Remediation of anthropogenic radionuclides

Tomo Suzuki-Muresan[1*], Danis Abdillahi[1], Tatsuki Kimura[2], Olivier Péron[1], Thierry Lebeau[3], Abdesselam Abdelouas[1], Fuminori Sakamoto[4*], Naofumi Kozai[4], Kazuya Tanaka[4], Bernd Grambow[1], Keiko Yamaji[5***], Misaki Hoshi[5], Hayato Masuya[6], Haruka Kato[5], Ayako Hoshino[5], Aoi Nikkesi[5]

[1] SUBATECH, Unité Mixte de Recherche 6457, École des Mines de Nantes, CNRS/IN2P3, Université de Nantes, BP 20722, 44307 Nantes cedex 3 France

[2] Kyoto University Graduate Scholl of Engineering, Department of Environmental Engineering, University of Kyoto

[3] LUNAM University, LPGN, UMR 6112, BP 92208, 44322 Nantes, France

[4] Advanced Science Research Center, Japan Atomic Energy Agency, Japan

[5] Graduate School of Life and Environmental Sciences, University of Tsukuba

[6] Tohoku Research Center, Forestry and Forest Products Research Institute

* Corresponding author: suzuki@subatech.in2p3.fr

** Corresponding author: sakamoto.fuminori@jaea.go.jp

*** Corresponding author: yamaji.keiko.fp@u.tsukuba.ac.jp

Abstract

New biological remediation methods of radio-Cs doped in simulated contaminated soil are studied. Three experiments have been conducted: (experiment 1) study on the bio-,phyto-extraction of Cs from doped illite; (experiment 2) study on the development of accumulation technique of ^{137}Cs using mushroom mycelium and minerals; (experiment 3) study on the effect of ^{137}Cs on symbiosis between *Clethra barbinervis* and root-endophytes under heavy-metal stress .

1 General introduction

Contamination by actinides (ANs) and fission products (FPs), collectively denoted as anthropogenic radionuclides (ARs), are of great concerns in many countries. For instance, the disposal of legacy wastes and the decontamination of polluted soils by ARs is a central issue in the US DOE sites such as the Hanford site [Hartman et al., 2006]. This site was established to produce military Pu and is today under environmental monitoring. Hanford site is faced to soil and groundwater contamination by ^{3}H, ^{129}I, ^{99}Tc… US DOE has developed a large program of cleanup to remediate and monitor the groundwater, and to reduce the contaminated zone. In Japan, the decontamination of polluted environments by ARs, mostly radioactive cesium, and the management and the disposal of excavated soils are emerging problems after the accident of the Fukushima Daiichi nuclear plant. In France the major public health challenges and environmental issues as well as the potential release of ARs from repository sites in environment for the wastes management are strongly examined.

Various decontamination strategies have been developed in general, including excavation, chemical leaching, bio-remediation, and phyto-remediation. Among them, bio- and phyto-remediation are attractive, as they are less invasive against surrounding environments. ARs readily react with solid phase in soil and rock and change their chemical forms. For instance, plutonium under reducing and environmental conditions is expected to be under the form of PuO_2 and immobile in soil. However, the presence of minerals (inorganic material), natural organic materials, microorganisms, and colloids may initiate the remobilization and its transportation within the environment [Mahara et al., 2001]. The contribution of micro-organisms on the decontamination of soils needs to be explored, notably when they are coupled with the phyto-extraction.

Therefore, the objective of the research is to find a new remediation scheme coupling bio- and phyto-remediation for soils contaminated by ARs. Various bio- and phyto-remediation techniques have been proposed; however, examples of such coupled schemes are scarce. This new technique could be a cheap and less invasive decontamination strategy, as similar mechanisms are widespread in nature, where plants absorb nutrients dissolved from soil minerals, using organic ligands secreted from their roots or coexisting microorganisms.

This work was structured within three parts:

- The bio-,phyto-extraction of Cs from doped illite,
- Development of accumulation technic of ^{137}Cs using mushroom mycelium and

minerals,

- Effect of ^{137}Cs on symbiosis between *Clethra barbinervis* and root-endophytes under heavy-metal stress

2 Bio-,phyto-extraction of Cs spiked-illite

Biological remediation, such as natural attenuation, bioaugmentation and phytoremediation, are used as friendly, cheap and low cost method to decontaminate polluted soils [Mirsal, 2008]. Bioaugmentation is based on the introduction of microorganisms in soil, and phytoremediation on the extraction of pollutants by a plant. Bioaugmentation assisted phytoremediation is a promising method for decontamination thanks to its synergic effect on mobilization of pollutant in soil directly accessible to the root of the plants and/or bioavailable in the rhizosphere [Lebeau et al., 2008; Sessitsch et al., 2013].

This clean-up method has given evidence for the decontamination of soil contaminated by heavy metals and petroleum hydrocarbons [Agnello et al., 2016; Lebeau et al., 2008]. This technique was tested to remove cesium from illite, considered as the major mineral clayey phase sorbing Cs, before applying on contaminated soil of Fukushima [Hazotte, 2016; Hazotte et al., 2016]. These authors performed bioaugmentation assisted phytoremediation experiments in presence of purified siderophores, added up to 250 $\mu mol \cdot L^{-1}$ in contact with Cs-spiked illite with 10 and 100 $mmol \cdot kg^{-1}$ [Hazotte, 2016]. A partnership Red Clover (*Trifolium pratense*) – Pyoverdine was put in direct contact with Cs-spiked soil in pot. The results showed a weak uptake of Cs, up to 1% in the aerial parts and 0.6% in the roots which was not enhanced with pyoverdine. The expected efficiency of the method of bioaugmentation assisted phytoremediation is not reached and the hypothesis of the phyto-toxicity of PVD regarding the roots of the Red Clover is raised.

