Effects of Soil Copper Concentration on Growth, Development and Yield Formation of Rice (*Oryza sativa*)

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Abstract: Pot experiments were conducted in 2002 and 2003 to investigate the effects of soil copper(Cu) concentration on growth, development and yield formation of rice by using the japonica cultivar Wuxiangjing 14 and hybrid rice combination Shanyou 63. The plant height, leaf number, elongated internode number and heading date of rice plants were not affected at soil Cu levels below 200 mg/kg, but affected significantly at above 400 mg/kg. The inhibitory effects on rice growth and development were increased with the increment of soil Cu levels. The grain yields decreased significantly with raising soil Cu levels. The main reasons for the grain yield reductions under lower soil Cu levels (100, 200 mg/kg) were mainly due to the decrease of number of spikelets per panicle, however, under higher soil Cu levels (more than 400 mg/kg), both panicle number and number of spikelets per panicle contributed to the yield loss. The decreases of panicle number by Cu stress were mainly attributed to slow recovery from transplanting, delayed tillering and reduced maximum tiller numbers. The reduction of number of spikelets per panicle under soil Cu stress resulted from the decreases of both shoot dry weight (SDW) at the heading date and the ratio of spikelets to SDW. Total biomass at maturity decreased significantly with the increase of soil Cu levels, while economic coefficient showed non-significant decrease except under soil Cu levels above 800 mg/kg.

Key words: rice; soil Cu concentration; growth and development; yield

Copper (Cu) level is one of the eight heavy metal manipulative indices in agricultural soil environment evaluation in China¹¹. The second and third soil quality standard in Cu concentrations were 100, 400 mg/kg (pH 6.5–7.5), respectively. Owing to quick development of modern industry and the widespread use of pesticides containing Cu additives, the concentration of Cu in agricultural soils around the world increased rapidly, and at some sites average soil Cu levels have already exceeded 1000 mg/kg²⁻⁴. Several studies have been reported about the effects of soil Cu stress on rice growth and development⁵⁻¹³. However, the mechanism of grain yield reduction by soil Cu stress remained unclear. Soil Cu levels designed in many previous experiments were low, generally less than 250 mg/kg, far below the third quality criterion of soil environment in China (400 mg/kg). To find out the reasons of grain yield loss under soil Cu stress, we conducted two pot experiments to investigate the effects of soil Cu concentration between 100 to 1000 mg/kg on rice growth and development and grain yield formation and to provide a basis for establishment of rice cultivation system in Cu contaminated areas.

**MATERIALS AND METHODS**

**Plant materials and Cu treatments**

*Experiment 1* The pot experiment was conducted at Agricultural and Forestry Bureau of Changzhou (30°41’ N, 119º50’ E) in 2002. The test rice cultivar was Wuxiangjing 14, an early-maturity late-season japonica cultivar. The soil for pot experiment was a silty loam with the alkali-hydrolysable N, P₂O₅, K₂O and total Cu contents 80.5, 10.2, 82.1 and 37.4 mg/kg, respectively. Ten-kilogram of soil was placed in each pot (26 cm in diameter and 28 cm in height). CuCl₂·3H₂O was added to the soil to obtain a series of soil Cu levels of 100, 150, 300, 500 and 1000 mg/kg, with 20 replicates. The thoroughly mixed soil...
was submerged in water for one month prior to the transplanting of seedlings. Seeds were sown in uncontaminated paddy field on 22 May, and seedlings were hand-transplanted into the pots (3 plants per pot) on 22 June. Fertilizers were applied as a basal dressing on 21 June (2.0 g N, 1.2 g P$_2$O$_5$, 2.0 g K$_2$O per pot), and at panicle initiation (PI) on 31 July (1.0 g N per pot). The pots were flooded with a water layer of 4 cm during the whole growth period.

