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Research Article

Effect of Exogenous Ferrous Sulfate Treatment on Edible Rice

¹Yan Shi, ¹Shanshan Dong, ¹Zhouping Liu, ²Keke Yi, ²Junming Wang, ¹Cheng Zhu and ¹Feijuan Wang

¹College of Life Sciences, China Jiliang University, 310018 Hangzhou, China

²Zhejiang Academy of Agricultural Sciences, 310021 Hangzhou, China

Abstract

Background and Objective: Cadmium is one of the heavy metal elements in the farmland and rice pollution in China. Rice is one of the main food crops in China, how to reduce the accumulation of Cd in rice to ensure the safety of rice Cd has become a hot topic recently.

Methodology: This experiment mainly study the effects of exogenous $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ on the accumulation of Cd in edible rice, "Yongyou 9th" as study materials was selected through soil application of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. The field experiments were conducted in a highly Cd polluted area (Taizhou, Zhejiang). Study on the effects of exogenous $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ on the agronomic characters of rice the Cd content in different parts of rice and the soil properties. **Results:** The results showed that soil application of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ did not significantly affect the agronomic traits and yield of rice but it can reduce the accumulation of Cd in edible rice, among them the Cd content decreased by 21.80% in rice, exogenous $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ treatment increased soil organic matter and soil pH value significantly but also reduce the content of soil available Cd. The results showed that exogenous $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ through influencing the physical and chemical properties of soil and the content of available Cd in soil, so as to reduce the absorption and accumulation of Cd in different parts of rice.

Conclusion: Therefore, the exogenous $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ can be used as a potential and effective control technology for Cd pollution in edible rice. To a certain extent, it can ensure the safety of edible rice.

Key words: Edible rice, safety, Cd pollution, ferrous sulfate

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Corresponding Author: Feijuan Wang, College of Life Sciences, China Jiliang University, 310018 Hangzhou, China

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cadmium is a highly toxic effect on crops can produce environmental pollutants¹. Due to industrial pollution, mining and chemical fertilizer, it has been released into the soil, air and water. Agricultural soil Cd pollution is becoming more and more serious² and the soil is the main source of Cd in plants. The Cd in soil is easily absorbed by plants, while Cd is easy to enter the body through the food chain, so it causes a serious threat to human health³. Nowadays, the problem of Cd pollution in crops and vegetables has aroused people's concern about^{4,5}.

Rice (*Oryza sativa* L.) is an important crop in the world and it is one of the crops that are more easily absorbed and accumulated by Cd. The Cd pollution has a strong concealment and danger. It brings greater risk and harm to food safety and people's health. In recent years, the content of Cd in rice and its processed products has been detected exceeded frequently. Researchers randomly survey found that nearly 10% of the city's sale of rice Cd exceeded⁶. In China, Cd contaminated farmland is more than 1.3×10^5 km², Cd contaminated rice⁷ up to 5×10^4 t. The Cd pollution in farmland soil seriously affects the agricultural environment quality and food safety in China and has become an important factor restricting China's pollution-free rice. Therefore, it is an urgent problem to limit the content of Cd in rice grain and edible part.

In order to effectively reduce the risk of heavy metal Cd, people are trying to find safe and efficient foreign substances to reduce the toxicity of heavy metals. Previous studies have indicated that exogenous organic acids, such as organic acid can improve the growth of plants under heavy metal stress, enhance the resistance of plants and weaken the toxic effects of heavy metals⁸. The study on the effect of the reduction of soil pollution on grain production by the specific soil improvement measures has also made positive progress⁹. Combined with other agricultural improvement measures to reduce heavy metal accumulation in rice. Shen¹⁰ and Wu *et al.*¹¹ through the application of calcium magnesium phosphate improved the growth of rice and decreased Cd content in brown rice. However, little study is about the application of ferrous sulfate to control the accumulation of Cd in rice. In this study, the absorption characteristics of Cd and the accumulation of Cd in rice grain and the

corresponding changes of soil properties were studied after ferrous sulfate treatment in order to reduce the content of Cd in rice by improving soil properties. The study provides a certain basis for the realization of the safe production of rice in the middle and light heavy metal contaminated soil and it has a certain significance to ensure food security, ecological security and people's health.