To verify this hypothesis, experiments are performed in this work based on the compartmentalization of Cs-spiked illite in contact with PVD enclosed in a dialysis membrane, the contact between PVD and plant being avoided, no Cs is able to diffuse across the membrane. Three main ongoing experiments are performed (Figure 1):

a. Test of Cs and PVD diffusion through the membrane of dialysis (Figure 1-1)
b. Desorption of Cs from spiked-illite in absence and presence of PVD by Red Clover phyto-extraction (Figure 1-2)
c. Desorption of Cs from spiked-illite in presence of PVD by KCFC (Figure 1-3)

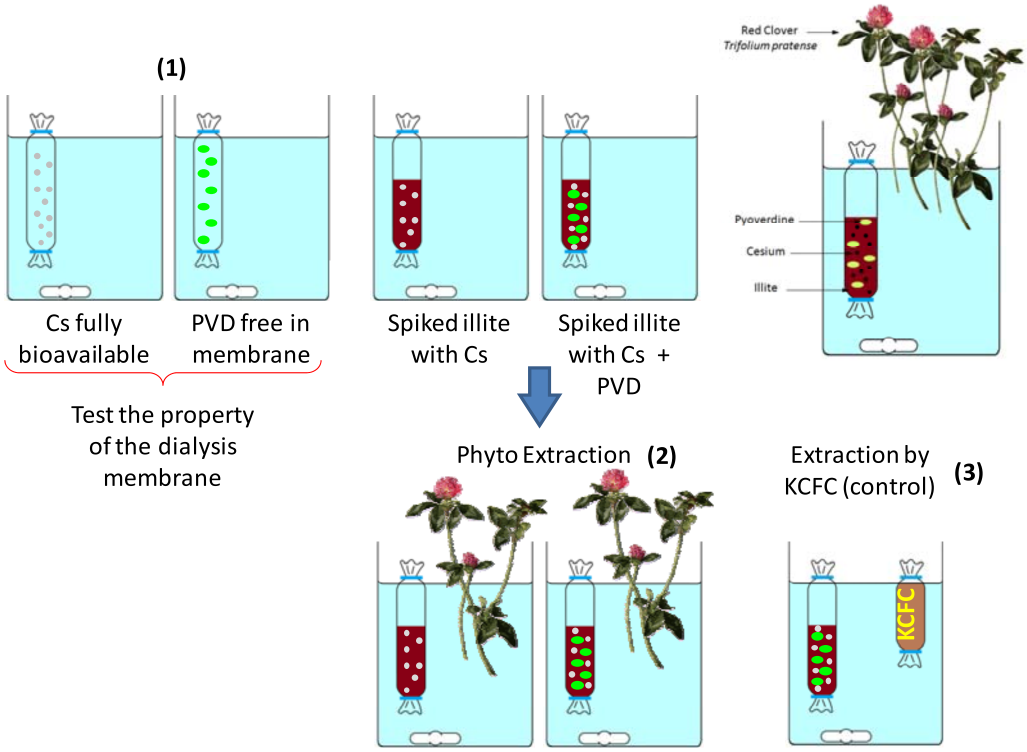

Figure 1: Principle of bioaugmentation assisted phytoremediation of Cs from spiked illite experiment.

2.1 Cesium doped illite

Prior starting the experiments, a stock solution of illite (100 g·L^{-1}) are prepared. Purified illite, kindly provided by BRGM, is put in contact with 100 mmol Cs·kg^{-1} of Illite. Aliquots are measured and almost 80% of Cs is adsorbed onto illite. This suspension is sterilized by tyndalisation method (3 cycles of 60 min at 70 °C, with 24 h of cooling at ambient temperature between each cycle) and constitutes the initial stock solution for the bioaugmentation assisted phytoremediation experiments.

2.2 Dialysis membrane performance

Two types of dialysis membrane from Spectrum Lab were tested: Spectra/Por 7 (c.o. 1000 Da) and Spectra/Por Float-A-Lyser® G2 (c.o. 100-500 Da). Their efficiency was assessed in terms of:

- Resistance against sterilization
- Good diffusion of Cs through the membrane
- Good retention of PVD inside the membrane

Spectra/Por 7 membrane presents a good resistance against the sterilization by autoclave

(121°C, 20 min) and by tyndalisation. No influence on the diffusion of Cs through the membrane was observed. However, the PVD is not retained by the membrane and diffuses into the solution, independently of the sterilization method (Figure 2). Therefore, a second dialysis membrane, sterilized Float-A-Lyzer and ready to use dialysis device, was selected to test its performance on the retention of PVD inside the membrane (Figure 3). The new membrane confines the PVD: no diffusion through the membrane was observed. In addition, 100% of cesium diffuse through the membrane among which 6% of diffused Cs are retained on the floatation ring.

