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Application of Sludge in a Chinese Cabbage (*Brassica Campestris ssp. Chinesis*) and Chicken Manure on Grain Protein Concentration, Grain Essential Amino Acid Concentration in Shanxi Province China

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Abstract The Chinese cabbage (*Brassica campestris ssp. chinesis*) experimental base of Shanxi agricultural in Shanxi Province, China. Organic fertilizer was the main plot, 0 and 1.5 t/ha (Chicken mature Organic fertilizer). Five treatments were set up ① Control CK (0g sludge + 0g chicken manure) ② Organic fertilizer "S" level (sludge + chicken manure 0g) ③ "SM60" level of organic fertilizer (sludge + organic fertilizer 60 g/kg) ④ "SM120" level of organic fertilizer (sludge + organic fertilizer 120 g/kg) ⑤ Organic fertilizer "SM180" level (sludge + organic fertilizer 180 g/kg), 4 replicates. The results showed that plant height, root length, aboveground dry weight and underground dry weight of Chinese cabbage increased firstly and then decreased in latosol, red soil and calcareous brown soil. The growth and biomass of Chinese cabbage in the three soils ranged from large to small, red soil. The contents of Cu and Zn in edible parts of Chinese cabbage in three soils showed an increasing trend. The enrichment coefficients of Cu and Zn in Chinese cabbage are quite different, and the enrichment coefficients of Zn are larger than that of Cu, both of which are less than 1. The content of organic matter in latosol, red soil and calcareous brown soil increased, and the average content of organic matter in each experimental treatment was from large small. The total Cu and Zn contents and available state contents in the three soils showed an increasing trend. The average Cu content of each treatment was red soil > calcareous brown soil > latosol, and the average Zn content was red soil. The condition of chicken manure, the content of essential amino acids was basically equal to that of non-essential amino acids, which increased the accumulation of essential amino acids in grains, inhibited the accumulation of non-essential amino acids in grains, promoted the balance of amino acids in grains, and thus improved the nutritional quality of Chinese cabbage.

Keywords: *municipal sludge, different acidic, protein, crop growth, chicken manure*

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1. Introduction

Chinese cabbage (*Brassica campestris ssp. chinesis*) originated in China, is the largest vegetable crop, and plays an essential role in all-year production, year-round vegetable supply, and market stabilization in China [1]. Chinese cabbage (*Brassica campestris ssp. chinesis*) was originally named Sinapis pekinensis Lour, and "Pëtƒái", which was produced in the Beijing region of China, was reported as a new species [2]. As a leaf vegetable, Chinese cabbage requires the application of a large amount of N fertilizer to the soil during its growth [2,3]. In addition, Chinese cabbage is also a vegetable that is prone to NO^{-3} accumulation [4]. In recent years, a great of research has been carried out on the effects of the N application rate and different application methods on the economic characteristics of Chinese cabbage, such as yield and quality. It has been shown that different Chinese cabbage varieties have different nitrogen utilization efficiencies (NUE), and different N fertilizer application rates and methods have essential effects on the economic properties of Chinese cabbage. A higher N application rate cannot improve the yield or quality of Chinese cabbage heads [5,6]. A systematic analysis of the genotype differences in 68 Chinese cabbage varieties was performed under two N levels and revealed significant genotype differences in 15 crucial agronomic traits and three NUE traits of Chinese cabbage [7]. Studies have shown that due to the long-term deficiency of sulfur fertilizer, sulfur deficiency in more than 30% of farmland soil in China has occurred. Current situation of urban sludge resource utilization municipal sludge is the solid organic waste produced in the sewage treatment process of municipal sewage treatment plants [8]. The including organic substances, inorganic substances and microorganisms, which is a very complex heterogeneous body [9]. At present, municipal sludge disposal mainly includes landfill, incineration, construction materials and land utilization and other disposal methods [10]. However, the disposal method that can be called resource utilization is mainly land use. Urban sludge contains a large number of organic substances and nutrient elements, which can improve the physical and chemical properties of soil, repair and improve soil, and have a long-term promoting effect on it [11,12]. Therefore, land use has become one of the most common ways of sludge treatment [13]. In the European Union, 50% of sludge is used for land [14]. While in the United States from 2000 to 2010. The land use of sludge increased to 70% of the total sludge. In Lithuania, sludge compost accounted for 26% of the total sludge, 14% of which was used for agriculture and 26% for composting [15]. A large number of research results show that urban sludge has good effects on yield increase [16]. The sludge application significantly increased the Cu and Zn contents above and below ground, and the accumulative amounts of Cu and Zn significantly increased [17]. Showed that when the application rate of sludge compost was greater than 20%, the biomass of green vegetables showed a downward trend [18].

The germination and growth of flax seeds were seriously inhibited when the amount of sludge was more than 25% [19]. Different amounts of sludge to yellow soil for 10 consecutive years, Pb, Ni, Cd and Cu exceeded the standard [20]. Applied 20% sludge to saline-alkaline soil, and soil Zn exceeded the standard [21]. Sludge compound fertilizer to loam brown soil where wheat was grown, and the contents of heavy metals Cu, Zn, Pb and Cd in wheat grains were increased [22]. Showed in the field experiment of tidal brown soil that Cu, Zn and Pb contents in maize grains were positively correlated with the corresponding heavy metal contents in soil. The organic matter in urban sludge can promote the growth and development of crops, while the heavy metals in it will accumulate in soil crops, thus having a great impact on human beings, animals and the ecosystem. We should make the advantages of urban sludge and avoid the disadvantages through scientific means, so as to realize the sustainable utilization of its resources [23,24].

This study provides a scientific basis Chinese cabbage (*Brassica campestris ssp. chinesis*) for the rational land use of municipal sludge and livestock manure, and also for the sustainable use of municipal sludge and chicken manure by different soils and crops. A transcript sequence analysis was also performed on the shoots and roots of Chinese cabbage seedlings to understand the expression patterns of the related transcription factors in Chinese cabbage under different chicken manure.

2. Materials and Methods

2.1. Site Description

The experiment was conducted in the Chinese cabbage (*Brassica campestris ssp. chinesis*) of the Experimental Station of College of Resources and Environment, Shanxi Agricultural University, of the Huaqiao Team in Chuanshan Town, Liujiang County, Liuzhou City, Guangxi Zhuang Autonomous Region, respectively. The grassy land (N19°02', E110°13) of Dashuiling Village, Xicun Village Committee, Tanmen Town, Qionghai City, Hainan Province. The collected soil was removed from rocks, animal and plant residues and other impurities, and mixed for pot experiments. At the same time, part of the soil was passed through 1 mm and 0.149 mm nylon sieve respectively for the determination of basic properties of soil samples. The physical and chemical properties of the tested soil in Table 1.

