田間施用雞糞生物炭對土壤與作物銅鋅濃度及團粒穩定度之影響

童曦儀(1)　**王尚禮**(2)　**程梅萍**(3)　**宋國彰**(4)　**賴鴻裕**(5)

摘　要

本研究將兩個熱裂解溫度(350 °C 和 600 °C)所製備的雞糞生物炭，施用在彰化露天栽培和桃園溫室栽培的土壤中，並分別連續種植四期作蕹菜(*Ipomoea aquatica* Forsk.)及三期作萵苣(*Lactuca sativa* L.)，以評估雞糞生物炭對土壤團粒穩定度及銅、鋅累積的影響。與化學肥料處理組比較，雞糞生物炭的處理可以顯著提升彰化土壤的團粒穩定度達 5~12%，而在桃園則無顯著的效果。若是以生物濃縮係數(作物體濃度與土壤濃度的比值)考慮兩種作物對於銅及鋅的累積能力，則萵苣對於銅及鋅的累積能力分別是蕹菜的 3.1~5.2 倍及 6.2~9.9 倍；與對照組比較，兩試驗區土壤及作物體中的銅及鋅濃度並未因添加雞糞生物炭而有顯著的增加，但與對照組及化學肥料處理組比較，雞糞生物炭處理卻會顯著降低兩種作物對鋅的生物濃縮係數，且鋅的生物濃縮係數會隨著採收期作的增加而降低，顯示雞糞生物炭的處理可以降低兩種作物對於鋅的累積能力。

（**關鍵詞**：生物濃縮係數、銅、雞糞生物炭、團粒穩定度、鋅）

Effects of consecutive in-situ applying poultry-litter biochar on the concentration of Cu and Zn in soils and crops, and soil aggregate stability

Prapasiri Tongsiri(1)　*Shan-Li Wang*(2)　*Mei-Ping Cheng*(3)　*Guo-Zhang Song*(4)　*Hung-Yu Lai*(5)

ABSTRACT

Two temperatures (350 °C and 600 °C) of pyrolytic poultry-litter biochars (PLBs) were in-situ applied in cropland of Changhua (CH) and greenhouse of Taoyuan (TY) at various amounts and consecutive cultivating four harvests of water spinach (*Ipomoea aquatica* Forsk.) and three harvests of

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(1) 國立中興大學水土保持學系博士班研究生
(2) 國立臺灣大學農業化學系教授
(3) 行政院農業委員會畜產試驗所研究員
(4) 國立中興大學水土保持學系助理教授
(5) 國立中興大學土壤環境科學學系教授；國立中興大學永續農業創新發展中心(Innovation and Development Center of Sustainable Agriculture; IDCSA; ORCID 0000-0002-9866-0706) (通訊作者 e-mail：soil.lai@nchu.edu.tw)

lettuce (*Lactuca sativa* L.). The objective of these experiments is to assess PLB's effect on wet-aggregate stability (WAS) and accumulation of copper (Cu) and zinc (Zn) in the soil and crop. Relative to treatment of chemical fertilizer, there was a 5-12% increase in WAS in CH under PLB treatments but not for TY. Bioconcentration factor (BCF), the ratio of shoot concentration to soil concentration, was used to compare the accumulation between different crops and heavy metals. Experimental result shows that the BCF of Cu and Zn of lettuce was 3.1-5.2 and 6.2-9.9 times higher than that of water spinach. The application of PLB did not significantly increased the concentrations of Cu and Zn in the soil and two crops compared to control. However, the PLB treatments significantly decreased the BCF of Zn of two crops compared to treatments of control and chemical fertilizer. The PLB treatments also decreased the accumulation of Zn of two crops because the BCF of Zn decreased with the increase of harvesting number.

(Key words：Bioconcentration factor; Copper; Poultry-litter biochar; Wet-aggregate stability; Zinc)

Introduction

At present, the increasing population makes more demand for agricultural products which also causing the production of great quantities of agricultural waste. The production of livestock manure was more than 32.33% of total agricultural waste or 2.3 million metric tons per year in Taiwan; however, the application of poultry litter or chicken manure to soil directly may leads to many environmental problems. Taiwan's climate was subtropical, with an average annual rainfall of about 2,500 mm [1]. Leaching and the degradation of organic matter decreased the soil fertility. The application of organic fertilizer, chemical fertilizer, or manure thus plays an important role in implementing the soil fertility. However, if the manure is not suitably applied, it will result in higher emissions of pollutants, such as ammonia, unpleasant smells and the accumulation of heavy metals (HMs). In livestock manure, copper (Cu) and zinc (Zn) are paid more attention than others because they are essential elements for plants and living organisms. If crops uptake HMs from contaminated soil, there is a great risk of HMs transferring to the human food chain through the consumption of these crops. Contaminated waste has caused in the contamination of sustenance crops. While waste contains HMs at low concentrations, they may be suitable for growing productivity in agricultural production, as they are important to living organism growth. Conversely, high concentrations of HMs have negative influences on the environment. HMs are considered as non-degradable or non-destroyable inorganic pollutants, changing their solubility will subsequently alter their bioavailability to plants. The total concentration of HMs in soils thus might not mirror their phytotoxicity and accessibility to plants. [2].