Experiment 2 The pot experiment was conducted at Yangzhou University (32°30′ N, 119°25′ E) in 2003. The test rice cultivars were Wuxiangjing 14 and Shanyou 63, a mid-maturity indica hybrid combination. The soil for the pot experiments was a loam, with alkali-hydrolysable N, P$_2$O$_5$, K$_2$O and total Cu contents 110.5, 16.4, 96.5 and 75.4 mg/kg, respectively. Ten kilogram of soil was placed in each pot (25 cm in diameter and 30 cm in height). CuCl$_2$·3H$_2$O as Cu source was added to the soil to establish a series of soil Cu levels of 100, 200, 400, 600, 800 and 1000 mg/kg, with 20 replicates. The thoroughly mixed soil was submerged in water for one month before rice seedlings were transplanted in. Seeds were sown in uncontaminated paddy field on 15 May, and seedlings were hand-transplanted into the pots (3 plants per pot) on 8 June. Fertilizers were applied as a basal dressing on 7 June (1.0 g N, 1.0 g P$_2$O$_5$, 1.0 g K$_2$O per pot), and at PI on 5 August (0.5 g N per pot). Water management was the same as Experiment 1.

Samples preparation and analysis methods

Leaf number on the main stem, tiller number and plant height were investigated every 8 d after transplanting. Heading date for each treatment was recorded when 50% of plants headed.

Four pots for each treatment (two replicates) were sampled at maturity to determine the dry matter of root, leaf blade, shoot (including leaf sheath) and panicle, as well as the elongated internode number on the main stem.

The samples harvested at maturity were also measured for yield components, i.e. panicle number, number of spikelets per panicle, 1000-grain weight, and filled grain percentage. Fertile spikelets were selected by water floating and counted by manual. The 1000-grain weight of fertile spikelets was determined after drying at 80°C in oven for 72 h.

RESULTS

Effect of Cu on growth and development of rice

Effect of Cu on plant height

Compared to the control, the plant height at maturity was decreased by 4.1, 6.6, 20.6, 27.4, 37.2 and 48.4% at soil Cu levels of 100, 200, 400, 600, 800 and 1000 mg/kg, respectively (Table 1). The plant height was decreased significantly with the increase of soil Cu levels ($r$=-0.993**). Multiple comparisons showed that only the Cu concentrations higher than 400 mg/kg resulted in a reduction in plant height in comparison with the control.

As showed in Fig. 1, highly significant differences existed in plant height at different growth stages between the control and the treatments of higher than 400 mg/kg. Such differences became larger with plant development, and then became smaller after the maximum differences appeared at 30, 42, 42 and 62 days after transplanting (DAT) for 400, 600, 800 and 1000 mg/kg treatment, respectively. The differences were also enlarged with increasing soil Cu levels. The above results indicated that the inhibitory influence of soil Cu treatments on plant height increased with plant development, but decreased as soil Cu concentrations. And the time for peak

<table>
<thead>
<tr>
<th>Character</th>
<th>75.4 (CK)</th>
<th>100</th>
<th>200</th>
<th>400</th>
<th>600</th>
<th>800</th>
<th>1000</th>
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</thead>
<tbody>
<tr>
<td>Plant height at maturity (cm)</td>
<td>101.8</td>
<td>97.6</td>
<td>95.1</td>
<td>80.8</td>
<td>73.9</td>
<td>63.9</td>
<td>52.5</td>
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<tr>
<td>Leaf number on the main stem</td>
<td>17.0</td>
<td>16.8</td>
<td>16.6</td>
<td>16.0</td>
<td>15.0</td>
<td>13.8</td>
<td>13.0</td>
</tr>
<tr>
<td>Elongated internode number on the main stem</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>5.5</td>
<td>5.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Heading date (month-day)</td>
<td>08-23</td>
<td>08-23</td>
<td>08-24</td>
<td>09-03</td>
<td>09-07</td>
<td>09-11</td>
<td>09-16</td>
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</table>
appearance was delayed with the increase of soil Cu levels. The result also suggested that plant height reduction became greater as soil Cu level increased.

