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Energy Sorghum Removal of Soil Cadmium in Chinese Subtropical Farmland: Effects of Variety and Cropping System

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Abstract: Planting energy sorghum to remove soil cadmium (Cd) has been selected as an effective phytoremediation method in subtropical farmland in China in recent years. Nevertheless, the effects of energy sorghum species and cropping systems on Cd removal by energy sorghum are still not fully understood. In the present work, biomass sorghum (BS) and sweet sorghum (SS) were planted for screening varieties and comparing the applicability of cropping systems to remove Cd from contaminated soils through batch field experiments. The results indicated that BS had a higher plant height (4.70–75.63%), lower water content in the shoot (4.78–13.49%), greater dry biomass yield (13.21–125.16%), and stronger Cd removal (average 45.71%) compared with SS. Significant differences ($p < 0.05$) were observed in the agronomic traits and Cd accumulation of energy sorghums with genetic regulation of varieties. Pearson correlation coefficients analysis and the structural equation model (SEM) showed that plant height was the crucial agronomic parameter affecting the dry biomass yield, and Cd concentration in the stem was the key factor for evaluating the Cd extraction ability of energy sorghums, which indirectly determined the removal of Cd by energy sorghum together. Furthermore, the regeneration cropping system was the most suitable because of the adaptation to climatic conditions of energy sorghums in subtropical regions of China, and its Cd removal efficiency increased by more than 49% compared with double cropping and single cropping systems, respectively. Our study provides valuable information for the phytoremediation of Cd-contaminated soil in Chinese subtropical farmland.

Keywords: energy sorghum; phytoremediation; Cd; regeneration cropping system

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1. Introduction

Heavy metal pollution of soil in farmlands has become a global environmental problem that hinders agricultural production and food safety [1,2]. In China, approximately 9.179 × 106 ha of Cd-contaminated field land present a risk to human health via the food chain [3]; it is urgently needed to minimize the Cd accumulation of soil in farmlands [4]. Phytoremediation is a promising cost-effective technology because of its low cost, ease of operation, and environmental friendliness [5,6], and planting with high biomass and improved phytoextraction potential for Cd removal has been widely accepted [7,8]. Energy sorghum, a C_4 crop with high biomass yield and heavy metal tolerance that can transfer heavy metals from the food chain to the energy chain [2,9], has been regarded as a promising crop for the phytoremediation of Cd-contaminated soils [8]. Moreover, due to the coupling of the phytoremediation of Cd-contaminated soils with bioenergy production,

energy sorghum presented an attractive option [10], which created excellent ecological values and economic benefits. Hence, further studies are needed into the phytoremediation of Cd contamination in soils by energy sorghum.

Energy sorghum varieties, the core of phytoremediation to remove Cd from contaminated soils [11,12], due to the significant differences in agronomic traits and Cd accumulation among different energy sorghums induced by genetic control of varieties [9], greatly determine the capacity of sorghum to remove Cd. Weitao Jia [13] investigated ninety-six sorghum genotypes to compare their potential for remediation of Cd contamination and classified them into five grades on the basis of plant growth parameters. Additionally, Zhiquan Liu [12] showed that various sorghum genotypes can be used for phytoremediation, safe production of food, feed, and bio-energy because of the vast difference in Cd accumulation and distribution in 126 sorghum field experiments. These previous studies highlighted the importance of the sorghum variety for Cd removal. Biomass sorghum (BS) and sweet sorghum (SS) are two main types of energy sorghums, which have been widely studied for the phytoremediation of Cd contamination in soils [2,9,14]. BS is characterized by long vegetative growth duration and large biomass accumulation [15,16] and possibly exhibits a greater potential to remove Cd contamination in soils than sweet sorghum (SS). However, what were the key factors controlled by varieties of those energy sorghums to predict the capacity of sorghums to remove soil Cd. Weitao Jia [13] considered root biomass as a biomarker to evaluate the sorghum's ability to extract Cd in a 2-week hydroponic system experiment. Our previous study [11] demonstrated that plant height (PH) was an important factor for screening potential energy sorghum based on the significant correlation among PH, dry matter weight, and Cd concentration in the shoot. The conflicting results may be attributed to the different conditions of the test and require further verification in field experiments from an application perspective [12,17]. As a result, it is necessary to conduct field tests for screening more valuable energy sorghum varieties and further analysis of key factors to predict the capacity of Cd removal from contaminated soils.

The cultivation of energy sorghum, a warm and drought-tolerant crop, is influenced by variations in temperature and precipitation [18,19]. Constrained by the long duration of rainfall and low temperature in spring in large parts of the subtropical region of China [20], the double cropping system for energy sorghum may be difficult to implement. However, based on previous research findings, sorghum has been shown to achieve higher agricultural productivity and economic benefits when rotated with other crops [21] or planted using a regeneration cropping system [22]. For instance, Sadafzadeh Elnaz [23] showed that intercropping sorghum with soybean enhances both the quantity and quality of forage obtained, surpassing those achieved in sorghum monoculture. The study conducted by Syuryawati [24] found that the regeneration season of sorghum resulted in a yield increase equivalent to the main crop while requiring less than 50% of the planting cost, thereby significantly enhancing economic benefits. Additionally, an increase in biomass yield indicates the potential for greater removal of Cd. Therefore, it is crucial to establish an appropriate cropping system for energy sorghum that is well-suited to the specific climatic conditions of its cultivation area in order to achieve optimal growth, maximize economic benefits, and enhance efficiency in removing Cd from contaminated soils.

Herein, we conducted a batch of field experiments on removing Cd contamination by energy sorghums in farmlands. The aims of this study were to: (1) compare the difference in Cd removal efficiency between BS and SS and analyze the key factor to predict energy sorghum's capacity for Cd removal; and (2) explore the most suitable cropping system for energy sorghums to remove soil Cd in Chinese subtropical farmland. To achieve these objectives, we conducted an analysis of agrological parameters and the capacity of different parts of mature sorghum to accumulate Cd. Furthermore, correlation analysis and structural equation modeling were employed to investigate the key factors and pathways influencing Cd accumulation. Our results are expected to provide novel insights into the variety selection and cropping systems of energy sorghum to remove soil Cd in practice.

