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Effects of Sound Wave and Water Management on Growth and Cd Accumulation by Water Spinach (*Ipomoea aquatica* Forsk.)

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Abstract: Vegetable contamination by cadmium (Cd) is of great concern. Water spinach (*Ipomoea aquatica*) is a common leafy vegetable in many countries and has a strong ability to accumulate Cd. The work was conducted to study the effects of sound wave, water management, and their combination on Cd accumulation and growth of water spinach, using the following three experiments: a hydroponic trial with the treatment of a plant acoustic frequency technology (PAFT) generator in test sheds, a hydroponic trial with three music treatments (electronic music (EM), rock music (RM), and classical music (CM)) in artificial climate boxes, and a soil pot trial with treatments of PAFT and EM under non-flooded and flooded conditions. The results showed that the hydroponic treatments of PAFT and EM significantly reduced the Cd concentrations in roots and shoots (edible parts) of water spinach by 22.01–36.50% compared with the control, possibly due to sound waves decreasing the root tip number per unit area and increasing average root diameter, root surface area, and total root length. Sound wave treatments clearly enhanced water spinach biomass by 28.27–38.32% in the hydroponic experiments. In the soil experiment, the flooded treatment significantly reduced the Cd concentrations in roots and shoots by 43.75–63.75%, compared with the non-flooded treatment. The Cd decrease and the biomass increase were further driven by the PAFT supplement under the flooding condition, likely related to the alteration in root porosity, rates of radial oxygen loss, extractable soil Cd, soil Eh, and soil pH. Our results indicate that the co-application of plant acoustic frequency technology and flooded management may be an effective approach to reduce Cd accumulation in water spinach.

Keywords: water spinach; cadmium; biomass; sound wave; water management; root characteristics

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1. Introduction

Cd contamination in farmlands has been a globally environmental problem and can lead to excessive accumulation of Cd within crops and quality decline in agricultural products [1–4]. Crop plants can uptake Cd from soil and deposit Cd into various organs, threatening human health via the food chain [1,5]. In recent years, vegetables contaminated by Cd and their food safety have attracted extensive attention [6,7]. Water spinach (*Ipomoea aquatica* Forsk.) is a common leafy vegetable in many countries [8] and has a strong ability to accumulate Cd in its shoots (edible parts) [9–11]. Thus, it is urgently needed to reduce Cd accumulation in vegetables for safe food production using cost-effective and environmentally compatible methods.

Conventional remediation methods may have some limitations in agriculture soils contaminated by heavy metals [3,12]. Chemical fixation and leaching could interfere with soil environment [13,14], and the phytoremediation needs a long time to generate the repair effects, affecting agriculture operations [15,16]. Traditionally physical technologies, e.g., soil removal and replacement, find it difficult to meet the requirements of crop safety due to Cd contamination [13,14]. Regarding other physical technologies, using sound wave may be an interesting method and is rarely reported on the remediation for soil contamination by heavy metals.

Spontaneous frequencies of plants are mainly concentrated in low-frequency sound waves, ranging from 20 to 2000 Hz. Owing to the theory and practice of acoustic biology, the action of sound waves on plants has been affirmed and applied in agricultural practice [17]. Sound wave technology of high and low frequencies has generated different effects on plants [18,19]. Sound wave treatments can increase the post-harvest shelf life of tomato fruits [20], enhance the immune responses of plants to diseases and insect pests, and promote plant growth with the reduced consumption of fertilizers [21]. Among sound wave technologies, plant acoustic frequency technology (PAFT) is a newly agricultural production technology [17] and can increase crop yields [21]. The working principle of PAFT is to apply a specific frequency sound wave matched with the frequency of crop self sound, so as to generate resonance, improve the electron flow rate in plant cells, enhance photosynthesis and respiration, and promote the absorption and transformation of elements by plants [21]. Different styles of music, such as classical, jazz, rock, or light music, may be also conducive to crop health. Nevertheless, information is lacking regarding the effects of sound waves on the uptake and accumulation of heavy metals (e.g., Cd) by plants.

Water spinach is a kind of wetland plant [22,23], but also a xerophytes plant, indicating that this plant can survive under different water conditions. Changes in root characteristics (e.g., number of root tips, root specific surface area, radial oxygen loss (ROL), root aerenchyma, and porosity) may affect heavy metal uptake by plants in a nutrient solution or a flooded environment [24–29]. It is noted that the agronomic measure of water management can influence the accumulation of heavy metals in rice [30–32]. Arao et al. (2009) [31] reported that flooding treatments decreased the concentrations of rice Cd and might be an effective method to control Cd in crops. Collectively, we hypothesize that the accumulation of Cd in water spinach may be reduced by the treatments of sound waves, water management, or their co-application.

Based on the aforementioned observations, the aim of this work was to study the effects of sound waves, water management, and their combination on Cd accumulation by water spinach and its growth, and to explore the possible mechanisms involved. Hydroponic and soil pot experiments were conducted to study the effects by determining Cd concentrations in shoots and roots, translocation of Cd from roots to aerial parts, plant biomass, chlorophyll content, total root length, number of root tips, root specific surface area, root porosity, rates of ROL, root aerenchyma, and soil properties (extractable Cd, Eh, and pH).

2. Materials and Methods

2.1. Experimental Design

Three experiments were set up in this work. A hydroponic trial was conducted to verify whether the PAFT treatment could reduce Cd uptake by water spinach and promote its growth in test sheds. A hydroponic trial was used to investigate the effects of three kinds of music (electronic music (EM), rock music (RM), and classical music (CM)) on Cd accumulation and growth of water spinach in artificial climate boxes. A soil pot trial was conducted to study the synergistic effects of sound wave treatments screened from the above trials and water management (non-flooded and flooded conditions) on the accumulation of cadmium in water spinach.

2.2. Seed Variety and Soil Preparation

Seeds of water spinach cultivar (*Ipomoea aquatica* Forsk. T-308), obtained from Guangzhou Aipunong Agricultural Technology Co., Ltd., China, were used in the hydroponic and soil experiments. According to the previous reports, T-308 was a variety of high Cd accumulation [27,33]. Water spinach seeds, disinfected in 30% H_2O_2 (w/v) solution for 15 min, were washed thoroughly with deionized water and then germinated in acid-washed quartz sands for 12 days. Uniform seedlings selected at the three cotyledons stage were used in the experiments.