Figure 1. Chicken manures are the best nutrients boost which can give into soil for roots growth. While all chicken manure have not equal composition. Organically chicken manure bags can buy. As well neighbors who raise chickens organically, ask them for some of the manure

Table 1. Some basic properties of experimental materials

Treatments	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Total nitrogen (%)	Available P (mg kg^{-1})	Available K (mg kg^{-1})	Organic Matter (%)	pH
Latosol	17.35	67.99	0.04	1.52	49.60	0.41	4.42
Red Soil	45.27	101.06	0.11	4.37	183.53	1.73	5.38
Calcareous cinnamon soil	37.89	101.03	0.08	25.57	199.40	1.37	8.32
Test sludge	448.70	386.80	0.98	74.59	230.0	33.2	7.42
Chicken manure	70.23	408.13	2.86	149.18	118.32	50.00	8.45

2.1.1. Experimental Design and Implementation

The experiment was carried out in the solar greenhouse pot of the experimental station of College of Resources and Environment, Shanxi Agricultural University in March 2016. Variety four Seasons Chinese Cabbage. Organic fertilizer was the main pot, 0 and 1.5 t/ha (Chicken mature Organic fertilizer). Five treatments were set up Control CK (0g sludge + 0g chicken manure) Organic fertilizer "S" level (sludge + chicken manure 0g) "SM60" level of organic fertilizer (sludge + organic fertilizer 60 g/kg) "SM120" level of organic fertilizer (sludge + organic fertilizer 120 g/kg) Organic fertilizer "SM180" level (sludge + organic fertilizer 180 g/kg), 4 replicates. Each pot of the experiment was filled with 780 g of soil, and the sludge was fully mixed into the soil of each treatment at the ratio of 18g/kg, and the corresponding amount of chicken manure was applied to different treatments according to the experimental design. After being thoroughly mixed, distilled water was poured to 70% of the field water capacity, and the plants were randomly placed in the greenhouse to stabilize for 2 days.

Then the cabbage was sown, and 10 plants (seedlings) were selected after the emergence of seedlings. Water supply should be paid attention to during the growth of Chinese cabbage and the consistency of all experimental treatments should be maintained. The experiment was finished in May 2016, and the cabbages were picked for use.

2.1.2. Test Sludge

The test sludge was collected from a sewage treatment plant in Yuncheng City, Shanxi Province. The sludge was decomposed sludge prepared by aerobic composting at high temperature. The sludge sludge conforms to the national Standard for the Control of Pollutants in Agricultural Sludge (GB-4284-2018).

2.1.3. Test chicken Manure

The test chicken manure was collected from Changzhi city, Jincheng city and other areas in Shanxi Province. Organic fresh chicken manure from Changzhi and Jincheng areas was collected, decomposed and dried to remove impurities such as rocks and soil particles, and then mixed evenly as organic fertilizer.

2.1.4. Measurement Items and Methods

Then the samples according to different treatment of the underground part and the ground part were loaded into kraft paper bags, and put into the oven, in the oven of 105°C for 30 minutes, after the completion of the control oven temperature of 70°C drying until constant weight, determination of the dry weight of the underground part and the ground part, ground with agate mortar 1 mm sieve, heavy metal content determination.

The plant height and root length of the cabbage were measured directly with a tape measure and the above/below dry parts were weighed with an analytical balance. The organic matter and total nitrogen, total phosphorus and total potassium of Chinese cabbage were determined by conventional methods. All heavy metal elements were determined by international standard method, where Cu, Zn, Pb, Cr, Ni HNO_3-$HCLO_4$ was used for elimination cooking and plasma emission spectrometry was used for determination. Cd was determined by graphite furnace atomic absorption spectrophotometry after HNO_3-$HCLO_4$ elimination cooking. As was boiled by HNO_3-HCl and determined by atomic fluorescence spectrophotometry. The total nitrogen, phosphorus and potassium of Chinese cabbage were determined by H_2SO_4-H2O_2 elimination Kjeldahl method, H_2SO_4-H_2O_2 elimination vanadium molybdenum yellow colorimetric method, and H_2SO_4-H_2O_2 elimination.

Figure 2. Four Seasons Chinese Cabbage under different chicken manure levels in the pot experiment College of Resources and Environment, Shanxi Agricultural University

2.1.5. Calculation Method

Bioconcentration factor (BCF): The ability of a plant to absorb a particular heavy metal from the soil and accumulate it in the plant. Calculation formula [25]

$$BCF = C_{plant} / C_{soil}$$

General Training Module, C_{plant}(Sum)C_{soil}

Represents the heavy metal concentration of a plant site and the corresponding heavy metal concentration of soil, based on the drying weight.

(Translocation factor, TF): The ability of a plant to move heavy metals from its roots to the ground. A formula to calculate [26]

$$TF = C_{shoot} / C_{root}$$

General Training Module, C_{shoot}, C_{root} Represents heavy metal concentrations in plant aboveground parts and plant roots, respectively.

2.1.6. Statistical Analysis

The different data were subjected to analysis of variance (ANOVA) as split-plot design using DPS and SAS 9.0. Graphics were constructed using Microsoft Excel 2010. Mean values were calculated and significance of the difference between treatments was tested by LSD (least significant difference) method at the significance level of P=0.05.

3. Results

3.1. Effects of Different Chicken Manure Chinese cabbage in Lateritic Soil

Plant height, root length, dry weight of aboveground and underground Bok choy in latosol were 10.5-20.4 cm, 10.9-15.4 cm, 2.24-13.05 g/ basin and 0.25-0.78 g/ basin, respectively seen from (Table 2). In addition, from the non-application of sludge and chicken manure treatment (CK) to the application of municipal sludge and with the increase of the application of chicken manure, all indexes showed a trend of increasing first and then decreasing, and all indexes reached the maximum value in the treatment of SM120.

CK compared to plant height and root length of Chinese cabbage treated with sludge (S) alone increased by 24.8% and 30.2%, respectively, while the dry weight of aboving-ground and underground parts also increased, but the difference was not significant. Furthermore, with the application and increase of chicken manure, the plant height, root length, dry weight of aboveground and underground parts of Chinese cabbage increased by 88.90%, 33.92%, 387.70% and 152.00% in SM60 treatment compared to CK, respectively. SM120 treatment increased by 94.99%, 42.21%, 483.89% and 212.00%, respectively, and SM180 treatment increased by 81.00%, 6.08%, 402.24% and 112.00%, respectively. There were significant differences in the indexes of all treatments except the root length of SM180 treatment.

S compared to plant height, aboveground and underground dry weight of Pak choy under SM60, SM120 and SM180 treatments were increased in different degrees, and the SM60 treatment was increased by 51.17%, 211.43% and 40.00%, respectively, compared with S treatment. S Compared to SM120 treatment increased by 56.04%, 272.86% and 73.33%, respectively. Compared with S treatment, SM180 treatment increased by 44.84%, 220.71% and 17.78%, respectively, among which, except for the underground dry weight of SM60 and SM180 treatment, the other treatment indexes were significantly different. Compared with S treatment, the root length of cabbage under SM60 and SM120 treatment also increased by 2.83% and 9.20%, respectively, but the difference was not significant. Compared with S treatment, the root length of cabbage under SM180 treatment significantly decreased by 18.54%.