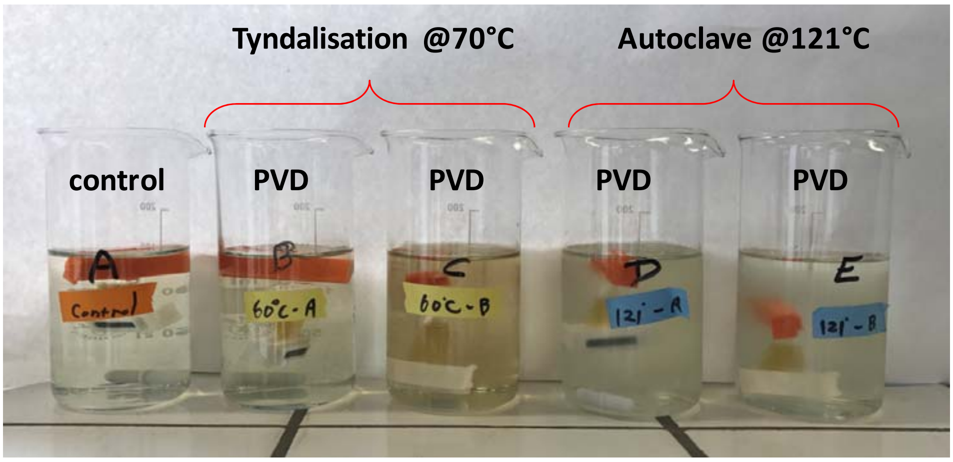

Figure 2: Diffusion test of PVD through Spectrapor7 membrane. Comparison between tyndalisation and autoclave sterilization method.

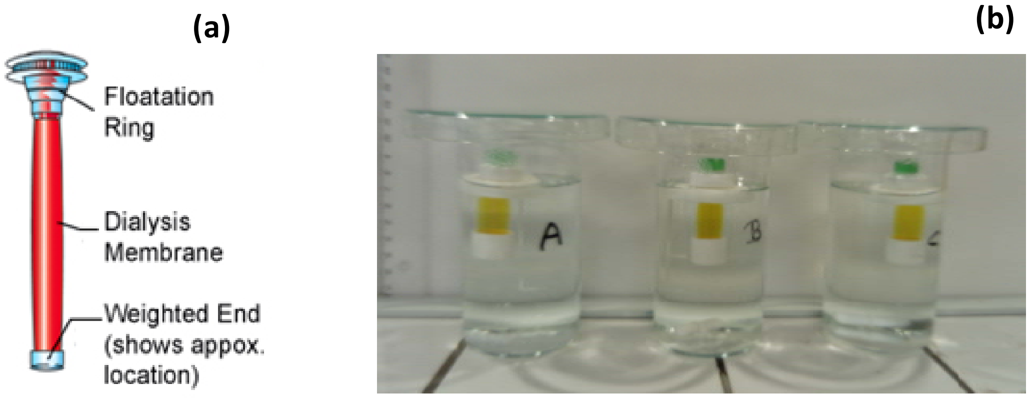

Figure 3: (a) Caracteristics of the Spectra/Por Float-A-Lyser® G2, (b) Diffusion test of PVD through Float-A-Lyser membrane (3 repetitions: A, B, C).

2.3 Plant growth

The culture of Red Clover (*Trifolium Pratense*) is performed in plant growth enclosure. The growth of plants are performed during 45 days at least and kept in adapted culture media until their use for the bio-,phyto-extraction experiments.

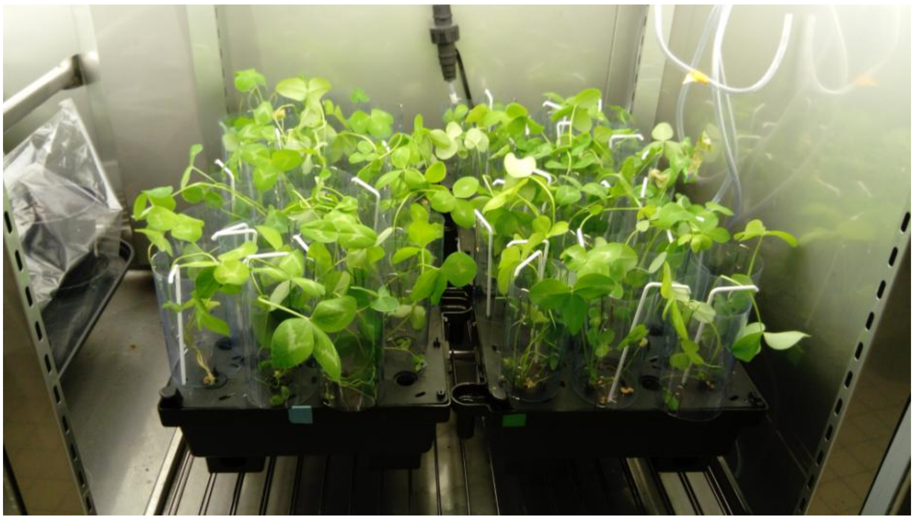

Figure 4: Growth of Red Clover (*Trifolium Pratense*) in phytotron.

2.4 Ion exchanger resin

KCFC (potassium cobalt ferrocyanide) is an ion exchanger resin which retains specifically Cs. KCFC is cleanded, washed and conditioned in NaCl 1×10^{-3} M. The pH value of this suspension is adjusted at 8.

As shown in Figure 1-3, KCFC is used to extract selectively Cs during the desorption of Cs from illite by PVD. The aim of this experiment is to control only the efficiency of the PVD on the desorption of Cs doped onto illite. KCFC is used as a sink for cesium and replace the role of plants during the extraction process.

2.5 Summary

Property tests on dialysis membrane of Spectr/Por Loat-A-Lyser® G2 are realized which presents good properties in terms of retention of PVD within the membrane and diffusion of Cs through it. All the elements for the bio-,phyto-extraction are prepared and ready to go: plants come to maturity, sterilized illite doped with Cs, cleaned and conditioned KCFC. The experiments are ongoing to verify the hypothesis of the phyto-toxicity of PVD regarding the roots of the Red Clover.