The pollution-free soils were taken from 0–20 cm topsoil on the campus of Hefei University, Anhui Province, China. The soil contained 3.2% organic matter, 1.6 g/kg total nitrogen, 1.1 g/kg total phosphorus, 4.1 g/kg total potassium, 0.09 mg/kg total Cd, and had a pH of 7.2. The air-dried soils were ground and passed through a 5 mm sieve. Subsequently, PVC pots were filled with 1.2 kg of the prepared soils. The soil in the pots was added with a treatment of 30 mg/kg Cd (solution of $CdCl_2 \cdot 2.5\ H_2O$), 28.6 mg/kg K (solution of KCl), and 76.3 mg/kg N (solution of $CO(NH_2)_2$), and balanced for 30 days in a glasshouse with the following conditions: temperature day/night 28/18 °C; day/night relatively humidity 60/80%. After soil equilibration, the final mean concentration of total Cd in the soil was 30.57 mg/kg, and the mean concentration of DTPA-extractable soil Cd was 11.25 mg/kg.

2.3. Hydroponic Experiments of Sound Wave Treatments

Uniform seedlings of T-308 were transplanted into 25% Hoagland's nutrient solutions for 3 days, and the solutions were added with 25 μM Cd ($CdCl_2 \cdot 2.5\ H_2O$). The first experiment was carried out in test sheds, including treatments of PAFT and silent environment (the control, CK_A), each with four replicates. In order to avoid interference, the experimental sheds were separated by 100 m. The fully automatic first-generation PAFT generator (BL01-2011, Figure 1) was produced by Beijing Lehe Tongfeng Agricultural Technology Co., Ltd. The frequency of the sound wave by the PAFT generator ranged from 0 to 2000 Hz. The audio loudness was 80 dB, measured by a sound level meter with an accuracy of ±1.5 dB and range of 30–130 dB (DECEMSMD1130, Delixi, China), and the effective maximum range of the audio was about 100 m away from the generator. A programmed timer was used to set the playback time, and the above audio was played for 3 h every day (8:30–11:30 a.m.). Water spinach seedlings were cultured for 35 days and then were harvested.

Figure 1. The fully automatic first-generation PAFT generator (BL01-2011).

The second experiment was carried out in four artificial climate boxes, including treatments of three kinds of music (EM, RM, and CM) and silent environment (the control, CK_M), each with three replicates. The EM was selected from Bandari's "Anne's Wonderland", which was quiet and elegant; "Don't cry" by Guns N' Roses was selected as the RM treatment, which was high-pitched and high frequency; the CM was Tchaikovsky's "1812 prelude", which was solemn and heavy. The volumes of three kinds of music were adjusted to the extent that human ears could not feel mutual interferences. The loudness of three types of music was 80 dB, measured by the sound level meter. The playback time was from 8:30 (a.m.) to 16:30 (p.m.). The Hoagland's nutrient solutions were changed every 4 days. In addition, 20 seedlings of water spinach were cultured for 3 weeks in each artificial climate box, with an inner dimension of 55 cm × 52 cm × 114 cm (length × width × height).

The temperature was maintained at 24 ± 4 °C in the growth chamber, the relative humidity was 65 ± 5%, and the photosynthetic photon flux (PPF) was 58 ± 5 μmol·m^{-2}·s^{-1}. The cultures were subjected to a day-to-night cycle, in which 12 h was light with aeration and 12 h was dark without aeration. The aeration rate was 50 mL/min and the concentration of CO_2 was 2% in the air.

After harvest, the plants were washed cleanly with distilled water. The morphological indexes, including plant growth, chlorophyll contents of shoots, and Cd concentrations in shoots and roots, were measured. The processing waveforms of PAFT and three kinds of music are shown in Figure 2.