Biochar, a fine-grained and porous material, is created from pyrolysis of organic wastes and can be considered as an alternative additive. It is produced by pyrolysis of biomass, including agricultural waste and biological materials, under oxygen-limited conditions. Different temperatures and periods of pyrolysis determine their physicochemical and biological properties [4]. The biochar has received considerable interest as a large-scale soil amendment to improve soil fertility, crop production, and nutrient retention and to serve as a recalcitrant carbon stock. However, the properties of the final product are also dependent upon the nature of the feedstock. Researchers have revealed that adding biochar to soil reduce soil fertility degradation, recovers crop yields, and recovers plant response to fertilizer. It has also been proposed that biochar may have the potential to decrease leaching of pollutants from agricultural soils [5]. Moreover, biochar has a relatively structured carbon matrix with a high degree of porosity and extensive surface area, so that it may perform as a surface sorbent which is like in some features to activated carbon and thus play a significant role in controlling pollutants in the environment.

Because of the essentiality of Cu and Zn in some feedstock, some biochars could have high concentrations of Cu and Zn. Bridle and Pritchard [6] found high concentrations of Cu, Zn, chromium (Cr), and nickel (Ni) in biochar produced from sewage sludge. The value of Zn, Cu, aluminum (Al), and iron (Fe) in pine chip and peanut were lower compared to the poultry-litter biochar (PLB), which pattern appears to be reverse to that experiential in the feedstock materials [7]. Many studies have revealed that HMs are potentially toxic to harvests, animals, and humans [8]. HMs may enter the human body through the breath of airborne dust, drinking water and crops grown on HM-contaminated soils [9].

The soil structure has significant impacts on nature related to soil and the environment. The capability of soil organic carbon in forming stable aggregates is correlated to its decay rate, which in turn is affect through its physical and chemical defense from microbial activity. Thus the structure formation is often slow by the stability of soil aggregates [10]. Soil aggregation, mostly responsible for soil structure, is essential for soil functioning and agricultural yield. Soil aggregates can physically stabilize soil organic matter (SOM) and protect it from decomposition [11]. Aggregation and related soil structure also have influence for the movement of water, energy and air in the soils as well as organism activities. Soil aggregate dynamics are influenced by a number of factors, including soil biota, root growth, soil mineralogy and texture, the availability of inorganic or mineral fraction, and environmental conditions. Due to different physical-chemical characteristics, the capacity of various particle size aggregates in adsorbing external materials (HMs, N, P, etc.) may be different. It is usually considered that fine size aggregates have a greater capacity to carry HMs than coarser aggregates because of larger specific surface area and more contents of organic matter, Fe, Mn, and Al oxides [12]. Soil aggregation maintains soil fertility because it decreases erosion and preservation and facilitates soil aeration as well as water infiltration. Moreover, soil aggregation prevents SOM from mineralizing because it physically decreases the accessibility of organic compounds for microorganisms and oxygen [13]. Generally, the size distribution and stability of soil aggregates are positively related with the multivalent cations and SOM.

The plant's ability to accumulate HMs from soils can be estimated using the bioconcentration factor, i.e. BCF, which is defined as the ratio of HM concentration in the plant tissue to that in the soil. We can compare the capacity of diverse plants in taking up HMs from soils and translocating them to the shoots using BCF. Tolerant plants tend to restrict soil to shoot transfers and also have much less accumulation in their biomass, while hyperaccumulators actively take up and translocate HMs into their aboveground biomass [14]. Bioconcentration and bioaccumulation endpoints can be assessed using quantitative structure-activity relations or the dangers that chemicals may pose to people and the environment and is a current focus of regulatory effort [15]. Chen et al. [16] found that plant-residue or agricultural waste derived biochar can act as effective surface sorbent, but their ability to treat mixed waste streams needs to be carefully evaluated on an individual basis.

Raw poultry litter was applied in the croplands directly by some farmers in Taiwan because of its higher contents of some essential nutrients; however, this practice can cause the pathogenic problems and also decrease the soil quality. In order to reuse poultry litter as a soil amendment in providing essential nutrients and also to increase the carbon sequestration in the soil, field experiments using PLB was conducted. Two most common cultivating leafy vegetables during experimental period were selected as study crops. In this study, two pyrolytic temperatures (350 ºC and 600 ºC) of PLB were in-situ applied in the cropland of Changhua (CH) and greenhouse of Taoyuan (TY) at various quantities and consecutive planting water spinach (*Ipomoea aquatica* Forsk.) and lettuce (*Lactuca sativa* L.). The objective was to evaluate PLB's effects on soil properties, growth exhibitions, and accumulation of Cu and Zn in these two crops grown in CH and TY areas. PLB's effects on the soil chemical properties already presented in Yu et al. [24], this paper only assesses PLB's effect on the WAS and the accumulation of Cu and Zn in the crops and soils.

