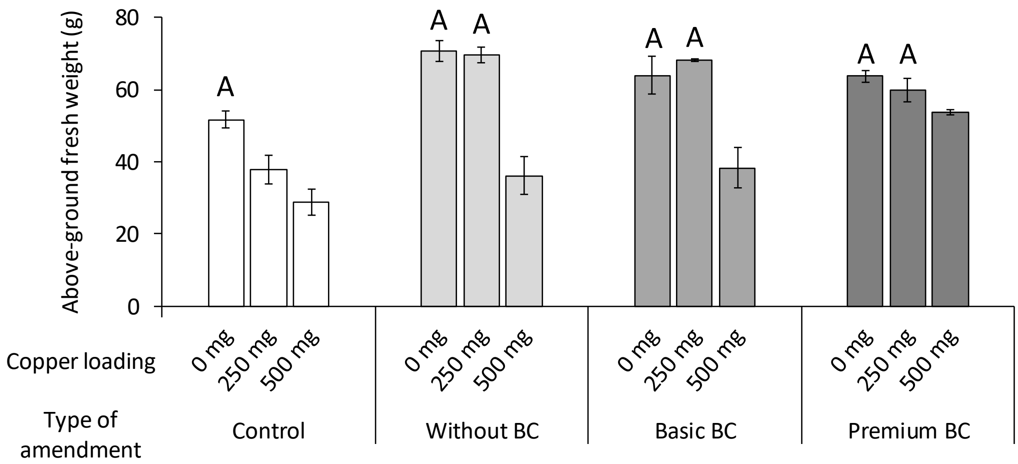


Figure 1. Above-ground fresh weight of Chinese cabbage cultivated in soil contaminated with copper at three different levels and amended with different types of compost compared to the control without compost. Treatments with letter A within the same compost treatment do not differ significantly from soil without copper (Dunnett test, $p \leq 0.05$). Error bars represent the standard error of the mean ($n = 3$).

Similar to the present results, other authors have also observed that the addition of biochar and compost improved plant growth and enhanced plant performance on copper contaminated soils (Buss et al. 2012; Jones et al. 2016). One possible reason therefore could be the reduction of copper bioavailability. As shown in several studies, biochars have great potential to immobilize heavy metals in soil by ion exchange, electrostatic interaction, complexation, precipitation and physical adsorption (Rizwan et al. 2016; Wang et al. 2018 and 2021). In addition, due to its high organic matter content, compost has similar sorption properties and can also be used for remediation of heavy metal polluted soils (Huang et al. 2016). In contrast to above-ground biomass, no effect of compost application on Cu concentration in plant tissue was found (data not shown). At first sight, this seems to contradict the assumption of a reduction in copper bioavailability. However, it has to be considered that the informative value of copper concentration in above-ground biomass of Chinese cabbage seems to be limited. Several authors found that copper is poorly translocated from roots (Shahbaz et al. 2010), which were not analysed in the current research. In follow-up-experiments, this will be done. Besides the reduction of copper bioavailability, ameliorating effects on soil characteristics such as water retention and soil structure are suspected causal for the improvement of plant growth on copper contaminated soils by biochar and compost application (Aggelides and Londra 2000; Ohsowski et al. 2012). However, since soil moisture was consistently maintained between 60% and 80% of maximum water capacity, enhanced water retention should be of minor importance in the present experiment. In addition to soil structure improvements, higher activity of soil microorganisms, as described in the following paragraph, might have contributed to the improved plant performance. The fact that only the premium biochar compost showed a significant remediation effect at 500 mg Cu addition can possibly explained by the higher pyrolysis temperature and the associated increased adsorption capacity of the premium biochar compared to the basic biochar (Liao et al. 2022). Moreover, the addition of premium biochar improved the composting process and thus compost quality (data not shown), which might enhance ameliorating effects.

*Effects of amendments on soil respiration*

The two-way ANOVA revealed significant effects of amendment type and copper loading on soil respiration rate as indicator of microbial activity without significant interactions between the two factors. Oxygen consumption was highest in the soil with biochar-free compost and lowest in the soil without compost as well as with basic biochar compost (Figure 2). These results are consistent with other studies, showing that the addition of compost enhances microbial activity and thus oxygen consumption in soils (Ros et al. 2003). However, the level of the effect strongly depends on the amount and quality of the applied organic material, which is confirmed by the present results. Since the same amount of compost was used, the differences in respiration rate are mainly due to the composition and, in particular, to the degradability of the organic material. Considering the high degradation stability of biochar, the biochar composts contain a lower proportion of degradable carbon sources compared to the biochar-free compost. Therefore, biochar composts are less degradable resulting in a lower respiration rate than the biochar-free compost. Although the composts partially increased the oxygen consumption in soil, they were not able to compensate the negative effects of copper. Thus, regardless of amendment type, both Cu additions of 250 and 500 mg $kg^{-1}$ resulted in a significant reduction of respiration rate, mirroring findings of other authors (e.g. Nwuche and Ugoji 2008).

