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Inhibitory effects of reactive X-3B red dye (RRD) on iron uptake by three crops

Qixing Zhou[1,4], Jiru Xu[2] & Yun Cheng[3]
[1]*Key Laboratory of Terrestrial Ecological Process, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, People's Republic of China.* [2]*Northern Ireland Public Health Laboratory, Belfast City Hospital, Belfast BT9 7AD, United Kingdom.* [3]*Department of Soil and Water, Florida University, U.S.A.* [4]*Corresponding author**

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Abstract

Previous field investigations and a pot-culture experiment treated with 0, 5, 10, and 20 mg kg^{-1} of reactive X-3B red dye (RRD), combined with chemical and statistical analyses, indicated that the amount of iron accumulated in seeds of soybean (*Glycine max*) and rice (*Oryza sativa*) and edible parts of watermelon (*Citrullus Vulgaris Schrad*) decreased with the increase in the amount of RRD added to various soils including burozem, aquorizem and krasnozem. There were significant negative relationships between the uptake of iron by the crops and the concentration of RRD added to the soils. Thus, chemical pollution of some organic dyes in agricultural soils may result in the deficiency of iron in crops. According to the extraction with 1.0 M NH_4OAc solution and the determination of enzyme activity, the adverse effect inhibiting the uptake of iron in crops was chemical chelation and immobilization of iron with RRD. The activity of iron reductase was also greatly affected.

Introduction

Since the synthesis and manufacture of a purple dye by W. H. Perkin in 1856 (Waring and Hallas, 1990), various types of synthetic dyes have been continuously developed and applied (McLaren, 1983; Waring and Hallas, 1990; CMCI, 1995). Currently, the annual consumption level of organic dyes in the world is 8.1 × 10^8 kg (CMCI, 1995; Wang, 1995). Organic dyes have been regarded as an important type of pollutants influencing ecosystems and food security in the world (Zhou and Wang, 1997; Aplin and Waite, 2000; Zhou, 2001). In recent years, organic dye pollution of the soil and water environment in China has been of increasing concern with the rapid development of rural textiles, printing, dyeing and synthetic dye industries of the country. According to our investigations from 1992 to 2000, the range of the concentration of organic dyes in polluted surface water and soils was 6–706 mg L^{-1} and 0.9–3114.0 mg kg^{-1}, respectively (Zhou, 2001). In particular, the concentration of reactive X-3B red dye (RRD) in polluted surface water and soils was up to 0.01–1.23 mg L^{-1} and 0.02–31.8 mg kg^{-1}, respectively (Zhou, 2001; Zhou and Huang, 2001). We estimate the area of agricultural soils polluted by organic dyes is annually increased at the rate of 1.3 × 10^5 ha in eastern Chinese coastal areas (Zhou and Huang, 2001). Unfortunately, there is no literature about soils contaminated with RRD in areas other than China, although organic dye pollution has taken place in the water and soils of other Asian countries such as India, Thailand and Japan according to our recent investigations.

Because some organic dyes and their degradation products are suspected human carcinogens, attention has been paid to research on the treatment of organic dyes in wastewater (Aplin and Waite, 2000; Hao et al., 2000; Kang et al., 2000). There is a large amount of literature about the decolorization of wastewater from printing, dyeing, and synthetic dye industry (Meehan et al., 2000; Panswad and Luangdilok, 2000). Be-

*FAX No: +86-24-83970436/83970300.
E-mail: Zhouqixing2003@yahoo.com/Zhouq@mail.sy.ln.cn

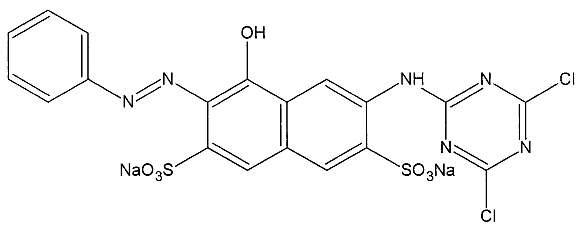
havior, transformation, and environmental effects of heavy metals such as mercury (Hg) and arsenic (As) and some organic pollutants such as hydrocarbons, PAHs, PCBs and DDT in soils, including adverse effects of these organic pollutants and heavy metals on plant growth and nutrient uptake, have also been well-documented (Wang, 1995; He et al., 1998; Sun et al., 2001; Zhou and Huang, 2001; Abadía et al., 2002; Liu et al., 2002; Song et al., 2002; Zhou, in press). However, few reports are available on behavior, transformation and environmental effects of organic dyes in soils and effects of organic dyes on plant growth and nutrient uptake (Wang, 1995; Stock et al., 2000; Sun et al., 2001). In particular, influences of organic dyes in agricultural soils on the quality of crops remain unclear.

