*Research article*

# Influence of Copper and Cadmium Soil Pollution in Soybeans: Uptake, Growth, and Yield Pollution

**CHOICHI SASAKI***
*Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki, Japan*
*Email: chsasaki@hirosaki-u.ac.jp*

**CHIHIRO KATO**
*Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki, Japan*

**NOBUHIKO MATSUYAMA**
*Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki, Japan*

**TAKEYUKI ANNAKA**
*Faculty of Agriculture, Yamagata University, Tsuruoka, Yamagata, Japan*

**KIICHI SASAKI**
*Aomori Prefectural Improvement Projects Federation, Aomori, Japan*

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**Abstract** Food produced on polluted farmland often damages human health. Studying the various mechanisms through which agricultural products are polluted is crucial. This study investigated the influence of combined copper (Cu) and cadmium (Cd) soil pollution on soybeans and the Cu and Cd uptake and resultant soybean growth and yield. Our models comprised a layer of 14 cm gravel at the bottom of plastic containers, a layer of 20 cm non-polluted soil on top, and another layer of 20 cm polluted soil on the top. Eight soybean plants were cultivated for each model. Here, we used a cadmium-polluted soil sample (approximately 1.81 mg $kg^{-1}$) taken from a paddy field. We maintained the Cu concentration in the soil at 100 mg $kg^{-1}$, 250 mg $kg^{-1}$, and 400 mg $kg^{-1}$. The soybeans were seeded in early June and harvested in early October. Cd concentrations in soybean seeds in three different models were 0.48 mg $kg^{-1}$, 0.53 mg $kg^{-1}$, and 1.30 mg $kg^{-1}$, respectively, while the Cu concentrations in soybean seeds in the three models were 10.53 mg $kg^{-1}$, 15.40 mg $kg^{-1}$, and 20.18 mg $kg^{-1}$ respectively. The levels of Cu and Cd pollution in the plants were highest in roots, medial in stems, and lowest in seeds. The growth and yield of soybeans were lower in models with a soil Cu concentration of 400 mg $kg^{-1}$ compared to models with a soil Cu concentration of 100 mg $kg^{-1}$. The models with soil Cu concentration at 400 mg $kg^{-1}$ yielded only three stumps. Thus, we conclude that changes in Cu concentration in soil polluted with both Cd and Cu have a considerable negative influence on the growth and yield of soybean plants.

**Keywords** soil pollution, Cadmium, rice, Copper, soybean

## INTRODUCTION

It has been argued that soil pollution by heavy metals results in damage to human health. Several studies describing mitigation techniques have been reported (e.g. Toikawa et al., 2020). Specific to select heavy metals, Itai-Itai disease is widely known to damage human health as caused by cadmium (Cd) pollution of soil and water (Kobayashi, 1978). Most studies related to Cd pollution mention the oral ingestion of rice but rarely refer to ingestion of soybeans. Recently, it has been recognized that Cd is easily absorbed by soybeans (MAFF, 2006), and the amount of Cd uptake after soybean ingestion is similar to that of ingesting polluted rice (Hasegawa, 2013). Therefore, people in countries such as Japan who consume large amounts of soybeans are concerned about the damaging impact of Cd on their health.

To address this problem, Haque et al. (2014) and Li et al. (2017) attempted to minimize soybean Cd uptake by regulating the groundwater levels of these heavy metals in their fields. In Japan, copper (Cu), Cd, and arsenic (As) have been identified as toxic substances in agricultural land. However, most studies on soil pollution by these heavy metals have been conducted on rice and rarely on soybeans.

The Bordeaux mixture, a mixture of copper sulfate and calcium carbonate, has been used for a long time in apple orchards in Japan, and severe Cu accumulation on soil surfaces has been reported by Aoyama (2009). The Cu concentration in the soil of some apple orchards in Aomori Prefecture was higher than 500 mg kg$^{-1}$ dry soil, whereas the paddy field soil criterion was 125 mg kg$^{-1}$ dry soil in Japan (Yamane et al., 1997). Therefore, there is a concern about excess Cu accumulation in the soils of paddy fields around apple orchards. Fan et al. (2018) mentioned the possibility that some apple orchards in the lowlands that were once converted from paddy fields might have been restored to paddy fields.