2. Materials and Methods

2.1. Sites Description and Energy Sorghums

The screening experiments were conducted in the field at Beishan town, Changsha County, Hunan Province (28°22′ N, 112°58′ E), China. The ratoon sorghum cultivation experiment was performed in the Changqing demonstration zone, Liling City, Hunan Province (27°41′ N, 113°32′ E), China. Both sites have sandy loam primarily developed from granite and plate shale, respectively, and are classified as Anthrosol (USDA Taxonomy). Two sites have a subtropical monsoon climate, with an average annual temperature of 17–18 °C and an average annual precipitation of 1200–1600 mm. Energy sorghum seeds (including BS and SS) were obtained from Hunan Longping High-Tech Arable Land Remediation Technology Co., Ltd., Changsha, China, which introduced hybrid sorghum varieties from NexSteppe Co., Ltd., New Castle, DE, USA.

2.2. Screening Experiments

51 sorghum masteries were used to screen for Cd phytoremediation (the codes for the different varieties are provided in Table S2), including 29 biomass sorghums (BSs) and 22 sweet sorghums (SSs). The screening experiments were conducted from 2016 to 2017 in Beishan town, Changsha County, China. After plowing and leveling, the drained experimental plot was conducted uniformly at 2.8 × 10 m in area for the cultivation of 51 sorghum masteries with a completely randomized block design. Four rows of sorghum were planted in each plot, at a distance of 0.7 m. In all experiments, double seed was sown by hand, and the planting density was 105,000 plants ha^{-1}. The seedling number was controlled at the seedling stage of 3–5 leaves. The growth period of these energy sorghums ranged from 110 to 140 days, and the field management of the whole growth period followed local agricultural practices. Each treatment was independently replicated three times.

After preliminary screening, the Cd removal, lodging resistance, and commercialization of different sorghums were compared, and four BSs (N5D61, N52K2562, N52K1009, and N51M4264) and four SSs (N32F2026, N31K2168, N31H2358, and N31G2174) were selected to further analyze the agronomic parameters and Cd absorption and distribution of sorghums in field experiments.

2.3. Ratoon Sorghum Cultivation

In order to improve the annual removal efficiency of Cd pollution in soil by energy sorghums, the ratoon sorghum cultivation experiment was conducted in Changqing demonstration zone, Liling City, Hunan Province, with two BSs (N5D61, N51M4264) and two SSs (N31K2168, N31G2174), from April to November 2017. 12 plots with an area of 31.5 m^2 (3.5 m × 9 m) were completely random designs for four energy sorghums. Each treatment was independently replicated three times. The regeneration cropping system was performed by cutting the stems of the main plants, leaving about 5–10 cm above the soil surface. The soil was then sufficiently watered to moisten the soil until new shoots appeared, and 50% of the dose of the main crop fertilizer was given after a month (70 kg ha^{-1} N, 60 kg ha^{-1} P_2O_5, 70 kg ha^{-1} K_2O).

2.4. Sample Collection and Analysis

Sample collection and biomass yield determination of sorghums in all experiments were conducted at the mature stage, which typically occurred between 100 and 130 days after sowing. All sorghum varieties of 2.8 square meters (2 rows × 2 m) in each plot were collected for biomass yield determination, and 3 sorghum plants were randomly selected to record fresh matter weight (FW), dry matter weight (DW), water content in the shoot (WC), plant height (PH), stem pitch number (SPN), and stem diameter (SD). In addition, 3 sorghum plants were randomly collected from each plot and divided into grain, leaf, stem, and root, and each part was weighed before and after oven drying at 65 °C for 72 h to estimate FW and DW, then pulverized for the determination of Cd.

Topsoil (0–20 cm) samples were collected before energy sorghum planting. Each soil sample was composited from more than 5 soil cores following an 'S'-shaped sampling. The sampled soils were air-dried and sieved through 1.0 and 0.149 mm meshes.

Total Cd in sorghums and soil samples was determined after the digestion with mixed acids of HNO_3-H_2O_2-$HClO_4$ (8:1:1) and aqua regia-$HClO_4$ (5:1), respectively, in an open system. Extractable Cd in the soil was determined by the DTPA (0.005 mol L^{-1} diethylenetriamine pentaacetic acid-0.10 mol L^{-1} triethanolamine-0.01 mol L^{-1} $CaCl_2$) extraction method with a soil-solution ratio of 1:2.5 (*w*/*v*) [25]. The concentrations of Cd in the digestion and extraction solutions were analyzed by inductively coupled plasma-optical emission spectrometry (ICP-OES720; Varian, Palo Alto, CA, USA). Soil pH was measured using a pH meter (Metro-pH 220, Mettler-Toledo Instruments Ltd., Shanghai, China) with a soil-water ratio of 1: 2.5 (*w*/*v*). The soil organic matter (SOM), cation exchange capacity (CEC), total nitrogen content, and available N were determined by K_2CrO_4-H_2SO_4 digestion, NH_4OAc extraction, the Kjeldahl method, and the alkaline diffusion method, respectively [26].

The bioaccumulation factor (BCF) (Equation (1) was calculated to evaluate the ability of the sorghum to accumulate Cd from the soil. The translocation factor (TF) (Equation (2) was calculated to determine the efficiency of sorghum in translocating Cd from below-ground tissues to above-ground tissues [8].

$$BCF = C/C_{soil} \quad (1)$$

$$TF = C/C_{root} \quad (2)$$

Equation (1): C_{soil}, Cd concentration of soil; C, including Cd concentration in root, stem, leaf, and grain; Equation (2): C_{root}, Cd concentration in root; C, including Cd concentration in stem, leaf, and grain.