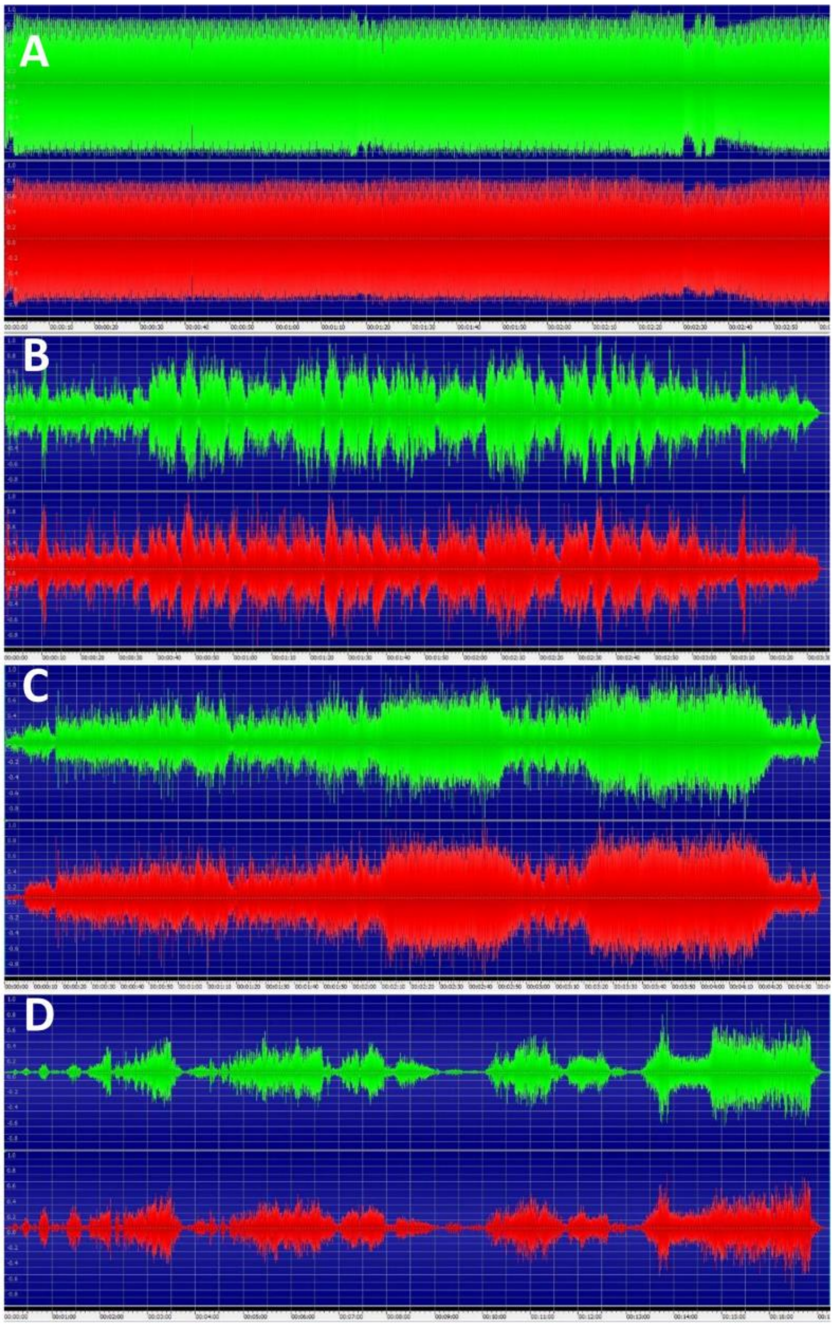

Figure 2. Acoustic characteristic diagram (**A**: PAFT; **B**: EM; **C**: RM; **D**: CM); The sampling rate of the frequency spectrum analysis software GoldWave was 44,100 Hz for the MPEG format of the sound samples, and the green and red waveforms represent the left and right channels, respectively.

2.4. Soil Experiment of Sound Wave and Water Management Combination

After the hydroponic experiments, the treatments of PAFT and EM, generating better effects on Cd decreases in water spinach, were selected to cooperate with water management in the soil pot experiment. Uniform seedlings of T-308 with three cotyledons were transplanted into the prepared soil pots above. After 10 days, the pots were treated with different types of water management (non-flooded and flooded (water surface 3 cm from soil surface)), and were placed in an experimental shed. Subsequently, the seedlings were treated with PAFT, EM, and silent environment (the control, CK), each treatment with four replicates. The treatments of PAFT and EM were conducted to play for 3 h (08:30–11:30 a.m.) every day. The seedlings were cultured for 35 days and then were harvested.

2.5. Sampling and Analytical Methods

The harvested plant samples were separated into roots and shoots. The chlorophyll contents in water spinach leaves were extracted with 95% ethanol and were determined using a spectrophotometer. Oven-dried plant tissues were digested in a mixture of HNO_3-$HClO_4$ (3:1, *v*/*v*), and Cd concentrations in roots and shoots were determined by inductively coupled plasma mass spectrometry (ICP-MS; iCAP-Q, Thermo Fisher, USA). Blanks, tea standard material (GBW-08303), and soil standard material (GBW-07435) (China Standard Materials Research Center, Beijing) were performed to meet the QA/QC requirements. Cd recovery rates were 90%. To estimate Cd translocation from roots to shoots, the transfer factor was calculated as follows: transfer factor (IF) = Cd concentration in shoot/root. Soil pH and soil Eh were measured with a pH/Eh meter (TM-39, Germany).

In order to observe the root morphology in the hydroponic experiments, a root scanner (std4800, WinRhizo) was used to measure root length, root tip number, root diameter, and root surface area [34,35]. Regarding the difference of root aerenchymas among the treatments in the soil experiment, fresh root cross-sections of 35-days-old plants were cut by hand with a sharp razor, at a distance of 7.0 cm from the root tip. These specimens were examined and photographed using a scanning electron microscope (SEM) (S520; Hitachi, Japan) [26,36]. Root porosity (% gas volume/root volume) of plants was measured by the pycnometer method [37]. The rates of ROL from roots were determined according to the Ti^{3+}-citrate method described by Mei et al. (2009) [38].

2.6. Statistical Analysis

Data were analyzed using the SPSS 22.0 statistical software package and were summarized as means ± standard errors (SE). A statistical comparison of treatment means was examined by one-way ANOVA, followed by Tukey-HSD tests.

3. Results

3.1. Effects on Cd Concentrations in Water Spinach

In the hydroponic experiment, the PAFT treatment significantly ($P < 0.05$) decreased the concentrations of Cd in roots and shoots (edible parts) compared with the control (Table 1). The transfer factors of Cd from roots to shoots were not affected significantly by the PAFT amendment (Table 1).

In the hydroponic experiment of music treatments, the treatments of EM and CM markedly ($P < 0.05$) reduced the Cd concentrations in roots compared with the control, by 36.50% and 28.76%, respectively (Table 1). In addition, the Cd concentrations in shoots decreased significantly ($P < 0.05$) by 22.01% after the EM treatment, but not after the treatments CM and RM. The transfer factors of Cd were not altered markedly by the music treatments (Table 1).

In the soil experiment, the co-application effects on the Cd concentrations in shoots and roots are shown in Figure 3. The results showed that the range of the Cd concentrations in shoots and roots were 2.9–10 mg/kg and 11–36 mg/kg, respectively. The flooded treatment significantly ($P < 0.05$) decreased the Cd concentrations in roots and shoots compared with the non-flooded condition. Under the flooded and non-flooded conditions, the PAFT treatment dramatically ($P < 0.05$) reduced the Cd concentrations in roots compared with the EM treatment and the control, and also significantly ($P < 0.05$) decreased Cd in shoots compared with the control. Significant differences were not observed in the Cd concentrations of the tissues between the EM treatment and the control under the flooded and non-flooded conditions.

Table 1. Cd concentrations (conc.mg/kg) in shoots and roots, and transfer factors of Cd from roots to shoots with and without sound waves in the hydroponic experiments.

Treatments	Cd Conc. in Roots	Cd Conc. in Shoots	Transfer Factors
CK_A	738.74 ± 17.84 a	55.74 ± 2.11 a	0.076 ± 0.005 a
PAFT	553.55 ± 23.38 b	38.62 ± 2.96 b	0.070 ± 0.007 a
CK_M	726.12 ± 78.35 a	50.21 ± 2.95 a	0.069 ± 0.009 a
EM	461.24 ± 22.90 c	39.16 ± 2.40 b	0.085 ± 0.007 a
RM	681.71 ± 79.08 ab	48.04 ± 0.75 a	0.070 ± 0.009 a
CM	517.27 ± 14.18 bc	43.05 ± 2.19 ab	0.083 ± 0.005 a

In each hydroponic experiment and in each parameter, different letters indicate significant differences among the different treatments ($P < 0.05$).