In conclusion, the plant height and biomass of pakchoi in latosol could be increased by applying municipal sludge (S) alone or with different amounts of chicken manure (SM60, SM120, SM180), but the increase of plant height, aboveground and underground dry weight of pakchoi in latosol first increased and then decreased with the increase of the application amount of chicken manure. The single application of municipal sludge (S) and the combined application of low and medium chicken manure (SM60 and SM120) also promoted the root growth of Chinese cabbage to a certain extent, but the high combined application of chicken manure (SM180) significantly inhibited the root growth of Chinese cabbage. In conclusion, in this experiment, based on the application amount of the tested sludge, the promotion effect of medium amount of chicken manure on the growth of pakchoi on the red soil was relatively best, while the promotion effect of high amount of chicken manure on the growth of pakchoi on the red soil was weakened, and even had an inhibitory effect, and the inhibitory effect on the underground part was significantly greater than that on the above-ground part.

3.2. Effects of Cu and Zn Contents in Edible Parts of Cabbage on Latosol

The content of Cu and Zn in aboveground part of Chinese cabbage in lateritic soil showed an increasing trend, and the content of Cu and Zn was relatively maximum in SM180 treatment (Figure 3). CK compared to Cu content of S, SM60, SM120 and SM180 increased by 38.84%, 85.44%, 86.60% and 117.09%, respectively. S compared to Cu content in Chinese cabbage SM60, SM120 and SM180 was significantly increased by 33.57%, 34.41% and 56.36%, respectively. However, SM60 compared to processing, the increase of SM120 processing was small and the change was not significant, but the SM180 processing increases significantly.

The content of Cu and Zn in aboveground part of Chinese cabbage was significantly increased by applying municipal sludge alone or with chicken manure in latolian soil. This may be mainly related to the content of organic matter in the soil and the acidity and alkalinity of the soil. Under acidic conditions, the binding ability of Cu and Zn ions with organic macromolecules in the soil was greatly weakened, resulting in the increase of available Cu and Zn contents.

Table 2. Effects of different amounts of chicken manure combined with municipal sludge on the growth of Pakchoi on latosol

Treatments	Plant height (cm)	Aboveground dry weight (g/pot)	Root length (cm)	Underground dry weight (g/pot)
CK	10.5±0.78d	10.9±1.08b	2.24±0.09c	0.25±0.03c
S	13.1±0.48c	14.1±1.41a	3.50±1.00c	0.45±0.03bc
SM_{60}	19.8±0.85b	14.5±0.83a	10.90±1.56b	0.63±0.13ab
SM_{120}	20.4±0.71a	15.4±0.86a	13.05±0.82a	0.78±0.09a
SM_{180}	19.0±1.37b	11.5±1.19b	11.23±1.96ab	0.53±0.03b

Table 3. Effects of different amount of chicken manure combined with municipal sludge on the growth of Pakchoi in calcareous cinnamon soil

Treatments	Plant height (cm)	Aboveground dry weight (g/pot)	Root length (cm)	Underground dry weight (g/pot)
S	10.8 ± 0.6 ^{d}	2.7 ± 0.1 ^{c}	11.0 ± 0.4 ^{bc}	0.2 ± 0.03 ^{c}
SM_{60}	14.5 ± 1.0 ^{c}	4.1 ± 0.2 ^{c}	13.5 ± 0.8 ^{ab}	0.4 ± 0.03 ^{b}
SM_{120}	18.8 ± 0.9 ^{a}	8.5 ± 0.8 ^{a}	14.8 ± 0.8 ^{a}	0.6 ± 0.06 ^{a}
SM_{180}	17.7 ± 0.9 ^{a}	7.7 ± 0.6 ^{a}	11.0 ± 0.7 ^{bc}	0.4 ± 0.05 ^{b}
S	16.0 ± 1.0 ^{b}	6.4 ± 0.5 ^{b}	8.8 ± 0.5 ^{c}	0.2 ± 0.03 ^{c}

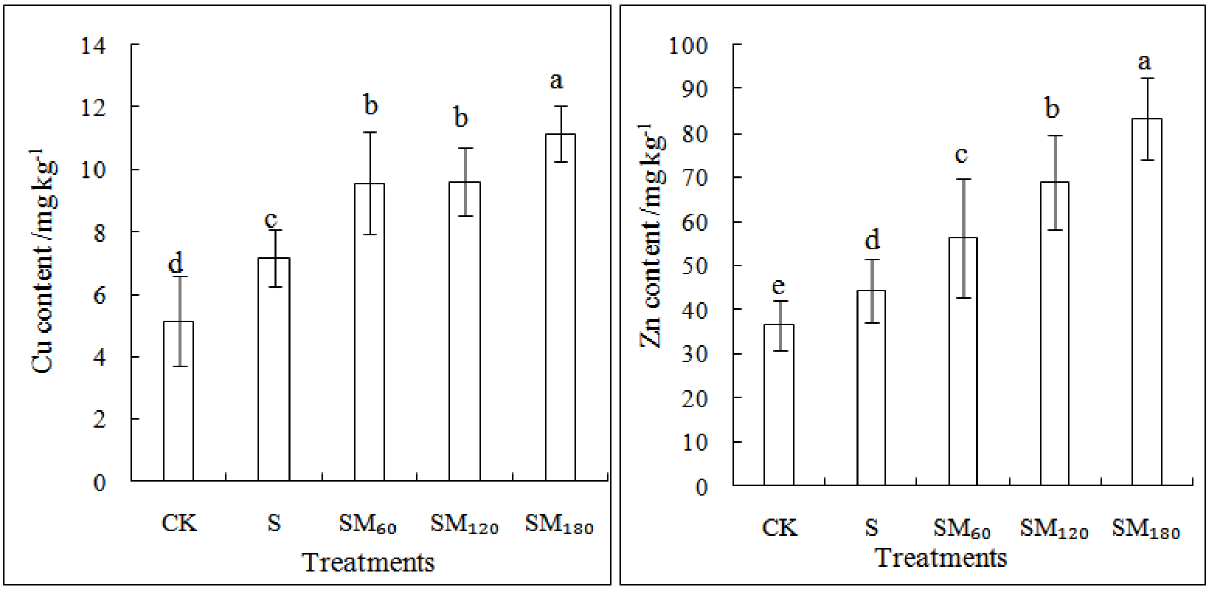

Figure 3. Effects of different amounts of chicken manure combined with municipal sludge on Cu and Zn contents of Pakchoi in latosol

