博士論文要約（Summary）

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タイトル	Influence of percolation patterns and concentrations of polluted soil on copper uptake, and growth and yield of rice plants in copper-polluted stratified paddy fields

Chapter1 General introduction

With modern industrial development, there is increasing environment pollution. The emission of “three wastes”, urban living garbage, pesticides with heavy metals, and unreasonable use of chemicals result in the soils polluted by heavy metals. Thus, pollutants are accumulated gradually in humans and animals, resulting in damages to their health accordingly. Therefore, people are more and more concerned about heavy metal accumulation in agricultural productions and pollutant control measures (Zhang et al., 2007).

people excavated numerous metal ores to satisfy the needs. However, the domestic production could not meet the fast growing needs, thus people had to import more metal ores from other countries. In this period of time, a large number of heavy metals were thrown away into the surrounding environment without treatment, resulting in extensive soil contaminations due to the pollutants, such as copper (Cu) (Arao et al., 2010).

It is reported that in apple orchards, Bordeaux mixtures, mixture of copper sulphate and calcium carbonate have been used for a long time and thus soil Cu concentrations in some orchards are as high as several hundred mg/kg (dry soil) (Aoyama, 2009) while the safety standard of paddy soil Cu concentrations in Japan is 125 mg/kg. Since apple farming is really hard work, elderly farmers, especially, tend to abandon their orchards. Some of the apple orchards in lowland had once been converted from paddy fields and there is a possibility that they will be restored to paddy fields, which require less labor. Therefore, it should be necessary and important to develop the technique of minimizing Cu uptake of paddy rice plants.

The purpose of this study is to elucidate whether the percolation patterns affects the growth, yield and copper absorption of rice plants. The stratified paddy field model was prepared with about 40 mg/kg, 70 mg/kg, 100 mg/kg, 150 mg/kg, 250 mg/kg, and 500 mg/kg Cu contaminated soil, while copper safety standard (125 mg/kg). Therefore, the objectives of the study were as follows:

1. to investigate Cu and Cd uptake of rice plants from polluted soil affected by percolation patterns
2. to investigate the effects of soil copper on growth and yield of paddy rice with open or close percolation pattern relationship between percolation patterns and paddy rice yield of the copper polluted field.

The results showed that the percolation pattern significantly changed the concentration of copper in brown rice, but did not affect its growth and yield.

Chapter2 Materials and methods

In this study, two types of stratified paddy field models were used for the experiment: the open-system percolation model and the closed-system percolation model. The percolation patterns were defined in Sasaki et al. (1992). Each stratified paddy field model was constructed in an iron box (30cm×50cm×70cm) filled with three layers of soil which was shown in Fig.1. The plow layer was from 0~10cm deep from the soil surface packed with non-polluted Kanagi soil (dry bulk density in puddling condition was 1.04 g/cm3). The plowsole was from 10~20cm deep with non-polluted and polluted soil (dry bulk density from 10~12.5cm [non-polluted Kanagi soil] and from 12.5~20cm deep [Cu mixed polluted Bunkyou soil] were 1.23 g/cm3 and 0.75 g/cm3, respectively). The subsoil was from 20~55cm deep with polluted Bunkyou soil and non-polluted gravel (dry bulk density at the depth from 20cm to 27.5cm [polluted Bunkyou soil] and from 27.5cm to 55cm [the gravel] was 0.75 g/cm3 and 1.40 g/cm3, respectively.) These subsoil layers were formed by compaction. Hereafter, the models are called O-40 , C-40, O-70, C-70, O-100 , C-100, O-150 , C-150, O-250 , C-250 and O-500 , C-500, where the numbers 40, 70, 100, 150, 250 and 500, mean soil Cu concentration as 49, 71, 95, 155, 247 and 519 mg Cu/kg, respectively. ('O' and 'C' stand for the open-system and the closed-system percolation, respectively). The ground water levels of the open-system and the closed-system percolation models were controlled at 57.5cm and 12.5~20cm depth, respectively. In the closed-system percolation models, the holes of the side walls of iron box were blocked in order to prevent the aeration. On the other hand, in the open-system percolation models, the holes of the side walls of the iron box were open in the lower part of the plowsole and the upper part of the subsoil in order to aerate those layers.

