



## Reducing Cadmium and Copper Uptake of Soybeans by Controlling Groundwater Level and its Impacts on Growth and Yield

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**Abstract** In Japan, cadmium (Cd), copper (Cu) and arsenic have been designated as specific harmful substances in the Agricultural Land Soil Pollution Act. Though the safety standards of these substances have not been provided for upland field crops in Japan, it is important to minimize the concentration of these contaminants in agricultural products for both international trading and our health. The objective of this study is to clarify the effects of controlling groundwater levels on Cd and Cu uptake and growth and yield of soybeans. For this experiment, three plastic containers were prepared, and then 14cm-thick of non-contaminated gravel, 15cm-thick of non-contaminated soil ( $0.12 \text{ mg Cd kg}^{-1}$ ;  $2.4 \text{ mg Cu kg}^{-1}$ ) and 25cm-thick of contaminated soil ( $2.24 \text{ mg Cd kg}^{-1}$ ;  $43.4 \text{ mg Cu kg}^{-1}$ ) were placed in this order from the bottom of each container. Then the groundwater level of each container was maintained as 5cm (GL5), 10cm (GL10) and 40cm (GL40) during the growing period. As a result, Cd and Cu concentration of soybean seeds of GL5, GL10 and GL40 were 0.25, 0.52 and  $1.07 \text{ mg Cd kg}^{-1}$ , respectively, and 5.08, 5.82 and  $9.96 \text{ mg Cu kg}^{-1}$ , respectively. Significant difference in both Cd and Cu concentration of soybean seeds were found among three models at 5% level. On the other hand, growth and yield of soybeans tended to decrease with the rise of the groundwater level. Noticeably, plant heights and weights of a hundred grains were significantly different among these three models. From the above, it can be concluded that controlling groundwater levels can reduce Cd and Cu uptake and affect growth and yield of soybeans.

**Keywords** soybeans, uptake of cadmium and copper, control of groundwater level, growth and yield

## **INTRODUCTION**

Soil contamination in arable lands is a threat to human health through the intake of agricultural produce. In Japan, since there has been some serious problems as seen in such grave impacts of heavy metals in the cases of itai-itai disease induced by cadmium (Cd) and a decrease of rice yield affected by copper (Cu) (Yamane et al., 1997), “Agricultural Land Soil Pollution Act” was passed into law in 1970. According to this law, Cd and Cu, and arsenic were designated as specifically harmful substances (Kobayashi, 1978). In 2010, the safety standard for Cd in brown rice was lowered to 0.4 mg kg<sup>-1</sup> in Japan, while other countries such as countries in the EU, China, Australia, etc., have stricter standards (MAFF, 2015). The safety standards for upland crops have not been provided yet in Japan. However, studies for reconsideration of Cd standards have been conducted as consumers’ interests in safety and security of food (MAFF, 2007).

In Japan, an expansion of growing areas of soybeans is one of the important counter- measures for increasing calorie based food self-sufficiency rate. Some countries provide the safety standard of Cd in soybean seeds as 0.2 mg kg<sup>-1</sup>, while in Japan, Cd concentration in soybean seeds which have been produced even in non-contaminated field often exceeds this value (MAFF, 2004 ). It is inferred, therefore, that soybeans produced in Cd-contaminated and semi contaminated fields may contain much more Cd. Thus there is real concern that Japanese domestic soybeans will not meet the standards of export-partner countries such as United States and Hong Kong etc. (Ishitsuka and Jindai, 2013) in international trading. As for Cu in soybeans, it is a future task for Japan to provide a safety standard value, while some other countries already have such, e.g., 20 mg kg<sup>-1</sup> in China (MAPRC, 2005).

As similar to the case of paddy fields, some techniques for reducing Cd uptake of soybeans e.g., soil dressing, chemical methods such as controlling pH, and phyto -remediation etc. have been evaluated recently (Akahane et al., 2013; Dong et al., 2007). These measures, however, cost much money and take a long time, and so they have not been implemented widely yet (Haque et al., 2014a, b). Furthermore, soil dressing needs a large amount of soil and so it causes a heavy environmental burden (MEGJ, 2010).

As a cost-saving and easy technique, Murakami et al. (2011) and Haque et al. (2014a, b) reported that the Cd uptake of soybeans decreased by controlling oxidation reduction potential of the plowed layer by changing the groundwater level since Cd becomes insoluble under a reduction condition.