In our previous field investigations, it was accidentally found that the concentration of iron in edible parts of watermelon and soybean seeds was decreased with the increase in the concentration of organic dyes in irrigation water and the accumulation of organic dyes in soils. This is a concern because iron is an essential element for human health and the long-term deficiency of iron in food, agricultural products and crops can result in anaemia of adults and mental retardation of children (Abadía et al., 2002; Buttriss and Hughes, 2002; Hooda et al., 2002). An experiment to verify the negative interrelationship between the uptake of iron by crops and enrichment of organic dyes in agricultural soils was carried out using the pot-culture method combined with chemical and statistical analyses.

Materials and methods

Tested organic dye

Reactive X-3B red dye (RRD) whose trade name is Basilen red M-5B (BASF) or Intracron red C-5B (CKC) was obtained from the Second Sheyang Factory of Synthetic Dye and its purity is 99.5%. The molecular formula of the dye is $C_{19}H_{10}C_{12}N_6Na_2O_7S_2$ and its structural formula is as follows:



Sampling

Three representative soil types, burozem (brunisolic soil), aquorizem (paddy soil) and krasnozem (red soil), were respectively sampled from a site (40°19′35″ N, 122°22′05″ E) of Yingkou County in Liaoning Province, a suburb area (29°59′08″ N, 120°35′29″ E) of Shaoxing in Zhejiang Province and a suburb area (23°10′55″ N, 113°16′15″ E) of Guangzhou in Guangdong Province. The sampling depths were 0–20 cm. Basic physical and chemical properties of these surface soils are listed in Table 1. Total concentration of iron in the soils was in the sequence krasnozem > burozem > aquorizem.

Pot-culture experiment

Doses of RRD added to these soils were chosen according to a previous investigation (Zhou, 2001). The known amount of soil was mixed with one of three doses of RRD and was put into pots marked with numbers. Control experiments were carried out without the addition of the dye to the soils. Thirty rice (*Oryza sativa*) seeds were sown in each pot filled with aquorizem samples, 30 soybean (*Glycine max*) seeds were sown in each pot filled with burozem samples, and 6 seedlings of watermelon (*Citrullus Vulgaris Schrad*) were planted in each pot filled with krasnozem samples. The three doses of RRD were 5, 10 and 20 mg kg^{-1} air-dried soil, respectively. In order to compare crops in all soils, the three species were sown in pots filled with krasnozem, burozem or aquorizem samples containing 20 mg kg^{-1} of RRD. All the treatments were replicated four times, and each replicate consisted of fresh soil samples whose air-dried weight equivalent was 2.5 kg.

According to the water demand of the crops, the soils in the pots planted with rice, soybean and watermelon were moistened using 100, 50, and 70 mL of tap water every three days. At the trifoliate (the first three leaves) stage, some rice and soybean seedlings were pulled out for analyses. Only 15 rice and soybean seedlings were grown to maturity in each pot. Two seedlings of watermelon in each pot were also pulled out for analyses. After the rice and soybean had matured, their seeds were harvested, washed, rinsed, air-dried and ground. Ripe watermelons were picked and their edible parts were air-dried and ground. The ground samples were then analyzed for total iron using atomic absorption spectrophotometry (AAS) after digestion in concentrated HNO_3/HCl_4 (Yang, 1993; NAU, 1996; Zhou, 1996).

