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Impact of effective microorganisms on the transfer of radioactive cesium into lettuce and barley biomass

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ABSTRACT

Soil microorganisms play an important role in determining the physical and chemical properties of soils. Soil microorganisms have both direct and indirect effects on the physical and chemical states of radionuclides and their availability for uptake by plant roots. Controlling the soil microorganisms to immobilize radionuclides is a promising strategy to reduce the content of radionuclides in the food chain. In this study, we evaluated the impact of effective microorganisms (EM) comprising lactic-acid bacteria, photosynthetic bacteria, and yeast on the transfer of ^{137}Cs into the aboveground biomass of barley and lettuce. The application of EM or fermented organic fertilizer (bokashi) alone to sod-podzolic sandy-loam soil significantly reduced the aggregated transfer factor of ^{137}Cs in barley by 37% and 44%, respectively. The combination of EM with bokashi or potassium fertilizer produced the largest reductions in ^{137}Cs transfer into barley biomass (50% and 63%, respectively). EM had a stronger effect on ^{137}Cs transfer into barley compared to lettuce. Laboratory experiments suggested that the effect of microorganisms on ^{137}Cs uptake can be attributed to a reduction in the proportion of bioavailable physicochemical forms of ^{137}Cs in the soils treated with EM and bokashi. This study, to the best of our knowledge, is the first to report the mechanism by which microbial fertilizers reduce the transfer of ^{137}Cs into plants.

1. Introduction

Large-scale nuclear programs for both peaceful and military applications have resulted in significant soil contamination with radioisotopes. This contamination prohibits safe agricultural production in some areas, particularly those affected by the incidents at the Mayak nuclear facility, the Chernobyl nuclear power plant (NPP), and the Fukushima NPP. High doses of mineral fertilizers are frequently applied at agricultural sites to reduce the accumulation of radionuclides in crops (Ageyets, 2001; Bogdevich et al., 2002; Kato et al., 2015; Yamaguchi et al., 2016). However, this approach has several drawbacks. For example, the cost of additional mineral fertilizers decreases the economic efficiency in comparison with non-contaminated areas, and the fertilizer application negatively affects the ecological state of the soil (Savci, 2012; Khan et al., 2014). Consequently, it is critical to develop new approaches to regulate the uptake of radioactive isotopes by plants.

Soil microorganisms have important effects on the physical and chemical properties of soils, various soil processes, and the physiological states of plants. Bacteria, fungi, and algae found in soils participate actively in mineral destruction and formation and indirectly affect the physical and chemical states of radionuclides (Roussel-Debet et al., 2005). In addition, radioisotopes can be absorbed on the cell walls and other cellular components of these microorganisms (Lloid and Renshaw, 2005). Thus, elucidating the effects of soil microbes on the transformation and bioavailability of radionuclides may help develop new approaches to regulating the flow of pollutants in agricultural ecosystems (Ehlken and Kirchner, 2002). There are two main ways for decreasing transfer of the man-made radionuclides into plants from soil using microorganisms. One of it is the biogeochemical transformation of pollutants into biologically inaccessible forms and another is bioextraction of pollutants (Tabak et al., 2005).

Microbial fertilizers can improve soil microbial diversity along with the physical and chemical properties of the soil, resulting in enhanced

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crop yield and quality. Effective microorganisms (commercially known as EM.1®, referred to as EM hereafter) (Higa and Parr, 1994), which consist of naturally occurring beneficial microorganisms such as lactic-acid bacteria, photosynthetic bacteria, and yeast (Higa, 2000), are one example of a microbial fertilizer. EM is used in both conventional and eco-friendly farming to improve soil fertility, increase crop productivity, and developing plant resistance to pests and disease (Olle and Williams, 2013; Javaid, 2006; Ndona et al., 2011). EM is also applied to improve compost quality, and the incorporation of EM enhances the efficacy of organic matter as a fertilizer (Hu and Qi, 2013).

This study evaluated whether the application of EM in liquid form (EM solution) or solid form (EM-bokashi) reduces the transfer of ^{137}Cs in the above-ground biomass of plants. EM's mechanism of action along with the effect of EM on the physicochemical form of ^{137}Cs in soil were also evaluated.

It is hypothesized that the application of EM in liquid form or in solid form or in combination with a potassium fertilizer reduces the transfer of ^{137}Cs in the above-ground biomass of crops due to the reduction in radionuclide percentage in the bioavailable physicochemical forms. If we verify the effect of EM on the bioavailability of ^{137}Cs in soil, we can confirm that its application will be a good ecofriendly method to perform agriculture in the territories that are contaminated with artificial radionuclides.