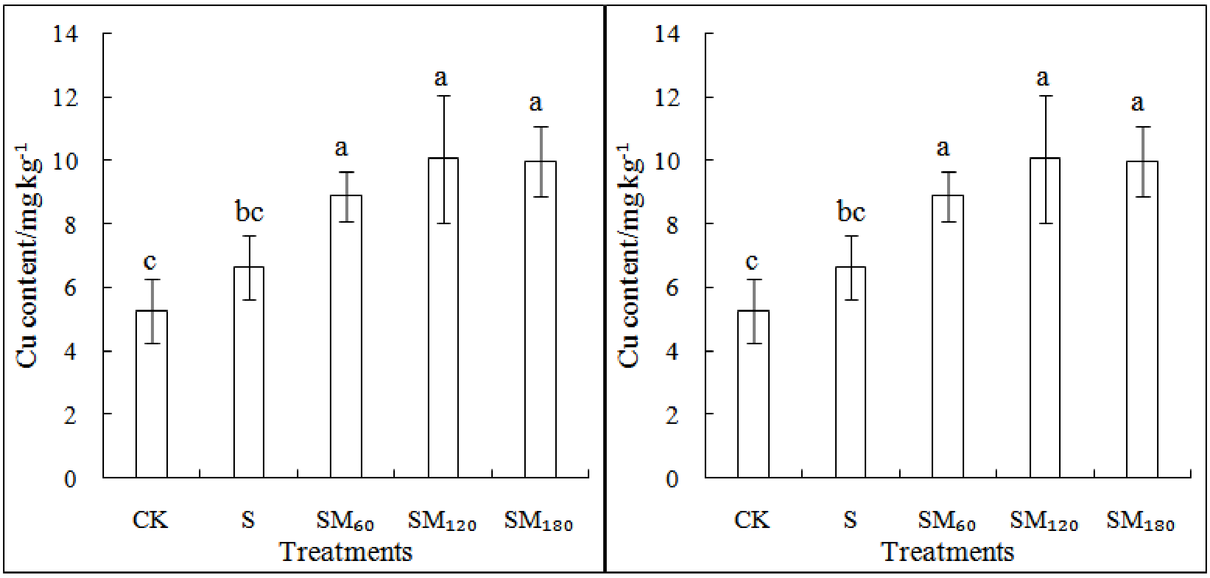

Figure 4. Effects of different amounts of chicken manure combined with municipal sludge on Cu and Zn contents of Pakchoi in red soil

3.3. Effects of Cu and Zn Contents on Edible Parts of Cabbage in Red Soil

The Cu and Zn contents in aboveground parts of Chinese cabbage in red soil showed a trend of first increasing and then decreasing, and Cu and Zn contents were relatively maximum under SM60 treatment (Figure 4).

Compared with CK, the Cu content of S. cabbages treated with S, SM60, SM120 and SM180 increased by 26.48%, 69.14%, 91.62% and 89.90%, respectively. There was no significant difference between S treatment and CK, but significant difference between SM60, SM120 and SM180 treatment and CK. The content of Zn in Chinese cabbage treated with S, SM60, SM120 and SM180 increased by 16.25%, 34.00%, 43.98% and 46.01%, respectively. The content of Zn in Chinese cabbage treated with SM60, SM120 and SM180 increased significantly, while that in S treatment did not.

Compared with S, Cu content in cabbage treated with SM60, SM120 and SM180 was significantly increased by 33.73%, 51.51% and 50.15%, respectively. The content of Zn in Chinese cabbage was significantly increased by 24.00% and 26.00% in SM120 and SM180 treatments and 15.00% in SM60 treatment, respectively, and the difference was not significant compared with S.

3.4. Effect on Cu and Zn Contents in Edible Parts of Cabbage in Calcareous Brown Soil

The Cu and Zn contents in the aboveground part of Chinese cabbage (Figure 5), in calcareous brown soil showed a trend of first increasing and then decreasing, and Cu and Zn were relatively maximum under SM60 treatment and SM120 treatment.

Compared with CK, Cu content in aboveground parts of Chinese cabbage increased by 9.07%, 30.07%, 31.74% and 16.23% in S, SM60, SM120 and SM180 treatments, respectively. Except for S and SM180 treatments, there were significant differences in Cu content. Zn content of S, SM60, SM120 and SM180 in aboveground parts of Chinese cabbage increased by 18.88%, 40.52%, 45.05% and 44.29%, respectively.

Compared with S, Cu content in aboveground part of Chinese cabbage increased by 19.26%, 20.79% and 6.56% in SM60, SM120 and SM180, respectively, but the increase was not significant in SM180. The content of Zn in the above-ground parts of Chinese cabbage SM60, SM120 and SM180 increased by 18.21%, 22.01% and 21.38%, respectively.

3.5. Effects on Cu and Zn Enrichment Coefficient of Cabbage in Latosol

Cu and Zn enrichment coefficients of aboveground part of Chinese cabbage in lateritic soil increased first and then decreased (Figure 6). Among them, the enrichment coefficient of Cu in S treatment is the relative maximum of 0.51, SM180 treatment is the relative minimum of 0.14, and the enrichment coefficient is less than 1. Compared with CK, Cu enrichment coefficient of S treatment is increased by 27.50%, while that of SM60, SM120 and SM180 treatment is decreased by 10.50%, 49.25% and 66.25%, respectively. The differences are all significant except SM60 treatment. S Compared to enrichment coefficients of Cu in SM60, SM120 and SM180 treatments are significantly reduced by 29.80%, 60.20% and 73.53%, respectively.

The enrichment coefficient of Zn in aboveground part of Chinese cabbage in red soil was 0.52 under S treatment and 0.28 under SM180 treatment. CK compared to enrichment coefficient of Zn in S treatment increased by 1.96%, and the difference was not significant. The enrichment coefficient of Zn in SM60, SM120 and SM180 treatments decreased by 5.88%, 35.29% and 45.49%, respectively. The difference was not significant in SM60 treatment, but significant in SM120 and SM180 treatment. Compared with S treatment, the enrichment coefficients of Zn in SM60, SM120 and SM180 treatments were reduced by 7.69%, 36.54% and 46.54%, respectively, among which, there was no significant difference between SM60 treatment and SM120 and SM180 treatment.

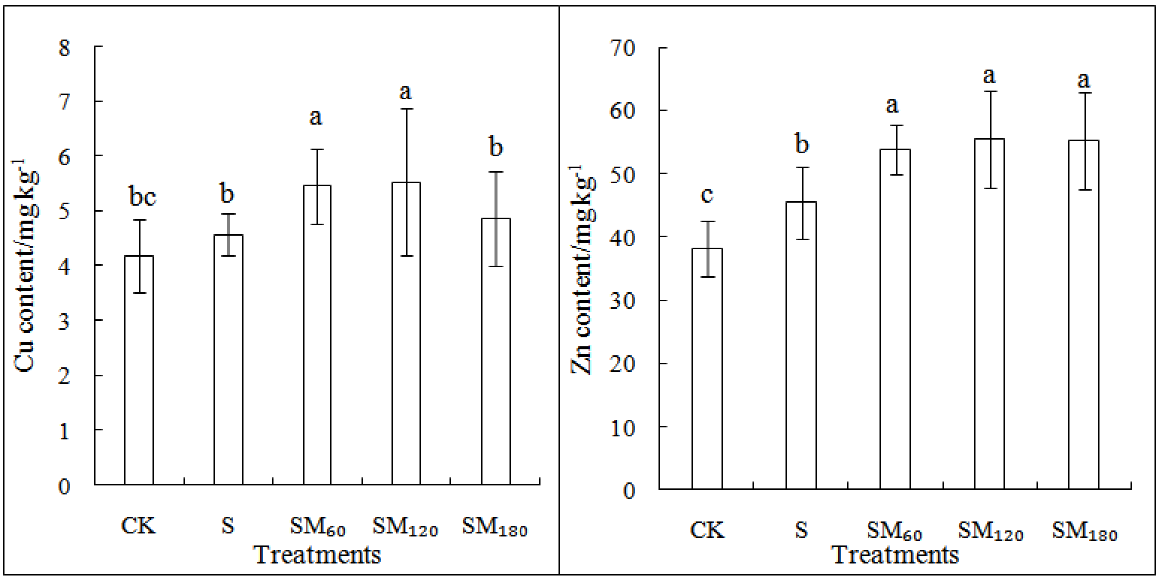

Figure 5. Effects of different amounts of chicken manure combined to municipal sludge on Cu and Zn contents of Pakchoi in calcareous cinnamon soil