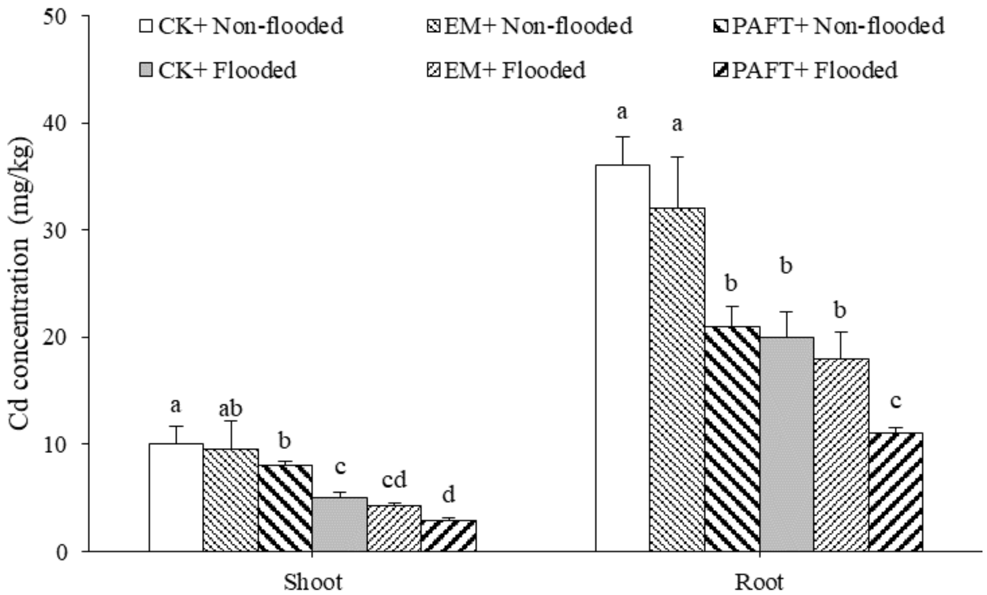

Figure 3. Cd concentrations in shoots and roots of water spinach in the soil pot experiment. In the same parameter, different letters above the bar chart indicate significant differences among the different treatments ($P < 0.05$).

3.2. Effects on DTPA-Extractable Cd, Eh, and pH in Soil

In the soil experiment, the co-application effects on DTPA-extractable Cd, Eh, and pH in soil are shown in Figures 4–6, respectively. The results indicated that the range of the DTPA-extractable soil Cd concentrations, soil Eh, and soil pH were 4.39–5.87 mg/kg, 76–96 mV, and 6.63–7.02 under the flooded condition, and 18.20–22.33 mg/kg, 386–399 mV, and 5.57–5.69 under the non-flooded condition, respectively. Compared with the non-flooded condition, the flooded treatment significantly ($P < 0.05$) decreased the extractable soil Cd and soil Eh by 74.36% and 77.31%, respectively (Figures 4 and 5), whereas it significantly ($P < 0.05$) increased soil pH by 22.12% (Figure 6). Furthermore, compared with the EM treatment and the control, the PAFT treatment markedly ($P < 0.05$) reduced the extractable Cd under the flooded and non-flooded conditions, and also significantly ($P < 0.05$) decreased soil Eh under the flooded condition.

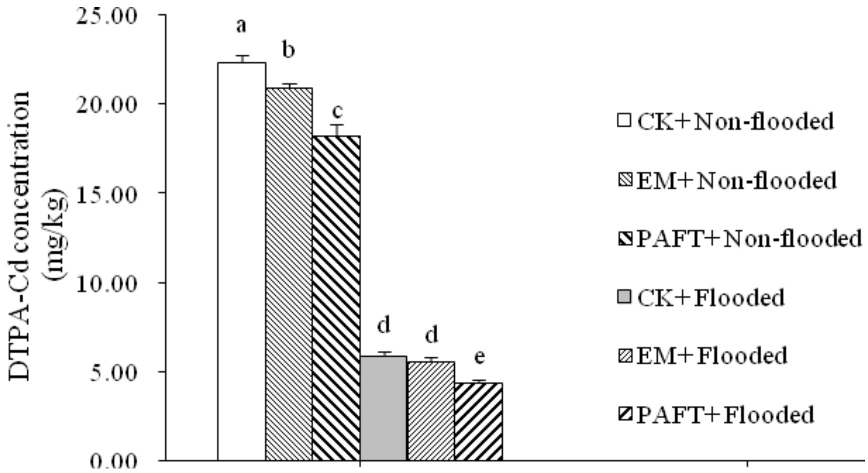

Figure 4. The DTPA-extractable Cd concentrations in the soil pot experiment. Different letters above the bar chart indicate significant differences among the different treatments ($P < 0.05$).

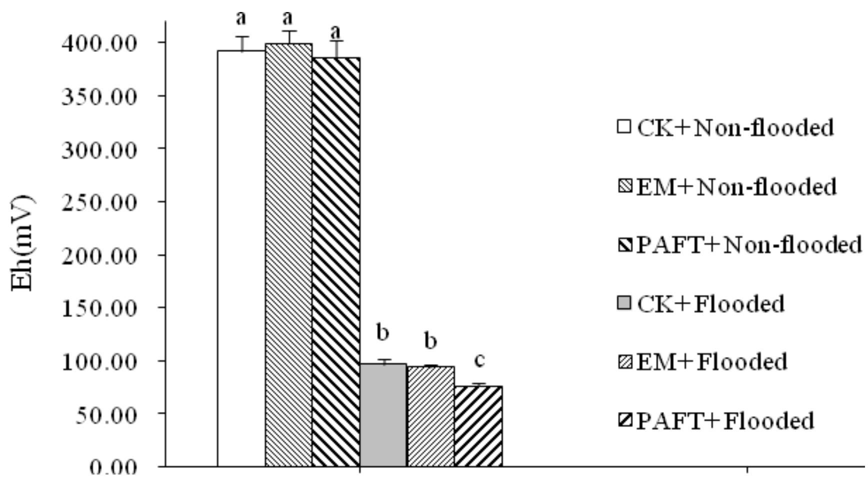

Figure 5. The Eh in soil in the soil pot experiment. Different letters above the bar chart indicate significant differences among the different treatments ($P < 0.05$).