Recently, soybeans have become a major crop for upland fields converted from paddy fields; therefore, Cu and Cd concentrations in soybeans are a major concern for consumers. Cd standards for soybeans have not yet been established. Haque et al. (2014) and Li et al. (2017) planted soybeans in Cd-polluted paddy soils and examined the growth, yield, and Cd concentrations in the plants. However, no study has examined the effects of the combination of Cd and Cu pollution in soil on soybeans.

## OBJECTIVE

The aims of this study were to clarify the effects of combined pollution of Cd and Cu in farmland on the growth and yield of soybeans and to measure the concentrations of those heavy metals in them.

## METHODOLOGY

### Soil Properties and Experimental Design

In this study, we used polluted soil samples collected from Eastern Japan (where the location is secret) and non-polluted soil samples from the Kanagi Farm of Hirosaki University, Aomori Prefecture. Those two soils were collected from those two sites following the practice by Toikawa et al. (2020). The polluted and non-polluted soils were placed in plastic containers (41 cm × 61 cm × 63 cm). In addition, gravel (particle size 2–4 mm) was filled up to 14 cm from the bottom of the containers to maintain uniform drainage. Cu and Cd concentrations in Kanagi soil were 3.70 mg kg$^{-1}$ and 0.14 mg kg$^{-1}$, respectively. However, Cu and Cd concentrations in the polluted soil were 12.2 mg kg$^{-1}$ and 1.81 mg kg$^{-1}$, respectively. The organic matter content was 3.6% in the Kanagi soil and 5.1% in the polluted soil.

The Cd concentration levels in the two types of soils were left as they were at the time of soil sampling, whereas the Cu concentration levels were changed to three levels by adding copper chloride ($CuCl_2.2H_2O$) to them: 100 mg kg$^{-1}$, which is the standard in non-polluted soil of paddy fields; 250 mg kg$^{-1}$, which is approximately twice the standard; and 400 mg kg$^{-1}$, which is approximately four times the standard. We called the three different models with three different Cu concentration levels, 100 mg kg$^{-1}$, 250 mg kg$^{-1}$, and 400 mg kg$^{-1}$, M-①、M-② and M-③, respectively. The thicknesses of the two kinds of soil layers which were exact copies of a polluted field on-site were: the polluted soil layer (density: approximately 0.80 mg kg$^{-1}$) of 0-20 cm and the non-polluted soil (density: 0.89 mg kg$^{-1}$) of 20-40 cm.

A self-recording temperature sensor and an electrode for measuring the redox potential (Eh, Central Science Co., Ltd. UC-203) were inserted into the side walls of the containers to measure the soil temperature and Eh. The groundwater level was set to 40 cm by using Mariotte bottle, which has been reported to maximize soybean yield (Arihara, 2000).

### Cultivation and Measurement Procedure

Soybeans were sown at four different places in plastic containers with five to six grains each. The soybean variety used in the experiment was Ryuhou (*Glycin Max* (L.) Merr. cv. Ryuhou). The prefecture in this area recommends the cultivation of it. Soybean seeding was performed on May 31, 2019, and June 1, 2020. Approximately two weeks after sowing, thinning was performed to leave two plants of average growth. Fertilizer recommended for Ryuhou was applied to soybean plants in a generally approved amount (Li et al. 2017). Water (2 L) was supplied to the soybeans every four days. Pest control was conducted as required. The experiments were conducted in a greenhouse at the Faculty of Agriculture and Life Sciences, Hirosaki University, Japan. The average daily temperature in the house ranged from 20 to 30 degrees.