Kanagi soil (Loam), 3.7 mg/kg Cu concentration, was sampled from a plow layer of the paddy field in Kanagi farm of Hirosaki University, Aomori prefecture. Bunkyou soil (Clay Loam) was made by adding a solution of $CuCl_2 \cdot 2H_2O$ to the soil which had been sampled from a plow layer of the paddy field on Bunkyou campus of Hirosaki University, Aomori prefecture, and both were mixed well. Cu concentrations in the soil of Bunkyo campus were originally 10.5 mg/kg. We produced six levels of Cu contaminated soil, Cu concentrations in which were either lower (49 mg/kg, 71 mg/kg, 95 mg/kg) or higher (155 mg/kg, 247 mg/kg, 519 mg/kg) than Japanese safety standard (125 mg/kg). These values are 10, 15, 20, 30, 50 and 100 times, respectively, as large as the average Cu concentration of non-contaminated paddy fields in Japan (4.47 mg/kg) (Asami, 2010). The organic matter (OM) content of the Kanagi soil(latitude:40° 54′ 8″ , logitude:140° 28′ 2.9″) and Bunkyou soil (latitude:40° 35′ 9.6″ , logitude:140° 28′ 28.2″) was 4.7% and 6.6%, respectively. The gravel, which contained 0.8 mg/kg of Cu, was used for the lower layer of the models since they were designed after the fashion of paddy fields near a river.

Chapter3 Results and Discussion

Irrigation water is very important for growth and yields of rice plants. In this study, irrigation water was continuously supplied. The water requirement rate was controlled by the impermeable layer of both percolation systems. The average Day water loss in depth of models O-40, C-40, O-70, C-70, O-100, C-100, O-150, C-150, O-250, C-250, O-500 and C-500 were 8.7, 10.7, 10.4, 12.3, 8.2, 10.6, 8.3, 11.8, 9.0, 14.0, 8.0 and 8.8 mm/day. The results show that the water loss of closed system was larger than that of open system. The average of water requirement rate was low at the beginning and the end of the experiment, while it became higher in the middle of the experiment period, from July to August.

The leaf, stems and roots growth of rice plants depend on many factors. Low soil temperature during the growing season may cause substantial reductions in the growth of plants in agronomic and native ecosystems.

In this study, different depths of soil temperature were recorded from sowing to harvesting of rice. Air temperature was also measured of the green house in both years. The average high and low temperature recorded in the green house was 28.0℃ and 18.3℃ respectively. The average temperature in the greenhouse was 2~3℃ higher than that of AMEDAS data of Hirosaki city

The temporal changes of Eh are shown in Fig.34~45. The plow layer of O-40, O-70, O-100, O-150, O-250 and O-500 became reduction layers (under -100mV) while the plowsole and the subsoil became oxidation layers (over 300mV). On the other hand, Eh values measured at the depths of C-40, C-70, C-100, C-150, C-250 and C-500 were gradually decreased after transplanting, and in due time all the layers became reduction layers as Eh values showed under 0 mV. This means that, in this study, the polluted soil layers in O-40, O-70, O-100, O-150, O-250 and O-500 were under oxidation condition while those layers in C-40, C-70, C-100, C-150, C-250 and C-500 were under reduction condition. It has been pointed out that the Cu uptake in rice is affected by the oxidation-reduction environment (Matsunaka, 2014) and, therefore, in this study, Cu solubility was probably high in the models of O-40, O-70, O-100, O-150, O-250 (Takaishi et al., 2015). We decided on the oxidation and reduction condition on the basis of Yamane (1982), who had defined the oxidation layer as Eh value as 300mV or more and reduction layer as < 300 mV.

The plant length was measured from transplanting to harvesting period. In 2016, the plant length of each model was almost equal, at 90cm~100cm. It has barely increased since 73 days. In 2017, the plant height of each model was almost equal, at 100cm~105cm level. It has barely increased since 70 days. In 2016 and 2017, the average plant length of closed system percolation was higher than that of open system percolation.

In 2016,the average number of stem of O-70, C-70, O-100, C-100, O-150, C-150 and O-250, C-250 were 9.4, 9.3, 8.8, 9.3, 8.9, 8.6, 10.0 and 8.3. In 2017, the average number of stem of O-40, C-40, O-500 and C-500 were 7.8, 8.7, 7.3 and 7.3. In Both 2016 and 2017 experiment, the number of stem has no significant difference between the open and closed system percolation models.

In 2016, the number of leaf of O-70, C-70, O-100, C-100, O-150, C-150 and O-250, C-250 were 14.3, 14.3, 14.0, 14.4, 14.0, 14.0, 14.3 and 14.0. In 2017, the number of stem of O-40, C-40, O-500 and C-500, were all 15.0. The number of leaves in each system was almost the same in 2016 and 2017.