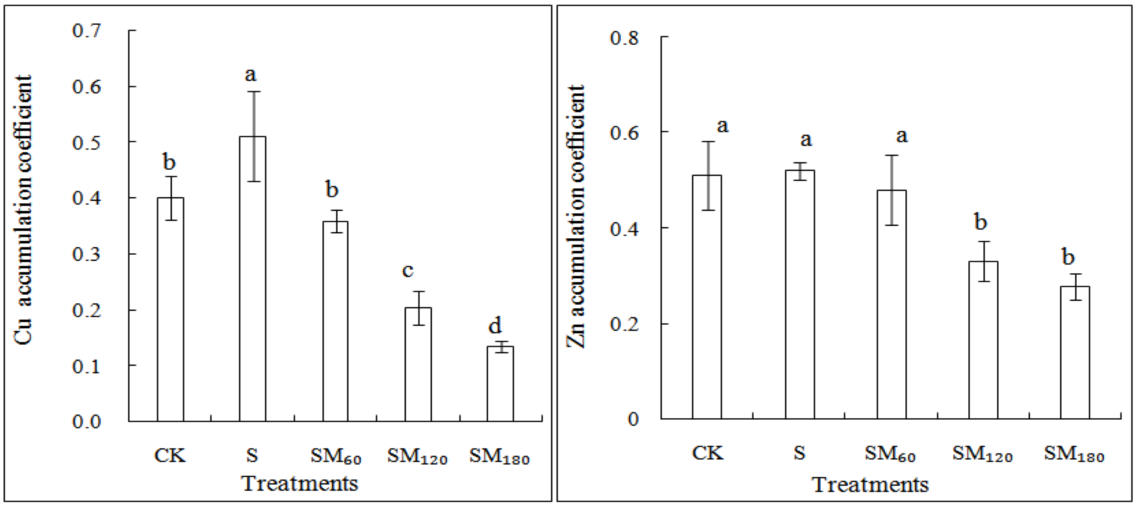

Figure 6. Effect of different amounts of chicken manure combined with municipal sludge on Cu and Zn accumulation coefficient of Chinese cabbage on latosol

3.6. Effects on Influence on Cu and Zn Enrichment Coefficient of Cabbage in Red Soil

The enrichment coefficients of Cu and Zn in the aboveground part of Chinese cabbage in red soil increased first and then decreased (Figure 7). Among them, the maximum enrichment coefficient of Cu in SM120 treatment is 0.20, the minimum value of CK treatment is 0.11, and the enrichment coefficient was less than 1. Compared with CK, Cu enrichment coefficient of S, SM60, SM120 and SM180 treatments increased by 27.27%, 63.64%, 81.82% and 27.27%, respectively. The difference of SM120 treatment was significant, while that of other treatments was not significant. S Compared to Cu enrichment coefficient of SM60, SM120 and SM180 treatments increased by 28.57% and 42.86%, respectively, while there was no change in SM180 treatment.

The enrichment coefficient of Zn reached the maximum value of 0.54 in S treatment and the minimum value of 0.31 in SM180 treatment. CK compared to enrichment coefficient of Zn in S treatment increased by 5.88%, while that in SM60, SM120 and SM180 treatments decreased by 5.88%, 21.57% and 39.22%, respectively. Among them, there were significant differences in SM120 and SM180 treatments, but no significant differences in other treatments. S Compared to enrichment coefficients of Zn in SM60, SM120 and SM180 treatments were reduced by 11.11%, 25.93% and 42.59%, respectively, with significant differences in all treatments except SM120.

3.7. Effects of Influence of Cu and Zn Enrichment Coefficient of Chinese Cabbage in Calcine Brown Soil

The enrichment coefficients of Cu and Zn in the aboveground part of Chinese cabbage in Calvary brown soil increased first and then decreased (Figure 8). The enrichment coefficients of Cu and Zn in the Calvary brown soil reached the maximum value of 0.22 in SM120 treatment and 0.55 in SM60 treatment, both of which were less than 1.

Compared with CK, Cu enrichment coefficients of S, SM60, SM120 and SM180 treatments increased by 36.36%, 81.82%, 100.00% and 72.73%, respectively, and the differences were significant in all treatments except S. Compared with S treatment, Cu enrichment coefficients of SM60, SM120 and SM180 treatments were significantly increased by 33.33%, 46.67% and 26.67%, respectively.

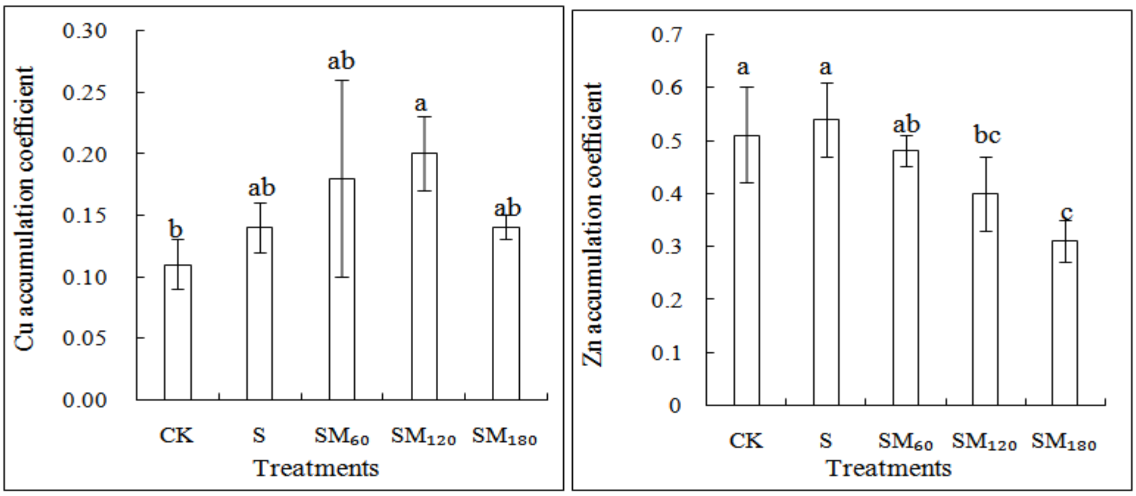

Figure 7. Effects of different amounts of chicken manure combined with municipal sludge on Cu and Zn accumulation coefficient of Chinese cabbage on red soil