**Effect of Cu on leaf number on the main stem**

Compared with the control, the leaf number on the main stem were reduced by 1.2, 2.4, 5.9, 11.8, 18.8 and 23.5% at soil Cu levels of 100, 200, 400, 600, 800 and 1000 mg/kg, respectively (Fig. 1). The leaf number on the main stem decreased significantly with the increase levels of soil Cu ($r=-0.975^{**}$). The multiple comparisons showed that there were significant decreases ($P < 0.01$ or $P < 0.05$) at soil Cu levels of 400–1000 mg/kg. The results indicated that the leaf number on the main stem was significantly influenced by soil Cu stress, and reduced with the increased levels of soil Cu.

As indicated in Fig. 1, there was no significant reduction in leaf number on the main stem for the treatments(100 and 200 mg/kg Cu) in comparison with the control. While the treatments(higher than 400 mg/kg Cu) showed a highly significant reduction. For a specific soil Cu level, its effect on leaf number on the main stem increased with plant development, and then decreased after reaching a peak; As the Cu concentrations increased, the plants tended to have less leaves on the main stem.

**Effects of Cu on elongated internode number on the main stem**

Compared with the control, the same elongated internode number on main stem at maturity was observed at soil Cu level of 100 or 200 mg/kg, but the number of elongated internodes decreased by 0.5, 1.0, 2.0, 2.0 unit internode at soil Cu levels of 400, 600, 800 and 1000 mg/kg, respectively (Fig. 1). The multiple comparisons showed that the elongated internode number on the main stem significantly decreased at 400–1000 mg/kg Cu levels. The results indicated that elongated internode number on the main stem was not significantly decreased under lower soil Cu levels, but significantly reduced at Cu levels higher than 400 mg/kg. The elongated internode number declined gradually with increased supply of Cu.

**Effects of Cu on heading date**

The heading dates of the plants exposed to the soil Cu levels of 200, 400, 600, 800 and 1000 mg/kg were delayed by 1, 11, 15, 19 and 24 d, respectively, whereas the heading date at soil Cu level of 100 mg/kg was similar with the control (Fig. 1). The heading date at soil Cu level of 100 or 200 mg/kg showed no difference with the control, but was significantly delayed at soil Cu levels of 400, 600, 800 and 1000 mg/kg ($P < 0.01$ or $P < 0.05$), indicating that the heading dates were not influenced under lower soil Cu levels, but severely delayed at higher soil Cu levels (above 400 mg/kg).

**Effect of Cu on grain yield and its components**

**Effect of Cu on rice grain yield**

Considering that consistent results were obtained from 2002 and 2003 experiments, we only analyzed
the experiment with Wuxiangjing 14 in 2003. Table 2 indicated that the yields were decreased by 10.1, 15.4, 37.0, 83.9, 89.3 and 96.2% at soil Cu levels of 100, 200, 400, 600, 800 and 1000 mg/kg, respectively. The toxic effect on rice yield significantly increased with increasing levels of Cu application. The results of ANOVA indicated that the differences among different soil Cu treatments reached significant level for yields ($F=1202.89^{**}$). The multiple comparisons showed that the yield at any soil Cu treatments was consistently and significantly lower in comparison with the control; No observed yield difference was detected between soil Cu treatments of 100 and 200 mg/kg, while both of them were significantly higher than those under 400, 600, 800, 1000 mg/kg Cu. The yield under soil Cu level of 400 mg/kg was significantly higher than those under soil Cu levels of 600, 800, 1000 mg/kg. The yield under soil Cu level of 1000 mg/kg showed a significant reduction of yield in comparison with the treatments (600 and 800 mg/kg), while no significant difference existed between the treatments of 600 and 800 mg/kg (Table 2). The above results indicated that grain yields were significantly influenced by Cu even at level of 100 mg/kg. The yield decreased sharply with the increasing doses of Cu.