Cu also becomes insoluble under reduction condition (Matsui and Okazaki, 1993) and thus it is expected that controlling the groundwater level is also effective in reducing the Cu concentration in crops. The effects of a shallow groundwater level, especially higher than 10cm, on the Cu uptake of crops have not been evaluated yet and neither the distribution of Cu in soybean plant bodies. Heretofore groundwater level was controlled by relief well and insoluble condition of Cu by pH control amendment was made (Matsui and Okazaki et al., 1993; Yamane et al., 1997).

Against this background, the objective of this study is to clarify the effects of the controlling groundwater level on Cd and Cu uptake and growth and yield of soybeans. To achieve the purpose, growing tests of soybeans were conducted by using Cd- and Cu- contaminated soil samples. During the experiments, the groundwater level was maintained as 5, 10 and 40cm to control the thickness of soil layer, in which Cd and Cu was soluble.

This study will help to clarify the effects of rice-soybean crop rotation in contaminated ill- and/or well-drained paddy fields on Cd and Cu uptake of soybeans and to develop techniques for reducing Cd and Cu uptake with a low cost.

## **MATERIALS AND METHODS**

### **Soil Properties**

In this experiment, we used soil sampled from the plow layer of a paddy field which had been contaminated with agricultural water from mine drainage. Non-contaminated soil had been sampled from the plow layer of the paddy field at Kanagi farm of Hirosaki University, Aomori prefecture, the northeastern part of Japan. Gravels had been collected from a nearby mountain, Mt.Iwaki, Aomori prefecture.

Chemical and physical properties of the soil samples are shown in Table 1. Soil textures of contaminated and non-contaminated soil were both clay loam. Cd concentration in contaminated soil, non-contaminated soil and gravels were 2.27, 0.12 and 0.13 mg kg<sup>-1</sup>, respectively while Haque et al. (2014a, b) had used contaminated soils of 3.39 and 1.57 mg kg<sup>-1</sup>. Cu concentration in the contaminated and non-contaminated soil in this study was 43.4 and 2.4 mg kg<sup>-1</sup>, respectively. The contaminated soils in this study contained seven times and twice as much Cd and Cu, respectively, as those in the average non-contaminated agricultural lands in Japan, the figures of which are 0.33 mg Cd kg<sup>-1</sup> and 24.8 mg Cu kg<sup>-1</sup> (Asami, 2010).

**Table 1 Physical and chemical properties of the soil samples**

Sample	Density (g cm <sup>-3</sup> )	Soil Texture	MgO *	Na <sub>2</sub> O *	CaO *	K <sub>2</sub> O *	Cu *	Cd *	T-N (%)	T-C (%)	C/N	OM (%)
Polluted soil	2.57	CL	224	96	2032	232	43.4	2.27	0.28	3.39	12.2	5.86
Non-polluted soil	2.54	CL	120	64	400	120	2.4	0.12	0.16	2.07	13.3	3.62
Gravel	2.68	-	147	18	539	58	1.5	0.13	0	-	-	0.05

Note: Soil texture is based on the International Soil Society classification. CL: Clay loam. Gravel diameter size 2-4mm.

\*mg kg<sup>-1</sup>

## Experimental Design

In this study, a growing test of soybeans was conducted with stratified field models. Three plastic containers (41×61×63 cm) were prepared and then they were packed with 14cm-thick of gravels (dry bulk density  $\rho_d=1.40$  g cm<sup>-3</sup>), 15cm-thick of non-contaminated soil ( $\rho_d=0.89$  g cm<sup>-3</sup>) and 25cm-thick of contaminated soil ( $\rho_d=0.80$  g cm<sup>-3</sup>) in this order from the bottom of each container. After packing the samples, oxidation reduction potential sensors were inserted at depths of 2.5, 7, 9, 12.5, 14.5, 22, 32, 37, 42cm. Soil temperature sensors were also buried at depths of 5, 15, 25 and 35cm in each container. During the experiment period, the groundwater level of each container was maintained as 5, 10, and 40cm; hereafter we call the models GL5, GL10 and GL40, respectively.