A survey of soybean growth and yield (plant height, leaf age, number of nodes, number of pods, and weight of 100 beans) was conducted based on the survey standards of Kodama et al., 2004. We measured the Cu and Cd concentrations in the beans, stems, and roots. Analyses of these concentrations were performed using atomic absorption spectroscopy after extraction with HCI and $HNO_3$ (MAFF, 1979). These data were tested for significance using the Tukey-Kramer method.

## RESULTS AND DISCUSSION

### Oxidation-reduction Potential

The redox potential (Eh) values were in the range of 300–400 mV in models M-① and M-③ as shown in Figs.1 and 2. Moreover, the redox potential of M-② became the same range as M-①. The presence of Cd and Cu is affected by the redox state of the soil (Matsunaka, 2014). Thus, it was inferred that the polluted soil layers in all three models were in an oxidized state and that soluble Cd and Cu were absorbed by soybeans.

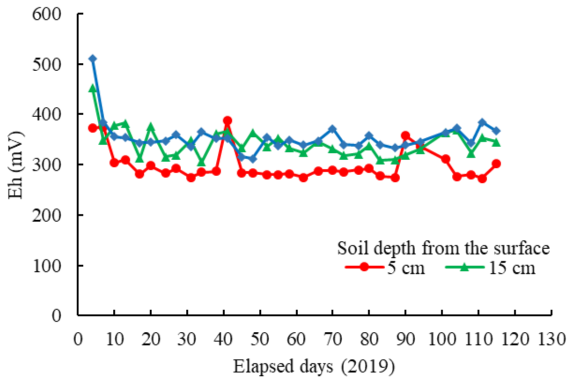


**Fig. 1 Temporal change of Eh value with M-①**

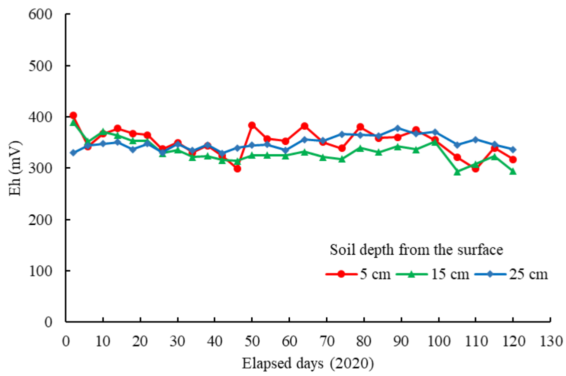


**Fig. 2 Temporal change of Eh value with M-③**

## Cd and Cu Concentrations in Soybean Plants

Table 1below shows the Cd and Cu concentrations in the plants.

**Table 1 Cd and Cu concentration in soybean plants in the three different models**

| Model | Seed-Cd* | Stem-Cd* | Root-Cd* | Seed-Cu* | Stem-Cu* | Root-Cu* |
|---|---|---|---|---|---|---|
| M-① | 0.48±0.05$^a$ | 1.07±0.07$^a$ | 2.55±0.17$^a$ | 10.53±0.48$^b$ | 5.63±0.97$^b$ | 81.16±18.66$^b$ |
| M-② | 0.53±0.19$^a$ | 2.77±0.70$^a$ | 3.83±1.07$^a$ | 15.40±2.43$^{ab}$ | 21.78±7.10$^a$ | 365.60±43.68$^a$ |
| M-③ | 1.30±0.73$^a$ | 7.40±5.76$^a$ | 29.23±26.43$^a$ | 20.18±0.62$^a$ | 21.87±12.10$^a$ | 374.27±140.93$^a$ |

*Notes: The two kinds of superscripts 'a' and 'b' indicates a statistically significant difference at a 5% level according to the Turkey-Kramer test; ± shows standard deviation. In all cases, three samples were used. * mg kg$^{-1}$*