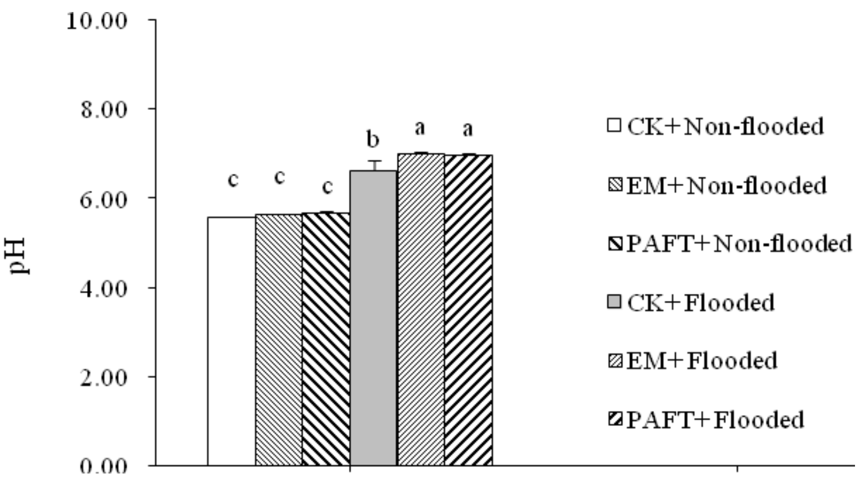

Figure 6. The pH in soil in the soil pot experiment. Different letters above the bar chart indicate significant differences among the different treatments ($P < 0.05$).

3.3. Effects on Growth of Water Spinach

In the hydroponic experiment, the PAFT treatment showed beneficial effects on the growth of water spinach (Table 2). Compared with the control, the PAFT treatment significantly ($P < 0.05$) enhanced the plant height, the longest root length, the shoot biomass, the root-shoot ratio (R/S), and the chlorophyll contents of leaves (Table 2).

Table 2. Growth and physiological index of water spinach with and without sound waves in the hydroponic experiments.

Treatments	Plant Height (cm)	Root Length (cm)	Shoot Biomass (g Plant^{-1} Fresh Weight)	Root-Shoot Ratio	Chlorophyll Contents (mg/g)
CK_A	12.59 ± 0.64 b	10.25 ± 0.63 b	27.28 ± 0.61 b	0.36 ± 0.03 b	1.43 ± 0.13 b
PAFT	17.45 ± 1.16 a	12.17 ± 0.85 a	36.76 ± 0.68 a	0.44 ± 0.03 a	1.97 ± 0.14 a
CK_M	10.63 ± 0.84 b	9.70 ± 0.26 c	13.23 ± 0.53 b	0.47 ± 0.03 a	0.83 ± 0.03 c
EM	14.40 ± 0.61 a	12.37 ± 0.64 a	18.30 ± 0.55 a	0.51 ± 0.03 a	1.47 ± 0.06 a
RM	12.37 ± 0.67 a	10.73 ± 0.34 b	13.00 ± 0.96 b	0.48 ± 0.02 a	1.13 ± 0.10 b
CM	10.43 ± 0.62 b	9.90 ± 0.25 c	16.97 ± 0.90 a	0.49 ± 0.01 a	1.36 ± 0.02 a

In each hydroponic experiment and in each parameter, different letters indicate significant differences among the different treatments ($P < 0.05$).

In the hydroponic experiment of the music treatments, the treatments of EM and CM significantly ($P < 0.05$) increased the shoot biomass by 38.32% and 28.27%, respectively, and also improved the chlorophyll contents of leaves compared with the control (Table 2). The treatments of EM and RM significantly ($P < 0.05$) increased the plant height and the longest root length compared with the control. There were no significant differences in the R/S among the four treatments. Collectively, the EM treatment showed better effects on the growth index compared to the RM and CM treatments (Table 2).

In the soil experiment, the combination of PAFT and flooded treatments significantly ($P < 0.05$) enhanced the shoot biomass compared with the control (Table 3), while there were no significant differences in the root length and the R/S among the six treatments (Table 3).

Table 3. Growth of water spinach in the soil pot experiment.

Treatments	Root Length (cm)	Shoots Biomass (g Plant^{-1} Fresh Weight)	Root-Shoot Ratio
CK + flooded	29.50 ± 3.70 a	8.54 ± 2.56 b	1.02 ± 0.13 a
PAFT + flooded	33.25 ± 4.99 a	20.71 ± 6.85 a	1.27 ± 0.44 a
EM + flooded	28.25 ± 1.26 a	15.13 ± 3.32 ab	1.07 ± 0.37 a
CK + non-flooded	33.50 ± 3.11 a	7.03 ± 0.79 b	1.11 ± 0.23 a
PAFT + non-flooded	35.75 ± 6.80 a	11.90 ± 4.19 b	1.01 ± 0.28 a
EM + non-flooded	32.50 ± 1.00 a	8.65 ± 1.84 b	1.12 ± 0.22 a

Different letters in the same column indicate significant differences among the different treatments ($P < 0.05$).

3.4. Effects on Root Morphology

In the hydroponic experiments, there were differences in the morphology of water spinach roots after sound wave amendments (Figure 7). Total root length, root surface area, number of root tips per unit area, and root average diameter were measured, as shown in Table 4. Compared with the control, the PAFT treatment significantly ($P < 0.05$) improved the total length of root, the root surface area, and the root average diameter, but reduced the number of root tips per unit area (Table 4). In the hydroponic experiment of music treatments, the treatments of EM and CM dramatically ($P < 0.05$) increased the total length and the surface area of root, and decreased the number of root tips per unit area compared with the control. The EM treatment exhibited more effects on the root length, the root surface area, and the root average diameter than the treatments of CM and RM (Table 4).