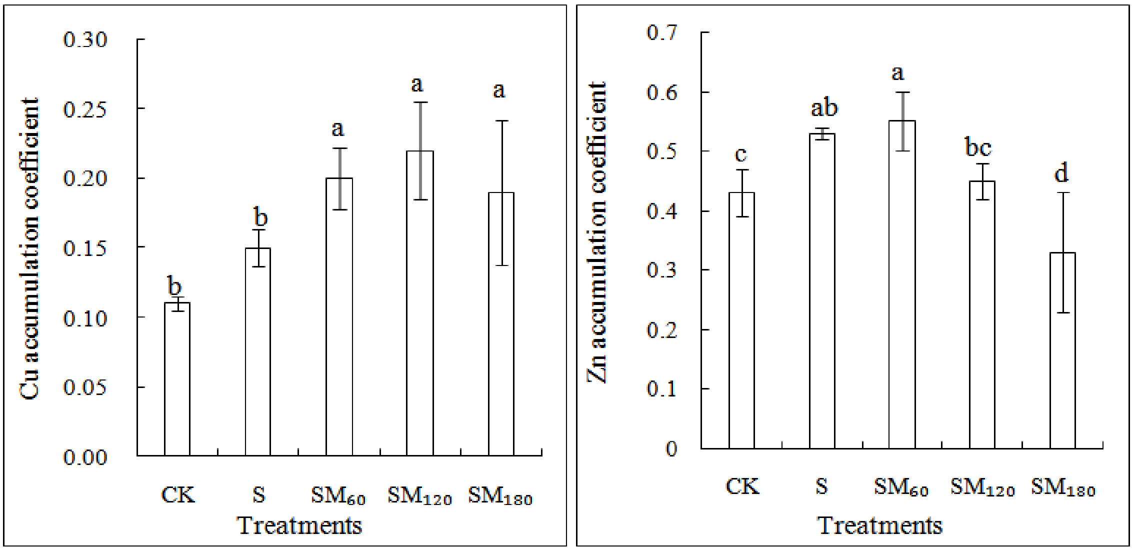

Figure 8. Effects of different amounts of chicken manure combined with municipal sludge on Cu and Zn accumulation coefficient of Chinese cabbage on calcareous cinnamon soil

The enrichment coefficient of Zn in the aboveground part of Chinese cabbage in calcareous brown soil reached the maximum value of 0.55 under SM60 treatment and the minimum value of 0.33 under SM180 treatment. Compared with CK, the enrichment coefficients of Zn in S, SM60 and SM120 treatments increased by 23.26%, 27.91% and 4.65%, respectively. Except for SM120 treatment, the other treatments had significant differences, and SM180 treatment significantly reduced by 23.26%. S Compared to enrichment coefficient of Zn increased by 3.77% in SM60 treatment, decreased by 15.09% and 37.74% in SM120 treatment and SM180 treatment, respectively. Except for SM180 treatment, the difference of other treatments was not significant.

3.8. Effects of Chicken Manure on Amino Acid Content and Amino Acid Balance in Chinese Cabbage Grains

The grain protein content, the content and proportion of amino acids that constitute protein are also important factors determining the nutritional quality of Chinese cabbage. We measured the content of 18 amino acids that constitute protein in Chinese cabbage grains (Table 4). The results showed that chicken manure had significant effects on the amino acids of grain protein. From the perspective of soil types, the content of most amino acids did not change with soil types, and a few amino acids were different with soil types, which may be related to the different supply capacity of soil chicken manure. Methionine, tryptophan and cysteine are essential amino acids, of which methionine and cysteine are sulfur amino acids. Aspartic acid, glutamic acid, glycine, threonine and phenylalanine increased first and then decreased with the amount of chicken manure. Histidine, tryptophan, leucine and lysine increased with the increase of chicken manure, and then slightly decreased, but did not reach a significant level. Serine, arginine, methionine, tyrosine, valine and cysteine continued to increase with the increase of chicken manure, while proline and isoleucine continued to decrease with the increase of chicken manure high quality dietary protein requires a proper balance between essential and non-essential amino acids. The amino acids that make up a protein should be mixed in the right proportions.

Table 4. Concentrations of 18 amino acids in Chinese cabbage grains under different chicken manure

Treatment	Asp	Glu	Ser	Gly	Arg	Ala	Pro	Met	His	Thr	Tyr	Val	Trp	Iso	Leu	Phe	Lys	Cys
Clay	5.10 ^{a}	16.20 ^{b}	3.60 ^{a}	2.93 ^{a}	4.30 ^{a}	3.40 ^{a}	11.4 ^{a}	3.00 ^{b}	2.40 ^{a}	2.90 ^{a}	2.90 ^{a}	3.30 ^{a}	1.20 ^{b}	2.80 ^{a}	9.90 ^{a}	3.20 ^{a}	2.20 ^{a}	3.70 ^{b}
Sandy	5.40 ^{a}	17.60 ^{a}	3.80 ^{a}	3. 10 ^{a}	4.60 ^{a}	3.60 ^{a}	12.1 ^{a}	3.20 ^{a}	2.50 ^{a}	3.10 ^{a}	3.00 ^{a}	3.50 ^{a}	1.30 ^{a}	2.90 ^{a}	10.50 ^{a}	3.40 ^{a}	2.40 ^{a}	4.0 ^{a}
CK	4.40 ^{b}	14.70 ^{c}	3.0 ^{c}	2.60 ^{c}	3.30 ^{d}	4.30 ^{a}	14.6 ^{a}	2.20 ^{c}	1.50 ^{c}	2.50 ^{b}	1.90 ^{d}	2.10 ^{c}	0.80 ^{c}	3.90 ^{a}	7.50 ^{b}	2.30 ^{d}	1.70 ^{c}	2.70 ^{e}
S	5.60 ^{a}	17.50 ^{ab}	3.10 ^{bc}	3.10 ^{abc}	4.0 ^{cd}	4.10 ^{a}	13.4 ^{a}	2.50 ^{c}	2.40 ^{b}	3.20 ^{a}	2.40 ^{c}	3.10 ^{b}	1.20 ^{b}	3.20 ^{b}	10.30 ^{a}	3.30 ^{bc}	2.10 ^{b}	3.50 ^{d}
SM_{60}	6.30 ^{a}	17.90 ^{ab}	3.8 0 ^{ab}	3.50 ^{a}	4.30 ^{bc}	3.50 ^{b}	11.6 ^{b}	3.20 ^{b}	3.00 ^{a}	3.50 ^{a}	2.90 ^{bc}	3.90 ^{a}	1.50 ^{a}	2.80 ^{bc}	11.40 ^{a}	4.0 ^{a}	2.60 ^{a}	3.90 ^{cb}
SM_{120}	5.70 ^{a}	18.10 ^{a}	3.90 ^{a}	3.20 ^{ab}	4.80 ^{ab}	3.30 ^{bc}	10.7 ^{b}	3.40 ^{b}	2.80 ^{ab}	3.40 ^{a}	3.30 ^{ab}	3.80 ^{a}	1.40 ^{a}	2.60 ^{c}	11.20 ^{a}	3.90 ^{ab}	2.60 ^{a}	4.20 ^{bc}
SM_{180}	4.70 ^{b}	16.90 ^{ab}	4.10 ^{a}	3.0 ^{abc}	5.0 ^{ab}	3.10 ^{bc}	10.0 ^{b}	3.60 ^{a}	2.60 ^{ab}	3.10 ^{a}	3.50 ^{a}	3.80 ^{a}	1.30 ^{ab}	2.50 ^{c}	10.60 ^{a}	3.30 ^{bc}	2.50 ^{a}	4.40 ^{ab}
ANVOA																		
Soil	ns	*	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	*	ns	ns	ns	ns	*
CM	**	**	**	*	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Soil × CM	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note: The different lowercase letters after the data indicate the significant differences (P < 0.05). ** indicate the significant at P < 0.01, * indicate the significant at *P* < 0.05, and ns indicate not significant (*P* > 0.05).