2.5. Statistical Analysis

Data were calculated using Excel 2019 (Microsoft Corporation, Redmond, WA, USA) and shown as mean ± standard error. A one-way ANOVA followed by Duncan's multiple range test ($p < 0.05$) was performed to examine the data differences. Pearson correlation analysis was used to determine the relationships among the tested indices. All statistical analyses were performed using SPSS (SPSS-AMOS for the structural equation model) software (version 25.0, IBM SPSS Inc., Armonk, NY, USA). All diagrams were made by ORIGIN 2021.

3. Results

3.1. Basic Properties of the Soils

The two soils used in this study were all contaminated with Cd due to long-term sewage irrigation in the past decades (Table 1). The total Cd concentrations in the two soils were 1.22 mg kg^{-1} and 039 mg kg^{-1}, respectively, which clearly exceeded the limit set by the National Soil Environmental Quality Standard of China [27]. The available Cd concentrations in the two soils were 0.60 mg kg^{-1} and 0.11 mg kg^{-1}, presenting high Cd availability in the soil.

Table 1. The basic properties of the soil in two experimental fields.

County	pH	SOM (g kg^{-1})	CEC $cmol^+$ kg^{-1}	Total Cd (mg kg^{-1})	Available Cd (mg kg^{-1})	Total N (g kg^{-1})	Available N (mg kg^{-1})
Changsha	5.6	18.72	6.37	1.22	0.60	2.12	178
Liling	6.5	36.4	15.9	0.39	0.11	1.88	162

Note: SOM, soil organic matter. CEC, cation exchange capacity. Total Cd, Total amount of cadmium in soil. Available Cd, DTPA extractable Cd in soil. Total N, total nitrogen content in soil. Available N, soil alkali-hydrolyzable nitrogen.

Soil pH levels were 5.6 and 6.5 for the two soils, while the cation exchange capacities (CEC) were 6.37 $cmol^+$ kg^{-1} and 15.90 $cmol^+$ kg^{-1}, respectively. The total nitrogen (N) concentrations in the two soils were 2.12 g kg^{-1} and 1.88 g kg^{-1}, while available nitrogen concentrations were 178 mg kg^{-1} and 162 mg kg^{-1}. The organic matter (OM) contents in the two soils were 18.72 g kg^{-1} and 36.4 g kg^{-1}, respectively.

3.2. Variation in Soil Cd Removal by Energy Sorghum Species

Biomass yield and Cd accumulation in shoots directly determine the ability of energy sorghum to remove Cd. The variations of DW, Cd concentration in shoots, and removal of Cd by sorghum were detected at the mature stage by frequency distribution and presented a skewed normal distribution according to the skewness and kurtosis values (Figure 1 and Table S1). The DW of BSs was 12.43 × 10^3–32.67 × 10^3 kg ha^{-1} with an average of 21.14 × 10^3 kg ha^{-1} (Figure 1a), which was significantly higher than that of SSs ($p < 0.05$). Moreover, the larger maximum, minimum, quarter, half, and three-quarters of DW and the smaller coefficient of variation value (Table S1) also indicated that BS was superior to SS in DW. Indeed, the average Cd concentration in BSs shoot was 1.86 mg kg^{-1}, which was slightly lower than that in the SS (2.43 mg kg^{-1}, Figure 1b). However, a 48.63% increase in DW (Figure 1a) resulted in an 18.98% higher average Cd removal in BS than in SS ($p < 0.05$, Figure 1c).

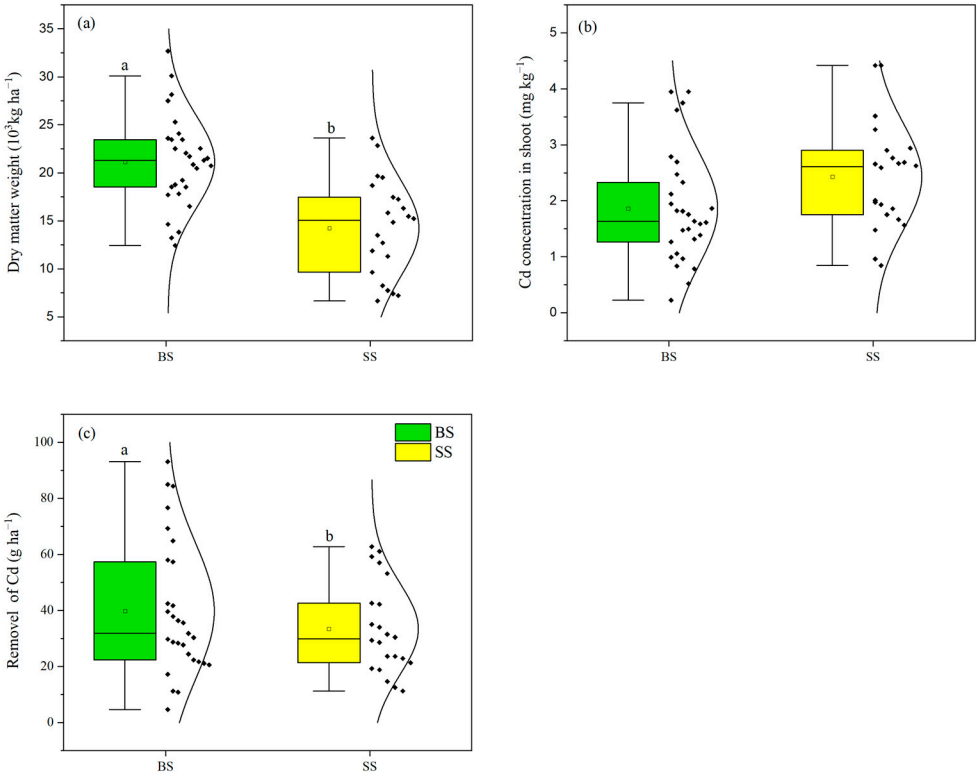


Figure 1. Dry matter weight (DW, (**a**)), Cd concentration (**b**) and removal of Cd (**c**) in shoot of biomass sorghum (BS, n = 29) and sweet sorghum (SS, n = 22). Different letters indicate significant differences between BS and SS, paired sample *t*-test, $p < 0.05$.