3 Development of accumulation technique of ^{137}Cs using mushroom mycelium and minerals

A large quantity of radioactive material was released to the environment from Fukushima Daiichi Nuclear Power Station due to the 2011 Tohoku earthquake and tsunami. Radioactive materials with short half-lives were serious problem for a few months after the accident. At the present time after more than six years from the accident, the radioactive materials with medium- and long-half-lives are dominant. Especially, ^{137}Cs with 30-year half-life gives various limitations to the residents' life. The decontamination of living zone such as residential district, public facilities, and cultivated land has been gradually carried out. However, the decontamination of the forest area remains almost untouched in Fukushima. The forest area in Fukushima accounts for more than 70% of the land area of Fukushima prefecture; therefore, in 2016 the Japanese government made a decision to decontaminate the forest areas near residential areas.

It is well known that mushrooms accumulate radioactive Cs. Some mushrooms accumulate radioactive Cs at high concentration. However, mushrooms can be harvested only twice (spring and autumn) in one year, and almost all of the mushrooms' fruit bodies don't grow much. On the other hand, fungal mycelia grow all the year except winter. Some of them grow quickly. Therefore, we tried to utilize fungal mycelia to accumulate radioactive Cs.

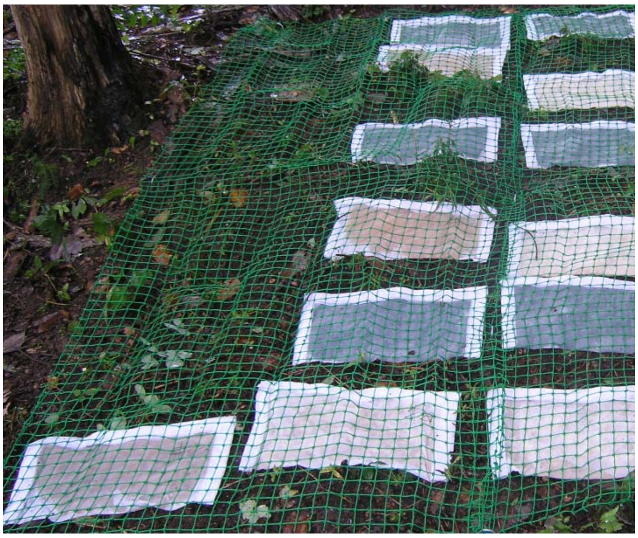

Figure 5: The bio-mat set up in the forest

We devised the method to estimate the radioactivity of ^{137}Cs accumulated to mycelium. The ability of ^{137}Cs accumulation were estimated for about 1500 strains of fungi by using the method. Then, we made the bag named "bio-mat" containing a fungal mycelium that accumulates ^{137}Cs efficiently. As a result, we confirmed the bio-mat accumulated ^{137}Cs. However, as the mycelium died, the accumulated ^{137}Cs began to diffuse to the environment. Because some minerals stably retain ^{137}Cs, we prepared the new bio-mat containing a mixture of some minerals. The mineral mixture named "Racoin" is our commercially available product. It contains zeolite, vermiculite and phlogopite. It was developed to prevent accumulation of ^{137}Cs in mushroom in the first place. The size of the new bio-mat is a quadrangle of 20 cm x 40 cm. The size of the first bio-mat was a quadrangle of 8 cm x 15 cm. The new bio-mat retained ^{137}Cs for a long period. In the field examination (Figure 5), we used the new bio-mat without mycelium as a reference. Surprisingly, we confirmed that the reference bio-mat also accumulated ^{137}Cs (Figure 6). Some kinds of wild fungi accumulate ^{137}Cs. Such fungi might have grown in the bio-mat with nutrition and accumulated ^{137}Cs.

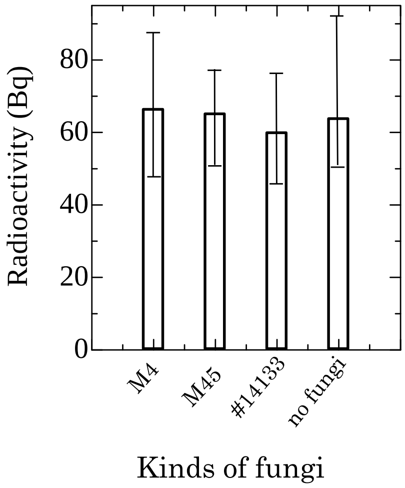

Figure 6: Radioactivity of ^{137}Cs in the new biomat containing Racoin.

To confirm the reproducibility, we tried supplementary examination last year. At the examination, we used the bio-mat of the 50 cm square size. The setting period was up to 150 days. As a result, the following things are suggested; 1) the existence of fungi does not influence on the accumulation rate of ^{137}Cs, 2) the amount of the accumulated ^{137}Cs increases with the setting period of the bio-mat, 3) approximately 0.5 weight % of Racoin is enough to add the mat under the present conditions.

4 Effect of ^{137}Cs on symbiosis between *Clethra barbinervis* and root-endophytes under heavy-metal stress

Abstract

Due to the accident at the Fukushima Daiichi nuclear power plant caused by tsunami in March 2011, large amounts of radioactive materials have been released into the atmosphere. Especially, ^{137}Cs contamination might have serious influences on environments because its half-life (30.2 years) is longer than other radionuclides. It has been reported that soil-fungal community have been altered by ionizing radiation, leading simpler community structure, such as high dominance of stress-tolerant melanized fungi. Generally, fungi play major roles in forest ecosystem and root endophytes have been demonstrated to alleviate various kinds of environmental stress to plants. Therefore, the change of endophytes community may affect the symbiosis interaction between root endophytes and plants. Our targeted tree, *Clethra barvinervis* Seib.et Zucc is known to be a tolerant tree against the high concentrations of heavy metals due to the root endophytes, which increase enhance heavy-metal tolerance. The purpose of this study is to clarify the effect of ^{137}Cs on species and chemical characteristics of root endophytes in *C. barvinervis*.