## Cultivation Procedure

We used *Ryuho* soy bean (*Glycine max* (L.) Merr.cv.*Ryuho*) as the breed variety of our cultivation experiment. The holes of 3 to 5 cm diameter at the four positions in each container were made, and then 5 to 6 grains were seeded in June 16, 2014. Thinning out was done in 14 days after seeding and left two of normal growth. Harvesting was conducted between late September and early October, which is the grain filling period in Japan. The groundwater level after seeding was 15cm, the ground water levels after germination were 5, 10 and 40cm. The authors used Marriott bottles to control the groundwater levels and checking of each groundwater level was carried out by using three groundwater pipes attached to the containers. The recommended amount of chemical fertilizer for *Ryuho* soy bean was added. 2L of irrigation water was supplied in 4-days intervals (equivalent to 2.0 mm d<sup>-1</sup>). Protection treatment was done when it was necessary.

## Measurement Method

Oxidation-reduction potential (ORP) electrodes were installed for measuring oxidation-reduction potential at arbitrary depths of the soil boxes. Measuring with the ORP meters (model UC-23, Central Kagaku co. Ltd) was conducted during the entire period of cultivation experiment. The growth and yield assessments such as plant height, leaf age, branching, pod number and 100-grains weight, were carried out based on the cultivation guideline of soybean in Akita Prefecture (Akita Prefecture, 2015). After harvesting, Cd and Cu were extracted with HCl and HNO<sub>3</sub> and their concentration in stem and leaf, root, grain and soil, was quantified by analyzing their specimens with the atomic absorption spectrophotometry (MAFF, 1979). Other physical and chemical properties of the soil was also measured with the standard methods in Japan. We found a significant difference in the results of growth and yield assessments by using the Tukey-Kramer method.

## RESULTS AND DISCUSSION

### Oxidation-Reduction Potential (Eh)

For a long time, the reduction condition of soil has been known to cause insolubility of Cd and Cu, this has been utilized to Cd uptake suppression (Yamane et al., 1997). That is, by increasing the solubility of Cd and Cu in the soil in the oxidation condition, the uptake of these heavy metals from plant roots is promoted. On the other hand, it is known that the uptake of these heavy metals is inhibited when their solubility is reduced in the reduction condition. In the oxidation-reduction potential (Eh), the oxidation layer and the reduction layer are defined as  $Eh \geq 300\text{mV}$  and  $Eh < 300\text{mV}$ , respectively (Iimura, 1981).

Measured Eh values on each of the groundwater levels are shown in Figs.1, 2 and 3. In the GL5 container (Fig.1), Eh values measured at the 2.5cm depth was an oxidation layer of about 600mV; however, below the 7cm depth Eh values indicated reduction condition. In the GL10 container (Fig.2), Eh values at 2.5cm and the 7cm depths indicated more than 500mV; however, Eh values measured below the 12cm depth was a reduction layer of  $Eh < -100\text{mV}$ . In the GL40 container (Fig.3), except for Eh values measured at the 42cm depth, all of the observation depths became oxidation layers of  $Eh \geq 500\text{mV}$ .