3.3. Agronomic Parameters Traits of Energy Sorghums

BSs were superior to SSs in PH and SPN, but there was no difference in SD. The PH of BS was all above 5.1 m, except for N5D61, which was 4.1 m high. On the contrary, only N31K2168 had a PH of 4.0 m, and the other SS were all lower to 3.5 m (Figure 2d). Therefore, it is obvious that BS was higher than SS. Similar results were observed for the SPN (Figure 2e). The SPN of N5D61 and N31K2168 were 21 pcs, and the SPN of other BS (>21 pcs) was greater than that of SS (<16 pcs). The PH of sorghums was positively

correlated with the SPN ($p < 0.01$, R = 0.98, Figure 3). Nevertheless, no difference was found in SD among all sorghums, which ranged from 20.00 to 23.50 mm in SD (Figure 2f).

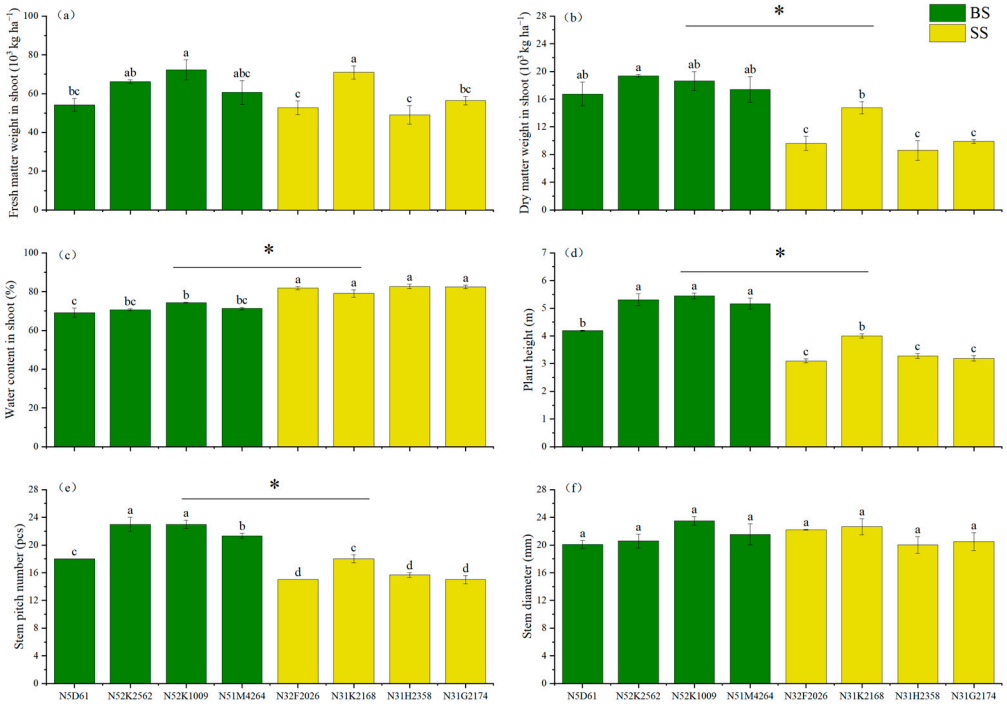


Figure 2. Fresh matter weight (FW, (**a**)), dry matter weight (DW, (**b**)), water content (WC, (**c**)), plant height (PH, (**d**)), stem pitch number (SPN, (**e**)), stem diameter (SD, (**f**)) of sorghum shoots. Different letters indicate significant differences between each variety and "*" indicate significant differences between BS and SS, LSD test, $p < 0.05$ (n = 24).

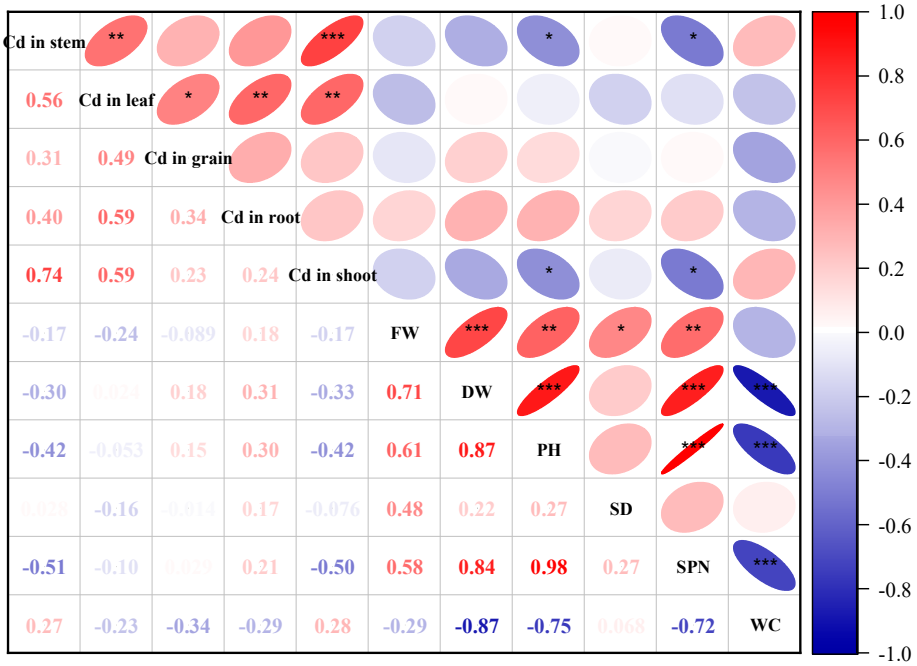


Figure 3. Pearson correlation coefficients in different agronomic parameters and Cd accumulation of sorghums. The degree of red represents the positive correlation, and the degree of blue represents the negative correlation. Significance levels are indicated: "*", $p < 0.05$, "**", $p < 0.01$, "***", $p < 0.001$.

