REDUCING CADMIUM ABSORPTION IN SOYBEAN WITH MIXING TILLAGE AND GROUNDWATER LEVEL CONTROL

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THE UNITED GRADUATE SCHOOL OF AGRICULTURAL SCIENCES
IWATE UNIVERSITY (HIROSAKI UNIVERSITY)
JAPAN

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A Thesis Submitted

By

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Abstract

With the development of modern industry and human activities, agricultural soil contamination occurs and poses a threat to crop production and human health. It is important to minimize the concentration of these contaminants in agricultural products for both international trading and our health. In this greenhouse experiment, we investigated the effect of groundwater level control and mixing tillage on the growth and yield, cadmium (Cd) and copper (Cu) uptake by soybean plants which were cultivated in contaminated soil. In the groundwater level control experiment, Growth tests of three models: GL5, GL10 and GL40 were conducted. “5”, “10” and “40” mean the groundwater level in the containers. Each of them was consisted of 15 cm thickness of non-contaminated subsoil (0.12 mg Cd kg^{-1}, 2.4 mg Cu kg^{-1}) and 25cm thickness of contaminated topsoil (2.27 mg Cd kg^{-1}, 43.4 mg Cu kg^{-1}). In the mixing tillage experiment, growth tests of eight models: C-01, C-02, L-10, M-10, H-10, L-20, M-20 and H-20 were conducted. Here, the “C-01” and “C-02” models consisted of 40cm-thick non-contaminated soil (0.14 and 0.13 mg Cd kg^{-1} respectively); “L”, “M” and “H” mean the Cd concentration in contaminated soil, 0.65, 1.20 and 1.73mg kg^{-1}, respectively; “10” and “20” mean the thickness of contaminated soil in the topsoil, 10cm and 20cm, respectively. As a result, in the groundwater level control experiment, Cd and Cu concentration of soybean seeds of GL5, GL10 and GL40 were 0.25, 0.52 and 1.07 mg Cd kg^{-1}, respectively, and 5.08, 5.82 and 9.96 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. From the above, it can be concluded that controlling groundwater levels can reduce Cd and Cu uptake and affect growth and yield of soybeans. In the mixing tillage experiment, Cd concentration in each model of the soybean seeds was in the order of C-02 (0.14 mg kg^{-1}) < C-01 (0.18 mg kg^{-1}) < M-10 (0.26 mg kg^{-1}) < L-10 (0.30 mg kg^{-1}) < M-20 (0.33 mg kg^{-1}) < L-20 (0.37 mg kg^{-1}) < H-10 (0.46 mg kg^{-1}) < H-20 (0.54 mg kg^{-1}). It reveals that soybean plants absorb more Cd when the soil Cd concentration is higher. Cd accumulation in the seeds of H-10 was higher than that in the seeds of L-20 and significantly higher than that in the seeds of M-20 at 5% level. The ratio of average Cd concentration in seeds, stems, and roots were about 1 : 2 : 7. There was no significant effect caused on soybean growth and yield by heavy metal concentration. It was proved that mixing tillage can effectively alleviate the Cd accumulation in the soybean seeds and that it had no significant influence on the soybean growth and yield.

Keywords

groundwater level control, mixing tillage, oxidation-reduction potential, cadmium and copper uptake, soybean, growth and yield

Chapter 1: Introduction

Current status of heavy metals contamination: Soil under natural conditions contains a small amount of heavy metals. However, owing to the global industrialization and various anthropogenic activities, such as the application of heavy metal containing sewage sludge and phosphate fertilizers, municipal waste disposal, leather working and the wide use of Nickel-Cadmium batteries, large amounts of potentially detrimental elements were released into the biosphere and then threaten the ecological environment and human health (Arao et al., 2010; Hillel, 2008; Matsui and Okazaki, 1993). Agricultural lands have been heavily contaminated by cadmium (Cd), copper (Cu) and arsenic, which were defined as the targeted hazardous substances for regulation in Japan. Japan and other developed countries have suffered a lot from the soil pollution during the 20th century. In Japan, as seen in such grave impacts of heavy metals in the cases of Itai-itai disease induced by Cd and a decrease of rice yield affected by Cu, there have been some serious problems regarding heavy metals. (Asami, 2010). It is still a long-term task for dealing with soil pollution both now and in the future. It was reported that average Cd concentration in the soil of Japan was 0.23 mg kg^{-1} in 1984 (MAFF, 2011) and higher values of 0.33 ~ 0.34 mg kg^{-1} by Takeda et al. (2004) and Yamazaki et al. (2009). Problems of heavy metals pollution are also emerging in many other countries (Asami, 2010; Hata, 2008). According to previous soil surveys done in China, more than 13330 ha of field in 11 prefectures were contaminated by varying degrees of Cd (Zhang and Huang, 2000); the probability of heavy metal pollution in soil of cultivated land was about 16.7% and the pollution probability of Cd was 25.2%, significantly exceeding pollution levels of the other heavy metals (Song et al., 2013). This serious situation can be clearly interpreted from the issue of “the Action Plan for Soil Pollution Prevention and Control” by the Ministry of Ecology and Environment of the People’s Republic of China (MEE) in 2016.

Hazard of Cd and Cu contamination: Agricultural product would be contaminated by soil contamination. It would affect growth and yield of crops and may adversely cause the disadvantage to human body through the food chain. Increasing evidence suggests that soil microorganisms which benefit soil environment are far more sensitive to heavy metal stress than soil animals or plants growing on the same soils (Giller et al., 1998). Cd is one of the most detrimental trace metals to both plants and animals. Cd accumulation in their bodies is a non-reversible process with a biological half-life about 20 years (Ryan, 1982). As well, it is estimated that half-life in soil varies from 15 to 1100 years (Kabata and Pendias, 1992). It is reported that Cd can induce a decrease in plant biomass and also can cause oxidative stress in pea plants (Dixit er al., 2001). Precious research also indicated that low Cd concentration in the soil might have positive impact on plant growth, the ecotoxicity of Cd to plant growth increases with the rise of Cd concentration level in soil (Cao et al., 2007; Liu et al., 2009, 2010a, 2010b; Wu and Zhang, 2002). An extreme case of chronic Cd toxicity can result in human’s health risks, such as osteomalacia and bone fractures (Kobayashi, 1978). The JECFA (Joint FAO/WHO Expert Committee on Food Additives) recommended PTWI (provisional tolerable weekly intake) of 7 μg kg^{-1} bw $week^{-1}$ (intake of Cd per kg of body weight per week). The committee evaluated the national dietary intake of Cd and its estimates of the mean national intake of Cd ranged from 0.7–6.3 μg kg^{-1} of body weight per week, and they also addressed that for some individuals, the total intake of Cd might exceed the PTWI because total food consumption for high consumers is estimated to be about twice the average amount of total food consumption for average person (JECFA, 2004). It has been reported that trace of Cu has promotion effect on the growth of plants, but it will be toxic to plants when the concentration is high (Chang et al., 2000; Huang et al., 1993; Kang et al., 1999). For example, an inhibition of crop growth and suppression of root absorption function were reported (FAMIC, 2017; Gao et al., 2014; Ishidzuka, 1940, 1942; Itou and Imure, 1971; Kang et al., 1999).

Discussion of heavy metals contamination managements: Measures and technologies for minimizing the absorption of heavy metals by agricultural crops have been proposed and discussed as follows: (1) soil dressing, addressing non-contaminated soil on the contaminated soil surface or replacing the contaminated topsoil with non-contaminated soil; (2) chemical washing with iron salts, such as $FeCl_3$; (3) use of low heavy metal absorption variety for cultivation; (4) phytoremediation by promising crops, for example, the rice cultivar of Japonica-Indica (Milyang 23); (5) pH adjustment (Akahane et al., 2013; Arao at al., 2010; Cheng, 2003; Dong at al., 2007; Matsuda, 1997; Sasaki K et al., 2016a; Singh et al., 2003; Wuana and Okieimen, 2011; Zheng et al., 2002; Yamada, 2007). However, these countermeasures are sometimes too expensive or time consuming, and some of them have residual effect also. Soil dressing has been widely applied in Japan for paddy field cultivation. However, it needs a large amount of soil from some other places to improve a local soil. Previous researches showed that Japan and Taiwan had tried to use mountain soil instead of farmland soil for soil dressing to protect farmland productivity and ecosystem (Arao et al., 2010; Huang and Nakayama, 2010), although the mountain soil is often sterile and contains inadequate organic matter for crop growth.

For upland soybean crops, it has been reported that when the groundwater level is around 40cm, soybean plants are likely to have the high yield (Shimada et al., 1995; Arihara, 2000). In the situation of paddy rice, groundwater level control management has been applied to reduce cadmium uptake by rice plants, and it was reported that thickness of applied soil layer in soil dressing can be thinned by adopting groundwater level control management (Sasaki et al., 2009, 2010, 2012). Some research also reported that groundwater level control can also reduce the Cd uptake in soybean plants (Haque et al., 2014a, 2014b; Murakami et al., 2011). However, some verification experiment is needed to support its effectiveness. In addition, mixing tillage is an engineering method to plow up and mix soil layers (Sano, 1981; Uchiyama, 1983), which was mentioned by the Ministry of the Environment, Japan as one of the countermeasures to deal with soil pollution (MEG, 2010). Some research reported that it had a positive effect on minimizing Cd absorption by brown rice (Huang and Nakayama, 2011; Sasaki et al., 2016b).

Current situation and prospect of soybean planting: As one of the world's fastest expanding crops, international demand for soybean has highly increased in recent years, driven by ever-increasing use of products ranging from cooking oil to animal feed (soybean cake). Soybean is classified as one of the oil plants by FAO, since it is mainly used for edible oil processing in most countries, especially in the western countries (Kawashima, 2016). For East Asia residents, soybean is also one of the important ingredients in the food culture and plenty of soybeans is processed into many kinds of delicious food traditionally. It contributes the basis of many distinct flavors, such as natto and tofu. However, previous researches have proved that soybean is one of the crops which heavy metals Cd and Cu can be easily accumulated to their seeds (Wang and Wu, 1998; MAFF, 2007). It is essential to minimize the Cd and Cu absorption of soybean plants from contaminated soil.

In Japan, the self-sufficiency of soybean was around 7% in recent years, and an expansion of growing area of soybeans is one of the important measures for increasing calorie based food self-sufficiency rate (MAFF, 2017a). It is proposed that the safety standard of Cd in soybean is 0.2 mg kg^{-1} in China and EU, while in Japan, Cd concentration in soybean seeds which have been produced even in non-contaminated field could exceed this value (MAFF, 2015). It was reported that 9.6% of soybean produced in Japan contains over 0.2 mg Cd kg^{-1} and an average concentration as 0.13 mg Cd kg^{-1} (MAFF, 2017b). Therefore, it is inferred that soybean produced in Cd-contaminated and semi contaminated fields can be more dangerous than that in non-contaminated field to absorb much more Cd. Thus, there is real concern that Japanese domestic soybeans and its products will not meet the standards of export-partner countries in international trading. As for Cu in soybeans, it is probably a future task for Japan to provide a safety standard value, while some other countries already have such, e.g., 20 mg kg^{-1} in China (MAPRC, 2005).

In China, soybean had turned into one of the net import crops from 1996, and the scale and deficit is showing an increasing trend in the past years and in the future (Lu, 2017). Self-sufficiency rate of soybean was 13.1% with an import volume of 8391 t in 2016, and it is predicted by the government agency that the import of soybean will be 8556 t in 2020 and 9600 t in 2026. As one of the five grains of China’s agriculture civilization and the most important plant protein resource, the situation of depending on foreign trade at a high degree may change the foundation of China’s food security

(Lu, 2017; Wang and Wang, 2015). China government should continue to take soybean self-sufficiency and agriculture sustainable development as the primary basis. In 2016, the government proposed the measure of “Maize transfer Bean” (Chinese pronunciation “Mi Zhuan Dou”) and adopted a series of items to adjust crop planting structure for increasing soybean production (MOA, 2016).

Previous studies about Cd and Cu contamination in soybean planting: For reducing cadmium uptake by soybean plants cultivated in contaminated field soil, MAFF (2007) suggested that it can be feasible by choosing low absorption soybean breeds, adjusting pH by fertilizing and avoiding soil drying and so on. For a long time, the reduction condition of soil has been known to cause insolubility of Cd and Cu, this have been utilized to Cd and Cu uptake suppression (Paul et al., 2011). In soils, redox measurements integrate both physical conditions and microbial activity and provide a relevant description of the water level (Dwire et al., 2006; Mars and Wassen, 1999). In order to seek a convenient and efficient method, previous researches also verified the effectivity of changing the cultivate soil redox condition by controlling the groundwater level (Haque et al., 2014a, 2014b; Murakami et al., 2011), even though that groundwater level around 40 cm is recommended for high yield soybean planting.

Objectives: From these backgrounds, we can understand that soil contamination has become a serious problem worldwide, and it resulted in lots of crop problems and social misfortunes. However, there was no efficient or economic method to remove harmful heavy metals completely from field soil at this moment. This fact will last for a long period of time and pose a threat to safe food production and human health. Meanwhile, soybean is becoming more and more important for the world trade and food consumption. It will be an important task for secure expanding soybean production with a deteriorating soil environment. Supposing mixing tillage as one of the efficient and convenient countermeasures to deal with this problem, the objective of this study is to evaluate the practicability of mixing tillage on decreasing Cd and Cu absorption and its effect on the growth and yield of soybean plant. In order to confirm the effectiveness of controlling groundwater level on reducing Cd and Cu uptake in soybean, groundwater level control experiment was conducted before the test of mixing tillage. Information provided in this study would be useful for developing cost saving and convenient techniques of reducing Cd and Cu uptake for soybean-planting. This experiment was conducted under directions of the cultivation guidance of soybean in Akita prefecture, Japan (2015).

Chapter 2 Materials and methods

2.1 Introduction

The experiment was conducted in a greenhouse (Latitude: 40°35′; Longitude: 140°28′) of Hirosaki University, Hirosaki, Aomori, Japan. Experimental field models were simulated for soybean planting.

In groundwater level control experiment, three models with different groundwater level were prepared. Three models had the same distribution of soil layers. It was proposed to investigate the effects of controlling groundwater level on the growth and yield, Cd and Cu uptake of soybean plant which was cultivated in the contaminated soil.

In mixing tillage experiment, five models and three models were prepared in each year of 2016 and 2017, respectively. Different model was prepared with different thickness of contaminated soil or different concentration of heavy metals in the soil. It was proposed to investigate the effects of mixing tillage on the growth and yield, Cd and Cu uptake of soybean plant which was cultivated in the contaminated soil. The following parts in this chapter will give an introduction in details.

2.2 Experimental design

Experimental planting device (61 × 41 × 63 cm) was made with thick plastic containers and it was shown in Fig. 1. At the bottom of each container, 14 cm-thickness of gravel was packed. Then each container was filled with non-contaminated soil and contaminated soil. Contaminated soils were placed as the upper layer since they were designed to imitate the remediated field by mixing tillage.

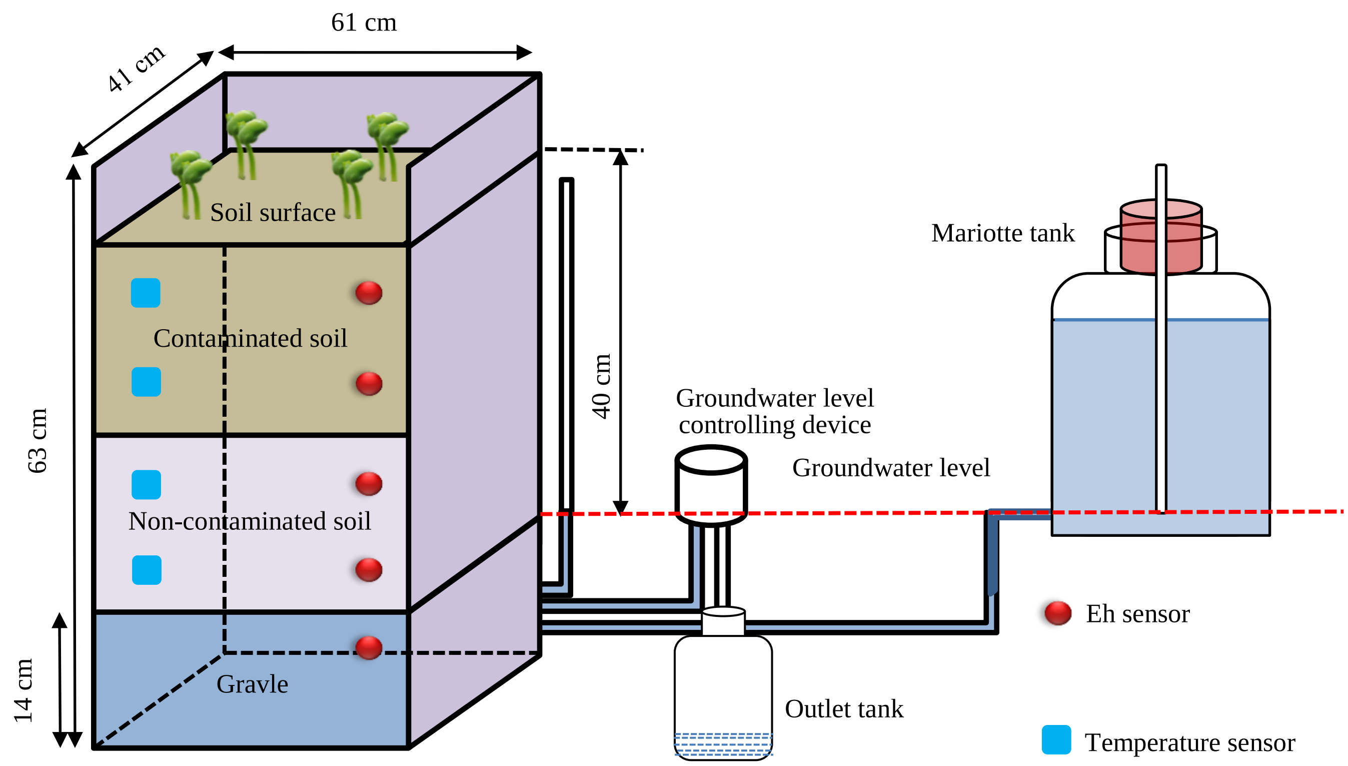


Fig. 1 Experimental planting device

2.2.1 Groundwater level control experiment

Three plastic containers were prepared and then they were packed with 14 cm-thick gravels, 15 cm-thick non-contaminated soil and 25 cm-thick contaminated soil in this order from the bottom of each container (Fig. 2). During the experiment period, the groundwater level of each container was maintained as 5, 10 and 40 cm; Hereafter, we call the three models as GL-5, GL-10 and GL-40 (Fig. 2), where “5”, “10” and “40” mean the groundwater level 5, 10 and 40 cm, respectively.

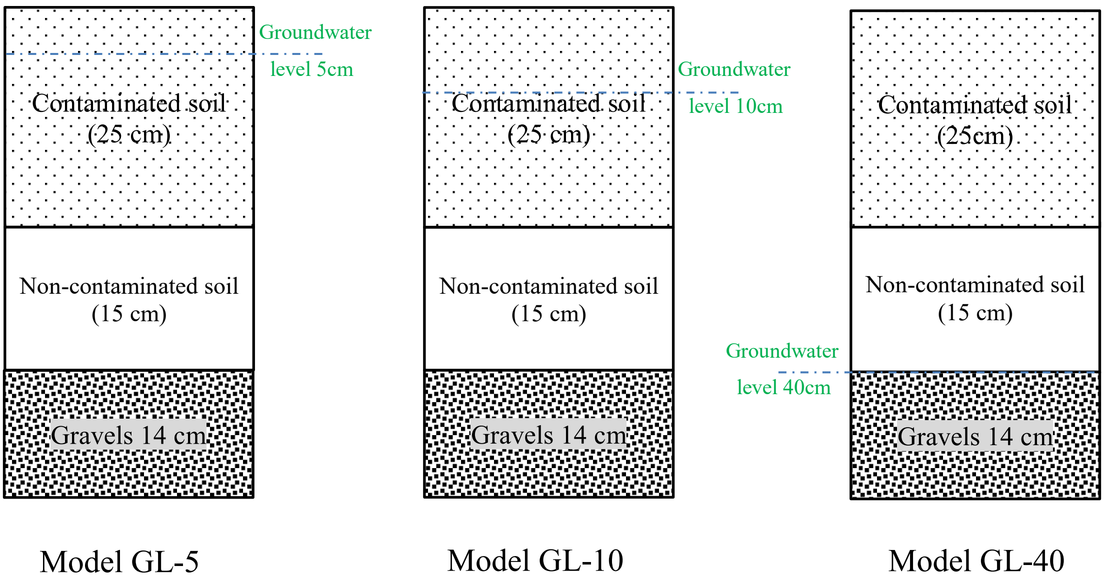


Fig. 2 Experimental design of soil layers in groundwater level control experiment

2.2.2 Mixing tillage experiment

In this experiment, there were three Cd concentration levels of contaminated soil and two different thickness of contaminated soil layer being designed in two years. In the year of 2016, four plastic containers were prepared with the combination of two different Cd concentration of soil-1.75 and 0.65 mg kg^{-1} and two different thickness of contaminated soil, 10- and 20 cm-thickness. In the year of 2017, two plastic containers were prepared with the combination of Cd concentration of soil-1.2 mg kg^{-1} and two different thickness of contaminated soil, 10- and 20 cm-thickness. “Control” model, which was consisted of 40 cm-thickness of non-contaminated soil was also prepared during the two years. Hereafter, we call the five models in the year of 2016 as C-01, L-10, H-10, L-20 and H-20, and we call the three models in the year of 2017 as C-02, M-10 and M-20 (Fig. 3), where: “L”, “M” and “H” stand for low, middle and high cadmium concentration, respectively; “10” and “20” mean the thickness of contaminated soil layer 10 cm and 20 cm, respectively.

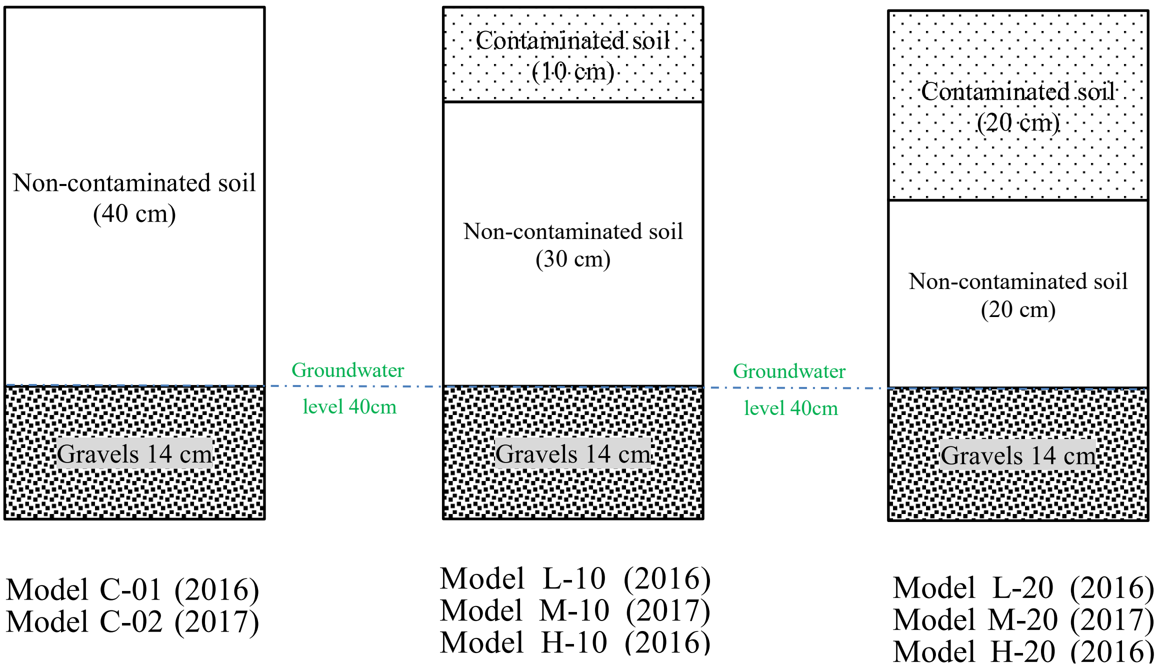


Fig. 3 Experimental design of soil layers in mixing tillage experiment

2.3 Soil materials

Soils used in this experiment were collected from the plow layer of the paddy field at two different places. Non-contaminated soil was collected from the Kanagi farm (Latitude: 40°54′ N; Longitude: 140°28′ E) which belongs to Hirosaki University, located in Goshogawara, Aomori Prefecture, Japan. Hereafter, we call it “Kanagi soil”. Contaminated soil was collected from a Cd contaminated paddy field in “X” Prefecture located in the Estern Japan, where the mine waste water had been used as irrigation water. Hereafter, we call it “X paddy field”. Both of the contaminated soil and non-contaminated soil used in this experiment were collected from the same paddy fields (Fig. 4).

(a) Kanagi farm (b) “X” paddy field

Fig. 4 Scene of soils collection

2.3.1 Soil properties

Soil properties in two experiments had some difference for the reason of soil collection in different years.

Groundwater level control experiment: Chemical and physical properties of the soil samples are shown in Table1. The 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^{-1}, respectively while Haque et al. (2014a, 2014b) had used contaminated soils of 3.39 and 1.57 mg kg^{-1}. Cu concentration in the contaminated and non-contaminated soil in this study was 43.4 and 2.4 mg kg^{-1}, 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^{-1} and 24.8 mg Cu kg^{-1} (Asami, 2010).