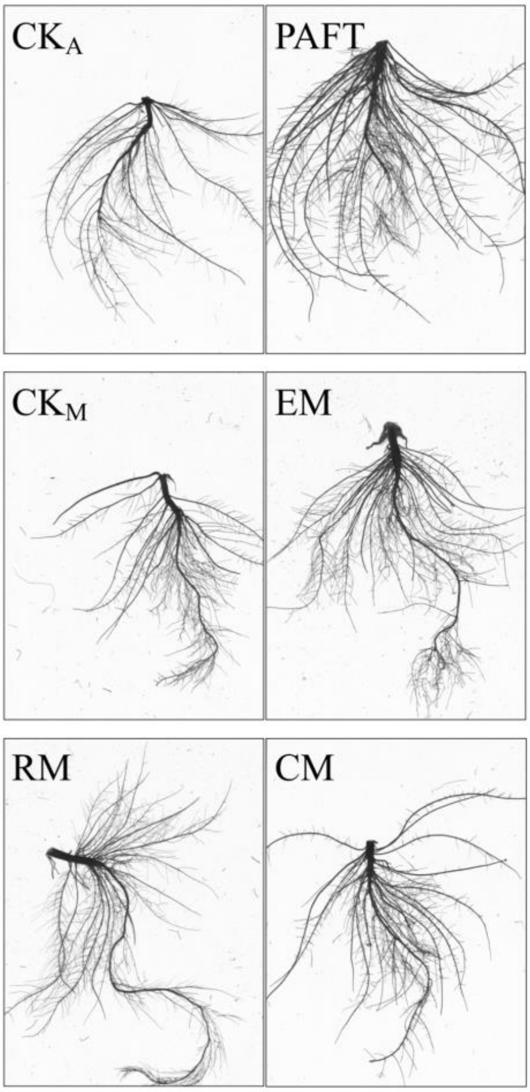

Figure 7. Root scanning of water spinach in the hydroponic experiments.

Table 4. Morphology index of water spinach roots with and without sound waves in the hydroponic experiments.

Treatments	Total Length (cm)	Surface Area (cm^2)	Average Diameter (mm)	Number of Tips /cm^2
CK_A	417.63 ± 32.55 b	57.23 ± 3.52 b	0.39 ± 0.01 b	17.23 ± 1.56 a
PAFT	596.15 ± 36.09 a	68.21 ± 3.21 a	0.43 ± 0.02 a	13.82 ± 0.58 b
CK_M	424.78 ± 24.35 c	53.53 ± 3.26 c	0.36 ± 0.01 b	22.99 ± 1.97 a
EM	544.67 ± 53.32 a	65.89 ± 6.91 a	0.48 ± 0.01 a	14.89 ± 0.81 b
CM	510.79 ± 58.17 ab	61.69 ± 6.71 b	0.44 ± 0.07 ab	15.84 ± 0.87 b
RM	488.67 ± 37.34 bc	55.89 ± 1.42 bc	0.37 ± 0.01 b	19.09 ± 1.13 a

In each hydroponic experiment and in each parameter, different letters indicate significant differences among the different treatments ($P < 0.05$).

3.5. Effects on Root Aerenchyma, Root Porosity, and Rates of ROL

In the soil experiment, the change in root characteristics varied among the treatments (Figures 8–10). The aerenchyma at the base of the root varied greatly between the flooded and non-flooded conditions, and the differences were also clear after further addition of sound waves (Figure 8). In order to compare the aerenchyma difference, the figures of the scanning electron microscope were analyzed by software Auto CAD. The percentage of aerenchyma in the whole cross-sectional area in the flooded condition (PAFT: 24%; EM: 19%; CK: 18%) was significantly ($P < 0.05$) higher than those in the non-flooded condition (PAFT: 14%; EM: 13%; CK: 11%), respectively. Under the flooded condition, the PAFT treatment significantly ($P < 0.05$) increased the proportion of aerenchyma compared with the EM treatment and the control; however, significant differences were not apparent in the proportion of aerenchyma among the three treatments under the non-flooded condition (Figure 8). In addition, the root porosity and the rates of ROL from roots in the flooded treatment were significantly ($P < 0.05$) higher than those in the non-flooded treatment (Figures 9 and 10). Under the flooded condition, the PAFT treatment resulted in higher root porosity and rates of ROL than the EM treatment and the control ($P < 0.05$), whereas significant differences were not observed among the three treatments under the non-flooded condition.

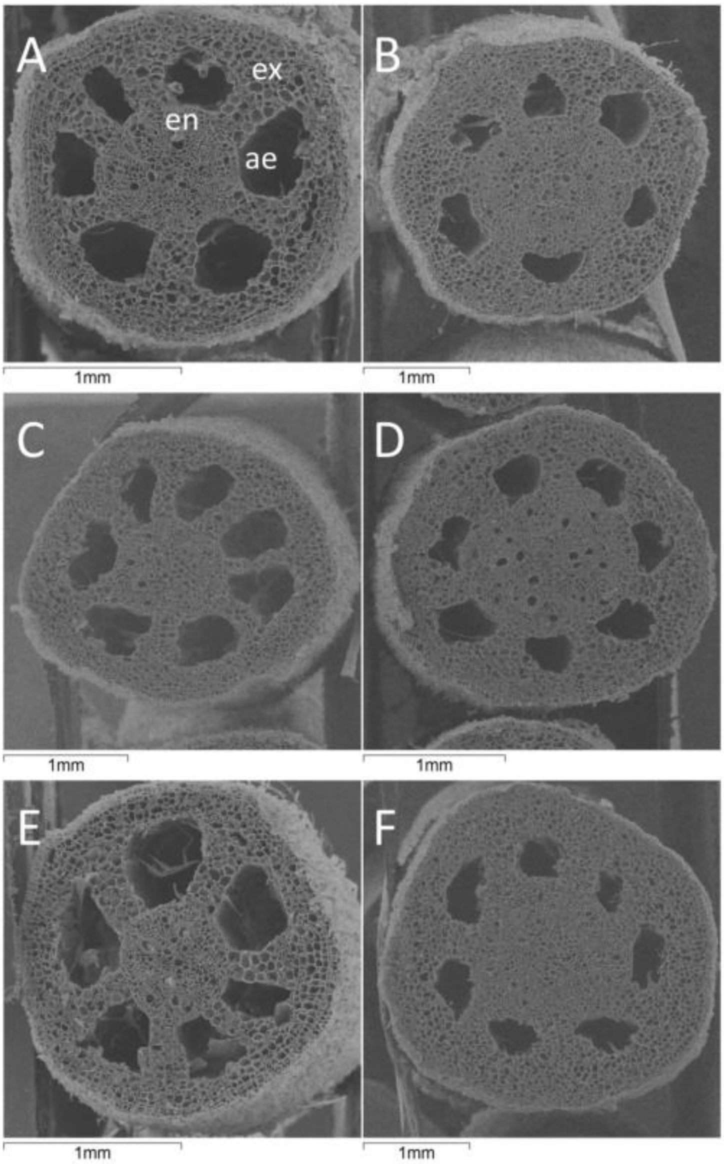

Figure 8. Cross-sectional scanning electron micrographs of the root (7 cm behind root tip, root length 8–9 cm), showing aerenchyma in the water spinach cultivar T-308 grown in the soil experiment. (ae: aerenchyma; en: endodermis; ex: exodermis; (**A**) CK + flooded; (**B**) CK + non-flooded; (**C**) PAFT + flooded; (**D**) PAFT + non-flooded; (**E**) EM + flooded; (**F**) EM + non-flooded).