Although the FW of BS was similar to that of SS, the DW of BS was significantly higher than that of SS because of the smaller WC. The FW of BS and SS were 49.29 × 10^3–82.50 × 10^3 kg ha^{-1} and 42.50 × 10^3–75.71 × 10^3 kg ha^{-1}, respectively (Figure 2a), and there was no significant difference between them ($p > 0.05$). However, the WC in shoot of BS was 69.0–74.0%, which was significantly lower than that of SS (79.0–83.0%, $p < 0.05$, Figure 2c). Consequently, BS had a larger DW (16.73–19.38 kg ha^{-1}, $p < 0.05$) than SS (8.61–14.78 kg ha^{-1}, Figure 2b).

Pearson correlation coefficients analysis (Figure 3) showed that the DW of sorghums was positively correlated with PH ($p < 0.01$, R = 0.87), SPN ($p < 0.01$, R = 0.84), and FW ($p < 0.01$, R = 0.71), but negatively correlated with WC ($p < 0.01$, R = 0.87). The structural equation model (SEM) was used to assess the direct and indirect effects of the explanatory variables on the DW of sorghums (Figure 4a). The SEM revealed that different sorghum varieties contributed to DW through two indirect pathways without significant direct effects.

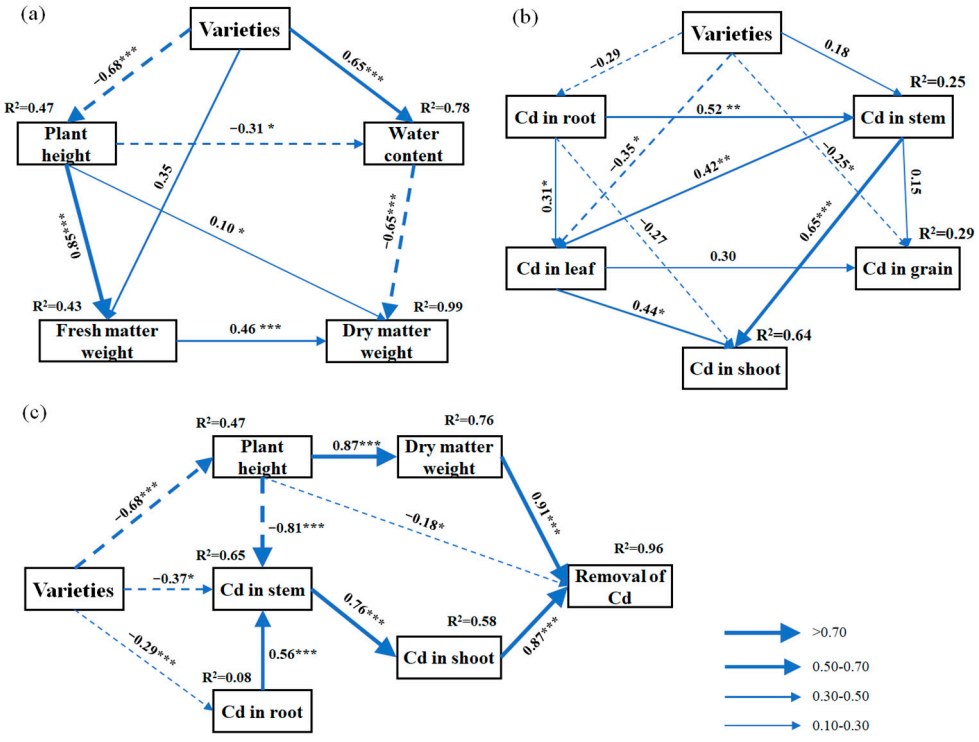


Figure 4. Structural equation models (SEM) representing hypothesized causal relationships among the varieties, including plant height, water content, fresh matter weight, dry matter weight (**a**), Cd in shoot and root, stem, leaf, and grain (**b**), and removal of Cd (**c**). The final SEM adequately fitted the data: (**a**), $\chi^2 = 0.365$, DF = 1, $p = 0.546$, CFI = 1.000, and RMSEA < 0.001; (**b**), $\chi^2 = 0.228$, DF = 2, $p = 0.892$, CFI = 1.000, and RMSEA < 0.001. (**c**), $\chi^2 = 12.300$, DF = 11, $p = 0.342$, CFI = 0.992, and RMSEA = 0.072. Arrows depict casual relationships: continuous and dashed arrows indicated positive and negative relationships, respectively. Significance levels are indicated: "*", $p < 0.05$, "**", $p < 0.01$, "***", $p < 0.001$.

3.4. The Cd Accumulation and Distribution in Energy Sorghums

The Cd concentrations in the root, stem, leaf, and grain of 8 sorghum varieties were 5.11–10.85, 2.48–6.32, 0.40–1.70, and 0.03–0.11 mg kg^{-1}, respectively (Figure 5). Despite the significant differences in Cd accumulation among sorghum cultivars, there was no difference in the distribution of sorghum organs, which were ranked as root, stem, leaf, and grain. Compared with the root and stem with higher Cd accumulation capacity (4.45–8.84 and 1.90–5.37), the grain showed low bioaccumulation because the BCF only ranged from 0.02–0.08 (Figure 6a). All grains of sorghums had Cd concentrations that were below the

maximum levels of contaminants in feeds (1 mg kg^{-1}), and only N5D61 grain exceeded the limit of the food safety standard (0.1 mg kg^{-1}) due to the restricted transport of Cd from other organs of sorghums to grain (Figure 6b). In addition, the results of the normalized distribution (Figure S1b) demonstrated that the stem contribution, which was greater than 90%, far outweighed that of the leaf (less than 10%) and grain (less than 0.5%).