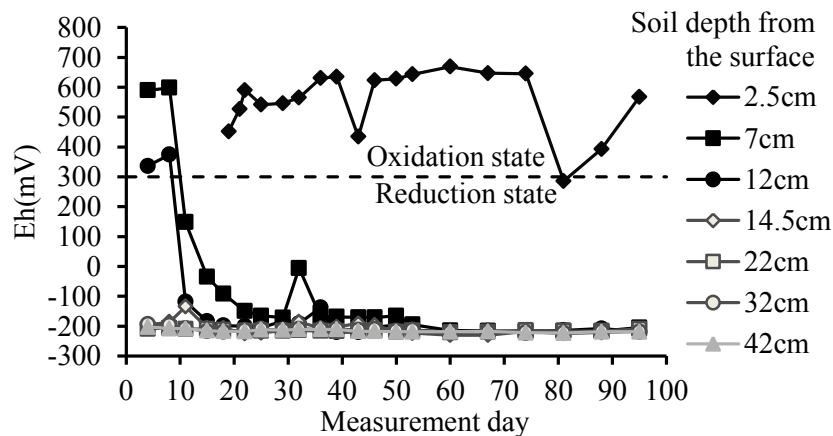
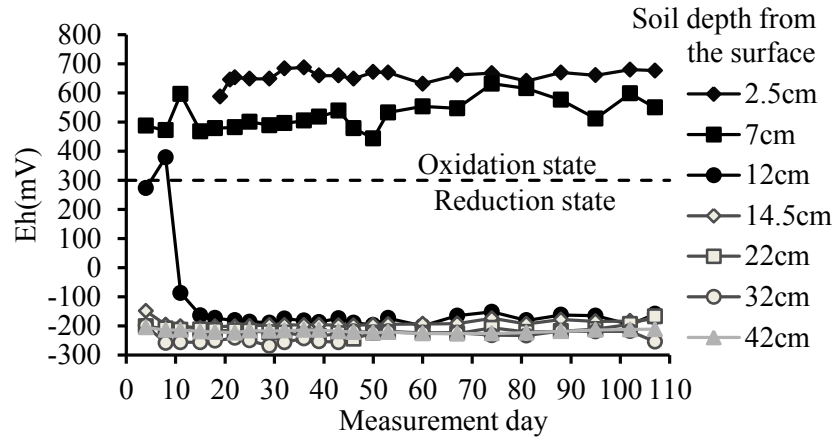
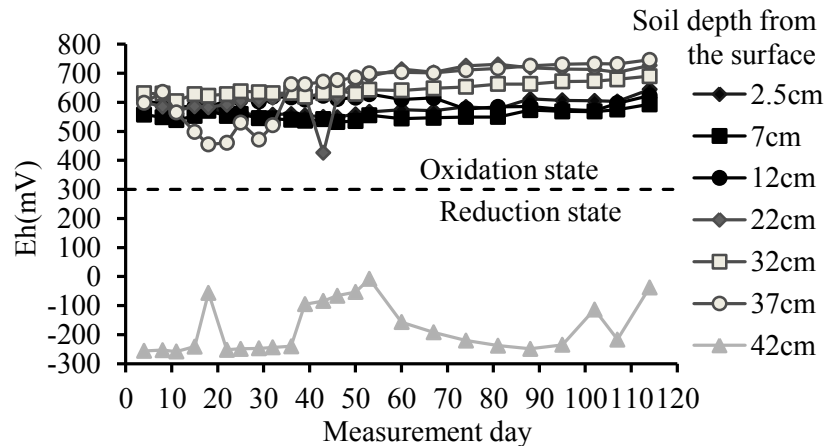


Fig.1 Temporal changes of Eh with GL5



**Fig.2 Temporal changes of Eh with GL10**



**Fig.3 Temporal changes of Eh with GL40**

From these results, it became apparent that controlling the upper part of the groundwater table to be an oxidation layer and the part below the groundwater table to be a reduction layer can be possible. It is inferred from this that thickness of supply layer of solubilized Cd and Cu are estimated as about 5cm for GL5, about 10cm for GL10, and about 40cm for GL40. From the temporal changes of Eh values measured at each level of the groundwater table, after clarifying the oxidation-reduction environment of the root zone, preparation for comparing values of the uptake behavior of Cd and Cu can be said to have been established. Although the values of heavy metal concentration in the contaminated soil were different, the vertical directions of Eh distribution controlled in the GL10 and the GL40 models were almost the same as the result of Haquet al. (2014a, b). In addition, it is estimated that the Eh values in the canonical paddy field depend on the soil crashing rate, the amount of organic matter, and soil pH.

### Cd and Cu Concentration in Soybean Plants

Cd concentration in soybean seeds was GL5 ( $0.25\text{mg kg}^{-1}$ ) < GL10 ( $0.52\text{mg kg}^{-1}$ ) < GL40 ( $1.07\text{mg kg}^{-1}$ ) and there were significant differences among the three treatments ( $p < 0.05$ ) (Table 2). Those values were three to ten times greater compared with Cd concentration in soybeans in non-polluted soil (Murakami et al., 2011). Cd concentration in stems was GL5 ( $0.28\text{mg kg}^{-1}$ ) < GL10 ( $0.45\text{mg kg}^{-1}$ ) < GL40 ( $1.48\text{mg kg}^{-1}$ ) and there were little significant difference between GL5 and GL10. However,