Mixing tillage experiment: The physical and chemical properties of soils are shown in Table 2. The soil texture of the contaminated and non-contaminated soil was Loam and Clay Loam, respectively. The organic matter content (OM) of the contaminated and non-contaminated soil was 5.15% and 3.64% respectively. Content of organic matters were analyzed by elemental analyzer (Model: vario EL cube, Elementar Analysensysteme GmbH, Germany). Cd and Cu concentration of the contaminated and non-contaminated soil were 1.73 and 0.14 mg Cd kg^{-1}, 12.2 and 3.7 mg Cu kg^{-1}, respectively, while in the year of 2017, Cd and Cu concentration of the contaminated and non-contaminated soil were 2.17 and 0.13 mg Cd kg^{-1}, 12.2 and 5.2 mg Cu kg^{-1}, respectively. We obtained 0.65 mg Cd kg^{-1}, 6.5 mg Cu kg^{-1} soil by mixing the contaminated soil and non-contaminated soil at an appropriate ratio of 1 : 2.6 in 2016.

Its soil texture was Clay Loam, and its OM turned to be 5.01%. We got 1.2 mg Cd kg^{-1}, 11.8 mg Cu kg^{-1} soil by mixing the contaminated soil and non-contaminated soil at an appropriate ratio of 1 : 0.91 in 2017.

Table 1 Physical and chemical properties of the soil samples (groundwater level control experiment)

Sample	Density (g cm^{-3})	Soil texture	K_2O *	Na_2O *	CaO *	MgO *	Cu *	Cd *	T-C (%)	T-N (%)	C/N	OM (%)
X paddy soil	2.57	CL	232	96	2032	224	43.4	2.27	3.39	0.28	12.2	5.86
Kanagi soil	2.54	CL	120	64	400	120	2.4	0.12	2.07	0.16	13.3	3.62
Gravel	2.68	-	58	18	539	147	1.5	0.13	-	0	-	0.05

Note: Soil texture is based on the International Union of Soil Sciences (IUSS) classification. L: Loam, CL: Clay Loam; Gravel diameter size 2-4 mm; OM=1.714T-C;
*: *mg kg*$^{-1}$

Table 2 Soil physical and chemical properties (mixing tillage experiment)

Sample	Density ($g\ cm^{-3}$)	Soil texture	K_2O *	CaO *	MgO *	Cu *	Cd *	T-C (%)	T-N (%)	C/N	OM (%)
X paddy soil	2.54	L	156	1574	226	12.2	1.73	2.99	0.25	12.0	5.15
Kanagi soil	2.58	CL	184	372	190	3.7	0.14	2.11	0.17	12.3	3.64
Gravel	2.68	-	-	-	-	0.8	-	0.04	0.01	7.2	0.05

*Note: Soil texture is based on the International Union of Soil Sciences (IUSS) classification. L: Loam, CL: Clay Loam; Gravel diameter size 2-4 mm; OM=1.714T-C; *: mg kg^{-1}*

2.3.2 Soil preparation

Soil water content ratio was detected at first in order to get appropriate amount of soil materials. Small stones and plant debris were removed by hands from both contaminated soil and non-contaminated soil (Fig. 5). Then water was added into the soil and mixed in a bowl. Soil balls (diameter: 10 ~ 12 cm) was made after the soil and water was well mixed as Fig. 6 shows. After that, wet soil balls were placed on the thin board in the greenhouse for drying (about one week) (Fig. 7). The soil balls were broken into coarse grained soil about 2 ~ 4 cm in diameter with hammer as shown in Fig. 8 for the construction of soil layers. Then fine soil grains were sieved out by size 4.75 mm sieve (Fig. 9)

Fig. 5 Remove of stones and plant debris

Fig. 6 Making of soil balls

Fig. 7 Drying of soil balls

Fig. 8 Breaking of soil

Fig. 9 Soil sieving

2.3.3 Soil layer construction

The filling procedure of soil layers were shown in figures followed. Firstly, 14 cm-thick gravel layer was filled with small gravels at the bottom of each container (Figs 10 & 11). Then on the gravel layer, non-contaminated Kanagi soil was packed

40 cm thickness of soil layer in Model C-01 and C-02, 30 cm thickness in Model L-10, M-10 and H-10, 20 cm thickness in Model L-20, M-20 and H-20. Finally, 10 cm thickness of top layer in Model L-10, M-10 and H-10 were filled with 0.5 mg Cd kg^{-1}, 1.2 mg Cd kg^{-1} and 1.7 mg Cd kg^{-1} contaminated soil, respectively. As well, 20 cm-thick top layers in Model L-20, M-20 and H-20 were filled with the contaminated soils. Rubber rammer (Fig. 12) was used to make the gravel layer and soil layer of each model in uniform bulk density (0.80 ~ 0.89 g cm^{-3}). After the contaminated soil layer's filling, the soil surface was about 9 cm lower than top of the plastic container (Fig. 13).

Fig. 10 Gravel filling

Fig. 11 Depth measuring

Fig. 12 Soil filling

Fig. 13 Depth measuring

2.3.4 Fertilizer application

Table 3 Fertilizers application

Fertilizer	Standard	Amount
Soybean special compound fertilizer No. 2 (N-P_2O_5-K_2O=5-15-15%)	100 kg ha^{-1}	2.5 g
Fused phosphate	800 kg ha^{-1}	20 g
Bio-fertilizer (contains rhizobia)	-	50 g

Before the top 20 cm soil of each Model was packed, the recommended amount of fertilizers (Table 3) were added in the soil and well mixed (Figs. 14 & 15). Additional fertilizer was not applied after soybean seeding.

Fig. 14 Fertilizers

Fig. 15 Fertilizers addition

2.4 Groundwater level management

Stable water supply is vital to plant growth, and constant groundwater level of all models is essential for the comparison of different models. In our experiments, marriott equipment was connected to the bottom of each container to control the groundwater level. Groundwater level was checked by the manometers attached to the bottoms of containers (Figs. 1 & 16).

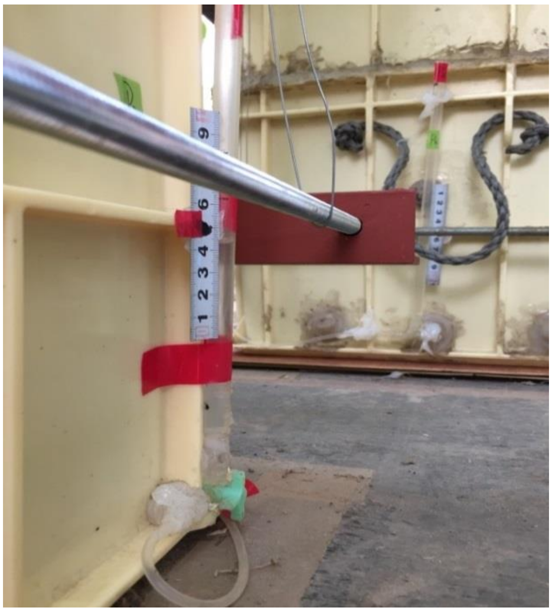


Fig. 16 Groundwater level check

Fig. 17 Soil surface after water supplying

For germination, it is necessary to make the soil moist enough before sowing (Fig. 17), so underground water (about 32 L) was supplied from the soil surface to dry soil in the containers until water could be seen in the manometers. After seeding, groundwater level management was carried out. Here, groundwater level was controlled at 40 cm for all models.

2.5 Soybean seeding and thinning out

Fig. 18 Photo taken before seeding (2017)

After the soil being moist completely (Fig. 18), soybean seeds were sown at 4th June, 2016, and 3rd June, 2017. Ryuho soybean (Glycine max (L.) Merr. cv. Ryuho) was used as the breed for this cultivation experiment (Fig. 19). It is one of the recommended varieties in Akita, Iwate and Yamagata Prefectures, Japan.

Holes of 3 to 5 cm diameter were made at four designated positions on the surface of each container, and then 5 seeds were planted in each position (Figs. 20 & 21). Thinning out was done after 16 days from seeding in both years, and two best growths were left at each position (Fig. 22).

Fig. 19 Seeds of Ryuho

Fig. 20 Soybean seeding

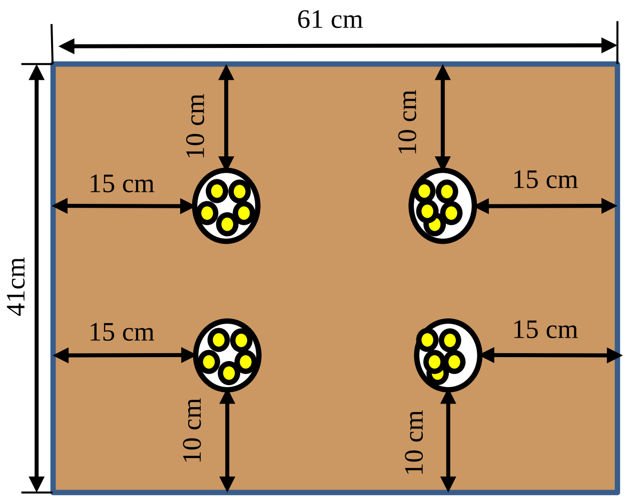


Fig. 21 Plane view of seeding positions in the container

Fig. 22 Thinning out

2.6 Cultural management practices

For normal growth of soybean plants and simulation of actual fields, some management were conducted throughout the whole growth period, such as weeding, watering, pesticides spraying and other measurements.

2.6.1 Weeding

Weeds can compete with soybean plants for water and nutrients and provide protection to the pests. Thus, hand weeding was done when it was necessary after seeding. Before soybean plants' leaves could cover the surface of containers, the weed grew vigorously, and this situation was suppressed after the soybean plants' leaves shaded the soil.

2.6.2 Watering

2 L of irrigation water was sprayed in 4-days intervals to every container to simulate the precipitation pattern of the northeast Japan region.

2.6.3 Pesticides

Pesticides were sprayed for the prevention and treatment of pests and diseases. Since pest of Tetranychus urticae happened badly during the leaf fall period in 2016 (Fig. 23), pesticide was applied twice in September to suppress the insect attack. A total of 5 times of pesticides spraying was done in 2016, and 4 times in 2017 (Table 4). Fig. 24 is showing the photo taken after pesticides spraying.

Table 4 Pesticide spraying in two years

2016		2017	
date	pesticide	date	pesticide
7/03	Mospilan (acetamiprid)	7/06	Marathon (emulsion)
8/05	Porebathon polysulfide	7/28	Porebathon polysulfide
8/24	Albelin (granule water solvent)	8/23	Marathon (emulsion)
9/07	Sumithion (emulsion)	9/08	Marathon (emulsion)
9/13	Acaricide	-	-

Fig. 23 Pest of Tetranychus urticae happened in 2016

Fig. 24 Photo taken after pesticides spraying

2.6.4 Other treatments

Soybean plants were tied with supporting sticks for making the plant straightly. In addition, soybean plants in each container were numbered from 1~8 after thinning out (Fig. 25).

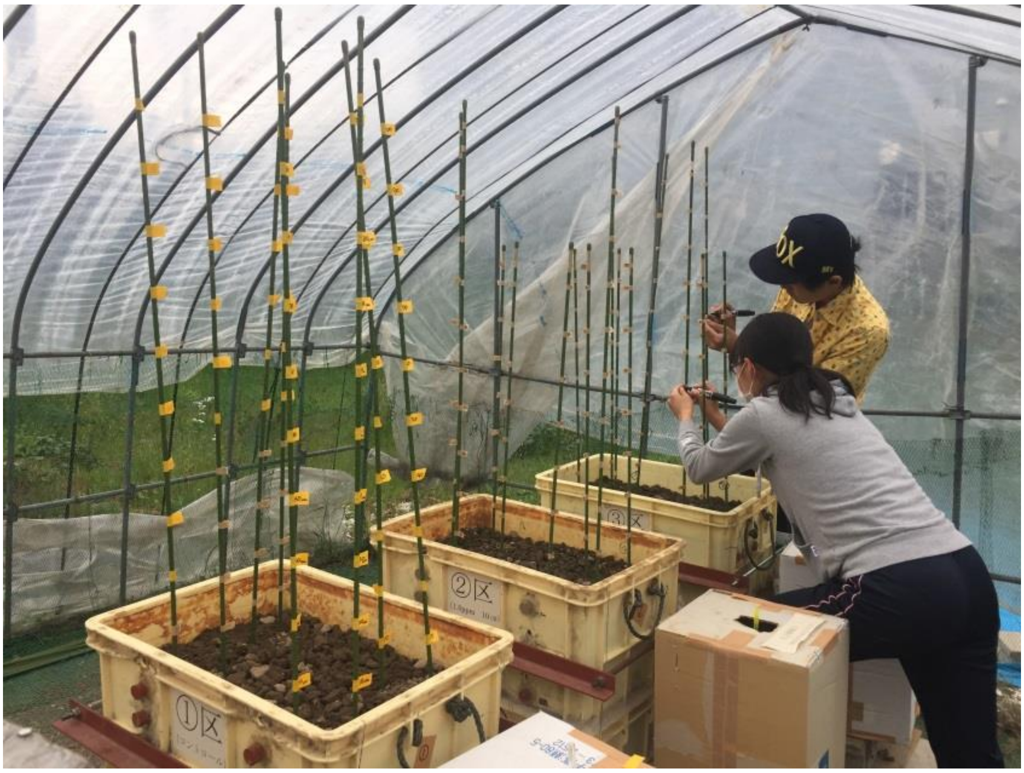


Fig. 25 Setting of supporting sticks and length label

2.7 Plant environmental monitoring

Before seeding, the oxidation-reduction potential electrodes were inserted for measuring the oxidation-reduction potential (Eh) and soil temperature sensors were also inserted for temperature measurement.

2.7.1 Oxidation-reduction potential (Eh) of soil

Before inserting the oxidation-reduction potential electrodes (Model UC-702E combination electrode, Central Kagaku Corporation, Japan) were checked for their function by using powders for ORP standard solutions (160-22 & 160-51, HORIBA Corporation, Japan) (Fig. 26). After checking, the Eh sensors were inserted. In groundwater level experiment, sensors were inserted at depths of 2.5, 7, 9, 12.5, 14.5, 22, 32, 37, 42cm, and in the mixing tillage experiment were at depths of 5, 15, 25, 35 and 45 cm of each container (the bottom of sensor is 4cm inside the soil). During the entire period of cultivation, measuring Eh was conducted by using ORP meter (Model UC-23, Central Science Co., Ltd.) (Fig. 27) twice per week. Multi recorder (Model ADL12, AS ONE Corporation, Japan) was applied in 2017 for Eh sensors in the soil layers of three models (Fig. 28). Eh value in soil indicates an oxidation condition when it is over 300 mV, and a reduction condition when it is under 300 mV. From previous researches, the Eh value can also indicate the groundwater level in the soil, as soil above groundwater level shows oxidation condition and soil below groundwater level shows reduction condition.

Fig. 26 Powders for ORP standard solutions

(a) Eh sensor (b) ORP meter

Fig. 27 Eh sensor and UC-23 digital pH/EC meter

Fig. 28 Multi recorder ADL12

2.7.2 Soil and air temperature

Soil temperature sensors (Model 203AT, SEMITEC Corporation, Japan) were inserted at depths of 5, 15, 25 and 35 cm of each container, and temperature data was automatically recorded by using ESPEC Thermo Recorder (Model TR-71U, T&D Corporation) (Fig. 29). Recorders were set (Fig. 30) in computer before logged with the temperature sensors. Recorder data was saved to the computer once a month. Greenhouse air temperature was also measured, since a temperature sensor was set beside the models in the greenhouse. Temperature sensor and its recorder used in this study were shown in the figures below.

Fig. 29 Temperature sensor and Thermo Recorder TR-71U

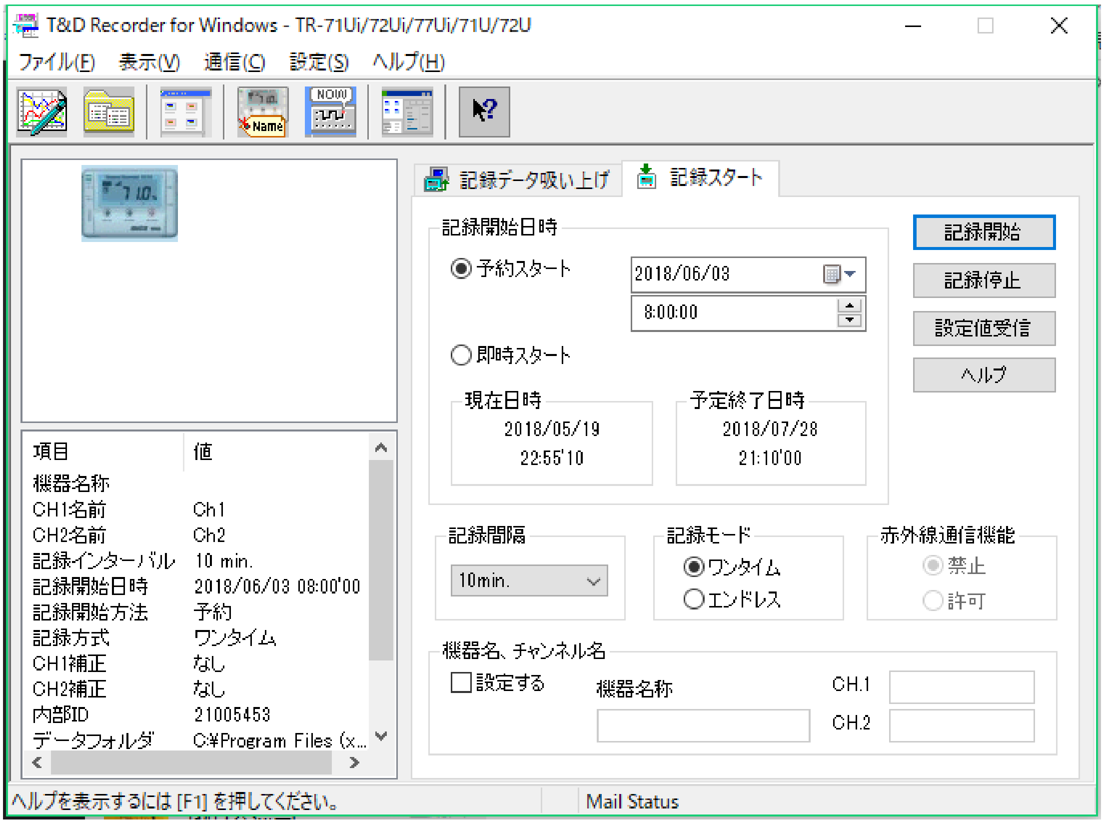


Fig. 30 Thermo Recorder set by software

2.8 Growth survey and yield investigation

During the growing period of soybean plants, total length, main stem length, leaf age and soil plant analyzer development (SPAD) were measured. In addition, stem diameter, branch number and yield investigations were measured after harvest.

2.8.1 Total length and main stem length

As shown in Fig. 30, total length means the height from soil surface to the top of the highest leaf. Main stem length means the height from soil surface to the top of main stem. Total length and main stem length were measured once a week from thinning out to harvest.

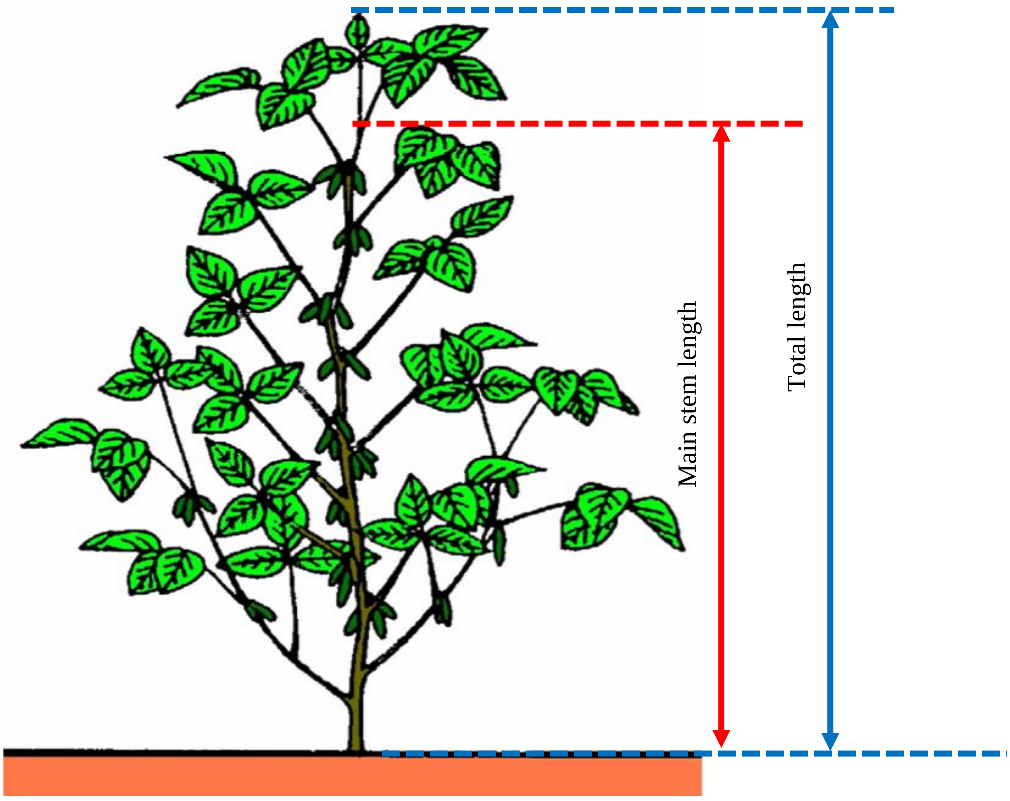


Fig. 31 Total length and main stem length

2.8.2 Leaf age

Leaf age represents the number of leaves on the main stem except for cotyledon and primary leaves. Its measurement was started after thinning out, and it continued once a week until the leaf age stopped increasing.

2.8.3 SPAD

SPAD value was measured once a week after the second trifoliate leaf on the main stem unfold entirely. Three leaflets of the second latest fully developed trifoliate leaf on the main stem were always being measured (Fig. 32) once a week until the harvesting period. Minolta SPAD-502 (KONIKA MINOLTA Corporation, Japan) was used in this experiment (Fig. 33). Fig. 34 shows the scene of SPAD measuring.

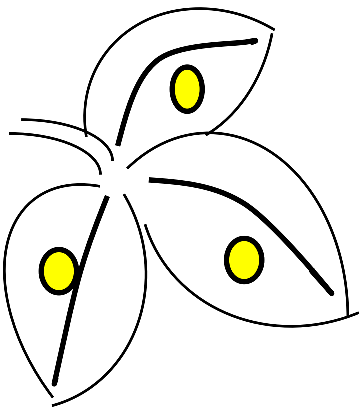


Fig. 32 SPAD measuring positions

Fig. 33 Minolta SPAD-502

Fig. 34 SPAD measurement

2.8.4 Harvesting of soybean plants

The life duration of soybean Ryuho is about 108 ~ 131 days (Akita 2015), and the harvest time comes when a clear sound can be heard from the dry pod. Soybean plants were cut by scissors 2 cm above the soil surface and then wrapped with paper, then soil in the containers were collected in order (Fig. 35). Soil samples were collected by soil samplers in every 10 cm soil layer of each container (Fig. 36 (a) & (b)). Root in the soil was picked up by blocks (Fig. 36 (c)). After harvesting, soil samples were analyzed for soil physical and chemical properties, including soil density, three phase distribution and soil solution pH etc. Wrapped soybean plants were put into net bags separately and hanged in the greenhouse for drying (Fig. 37). After about one month drying, the net bags were moved to the laboratory for further investigations, such as main stem length, branch number, node number and yield.

(a) Cutting of stem

(b) Collecting soil block

(c) Appearance of soil block

(d) Scene of soil collection

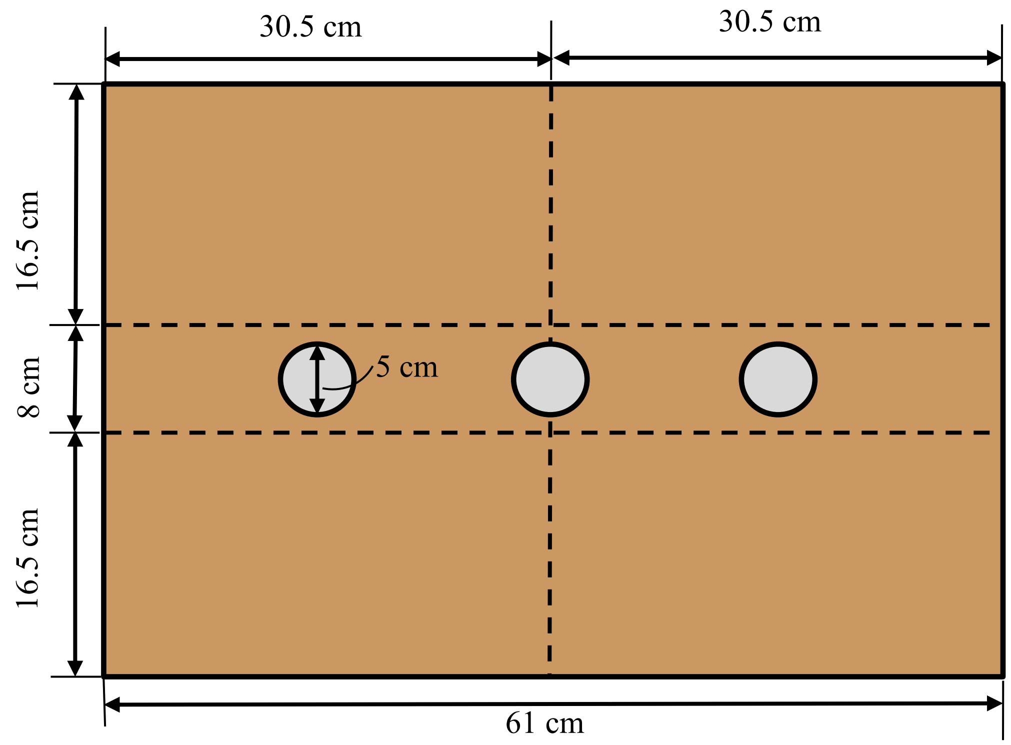
(e) Wrapping paper for soybean plant

(f) Putting into net bags

Fig. 35 Harvesting

(a) Soil sampling

(b) Soil sample cleaning



(c) Sampling positions

Fig. 36 Collecting of soil samples

Fig. 37 Drying of harvested soybean

2.8.5 Stem diameter

Stem diameter was measured by vernier caliper after the soybean plant was cut (Fig. 38). The value represents average of the longest diameter and the shortest diameter at the position 2 cm above soil surface.