Materials and Methods

Basic properties of two PLBs were analyzed before in-situ application. The pH of 350-PLB and 600-PLB were slightly to moderately alkaline and these two PLBs had high electrical conductivity (EC) values (Table 1). The total concentrations of Cu and Zn were also determined [17] because poultry litter always contain high concentrations of these two HMs. Analytic result shows that the total concentration of Cu and Zn of these two PLBs was in the levels of 90-130 and 800-1100 mg/kg, respectively.

Two field experiments were conducted in CH and TY from June 2016 to July 2017. Totally 15 treatments were used with four replicates and consecutive planting four harvests of water spinach (*I. aquatica* Forsk.) in CH outdoor and three harvests of lettuce (*L. sativa* L.) in the greenhouse of TY. These treatments were showed as followings. According to suggestion of Council of Agriculture of Taiwan (COA Taiwan), the recommended amount of N, P_2O_5, and K_2O for water spinach and lettuce is 120, 60, and 120 kg/ha, respectively. Experimental treatment was based on the recommended amount of P_2O_5 of the COA Taiwan. The 15 treatments are listed below Table 2.

Table 1. The basic properties of two PLB used in this study

Soil property	Unit	Pyrolysis temperature	
		350 ºC	600 ºC
pH	----	8.13	7.78
EC	dS/m	10.07	16.18
Cu	mg/kg	91	126
Zn	mg/kg	803	1,061

Treatments of 0.5% and 1% of PLB were applied only before the first cultivation and other amendments were applied before each cultivation. The area of each block was 4 m^2 and totally 60 blocks were planted. Different amendments were incorporated with surface soil (0-15 cm). Three samples per block of surface soil (0-15 cm) were collected and homogenized as a represented sample before each cultivation. These soil samples were coded as CH X-1 and TY X-1, i.e. CH 1-1, CH 2-1, CH 3-1 and CH 4-1 for the 1st, 2nd, 3rd, and 4th cultivations, respectively. TY 1-1, TY 2-1, and TY 3-1 for the 1st, 2nd, and 3rd cultivations, respectively. Commercial seeds of water spinach and lettuce got from Known-You Seed Co., Ltd. was sown in each block. The soil water of CH was supplied using surrounding irrigation channels and sprinkler for CH and TY, respectively. The weeds were pulled up during experimental periods.

At least 20 plants of mature water spinach and lettuce per block were harvested after growing for 32-35 days. The harvested shoots were rinsed with tap water and deionized water, further oven dried at 65 °C for 72 hr., and then ground into powder with a grinder. The powder was digested using $HNO_3/HClO_4$ [18], filtered using Whatman No.42 filter papers, and then quantified to 25 ml. The total concentrations of Cu and Zn in the filtered solutions were analyzed using an inductively coupled plasma optical emission spectrometer (ICP-OES; PerkinElmer Avio200, MA, USA). Besides plant, three samples per block of surface soil (0-15 cm) were also collected and homogenized as a represented sample for analysis after each cultivation. These soil samples were coded as CH X-2 and TY X-2, i.e. CH 1-2, CH 2-2, CH 3-2 and CH 4-2 for the 1st, 2nd, 3rd, and 4th cultivations, respectively. TY 1-2, TY 2-2, and TY 3-2 for the 1st, 2nd, and 3rd cultivations, respectively. After air drying, grinding, and passing through 100 mesh stainless steel sieves, the soils were digested using HNO_3/HCl, filtered using Whatman No.42 filter papers, and then quantified to 100 ml. The total concentrations of Cu and Zn in the filtered solutions were analyzed using an ICP-OES (PerkinElmer Avio200, MA, USA). Besides heavy metal analysis, the wet aggregate stability of soil samples was analyzed according to the method of Kemper and Rosenau [23].

The statistical analysis was performed using SAS (Statistical Analysis System). A one-way analysis of variance (ANOVA) was performed to detect differences in soil and crops between treatments. The least significant difference (LSD) test was used to identify significant differences between means. A value of $p < 0.05$ denoted statistical significance.

Table 2. The total detail 15 treatment in Changhua and Taoyuan.