We isolated root endophytes from *C. barvinervis* seedlings growing in 6 different study sites, showing different concentrations of ^{137}Cs and heavy metals in soil. The following 8 species were isolated from *C. barvinervis* roots growing in all study sites: *Acephala, Colletotrichum, Cryptosporiopsis, Lachnum, Leptodontidium, Phialocephala, Rhizoscyphus,* and *Rhizodermea.* We analyzed chemical characteristics of these root endophytes, such as production of Zn-chelating compounds (detoxicants of Zn) and melanin (detoxicants of heavy metal as well as defensive compounds against radiation). Finally, we clarified the effect of ^{137}Cs on species and chemical characteristics of root endophytes using statistical analyses; non-metric multidimensional scaling (nMDS) and generalized linear mixed model (GLMM).

nMDS analysis showed that the appearance numbers of root endophytes and their chemical characteristics were influenced by ^{137}Cs and heavy metal (Cu, Zn, and Pb) concentrations in root-zone soil. In contrast, according to GLMM analysis, the appearance numbers of each fungal species were not significantly influenced by ^{137}Cs and heavy metal. GLMM analysis also clarified that Zn-chelating compounds production by *Colletotrichum* or melanin production by *Acephala* showed significantly positive correlation with ^{137}Cs concentration in root-zone soil. Our results indicate that ^{137}Cs would influence microflora of root endophytes in *C. barbinervis*, but in mine sites, the microflora would be mainly influenced by heavy metals. Additionally, microbial metabolism related to heavy metal tolerance mechanism would be enhanced by ^{137}Cs in

Colletotrichum and *Acephala* species. In conclusion, it suggests that ^{137}Cs would not influence remarkably symbiosis between *C. barbinervis* and root endophytes under heavy-metal stress.

4.1 Research Objectives

Due to the accident at the Fukushima Daiichi nuclear power plant caused by tsunami in March 2011, large amounts of radioactive materials have been released into the atmosphere. Especially, ^{137}Cs contamination might have serious influences on environments because its half-life (30.2 years) is longer than other radionuclides. It has been reported that soil-fungal community have been altered by ionizing radiation, leading simpler community structure, such as high dominance of stress-tolerant melanized fungi by several researches after the Chernobyl Nuclear Power Plant accident [Zhdanova et al., 2004; Zhdanova et al., 1994; Zhdanova et al., 2000]. Generally, fungi play major roles in forest ecosystem and root endophytes have been demonstrated to alleviate various kinds of environmental stress to plants [Mandyam and Jumpponen, 2005; Rodriguez et al., 2009]. Therefore, the change of endophytes community may affect the symbiosis interaction between root endophytes and plants. Our targeted tree, *Clethra barvinervis* Seib. et Zucc is known to be endemic tree species in Japan, to be observed in old mine sites [Hiroi, 1974] and to be a tolerant tree against the high concentrations of heavy metals due to the root endophytes, which increase enhance heavy-metal tolerance [Yamaji et al., 2016]. The purpose of this study is to clarify the effect of ^{137}Cs on species and chemical characteristics of root endophytes in *C. barvinervis*.

4.2 Research Contents

4.2.1 Elemental analysis of plants and root-zone soil

Five to ten year-old *C. barvinervis* seedlings (10 seedlings per each site) were collected from 6 different study sites, showing different concentrations of ^{137}Cs and heavy metals in soil (collection time; July-September, 2012-2015). Zn, Cu, Ni, Pb and Cd in air-dried root-zone soil (50 x 50 x 50 mm) were analyzed by ICP-OES after acid-digestion, HNO_3-$HClO_4$ (1:4 v/v). Plant materials carefully washed with running water and deionized water, were separately dried. After the ground materials were pyrolyzed in concentrated HNO_3, heavy metals as described above were quantified by ICP-OES. ^{137}Cs in the root-zone soil and plants were analyzed with Ge semiconductor detector before the acid digestion. These data were used for the statistical analysis.

4.2.2 Root endophytes isolation

Roots were sterilized using 70% ethanol and 15% hydrogen peroxide. 1,500 root pieces per each site were incubated on 1% malt extract agar for one month. Fungal detection rate (%) was calculated using the following formula; Detection rate (%) =Nd/Nt x 100. Nd; the number of root pieces from which the fungus was detected. Nt; total root pieces for the isolation. Identification of fungal species were done by DNA analysis as well as microscopic observation.

4.2.3 Production of Zn-chelating compounds (detoxicants of Zn) by root endophytes

Because Zn accumulation was highly found in *C. barbinervis* roots [Yamaji et al., 2016], *C. barvinervis* would be tolerant to high concentration of Zn. As chemical characteristics of root endophytes, we considered that root endophytes might produce Zn-chelating compounds to detoxify Zn in plant tissues or in the rhizosphere [Gadd, 1993]. Seven fungal species were used for this production test; *Rhizodermea, Rhizoscyphus*, *Lachnum*, *Colletotrichum*, *Cryptosporiopsis, Phialocephala,* and *Acephala*. Six strains per each fungal species from each study site were used (36 strains per each fungus in total). According to [Martino et al., 2003], insoluble ZnO and $Zn_3(PO_4)$ were mixed with medium and root endophytes were inoculated onto the medium. After 7-days incubation, clear zone was formed by Zn-chelating compounds on the medium. Zn-chelating activity was calculated using the following formula; Zn-chelating activity =(Cd-Ed)/Ed. Cd; the diameter of clear zone. Ed; the diameter of root endophytes.