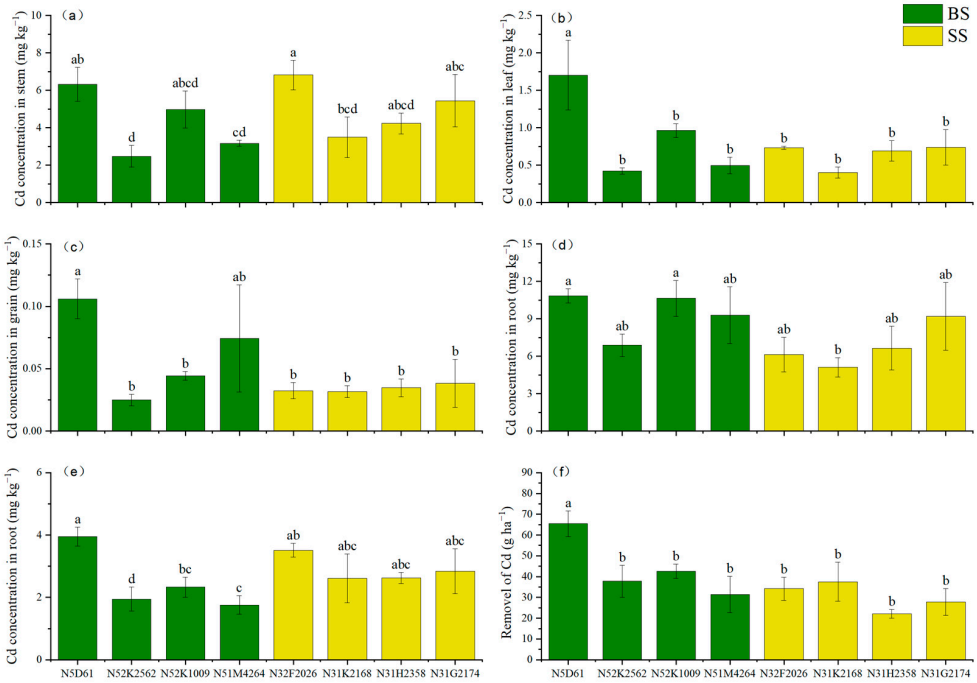


Figure 5. Cd concentration in stem (**a**), leaf (**b**), grain (**c**), root (**d**), shoot (**e**) and Cd removal in the shoot of sorghums (**f**). Different letters indicate significant differences between each variety, LSD test, $p < 0.05$ (n = 24).

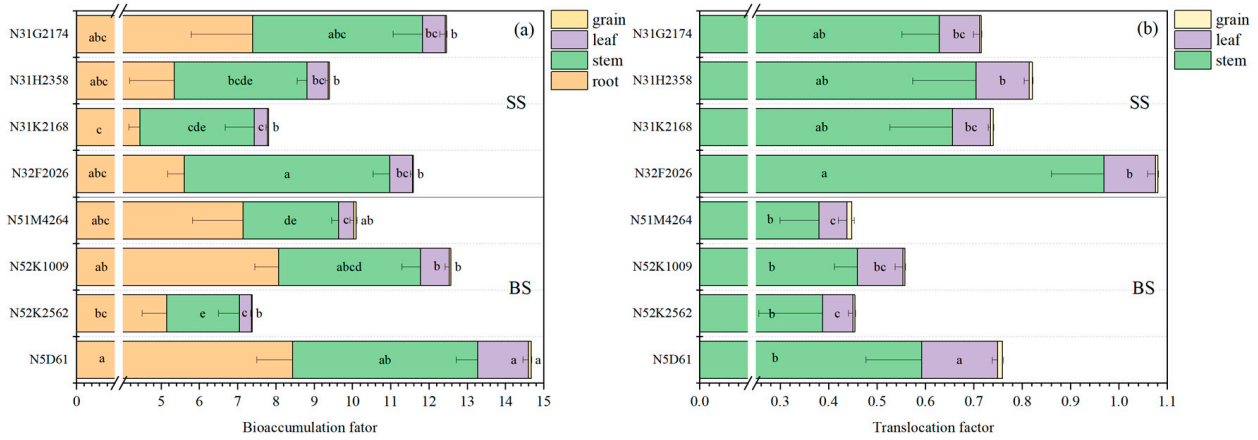


Figure 6. Bioaccumulation factor (BCF, (**a**)) and translocation factor (TF, (**b**)) of sorghums. Different letters indicate significant differences between each variety, LSD test, $p < 0.05$ (n = 24).

There were significant differences in Cd uptake and transport among 8 sorghums ($p < 0.05$), and N5D61 (BS) and N32F2026 (SS) were two representative sorghums with strong Cd accumulation. N5D61 not only had higher Cd concentrations in the root, stem, leaf, and grain than the other sorghums (Figure 5), but also ranked the highest in DW (Figure 2b); consequently, the removal of Cd by N5D61 (65.43 kg ha^{-1}) was significantly greater than that of the other sorghums ($p < 0.05$, Figure 5f). The weaker DW significantly caused a 47.78% difference in Cd removal compared with N5D61, despite the comparable Cd accumulation exhibited by N32F2026 (Figure 5). According to the findings of the BCF

and TF analysis (Figure 6), N32F2026 had a TF value of Cd from root to stem of 0.97, which was significantly higher than that of the other sorghums. The BCF of root, stem, and leaf in N5D61 were 8.44, 4.84, and 1.32, respectively, all ranking first among 8 sorghums. Thus, Cd accumulation in shoots was higher than that in other sorghums.

Pearson correlation coefficients analysis (Figure 3) showed that the Cd concentration in the shoot was positively correlated with that in the stem ($p < 0.001$, R = 0.74) and leaf ($p < 0.01$, R = 0.59), and there was no correlation with that in the grain ($p > 0.05$). Moreover, SEM further clarified the major pathways by which varieties of energy sorghums affect Cd accumulation in shoots with an adequate fit. Sorghum Cd was absorbed by the root from the soil, transported to the stem and leaf, and then entered the grain. Although the Cd in the roots and grain has no direct effect on the Cd in the shoot, the Cd in the root will indirectly promote the Cd accumulation in the shoot through Cd transportation to the stem and leaf. Obviously, the Cd in the stem and leaf were the two factors directly contributing to Cd accumulation in the shoot, and the Cd in the stem also had an indirect effect through an increase in Cd transportation (Figure 4b).