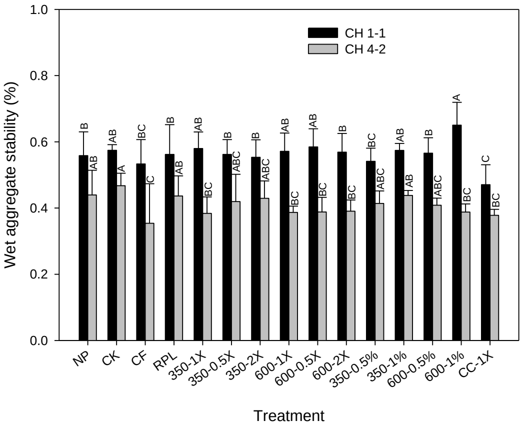
Treatment	Material applied (kg/ha)					Note
	N	P_2O_5	K_2O	350 °C PLB	600 °C PLB	
NP	0	0	0	0	0	No amendments and no crops
CK	0	0	0	0	0	No amendments but planted crops
CF	120	60	120	0	0	Chemical fertilizer: urea and monobasic potassium phosphate were applied
RPL	0	60	0	0	0	Raw poultry litter bought from market was applied
350-1X	0	0	0	60	0	350-1X, 350-0.5X, and 350-2X: applied 350ºC PLB as P_2O_5 = 60, 30, and 120 kg/ha, respectively
350-0.5X	0	0	0	30	0	
350-2X	0	0	0	120	0	
600-1X	0	0	0	0	60	600-1X, 60-0.5X, and 600-2X: applied 600ºC PLB as P_2O_5 = 60, 30, and 120 kg/ha, respectively
600-0.5X	0	0	0	0	30	
600-2X	0	0	0	0	120	
350-0.5%	0	0	0	1×10^4	0	350-0.5% and 350-1%: applied 10 and 20 ton/ha of 350ºC PLB, respectively
350-1%	0	0	0	2×10^4	0	
600-0.5%	0	0	0	0	1×10^4	600-0.5% and 600-1%: applied 10 and 20 ton/ha of 600ºC PLB, respectively
600-1%	0	0	0	0	2×10^4	
CC-1X	0	60	0	0	0	applied poultry-litter compost bought from market

Results and discussion

1. WAS

In the beginning of experiments in CH 1-1, there was no significant difference between CF and all PLB treatments except 600-1%. However, the WAS of all PLB treatments in CH4-2 was higher or significantly ($p < 0.05$) higher than that of CF after four consecutive cultivations (Figure 1). This phenomenon is especially true in the treatments of RPL, 350-0.5X, 350-2X, and 350-1%. The WAS of CH 4-2 of forgoing treatments was approximately 1.2- to 1.6-fold higher compared with CF. This result is consistant with a previous study which showed that biochar addition can significantly affect the form of soil water retention functions [19]. Because of the improvement in WAS under 350-PLB treatments, the remaining water contents should be thus increased. On the contrary, there was no significant influences of various treatments on the WAS in TY. Although 350-2X and 600-0.5% had higher WAS compared with CK and CF, however, these differences were not statistic significant and also disappeared after three consecutive cultivations.

Average of WAS of eight and six soil samples were calculated to assess the effect of different amendments on the WAS. Among these fifteen treatments in CH, CF and CC-1X had lower WAS compared with NP and CK. All of the WAS under PLB treatments was higher than CF, especially in the treatments of RPL, 350-1X, 350-1%, and 600-0.5%. Soil aggregate is the essential structural element of soils and its ability in absorbing and moving HMs in the soil environment of different size fractions are various [12]. The SOM could decrease bioavailability of HMs due to its greater stability against microorganism degradation. By the way, the accumulation of HMs of plants was determined by the intergraded effect of the soil constituents including SOM and the environmental factors such as pH, temperature, and moisture condition [27]. Moreover, when HMs adsorption effectiveness also decreased with increasing biochar application, suggesting that aggregation is a factor that needs to be explained for when developing HM removal plans using biochar [16]. Aggregation is influenced by the chemical composition of organic residues added to soils [20].



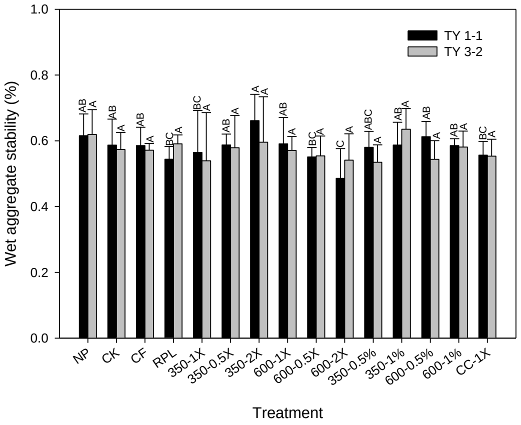


Figure 1. Effects of various treatments on wet aggregates stability of soils in Changhua (CH) and Taoyuan (TY) area. For the same harvest, repeat letter on each bar is not significant differences between treatments. Meanings of abbreviations are the same as Table 2.).

2. Soil Cu and Zn

There was no significant difference between the total concentration of Cu and Zn in the soil among different treatments in CH 1-1 (Figure 2) and TY 1-1 (Figure 3). At the end of field trail, there was also no significant difference among treatments (Figure 2). The total concentrations of Cu and Zn in CH 1-1 for all treatments was in the levels of 105-145 and 170-235 mg/kg, respectively. After four consecutive cultivations, the total concentration of Cu and Zn in CH 4-2 for all treatments was in the levels of 105-140 and 110-145 mg/kg, respectively. The Zn concentrations in CH 4-2 was only 60-65% of that in CH 1-1. For TY, there was also no significant difference of Cu and Zn concentrations amended with various treatments (Figure 3). The concentrations of Cu and Zn in the topsoil amended with various treatments did not changed drastically even after three consecutive cultivations. In the soils of TY

1-1 and TY 3-2, the total concentrations of Cu and Zn was in the levels of 17-22 and 30-40 mg/kg, respectively.