Table 1. Physical and chemical properties of the tested surface soil samples

Soil type	pH (H_2O)	Organic matter (%)	Clay content (%) <0.001 mm	Clay content (%) <0.01 mm	CEC [Meq $(100g)^{-1}$ soil]	Total Fe (%)
Burozem	6.5	2.31	10.3	18.0	12.05	4.55
Aquorizem	6.8	2.97	24.1	38.6	19.67	4.01
Krasnozem	5.3	2.37	17.2	32.7	8.71	6.49

Chemical extraction

Ten g of air-dried soil samples were placed in bottles with 40 mL of 1.0 N ammonium acetate solution (pH 4.8), and the samples were shaken for 30 min on a reciprocating shaker at 150 rev min^{-1} and 25 °C. The soil to extracting solution ratio was 1:4. The suspensions were filtered through 0.45 μm pore size membrane filter. The filtrates were analyzed for iron using the AAS method.

Iron-reductase determination

The activity of iron reductase was determined according to Zhang et al. (1988). The procedure was: 30 mg iron oxide was thinly ground and 2.5 g air-dried soil passed through a 0.25 mm sieve was placed in vacuum bottles. After they were fully mixed, 3.5 mL of distilled water and 3.5 mL of 1.0% (W/V) glucose solution were added. The bottles were capped and the air removed with a vacuum pump and shaken several times. Controls were soil samples without the addition of iron oxide. All the samples were cultured for 48 h in an incubator at 30 °C. Then 45 mL of 1 N H_2SO_4 solution was added, and the samples were shaken for 10 min on a reciprocating shaker at 150 rev min^{-1} at room temperature. The suspensions were filtered and the filtrates were analyzed for iron using the AAS method.

Statistical analyses

Four replicates were used to determine concentrations of iron in seeds of soybean and rice and edible parts of watermelon, and the chemically extracted iron from the tested soils, and the activity of iron reductase in the tested soils, and their standard deviations (SD). Regression analysis was used to test the relationships between the uptake of iron by the crops and the concentration of RRD added to the tested soils, chemically extracted iron of the soils and the activity of iron reductase in the soils. All the statistical analyses were carried out using Microsoft EXCEL 97.

Results and discussion

Uptake of iron in crops

Adverse effects of RRD on the absorption of iron by the three crops were observed under the pot-culture conditions. The mean concentration of iron accumulated in seeds of soybean and rice and edible parts of watermelon was decreased in the soils treated by RRD significantly (Table 2), compared with the controls. The uptake of iron by the crops was strongly inhibited when the concentration of RRD added to the soils was 20 mg kg^{-1}. The mean concentration of iron accumulated in seeds of soybean growing on burozem, rice growing on aquorizem, and edible parts of watermelon growing on krasnozem, was only 77.1, 25.7 and 10.8 mg kg^{-1}, respectively. Under the control conditions, the mean concentration of iron accumulated in seeds of soybean growing on burozem and rice growing on aquorizem and edible parts of watermelon growing on krasnozem was 126.3, 45.7 and 36.4 mg kg^{-1}. The accumulation of iron in seeds of soybean growing on burozem mixed with RRD had the most reduction among all the treatments. Thus, planting of soybean should be avoided on burozem land contaminated with RRD.

The concentration of iron in plant tissues decreased with the increase in concentration of RRD added to these soils. Significant negative linear relationships are expressed by regression equations in Figure 1, where $Y_c(1)$, $Y_c(2)$ and $Y_c(3)$ are the concentration (mg kg^{-1}) of iron accumulated in seeds of soybean growing on burozem, rice growing on aquorizem, and edible parts of watermelon growing on krasnozem, respectively; X_1, X_2 and X_3 are the concentration (mg kg^{-1}) of RRD added to burozem, aquorizem and krasnozem, respectively. This trend was consistent with our previous investigations.

Table 2. Absorption of iron in seeds of soybean and rice and edible parts of watermelon under the tested conditions, expressed as mg kg^{-1} air-dried tissue

Crop tissue	Concentration of RRD added to soils (mg kg^{-1})			
(Soil type)	0	5	10	20
Soybean seed (Burozem)	126.3 ± 15.34	104.8 ± 15.36	89.1 ± 9.99	77.1 ± 13.07
Rice seed (Aquorizem)	45.7 ± 6.45	37.1 ± 4.28	31.1 ± 4.93	25.7 ± 5.06
Edible parts of Watermelon (Krasnozem)	36.4 ± 6.73	26.8 ± 6.50	18.3 ± 4.60	10.8 ± 4.11