Fig. 38 Stem diameter measured by vernier caliper

2.8.6 Branch number and node number

A branch on the main stem which has more than one node can be counted as one branch. Node number values the amounts of nodes on the main stem. Branch number and node number can be counted clearly when the leaves and pods were removed from the dried plants.

2.8.7 Yield investigations

Yield components investigation in this experiment contains pod number and quantity (such as total seed weight and hundred-seed weight) and quality of the seeds. Pod and seed counting is shown in Fig. 39. Good seeds and diseased seeds of each soybean plant were separated. Some good seeds were oven dried (110℃, 24 h) for testing the moisture content of soybean seeds. Finally, the seeds weight was calculated by a moisture content of 15%.

(a) Seeds extraction and pods classification

(b) Seeds number countering

Fig. 39 Soybean seeds collection

2.9 Root distribution

Roots were cleaned out of the soil blocks as shown in Fig. 40, and then dried in oven (70℃, 7 days) (Fig. 41). Dry root weight of every 10 cm-thick soil block was weighed and total weight of every soybean plant was calculated finally.

Fig. 40 Root washing

Fig. 41 Root after dried in oven

2.10 Water quality parameters

In this experiment, water qualities of supplied water and outlet water were analyzed, such as pH, K^{+}, Ca^{2+}, Mg^{2+}, Cu and Cd concentration.

pH: pH/COND METER D-54, HORIBA Corporation;

K^{+}: JIS K 0102(2016)-49.3 Ion chromatograph method;

Ca^{2+}: JIS K 0102(2016)-50.4 Ion chromatograph method;

Mg^{2+}: JIS K 0102(2016)-51.4 Ion chromatograph method;

Cu: JIS K 0102(2016)-52.5 ICP mass spectrometry method;

Cd: JIS K 0102(2016)-55.4 ICP mass spectrometry method.

2.11 Heavy metal analysis

Determination of Cd and Cu concentration in the roots (depth 0 ~ 10 cm), stems, seeds and soil were carried out by analyzing their specimens in the Atomic Absorption Spectrophotometry method.

2.12 Statistical analysis

Turkey-Kramer test (Yanai, 2011) was adopted at a 5% significance level to testify the Cd and Cu uptake in the roots, stems, seeds and the growth and yield of all models.

Chapter 3: Results and discussion

3.1 Introduction

The growth condition, the results of investigations after harvest, water and soil analysis and heavy metal concentration in different parts of soybean plants will be explained in this chapter.

3.2 Groundwater level control experiment

3.2.1 Groundwater level

In this experiment, groundwater levels in three containers were maintained at 5, 10 and 40cm from the soil surfaces. Before germination, groundwater level in Models GL-5 and GL-10 were adjusted to 15cm for avoiding moisture damage. Fig. shows the temporal changes of groundwater level with three models.

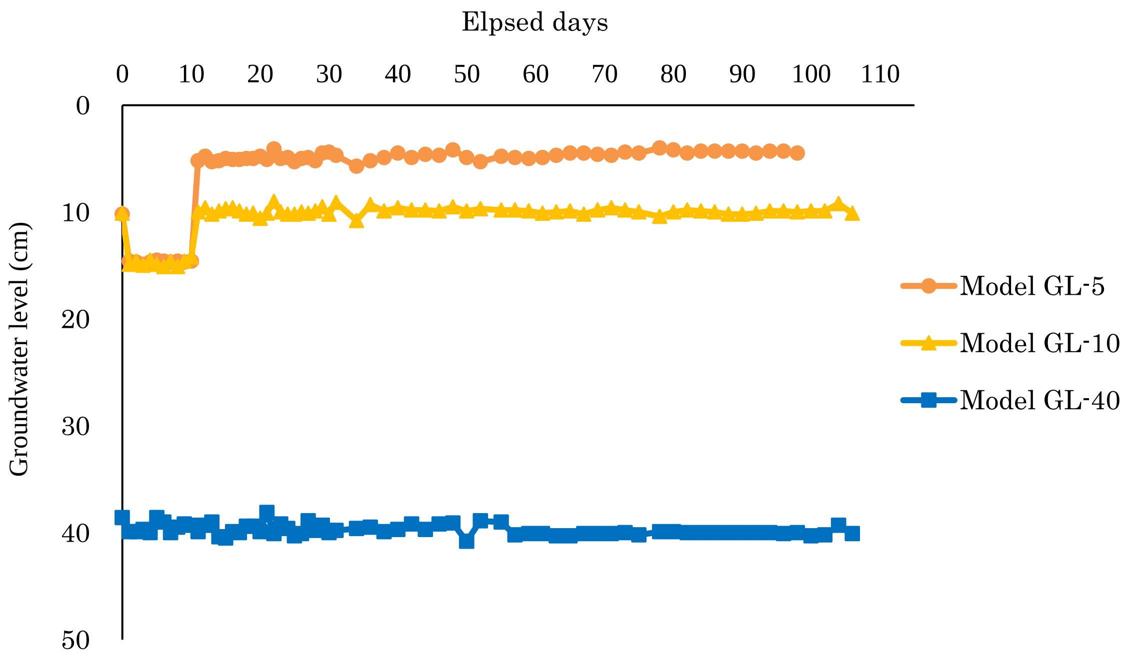


Fig. 42 Temporal changes of groundwater level in groundwater level control models

3.2.2 Oxidation-reduction potential (Eh)

Oxidation-reduction potential is also known as Eh, and the oxidation layer and the reduction layer are defined as Eh ≧ 300 mV and Eh ＜ 300 mV, respectively (Iimura, 1981). It has been reported that under the reduced state, sulfate ion (SO_4^{2-}) can be reduced to sulfide ion (S^{2-}), and the sulfide ion produced will precipitate with Cd^{2+} and Cu^{2+} ion as cadmium sulfide and copper sulfide (CdS, CuS), which are hardly soluble in the water (Arao et al., 2010; Dong et al., 2007). In this way the Cd and Cu concentration in the soil solution can be lowered under reduction condition. It was reported that oxygen, moisture content, organic matter and temperature are supposed to be the factors that determine reduction and oxidation (Mars and Wassen, 1999).

Measured Eh values on each of the groundwater levels are shown in Figs. 43, 44 and 45. In the GL-5 model (Fig. 43), Eh values measured at the 2.5 cm depth was an oxidation layer of about 600 mV; however, below the 7 cm depth Eh values indicated reduction condition. In the GL-10 model (Fig. 44), Eh values at 2.5 cm and the 7 cm depths indicated more than 500 mV; however, Eh values measured below the 12 cm depth was a reduction layer of Eh ＜ -100 mV. In the GL-40 model (Fig. 45), except for Eh values measured at the 42 cm depth, all of the observation depths became oxidation layers of Eh ≧ 500 mV.

From these results, it became apparent that controlling the upper part of the groundwater level to be an oxidation layer and the part below the groundwater level 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 5 cm for GL-5, about 10 cm for GL-10, and about 40 cm for GL-40. 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 GL-10 and the GL-40 models were almost the same as the result of Haque et al. (2014a, 2014b). 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.

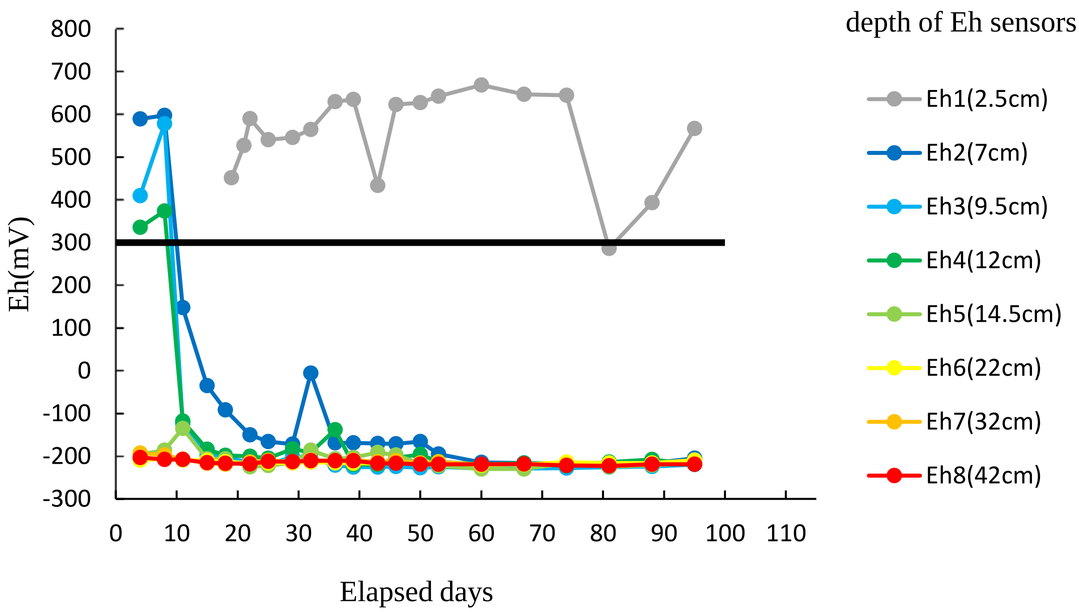


Fig. 43 Eh value of soil layer of 5cm groundwater level model

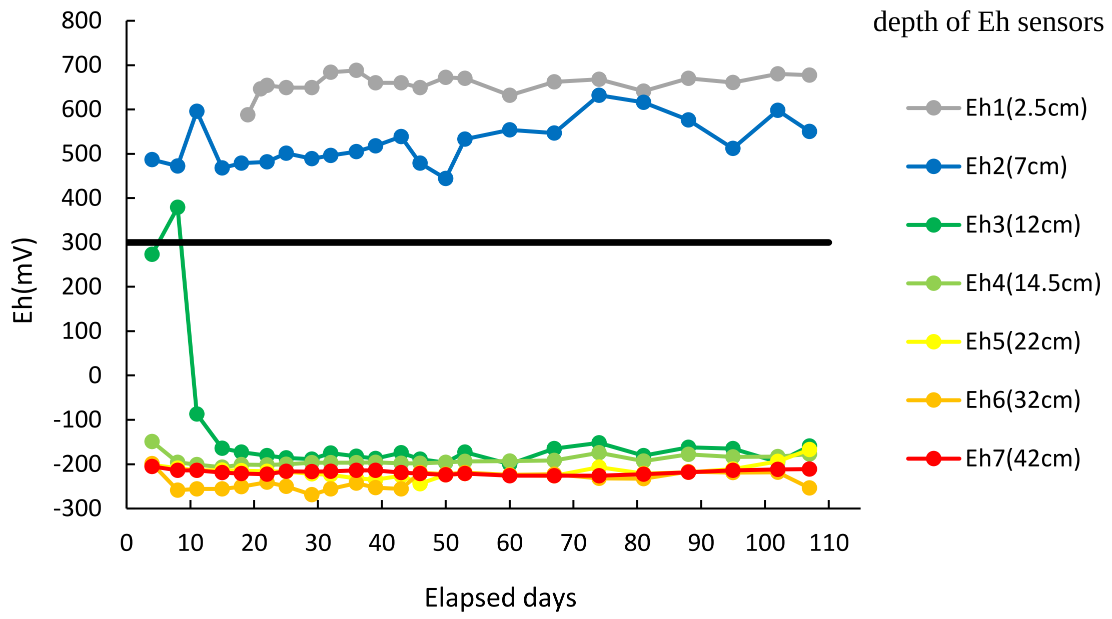


Fig. 44 Eh value of soil layer of 10cm groundwater level model

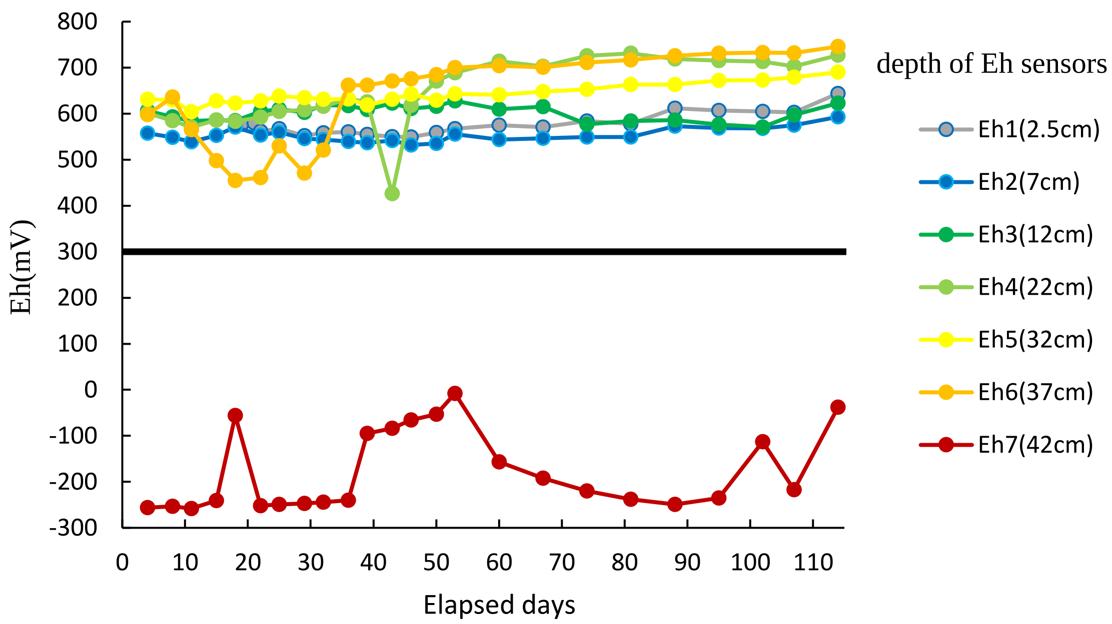


Fig. 45 Eh value of soil layer of 40cm groundwater level model

3.2.3 Growth and yield of soybean plants

Soybean growth and yield components are indicated in Table 5. Average stem heights (n = 8) were GL-5 (59.7 cm) < GL-10 (66.8 cm) < GL-40 (78.8 cm). Significant differences were recognized among the three models. Murakami et al. (2011) and Haque et al. (2014a, 2014b) 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 GL-10 and GL-40. However, there were significant differences between GL-5 and GL-10, and also between GL-5 and GL-40. The averages of good seed weight per plant were GL-5 (20.5 g) < GL-10 (36.2 g) < GL-40 (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 GL-5 and GL-10 models lowered by about 36 % and 64 %, respectively, compared with that of GL-40 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 5 Growth and yield components of soybean in three groundwater level models

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

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).

3.2.4 Heavy metals in soybean plants

Cd and Cu concentrations in the seeds, stems and roots of soybean plants in each model were measured.

Table 6 Cd and Cu concentration in soybean of three groundwater level models

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

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

3.2.4.1 Cd concentration in soybean

Cd concentration in soybean seeds was GL-5 (0.25 mg kg^{-1}) < GL-10 (0.52 mg kg^{-1}) < GL-40 (1.07 mg kg^{-1}) and there were significant differences among the three treatments ($p < 0.05$) (Table 6). Those values were three to ten times greater compared with Cd concentration in soybeans in non-contaminated soil (Murakami et al., 2011).

Cd concentration in stems was GL-5 (0.28 mg kg^{-1}) < GL-10 (0.45 mg kg^{-1}) < GL-40 (1.48 mg kg^{-1}) and there was little significant difference between GL-5 and GL-10. However, there was significant difference between GL-5 and GL-40, and also between GL-10 and GL-40 ($p < 0.05$). Cd concentration in roots was GL-10 (3.54 mg kg^{-1}) < GL-5 (4.92 mg kg^{-1}) < GL-40 (5.80 mg kg^{-1}) and there was a significant difference between GL-10 and GL-40 ($p < 0.05$). The trend of Cd concentration in soybean plants were seed < stem < root as found by Haque et al. (2014a, 2014b). In this experiment, Cd accumulated at a same level in the seeds and stems when the groundwater level was at a high level, and accumulated more in the stem than the seeds when the groundwater level was at a low level (Fig. 46). Cd concentration in seeds was about 1/10 compared with the one in roots in the case of GL-5 and GL-10. Cd concentration in seeds was about 1/5 compared with that of roots in the case of GL-40. It is thought that Cd concentration in soybean plants is affected by the groundwater level. Cd concentration in seeds was 0.25 mg kg^{-1} in the GL-5 treatment and it was close to the standard Cd content in non-contaminated 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.

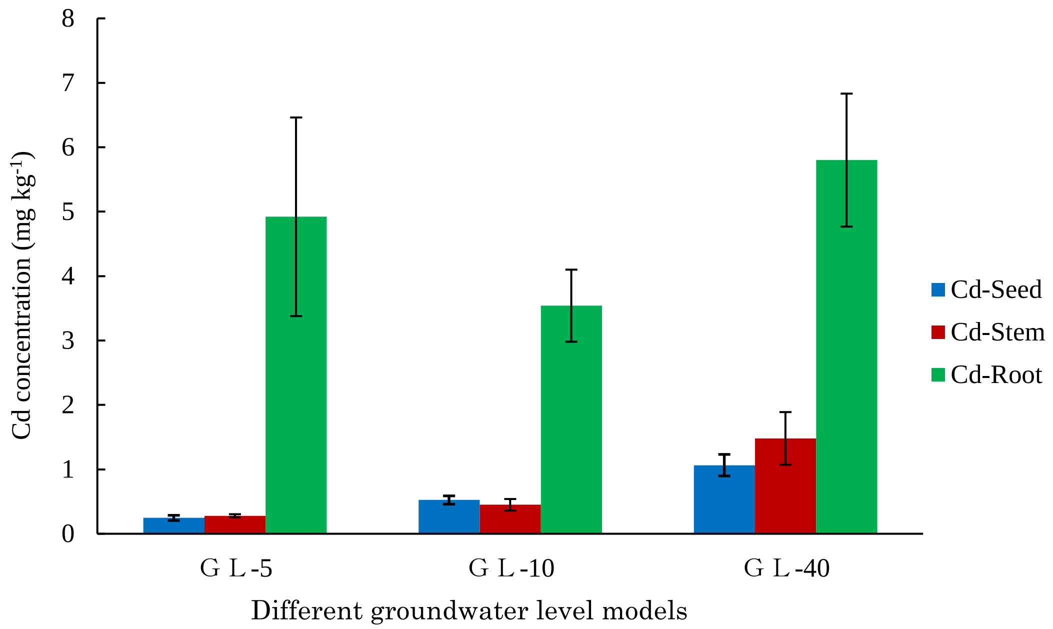


Fig. 46 Cd concentration in soybean plant with groundwater level control models

3.3.4.2 Cu accumulation

Table 6 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 GL-5 (5.08 mg kg^{-1}) < GL-10 (5.82 mg kg^{-1}) < GL-40 (9.96 mg kg^{-1}) and there were significant differences among the three treatments at $p < 0.05$. Cu concentration in stems was GL-5 (2.45 mg kg^{-1}) < GL-10 (2.76 mg kg^{-1}) < GL-40 (5.58 mg kg^{-1}) and there was little significant difference between GL-5 and GL-10. However, there were significant differences between GL-5 and GL-40, and also between GL-10 and GL-40 ($p < 0.05$). Cu concentration in roots was GL-40 (14.06 mg kg^{-1}) < GL-10 (39.48 mg kg^{-1}) < GL-5 (50.04 mg kg^{-1}) and there was little significant difference between GL-5 and GL-10. However, there was significant difference between GL-5 and GL-40, and also GL-40 between GL-10 and GL-40 ($p < 0.05$). The trend of Cu concentration in soybean plants was stem < seed < root (Fig. 47). 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 GL-5 and GL-10 was twice higher than that of GL-40. The morphological characteristic of roots is that there are many thick roots in the GL-40 treatment and many fine root mat in the GL-5 and GL-10 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 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.

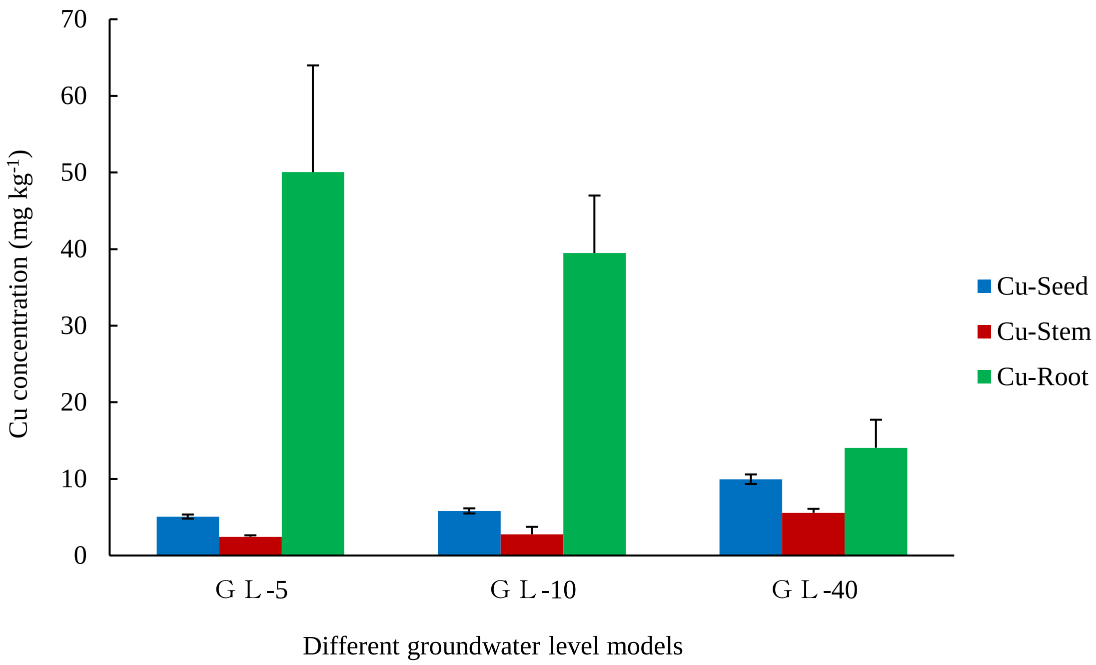


Fig. 47 Cu concentration in soybean plant with groundwater level control models

3.3 Mixing tillage experiment

3.3.1 Soil and air temperature

Greenhouse air temperature and different depths of soil temperature were measured and recorded from seeding to harvesting. For comparison, temperature of depths 10 cm and 40 cm in the field of Hirosaki University were also recorded (Figs. 48 & 49). Figs. 50 ~ 57 show the temporal changes of daily average of container soil temperatures, field soil temperatures and greenhouse temperatures in all eight models, respectively. In each model, daily average temperature of soil (depth 5 cm, 15 cm, 25 cm and 35 cm) in containers was between 12.6 ~ 31.9°C. Daily average temperature of field soil (depth 10 cm and 40 cm) was between 14.5 ~ 27.0°C. Soil daily average temperatures had the same trend with greenhouse daily average air temperatures (2016: 16.8 ~ 31.9°C; 2017: 13.1 ~ 30.2°C) for all models in two years, instead of having similar trend with the daily average temperature of field soil. It was because that the plastic containers could transmit heat quickly from the air to the inner soil.

With comparing the daily average temperature data in two years, we can see that before the 50^{th} day from seeding, the soil temperatures of 2016 models were higher, and the soil temperatures of 2017 models became higher after the 50^{th} day. It means that soybean plants in 2016 grew under a higher temperature condition before the flowering stage than soybean plants in 2017, and after that it turned to be the opposite. However, the difference value was little, we consider that this condition have no obvious influence on the growths of soybean plants in the two years..

Summary of data in Figs. 50 ~ 57 was shown in Table 7. Cadmium was absorbed by soybean root, and Matsunami et al. (2013) had reported that, Ryuho soybean, which is recognized as one of the low Cd absorption breeds, can bear a rooting zone temperature change and its root can maintain stable water uptake ability, bleeding rate and stomatal conductance within temperature 15℃ ~ 35℃.