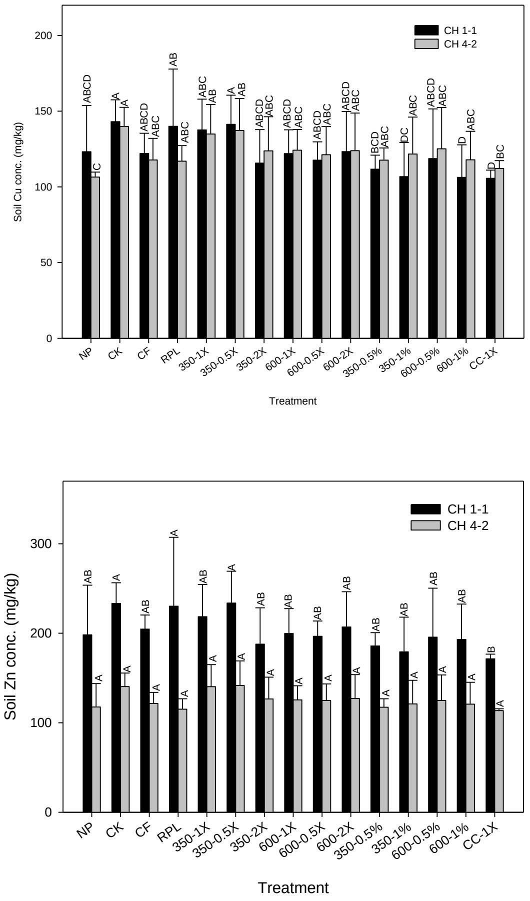


Figure 2. Effects of various treatments on the concentrations Cu and Zn in the soils of Changhua (CH). (For the same harvest, repeat letter on each bar is not significant differences between treatments. Meanings of abbreviations are the same as Table 2.).

The Soil and Groundwater Pollution Remediation Act (SGWPR Act) was announced in 2000 by the Environmental Protection Agency of Taiwan. The monitoring and controlling standards of Cu for croplands is 120 and 200 mg/kg, respectively. For Zn, they are 260 and 600 mg/kg, respectively. The two PLBs used in this study had moderate concentration of Cu (90-130 mg/kg) and high concentration Zn (800-1100 mg/kg). In an ideal situation, the maximum change in the concentration of Cu and Zn even in the treatment of 1% of PLB will less than 1.5 and 11 mg/kg, respectively. The maximum concentration of Zn in the CH 1-1 was close to 234 mg/kg, which is approach to the monitoring standard for cropland, i.e. 260 mg/kg. We suggest the farmers shall take Zn into consider when using these PLB even though PLB did not significantly alter the total concentrations of Zn in the topsoil.

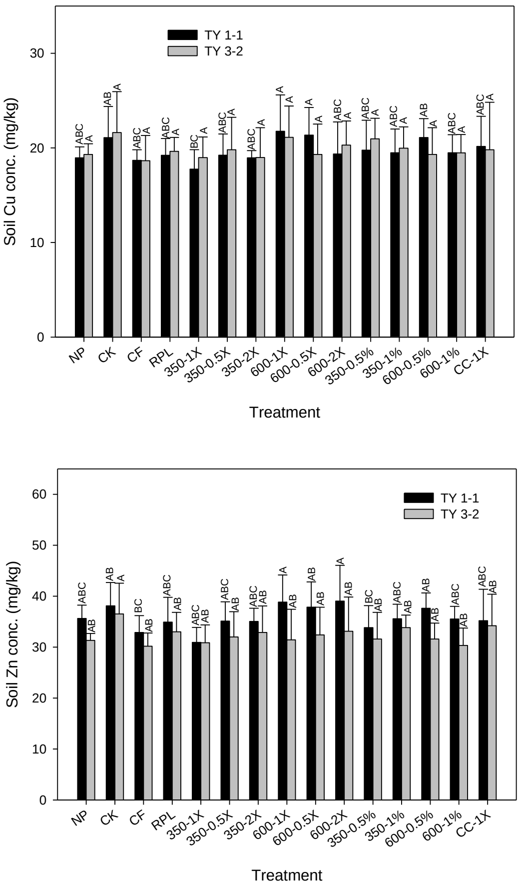


Figure 3. Effects of various treatments on the concentrations Cu and Zn in the soils of

Taoyuan (TY). (For the same harvest, repeat letter on each bar is not significant differences between treatments. Meanings of abbreviations are the same as Table 2.).