Table 3. Chemically extracted iron from the tested soils using 1.0 N NH_4Ac solution under various treatments ($n = 16$), expressed as mg kg^{-1} air-dried soil

Soil type	Concentration of RRD added to soils (mg kg^{-1})			
	0	5	10	20
Burozem	6.38 ± 0.71	5.42 ± 0.88	4.67 ± 0.60	3.98 ± 0.97
Krasnozem	3.52 ± 0.46	3.12 ± 0.58	2.56 ± 0.38	1.69 ± 0.53
Aquorizem	8.94 ± 0.86	7.80 ± 0.89	6.59 ± 0.80	5.22 ± 1.22

Soil types affected the accumulation of iron. Iron uptake by the crops growing on aquorizem was the highest among the three soil types. The mean concentration of iron in seeds of soybean and rice and edible parts of watermelon from aquorizem without the addition of RRD was 131.7, 45.7 and 38.0 mg kg^{-1}, respectively. When the concentration of RRD added to the soil was 20 mg kg^{-1}, the mean concentration of iron in seeds of soybean and rice and edible parts of watermelon from aquorizem was still the highest among the three soil types, equal to 79.0, 25.7 and 12.4 mg kg^{-1}, respectively. Iron uptake by the crops growing on krasnozem was the lowest among the three soil types. The mean concentration of iron in seeds of soybean and rice and edible parts of watermelon from krasnozem without RRD was 108.5, 41.7 and 36.4 mg kg^{-1}, respectively. When RRD was added to the soil at 20 mg kg^{-1}, the mean concentrations were still lower, only 71.5, 22.0 and 10.8 mg kg^{-1}, respectively. Iron uptake by the same type of the crops growing on the different soils was in the sequence aquorizem > burozem > krasnozem, and was not dependent on the concentration of total iron in the tested soils.

The decrease in iron in seeds of rice and edible parts of watermelon due to the effect of 20 mg kg^{-1} RRD, was the greatest on krasnozem among the tested soil types. The decrease in iron in seeds of soybean growing on aquorizem mixed with 20 mg kg^{-1} of RRD was the highest among the tested soil types. Rice and watermelon should not be planted in krasnozem contaminated by RRD, and soybean should not be planted in aquorizem contaminated by RRD.

The absorption of iron depended on the crops themselves. Soybean plants displayed the highest ability to absorb iron from the tested soils. The accumulation of iron in watermelon was the lowest among the crops. The decrease in the accumulation of iron in edible parts of watermelon growing on the soils due to 20 mg kg^{-1} of RRD was the highest among the tested crops. According to the experimental data, iron in edible parts in watermelon was most easily affected by RRD. Thus, watermelon crops should not be grown on land contaminated with RRD.

Chemical mechanisms

Usually the bioavailability of iron in soils can be accurately quantified using chemical extraction (NAU, 1996). Extraction of burozem, aquorizem and krasnzem with 1.0 N NH_4Ac solution without the addition of RRD was 6.38, 8.94 and 3.52 mg kg^{-1}, respectively. Chemically extracted iron of the soils decreased (Table 3) after RRD was added to the soils. At 20 mg kg^{-1} of RRD chemically extracted iron in burozem, aquorizem and krasnzem was reduced to 3.98, 5.22 and 1.69 mg kg^{-1}, respectively. There were significant negative linear relationships between chemically extracted iron and the concentration of RRD added to the soils described using regression

Table 4. The activity of iron reductase in the tested soils under various treatments ($n = 16$), expressed as mg (Fe_2O_3) g^{-1}(soil)

Soil type	Concentration of RRD added to soils (mg kg^{-1})			
	0	5	10	20
Burozem	1.58 ± 0.18	1.42 ± 0.13	1.29 ± 0.13	1.15 ± 0.17
Krasnozem	1.32 ± 0.16	1.19 ± 0.05	1.04 ± 0.15	0.85 ± 0.07
Aquorizem	2.27 ± 0.21	2.09 ± 0.14	1.88 ± 0.18	1.69 ± 0.23