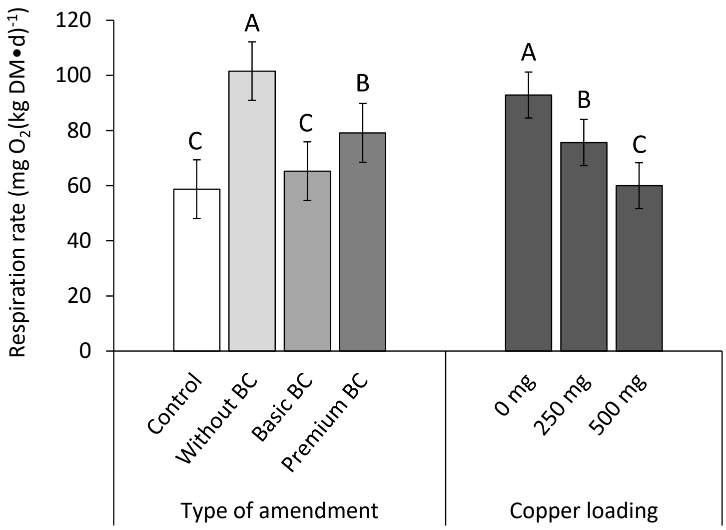


Figure 2. Respiration rate in soil amended with different types of compost and contaminated with copper at three different levels. Treatments with the same letter within type of amendment and copper loading, respectively, do not differ significantly (Tukey test, $p \leq 0.05$). Error bars represent the standard error of the mean (type of amendment: n = 9; copper loading: n = 12)

*Effects of amendments on earthworm avoidance behavior*

For the control soil without compost, already at a Cu load of 50 mg $kg^{-1}$, approximately 70% of the worms avoided the contaminated section (Figure 3). If copper load was doubled to 100 mg $kg^{-1}$, it were even more than 90%. These results reflect studies of Van Zwieten et al. (2004b), who already observed an avoidance response against copper at concentrations between 4 and 34 mg $kg^{-1}$. By incorporating compost into the soil, the avoidance response towards the contaminated section was partially reversed. Similar results were found by Neaman et al. (2012), who were able to improve soil quality and thus to enhance earthworm reproduction by incorporating compost into a copper and arsenic polluted soil. Therefore, the positive effects of compost in the present experiment were possibly caused by the general improvement of living conditions for earthworms. Additionally, the potential reduction in bioavailability of copper may be another reason for the lower sensitivity to the contaminated sections. In contrast to other studies (e.g. Sanchez-Hernandez et al. 2019), in which avoidance response was triggered by high amounts of fresh biochar, no correlation between application rate and earthworm behavior was evident in the present study.

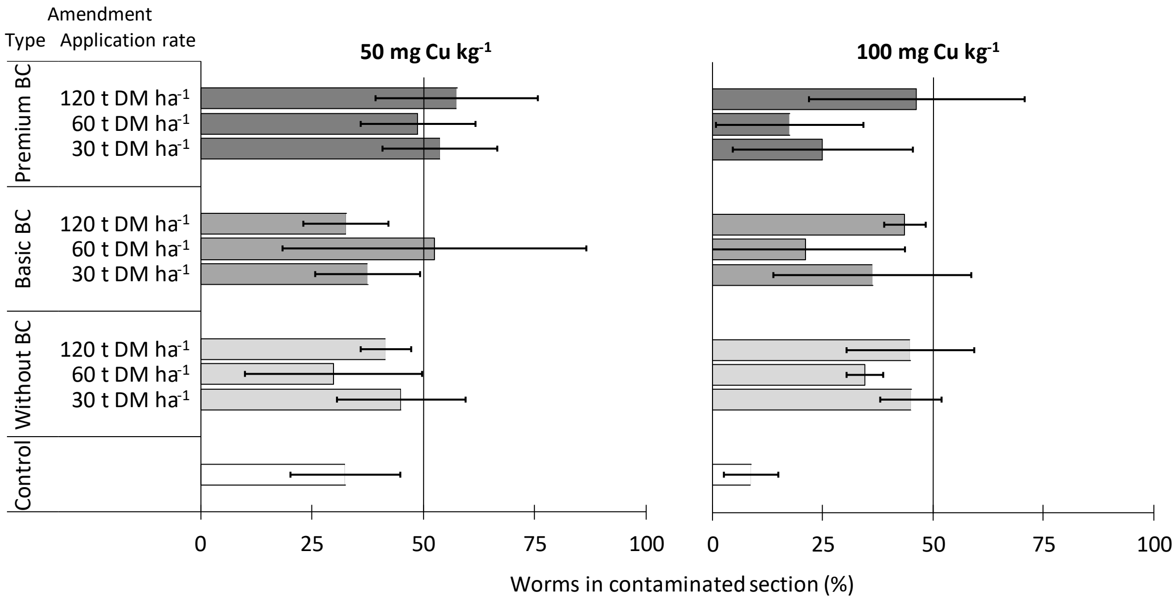


Figure 3. Avoidance behavior of earthworms against copper contaminated soils amended with different types and various amounts of compost compared to the control without compost. Error bars represent the 95% confidence interval (CI) of the mean (n = 4). If vertical line is within the CI of a mean, there is no significant avoidance behavior.

## 4. Conclusions

Regardless of biochar addition, the application of compost to copper-polluted soil significantly reduced the phytotoxic effects on Chinese cabbage and partly enhanced oxygen consumption of soil microorganisms. Additionally, copper-induced avoidance behavior of earthworms was decreased. However, a positive effect of biochar was only partly observed in plant growth. Therefore, the use of biochar-free compost might already be a suitable approach to reduce toxic effects of copper in soil. Since the experiments were conducted under controlled environmental conditions with artificially Cu-spiked soils, the efficacy of such composts has to be validated under field conditions on copper-polluted soils of hop gardens, orchards and vineyards, respectively.

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