3. Shoot Cu and Zn

The shoot Cu concentrations of the four harvests of water spinach was approximately 16-38 mg/kg (Figures 4 and 5). Because the vegetables only concentrated small amount of Cu in their shoots [20], different treatments have no significant influence on the accumulation of Cu for each harvest. Among these four harvests, the first harvest had the highest shoot Zn concentration in general. This phenomenon was possible resulted from the difference of temperature during one-year experiment. In TY and relative to CK, all of the treatments did not significantly affected the accumulation of Cu and Zn in the shoots of lettuce. The Cu and Zn concentrations in the shoots of lettuce was approximately ND (not detectable) -16.8 and 27-102 mg/kg, respectively.

Average concentrations of four and three harvests of water spinach and lettuce was calculated and assess their accumulation of Cu and Zn under different amendments. The accumulation of Cu and Zn of water spinach and lettuce grown in CH and TY was quite different. In CH, the average concentrations of Cu and Zn in the shoots of water spinach was similar and was in the levels of 24-29 and 24-49 mg/kg, respectively. In TY, however, the lettuce accumulated higher concentration of Zn than Cu in the shoots and they ranged from 55-73 and 12-14 mg/kg, respectively. Even through that, various treatments did not significantly affect the accumulation of Cu and Zn in the shoots of water spinach and lettuce. Although biochars were capable to form complex with HMs and thus reduce their bioavailability [3], but their immobilizing effect on Cu and Zn was not found in this study.

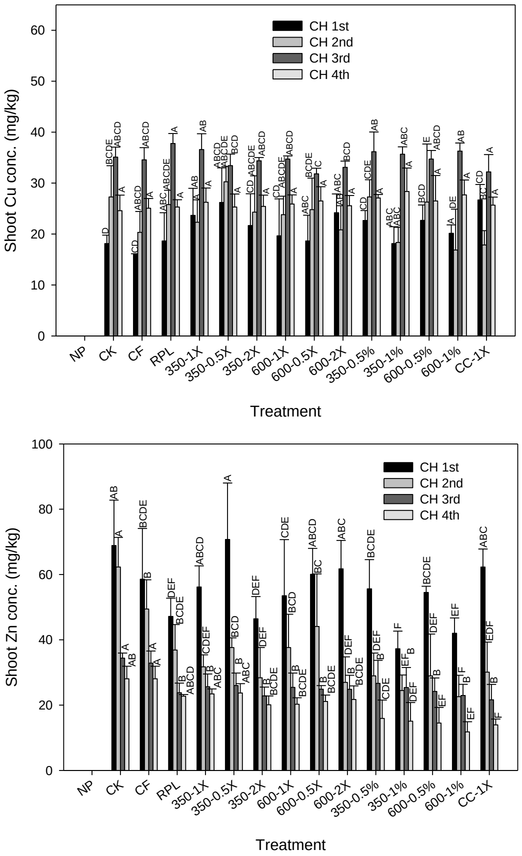


Figure 4. Effects of various treatments on the concentrations Cu and Zn in the shoots of water spinach grown in the soils of Changhua (CH) (For the same harvest, repeat letter on each bar is not significant differences between treatments. Meanings of abbreviations are the same as Table 2.).

4. BCF of Cu and Zn

The bioconcentration factor (BCF = shoot HM concentration to soil HM concentration) represents the ability of the plant to uptake HMs from the representing soil [13]. Table 3 shows the BCF of Cu and Zn of two crops grown in CH and TY. Experimental result reveals that the BCF is depend on species of crop and HM. For water spinach, the average BCF of Cu and Zn was all ranged from 0.1 to 0.3. Relative to water spinach, the lettuce had higher values of BCF and the average BCF of Cu and Zn was in the levels of 0.7-0.9 and 1.4-2.0, respectively. Relative to CF, PLB amendments decreased the BCF of Zn especially in the treatment of 350-1% and 600-1%. However, the application of PLB increased the BCF of Cu in general. Forgoing results reveals that the lettuce species used in this study has high accumulating capacity of Zn. The application of PLBs; however, could restrict the translocation of Zn from soil to the shoot of water spinach and lettuce. Under PLB amendments; moreover, the BCF of Zn of 3rd harvest lettuce decreased compared with 1st harvest which was possibly resulted from the decrease of bioavailability of soil Zn. Maximum Zn concentration, i.e. 101.6 ± 43.3 mg/kg, was observed in the edible organism of lettuce grown in CK. Even through these two PLBs had high concentrations of Zn; however, the lettuce grown under PLB treatments accumulated less than 85 mg/kg of Zn in the edible organs. In agreement with Chen et al. [16], PLB treatments could decreased the BCF of Zn of lettuce compared with CK because of adsorption.