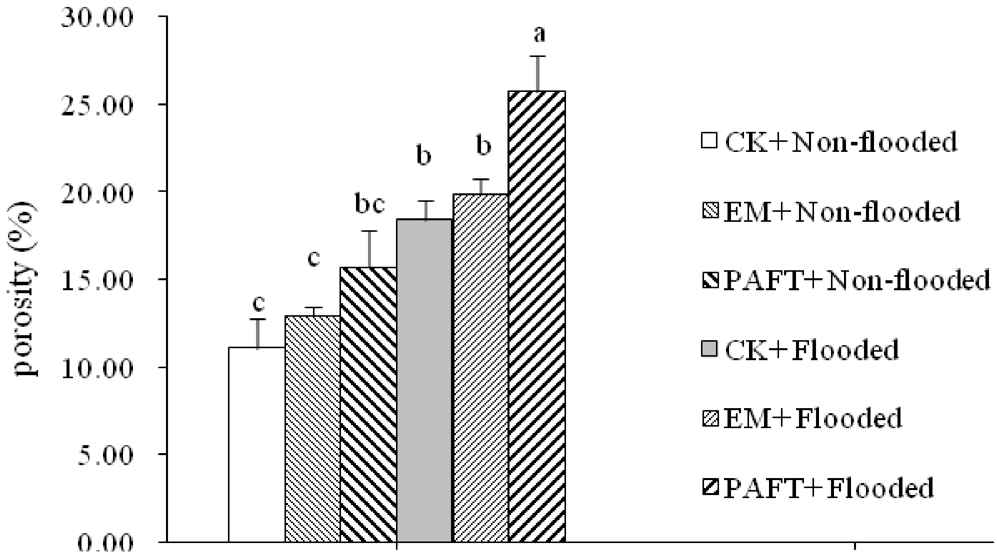

Figure 9. Root porosity in the soil pot experiment. Different letters above the bar chart indicate significant differences among the different treatments ($P < 0.05$).

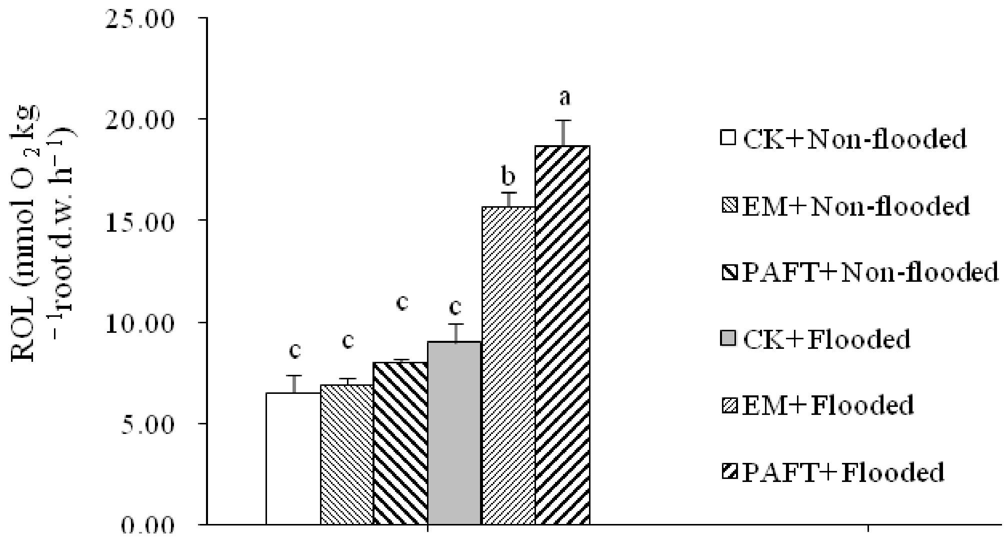

Figure 10. Rates of ROL in the soil pot experiment. Different letters above the bar chart indicate significant differences among the different treatments ($P < 0.05$).

3.6. Correlations between the Parameters

The linear regression analysis revealed that the DTPA-extractable soil Cd was negatively correlated with soil pH (R = −0.941, $P < 0.01$), root ROL (R = −0.789, $P < 0.01$), and root porosity (R = −0.876, $P < 0.05$), but the extractable Cd was positively correlated with soil Eh (R = 0.973; $P < 0.01$), Cd concentrations in roots (R = 0.851, $P < 0.01$), and Cd concentrations in shoots (R = 0.895, $P < 0.05$) (Table 5). The concentrations of Cd in roots and in shoots were negatively correlated with soil pH (R = −0.764 and −0.850, $P < 0.01$), root ROL (R = −0.780 and −0.823, $P < 0.01$), and root porosity (R = −0.846 and −0.831, $P < 0.05$), respectively, but they were positively correlated with soil Eh (R = 0.771 and 0.891, $P < 0.01$), respectively, (Table 5). Significant positive correlation was also observed between Cd in roots and Cd in shoots (R = 0.855, $P < 0.01$) (Table 5).

Table 5. Correlation coefficients (R) between the Cd concentrations (mg/kg) in roots and shoots, the DTPA-extracted Cd concentration, and soil pH, Eh (mV), rates of ROL (mmol O_2 kg^{-1} root d.w. h^{-1}) and root porosity (%), respectively, in the soil pot experiment (** $P < 0.01$).