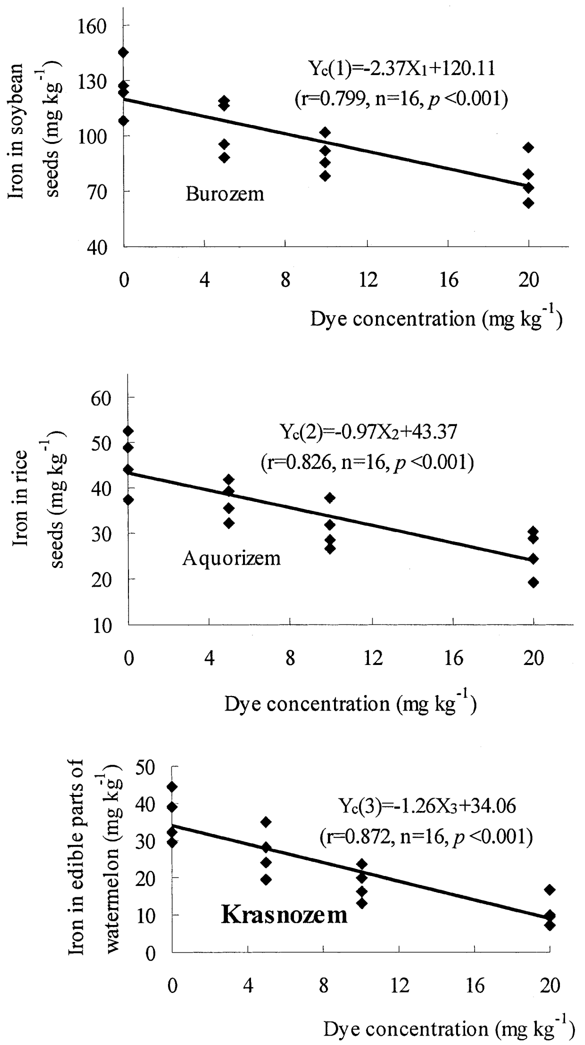


Fig. 1. Negative linear relationships between uptake of iron in the crops and concentration of RRD added to the tested soils.

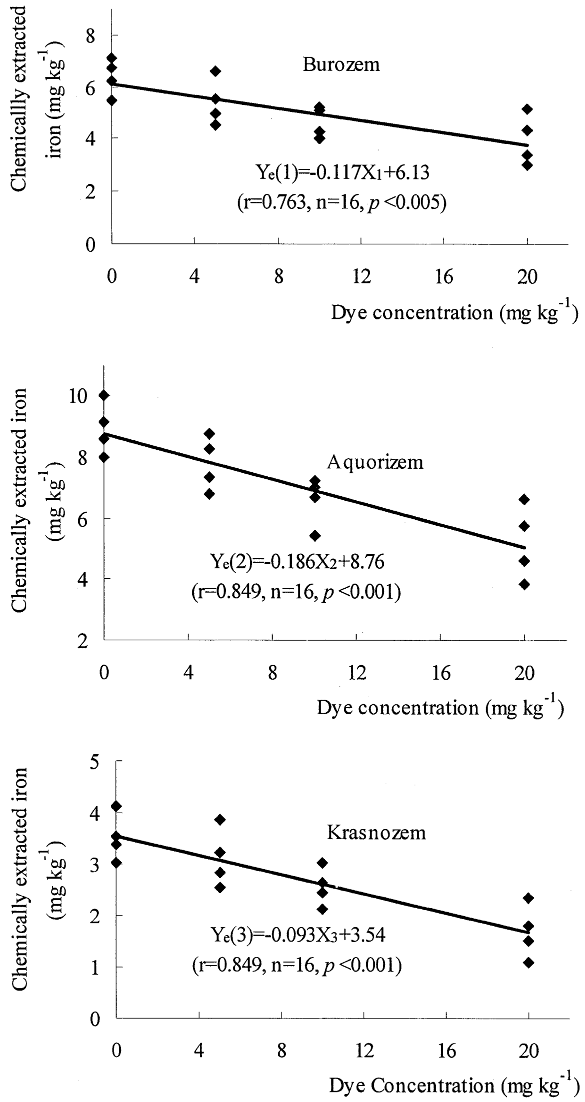


Fig. 2. Negative linear relationships between chemically extracted iron of the soils and the concentration of RRD added to the tested soils.