Table 5. Effects of chicken manure on the concentrations of different types of amino acids in Chinese cabbage grains

Treatment	Essential amino acid (mg g^{-1})	Non-essential amino acid (mg g^{-1})	Total amino acid (mg g^{-1})	Essential amino acid Total amino acid (%)
Clay	37.50 ^{b}	46.80 ^{b}	84.30 ^{b}	44.30 ^{a}
Sandy	39.80 ^{a}	50.20 ^{a}	90.00 ^{a}	44.10 ^{a}
CK	28.80 ^{c}	46.70 ^{bc}	75.50 ^{d}	38.10 ^{c}
S	37.00 ^{b}	50.70 ^{a}	87.70 ^{bc}	42.20 ^{b}
SM_{60}	42.60 ^{a}	50.80 ^{a}	93.40 ^{a}	45.60 ^{a}
SM_{120}	42.40 ^{a}	49.50 ^{b}	92.00 ^{b}	46.20 ^{a}
SM_{180}	41.00 ^{a}	46.90 ^{bc}	88.90 ^{c}	46.60 ^{a}
		ANVOA		
Soil	**	**	**	ns
CM	**	**	**	**
Soil × CM	ns	ns	ns	ns

Note: The different lowercase letters after the data indicate the significant differences ($P < 0.05$). ** indicate the significant at $P < 0.01$, and ns indicate not significant ($P > 0.05$).

Table 6. Concentrations of essential amino acid in protein of Chinese cabbage grains at different chicken manure

Treatment	His	Iso	Leu	Lys	Met + Cys	Phe + Tyr	Thr	Trp	Val
				Clay soil					
CK	18	51	85	20	50	56	27	10	25
S	23	44	103	20	58	62	31	11	34
SM_{60}	26	36	102	23	63	65	31	13	40
SM_{120}	26	35	107	26	72	76	34	13	43
SM_{180}	26	35	103	24	78	60	31	13	42
				Sandy soil					
CK	19	45	102	20	52	53	28	11	26
S	25	41	107	22	65	60	33	13	33
SM_{60}	29	37	109	25	68	65	34	14	39
SM_{120}	29	37	116	26	78	72	35	14	39
SM_{180}	28	36	116	28	88	73	33	15	41
RP	15	30	59	45	22	38	23	6	39

In order to explore the effect of chicken manure on the amino acid balance of Chinese cabbage grains, amino acids were divided into essential amino acids and non-essential amino acids for analysis (Table 5). The contents of essential amino acids, non-essential amino acids and total amino acids in aeolian sandy soil were higher than those in black soil, which may be due to the enrichment effect of Chinese cabbage grain yield in aeolian sandy soil. In terms of fertilizer application, essential amino acids continued to increase with the increase of chicken manure, non-essential amino acids increased first and then decreased with the increase of chicken manure, and total amino acids also increased first and then decreased with the increase of chicken manure, which was consistent with the change trend of protein. In particular, the essential amino acids under SM60 treatment reached the highest level 42.6 mg g^{-1}, which was significantly higher than that under CK treatment and SM60 treatment, but not significantly different from that under SM120 treatment, SM120 treatment, indicating that excessive chicken manure did not reduce the content of essential amino acids in Chinese cabbage. Similarly, non-essential amino acids.

It also reached the highest value in SM60 treatment, 50.8 mg g-1, which was not significantly different from that in S and SM120 treatment, but significantly higher than that in SM180 treatment (Table 6) indicating that the accumulation of non-essential amino acids was inhibited by chicken manure of more than 90kg ha^{-1}.

The proportion of essential amino acids in total amino acids can be used to measure the equilibrium state of grain amino acids. Chicken manure significantly increased the proportion of essential amino acids in total amino acids, promoted the balance of amino acids in grains, and improved the nutritional quality of Chinese cabbage. Specifically, the essential amino acids in the SM60 treatment accounted for 45.6% of the total amino acids, nearly half of the total amino acids, while the essential amino acids in the treatment without chicken manure treatment accounted for only 38.1% of the total amino acids, significantly lower than that in the SM60 treatment. In addition, the proportion of essential amino acids in total amino acids in SM60 treatment was not significantly different from that in SM120 treatment, SM180 treatment indicating that application of sulfur did not affect the proportion of essential amino acids in total amino acids and had no adverse effect on the amino acid balance in corn grains.

4. Discussion

4.1. Effects of Different Chicken Manure on the Growth of Chinese Cabbage

When urban sludge and different amounts of chicken manure were applied to potted cabbage in latosoil, red soil and calcareous brown soil, the plant height, root length and aboveground/underground dry weight of cabbage in the three soils increased first and then decreased with the increase of chicken manure application rate. These results indicated that the promoting effects of municipal sludge and chicken manure on cabbage yield did not increase with the increase of chicken manure application. Urban sludge and chicken manure contain nutrient elements and organic matter needed by plants, so they can promote the growth and development of crops, but the increase of nutrient elements will affect the uptake of Chinese cabbage root [27] showed that the yield of green vegetables increased significantly with the increase of sludge compost application rate [28]. The sludge after acid treatment had a significant promoting effect on the growth of spinach. The yield of rape could be significantly increased when the application rate of sludge was 5-8 g kg^{-1}. Pot experiments that sludge compound fertilizer could significantly improve vegetable yield [29]. The mixed application of domestic sludge and phosphate fertilizer could significantly improve the yield of cabbage. The single application of domestic sludge and the mixed application of domestic sludge and urea could increase the yield of celery (*Apium graveolens*) by about 15% [30]. The addition of 10%, 20% chicken manure was most conducive to the growth of tomato that chicken manure could significantly increase plant height, root length and biomass of amaranth [32]. But although city sludge contains a lot of crops needed nutrient elements and organic matter, at the same time and is harmful to crops of heavy metals in sewage sludge, pathogens, such as organic pollutants, would have a negative effect on crop growth and yield, and livestock manure contains antioxidants, mould inhibitors and antibiotics such as organic material, the improper use of microbial toxic, Adverse effects on soil, thereby inhibiting crop growth [33]. When the application rate of sludge was more than 10%, the germination rate of green vegetables decreased to different degrees. When the application rate of sludge compost was more than 20%, the biomass of green vegetables showed a downward trend [34]. The dry weight of root, stem, leaf and fruit of green pepper increased first and then decreased with the increase of sludge compost application rate [35]. The yield of okra increased first and then decreased with the increase of organic fertilizer application [36]. With the increase of zinc concentration, plant height, fresh weight and root length of cabbage under chicken manure treatment showed a decreasing trend. High sludge application rate and heavy metal content can inhibit or even poison plant growth [37]. In conclusion, when urban sludge and different amounts of chicken manure are applied to potted cabbage, the more the amount of chicken manure is applied, the more beneficial it is to the growth of cabbage. On the contrary, the harmful substances in urban sludge and chicken manure will inhibit the growth of cabbage. In addition, the application amount of chicken manure corresponding to the maximum biomass of Chinese cabbage was different in different acid-alkaline soils. Latosoil and calcareous brown soil were more sensitive to the application amount of chicken manure than red soil. In agricultural activities, we should pay close attention to the fertilization situation of different types of soil. At the same time, attention should be paid to the application amount of the two in the process of agricultural fertilization to avoid harmful substances on crops and the environment.