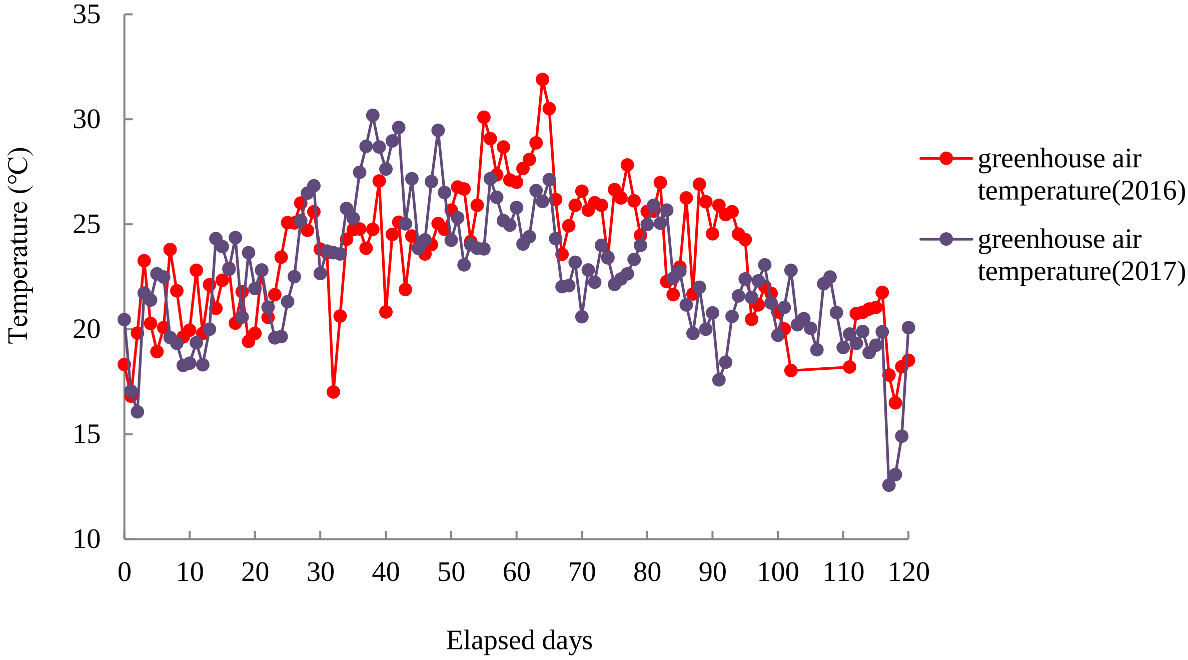


Fig. 48 Temporal changes of greenhouse air temperature in two years

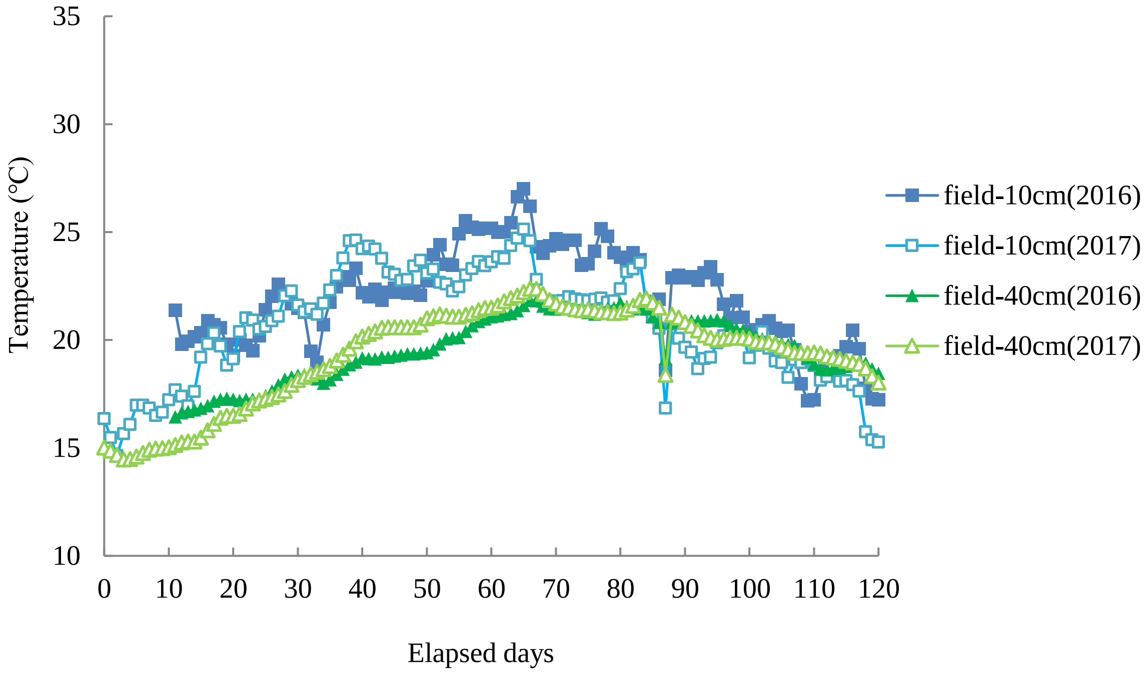


Fig. 49 Temporal changes of field temperature in two years

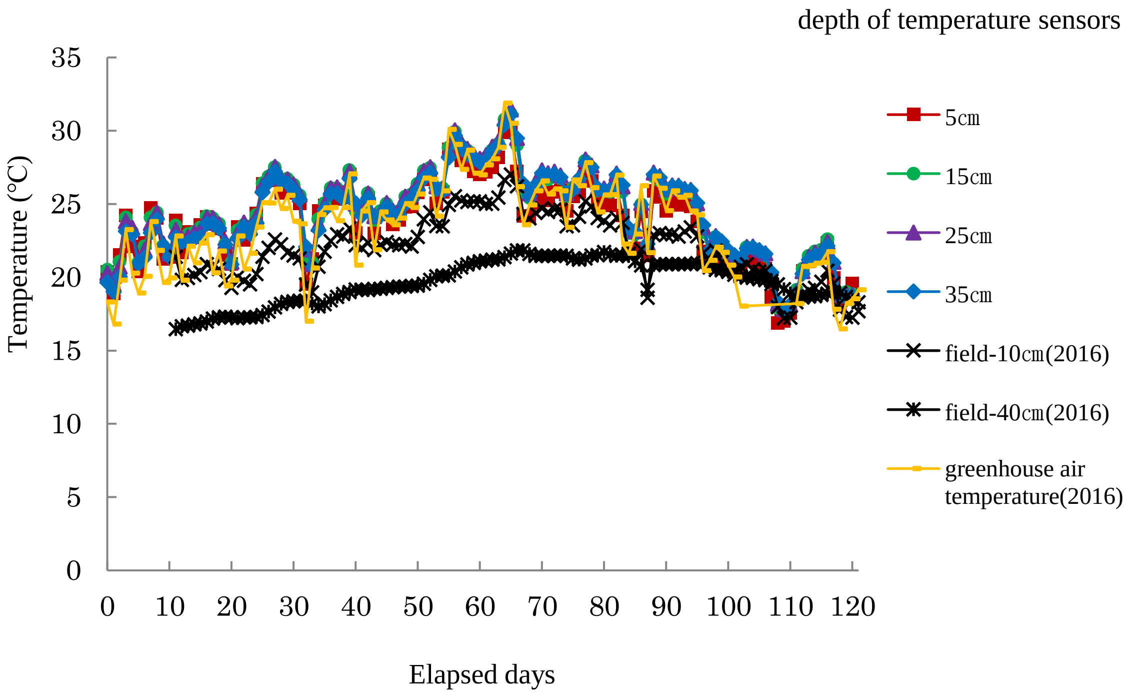


Fig. 50 Temporal changes of temperature with model C-01

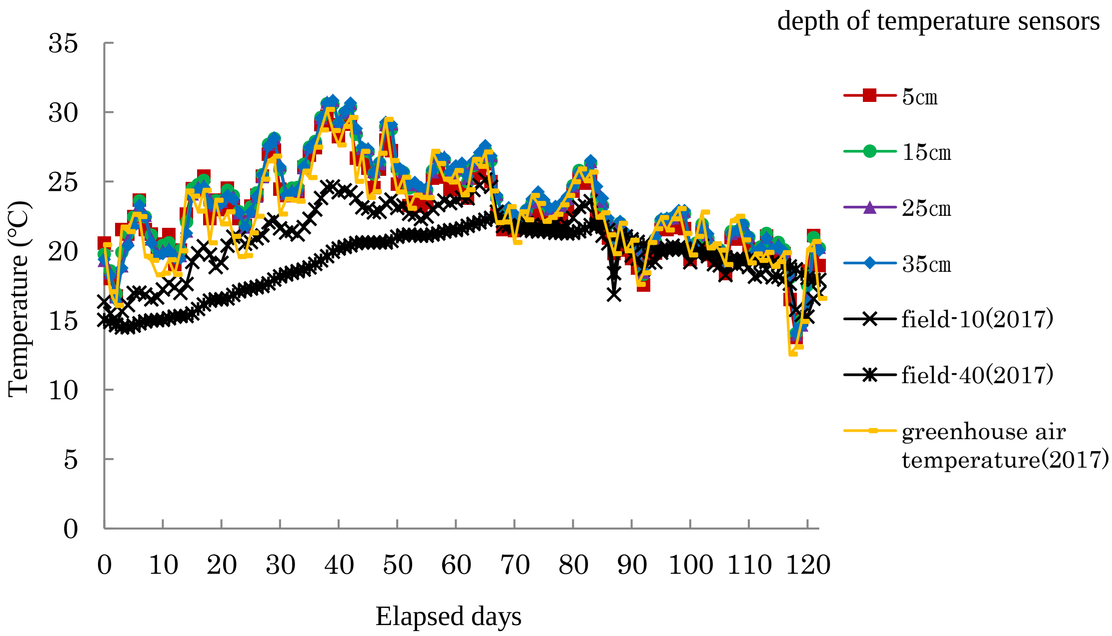


Fig. 51 Temporal changes of temperature with model C-02

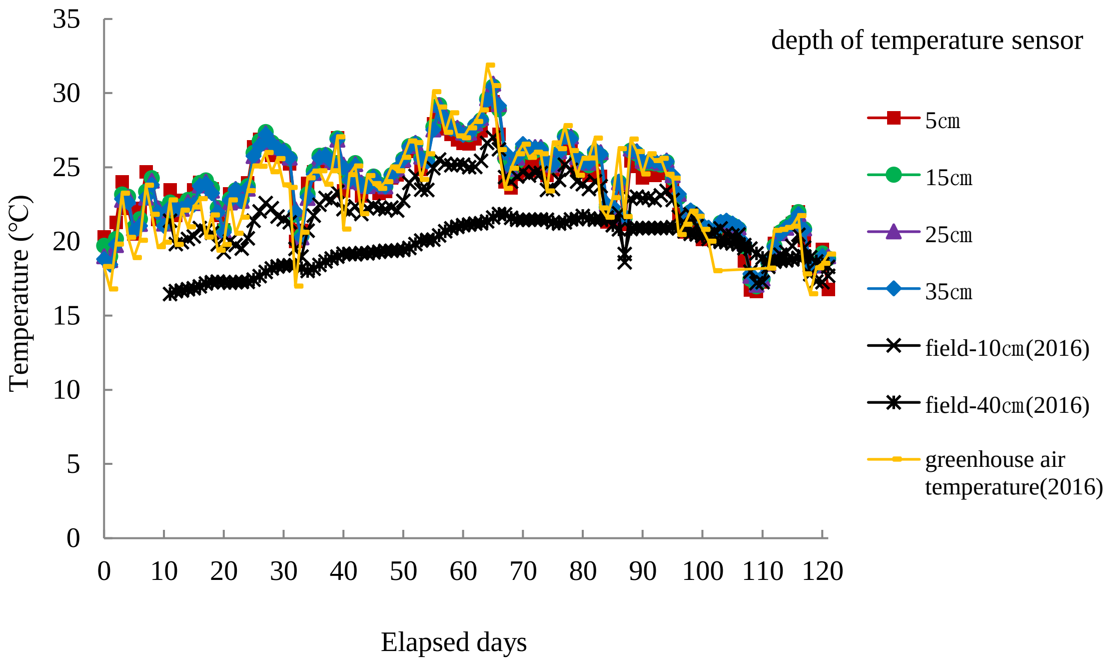


Fig. 52 Temporal changes of temperature with model L-10

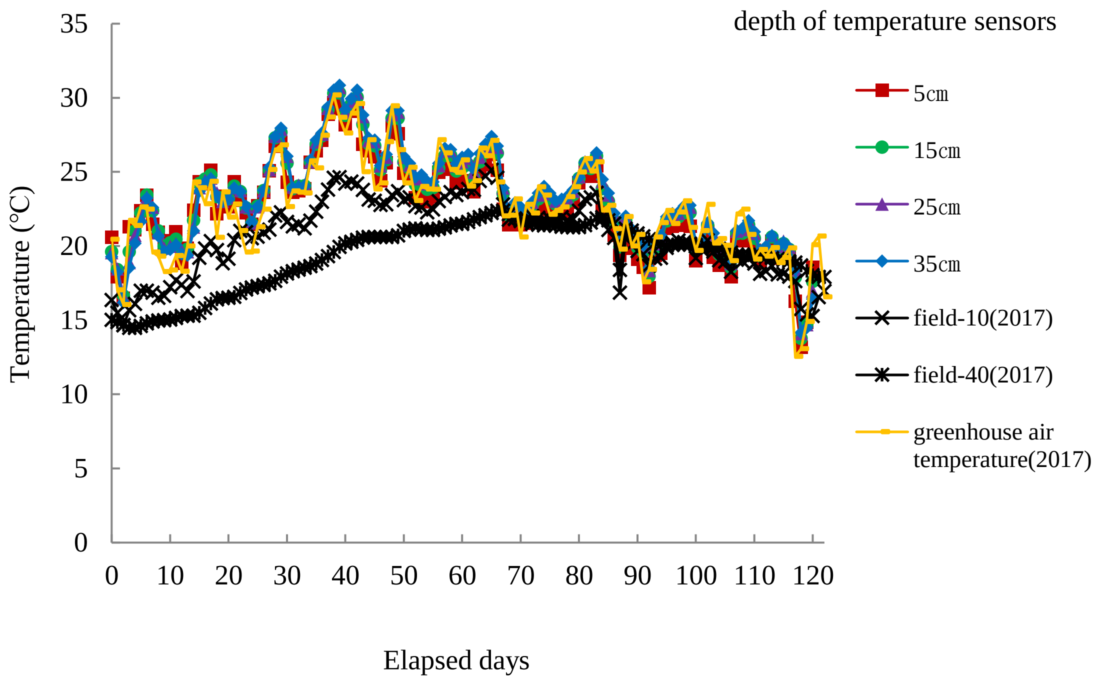


Fig. 53 Temporal changes of temperature with model M-10

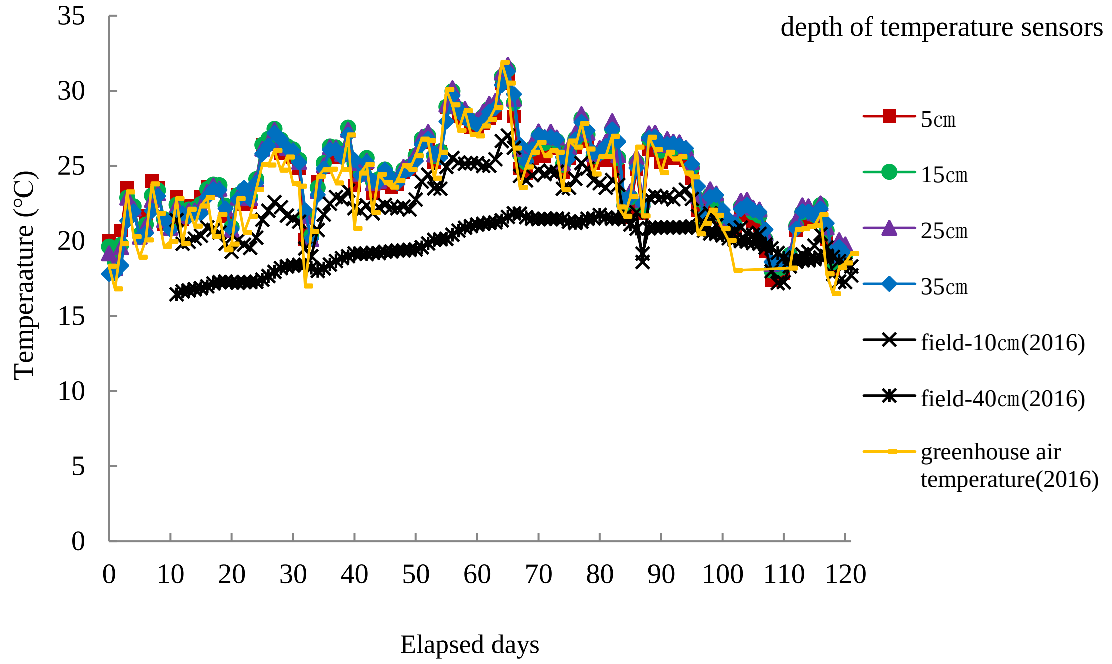


Fig. 54 Temporal changes of temperature with model H-10

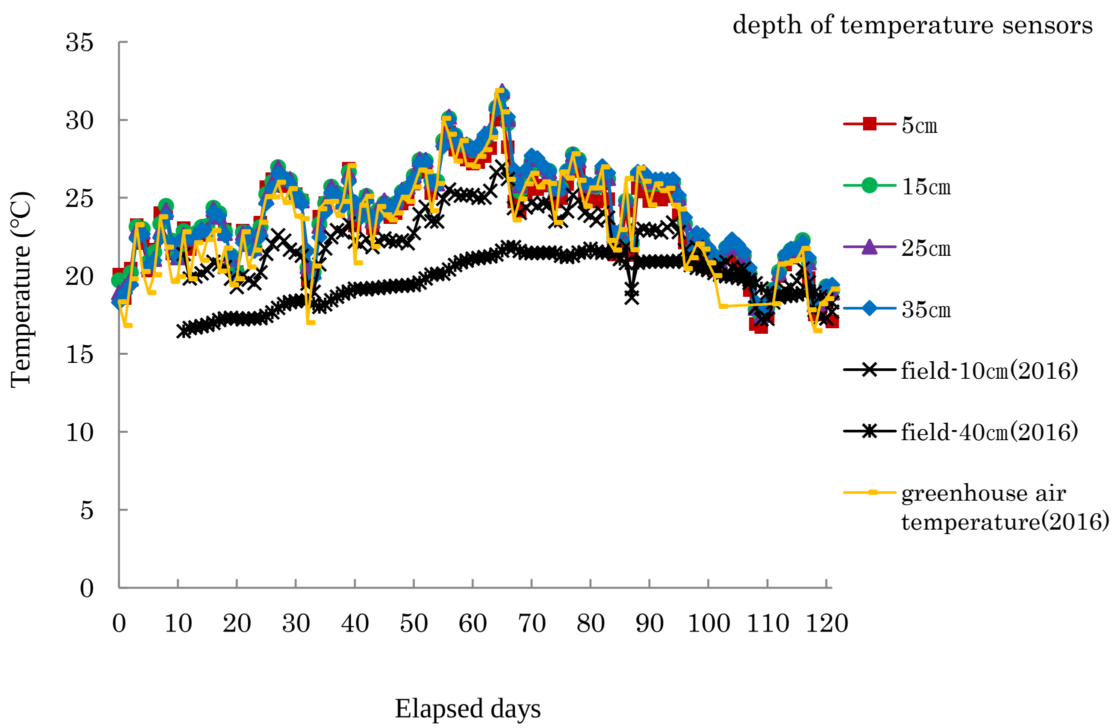


Fig. 55 Temporal changes of temperature with model L-20

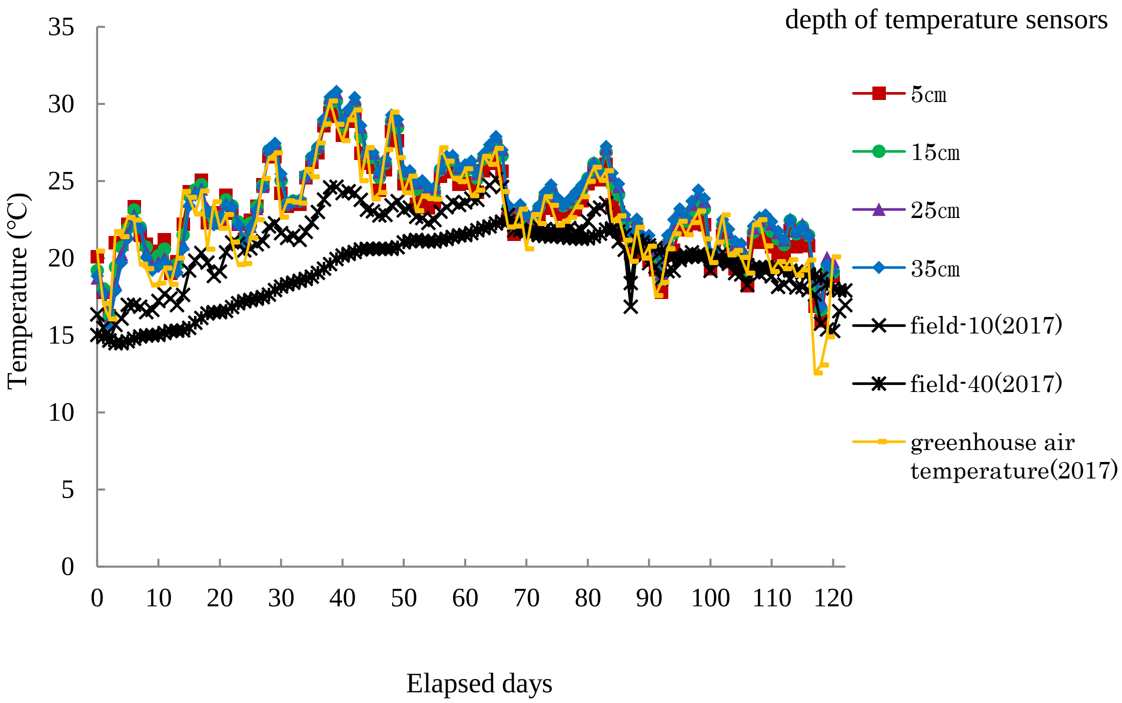


Fig. 56 Temporal changes of temperature with model M-20

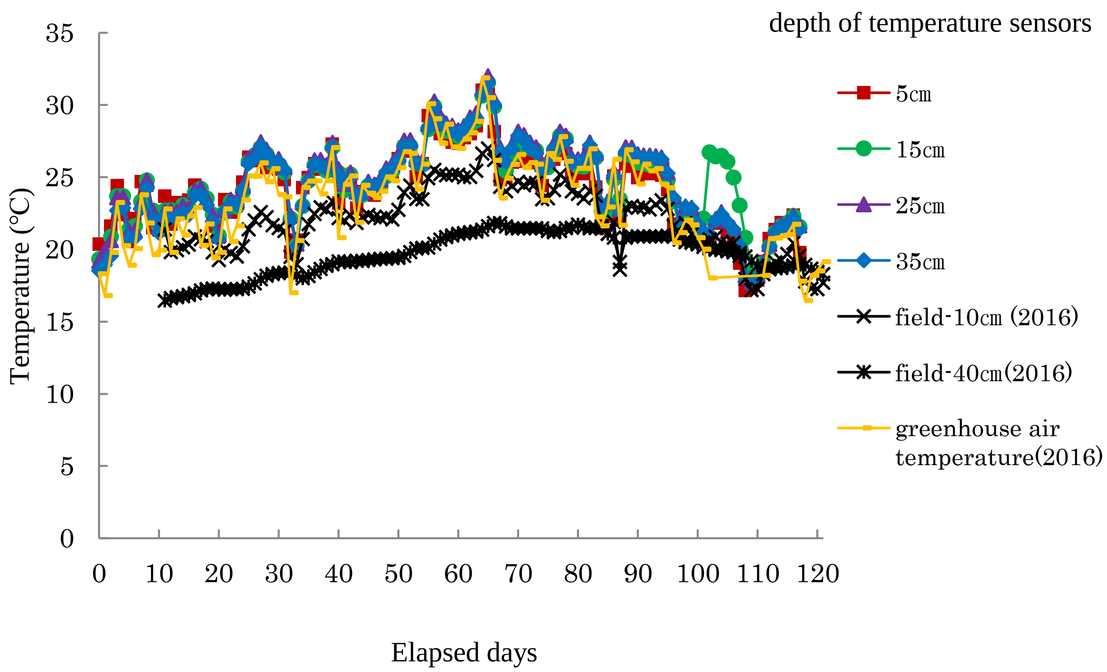


Fig. 57 Temporal changes of temperature with model H-20

Table 7 Soil and air temperature data in both two years

Location	Maximum (℃)	Minimum (℃)	Average (℃)
C-01 (2016)	31.4	16.9	24.3
C-02 (2017)	30.9	13.8	23.2
L-10 (2016)	30.6	16.6	23.8
M-10 (2017)	30.8	13.2	23.0
H-10 (2016)	31.7	17.4	24.2
L-20 (2016)	31.8	16.7	24.1
M-20 (2017)	30.8	15.7	23.4
H-20 (2016)	32.0	17.2	24.6
Field soil (2016)	27.0	16.6	20.3
Field soil (2017)	25.1	14.5	19.2
Greenhouse air (2016)	31.9	16.5	23.6
Greenhouse air (2017)	30.2	12.6	22.5

3.3.2 Groundwater level

In this experiment, groundwater level in each container was maintained at 40 cm from the soil surface. Figs. 58 and 59 show the temporal changes of groundwater level with all models, and all of them were around 40 cm from seeding to harvesting.