there was significant difference between GL5 and GL40, and also between GL10 and GL40 ( $p < 0.05$ ). Cd concentration in roots was GL10 ( $3.54\text{mg kg}^{-1}$ )  $<$  GL5 ( $4.92\text{mg kg}^{-1}$ )  $<$  GL40 ( $5.80\text{mg kg}^{-1}$ ) and there was a significant difference between GL10 and GL40 ( $p < 0.05$ ). The trend of Cd concentration in soybean plants were seed  $<$  stem  $<$  root as found by Haque et al. (2014a, b). Cd concentration in seeds was about 1/10 compared with the one in roots in the case of GL5 and GL10. Cd concentration in seeds was about 1/5 compared with that of roots in the case of GL40. It is thought that Cd concentration in soybean plants is affected by the groundwater level. Cd concentration in seeds was  $0.25\text{mg kg}^{-1}$  in the GL5 treatment and it was close to the standard Cd content in non-polluted soil (MAFF, 2004). There is a high possibility that Cd concentration in soybean seeds can be suppressed when soybeans are cultivated in poorly drained Cd-contaminated paddy field. From these results, it was considered that the control of the groundwater level had the effect of reducing the Cd concentration in soybean seeds.

Table 2 shows Cu concentration in soybean plants. Cu also change the solubility by oxidation-reduction potential as well as Cd. Cu concentration in soybean seeds was GL5 ( $5.08\text{mg kg}^{-1}$ )  $<$  GL10 ( $5.82\text{mg kg}^{-1}$ )  $<$  GL40 ( $9.96\text{mg kg}^{-1}$ ) and there were significant differences among the three treatments at  $p < 0.05$ . The average Cu concentration in edible soybeans in Japan, the United State and China is  $0.97\text{-}1.07\text{ mg kg}^{-1}$  (MEXT, 2016). The Cu concentration found in our current study was about five times higher than the above figure. One reason may have been that the Cu concentration in the soil we used this time was twice higher than the average Cu concentration in typical Japanese soil. Cu concentration in stems was GL5 ( $2.45\text{mg kg}^{-1}$ )  $<$  GL10 ( $2.76\text{mg kg}^{-1}$ )  $<$  GL40 ( $5.58\text{mg kg}^{-1}$ ) and there was little significant difference between GL5 and GL10. However, there were significant differences between GL5 and GL40, and also between GL10 and GL40 ( $p < 0.05$ ). Cu concentration in roots was GL40 ( $14.06\text{mg kg}^{-1}$ )  $<$  GL10 ( $39.48\text{mg kg}^{-1}$ )  $<$  GL5 ( $50.04\text{mg kg}^{-1}$ ) and there were little significant difference between GL5 and GL10. However, there was significant difference between GL5 and GL40, and also between GL10 and GL40 ( $p < 0.05$ ). The trend of Cu concentration in soybean plants was stem  $<$  seed  $<$  root. Cu concentration in seeds, however, was higher than that in stems unlike Cd concentration in the plant. It is thought to be due to transfer characteristics of Cu in soybean plants. Cu concentration in roots in GL5 and GL10 was twice higher than that of GL40. The morphological characteristic of roots is that there are many thick roots in the GL40 treatment and many fine root mat in the GL5 and GL10 treatments. The different root morphology may have affected the uptake of nutrient by soybeans. Cu concentration in soybeans at low groundwater levels was relatively high. Cu concentration in soybeans was higher than that in brown rice (Paul et al., 2011). Soybean seeds tend to accumulate more Cu than brown rice. Cu concentration in soybeans is defined as less than  $20\text{mg kg}^{-1}$  in China (MAPRC, 2005). On the other hands, there is no regulation value of soybean Cu content in Japan. However, those results of ours are valuable data because soybeans are important food and Cu in this food is detrimental for human health. From the above results, the control of the groundwater level is considered to be effective in reducing Cd concentration and Cu concentration in soybean seeds.