3.5. Regeneration Cropping System

The growth of ratoon crops was weaker than that of the main crop. Compared with the main crop, the SD of four sorghums (N5D61, N51M4264, N31K2168, and N31G2174) decreased by 23.07%, 25.06%, 21.77%, and 29.74% (Figure S2d), respectively. The PH and SPN of BS in the regeneration cropping system decreased significantly by 29.68%, 38.15%, and 16.67%, 30.77%, respectively ($p < 0.05$), but no difference occurred in SS (Figure S2b,c). Due to the thinner SD and smaller PH, the FW of ratoon crops of BS decreased above 50% (Figure 7a), and the increased WC (15.15% and 26.80%, Figure S2a) further led to a significant decrease in DW (63.45% and 78.12%, Figure 7b). However, the DW of SS showed no difference (N31G2174) or significant increase of 44.97% (N31H2358) between main and ratoon crops because of the reduction in WC in the shoot, in spite of the slight decrease in FW (Figure 7).

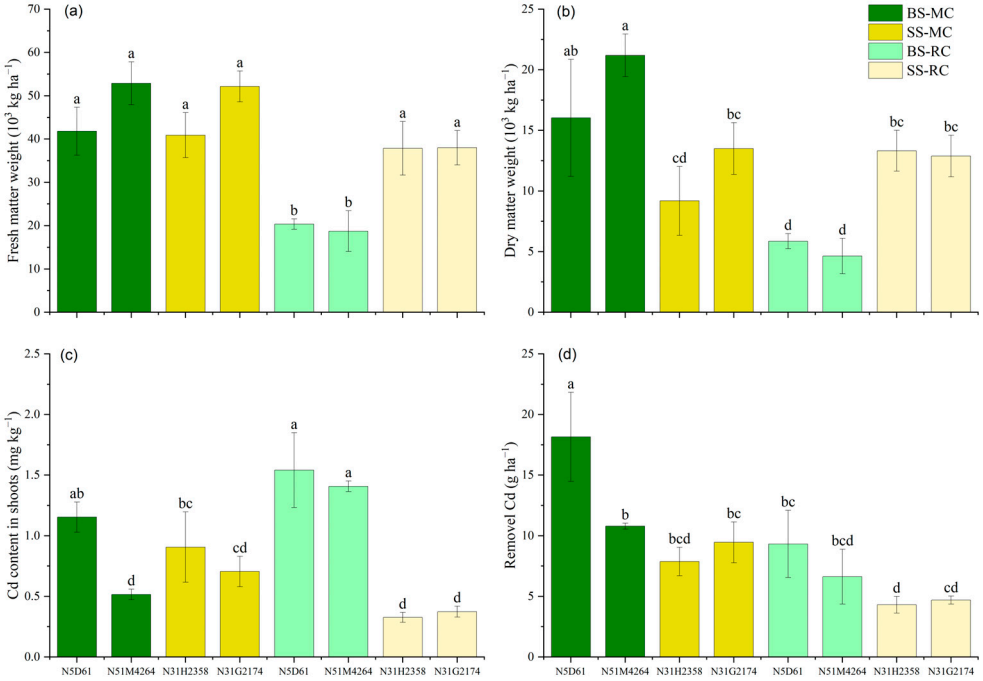


Figure 7. Fresh matter weight (FW, (**a**)), dry matter weight (DW, (**b**)), Cd concentration in shoot (**c**), and removal of Cd (**d**) of the main corps (MC) and ratoon crops (RC) of sorghums, Different letters indicate significant differences between each variety, LSD test, $p < 0.05$ (n = 24).

In the regeneration cropping system, there were opposite results in shoot Cd contents between BS (N5D61 and N51M4264) and SS (N31K2168 and N31G2174). As shown in Figure 7, compared with the main crops, the Cd concentration in the shoots of BS ratoon crops increased by 34.32% and 172.87%, yet that of SS decreased by 63.97% and 46.95%. The removal of Cd by ratoon crops of sorghum was 9.33, 6.63, 4.31, and 4.70 g ha^{-1}, which accounted for 51.35%, 61.41%, 54.76%, and 49.63% of the main crops, respectively.

4. Discussion

4.1. Biomass Sorghum Is Prior to Sweet Sorghum in Cd Removal

Sorghum exhibits high tolerance to Cd and can grow normally in Cd-contaminated soil [13,28]. In fact, the growth of energy sorghum (BS and SS) was not affected by Cd stress when planted in soil with Cd contents of 0.39 and 1.2 mg kg^{-1}, and the accumulation of Cd in sorghums increased with the increase in Cd concentration in soil (Figures 3e and 6c). Hence, using sorghum as a viable crop for phytoremediation of cadmium holds promise [5]. Xiaoming Zhao et al. showed that the concentration of Cd in six sorghum varieties reached 19.6-148 g ha^{-1} [2]. Similar results were observed in our study, where the maximum Cd removal of BS and SS could reach 93.09 and 62.78 g ha^{-1} $year^{-1}$, respectively (Figure 1c), which indicated that energy sorghum, especially BS, was a promising phytoremediation [5,8]. These BSs, introduced from the United States, are highly photoperiod-sensitive, late-flowering energy sorghum hybrids that exhibit a long duration of vegetative growth and high biomass accumulation [15,29]. The BS variety exhibited significantly higher cellulose, hemicellulose, and lignin content in the stem compared with SS [30] due to its breeding focus on maximizing cellulosic content, resulting in increased yields of dry biomass. In our study, BS had a lower WC of 4.78–13.49% (Figure 2c), a higher DW of 13.21–125.16% (Figure 2b), and a higher Cd tolerance in roots (Figure 6a), which was prior to SS removing Cd from soil [11]. Furthermore, we must pay attention to Cd accumulation in roots, which accounted for 30–40% of the whole sorghum (Figure S1a); it is necessary to deal with the roots while removing the shoots of sorghum.