2. Materials and methods

2.1. Experimental plot

A field stationary experiment was performed in the arable lands of the Open Joint Stock Company Khalch in the Vetka district of the Gomel region, Republic of Belarus. The average density of ^{137}Cs contamination in the area is 149 kBq·m^{-2}.

The field experiment was conducted on a sod-podzolic sandy-loam soil developed on fluvioglacial sands. The soil is characterized by a low absorption capacity and low concentration of nutrients. The agrochemical indices of the soil within the experimental plot are summarized as follows: pH = 6.1; average content of mobile potassium (K_2O; 178 mg kg^{-1}); high content of mobile phosphorus (P_2O_5; 340 mg kg^{-1}); average content of exchange calcium (Ca; 726 mg kg^{-1}); average content of exchange magnesium (Mg; 281 mg kg^{-1}); and average content of soil organic matter (2.14%).

The meteorological conditions during the experiments were favorable for the cultivation of crops.

2.2. Experimentally tested microbial materials

The EM used in the experiments was supplied by EM Research Organization (Japan). EM comprises mixed cultures of lactic acid bacteria (*Lactobacillus casei*), yeast (*Saccharomyces cerevisiae*), and photosynthetic bacteria (*Rhodopseudomonas palustris*), and the total number of these microorganisms is maintained at a stable density of approximately 1 × 10^{8} CFU/mL. For application to soil, EM was prepared into two forms: liquid form (EM solution) and solid form (EM-bokashi). EM solution was prepared by mixing EM with sugar cane molasses and water with a ratio of 1:1:20 (v/v). The mixed ingredients were transferred to a plastic container, which was closed tightly with a plastic lid and incubated for 20–25 d at 35 °C ± 2 °C to promote fermentation. EM solution was considered ready to use when it produced a pleasant fermentation smell, and the pH was below 3.5.

In Japanese, bokashi refers to fermented organic matter. EM-bokashi is an anaerobic fermentation product made from solid agricultural byproducts inoculated with EM. In EM-bokashi, bokashi serves as the growth medium for the microorganisms and provides a suitable microenvironment for EM in the soil. EM-bokashi was prepared according to the method described by Higa (1991). A mixture of 0.4 L of EM, 0.4 L of sugar cane molasses, and 4 L of chlorine-free tap water was added to 10 kg of wheat bran and mixed manually until homogeneity was achieved. The mixture was then placed in a plastic bag, which was hermetically sealed and kept under dark and warm conditions for 30 d. After the 30-d fermentation period, the EM-bokashi had a sweet-sour smell. The EM-bokashi was dried at room temperature before application.

2.3. Crops and experimental layout

Two kinds of crops, barley (Burshtyn variety) and lettuce (Odessky kucheryavets variety) were used in the experiments. The field stationary experiment was established according to the recommendation of Dospekhov (1979). The experimental barley and lettuce were divided into the following six treatments:

1. Control
2. Potassium fertilizer (KCl)
3. EM
4. Bokashi
5. EM + Bokashi
6. EM + KCl

This experiment design included 12 options (2 crops × 6 treatments). Each option was repeated three times, for a total of 36 experimental plots. The size of each plot was 5.1 m^{2} (2.7 m × 1.9 m), and the analyzed plot size was 4 m^{2} (2.4 m × 1.7 m). The experimental plots for each crop were arranged spatially using a completely randomized design.

Soil tillage included plowing to the depth of the plowing horizon in autumn, harrowing in early spring, cultivation with tandem-disk harrowing, and pre-sowing cultivation via the packing of soil.

During pre-sowing cultivation, KCl and bokashi were applied at doses of 20 and 400 g m^{-2}, respectively. The dosage of KCl was based on national guidance for farming on soils contaminated with radionuclides considering the soil type and agrochemical properties. EM was applied to the soil surface and above-ground parts of plants four times: at the beginning of sowing and in two- or three-week intervals after the emergence of sprouts. The EM concentration was 1% for the first application and 0.1% for remaining applications, and 2 L m^{-2} of EM solution was applied to each plot.

Sowing was carried out at the optimum time recommended for the different crops. Lettuce was seeded at a seeding rate of 3–4 g m^{-2} and planted in rows with a row spacing of 25 cm. The lettuce plots were weeded and thinned by hand to obtain 20 plants per m^{2}. Harvesting was performed manually in each plot after 75 d of cultivation.

Barley was sowed manually at a seeding rate of 400 plants per m^{2} in rows with a row spacing of 30 cm. Weeding was performed manually when necessary. Harvesting was carried out when the plants were completely ripe.

2.4. Sampling and measurements

Soil and plant samples were collected to evaluate ^{137}Cs transfer at harvest. The soil core samples were taken from the topsoil (depth = 0–20 cm) using an iron sampler Ø39 mm × 200 mm (volume: 248 cm^{3}). Five soil samples were picked in each plot. The samples were dried, sifted (1-mm sieve), and homogenized before measurement of ^{137}Cs content.