4.2.4 Production of melanin by root endophytes

Melanin is known to be detoxicants of heavy metal [Martino et al., 2003] as well as defensive compounds against radiation [Dadachova and Casadevall, 2008]. The following 7 fungal species were used; *Rhizodermea, Rhizoscyphus*, *Lachnum*, *Colletotrichum*, *Cryptosporiopsis, Phialocephala,* and *Acephala* (same strains were used in 2.2).

Root endophytes grown on potato-dextrose agar for 3 weeks were used for melanin extraction with 1M NaOH [Gadd, 1982]. According to [Gadd, 1982], melanin production was measured at 405 nm.

4.2.5 Statistical analysis

Clarification of the effect of ^{137}Cs on microflora and chemical characteristics of root endophytes using non-metric multidimensional scaling (nMDS) and generalized linear mixed model (GLMM). the vegan package of R [Oksanen et al., 2015] with 10000 permutations was used to examine differences.

4.3 Research results

4.3.1 Root endophytes isolation

The following 8 species were isolated from *C. barvinervis* roots growing in all study sites: *Acephala, Colletotrichum, Cryptosporiopsis, Lachnum, Leptodontidium, Phialocephala, Rhizoscyphus,* and *Rhizodermea.* These root endophytes were used for the production test of Zn-chelating compounds.

4.3.2 Production of Zn-chelating compounds and melanin by root endophytes

Results of Zn-chelating compounds production were shown in Fig. 1. *Colletotrichum, Cryptosporiopsis* and *Acephala* isolated from all sites produced Zn-chelating compounds. *Rhizoscyphus, Lachnum* and *Phialocephala* did not show the production. Results of melanin were shown in Fig. 2. All strains of *Acephala, Phialocephala, Cryptosporiopsis* and *Colletotrichum,* and several strains of *Rhizodermea* produced melain. *Rhizoscyphus* and *Lachnum* produce very small amounts of melanin. Strains show different patterns of melanin production even among each fungal species.

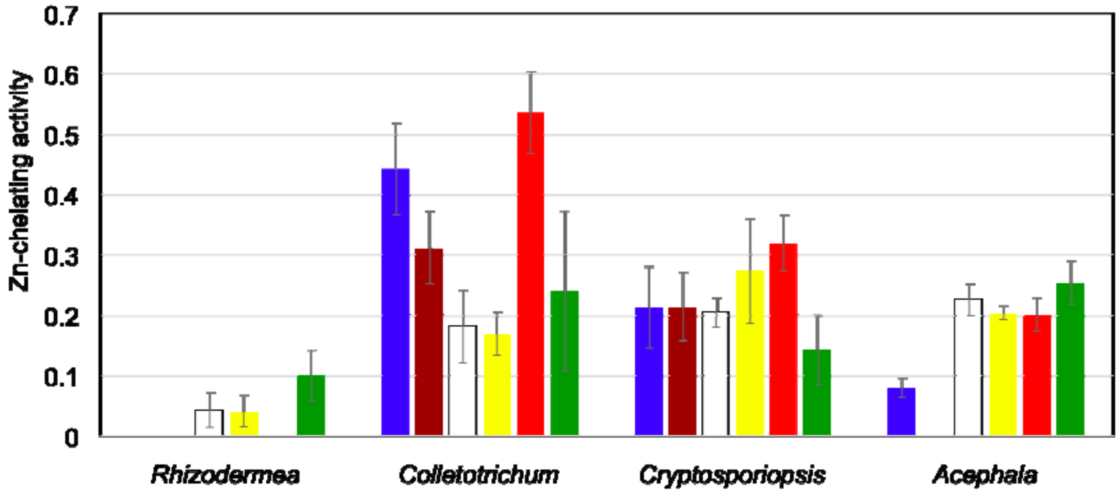

Fig. 1 Production ability of Zn-chelating compounds by root endophytes

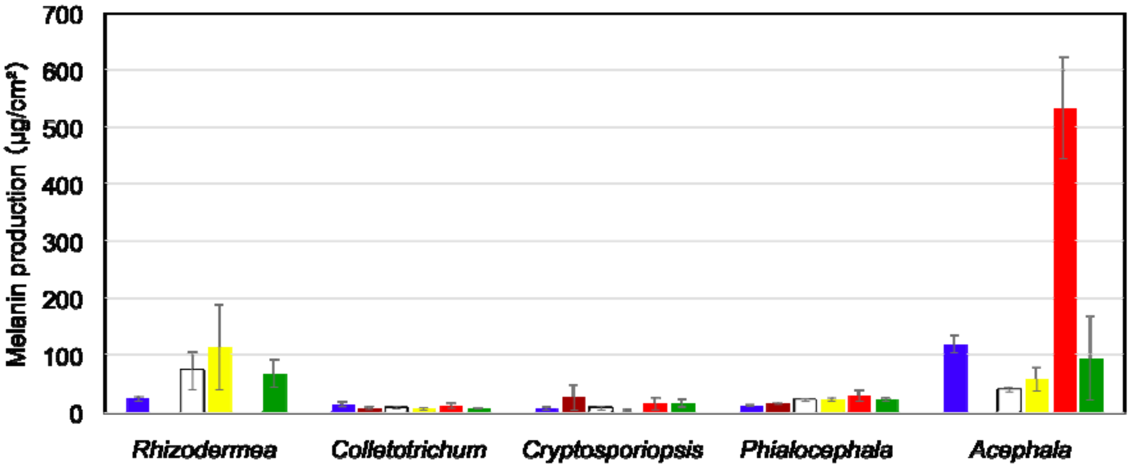

Fig. 2 Production ability of melanin by root endophytes

Data was shown as average ± SE. Different colors means different study area; blue, brown and white bar graphs indicate old mine sites (high concentrations of heavy metals). Blue and white bar graphs also indicate moderate concentrations of ^{137}Cs. Yellow and orange bar graphs indicate Fukushima study sites, which show high concentrations of ^{137}Cs. Green bar graphs means natural forest, which did not contain high concentrations of ^{137}Cs and heavy metals.