Parameters	DTPA-Cd	Roots-Cd	Shoots-Cd	Eh	pH	Root ROL	Root Porosity
DTPA-Cd	1	0.851 **	0.895 **	0.973 **	−0.941 **	−0.789 **	−0.834 **
Roots-Cd		1	0.855 **	0.771 **	−0.764 **	−0.780 **	−0.846 **
Shoots-Cd			1	0.891 **	−0.850 **	−0.823 **	−0.831 **

4. Discussion

Different agriculture technologies have attempted to solve vegetable contamination by heavy metals. A previous study indicated that the agri-wave technology could affect the concentrations of nutrient elements in spinach [18]. In the present study, the Cd concentrations in shoots (edible parts) and roots of water spinach evidently decreased after the sound wave amendments of PAFT and EM (Table 1), suggesting that the sound wave technology seemed to mitigate the accumulation of Cd in vegetable plants. Plant roots play a most vital role in the uptake of Cd by the whole plant, and the activity of Cd in roots has been demonstrated in different plants [1,39,40]. Jia et al. (2003) [41] reported that sound stimulation enhanced the activity of plant roots. Cd concentrations in shoots are determined greatly by Cd entry into the root [42]. Generally, the entry of metals into a root depends on its morphology and anatomy, and environmental adaptability [1,43,44]. It is noted that both the number of root tips and the total surface area in a root system may influence the amount of Cd deposited in roots [25]. Zheng et al. (2011) [35] reported that the root length, the root hair length, and the root density were significantly correlated with Cd uptake in wild-type barley. Xiao et al. (2021) [44] also reported that the numbers of root tips per surface area of Cd high-accumulated cultivars were greater than that of low-accumulated cultivars. In this study, the treatments of PAFT and EM markedly promoted the total length of the root, root surface area, and root average diameter, but decreased the Cd concentrations in roots and shoots of water spinach (Table 1, Table 4, and Figure 7). This suggests that the mechanisms underlying the decrease in plant Cd caused by sound waves are likely to be the alteration of root morphology.

Previous studies have demonstrated that water management could markedly affect the uptake of Cd by plants. Arao et al. (2009) [31] and Mei et al. (2020) [28] reported that the flooding condition decreased the Cd concentrations in rice grains, which is in agreement with our results (Figure 3). A decrease in Cd in plant shoots under flooded conditions may be related to several factors, e.g., Cd bioavailability, Eh, and pH in the rhizosphere [45]. In this study, the flooded treatment had greater effects on Eh, pH, and DTPA-Cd in the soil compared with the non-flooded treatment (Figures 4–6). As reported, soil Cd was consistently lower after flooding than in aerobic conditions, when the soil Eh decreased below −200 mV after flooding [31]. Similarly, soil Eh was lower in the flooded treatment than in the non-flooded treatment (Figure 5), and DTPA-Cd clearly correlated with soil Eh and pH (Table 5). In addition, the flood treatment increased the root porosity and the rates of ROL, and resulted in a highly-developed aerenchyma of water spinach (Figures 8–10). The possibilities detailed below can be envisaged to explain the effects of the root porosity and the rates of ROL changes on the decrease in Cd. Water spinach has been considered as a wetland plant. Wetland plants include rice with higher root porosity and ROLs can result in greater abilities to modify their rhizosphere, thereby reducing the accumulation of toxic elements (Cd, lead, and arsenic) in aerial parts [28,46]. Mei et al. (2009) [38] reported higher root porosity and ROL reduced arsenic uptake by rice. In agreement with our results, water spinach cultivars of low Cd accumulation had a highly developed aerenchyma in the roots [28].

Furthermore, the co-application of PAFT and flooded treatments generated better effects on Cd decreases in roots and shoots of water spinach compared with the single treatment (Figure 3), which might be due to root characteristics (root morphology, root porosity, and rates of ROL) and soil properties (extractable Cd, Eh, and pH), altered in response to this stacking condition (Table 5). As reported, the root porosity was negatively correlated with grain Cd in plants [28], and root anatomy affected Cd accumulation by plants [43,44]. Low-Cd accumulated plants, e.g., rice [43] and water spinach [27], tended to develop larger root porosity. It is well known that sound waves propagate faster in water (1500 m/s) than in air (340 m/s). Therefore, sound wave treatment might have a greater impact on the variation in histological structures of roots under the flooding condition, but not for the non-flooding condition.

Apart from the Cd decrease in water spinach, sound wave treatments used in the hydroponic experiment increased the shoot biomass, especially after the treatments of PAFT and EM (Table 2), which is similar to the results of the research on lettuce and spinach [18]. Previous studies have demonstrated that after sound stimulation, the content of indoleacetic acid (IAA) and polyamine compounds (PAS) in plants increased, which could regulate plant cell division and plant morphogenesis and promote the formation of vascular bundles, leaves and flower buds [47–49]. The PAFT and EM treatments significantly increased the total root length and the average area of roots (Figure 7 and Table 4), indicating that sound wave application can promote the growth of roots. Exogenous stimulation can lead to lateral root induction, generating a phenotype that has been attributed to the auxin accumulation in roots [50]. In agreement with our results, the PAFT amendment increased the numbers of leaves and flowers, the chlorophyll contents, and the yields in some plants, e.g., tomato, lettuce, and spinach [21]. Chlorophyll is the main pigment for plant growth, used to absorb solar energy during photosynthesis, and its contents directly influence the light and the intensity of leaves. In this experiment, the PAFT generator promoted the chlorophyll contents in water spinach leaves (Table 1), thereby improving photosynthesis and increasing the biomass. Among the three types of music, the EM treatment had great effects on the photosynthesis of water spinach (Table 2). It can be explained that the "stimulation" of music treatments can affect the yield of water spinach and water spinach may produce the physiological changes in the process of "enjoying" music, leading to the changes in growth capacity. In the soil culture environment, the combination of the PAFT treatment and the flooded condition generated the best effects on the shoot biomass among all the treatments, suggesting that sound wave treatments can further increase the yield of water spinach on the basis of water management.

5. Conclusions

This study demonstrated that sound wave treatments effectively enhanced water spinach biomass, and the treatments of PAFT and EM significantly reduced the Cd concentrations in roots and shoots (edible parts) of water spinach. The flooded treatment markedly decreased Cd in roots and shoots compared with the non-flooded condition, and the effects were further strengthened after the superposition of the PAFT treatment. The possible mechanism behind the effects on Cd in water spinach is likely to be that these treatments altered the root characteristics (root morphology, root porosity, and rates of ROL) and soil properties (extractable Cd, Eh, and pH). Our results suggest that the co-application of plant acoustic frequency technology and flooded management may be an effective way to reduce Cd accumulation in water spinach on the basis of promoting its yield. It is expected that the combination of sound wave and water management could be applied for vegetable cultivation in slightly Cd-contaminated soils and in hoop houses. Further investigations of field trials are needed to evaluate the effectiveness of the combined scheme.

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