4.2. Key Factors of Cd Accumulation Controlled by Sorghum Varieties

High biomass yield is the advantage of energy sorghum to remove soil Cd and may be affected by the agronomic parameters controlled by varieties, such as PH, SD, WC, and growth stages [13,16]. Herein, the Cd content in shoots of N5D61 and N32F2026 was found to be comparable (Figure 5e), but their Cd accumulation differed by more than one time (Figure 5f) due to the vast difference in DW (Figure 2b). Interestingly, DW was closely related to PH and WC (Figure 3), which indicated that PH and WC were the two factors that led to the difference in DW among sorghums (Figure 2c,d). Furthermore, the SEM (Figure 4a) showed that the PH not only had a direct positive effect on DW, which was consistent with the previous study [31], but also indirect positive effects through the increasing FW and decreasing WC. This may be because PH is a vital trait of plant architecture that can be used to predict sorghum yield [32]. Thus, PH is the key agronomic parameter affecting DW and further Cd removal from energy sorghums.

Cd accumulation in energy sorghum was affected by several physiological processes, including root uptake, sequestration in vacuoles, and transport in the xylem and phloem [8,33]. Herein, the BCF of root (Figure 6a), TF of stem, and leaf (Figure 6b) among 8 sorghums varied largely, which contributed to the difference in Cd accumulation in the shoot (Figure 5e), which can be attributed to the differences in Cd tolerance and transportation of energy sorghum [9,34]. Previous studies have shown that the root was the organ directly exposed to Cd in soil, which reflects the Cd tolerance of sorghum [35], and root biomass was recommended as a biomarker for evaluating the Cd extraction ability of sorghum genotypes [13]. However, this was a conclusion based on two weeks of hydroponics at the seedling stage, which differed from phytoremediation under field conditions [12]. Most importantly, the accumulation of Cd in the shoots of sorghum varied with the growth stage and reached the highest at the heading stage, so it was not suitable to consider root

biomass as a biomarker at the seeding stage for assessing the Cd extraction ability of energy sorghums in phytoremediation [11]. In our study, the Cd concentrations in the stem and leaf were the key factors affecting the Cd accumulation in the shoot rather than the Cd in the root, in spite of the indirect effect of the root through affecting Cd accumulation in the stem and leaf (Figure S1 and Figure 4b,c). Moreover, the stem not only contributed to the Cd content in shoots directly but also produced an indirect effect by increasing the Cd content in the leaf (Figure 4b) and finally promoted the removal of Cd from sorghum together with DW (Figure 4c). Therefore, the Cd concentration in the stem is the key factor for evaluating the Cd extraction ability of sorghum.

4.3. Advantages of the Regeneration Cropping System

Energy sorghum is a C_4 grass native to tropical or subtropical environments that is particularly resistant to drought and high temperatures but sensitive to cold temperatures [36,37]. When the soil temperature is below 15 °C or under water stress, several early developmental processes, including emergence, seedling vigor, and general metabolism, are affected, and the biomass yield at the mature stage ultimately decreases [18,19]. The subtropical regions of China are warm and light resource areas, with lower soil temperatures and more rain in the spring [38]. The growth period of most energy sorghums is generally longer than 120 days [12,39]; we had to sow in the spring with the double cropping system. Obviously, it is susceptible to cold stress and often exhibits poor early-season vigor and reduced competitive ability against weeds, owing to low temperatures and water stress after sowing [18,36]. Nematpour et al. and Mirahki et al. investigated that delay in planting shortens the growing season, overshadows the vegetative and reproductive phases, and ultimately reduces sorghum yield [40,41]. The lower dry biomass yield caused by the non-synchronization of plant growth stages with environmental conditions [42] could directly affect Cd accumulation in sorghum. These facts highlighted that it is not feasible to increase the annual removal of Cd by energy sorghum with the double cropping system in subtropical regions of China.

The regeneration cropping system is also a common planting pattern for improving agricultural production efficiency [22,43,44]. Compared with the double cropping system, the growth period of the regeneration cropping system of sorghum had a certain reduction [45], which made it possible to plant the main corps without chilling and water stress and obtain a better DW [43]. Furthermore, a lower cost of seeds, supplies, and manpower increased the economic benefits of phytoremediation [45]. Additionally, the agronomic and reproductive traits of ratoon sorghum are diminished compared with the main crop [46,47], while no reduction in Cd absorption was observed in this study (Figure 7c). Most importantly, the ratoon corps of energy sorghums can increase annually by approximately 50% removal of Cd (Figure 7d), which just adds a little fertilizer [22,24], compared with a single cropping system. Thus, considering the Cd removal efficiency, economic benefits, and rainfall-heat conditions in subtropical regions of China, it is advised to plant energy sorghum with the regeneration cropping system to remove Cd in subtropical farmlands of China.

5. Conclusions

A batch of field experiments was conducted to investigate the effects of variety and cropping system on Cd removal by energy sorghum in Chinese subtropical farmland. Biomass sorghums and sweet sorghums were both promising crops for Cd removal, but the agronomic traits and Cd accumulation varied significantly. Compared with sweet sorghum, biomass sorghum had a lower water content in the shoot, a higher plant height, and a higher dry biomass yield, which were more efficient in removing Cd from the soil. Plant height and stem Cd content were the key factors affecting the dry biomass yield and Cd accumulation in the shoots of energy sorghum, respectively, which indirectly determined the removal of Cd by energy sorghum together. Furthermore, the regeneration cropping system was an optimal planting pattern with lower cost than the double cropping

system, more suitable to natural conditions for agricultural production, and higher annual Cd removal efficiency than the single cropping system. Collectively, our study provides valuable information on the selection of varieties and cropping systems for the practical application of energy sorghum to remove Cd from contaminated soils.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13102487/s1, Table S1: Variation of agronomic characteristics and Cd removal in sorghum; Table S2: The variety codes of sorghums; Figure S1: Distribution of Cd removal in the different organs of whole sorghum and shoot; Figure S2: Water content in shoot (WC), plant height (PH), stem pitch number (SPN), stem diameter (SD) of the main corps (MC), and ratoon crops (RC) of sorghums.

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