Data was shown as average ± SE. Different colors means different study area; blue, brown and white bar graphs indicate old mine sites (high concentrations of heavy metals). Blue and white bar graphs also indicate moderate concentrations of ^{137}Cs. Yellow and orange bar graphs indicate Fukushima study sites, which show high concentrations of ^{137}Cs. Green bar graphs means natural forest, which did not contain high concentrations of ^{137}Cs and heavy metals.

4.3.3 Statistical analysis

nMDS clarified ^{137}Cs and heavy metals (Cu, Zn, and Pb) in the root-zone soil influence the microflora of root endophytes (Fig. 3). Microflora in mine sites (red circle; Fig. 3) would be influenced by mainly heavy metals. Chemical characteristics of root endophytes would be also influenced by these environmental factors (data not shown). GLMM analysis clarified that ^{137}Cs induces productions of Zn-detoxicants and melanin. For example, production of Zn-chelating compounds by *Colletotrichum* strains showed positive correlation with ^{137}Cs in root-zone soil ($p<0.05$). Production of melanin by *Acephala* strains showed positive correlation with ^{137}Cs in root-zone soil ($p<0.05$).

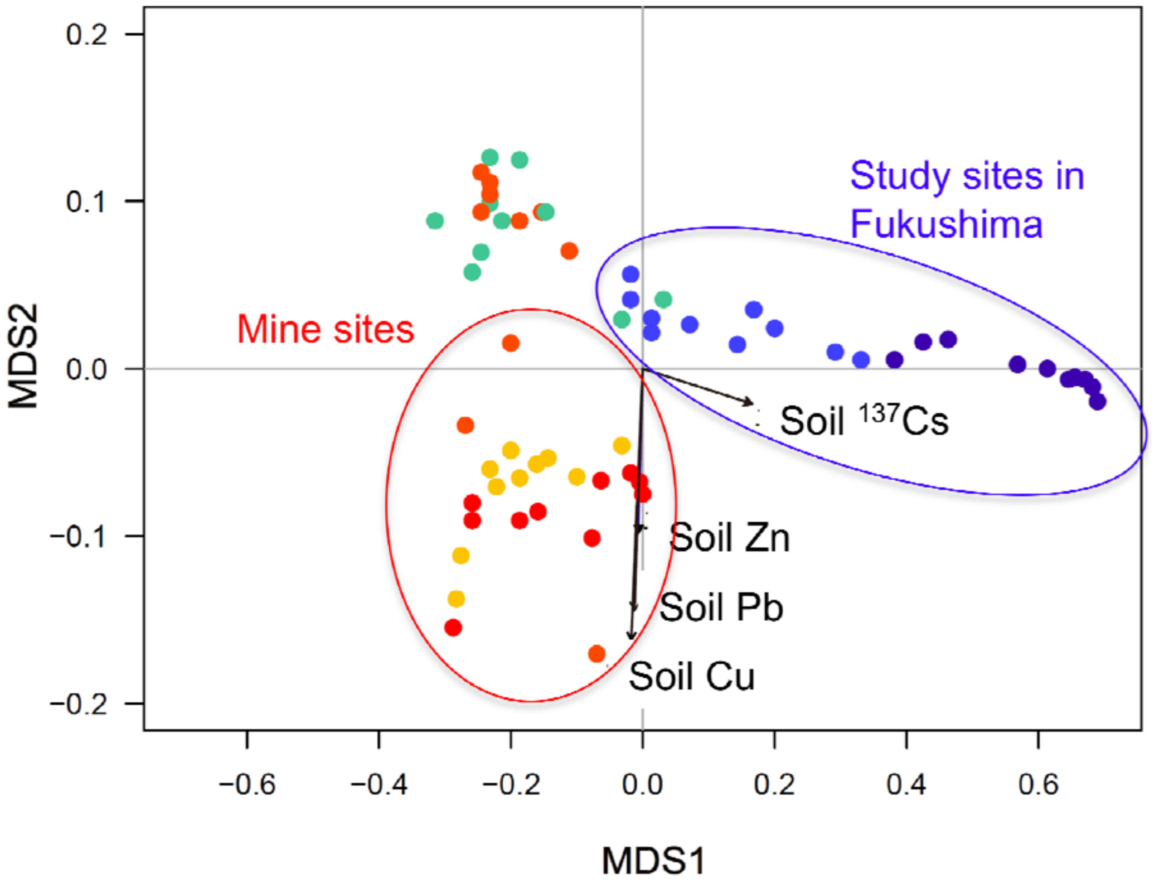

Fig. 3 Result of nMDS analysis

Red, yellow and orange spots mean mine sites. Blue and indigo blue spots indicate Fukushima study sites, which show high concentrations of ^{137}Cs.Green spots mean natural forest, which did not contain high concentrations of ^{137}Cs and heavy metals.