MATERIALS AND METHODS

Test material: Yongyou 9th (High Cd accumulation of late rice, hybrid rice and whole growth period is 114 day), the seedlings provided by the Taizhou Academy of Agricultural Sciences test base. Field experiment was carried out in Zhejiang province with high heavy metal polluted area (Taizhou). The area is mainly affected by the pollution of Cd (Cd national standard is less than 0.20 mg kg^{-1}), the soil properties are shown in Table 1.

Layout and design of rice field planting: In the rice before transplanting, basal application of exogenous substance $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, CK means not to use any modifiers, water management is the conventional management. Based on the amount of ferrous sulfate, preferably 0.93 kg mu^{-1} . The field layout, each treatment area is $1.20 \times 1.80 \text{ m}$, treatment interval is 0.40 m , plant spacing is 0.30×0.20 , 50 Cong.

Sample collection and processing: Respectively in rice transplanting and maturity by five random sampling method to take soil samples, the sampling depth was 0-20 cm, each take 1.00 kg soil, after mixing, 1 kg is selected as the mixed sample by four point method and the mixed soil samples were air dried in the laboratory, after crushing with 2.50 mm nylon sieve to remove plant residues in soil and gravel, then with 100 purpose nylon sieve screening, take 200 g stored in dry place standby. Rice samples were collected at maturity stage, the rice samples with different treatments were collected and washed with tap water. The roots, stems, leaves and ears were divided into different parts. The grain samples were washed with distilled water, dried in the sun, hulling and milling into powder after bagging stored in dry place standby. Other parts of the sample sealed and arranged in 103°C oven fixing 1 h and then loaded into a sealed bag and stored.

Table 1: Soil physical and chemical properties

Soil	Texture	pH value	Total Cd	Available Fe	Organic matter	Available P	Available K	Available N
					(mg k g ⁻¹)			
Paddy soil	Loamy soil	5.08 ± 0.12	0.92 ± 0.08	120.85 ± 6.08	35.29 ± 2.38	10.23 ± 1.98	84.13 ± 7.41	78.32 ± 0.56

All data presented are the mean values of 6 replications

Agronomic analysis of rice samples: Agronomic traits including plant height, ear length, seed setting rate and 1000 grain weight were studied.

Determination of physical and chemical properties of soil:

The soil organic matter, nitrogen, effective phosphorus and potassium content were measured, respectively. The soil pH was measured by the glass electrode method and the soil and water ratio was 2.5:1 (NY/T 1121). The soil organic matter content is determined by the potassium dichromate method¹². Soil alkali and nitrogen is measured by alkali solution diffusion method, the content of available phosphorus in soil was determined by Mo-Sb colorimetric method. Soil available potassium content was determined by atomic absorption spectrophotometer, soil catalase activity was measured by using the potassium permanganate titration method¹³. The soil available Cd was extracted by 0.05 mol L⁻¹ Na-EDTA solution and determined by atomic absorption spectrometry. Soil available Fe was extracted by DTPA extraction (NY/T 890) and then was determined by atomic absorption spectrometry.

Analysis of Cd and Fe in soil and rice samples: Weigh 0.2 g of soil samples in the digestive tube, adding 6 mL GR HNO₃ and 2 mL HF, microwave digestion instrument (CEM-MARS) was used to remove the acid to 1 mL with ultra pure water to 25 mL. Weighing 0.30 g of rice samples in the digestive tube, adding 6 mL of HNO₃ and 0.20 mL H₂O₂, the same static 2-3 h later, using microwave digestion instrument digestion, catch

acid to 1 mL after the use of ultra pure water to 25 mL to obtain a clear liquid, stored at 4°C to prepare for further determination. Roots, stems, leaves and digestive methods are the same as in rice. Determination of the content of Cd and Fe in all tested liquids by atomic absorption spectrometry (AA-7000). At the same time, the soil and rice samples were spiked recovery test. The recovery rate of Cd and Fe were 98.50 and 102.30%, respectively.

Data processing method: Data processing and analysis of this study was carried out by using Excel 2003 and SPSS (Product and service solutions statistical, 18.0).