**(i) Cd concentration:** Cd concentrations in soybean seeds were M-① (0.48 mg kg$^{-1}$) < M-② (0.53 mg kg$^{-1}$) < M-③ (1.30 mg kg$^{-1}$). However, these data varied so widely that there were no significant differences. The value of M-① (0.48 mg kg$^{-1}$) was less than half of the value (1.07 mg kg$^{-1}$) that Li et al. (2017) had reported by conducting a similar experiment. As the Cd concentrations in the sample soils used in the two experiments were almost the same, the difference in the Cd concentrations in seeds between the two experiments are most likely to have resulted from different Cu concentrations in the soil samples used in the two experiments. That is, the Cu concentration in the soil sample was 43.3 mg kg$^{-1}$ in the experiment conducted by Li et al. (2017), which is less than half that (100 mg kg$^{-1}$) in our experiment. The Cd concentration we found in seeds in our experiment, 0.48 mg kg$^{-1}$, was well over four times of 0.11 mg kg$^{-1}$, which the Japanese Ministry of Agriculture, Forestry and Fisheries (2024) specifies as the mean Cd concentration in soybean seeds cultivated in non-polluted soils. The Cd concentration in the polluted soil sample in our experiment was 1.81 mg kg$^{-1}$, which was approximately five times higher than that in the non-polluted upland soil (0.373 mg kg$^{-1}$) reported by Asami (2010).

The Cd concentrations in soybean seeds and soils were positively correlated. Additionally, the Cd concentrations obtained in our experiment did not satisfy the standard value of 0.1 mg kg$^{-1}$ set by the Codex Alimentarius Commission (MAFF, 2023).

Cd concentrations in stems were M-① (1.07 mg kg$^{-1}$) < M-② (2.77 mg kg$^{-1}$) < M-③ (7.40 mg kg$^{-1}$), which were similar to those in seeds, and they demonstrated no significant difference between them, compared to the Cd concentration in seeds. The Cd values in the stems in the experiment by Li et al. (2017) had been 1.48 mg kg$^{-1}$, whereas in our experiment they were much higher than in both our M-② and M-③. This difference can be attributed to the higher Cu concentrations in the soil, especially of the M-③, in which the Cu concentration was 400 mg kg$^{-1}$; a remarkable increase in Cd concentration was observed there.

Cd concentrations in the roots followed the same order as Cd concentrations in the seeds and stems, showing no significant differences among the three models. In M-③ Cd concentrations in seeds, stems and roots were far higher than those in the M-① and M-②. This difference was thought to have resulted from the fact that the Cu concentrations in M-③ soils were much higher than in the cases of the M-① and M-②, which may have affected the amount of Cd uptake from the roots.
The trend in Cd concentration in the soybean plants was as follows: seeds < stems < roots. In all three models, the same results were obtained by Haque et al. (2014) and Li et al. (2017). Toikawa et al. (2020), who experimented with paddy rice, reported that higher Cu concentrations in the soil caused lower Cd concentrations in brown rice. However, in the case of soybeans, we did not observe such a result.

**(ii) Cu concentration:** The Cu concentration in soybean seeds ranged in all three models from 10 mg kg$^{-1}$ to 20 mg kg$^{-1}$ and was in the order M-① (10.5 mg kg$^{-1}$) < M-② (15.4 mg kg$^{-1}$)< M-③ (20.2 mg kg$^{-1}$), suggesting a positive correlation between the Cu concentrations in soybean seeds and soils, and there was a significant difference ($p$<0.05) between the M-① and M-③. In addition, Cu concentration in seeds in the M-① was almost the same as that found by Li et al. (2017), 9.96 mg kg$^{-1}$.

The Cu concentration in stems was 5.63 mg kg$^{-1}$ in M-①, while those in M-② and M-③ were 21.8 mg kg$^{-1}$ and 21.9 mg kg$^{-1}$, respectively. There was a significant difference between M-① and the other two models ($p$<0.05). The Cu concentration in roots was 81.2 mg kg$^{-1}$ in M-① while those in M-② and M-③ were 365.6 mg kg$^{-1}$ and 374.3 mg kg$^{-1}$, respectively. There was a significant difference between M-① and the others ($p$<0.05). The above-mentioned Cu concentrations were approximately 6 times in the case of M-① and 26 times in the case of M-② and M-③, greater than that found by Li et al. (2017), which was 14.11 mg kg$^{-1}$, suggesting an unusual Cu uptake mechanism in soybean plants that should be studied further.