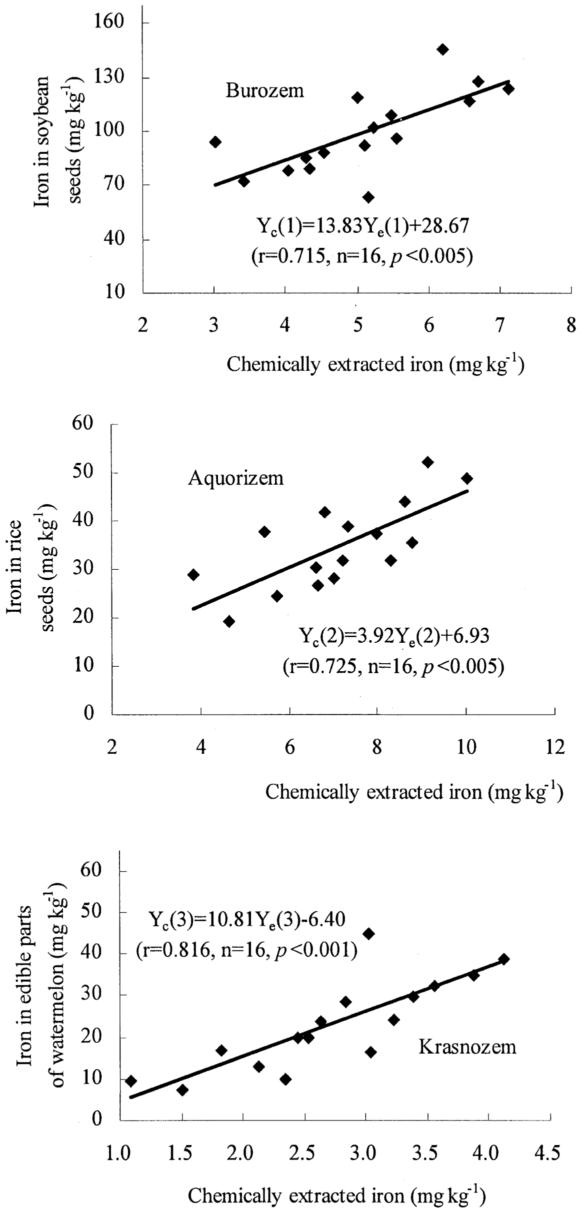


Fig. 3. Positive relationships between uptake of iron in the crops and chemically extracted iron of the soils.