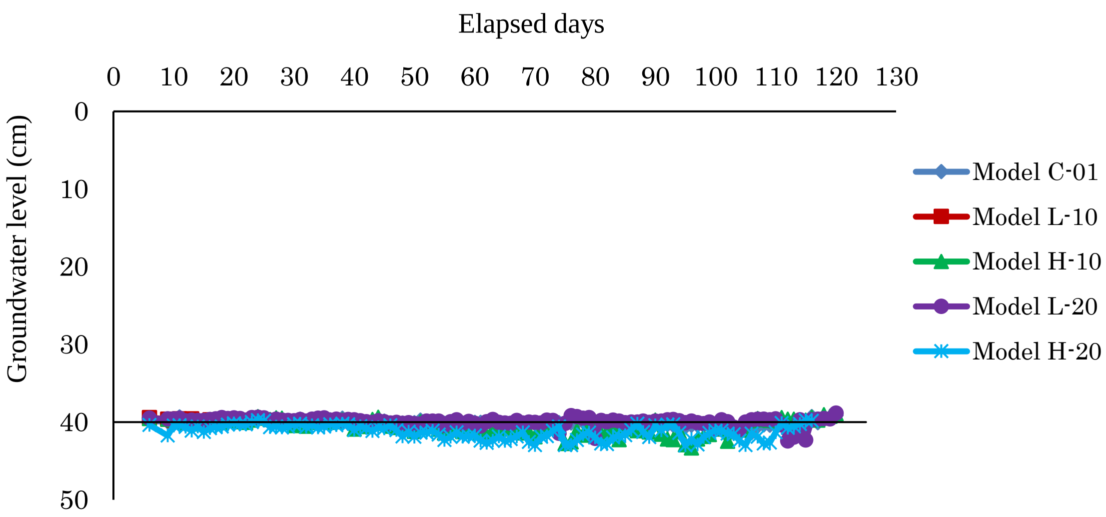


Fig. 58 Temporal changes of groundwater level with Models in 2016

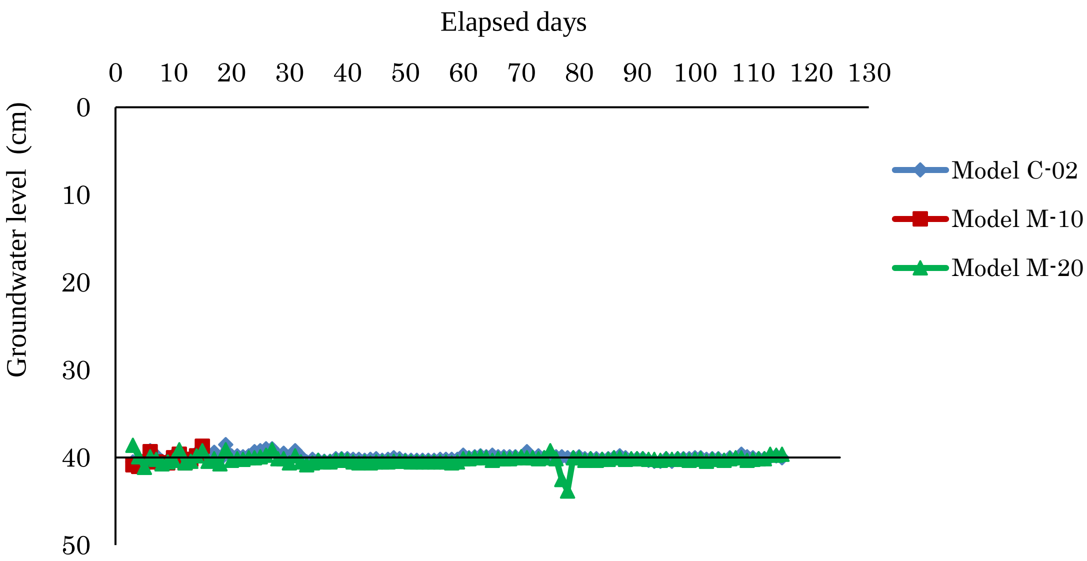


Fig. 59 Temporal changes of groundwater level with Models in 2017

3.3.3 Oxidation-reduction potential (Eh)

Water management such as groundwater level control and irrigation is a cost-effective method to control the soil Eh value for alleviating Cd and Cu absorption for soybean. However, the low Eh value (reduction condition) with a high groundwater level has considerable influence on the yield of soybean (Haque et al., 2014a, 2014b; Li et al., 2017; Murakami et al., 2011). In this experiment, the whole soil layer was maintained to be oxidation condition (Eh value > 300 mV, from the surface to 40 cm depth in all eight treatments, which was actually similar with well drained field condition.

Measured Eh values of eight models are shown in Figs. 60 to 67. In 2016, Eh values at the depth of 5, 15, 25 and 35 cm were over 300 mV evidently (300 ~ 768 mV) for the whole experiment period, while those in 2017 were varied from 300 mV to 400 mV. One possible reason of this difference was the different pattern of three-phase distribution of the soil between two years (Chapter 3.7.2). In 2017, the ratio of liquid phase and gas phase was lower than those in 2016. Eh value of 45 cm depth (under free water surface, gravel) was more than 300 mV in 2016. It could be also explained by a low content of organic matter in the gravel layer (Paul et al., 2011; Sasaki et al., 2001) and flow of supplied dissolved oxygen water into the groundwater. On the other hand, Eh value of 45 cm depth in 2017 was gradually decreased to reduction condition.

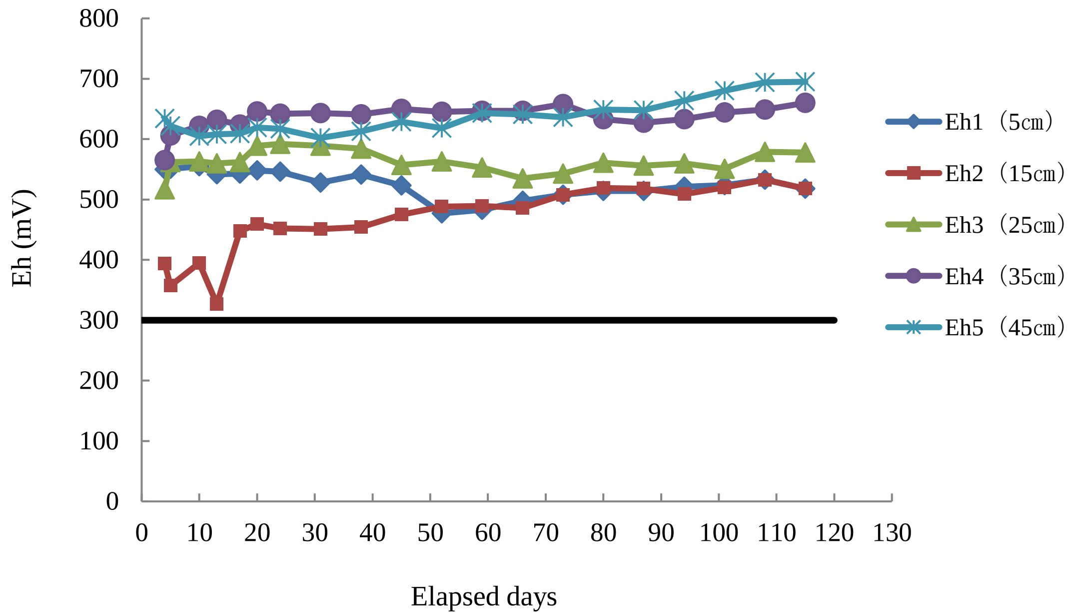


Fig. 60 Temporal changes of Eh with model C-01

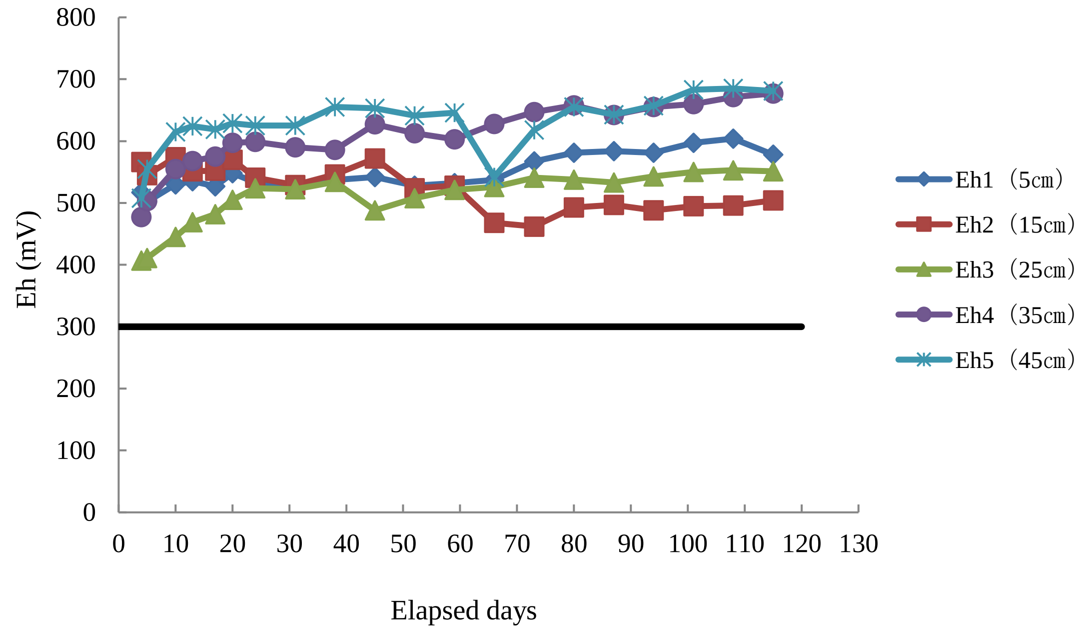


Fig. 61 Temporal changes of Eh with model L-10

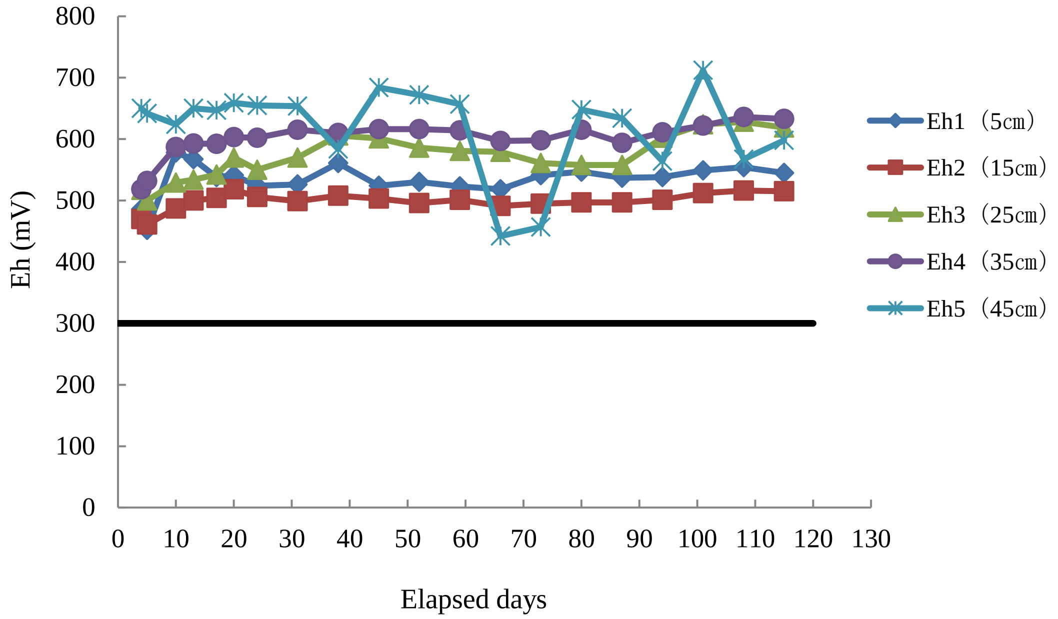


Fig. 62 Temporal changes of Eh with model H-10

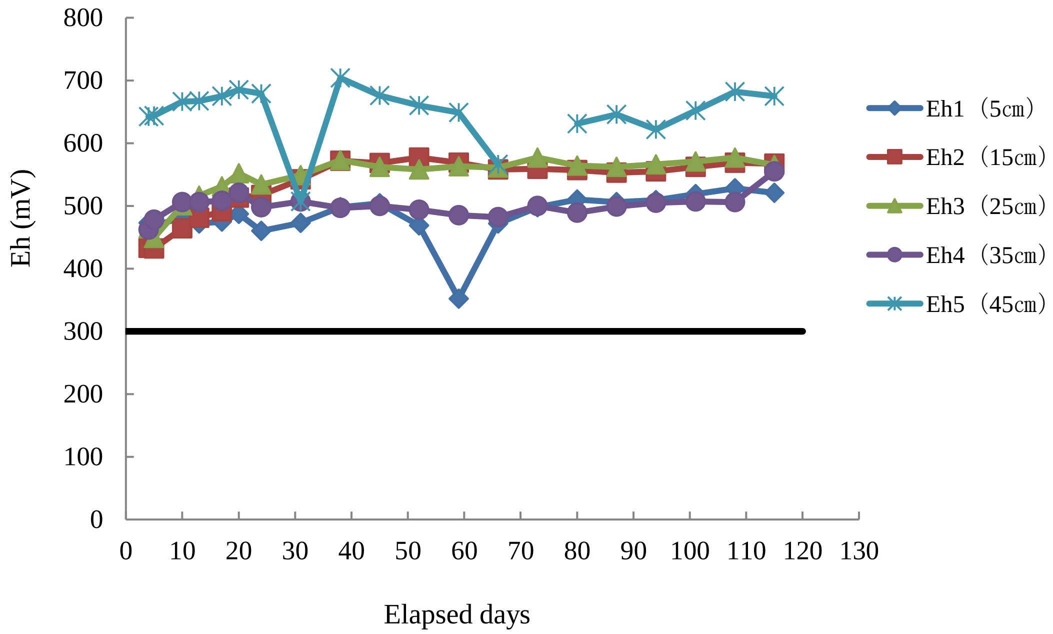


Fig. 63 Temporal changes of Eh with model L-20

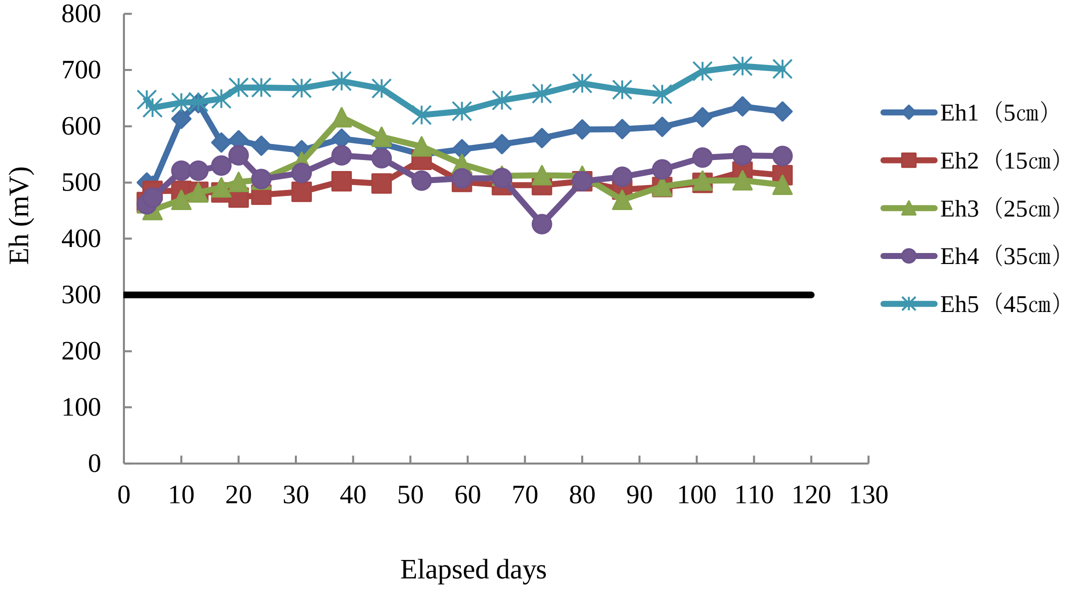


Fig. 64 Temporal changes of Eh with model H-20

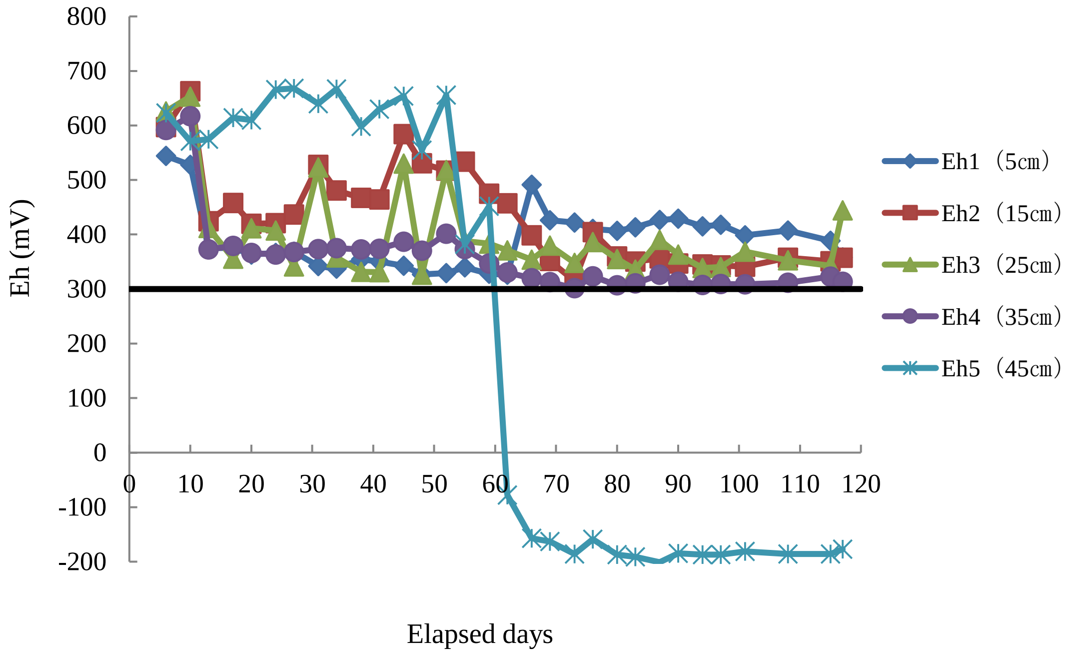


Fig. 65 Temporal changes of Eh with model C-02

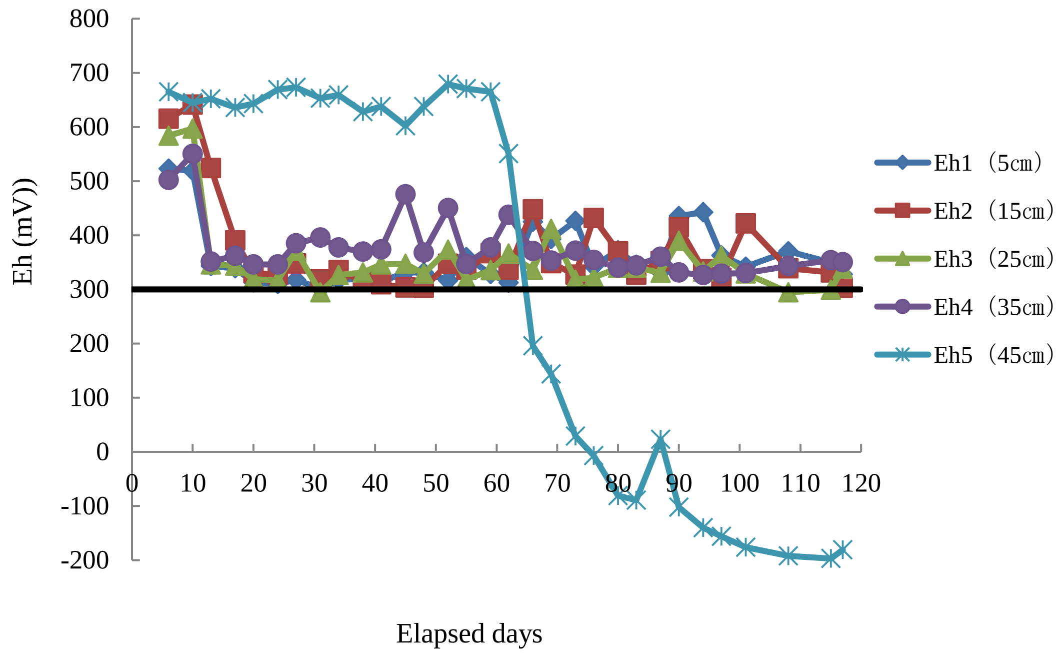


Fig. 66 Temporal changes of Eh with model M-10

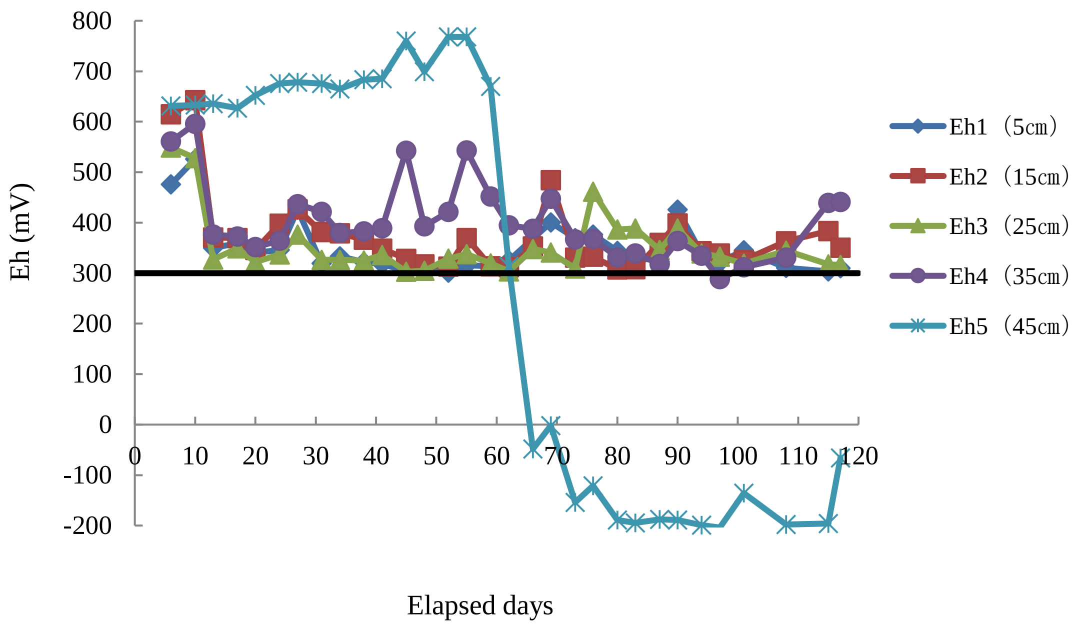


Fig. 67 Temporal changes of Eh with model M-20

3.3.4 Growth and yield of soybean plants

Germination began at the 5th day after seeding in both years (June 9th 2016 and June 8th 2017). When the thinning out was carried out, the germination percentages were 85% in 2016 and 95% in 2017. Flowers started to bloom at the 44th day (July 18th) and all soybean plants flowered at the 53rd day (July 27th) after seeding in 2016. In the year of 2017, flowers started to bloom at the 49th day (July 22th) and full flowered at the 54th day (July 27th) after seeding. Harvesting was carried out among the period of later September and early October in both years (Sept. 29th, Oct. 4th and 5th in 2016; Sept. 29th, Oct. 2nd and 3rd. in 2017). Fig. 68 shows the scenes before harvesting in both two years.

(a) Year of 2016 (b) Year of 2017

Fig. 68 Scenes before harvesting

3.3.4.1 Total length and main stem length of soybean plants

Temporal changes of total length and main stem length of soybean plants were shown in Figs. 69 and 70. After about 60 days from seeding, main stem length of all soybean plants in eight models stopped growing, and the growing of total lengths stopped about 10 days later. When the harvesting was approaching, the total length turned to have a decrease, it means that the latest leaf on the top of stem fell off.

In the year of 2016 and 2017, the values of stem length and total length before harvesting were 60 ~ 76 cm and 108 ~ 124 cm, 64 ~ 84 cm and 111 ~ 138 cm, respectively. We can conclude that the latest leaves in both years soybean plants were in a similar size with a length about 50 cm. However, the main stem lengths of both years were much less than the previous research data (Haque at al., 2014a, 2014b), in which the average main stem height of Ryuho soybean plants grown under groundwater level 40 cm were 111.6 cm and 99.2 cm, respectively. It might because of the air temperature during the growth period were higher in the years of 2012 and 2013 than the years of 2016 and 2017. Main stem length in this experiment was similar to the data from Li et al. (2017) and the cultivation guidance of soybean in Akita prefecture of Japan (2015), in which the average main stem height of Ryuho soybean plants were 78.8 cm and 68.0 cm, respectively.

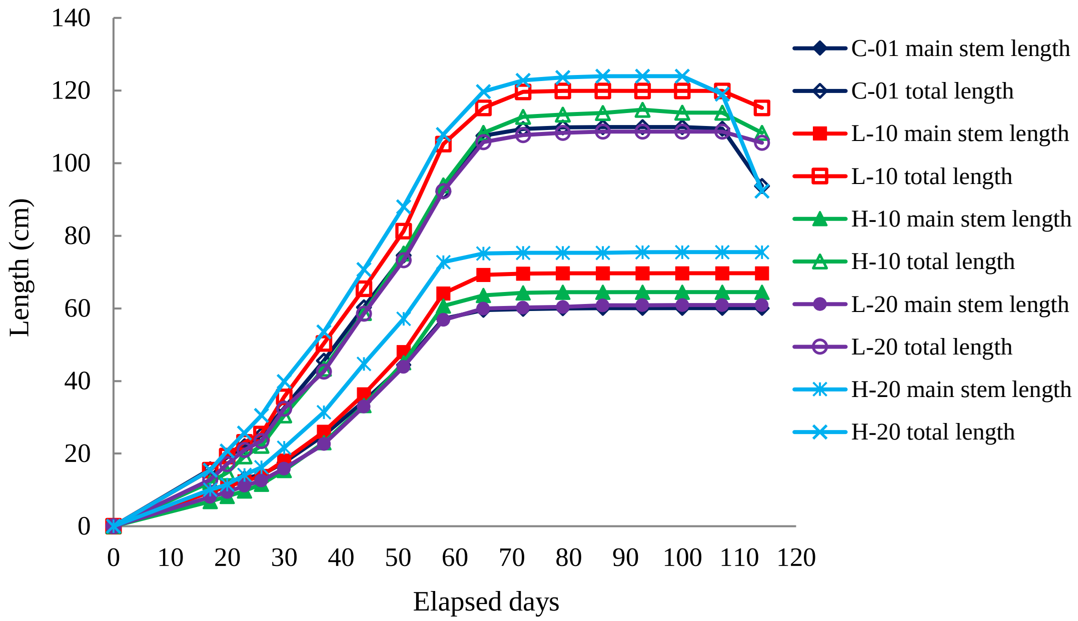


Fig. 69 Temporal changes of main stem length and total length (2016)

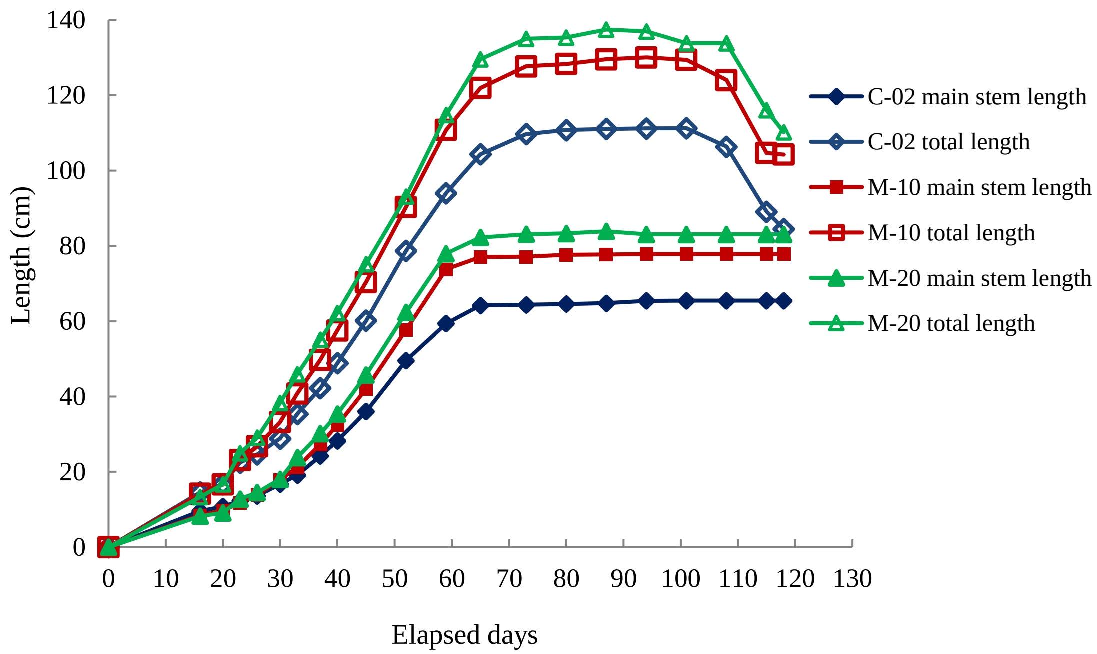


Fig. 70 Temporal changes of main stem length and total length (2017)

3.3.4.2 Leaf age of soybean plants

In the years of 2016 and 2017, the ranges of soybean plants leaf age were 12 ~ 14 and 13 ~ 15, respectively. The latest leaf was fully stretched about 65 days later after seeding in both years. Temporal changes of leaf age with each model were shown in Fig. 71 and 72.