**Effect of Cu on yield components**

**Effect of Cu on panicle number**

ANOVA test indicated that there were significant differences in panicle number among different soil Cu treatments in 2003 ($F=120.02^{**}$). The multiple comparisons showed that no notable differences were observed for panicle number between the control and soil Cu treatments of 100 or 200 mg/kg. However, plants under lower levels of Cu treatments(100 and 200 mg/kg) generally produced significantly more panicles than those under higher soil Cu levels (400–1000 mg/kg). The panicle number under soil Cu level of 400 mg/kg was significantly higher than those at soil levels of 600, 800, 1000 mg/kg. The panicle number at soil Cu level of 600 mg/kg showed no significant difference with that at soil Cu level of 800 mg/kg, but significantly higher than that at soil Cu level of 1000 mg/kg. No difference was detected for panicle number between soil Cu treatment of 800 and 1000 mg/kg (Table 2). The above results indicated that panicle number had no obvious change under lower levels of soil Cu stress, but was significantly reduced at higher Cu levels.

The panicle number is determined by the time of tiller occurrence and productive tiller ratio. The results indicated that seedlings showed no obvious toxic symptoms after transplanting under soil Cu levels below 100 mg/kg. However, the seedling leaves turned yellow, and the seedling recovery from transplanting was delayed obviously with increased levels of soil Cu. As showed in Fig. 2, with the increasing levels of soil Cu, tillering was delayed (or even no tiller emerged), the maximum tiller numbers were reduced, and the time when maximum tiller numbers were reached was postponed. However, the productive tiller rates were greatly elevated under Cu stress. The results indicated that the decreases of panicle number(except for higher soil Cu treatment) were caused by the inhibitory effects on tillering.

**Effect of Cu on number of spikelets per panicle**

ANOVA test indicated that there was a significant difference in number of spikelets per
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Number of spikelets per panicle are the product of shoot dry weight (SDW) at heading and spikelet number-SDW ratio. As showed in Fig. 3-A, number of spikelets per panicle correlated significantly \( (P < 0.01) \) and positively with the SDW at heading, with the correlation coefficients of 0.952** in 2002, 0.966** in 2003, and 0.915** in the years 2002 and 2003, indicating that the number of spikelets per panicle increased with the increase of SDW at heading. Fig. 3-B indicated the number of spikelets per panicle also were significantly \( (P < 0.01) \) and positively correlated with the ratio of spikelet number-SDW, with the correlation coefficients of 0.914** in 2002, 0.747** in 2003, and 0.781** in both 2002 and 2003, indicating that spikelets per panicle increased with the increase of spikelet number-SDW ratio. Direct effects of SDW at heading \( (X_1) \) and spikelet number-SDW ratio \( (X_2) \) on number of spikelets per panicle \( (Y) \) were determined based on a stepwise regression. The results revealed that the two parameters both significantly affected the number of spikelets per panicle, according to data in 2002, 2003 or 2002–2003. Direct path coefficients of \( X_1 \) to \( Y \) were 0.608 and 0.459 in 2002, 0.797 and 0.307 in 2003, and 0.702 and 0.449 in 2002–2003, respectively, indicating that the reduction of SDW at heading, caused by Cu stress, primarily determined the number of spikelets per panicle compared to spikelet number-SDW ratio.

The decreases of SDW at heading, which resulted from delayed tillering and following higher
ratio of small productive tiller (Fig. 2), together with decreased spikelet number-SDW ratio, were responsible for reduced spikelets per panicle under soil Cu stress.

Effect of Cu on filled grain percentage and 1000-grain weight

Soil Cu stress had no obvious effect on filled grain percentage of both japonica rice cultivar Wuxiangjing 14 and indica rice cultivar Shanyou 63 (Table 2). 1000-grain weight of Wuxiangjing 14 showed no difference under low soil Cu stress, but decreased under high Cu levels (Table 2). For Shanyou 63, 1000-grain weight decreased with the increment of soil Cu levels (data not shown).