**Table 2 Cd and Cu concentration in soybean of three different groundwater levels**

Model	Seed-Cd	Stem-Cd	Root-Cd	Seed-Cu	Stem-Cu	Root-Cu
GL 5cm	$0.25\pm 0.04^a$	$0.28\pm 0.02^a$	$4.92\pm 1.54^{ab}$	$5.08\pm 0.27^a$	$2.45\pm 0.19^a$	$50.04\pm 13.93^a$
GL 10cm	$0.52\pm 0.06^b$	$0.45\pm 0.09^a$	$3.54\pm 0.56^a$	$5.82\pm 0.33^b$	$2.76\pm 0.98^a$	$39.48\pm 7.51^a$
GL 40cm	$1.07\pm 0.17^c$	$1.48\pm 0.41^b$	$5.80\pm 1.03^b$	$9.96\pm 0.62^c$	$5.58\pm 0.51^b$	$14.11\pm 3.66^b$

Note: Small letter indicates significant difference at 5% level according to Turkey-Kramer test;  $\pm$  shows standard deviation. For Seed Cd analysis ( $n=8$ ); for other cases ( $n=5$ ). Unit:  $\text{mg kg}^{-1}$

### Soybean Yield and Its Components

Soybean yield and its components are indicated in Table 3. Average stem heights ( $n=8$ ) were GL5 (59.7

cm) < GL10 (66.8 cm) < GL40 (78.8 cm). Significant differences were recognized among the three models. Murakami et al. (2011) and Haque et al. (2014a, b) reported that in excess soil moisture condition, soybean plants do not grow well after the germination and result in low yield. A similar result was obtained in this study that the high groundwater level conditions suppress the growth of the stem.

The averages of the stem diameter and the number of seeds per pod (seed/pod) did not show any significant difference. The average of the branch number showed little significant difference between GL10 and GL40. However, there were significant differences between GL5 and GL10, and also between GL5 and GL40. The averages of good seed weight per plant were GL5 (20.5 g) < GL10 (36.2 g) < GL40 (56.3 g), showing significant differences among them. Averages of 100 seeds weight also showed a similar trend as the good seed weight. The good seed weights of GL5 and GL10 models lowered by about 36 % and 64 %, respectively, compared with that of GL40 model, which suggested that even a slight difference in the groundwater level can bring a critical effect on the soybean yield under a high groundwater level condition. According to Shimada et al. (1995), it is important to control the groundwater level according to the rain condition in actual paddy fields. Arihara (2000) reported that the soybean yield was highest in the fields with a 40-50 cm groundwater level. His finding was based on the investigation on the relationship between groundwater level and soybean yield.

The soybean yield in this experiment using Cd contaminant soil was higher under the low groundwater level condition and lower under the high groundwater level condition.

**Table 3 Soybean yield components of three different groundwater levels**

Model	Stem height (cm)	Stem diameter (cm)	Branch No.	Seed/Pod	100 seed wt. (g)	Good seed wt. (g)
GL 5cm	59.7±6.3 <sup>a</sup>	7.1±0.9 <sup>a</sup>	3.6±0.9 <sup>a</sup>	1.6±0.2 <sup>a</sup>	28.3±2.3 <sup>a</sup>	20.5±8.9 <sup>a</sup>
GL 10cm	66.8±3.8 <sup>b</sup>	7.6±0.8 <sup>ab</sup>	5.1±0.8 <sup>b</sup>	1.6±0.2 <sup>a</sup>	37.2±2.1 <sup>b</sup>	36.2±6.3 <sup>b</sup>
GL 40cm	78.8±5.2 <sup>c</sup>	8.5±0.7 <sup>b</sup>	5.5±1.1 <sup>b</sup>	1.6±0.1 <sup>a</sup>	40.0±1.8 <sup>c</sup>	56.3±4.7 <sup>c</sup>

*Note: Small letter indicates significant difference at 5% level according to Turkey-Kramer test; ± shows standard deviation. Seed weight at 15% moisture. In all case (n=8).*

## CONCLUSION

Soybean was cultivated in containers using Cd contaminant soil (concentration: 2.27 mg kg<sup>-1</sup>, thickness: 25 cm) under groundwater levels of 5 cm, 10 cm, and 40 cm with measuring the oxidation-reduction potential in the soil. Soil was in oxidation condition above the groundwater level and in reduction condition below it. Cd and Cu concentrations of stems and seeds were found to be lowered under a higher groundwater level condition. Under a higher groundwater level condition, Cd concentration in roots showed a low value but that of Cu showed the opposite result. It was confirmed, as earlier studies had reported, that growth and yield of soybeans were good when the groundwater level was low. Controlling groundwater level can be one of the simplest and most economical methods for reducing the Cd and Cu uptake by soybean plants. When we apply this method on site, relief well will be available for controlling ground water level. This can help minimizing toxic metals uptake from polluted soils of soybean cultivated fields with high groundwater level in developing countries.

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