RESULTS

Effects of exogenous ferrous sulfate on agronomic characters of rice:

Table 2 shows that, after treatment with exogenous ferrous sulfate, the mature period of rice plant height, panicle length, dry weight, thousand-grain weight and seed setting rate did not change significantly. Exogenous ferrous sulfate treatment did not affect the growth and agronomic characters of rice plants.

Effects of exogenous ferrous sulfate on Cd content in different parts of rice:

From Fig. 1, it can be know that compared to the control group, the content of heavy metals Cd in rice grain was significantly decreased by exogenous ferrous sulfate treatment, the Cd content in grains decreased by 0.05 kg mg⁻¹. The relative decline of Cd content in rice grains was 21.80% after exogenous ferrous sulfate treatment. It can be explained that the exogenous application of ferrous sulfate can reduce the Cd content of rice grain and control the accumulation of Cd in rice grain.

As can be seen from Fig. 2, the accumulation characteristics of Cd in different parts of rice were as follows: Root>stem>leaf. Compared with the control group, after treatment of exogenous ferrous sulfate, the contents of Cd in root, stem and leaf of rice were decreased in different degrees. The treatment of exogenous ferrous sulfate reduced the accumulation of Cd in different parts of rice. Combined with the characteristics of Cd accumulation in rice grains treated with exogenous ferrous sulfate, the effect of ferrous sulfate on the accumulation of Cd in different parts of rice was also found.

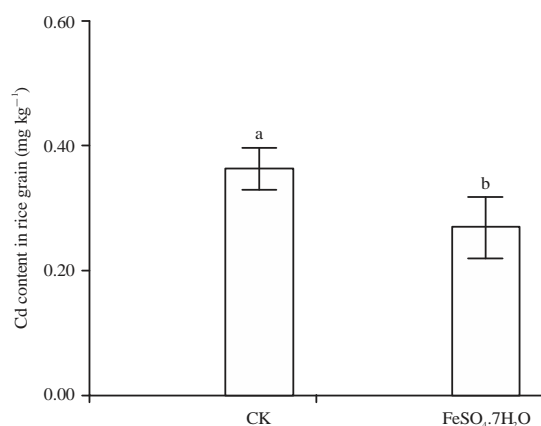


Fig. 1: Cadmium concentration in rice grain after exogenous substance treatment

Table 2: Agronomic characters of rice plants after exogenous substances treatment

Treatments	Plant height (cm)	Panicle length (cm)	Dry weight (g)	Seed setting rate (%)	Thousand grain weight (g)
CK	111.86±5.59 ^a	22.83±0.83 ^a	228.78±4.26 ^a	92.70±1.32 ^a	27.89±0.43 ^a
FeSO ₄ ·7H ₂ O	110.70±3.01 ^a	23.67±0.67 ^a	231.67±15.44 ^a	94.10±0.38 ^a	28.42±0.09 ^a

All data presented are the mean values of 6 replications. Within each column the values followed by the same letter have no difference at the 5% level

Changes of soil properties after treatment with exogenous ferrous sulfate:

From Table 3, it can be found that, after soil application of ferrous sulfate, the content of soil organic matter and soil pH value were significantly increased, soil organic matter relative to the control group increased about 10.10 mg kg⁻¹, pH relative increase of about 0.50, at the same time, soil available Fe content increased significantly and soil enzyme activity did not occur significant changes. That is to say, the application of ferrous sulfate to the soil can affect the partial properties of the soil, so as to affect the accumulation of Cd in rice.

Effects of exogenous ferrous sulfate on the availability of Cd in soils:

As can be seen from Fig. 3, the total Cd content in the tested soil was about 0.92 mg kg⁻¹ and the total Cd content in the soil was not significantly different after the ferrous sulfate application. After soil application of ferrous sulfate, the content of available Cd in soil decreased significantly and the content of available Cd in the control group was about 0.30 mg kg⁻¹, the content of available Cd decreased by about 0.07 mg kg⁻¹. It can be speculated that the soil could be activated by partial Cd in the soil, so as to reduce the absorption of Cd in rice.