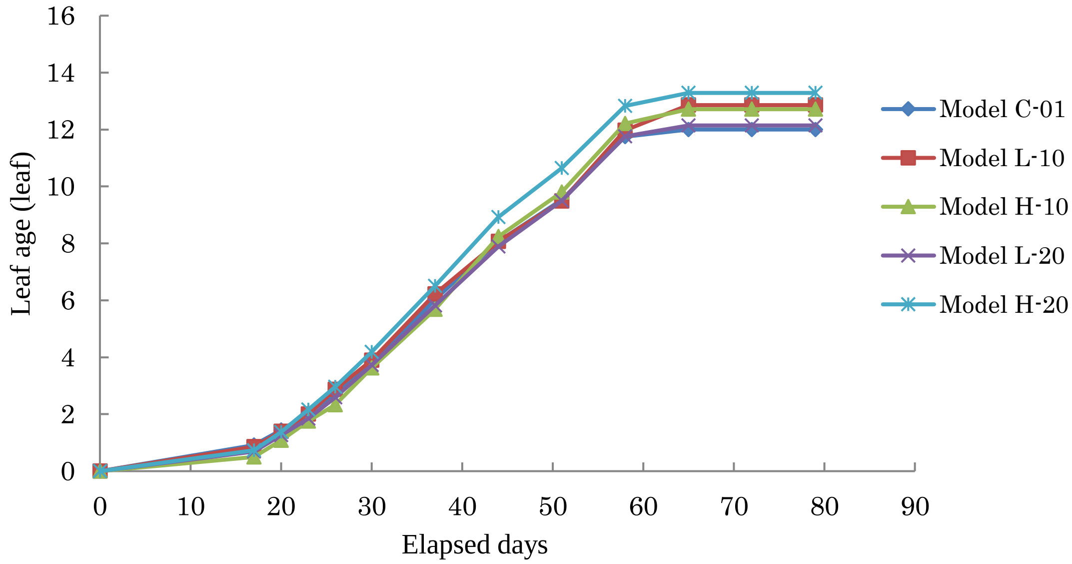


Fig. 71 Temporal changes of leaf age (2016)

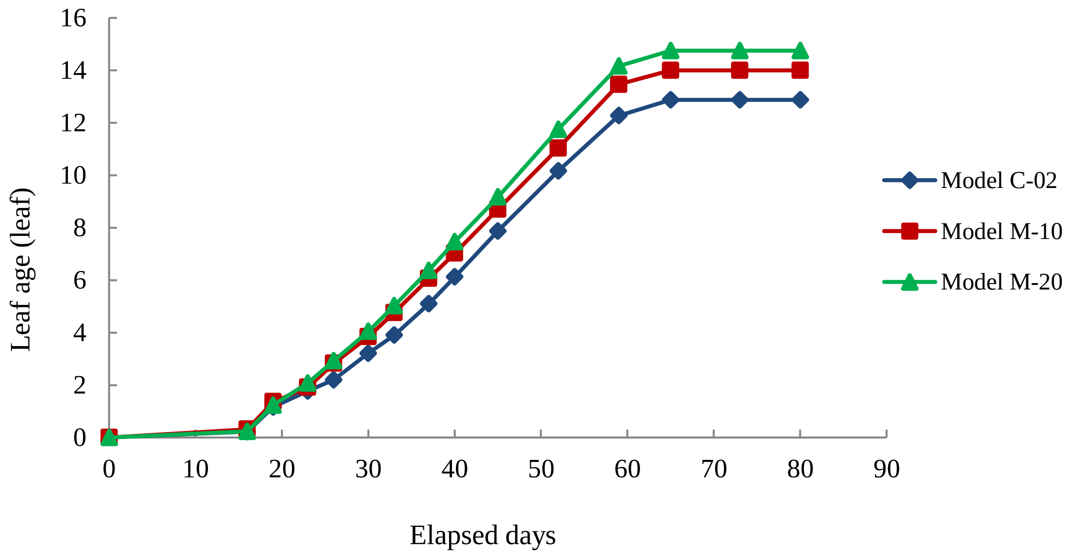


Fig. 72 Temporal changes of leaf age (2017)

3.3.4.3 SPAD value of soybean leaves

The chlorophyll meter provides a simple, quick, portable and non-destructive method for estimating plant leaf chlorophyll content. The chlorophyll content in plant leaf depends on some factors such as nitrogen level in soil, water availability, oxidation-reduction potential of soil, soil and air temperature. Previous researches reported that the photosynthesis of plant leaf could be affected by Cd (Santa et al., 1999; Zhang et al., 2006) and the SPAD value would increase with the increase of N content, and would decrease with the increase of Cd content in solution (Du et al., 2009). In 2016, the SPAD value was stable and around 40 from the beginning to the 60^{th} day after seeding (Fig. 73). However, the SPAD value went down at the beginning and then gradually became higher in 2017 (Fig. 74). It could be explained by the different trend of air and soil temperature in two years. At the time around the 90^{th} day in two years, the SPAD value came to be the highest (51.9 in 2016 and 54.8 in 2017). Since the damage by insects, model C-02 had a lower value than the others. SPAD values of model H-20 and model M-20 rapidly decreased in 2016 and 2017, respectively. It might be resulted from the high Cd concentration in the two models.

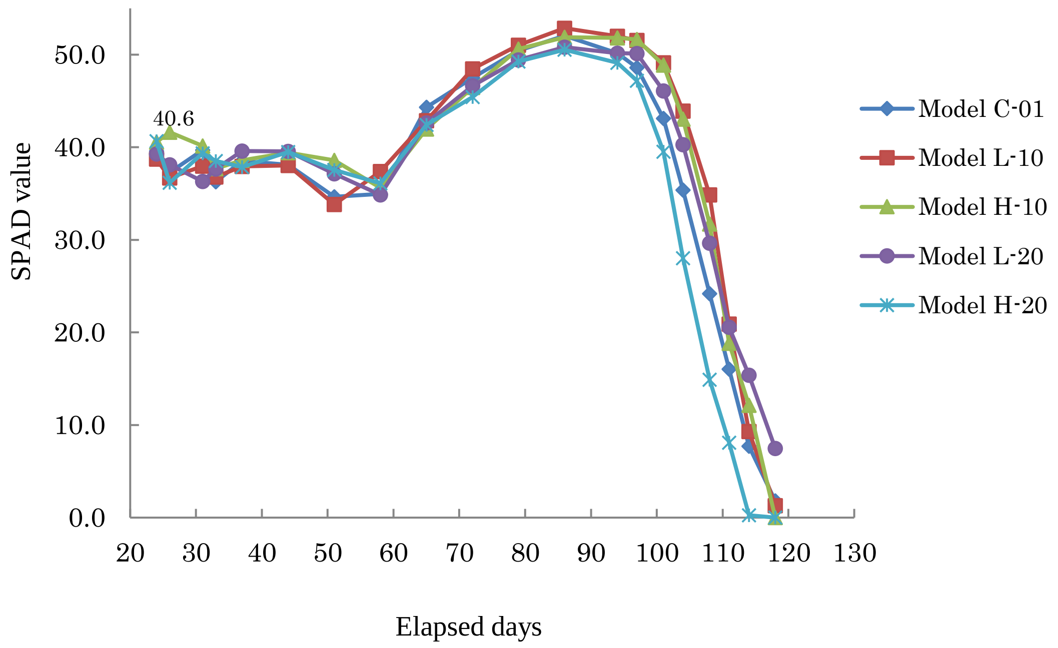


Fig. 73 Temporal changes of SPAD (2016)

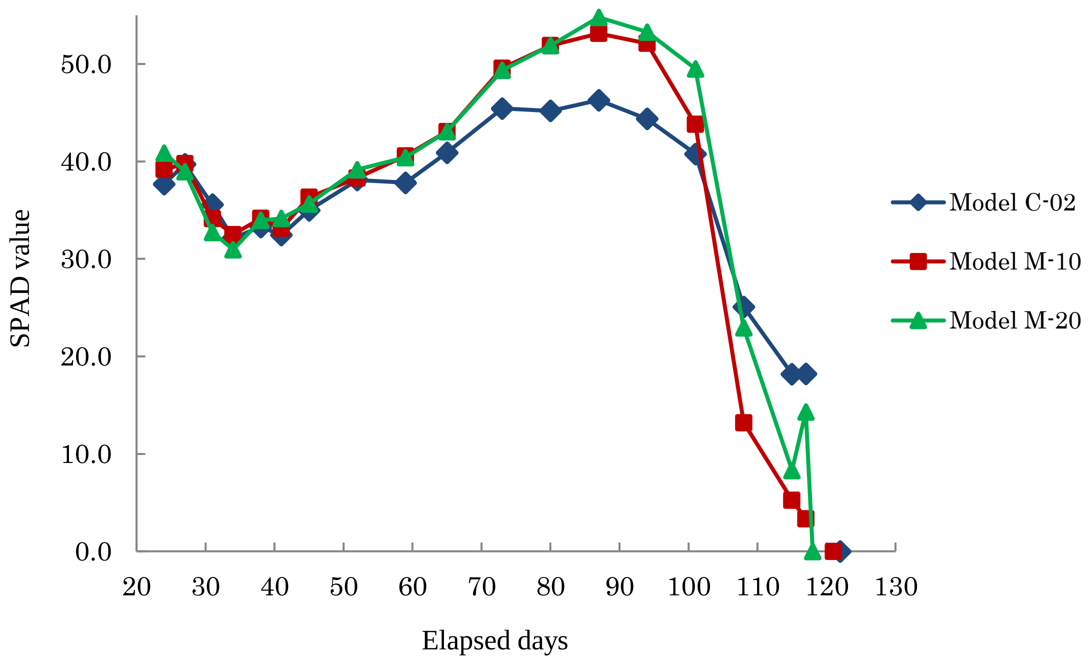


Fig. 74 Temporal changes of SPAD (2017)

3.3.4.4 Comparison of soybean yield components

Soybean growth state and yield components of eight different models in two years were shown in Table 8.

Table 8 Growth and yield components of soybean in eight mixing tillage models

Model	Growth			Yield		
	Stem diameter (mm)	Stem length (cm)	Branch No.	Seed/Pod	Good seed wt. (g)	100 seed wt.(g)
C-01	8.7 ± 0.8^{a}	58.2 ± 3.2^{a}	3.3 ± 0.5^{ab}	1.7 ± 0.1^{a}	43.0 ± 10.1^{a}	35.4 ± 0.9^{ad}
C-02	8.8 ± 0.7^{a}	61.7 ± 4.7^{a}	3.3 ± 0.5^{a}	1.7 ± 0.2^{a}	30.9 ± 7.7^{a}	37.3 ± 2.1^{a}
L-10	9.5 ± 1.3^{ab}	69.2 ± 4.3^{b}	4.1 ± 1.5^{a}	1.7 ± 0.2^{a}	56.0 ± 10.1^{b}	37.5 ± 1.9^{b}
M-10	9.7 ± 1.2^{ab}	75.0 ± 1.1^{b}	4.0 ± 1.2^{a}	1.9 ± 0.1^{a}	54.4 ± 9.4^{b}	39.4 ± 0.8^{b}
H-10	9.4 ± 1.5^{ab}	62.8 ± 5.3^{a}	2.4 ± 1.8^{ab}	1.7 ± 0.2^{a}	48.3 ± 19.7^{ab}	37.5 ± 1.0^{b}
L-20	10.0 ± 1.8^{ab}	58.8 ± 4.2^{a}	3.0 ± 1.2^{ab}	1.9 ± 0.1^{b}	49.6 ± 18.7^{ab}	36.4 ± 1.5^{ab}
M-20	11.0 ± 0.8^{b}	80.6 ± 3.9^{c}	5.3 ± 1.1^{b}	1.8 ± 0.2^{a}	64.5 ± 5.7^{c}	38.8 ± 1.0^{ab}
H-20	9.9 ± 1.2^{ab}	73.0 ± 1.9^{b}	2.4 ± 1.1^{a}	1.6 ± 0.2^{a}	44.6 ± 7.6^{a}	33.5 ± 2.9^{cd}

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 = 7).

As for stem diameter, it ranged from 8.7 mm to 11.0 mm. Model M-20 had a significant thicker stem than models C-01 and C-02, and there was no significant difference among other models.

Stem lengths here were measured after the soybean plants were dried. Average stem length of model M-20 was 80.6 cm and it was significantly longer than that of other models. Obvious significant difference was not shown in the stem height either among models C-01, C-02, H-10 and L-20, or models L-10, M-10 and H-20.

Except for that model M-20 had a significant higher branch number and model L-20 had a significant higher seed number in one pod, obvious significant difference was not shown in the branch number and the number of seeds per pod (seed/pod) among other models. According to the cultivation guidance of soybean in Akita prefecture Japan, the average of stem length and branch number are 68.0 cm and 4.0, respectively, for Ryuho in the cultivated land (Akita prefecture, 2015). It was mentioned in the guidance that organic matters in the soil can affect stem length of Ryuho significantly, and stem length would be suppressed easily when Ryuho was planted in the unproductive soil. In this experiment, the averages of stem length and branch number were 67.4 cm and 3.5, respectively.

Table 9 Seed investigation of one soybean plant in mixing tillage model

Model	Total seed No.	Good seed No.	Disabled seed No.	Total seed Wt. (g)*	Disabled seed Wt. (g)*	Good seed Wt. (g)*
C-01	125	122	3	43.68	0.68	43.00
C-02	86	83	3	31.23	0.78	30.45
L-10	155	149	6	57.57	1.54	56.03
M-10	143	138	5	53.54	0.42	53.12
H-10	134	128	6	50.19	1.78	48.40
L-20	144	137	7	51.05	1.68	49.38
M-20	173	166	7	64.01	0.48	63.53
H-20	137	134	3	45.34	0.61	44.73

*Note: *: weight at 15% moisture. In all case (n = 7).*

Table 9 shows that good seed weights of models C-01 and C-02 were the lowest among all treatments. This result can be explained by the low content of organic matter in the non-contaminated soil (Table 1). Furthermore, C-02 was the only model of which average good seed weight was less than 40 g with one plant. It is because that in the year of 2017, model C-02 was attacked by insect pest so that it had a poor growth and its SPAD value was lower than other models (Fig. 67). Significant difference was shown between H-20 and L-10, and between H-20 and M-10, and there was a trend of L-10 > M-10 > L-20 > H-10 > H-20 (Table 6). It might result from the toxicity of heavy metals (Das et al., 1998; Gao et al., 2014; Wuana et al., 2011). However, good seed weigh of M-20 was the highest. It might be because the container of model M-20 was set at the south site and it might get more sunshine then other models.

For the same reasons, 100 seed weight of H-20 had a small value in this experiment, and a trend of M-10 > M-20 > L-10 = H-10 > L-20 > H-20 (Table 6) was shown in the Table 6. Significant difference was shown between H-20 and the other contaminated treatments.

According to these indexes of the quantity and quality of seeds, it is suggested that mixing tillage can sustain the yield of soybean in the contaminated fields by this experiment. As well, the content of organic matter in the soil could promote the yield of soybean to balance the toxicity effect from heavy metals. In practical, mountain soil is supposed to be used for mixing tillage, but the mountain soil is often sterile and containing little humus. Therefore, restoring soil fertility by applying adequate organic fertilizers should be implemented simultaneously.

3.3.5 Water quality parameters

3.3.5.1 pH

For well understanding the growing condition of soybean plants in this experiment, we measured the pH of supplied drained water sampled from the three models in the year of 2017. Samples were collected eight times from July to September. From July 18^{th} to Sept. 5^{th}, a trend of pH value Model C-02 < Model M-10 < Model M-20 < Supplied water was observed, and after that Model C-02 < Supplied water < Model M-10 < Model M-20 (Table 10). This condition may result from the low pH value of Kanagi soil solution (Chapter 3, 3.7.1). It also has been reported that some plants can passively or actively change H^+ excretion under heavy metal stress. Tu et al. (1989) observed that Cd has an inhibitory action on H^+ excretion.

Table 10 pH values of supplied water and drainage in three models in 2017

Sampling date	7/18	7/25	8/22	8/29	9/05	9/12	9/19	9/26
Supplied water	6.38	6.05	6.49	6.45	6.47	6.11	6.00	6.25
Model C-02	6.16	5.64	5.86	6.15	6.19	5.96	5.94	6.13
Model M-10	6.30	5.90	6.17	6.38	6.37	6.20	6.04	6.28
Model M-20	6.33	6.00	6.26	6.38	6.37	6.32	6.17	6.38

3.3.5.2 K, Ca, Mg, Cu and Cd contents

For protecting people's health, environmental water quality standard was changed to 0.003 mg Cd L^{-1} from 0.01 mg Cd L^{-1} (MOE, 2014) by Japan government. In China, standards for irrigation water quality set the Cd concentration limitation as 0.01 mg L^{-1}, and Cu concentration was recommend being less than 0.5 mg L^{-1} for paddy field and less than 1 mg L^{-1} for upland field (AQISQ & SAC, 2006). Previous researches reported that paddy soil has a high absorbent capacity of Cd from cadmium contaminated water in both river water and soil salt solution conditions, and proposed to reset the Cd standard for water quality to 0.001mg L^{-1} (Itou and Imure, 1974). From tables 11 & 12, we can see that the supplied water was clean, and the erosion of water did not wash Cd away from contaminated soils, in this experiment.

Table 11 Metal elements content in supplied water and drainages in 2016

	Supplied water	C-01 drainage	L-10 drainage	H-10 drainage	L-20 drainage	H-20 drainage
Cd^{2+}	-	< 0.0003	< 0.0003	< 0.0003	< 0.0003	< 0.0003
Cu^{2+}	< 0.01	-	-	-	-	-
K^{+}	< 1.0	2.5	2.4	3.0	2.9	2.9
Ca^{2+}	7.3	8.9	9.1	8.7	8.3	10.4
Mg^{2+}	2.3	2.8	2.8	2.6	2.6	3.0

Note: unit: mg L^{-1}

Table 12 Metal elements content in supplied water and drainages in 2017

	2017/07/18				2017/08/29			
	Supplied water	C-02 drainage	M-10 drainage	M-20 drainage	Supplied water	C-02 drainage	M-10 drainage	M-20 drainage
Cd^{2+}	0.0022	< 0.0003	< 0.0003	< 0.0003	0.0023	< 0.0003	0.0006	< 0.0003
Cu^{2+}	0.003	0.001	0.001	0.002	0.003	0.001	0.003	0.003
K^{+}	< 1.0	1.8	1.3	1.6	1.8	1.7	< 1.0	< 1.0
Ca^{2+}	7.9	6.3	5.5	6.2	8.9	13.9	9.7	9.1
Mg^{2+}	2.4	2.0	1.8	2.0	2.4	4.6	2.6	2.4

Note: unit: mg L^{-1}

3.3.6 Soil properities (physical and chemical)

3.3.6.1 pH of soil solution

In this experiment, pH values of original soils and harvested soils were measured. The results of pH values were shown in Table 13. In Table 13, it shows that pH of original X paddy and Kanagi soils were 5.86 and 4.55, respectively. pH of Kanagi soils after harvesting in C-01, C-02, 10 ~ 40cm of Models L-10, M-10 and H-10, and 20 ~ 40cm of Model L-10, M-20 and H-20 were mostly under 5.0. pH of other soil layers was mostly over 5.0. pH of gravel layers of eight models ranged from 5.72 ~ 6.41, higher than soil layers but similar to the supplied water. Soybean plants are suitable for growing in such an environment with soil pH 5.5 ~ 7.0 (MAFF, 2007). Therefore, it is necessary to adjust the soil pH when its pH value was too low or too high.

Solubility of most heavy metals is affected by soil pH. In general, solubility and activity of heavy metals increase with the decrease in soil pH. Previous research observed a marked decrease in exchangeable Cd (free ion Cd^{2+}) in soil when soil pH increased from 4.55 to 7.0 (Xian, 1989). MAFF (2007) recommended that soil pH over 6.0 could suppress Cd absorption by soybean plants. The addition of lime into pinaster rhizosphere could significantly reduce the solubility of Cu, Pb and Cd ions in soil (Helmisaari et al. 1999). Lime application in acid soil may reduce the absorption of Cd in cabbage by 43% (Yang and Yang, 1996) and reduce Cd concentration in wheat grain by 50% (Lu et al., 1992). Consequently, it may be potential to alleviate Cd and Cu toxicity in Cd-Cu-contaminated acid soil to promote soil pH by lime or lime substances application.

Table 13 pH values of soil solutions after harvesting in mixing tillage models

Depth from soil surface	C-01	C-02	L-10	M-10	H-10	L-20	M-20	H-20
0 ~ 10cm	4.81	4.77	4.93	5.07	5.26	5.27	5.16	5.32
10 ~ 20cm	4.82	4.81	4.90	4.42	4.72	5.10	4.82	5.40
20 ~ 30cm	4.88	4.78	4.98	4.41	4.77	4.92	4.47	4.73
30 ~ 40cm	5.15	4.85	5.17	4.78	5.09	5.22	4.72	4.96
40 ~ 50cm	5.97	5.72	5.77	5.95	5.76	5.95	5.82	6.41

3.3.6.2 Three-phase distribution of soil

Three-phase distribution of soils in 0 ~ 10 cm, 10 ~ 20 cm, 20 ~ 30 cm and 30 ~ 40 cm layers were measured with eight models. Three phases meter (Model DIK-1120, DAIKI RIKA KOGYO CO., LTD.) was used to measure three-phase distribution of soils (Fig. 75). Constant head permeability test was also carried out as shown in Fig. 76. Three-phase distribution conditions of model C-01, C-02, L-10, M-10, H-10, L-20, M-20 and H-20 were shown in Figs. 77 ~ 84, respectively. As depth of soil being deeper, liquid phase ratio became higher and gas phase ratio became lower for all models. Comparison among eight models by depth was shown in Figs. 85 ~ 88. In 0~10 cm layer, Models C-02, M-10 and M-20 had a low liquid phase ratio, and all of this three models were carried out in 2017. Model M-10 had a high solid phase ratio and low gas phase ratio in the depth of 30 ~ 40 cm. Previous researches found the highest values of solid and liquid phases and low value in gas phase with puddled treatment; conventional tillage treatment produced the highest value in gas phase and the lowest values of both solid and liquid phases with the top soil 0~10 cm (Rahman et al., 2003, 2008). It is consistent with the condition of high gas phase values in this experiment.