4.4 Conclusion

^{137}Cs would influence microflora of root endophytes in *C. barbinervis*, but in mine sites, the microflora would be mainly influenced by heavy metals. Microbial metabolism related to heavy metal tolerance would be induced by ^{137}Cs in *Colletotrichum* and *Acephala* species. Under mine sites together with radio Cs, heavy-metal stress tolerance of *C. barbinervis* enhanced by root endophytes would be maintained.

5 Acknowledgement

The authors acknowledge the financial support from REIMEI project. We would like to address our sincere gratitude to Nolann Longeard from Subatech laboratory for experimental tests.

6 References

Agnello, A. C., Bagard, M., van Hullebusch, E. D., Esposito, G. and Huguenot, D. [2016]. Comparative bioremediation of heavy metals and petroleum hydrocarbons co-contaminated soil by natural attenuation, phytoremediation, bioaugmentation and bioaugmentation-assisted phytoremediation. *Science of The Total Environment*. Vol. 563â€“564 p. 693-703.

Dadachova, E. and Casadevall, A. [2008]. Ionizing radiation: how fungi cope, adapt, and exploit with the help of melanin. *Current opinion in microbiology*. Vol. 11 (6) p. 525-531.

Gadd, G. M. [1982]. Effects of media composition and light on colony differentiation and melanin synthesis in Microdochium bolleyi. *Transactions of the British Mycological Society*. Vol. 78 (1) p. 115-122.

Gadd, G. M. [1993]. Interactions of fungip with toxic metals. *New Phytologist*. Vol. 124 (1) p. 25-60.

Hartman, M. J., Webber, W. D. and Morasch, L. F. [2006]. Hanford Site groundwater monitoring for fiscal year 2005. Pacific Northwest National Laboratory, United States Department of Energy, PNNL-15670, p.

Hazotte, A. [2016]. Rôle de métabolites bactériens dans la mobilisation du césium dans une illite dopée: étude mécaniste et application à la phytoextraction. Université de Nantes, Thèse, p.

Hazotte, A., Péron, O., Abdelouas, A., Montavon, G. and Lebeau, T. [2016]. Microbial mobilization of cesium from illite: The role of organic acids and siderophores. *Chemical Geology*. Vol. 428 p. 8-14.

Hiroi, T. [1974]. Phytosociological research in copper mine vegetation. *J Hum Nat Sci*. Vol. 38 p. 177-226.

Lebeau, T., Braud, A. and Jezequel, K. [2008]. Performance of bioaugmentation-assisted phytoextraction applied to metal contaminated soils: A review. *Environmental Pollution*. Vol. 153 (3) p. 497-522.

Mahara, Y., Kudo, A. and Kudo, A. [2001]. Plutonium mobility and its fate in soil and sediment environments. Radioactivity in the Environment, Elsevier. **Volume 1**: 347-362.

Mandyam, K. and Jumpponen, A. [2005]. Seeking the elusive function of the root-colonising dark septate endophytic fungi. *Studies in Mycology*. Vol. 53 p. 173-189.

Martino, E., Perotto, S., Parsons, R. and Gadd, G. M. [2003]. Solubilization of insoluble inorganic zinc compounds by ericoid mycorrhizal fungi derived from heavy metal polluted sites. *Soil Biology and Biochemistry*. Vol. 35 (1) p. 133-141.

Mirsal, I., *Soil pollution: origin, monitoring & remediation*, Springer Science & Business Media, (2008), 312 p p.

Oksanen, A. J., Blanchet, F. G. R. and al., e. [2015]. vegan: Community ecology package for R, version 2.3-2. . *Website https://cran.r-project.org/web/packages/vegan/ index.html*. Vol. p.

Rodriguez, R. J., White Jr, J. F., Arnold, A. E. and Redman, R. S. [2009]. Fungal endophytes: diversity and functional roles. *New phytologist*. Vol. 182 (2) p. 314-330.

Sessitsch, A., Kuffner, M., Kidd, P., Vangronsveld, J., Wenzel, W. W., Fallmann, K. and Puschenreiter, M. [2013]. The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. *Soil Biology and Biochemistry*. Vol. 60 p. 182-194.

Yamaji, K., Watanabe, Y., Masuya, H., Shigeto, A., Yui, H. and Haruma, T. [2016]. Root Fungal Endophytes Enhance Heavy-Metal Stress Tolerance of Clethra barbinervis Growing Naturally at Mining Sites via Growth Enhancement, Promotion of Nutrient Uptake and Decrease of Heavy-Metal Concentration. *PloS one*. Vol. 11 (12) p. e0169089.

Zhdanova, N. N., Tugay, T., Dighton, J., Zheltonozhsky, V. and McDermott, P. [2004]. Ionizing radiation attracts soil fungi. *Mycological research*. Vol. 108 (9) p. 1089-1096.

Zhdanova, N. N., Vasilevskaya, A. I., Artyshkova, L. V., Sadovnikov, Y. S., Lashko, T. N., Gavrilyuk, V. I. and Dighton, J. [1994]. Changes in micromycete communities in soil in response to pollution by long-lived radionuclides emitted in the Chernobyl accident. *Mycological research*. Vol. 98 (7) p. 789-795.

Zhdanova, N. N., Zakharchenko, V. A., Vember, V. V. and Nakonechnaya, L. T. [2000]. Fungi from Chernobyl: mycobiota of the inner regions of the containment structures of the damaged nuclear reactor. *Mycological Research*. Vol. 104 (12) p. 1421-1426.