The levels of Cu pollution in M-① were highest in roots, medial in seeds, and lowest in stems, while they were highest in roots, medial in stems, and lowest in seeds in both M-② and M-③, which was the same order as the Cd concentration in them. The Cu concentration distribution in soybean plants in M-② and M-③ was different from those reported by Haque et al. (2014) and Li et al. (2017), which may suggest a possible effect of higher Cu concentration in soil in our experiment on Cu transfer in the plant. Therefore, the threshold Cu concentration in the soil for the transfer process should be further studied.

In Japan, there is no regulatory value for Cu concentration in soybeans, while in China it is no more than 20 mg kg$^{-1}$ (Li et al., 2017). The Cu concentration in seeds in our M-③, 20.18 mg kg$^{-1}$, does not satisfy that regulation value.

### Soybean Yield and its Components

Table 2 lists the soybean yield and its components. In M-③, the number of stumps whose soybean grains reached the harvest was three out of eight. For the sake of comparison, we experimented with another model which was under the same experimental conditions as in this study but in which Cu concentration in the polluted soil was 500 mg kg$^{-1}$; the results demonstrated that the number of stumps whose soybean grains reached the harvest was zero.

Based on these results, the number of stumps for statistical analysis was set to three. The main stem length, the number of main stem nodes, and the stem diameter were significantly decreased in M-③ compared with the other two models. The M-③ stem thickness was approximately 50% compared to the other two models. This may have resulted from the possibility that Cu concentration became over 400 mg kg$^{-1}$ during the growing stage.

**Table 2 Soybean yield components of the three different models**

| Model | Stem height (cm) | Stem diameter (mm) | Node No. | Seed/Pod | 100 seed wt. (g) | Good seed No. (g) |
|---|---|---|---|---|---|---|
| M-① | 73.3±2$^{-1a}$ | 12.0±0.8$^{a}$ | 16.7±0.6$^{a}$ | 1.8±0$^{-1a}$ | 32.2±1$^{-1a}$ | 197.7±16.0$^{a}$ |
| M-② | 87.7±7.2$^{a}$ | 11.9±2.0$^{a}$ | 18.0±0.0$^{a}$ | 1.4±0$^{-1b}$ | 25.3±8.8$^{a}$ | 86.7±96.7$^{a}$ |
| M-③ | 52.0±7.0$^{b}$ | 6.1±3.2$^{b}$ | 14.0±1.0$^{b}$ | 1.5±0$^{-1b}$ | 28.4±4.9$^{a}$ | 72.0±81.6$^{a}$ |

*Notes: The two kinds of superscripts 'a' and 'b' indicates a statistically significant difference at a 5% level according to the Turkey-Kramer test; ± shows standard deviation. In all cases, three samples were used. * mg kg$^{-1}$*

The number of seeds in a pod in M-① was significantly more than those in both M-② and M-③. The magnitude relationship between the number of good seeds and the weight of 100 seeds was M-③ < M-①; however, no significant difference was confirmed among the three models. This is perhaps due to considerable variations in the measured values. Compared to the results of Li et al. (2017), the stem height, stem diameter, number of soybean seeds per pod, and weight of 100 seeds tended to be lower. Thus, it can be concluded that an increase in the Cu concentration in the soil has an undeniable effect on growth and yield. However, further studies are required to confirm these findings.

## CONCLUSION

The Cu concentration has a significant influence on the growth and yield of soybeans. An increase in Cu concentration in the soil of combined pollution has a significant influence on soybean growth and yield, as well as on Cd and Cu concentrations in soybean plants.

Through our research, we learned that introducing soil dressing can be very effective in alleviating Cu pollution in soil. It is not soybean plants alone, but many other plants are potentially polluted by Cu in soil. Therefore, it is important for us to extend our research to those other plants as well.