Fig. 75 Three-phase distribution meter

Fig. 76 Permeability experiment

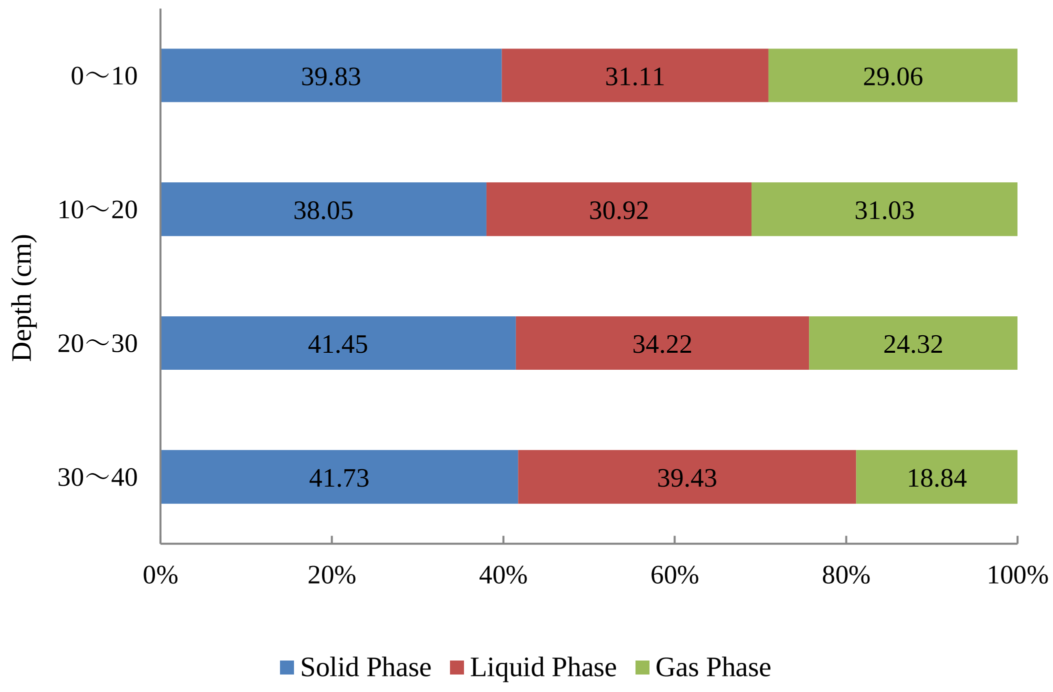


Fig. 77 Three-phase distribution of soil with model C-01

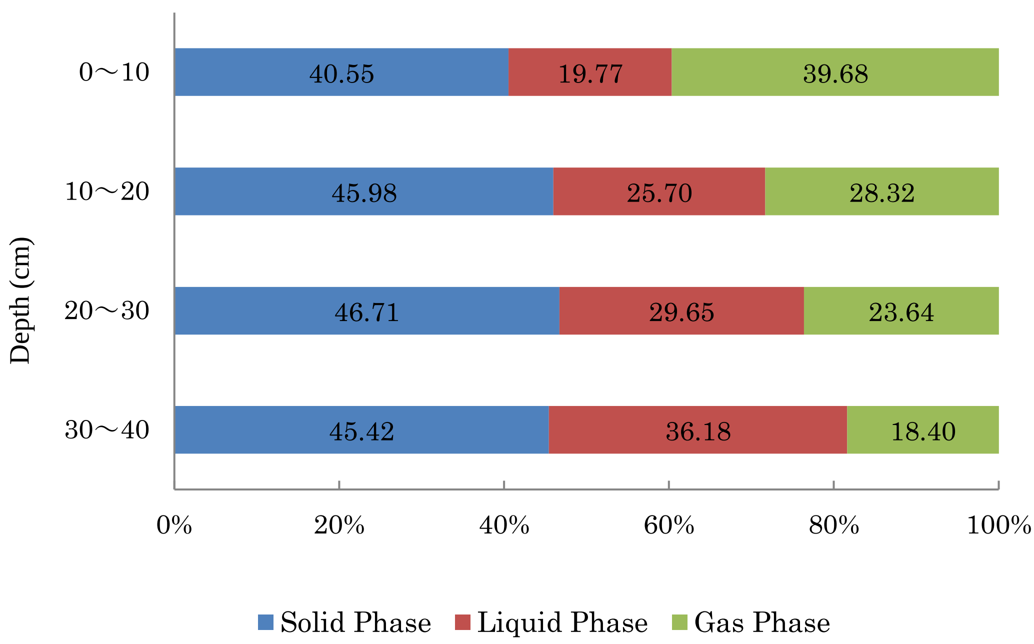


Fig. 78 Three-phase distribution of soil with model C-02

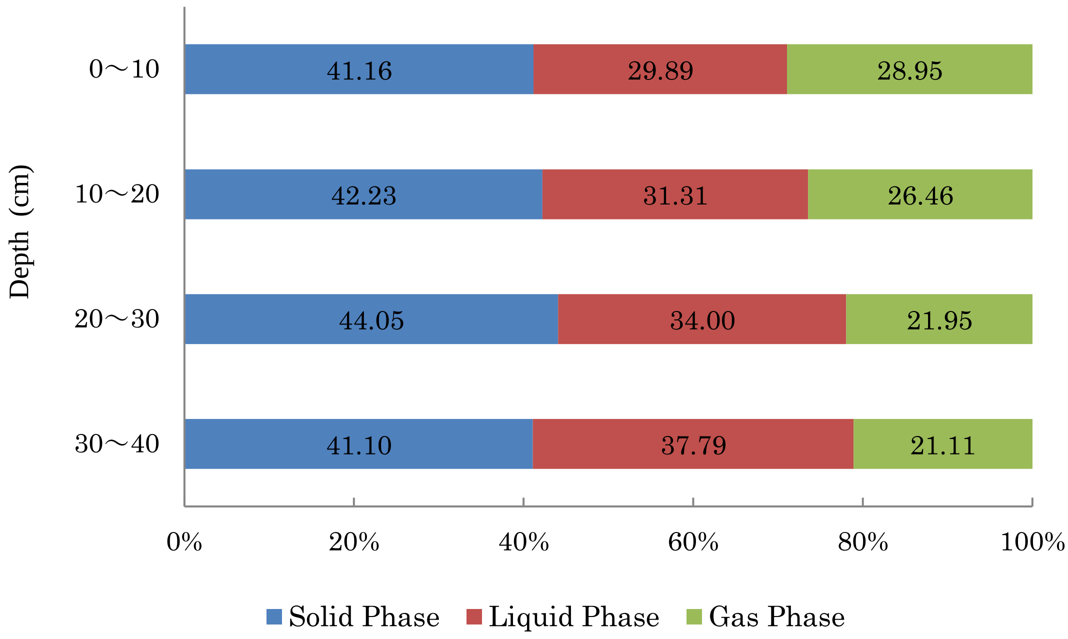


Fig. 79 Three-phase distribution of soil with model L-10

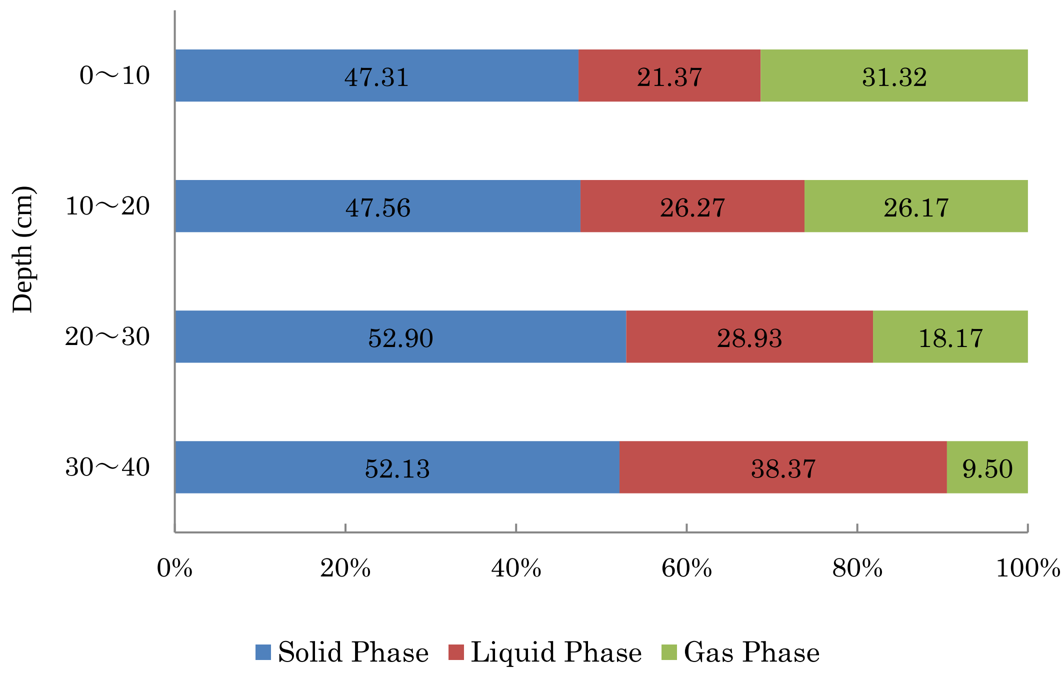


Fig. 80 Three-phase distribution of soil with model M-10

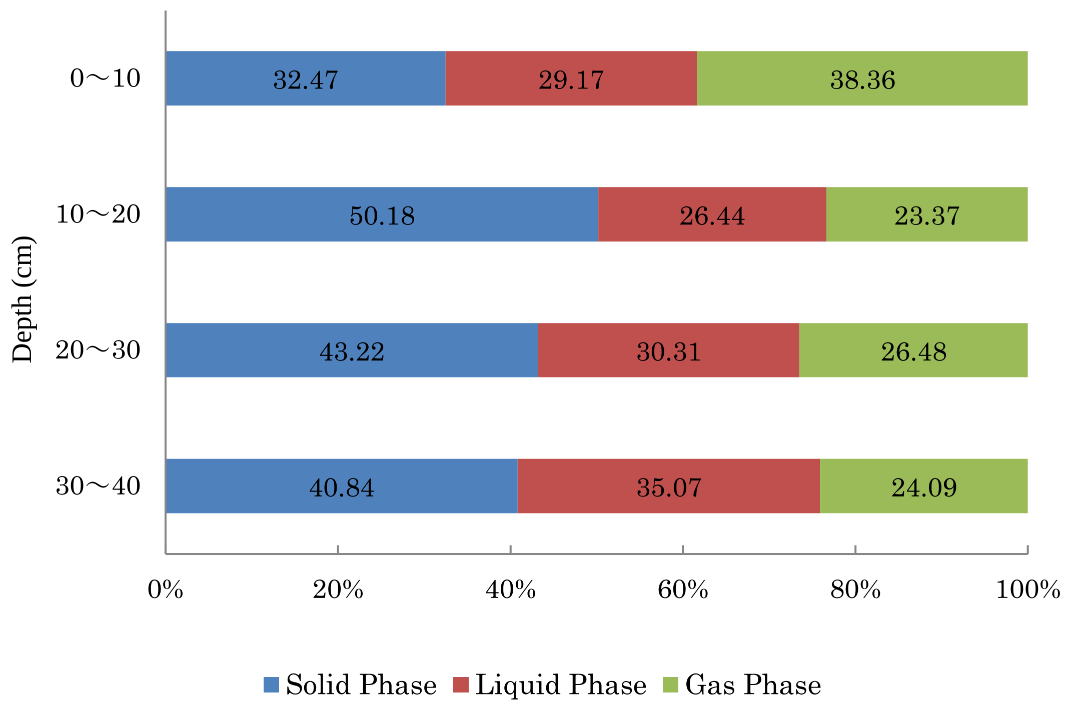


Fig. 81 Three-phase distribution of soil with model H-10

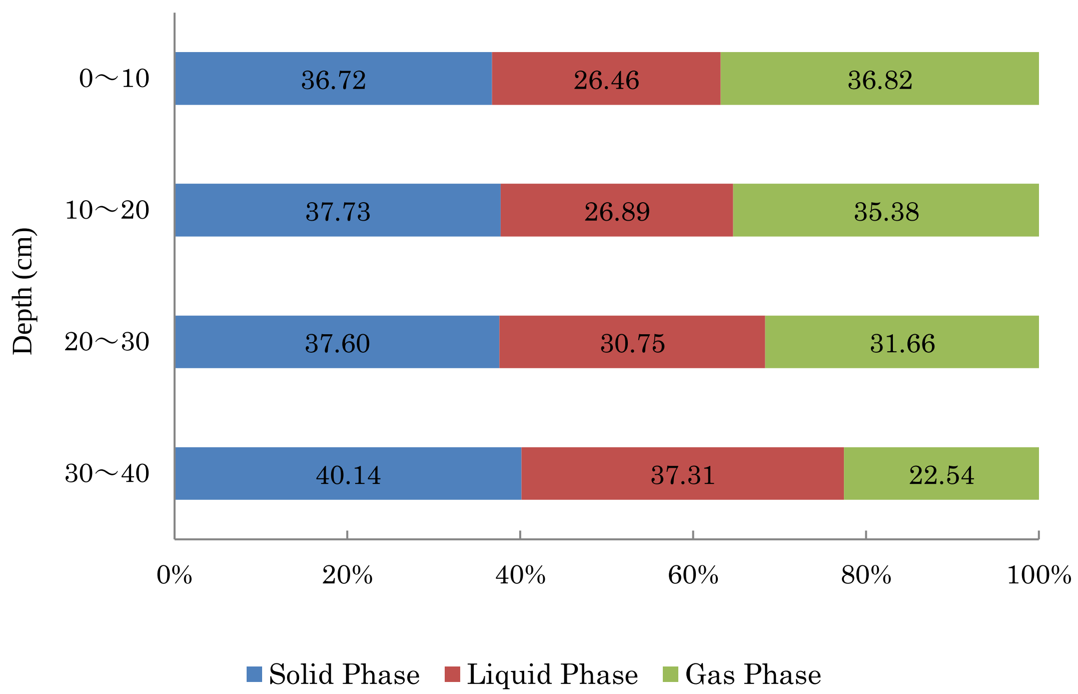


Fig. 82 Three-phase distribution of soil with model L-20

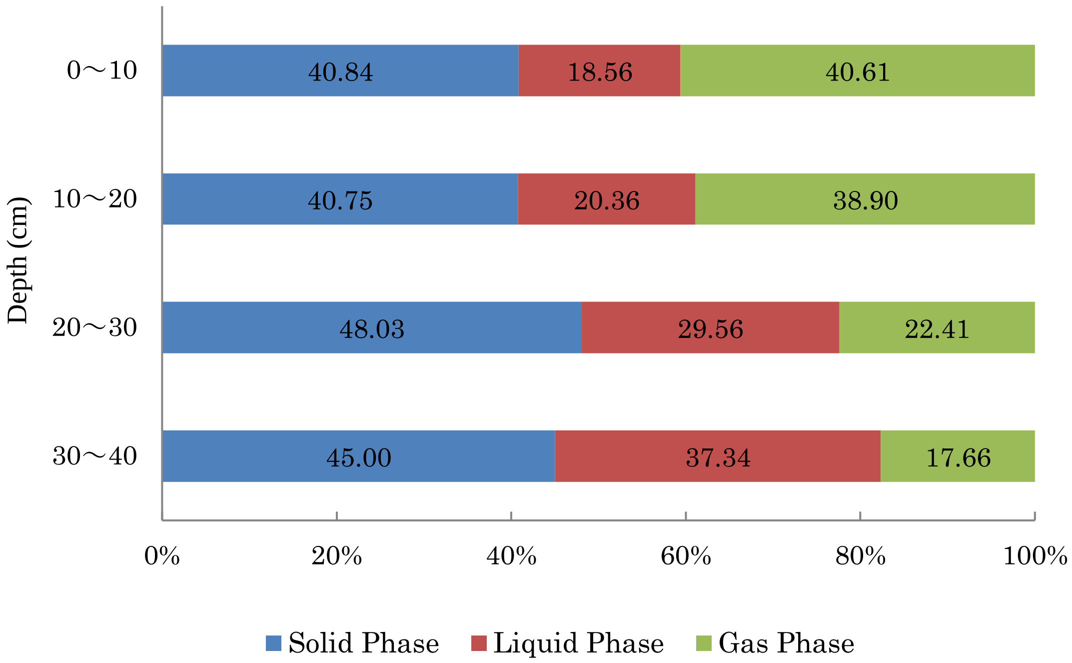


Fig. 83 Three-phase distribution of soil with model M-20

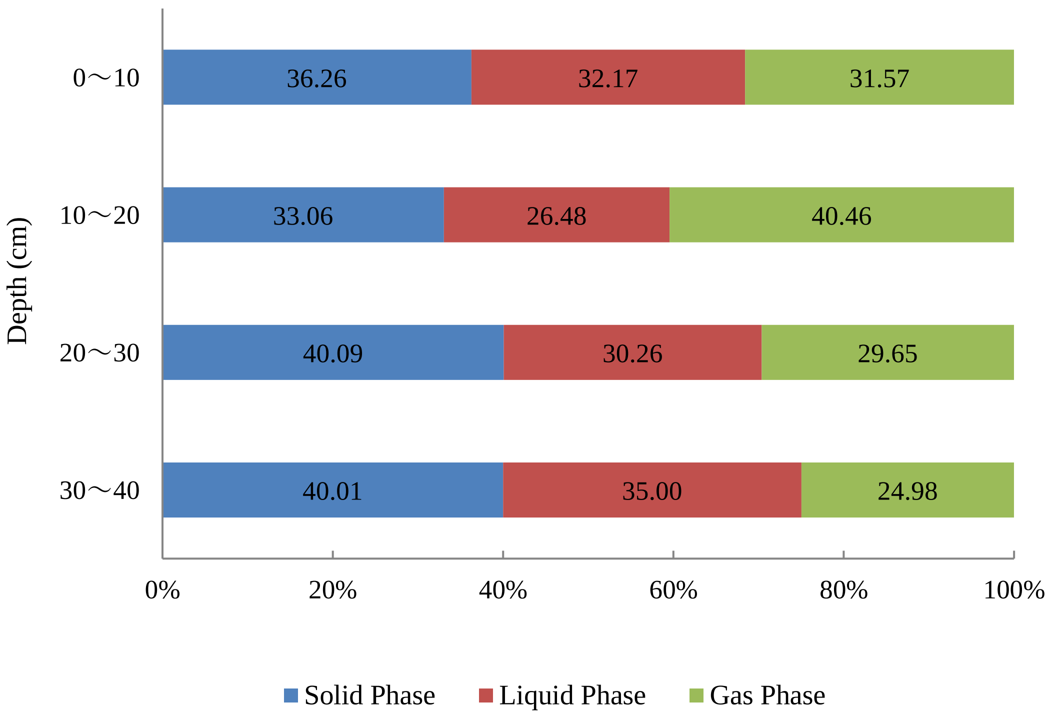


Fig. 84 Three-phase distribution of soil with model H-20

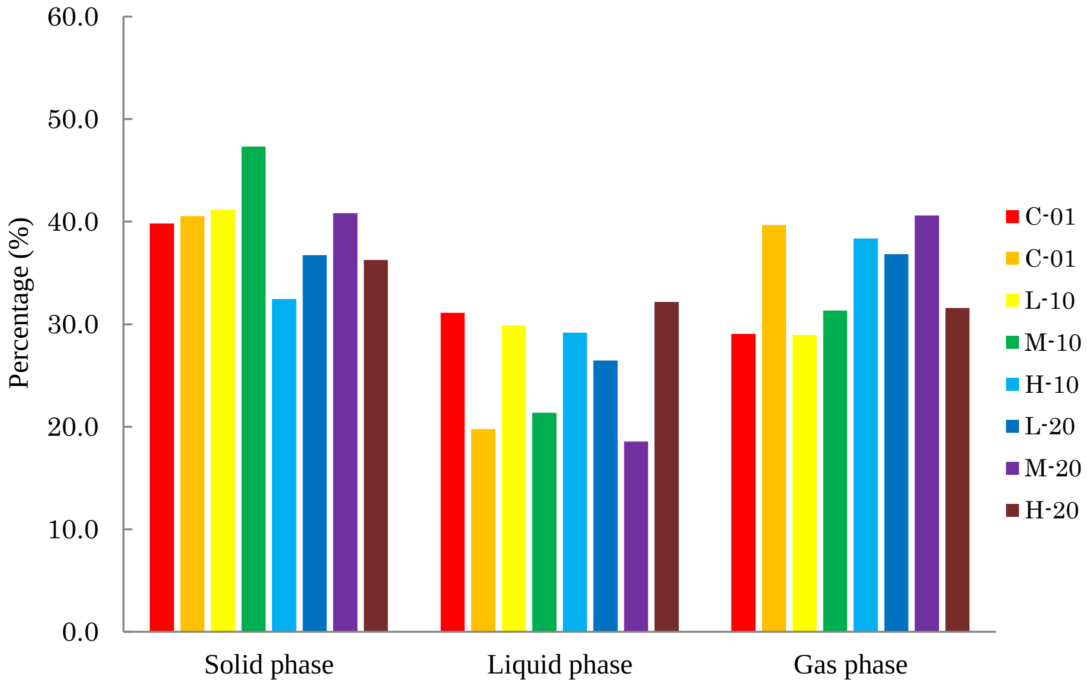


Fig. 85 Three-phase distribution of soil in 0~10cm with eight models

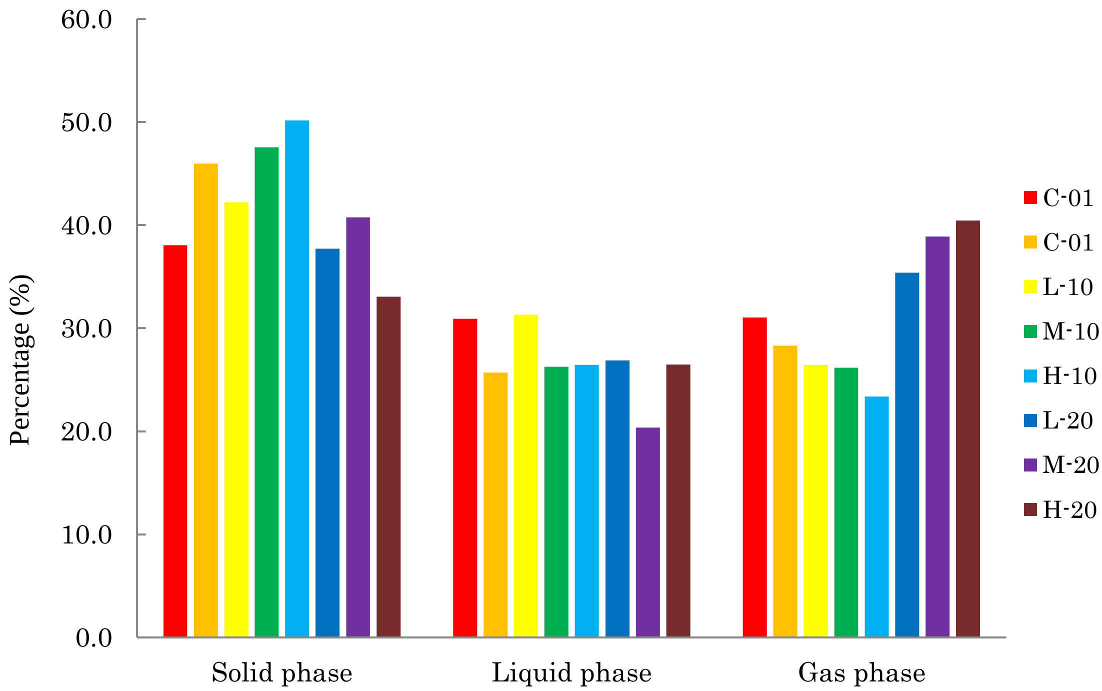


Fig. 86 Three-phase distribution of soil in 10~20cm with eight models

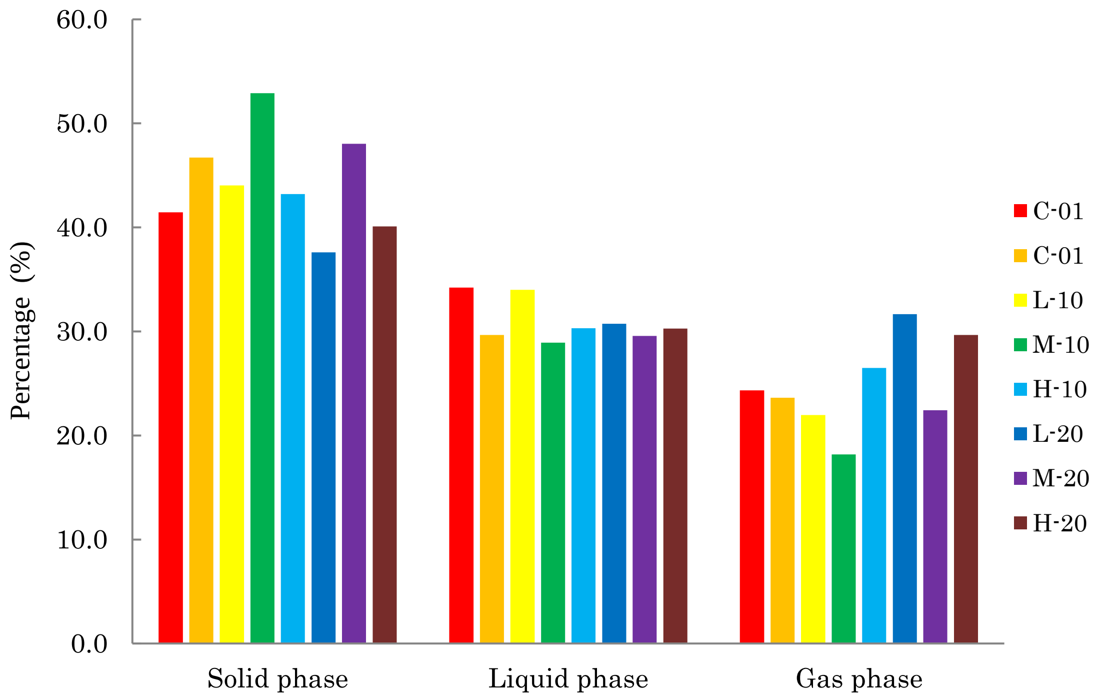


Fig. 87 Three-phase distribution of soil in 20~30cm with eight models

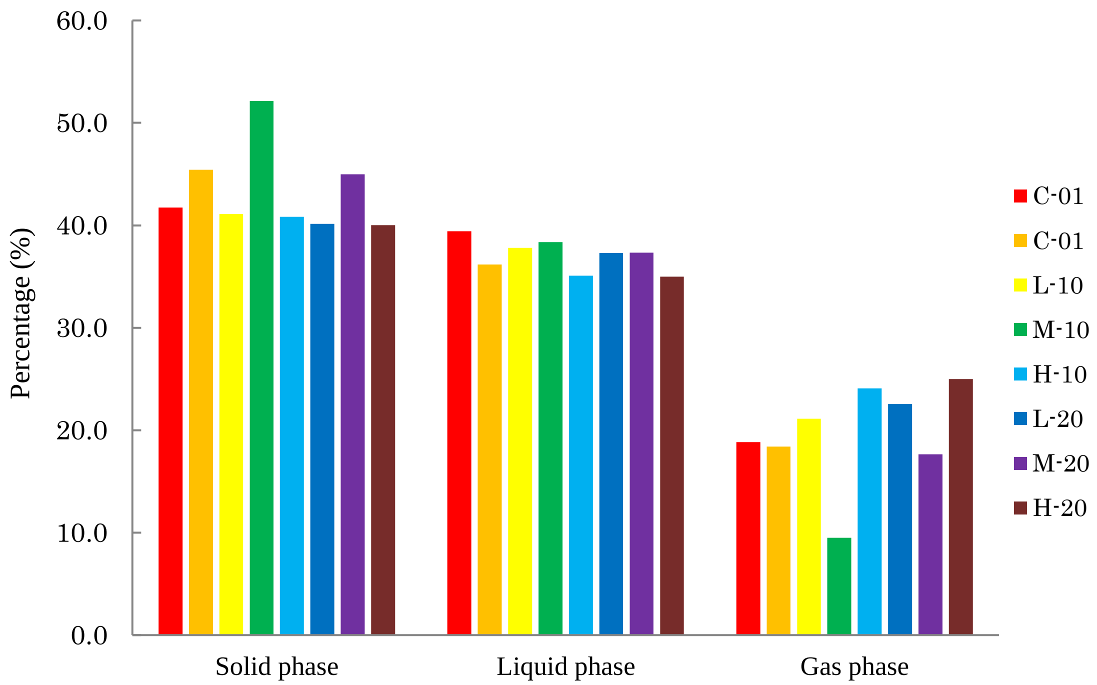


Fig. 88 Three-phase distribution of soil in 30~40cm with eight models

3.3.7 Root distribution of soybean plants

Root weight of 0~10cm soil included. 2 cm stem connected to the main root. Fig. 89 shows the root shape in different soil depth. Figs. 90 show the morphologies of wet roots in soil and gravel. Total root dry weight of one soybean plant in this experiment ranged from 5.12g to 9.78g (Table 14). Soybean plants in C-01, L-10, H-10 and L-20 have significant lower total weight than that in other models. It may be resulted from that in the year of 2016, roots of soybean plants in Models C-01, L-10, H-10 and L-20 were conserved in drying oven for about one month until cleaning and washing of dried root may lead to serious loss of root. However, root distributions by depth of each model nearly have the same trend and similar ratio (Fig. 91). Root in depth 0~10cm occupied about 50% for all models.