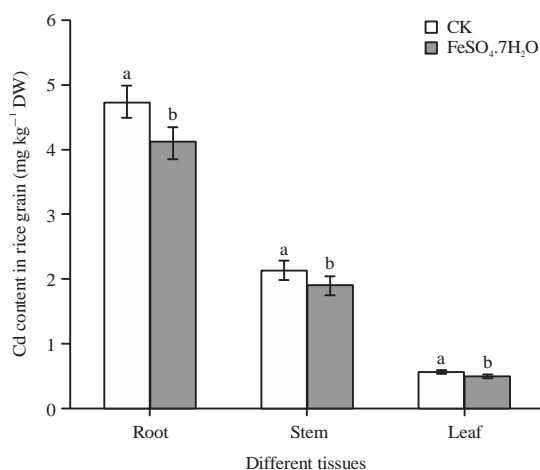


Fig. 2: Concentration Cd in different tissues after exogenous substance treatment

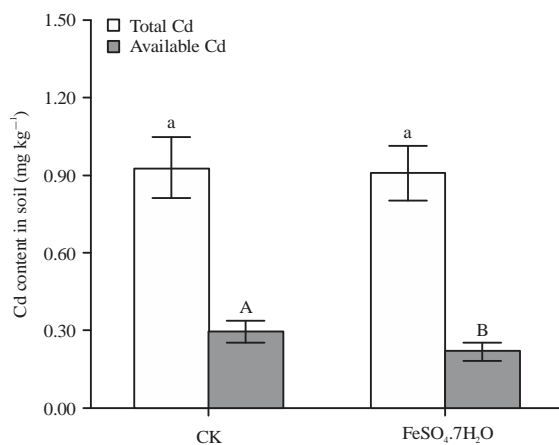


Fig. 3: Available Cd concentration in soil after exogenous substance treatment

Effect of exogenous ferrous sulfate on Fe content in rice:

It can be seen from Fig. 4 in rice, compared with the control group, the Fe content in rice was significantly increased after the exogenous application of FeSO₄·7H₂O, the Fe content was increased by 5.94 mg kg⁻¹ or so. The FeSO₄·7H₂O were applied to promote the absorption of Fe elements and to a certain extent, the nutritional quality of rice was improved.

DISCUSSION

The experimental results show that the exogenous ferrous sulfate treatment effects on agronomic traits of rice is not significant, soil application of ferrous sulfate reduced the accumulation of Cd in rice but also reduces the Cd content in different parts, namely the soil application of ferrous sulfate can tackle heavy metals from soil to root or root to shoot migration.

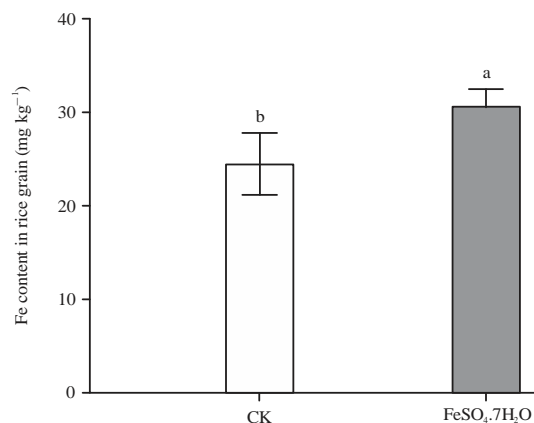


Fig. 4: Iron concentration in rice after FeSO₄·7H₂O treatment

Table 3: Change of soil organic matter content, available Fe concentration, soil pH and enzyme activity

Treatments	Soil organic matter (mg kg ⁻¹)	pH value	Available Fe concentration (mg kg ⁻¹)	Enzyme activity in soil (μmol L ⁻¹)
CK	37.22±0.16 ^b	5.10±0.05 ^b	117.12±7.16 ^b	1.62±0.01 ^a
FeSO ₄ ·7H ₂ O	46.31±0.38 ^a	5.51±0.03 ^a	133.05±8.38 ^a	1.59±0.03 ^a

All data presented are the mean values of 6 replications. Within each column the values followed by the same letter have no difference at the 5% level