The chlorophyll meter provides a quick, portable, simple, and non-destructive method for estimating leaf chlorophyll content. In September 15, 2016, the average SPAD value in O-70, C-70, O-100, C-100, O-150, C-150 and O-250, C-250 were 31.6, 34.6, 30.9, 36.2, 31.2, 35.1, 34.8, and 31.9, respectively. In September 14, 2017, the average SPAD value in O-40, C-40 and O-500, C-500 were 33.0, 38.5, 34.8 and 35.8, respectively. In 2016, the SPAD peaked around 24th day, and the highest value was 46.1 in early blooming stage of open and closed system percolation models but that value was gradually decreased with the increasing cultivation period. In 2017, the SPAD peaked around the 35th day, and the highest value was 46.1 in early blooming stage of open and closed system percolation models, but that value also was gradually decreased with the time. In both 2016 and 2017, the average SPAD in closed system percolation was higher than the open system percolation. This may be due to the different effects of Oxidation Reduction state in the soil

The yellowish parts of rice plants were measured in two kinds of percolation systems. In the experiments in 2016 and 2017, the percentage of the Yellow portion of the leaves was first dried in closed system. In 2016, at the time of harvesting, the yellowish part of leaf in the closed system was about 59%, while that in the open system was about 33%, and similar results were found in 2017. The results showed that the photosynthetic capacity of rice plants varied greatly in two system at maturity.

The results of Cu concentrations in rice plants values of the open system were larger than those of the closed system. Regarding Cu concentration in stems and leaves except for O-100 and C-100, the values of the open system were greater than those of the closed system. No significant differences were found in stems and leaves of models between different percolation types. The relationship of copper concentration between in soil and in rice grains of open and closed system. Cu concentration in rice grains of open system were higher than those of close system regardless of soil Cu concentration. Copper concentration in rice grains did not increase significantly with the rise of soil Cu concentration. The relationship of copper concentration between in soil and in stem and leaves. The values of open system are always above those of the closed system. Even if Cu concentration in soil increased, that in stems and leaves did not change significantly. The graph of copper concentration in soil and in root. Cu concentration in root increased with the increase of copper concentration in soil.

Chapter 4 Summary and conclusion

In this study, we investigated the effects of percolation patters and Cu concentration of polluted soil on growth and yield of rice plants and Cu uptake by using stratified paddy field models. From the experiments, we reached following conclusions (Fan et al, in press)

Rice Grains: In our study, Cu concentration in rice grains of open system were higher than those of close system regardless of soil Cu concentration. Copper concentration in rice grains did not increase significantly with the rise of soil Cu concentration.

Stems and Leaves: It confirmed that statistically significant differences in the Cu concentrations in stems and leaves were between O-150 (1.8 mg/kg) and C-150 (1.1 mg/kg), O-250 (1.6 mg/kg) and C-250 (0.8 mg/kg). However, there was no significant difference between O-70(1.5 mg/kg) and C-70(1.2 mg/kg), O-100(1.2 mg/kg) and C-100(1.2 mg/kg). This discrepancy might be caused by the soil Cu concentrations, but this requires further elucidation. the open system is always above the closed system. As Cu concentration in soil increased that in stems and leaves did not change significantly.

Roots: Cu concentrations in roots were 2~3 times the larger than those in soil. This may be due to the absorption of Cu in soil for the whole growing period by roots. Here, further discussion is needed to clarify its mechanism as in the case of stems and leaves.

Cu concentrations in the rice plants were in the order of roots > brown rice > stems and leaves. Their ratio was about 1:3:15. This order was similar to those of Shibuya (1979) and Paul et al. (2011b) who used rice plants, and to those of Li et al. (2017) who used soybeans. copper concentration in root increased with the increase of copper concentration in soil.

Unlike the rice grains, and stems and leaves, Cu concentration in roots significantly increased with the increase of soil Cu concentration.

Chapter5 Future Research Plan

From the above, here are further problems that should be discussed in future research.

① Contaminated apple orchards

More than 1000 mg/kg of high concentrations of copper contaminated soil experiment study is necessary

② Compound contaminated soil

About the influence of different infiltration patterns of Cd and Cu on the growth and harvest of rice and the absorption of Cu and Cd

③ Other heavy metal pollution

The percolation pattern can be evaluated with arsenic, Zinc, Nickel and Cobalt polluted soil.