The HM concentrations in plants differ from vegetable species and limited upward movement from roots into shoots can be considered as one of the tolerance mechanism [14]. Comparable Cu and Zn concentrations were found the value of root more than the shoots. Investigation of Stoltz and Greger [26] presented that Zn concentrations of 68-1630 mg/kg in vegetable biomass while those by Shu et al. [25] exhibited 66-7607 mg/kg in vegetable biomass. Vegetable uptake of HMs from soil occurs also tolerantly with the mass flow of water into the roots. Under usual growing conditions, vegetables can potentially accumulate certain HM ions in an order of size larger than the surrounding means. The mixture of high soil pH in the study site may limited the availability of Cu and Zn in the soil, resulting in low plant uptake of these HMs [22]. Similarly, the value of Cu concentrations in the roots were greater than those in the shoots, reported by described Stoltz and Greger [26], which were lower than those in our investigation. Since Zn and Cu are essential nutrients for plant systems, higher translocation from roots to shoots is clear [14]. Vegetables can immobilize HMs through absorption and accumulation by roots. This progression decreases HM mobility and leaching into groundwater, and also decreases HM bioavailability for entry into the food chain. By using HMs tolerant vegetable species for stabilizing contaminants in soil, it could also allow enhanced conditions for natural mitigation or stabilization of contaminants in the soil. HMs accumulated in the roots are considered moderately stable as far as discharge to an environment is concerned. Nevertheless, investigations are needed concerning the income benefits of nutrition roots and the potential release of HMs from decaying roots. Likewise, influences of plant–mycorrhizae relations, which might involve the HMs uptake and translocation which needed additional investigation.

5. PLB’s effect on WAS and recommended application rate

Using WAS's results is helpful for us to understand the PLBs' stabilization effect on aggregate formation as shown in Table 4. Experimental results showed that the WAS in CH 4-2 were 8-24% higher in the PLBs' treatments than in the CF treatment. Similarly, it was found that the treatments of 350-0.5X, 350-2X, 350-0.5%, 350-1%, and 600-0.5% had higher WAS among these 10 PLB treatments. While, there were no significant changes on the WAS of TY 3-2 under various PLBs treatments compared with CK and CF. These results propose that the addition of PLB effectively improved the formation of aggregates and thus led to soil structure improvement. Preceding studies directed that soil aggregates are formed through a combination of sand, silt, and clay particles by the electrostatic repulsive force and van der Waals attractive force and their stabilization by organic and inorganic materials. Biochar with high levels of carboxylic groups, oxygen/carbon (C) ratio, and CEC functional groups applied to the soil could contribute to the exchange between soil mineral particles and biochar surfaces. Also, the cation bond produced by aromatic compounds on biochars surfaces could increase the contact of soil mineral surfaces. This had two major mechanical properties contribute to aggregate including aggregate hydrophobicity, which decelerate the rate of water penetration in aggregate porosity and decreases its migration, and internal aggregate cohesion [28]. Moreover, the addition of biochars was evidenced to increase the soil water retention due to the great inside surface area and porous structure [20]. In general, the application of biochar is helpful to increase the strength of soil aggregation, this might be also results in the decrease of bulk density [30].

Experimental result also revealed that the BCF is depended on species of crop, SOM, and species of HMs. Relation of water spinach planted in CH, the lettuce grown in TY had greater values of BCF (Table 4). Relative to CF, PLB amendments decreased the BCF of Zn especially in the treatments of 350-0.5%, 350-1%, 600-0.5%, 600-1%, and CC-1X in CH 4-2. However, the application of PLB did not significantly change the BCF of Cu in CH 4-2 and TY 3-2 in general. Also, the results display that the lettuce species used in this study has high accumulating capacity of Zn. Electrostatic adsorption is the main adsorption mechanism and the absorption ability and stability of biochar is important to its adsorption performance. The C attached to the minerals in this fraction possibly forms a coating on their surface and displays a banded structure, which could have a part in the fate of the SOM-complexed Cu. This is supposed to increase the correlation between Cu and C. Thus, it is considered that the correlation between Cu and C does not depend on the total amount of C but on the decomposition state and kind of PLBs. Although SOM has a high correspondence for Cu, the amount of Cu retained by OM is limited due to its low content in the soil. It is supposed that more important contribution to the binding capacity of Cu could be assigned to the clay and silt fractions. Consequently, the adsorption of biochar might eventually increase their persistence in the environment because they will be protected from microbial degradation. Biochars are able to complex HMs ions on their surfaces and then decrease bioavailability, which get a concentrated risk. Moreover, the PLB's treatments decreased BCF of Zn in TY 3-2 compared with CF. Soils amended with biochar might modify some chemical properties such as cation exchange capacity and soil acidity, providing circumstances that are appropriate for HM immobilization and subsequently reducing their availability to plants [29]. Also, the accumulation of HMs of plants was determined by the intergraded effect of the soil composition including SOM and environmental factors such as pH, temperature, and moisture condition. HMs adsorption effectiveness also decreased with increasing biochars application. Nevertheless, based on the findings of our study, an increase of plant growth by biochar application did not significantly enhanced Cu uptake by water spinach and lettuce. In contrast, several studies found that the application of biochar stimulated Cu plant uptake, but it tended to reduce Zn uptake in shoots [26]. Regarding the effect of biochars, it can be seen that the uptake of Cu and Zn of plants depends on heterogeneity in response to different types of soil and species of plants. According to the results in this study and previous study [29], which assess the same PLBs’ effect on the soil properties, 350-0.5% is the most suitable treatment from sustainable viewpoint. This treatment could improve the strength of WAS, decrease the BCF of Zn in CH, and enhance the concentrations of essential nutrients. Moreover, it could increase the C sequestration and thus alleviate the stress of greenhouse effect compared with CK and CF treatments. However, it was not significantly increase the concentrations of Cu and Zn in these studies.