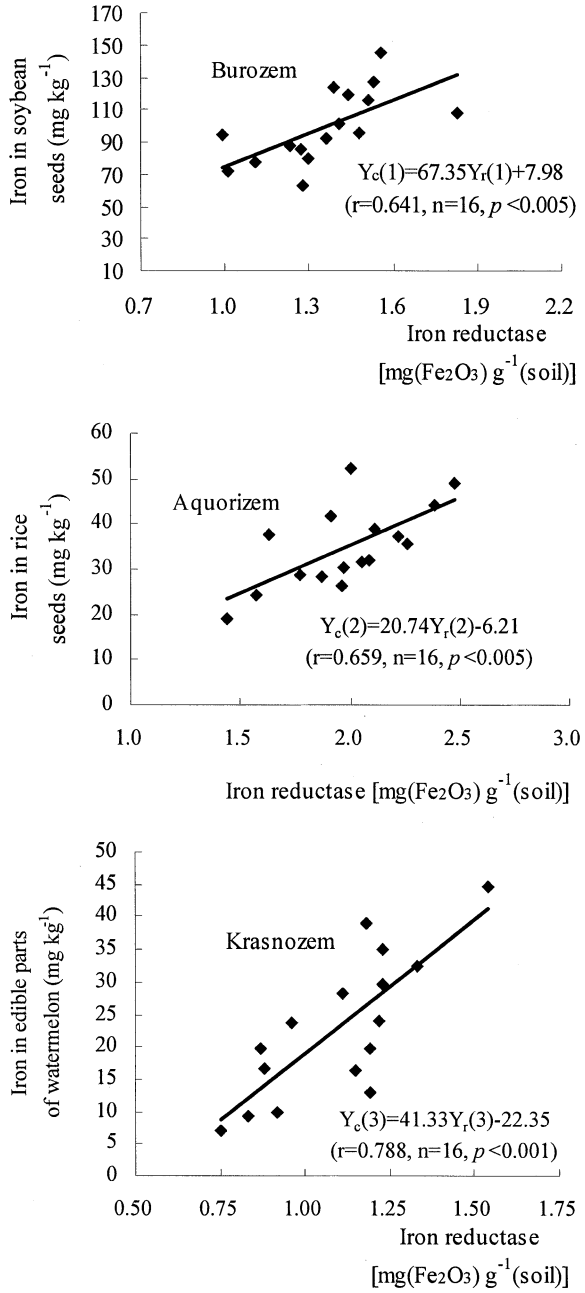


Fig. 4. Positive relationships between the uptake of iron by crops and the activity of iron reductase in the soils.

equations in Figure 2, where $Y_e(1)$, $Y_e(2)$ and $Y_e(3)$ are the concentration of iron extracted from burozem, aquorizem and krasnozem, respectively.

In principle, some chemically extracted iron, such as Fe^{2+}, could be chelated and immobilized by RRD. The possible chemical mechanism is

$$Fe^{2+}\ (\text{Available Fe}) + R_{Dye}{}^{2-} \rightarrow [R_{Dye} \cdots\cdots Fe]^0\ (\text{Unavailable Fe}) \quad (1)$$

Thus, chemical chelation and immobilization of iron by RRD restricts the bioavailability of iron in soils after an addition of the dye.

Regression analysis indicated that the uptake of iron by the crops was linearly correlated with chemically extracted iron (Figure 3). According to these relationships, the decrease in uptake of iron by the crops could result from the reduction of chemically extracted iron by RRD added to the soils.

Extraction with 1.0 N NH_4OAc suggested that chemically extracted iron of aquorizem was the highest among the three soils. Chemically extracted iron of krasnozem was the lowest among the three soils even though it had the highest total iron. Moreover, there was no significant correlation between the uptake of iron by the crops and total iron. Only chemically extracted iron was significant to explain the relationships between iron uptake by crops and the bioavailability of iron in soils, and the inhibition of RRD on the uptake of iron by the crops.

Biochemical mechanisms

In soils, Fe^{3+} often occurs in insoluble forms [Fe_2S_3, Fe_2O_3, $Fe(OH)_3$, $Fe_2(SO_4)_3$ and $FePO_4$] and that are unavailable to crops (He et al., 1998). Due to the catalysis of iron reductase, Fe^{3+} can be reduced to Fe^{2+} (Gaspard et al., 1998). For example:

$$Fe_2S_3\ (S) + 6H^+ + 2e \xrightarrow{\text{Iron rductase}} 2Fe^{2+} + 3H_2S^- \quad (2)$$

Generally speaking, Fe^{2+} can be easily utilized by crops (NAU, 1980; Zhang et al., 1988). A decrease in the activity of iron reductase in soils can inhibit the transformation from Fe^{3+} to Fe^{2+}, and the uptake of iron by crops.

Table 4 indicates the activity of iron reductase was decreased by increasing the concentration of RRD added to the soils. When the concentration was 5 mg kg^{-1}, the mean activity of iron reductase in burozem, krasnozem and aquorizem was respectively reduced to 89.9, 90.2 and 92.1% of the mean activity without RRD. When the concentration was increased to 10 mg kg^{-1}, the mean activity of iron reductase in the three soils was further decreased to 81.6, 78.8 and 82.8% of the mean activity under the control conditions, respectively. When RRD was added to the soils at 20 mg kg^{-1}, the mean activity of iron reductase in the three soils was only 27.2, 35.6 and 25.6% of the mean activity under the control conditions, respectively.

The uptake of iron by crops decreased with the decrease in the activity of iron reductase in the soils. The significant positive linear relationships between iron uptake by the crops and the activity of iron reductase was described using regression equations in Figure 4, where $Y_r(1)$, $Y_r(2)$ and $Y_r(3)$ are the mean activity of iron reductase in burozem, aquorizem and krasnozem, respectively. These linear relationships indicate that the decrease in the uptake of iron by the crops was closely related to the inhibition of iron reductase caused by RRD added to the soils. In this sense, the activity of iron reductase in the soils is a good indicator for the bioavailability of iron in the soils.

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