Fig. 89 Shape and distribution of two soybean plants' roots in model H-10

Fig. 90 Wet root in soil and gravel

Table 14 Dry root weight distribution in soil layers of eight models

Model	Depth from the soil surface (cm)					Total dry root weight of one plant (g)
	0~10*	10~20*	20~30*	30~40*	40~50*	
C-01	15.99	4.73	4.70	4.19	1.15	5.12
C-02	28.82	8.01	7.23	8.70	5.85	9.77
L-10	19.24	6.75	4.84	3.65	1.42	5.98
M-10	31.49	4.48	5.45	7.27	3.13	8.64
H-10	24.38	4.11	2.87	2.90	2.40	6.11
L-20	23.50	6.48	4.29	4.26	1.50	6.67
M-20	33.47	6.17	5.32	7.50	4.15	9.44
H-20	30.25	14.00	4.71	7.50	2.24	9.78

Note: *: *n* = 6

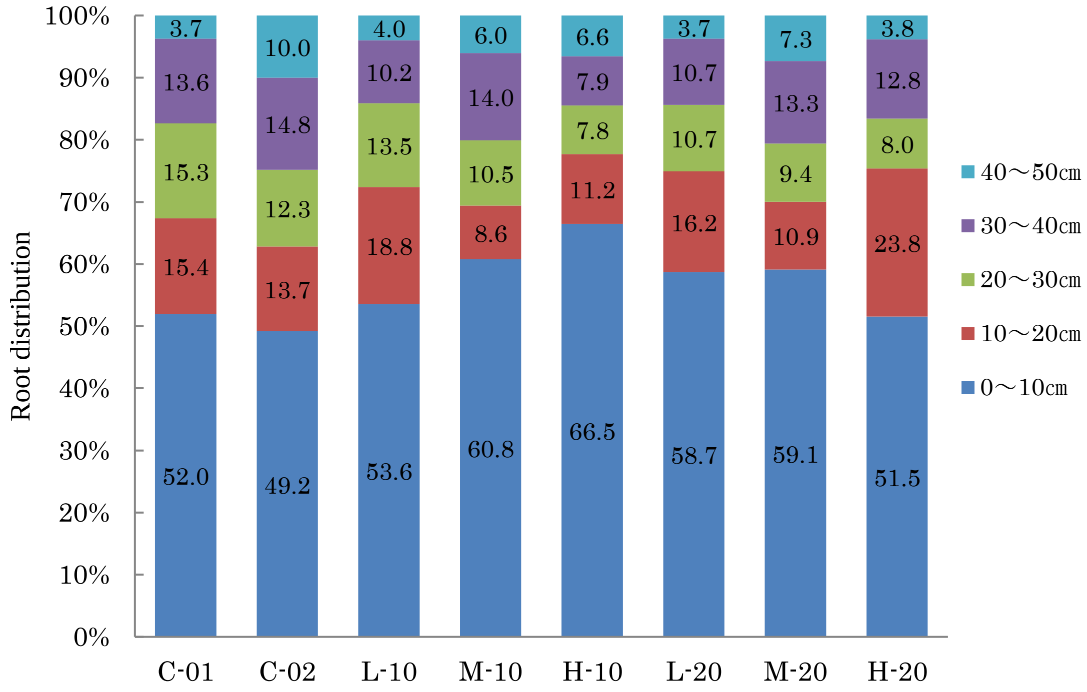


Fig. 91 Root distribution of Soybean plants (2016 and 2017)

3.3.8 Heavy metal accumulation in soybean plants

Cd and Cu concentrations in the seeds, stems and roots of soybean plants in each model were analyzed.

3.3.8.1 Cd accumulation

Comparisons of Cd concentrations in soybean seeds, stems and roots were shown in Table 15 and Fig. 92.

Cd concentration in soybean seeds: Cd concentration in soybean seeds of each model was in the order of C-02 < C-01 < M-10 < L-10 < M-20 < L-20 < H-10 < H-20. There were significant differences between control models and experimental models. Cd accumulations in the seeds of all experimental models were higher than

0.2 mg kg^{-1} which is the safety standard of Cd in soybean in China and EU. It is verified that soybean plant can absorb Cd easily. Thus, it is necessary to improve safe soybean production. Previous researches reported that Cd can be accumulated in soybean plants easier than any other crops and upland crops can absorb Cd more easily than lowland crops (MAFF, 2007; Nakagawa and Tsunematsu, 2002).

Significant differences were also found between L-10 and H-10, M-10 and H-10, L-20 and H-20, and between M-20 and H-20. Cd concentrations in seeds of models H-10 and H-20 were significantly higher than any other models. Those significant differences could be explained that soybean seeds would accumulate more Cd when the cultivated soil had a higher Cd concentration level. It is shown in Fig. 87, in which the data was collected form this experiment and pervious researches (Haque et al., 2014a, 2014b), and all the collected data of Cd accumulation in soybean plants were obtained under a similar condition (contaminated soil 20~25cm and groundwater level 40cm). Previous research has reported that rice seeds can accumulate more cd when the soil contains more Cd (Itou and Imura, 1975). For models H-10, L-20 and M-20, Cd accumulation in the seeds of H-10 was higher than that in the seeds of L-20 and significantly higher than that in the seeds of M-20. Thus it demonstrates that the method of mixing tillage can effectively reduce Cd concentration in the soybean seeds when cultivated in the contaminated soil. However, there were no significant difference among the models of L-10, M-10, L-20 and M-20, except for that significant difference between L-20 and M-10. Lower Eh value in modes of 2017 than that in models of 2016 may describe this situation. It was also suspected that when the soil Cd concentration was at a relatively low level, Cd accumulation in soybean seeds increase relatively slow. Significant differences were not found between L-10 and L-20, M-10 and M-20, and between H-10 and H-20. This could be illustrated by that the soil Cd in the upper 10cm layer has more obvious effect on Cd uptake than that in the underlying layer. Furthermore, the root distribution in the eight models (Fig. 85) revealed that the average root weight in the soil layer 0 ~ 10cm of all models had a high proportion over 50% of the total root weight. This distribution could also explain the important role of top 10cm filed soil to low Cd concentration soybean planting.

Cd concentration in soybean stems and roots: Cd concentration in soybean stems and roots had a similar trend as the case of Cd concentration in the seed. Soybean plants in control models had a lower stems and roots Cd accumulation than that in experimental models. Meanwhile, models H-10 and H-20 had a higher Cd absorption for stems and root than that in other experimental models. High Cd concentration of the soil can lead to a high Cd accumulation in stems and roots of soybean plants (Fig. 93). This agrees with the result from Cao et al. (2007), in which Cd concentration in roots and shoots was positively related with Cd concentration in soil for maize and soybean. In addition, if we want to harvest the soybean with Cd concentration in seeds under safety level 0.2 mg kg^{-1}, Cd concentration in the soil should be lower than 0.31 mg kg^{-1}, according to Fig. 93.

Dixit et al. (2001) reported that for pea plants, Cd accumulated immediately in roots, later in the stem and finally in the leaves, and maximum accumulation of Cd occurred in roots followed by stems and leaves. Cadmium accumulated in soybean leaves and pods can potentially move to the seeds from seed filling period to mature period (Oda et al., 2004; Yada et al., 2004). In this experiment, Cd concentration in soybean plants was in the order of seed < stem < root, and it was clearly shown in

Fig 87. This order is similar to previous researches for soybeans and other crops such as corn, rice and wheat (Gao et al., 2006; Liu et al., 2010a; Wang and Wu, 1998). The ratio of average Cd concentration in seeds : stems : roots was about 1 : 2 : 7 in this experiment regardless of the models. From Fig. 87, we can interpret that Cd accumulation in soybean seeds, stems and roots positively correlate with soil Cd concentration. According to approximation curves in Fig. 87, Cd concentration in soybean roots increased the fastest, then was stems and seeds. This correlation still presents at high Cd concentration environment for pea roots, stems and leaves (Dixit et al., 2001), and soybean roots and shoots (Cao et al., 2007). For example, when Cd concentration in the soil is 640 mg kg^{-1}, the concentration in the soybean roots and be about 150 mg kg^{-1}. This fact is different from the trend of Cu and Zn accumulation in other crops as their accumulations can reach a peak and have no rise when the Cu and Zn concentration in the soil get a really high level (Fan et al., 2018; Shibuya, 1979). This condition may result from the different transport characteristics of Cd and Cu in the soybean plant (Iwasaki, 1990; Oda and Arao, 2006). From the viewpoint of phytoremediation, it might be necessary to clean up the soybean roots from the topsoil layer of 10 ~ 20 cm depth from the surface after harvesting instead of discarding them in soil of the contaminated filed, since Cd concentrations of roots in all the experimental models were at a high level which was more than 2.0 mg kg^{-1}.

In addition, Table 16 shows that Cd concentrating rate from contaminated soil to soybean seeds, stems and roots becomes lower when the Cd concentration in contaminated soil is higher.

Table 15 Cd concentration in different parts of soybean plants

Model	Cd-Seed	Cd-Stem	Cd-Root
C-01	0.18±0.02^{ab}	0.43±0.07^{a}	0.64±0.06^{a}
C-02	0.14±0.01^{a}	0.33±0.04^{a}	0.95±0.10^{ab}
L-10	0.30±0.05^{cd}	0.66±0.11^{ab}	2.17±0.92^{bcd}
M-10	0.26±0.04^{bc}	0.5±0.11^{ab}	2.75±0.56^{cde}
H-10	0.46±0.08^{ef}	0.91±0.16^{b}	3.59±0.92^{de}
L-20	0.37±0.03^{de}	0.91±0.28^{bc}	2.44±0.58^{bcd}
M-20	0.33±0.06^{cd}	0.51±0.10^{ab}	2.05±0.39^{abc}
H-20	0.54±0.06^{f}	1.13±0.46^{c}	4.15±1.49^{e}

Note: Small letter indicates significant difference at 5% level according to Turkey-Kramer test; ± shows standard deviation.

Table 16 Cd concentrating rate (concentration in soybean/concentration in topsoil)

Model	Cd-Seed	Cd-Stem	Cd-Root
C-01	1.29	3.07	4.57
C-02	1.08	2.54	7.31
L-10	0.46	1.02	3.34
M-10	0.22	0.42	2.29
H-10	0.27	0.52	2.08
L-20	0.57	1.40	3.75
M-20	0.28	0.43	1.71
H-20	0.31	0.65	2.40

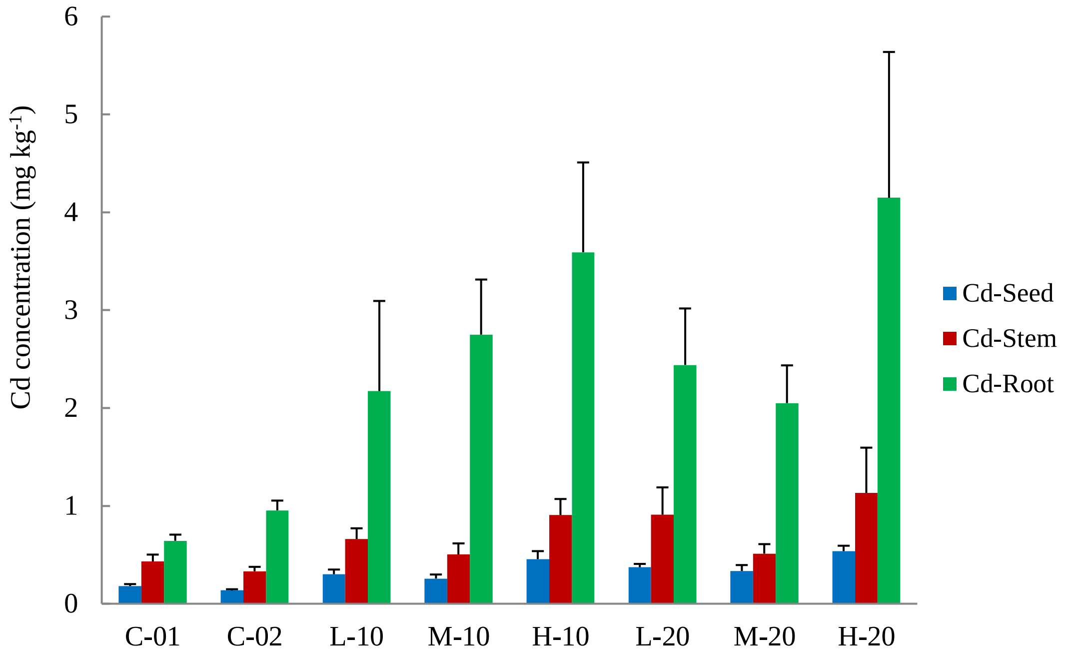


Fig. 92 Cd concentration in seed stem and soot

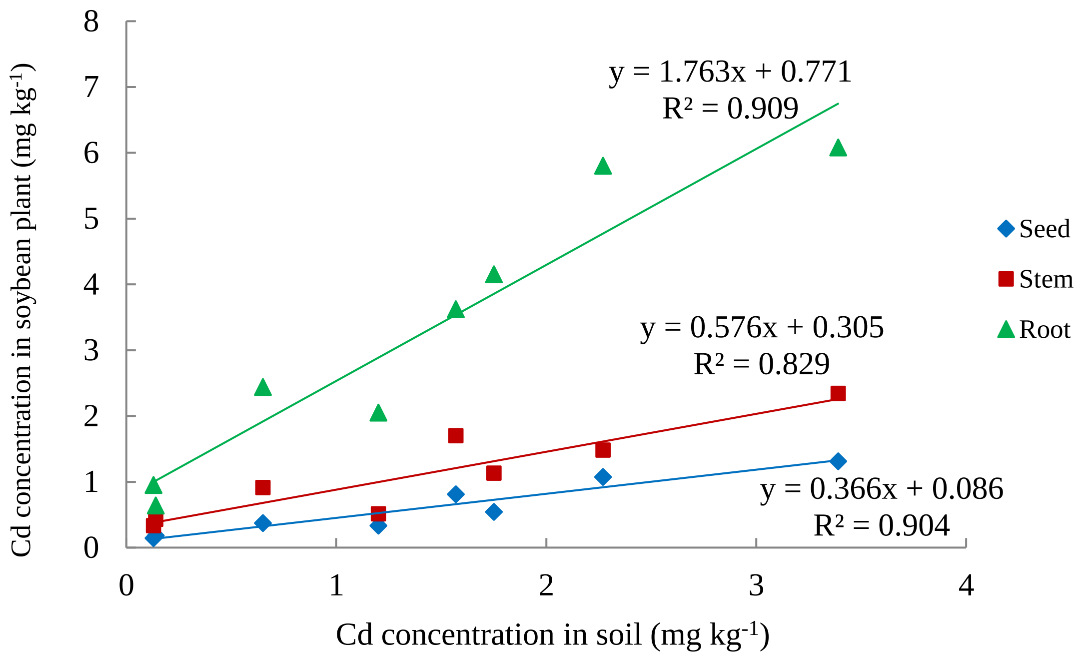


Fig. 93 Relationship between Cd concentration in soil and in soybean plant

3.3.8.2 Cu accumulation

Cu concentrations in different parts of soybean plants in this experiment were shown in Table 17. The trend of Cu concentration in soybean seed was L-10 < M-20 < C-01 < H-10 < M-10 < L-20 < C-02 < H-20. Cu accumulation in the seeds of H-20 was higher than that in the seeds of any other model. However, obvious significant difference was not found among them and Cu concentration of all models ranged from 9.12 ~ 11.20 mg kg^{-1}. In Japan, the average Cu concentration of homegrown and imported edible soybeans is 9.7-11.1 mg kg^{-1} (MEXT, 2016) and the safety standard value in China is 20 mg kg^{-1}. Soybeans would pose certain risk to human health when they are cultivated in a higher Cu containing soil than the ones used in this experiment. There was no significant difference of Cu concentration in the stem or seed among all treatments at $p < 0.05$. Cu concentration in the soybean plant of this experiment had the same trend with previous study on soybean and other crops (Huang et al., 1993; Li et al., 2017; Wang and Wu, 1998), stem < seed < root. This trend can be interpreted in Fig. 94, and it was different from Cd uptake situation. Maybe the different transport characteristics of Cd and Cu in the soybean plant can explain this distinct status (Iwasaki, 1990; Oda and Arao, 2006). The ration of average Cu concentration in seeds : stems : roots was about 2 : 1 : 5 in this experiment.

In addition, Table 18 shows that Cu concentrating rate from contaminated soil to soybean seeds, stems and roots becomes lower when the Cu concentration in contaminated soil is higher.

Table 17 Cu concentrations in different parts of soybean plants

Model	Cu-Seed	Cu-Stem	Cu-Root
C-01	9.55 ± 0.32^{ab}	4.33 ± 0.70^{a}	26.41 ± 3.95^{a}
C-02	10.88 ± 0.62^{c}	9.38 ± 1.93^{b}	36.49 ± 11.30^{a}
L-10	9.12 ± 0.77^{a}	4.99 ± 1.08^{a}	39.68 ± 23.03^{a}
M-10	9.64 ± 0.65^{ab}	6.11 ± 2.37^{a}	26.15 ± 4.80^{a}
H-10	9.61 ± 0.83^{ab}	4.88 ± 1.03^{a}	31.00 ± 18.32^{a}
L-20	10.47 ± 0.74^{bc}	5.68 ± 0.91^{a}	26.65 ± 12.40^{a}
M-20	9.52 ± 0.29^{ab}	4.87 ± 1.01^{a}	15.34 ± 2.49^{a}
H-20	11.20 ± 0.88^{c}	4.18 ± 0.31^{a}	20.54 ± 13.44^{a}

Note: Small letter indicates significant difference at 5% level according to Turkey-Kramer test; ± shows standard deviation.

Table 18 Cu concentrating rate (concentration in soybean/concentration in topsoil)

Model	Cu-Seed	Cu-Stem	Cu-Root
C-01	2.58	1.17	7.14
C-02	2.09	1.80	7.02
L-10	1.40	0.77	6.10
M-10	0.82	0.52	2.22
H-10	0.79	0.40	2.54
L-20	1.61	0.87	4.1
M-20	0.82	0.41	1.30
H-20	0.92	0.34	1.68

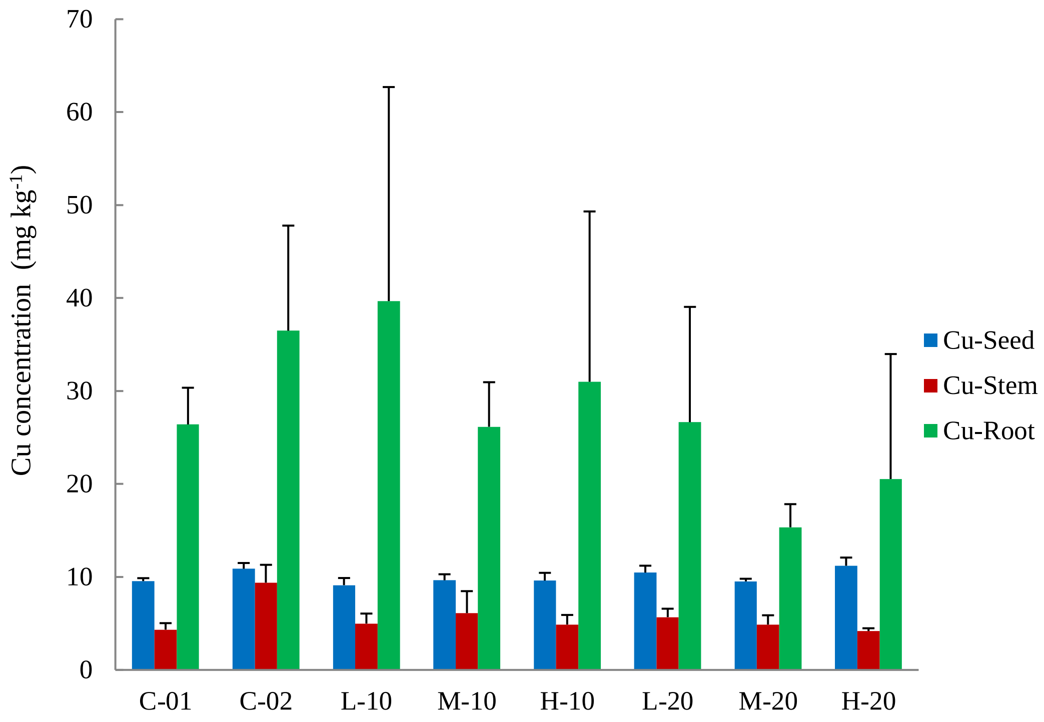


Fig. 94 Cu concentration in seed stem and root

Chapter 4: Summary and conclusion

This study was conducted for the search of a convenient and feasible method to deal with the threat of safe soybean planting.

From the experiment of reducing Cd and Cu concentration by controlling the groundwater, we learnt that controlling groundwater level can lead to the change of redox condition in the soil and then affect the Cd and Cu absorption by the soybean plants significantly. High groundwater level can effectively limit the Cd and Cu concentration in soybean seeds. However, it can also result in very low soybean yield at the same time.

In this mixing tillage experiment, eight experimental models were designed and conducted in two years to investigate the effects of mixing tillage on the contaminated soil for soybean cultivation at a 40 cm groundwater level. All of the soil layers in each model were under oxidation condition through the cultivation period.

Growth and yield: During all the growth period, **s**oil layers of each model were under oxidation condition according to the Eh values, and soybean plants growth parameters such as stem length, leaf age and SPAD had similar trends of all models. Good seeds weights of eight models were in the order of C-02 (30.9 g) < C-01 (43.0 g) < H-20 (44.6 g) < H-10 (48.3 g) < L-20 (49.6 g) < M-10 (54.4 g) < L-10 (56.0 g) < M-20 (64.5 g). Little difference was seen in growth and yield among the eight models. However, soybean plants in control models had a worse growth and lower yield than those in experimental models. It may be resulted from the low organic content of the non-contaminated soil in this experiment. Seeds yield of H-20 was lower than any other models. It proved that high level and deep depth of contaminated soil can result a low yield of soybean seeds.

Cd concentration in soybean plants: Cd accumulation in the soybean seeds of each model was in the order of C-02 (0.14 mg kg^{-1}) < C-01 (0.18 mg kg^{-1}) < M-10 (0.26 mg kg^{-1}) < L-10 (0.30 mg kg^{-1}) < M-20 (0.33 mg kg^{-1}) < L-20 (0.37 mg kg^{-1}) < H-10 (0.46 mg kg^{-1}) < H-20 (0.54 mg kg^{-1}), and Cd accumulations in the seeds of all experimental models were higher than 0.2 mg kg^{-1}. It can be interpreted that soybean seeds can absorb Cd easily and they would accumulate more Cd when the cultivated soil had a higher Cd concentration level.

Cd accumulation in the seeds of H-10 was higher than that in the seeds of L-20 and significantly higher than that in the seeds of M-20. This result showed that mixing tillage can significantly alleviate the Cd accumulation in the soybean seeds.

The trend of Cd distribution in soybean parts, or seeds, stems and roots, was approximately 1 : 2 : 7 in this experiment. Cd accumulation in soybean plants has an increasing trend when the Cd concentration as the soil become higher and higher.

Cu concentration in soybean plants: Obvious significant difference was not found among all models and Cu concentration of them ranged from 9.12 ~ 11.20 mg kg^{-1}. Cu concentration in soybean plants of this experiment had the trend as stem < seed < root. This is different from the situation of Cd.

From the above discussion, it can be concluded that mixing tillage can have significant effect on reducing Cd uptake in soybean plants. In addition, mixing tillage can probably avoid the influence from Cd or Cu to growth and yield of soybean plants in the severe heavy metals contaminated areas at the same time.

Chapter 5: Future plan

◎ For the results being more persuasive, more experimental data and practice were needed to be repeated. Studies on new methods and other heavy metals in soybean planting should be carried out.

◎ In this experiment, Cd concentration in the cultivating soil was controlled. Cu concentration controlling was needed to be added in future research. As the soil standard concentration of Cu is 125 mg kg^{-1}, Cu concentration gradient arounds 125 mg kg^{-1} is recommended, such as 50, 75, 100, 125, 150 and 175 mg kg^{-1}.

◎ Greenhouse cultivation should be finally transferred to real field, in there the effectiveness and feasibility of mixing tillage could be finally examined.

◎ A better result may occur when applying methods of mixing tillage and groundwater level control together, especially in the severe contaminated areas with heavy metals distributed deeply in soil. Furthermore, it will be convenient when being implemented in the rotational upland field as it has perfect water control system.

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