Table 3. Bioconcentration factor of Cu and Zn of water spinach and lettuce grown in Changhua and Taoyuan.

Treatment	**Changhua: water spinach**				**Taoyuan: lettuce**			
	Cu		**Zn**		**Cu**		**Zn**	
	1st	**4th**	**1st**	**4th**	**1st**	**3th**	**1st**	**3th**
NP	----	----	----	----	----	----	----	----
CK	0.13	0.18	0.30	0.20	ND[*]	0.78	2.67	1.18
CF	0.13	0.21	0.29	0.23	ND	0.79	2.45	1.33
RPL	0.13	0.22	0.20	0.20	ND	0.74	2.31	1.15
350-1X	0.17	0.19	0.26	0.17	ND	0.82	2.17	1.31
350-0.5X	0.19	0.18	0.30	0.17	ND	0.78	2.12	1.28
350-2X	0.19	0.21	0.25	0.16	ND	0.77	1.88	1.20
600-1X	0.16	0.21	0.27	0.16	ND	0.71	2.06	1.34
600-0.5X	0.16	0.22	0.31	0.17	ND	0.80	1.97	1.14
600-2X	0.20	0.21	0.30	0.17	ND	0.71	2.04	0.89
350-0.5%	0.20	0.23	0.30	0.14	ND	0.71	2.51	0.99
350-1%	0.17	0.23	0.21	0.12	ND	0.72	2.39	0.82
600-0.5%	0.19	0.21	0.28	0.12	ND	0.77	2.03	0.98
600-1%	0.19	0.23	0.22	0.10	ND	0.78	2.19	0.92
CC-1X	0.25	0.23	0.36	0.12	ND	0.76	2.37	0.85

* not detectable

Table 4. Percentages of PLB’s influences on WAS and BCF of water spinach and lettuce compared with CF.

Treatment	WAS				BCF							
					Cu				Zn			
	CH 4-2	%*	TY 3-2	%*	CH 4-2	%*	TY 3-2	%*	CH 4-2	%*	TY 3-2	%*
NP	0.44	124	0.62	108	----	----	----	----	----	----	----	----
CK	0.47	132	0.57	100	0.18	83	0.78	99	0.20	87	1.18	88
CF	0.35	100	0.57	100	0.21	100	0.79	100	0.23	100	1.33	100
RPL	0.44	123	0.59	103	0.22	102	0.74	94	0.20	85	1.15	86
350-1X	0.38	108	0.54	94	0.19	91	0.82	103	0.17	72	1.31	99
350-0.5X	0.42	119	0.58	101	0.18	87	0.78	98	0.17	72	1.28	96
350-2X	0.43	121	0.60	104	0.21	97	0.77	98	0.16	69	1.20	90
600-1X	0.39	109	0.57	100	0.21	98	0.71	90	0.16	70	1.34	100
600-0.5X	0.39	110	0.55	97	0.22	103	0.80	102	0.17	73	1.14	86
600-2X	0.39	110	0.54	95	0.21	97	0.71	90	0.17	74	0.89	67
350-0.5%	0.41	117	0.53	94	0.23	108	0.71	89	0.14	59	0.99	75
350-1%	0.44	124	0.64	111	0.23	109	0.72	92	0.12	54	0.82	62
600-0.5%	0.41	115	0.54	95	0.21	99	0.77	98	0.12	50	0.98	73
600-1%	0.39	110	0.58	102	0.23	110	0.78	99	0.10	42	0.92	69
CC-1X	0.38	107	0.55	97	0.23	107	0.76	96	0.12	53	0.85	64

* The proportion between WAS and BCF in various treatments to CF

Conclusions

Besides the benefit effects of PLB on raising soil pH and contents of available nutrients which had presented previously, experimental results of this study further demonstrated its beneficial effect on WAS and BCF. The consecutive in-situ application of two PLBs increased the WAS in the cropland in CH but not in the greenhouse in TY compared with control in general. Even through the two PLBs have high concentrations of Zn, the accumulation of Cu and Zn in the soils and two tested crops were not significantly different among different treatments. Relative to water spinach, lettuce had higher BCF regardless of different treatments. The application of PLB decreased the BCF of Zn in general compared with treatments of control and chemical fertilizer.

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