4.2. Effects of Different Cu and Zn Contents in Edible Parts of Chinese Cabbage

Urban sludge with different amount of chicken manure applied into laterite, red soil and calcareous soil in brown Chinese cabbage plants, with the increase of applying manure, content, three kinds of pakchoi edible parts of Cu in the soil, zinc content change trend, embodied in chicken manure significantly reduces the Cu content on the yield of pakchoi edible part, significantly increased the content of zinc; In addition, the Cu content in the edible part of Chinese cabbage on red soil was increased, among which the SM60 and SM120 treatments increased significantly, while the SM180 treatments increased not significantly, while the Zn content was decreased or increased, but the changes were not significant. The SM60 and SM180 treatments decreased, while the SM120 treatment increased. In general, the influence of the Cu content in the edible part of Chinese cabbage was greater than the Zn content. The contents of Cu and Zn in edible parts of Chinese cabbage in clime brown soil were also increased. Except for SM60 and SM120, the contents of Zn in other treatments were increased significantly, and the increasing amount of Cu and Zn was increasing continuously, in which the increasing amount of Cu was greater than that of Zn. The contents of Cu and Zn in crops increased or decreased when chicken manure was added to the soil [38]. Application of chicken manure could significantly increase the content and absorption of Cu and Zn in Tongcai. The application of chicken manure organic fertilizer in the damp soil could increase the Cu and Zn contents of amaranth plants [39]. Manure organic fertilizer could increase the contents of Cu, Zn, Cd and Pb in edible parts of crops. Adding chicken manure could reduce the contents of Cd, Cu, Pb and Zn in plants [40]. The application of chicken manure biochar into soil could reduce the absorption of Cu and Zn by plants [41]. Chicken manure could significantly reduce the contents of Cd and Pb in the aboveground parts of Chinese cabbage on potted plants [42]. The combination of chicken manure and phosphate powder into the soil, and the contents of Pb and Cd in the cabbage shoot were significantly reduced. The main reason is the result of soil organic matter and pH value. The application of urban sludge and chicken manure to the three kinds of soils increased the pH value of latosoil and red soil, and decreased the pH value of calcareous brown soil, but the average pH value of each treatment of latosoil was less than 7, the pH value of red soil SM60, SM120 and SM180 treatments was more than 7, and the pH value of each treatment of calcareous brown soil was more than 7.5. In alkaline soil, the soluble organic matter will stop the heavy metals to the difficult soluble carbonate form or hydroxyl form transformation, among them, the biggest impact is Cu, it makes easy to soluble Cu in the soil solution concentration increased, activity, make its migration ability increases, the biological effectiveness also will increase [43]. Therefore, the Cu content of cabbage in edible part of latosol decreased, while the Cu content of cabbage in red soil and calcareous brown soil increased. The influence of Cu content on edible parts of cabbage in three soils was greater than that of Zn.

4.3. Effects of Different Cu and Zn Enrichment in Edible Parts of Chinese Cabbage

The Cu enrichment coefficient of edible part of cabbage in latosoil decreased significantly, but increased significantly in callime brown soil, and increased significantly in SM60 treatment, but not in SM120 and SM180 treatment. The enrichment coefficient of Zn in latosol and red soil decreased significantly. In calcareous brown soil, Zn enrichment coefficient decreased in SM60 and SM120 treatments, while increased in SM180 treatments, but there was no significant difference among all treatments [44]. The Cu enrichment coefficient increased by 36.4% and the Zn enrichment coefficient decreased by 147.4% after the application of chicken manure in alkaline soil. Other studies have shown [45]. The soil is alkaline and the content of organic matter is high, the Cu in the soil basically exists in the soluble form, which increases the bioavailability of Cu and the absorption of Cu by cabbage, and the enrichment coefficient increases accordingly. Because urban sludge and chicken manure contain a large amount of organic matter [46]. Organic matter reduces the bioavailability of Zn through physical adsorption and chemical complexation (chelation) [47]. At the same time, the content of organic matter in urban sludge and chicken manure is high, and organic matter has a larger specific surface area and more functional groups, which has a stronger adsorption capacity for Zn ions than other cations. In addition, humic acid in organic matter is easy to form complex (chelate) complexes with Zn in soil, thus reducing the absorption of Zn by plants.

5. Conclusion

This study concludes that the response of Chinese Cabbage (*Brassica Campestris ssp. Chinesis*) The best enrichment degree of heavy metals Cu and Zn in Chinese cabbage directly affects the quality of Chinese cabbage and human health. The Cu and Zn contents in the edible part of cabbage in latosol could be significantly increased by the single application of municipal sludge. Compared with the single application of municipal sludge, the synergistic application of municipal sludge and different amounts of chicken manure to latosol could significantly reduce the content of Cu in cabbage. The decrease of Cu in cabbage increased continuously. The enrichment coefficients of Cu and Zn increased with the single application of municipal sludge in red soil, with significant difference in the former and insignificant difference in the latter. And for Zn enriched. Specifically, the contents of glutamic acid, methionine, tryptophan and cysteine in Aeolian sandy soil were significantly higher than those in black soil, but there was no significant difference in the contents of other amino acids. Among the four amino acids, glutamate was the most abundant non-essential amino acid in protein content has an important influence on protein content. The essential amino acids in the SM60 treatment accounted for 45.6% of the total amino acids, nearly half of the total amino acids, while the essential amino acids in the treatment without chicken manure treatment accounted for only 38.1% of the total amino acids. However, the characteristics of urban sludge and chicken manure, the absorption and enrichment mechanism of Cu and Zn by the edible part of Chinese cabbage needs to be further studied in the future.

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