Comparison of yield components under soil Cu stress indicated that number of panicles was affected the most severely, followed by number of spikelets per panicle, while both filled grain percentage and 1000-grain weight changed little under Cu stress. Further analysis showed that the yield loss under lower levels of soil Cu (100 or 200 mg/kg) was mainly attributed to the decreased spikelet number per panicle, while both panicle number and spikelet number per panicle were the main components contributed to yield reductions under higher levels of soil Cu (more than 400 mg/kg), with panicles playing a more important role than spikelet number per panicle (Table 2 and 3).

Effect of Cu on total biomass and economic coefficient

Our investigation showed that the total biomass under different soil Cu treatments from 100 to 1000 mg/kg were all significantly lower ($P < 0.01$ or $P < 0.05$) as compared to the control (Fig. 4). No difference existed in the total biomass between soil Cu treatments of 100 and 200 mg/kg, but both of them were significantly higher than those under the other soil Cu treatments (above 400 mg/kg). The total biomass at soil Cu level of 400 mg/kg was significantly higher than that under soil Cu levels of 600, 800, 1000 mg/kg. No significant difference was detected for total biomass between soil Cu treatment of 600 and 800 mg/kg, but both of them were significantly higher than those at soil Cu level of 1000 mg/kg. The results indicated that the total biomass was significantly influenced at soil Cu level of 100 mg/kg, and decreased with the increasing levels of Cu application.

The results highlighted that economic coefficient showed some reductions with the increased levels of soil Cu. However, compared to the control, economic coefficient showed no change under soil Cu stress between 100 to 600 mg/kg, but showed significant decreases under soil Cu levels of 800 and 1000 mg/kg (data not shown). The results suggested that there were no significant impacts on economic coefficients within a wide range of soil Cu concentration except under extreme high soil Cu levels (>800 mg/kg).

DISCUSSION

Effect of Cu on rice growth and development

Several researchers have obtained the different results about the response of plant height to soil Cu stress in previous reports. Kang et al [7] reported that

![Fig. 4. Effect of soil Cu concentration on total biomass production and economic coefficients in 2003.](image)

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<tr>
<td></td>
<td>1→Y</td>
<td>2→Y</td>
<td>1→Y</td>
</tr>
<tr>
<td>SDW (X1, 1)</td>
<td>0.608</td>
<td>0.344</td>
<td>0.797</td>
</tr>
<tr>
<td>Spikelet number-SDW ratio (X2, 2)</td>
<td>0.455</td>
<td>0.459</td>
<td>0.439</td>
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</table>
the plant height of rice subjected to soil Cu levels of 90.33 – 241.5 mg/kg was significantly higher than the control (37.33 mg/kg); Chen et al. [14] found no significant effect of soil Cu levels of 500 and 1000 mg/kg on rice plant height. Su et al. [8] and Hu et al. [5] observed that rice plant height decreased sharply at soil Cu levels between 14.5 to 250 mg/kg and between 17.0 to 151.6 mg/kg, respectively. Our present research exhibited that the plant height at maturity was decreased by 4.2 and 6.6% at soil Cu levels of 100 and 200 mg/kg, respectively, but decreased significantly by 20.6, 27.5, 37.2 and 48.4% at soil Cu levels of 400, 600, 800 and 1000 mg/kg, respectively (Table 1). The rice plant height was reduced greatly with the increasing levels of soil Cu \((r=-0.993^{**})\). Further analysis showed that no significant differences existed for plant height at different growth stages between the control and soil Cu treatment of 100 or 200 mg/kg, but large differences were observed between the control and soil Cu levels above 400 mg/kg. Maximum differences for plant height between the control and soil Cu treatments increased with the rice development and then decreased after maximum differences were reached. The differences were increased with the increase of soil Cu levels.