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## REFERENCES

Aoyama, M. 2009. Characteristics of the apple orchard soils and the effects of compost. Open Lecture in Teaching and Research Center for Bio-coexistence, Faculty of Agriculture and Life Science, Hirosaki University, Textbook of 'Science of Apple', 35-42. (in Japanese)

Arihara, J. 2000. Soybean. Nobunkyou, Tokyo, Japan, 101-110. (in Japanese)

Asami, T. 2010. Toxic heavy metal pollution in soils of Japan. Agune-gijutus Center, Tokyo, Japan, 3-8. (in Japanese)

Fan, J., Sasaki, C., Kato, C., Matsuyama, N., Annaka, T., Endo, A., Li, S. and Sasaki, K. 2018. Influence of percolation patterns on copper uptake, and growth and yield with copper-polluted stratified paddy fields. International Journal of Environmental and Rural Development 9 (1), 101-108, Retrieved from DOI https: //doi.org/10.32115/ijerd.9.1_101

Hasegawa, S. 2013. Soil and agricultural land. Youkenndou, Tokyo, Japan, 153-157. (in Japanese)

Haque, M.Z., Sasaki, C. Matsuyama N., Annaka T. and Sasaki, K. 2014. Effect of groundwater level on cadmium uptake and yield of soybean from cadmium polluted soils. International Journal of Environmental and Rural Development, 5 (1), 107-113, Retrieved from DOI https://doi.org/10.32115/ijerd.5.1_107

Kodama, T., Sato, U. and Sasaki, K. 2004. Cultivation guideline of soybean in Akita prefecture. Department of Agriculture, Forestry and Fisheries, Akita, Japan, 113-121. (in Japanese)

Kobayashi, J. 1978. Pollution by cadmium and the Itai-Itai disease in Japan. In Oehme, F.W. (Ed.), Toxicity of Heavy Metals in the Environment, 1, Marcel Dekker, New York, USA, 47-158, Retrieved from URL https://www.scirp.org/reference/referencespapers?referenceid=1299708

Li, S., Sasaki, C., Kato, C., Matsuyama, N., Annaka, T., Endo, A. and Sasaki, K. 2017. Reducing cadmium and copper uptake of soybeans by controlling groundwater level and its impacts on growth and yield. International Journal of Environmental and Rural Development, 8 (1), 77-84, Retrieved from DOI https:// doi.org/10.32115/ijerd.8.1_77

Matsunaka, T. 2014. Basic soil science. Nobunkyo, Japan, 327-333. (in Japanese)

Ministry of Agriculture, Forestry and Fisheries of Japan (MAFF). 1979. The foundation of environmental paddy field and investigation of paddy field, water quality and crop analysis method. National Conference of Paddy Field Preservation, Tokyo, Japan, 113-115.

Ministry of Agriculture, Forestry and Fisheries of Japan (MAFF). 2006. Manual for establishing techniques to suppress cadmium absorption in soybeans. Ministry of Agriculture, Forestry and Fisheries of Japan, Retrieved from URL https://www.maff.go.jp/j/syouan/nouan/kome/k_cd/2_taisaku/attach/pdf/01_tec-10. pdf (in Japanese)

Ministry of Agriculture, Forestry and Fisheries of Japan (MAFF). 2024. Standards for cadmium in food. Ministry of Agriculture, Forestry and Fisheries of Japan, Retrieved from URL https://www.maff.go.jp/j/ syouan/nouan/kome/k_cd/04_kijyun/01_int.html (in Japanese)

Toikawa, Y., Sasaki, C., Kato, C., Matsuyama, N., Endo, A., Fan, J. and Annaka, T. 2020. The influence of percolation patterns on copper and cadmium uptake, Growth and yield of rice plants in copper- and cadmium-polluted stratified paddy fields. International Journal of Environmental and Rural Development, 11 (1), 8-17, Retrieved from DOI https://doi.org/10.32115/ijerd.11.1_8

Yamane, I., Hamada, R., Yoshinaga, N. et al. 1997. Soil science. Bunneidou, Tokyo, Japan, 196-204. (in Japanese)