博士論文要約（Summary）

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タイトル	Reducing cadmium absorption in soybean with mixing tillage and groundwater level control.

Chapter 1: Introduction

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.

Chapter 2 Materials and methods

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. Experimental planting device (61 × 41 × 63 cm) was made with thick plastic containers. 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. 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” .

In groundwater level control experiment, three models with different groundwater level were prepared. 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. 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. 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. 1), where “5”, “10” and “40” mean the groundwater level 5, 10 and 40 cm, respectively.

In mixing tillage experiment, five models and three models were prepared in each year of 2016 and 2017, respectively. In the year of 2016, four models were prepared with the combination of two different Cd concentration, 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 models 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, 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. These experiments were designed 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.

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. Oxidation-reduction potential electrodes were inserted for measuring the oxidation-reduction potential (Eh) and soil temperature sensors were also inserted for temperature measurement. 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. Root distribution, water qualities of supplied water and outlet water were also analyzed. Cd and Cu concentration of the roots (depth 0 ~ 10 cm), stems, seeds and soil were analyzed with the Atomic Absorption Spectrophotometry method. Turkey-Kramer test 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

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

(1) Groundwater level control experiment

In this experiment, groundwater levels in three containers were maintained at 5, 10 and 40cm from the soil surfaces. In the GL-5 model, 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, 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, except for Eh values measured at the 42 cm depth, all of the observation depths became oxidation layers of Eh $\geqq$ 500 mV. From these results, the groundwater level could be regulated as we planned.

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. 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.

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 ($p < 0.05$). 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. It can be concluded that the control of the groundwater level had the effect of reducing the Cd concentration in soybean seeds.

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 between GL-10 and GL-40 ($p < 0.05$). The trend of Cu concentration in soybean plants was stem < seed < root. It can be said that the control of the groundwater level is also effective in reducing Cu concentration as well as Cd in soybean seeds.

(2) Mixing tillage experiment

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. In each model, the daily average temperature of soil (depth 5 cm, 15 cm, 25 cm and 35 cm) in containers was between 12.6 ~ 31.9℃ while that of field soil (depth 10 cm and 40 cm) was between 14.5 ~ 27.0℃. The trend of the daily average aoil temperatures was similar to that of greenhouse air temperature (2016: 16.8 ~ 31.9℃; 2017: 13.1 ~ 30.2℃) for all models in two years. In this experiment, groundwater level in each container was maintained at the depth of 40 cm from the soil surface.

Eh values at the depths 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. In 2017, the portions of liquid phase and gas phase were lower than those in 2016. Eh value at the depth of 45 cm (below free water surface, gravel layer) was more than 300 mV in 2016. It could be also explained by a low content of organic matter in the gravel layer and flow of supplied dissolved oxygen water into the groundwater. On the other hand, Eh value at the depth of 45 cm in 2017 gradually decreased to reduction condition.

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. The ranges of soybean plants leaf age were 12 ~ 14 and 13 ~ 15, respectively, in 2016 and 2017. In 2016, the SPAD values were stable and around 40 from the beginning to the 60th day after seeding. While they went down at the beginning and then gradually became higher in 2017.

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. 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. 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. It might be resulted from the toxicity of heavy metals. 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 was obtained. Significant difference was shown between H-20 and the other contaminated treatments. According to these indice 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.

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.

Total dry root weight of one soybean plant in this experiment ranged from 5.12g to 9.78g. Root distributions by depth of each model nearly have the same trend and similar ratio. Root in depth 0~10cm occupied about 50% for all models.

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. 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. Cd concentration in soybean stems and roots had a similar trend as the case of Cd concentration in the seed. Soybean plants in controlled 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. In this experiment, Cd concentration in soybean plants was in the order of seed < stem < root, and Cd accumulation in soybean seeds, stems and roots positively correlate with soil Cd concentration.

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}. 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, stem < seed < root, and the ration of average Cu concentration in seeds : stems : roots was about 2 : 1 : 5.

Chapter 4: Summary and conclusion

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.

Mixing tillage method 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.