There was no research concerning the effects of soil Cu concentrations on elongated internode number, leaf number on the main stem and heading date of rice plant. This experiment indicated that, compared to the control, the above mentioned growth parameters had no changes or small changes under soil Cu levels of 100 and 200 mg/kg, significantly changes under Cu levels of above 400 mg/kg. The toxic effect of Cu on the plant growth was increased with the increasing levels of Cu application.

Influences of Cu stress on the plant height and leaf number became larger with the advance of growth stages, then became smaller. The influences increased with the raising levels of soil Cu (Fig. 1). Such phenomena suggested that rice plants showed a gradual adaptation to soil Cu stress, and exhibited compensation ability at middle and late growth stages.

### Effect of Cu on grain yield and its causes

The present investigation showed that the grain yield decreased significantly with the increase of soil Cu levels. The result was highly consistent with Yang et al. [15], Chen et al. [14], Su et al. [8], Kang et al. [7] and Hu et al. [5]. The limited data on the reasons of rice yield reductions by Cu stress were derived from rice plants, which indicated that the decrease of panicle number [5, 6], unfilled grain number [15] or reduction of both panicle number and 1000-grain weight [7, 8, 14] contributed to the grain yield decrease. The present study indicated that grain yield reductions under lower soil Cu levels (100, 200 mg/kg) mainly resulted from the decrease of spikelet number per panicle, but under higher soil Cu levels (above 400 mg/kg), the grain yield reductions were mainly caused by the decreases of both panicle number and number of spikelets per panicle, with panicle number playing a more important role than spikelet number per panicle (Table 1).

Hu et al. [5] presented that the decreases of panicle number by soil Cu stress were caused by the reduction of tiller number in rice. The present research showed that the reduction of panicle number were mainly attributed to severe inhibition of tiller occurrence under higher soil Cu stress. Excess Cu concentration led to slow recovery from transplanting, delayed tillering and reduced maximum tiller numbers. Though the productive tiller rate increased under soil Cu stress, as the decrease in maximum tiller number was greater than this increase, panicle number decreased with elevated soil Cu concentration (Fig. 1). However, further research is necessary to clarify inhibition mechanisms on tiller occurrence under soil Cu stress.

Few studies had paid attention to the reason of spikelets reduction per panicle under soil Cu stress. This research indicated that the number of spikelets per panicle correlated significantly \((P<0.01)\) and positively with the SDW at heading and spikelet number-SDW ratio (Fig. 3). The multiple regression analysis showed that SDW at heading and spikelet number-SDW ratio all significantly affected the number of spikelets per panicle. The number of spikelets per panicle increased with the increase of SDW at heading and spikelet number-SDW ratio.
Path analysis showed that, according to data of 2002, 2003 or 2002 and 2003 combined, direct path coefficients of SDW at heading to number of spikelets per panicle were much larger than that of spikelet number-SDW ratio to spikelet number per panicle (Table 3), indicating that the decrease of spikelet number per panicle due to higher soil Cu stress were caused by the reduction of both SDW at the heading date and spikelet number-SDW ratio, the decrease of SDW at heading resulting from delayed tiller occurrence and higher rate of small productive tiller (Fig. 2).

**Effect of Cu on total biomass and economic coefficient**

Rice grain yield is a function of total biomass and economic coefficient: in our study, the former was more sensitive to soil Cu stress than the latter. The total biomass decreased significantly with raising soil Cu levels, which is in accordance with the results of the previous studies \(^7,8\). The biomass responses to soil Cu stress are probably associated with its inhibition on photosynthesis, because excessive Cu could restrain chlorophyll synthesis or result in chlorophyll decomposition \(^16\). There was little information about the effect of soil Cu stress on economic coefficient of rice plants. In present study no obvious changes in economic coefficient were observed under soil Cu levels below 600 mg/kg, only when reached 800 mg/kg or above, did the economic coefficient decrease significantly. The results suggested that there was no significant impact on economic coefficient within a wide range of soil Cu levels except under extreme high soil Cu levels (above 800 mg/kg).

**ACKNOWLEDGEMENTS**

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