The experimental results show that, after ferrous sulfate treatment, the pH value of soil was up the content of soil organic matter increased significantly, the content of available Cd in soil was significantly lower than that in control group. It is that the soil could change some properties of soil and reduce the absorption of rice to Cd, so that the accumulation of Cd in rice could be reduced. At the same time, the study showed that the soil heavy metal speciation and availability and the absorption capacity of plants to heavy metals are affected by soil pH¹². Soil pH value can regulate the chemical speciation and adsorption-desorption behaviors of Cd in soil¹³. With increase of soil pH value, the negative charge of soil colloid increases and the competition ability of H⁺ ion is weakened, heavy metals are combined firmly, eventually with the hydroxide, carbonate and phosphate insoluble form of increasing, thus reducing the Cd. The results of previous study also showed that the soil pH can affect the bioavailability of heavy metals in soil and the absorption of Cd in rice^{3,13}. It can be concluded that exogenous application of ferrous sulfate improved the soil pH, the soil colloids increased negative charge, to weaken the competition competence of H⁺ ions. The Cd was firmly with the hydroxide, carbonate and phosphate insoluble form, soil available Cd content decreased, thus affecting the biological availability of soil Cd and ultimately affect the absorption of Cd in rice.

Organic matter is one of the main factors that affect the exchangeable capacity of soil Cd. The study of Hettiarachchi¹⁴ showed that with the decrease of soil organic matter content, the adsorption capacity of Cd decreased significantly. The study of Singh and Pandeya¹⁵ also pointed out that the soil organic matter can significantly reduce the soil heavy metal elements and then reduce the amount of heavy metals in the crop. In this experiment, the application of exogenous affect the soil organic matter content, with the increase of soil organic matter content, the ability of the soil to adsorb heavy metals increased and the content of available Cd in the soil decreased, namely the bioavailability of Cd was decreased and the content of heavy metal Cd in rice was decreased. Researchers also found that after taking the organic matter into the contaminated soil, the bioavailability of heavy metals in soil reduced, the accumulation of heavy metals in plant body also reduced¹⁶.

Some studies have showed that Cd can compete with Ca²⁺, Fe²⁺ and other cationic competitive adsorption sites, interacting with their binding sites into the plant body and ultimately affect the Cd content of rice¹⁷. In this experiment,

after the application of ferrous sulfate, the content of soil Fe²⁺ increased, may inhibit the Cd competitive adsorption site, the adsorption sites of Cd²⁺ reduced, thus reducing the Cd into the plant body, so that the content of Cd in different parts of rice and rice was decreased.

Under a certain pH value, iron oxides are fixed in the soil by precipitation and adsorption¹⁸ Cd²⁺. In the presence of a large amount of reducing substances (such as Fe²⁺, Mn²⁺) oxidation ($4\text{Fe}^{2+} + \text{O}_2 + 10\text{H}_2\text{O} = 4\text{Fe}(\text{OH})_3 + 8\text{H}^+$), resulting in the formation of iron oxides in the root of the formation of iron film¹⁹. The root surface of rice is formed by iron oxide film, which can affect the bioavailability of Cd element in soil by adsorption and co-precipitation²⁰ and the experimental soil of ferrous sulfate happen to provide the soil with Fe²⁺ can inhibit the reduction of soil. The Cd adsorption or the occurrence of coprecipitation with Fe(OH)₃, while the amount of addition of Fe²⁺ increased the iron oxides in soil and root surface iron film, by adsorption and precipitation of the Cd in the soil is fixed, thus reducing the soil Cd behavior and bioavailability, reduce the uptake of Cd in rice roots.

CONCLUSION

In the height of Cd contaminated soil, soil applying FeSO₄·7H₂O can reduce the Cd content in rice and not affect the yield of rice. Exogenous FeSO₄·7H₂O reduced the content of Cd in rice by 21.80%. Compared with the control group, FeSO₄·7H₂O decreased the Cd content in root, stem and leaf of rice. Exogenous FeSO₄·7H₂O treatment also significantly reduced the soil available Cd content, after treatment of exogenous FeSO₄·7H₂O, the soil organic matter and soil pH value were significantly increased and the bioavailability of heavy metals in soil decreased, rice uptake and accumulation of Cd content also decreased. The results showed that soil FeSO₄·7H₂O were effective methods to prevent and control rice Cd uptake.

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