The sampling of barley was performed in a phase of a complete ripeness. Grain and straw of barley were harvested from the analyzed areas with volumes of not less than 450 mL after compaction. The plant samples were washed, crumbled, and dried. Air-dried biomass was homogenized to enhance the reliability of the results.

Measurements of ^{137}Cs specific activity were carried out using a Canberra Packard gamma-spectrometer with an extended range coaxial Ge detector (Canberra XtRa). The measurement range of γ-radiation was 40–10,000 keV. The relative efficiency of spectrum registration at 1.33 MeV was 22.4%. The times of the measurements of γ-spectrometry were 2 and 12–24 h for soil and for plant samples, respectively. The relative error in the measurements of ^{137}Cs specific activity ranged from 5% to 10%.

Plant and soil radioactivity were expressed as the specific activity per dry weight (in Bq·kg^{-1}). The above-ground part aggregated transfer factor (T_{ag}) of ^{137}Cs from soil to plants was calculated as follows: T_{ag} ($m^2 \cdot kg^{-1} \cdot 10^{-3}$) = the mass activity density in the above-ground plant biomass (Bq·kg^{-1} dry mass)/the unit area activity density in the soil (kBq·m^{-2}). The above-ground part concentration ratio (F_v) of ^{137}Cs from soil to the compartments of plants was calculated as the ratio of the activity concentration in the above-ground plant biomass (Bq·kg^{-1} dry mass) to that in the soil biomass (Bq·kg^{-1} dry mass). The activity, T_{ag}, and F_v for ^{137}Cs were determined as mean values over all plots.

2.5. Physicochemical forms of ^{137}Cs in the soil

A laboratory experiment was carried out to evaluate influence of EM and bokashi on the physicochemical forms of Cs in soil. The distribution of the physicochemical forms of Cs with different degrees of biological accessibility was assessed using the successive extraction method reported by Tessier et al. (1979) with some modifications. The soil used in the laboratory experiment was sod-podzolic sandy-loam soil sampled from the top 20-cm layer of former agricultural land in the exclusion zone of the Chernobyl NPP. The specific activity of the soil samples in the dry state was 10.2 ± 0.2 Bq/g; this allows the assessment of physicochemical forms of Cs that contribute less than 1% to total activity. The soil used in the experiment had a low humus content (1.5%), high phosphorus content (117 mg/kg), and very low calcium and magnesium contents (417 and 57.8 mg/kg, respectively).

The soil samples were sifted using a 1-mm sieve and mixed thoroughly. Subsequently, 100-g soil samples were added to plastic containers. The experiments were conducted with three treatments: EM, Bokashi, and Control. For the EM treatment, a 1% aqueous solution of EM was applied on the soil once a week up to full water capacity of the soil. For the Bokashi treatment, bokashi was mixed with the soil at a ratio of 1:20 (v/v). For the Bokashi and Control treatments, water was added to the soil once per week. All treatments included four replicates, and the experimental duration was four months.

After the four-month experimental period, 5 g (dry weight) of soil from each container was sampled and placed in a thermostable glass. Physical-chemical forms of ^{137}Cs were successively extracted from the sample with freshly prepared reagents (Table 1). The amount of the radionuclide in the extracted solution from each step was measured after the filtration, and the separated precipitate was used for the extraction of the next fraction except for a “non-extractable” fraction where the extract was not obtained. The sediment was washed three times with distilled water (20–30 mL) after the separation of each fraction.

In all cases, the volumes of the obtained fractions were increased to 100 mL with distilled water to measure the activity of ^{137}Cs using γ-spectrometry in a PET container with an inner size of Ø70 mm × 26 mm (volume 100 cm^3). Undecomposed sediment was taken from the filter and placed into a plastic container, then water was added in the container up to 100 mL for a more uniform distribution of the radionuclide in the volume.

The obtained data were processed using analysis of variance in Microsoft Excel (2016). The data are expressed as mean ± standard deviation. The statistical significance between each treatment and the control was examined by non-parametric *t*-test ($p < 0.05$).

Table 2
Effects of KCl, bokashi, and EM application on the productivity of lettuce.

Treatments	Plant height, cm	Plant weight, g/plant	Crop yield, t/ha
Control	15.6 ± 0.2	50.3 ± 0.9	10.1 ± 0.4
KCl	18.3 ± 0.1*	52.0 ± 0.3	11.4 ± 0.2*
EM	25.3 ± 0.1*	73.7 ± 0.3*	14.0 ± 0.2*
Bokashi	22.5 ± 0.1*	55.3 ± 0.1*	11.1 ± 1.5
EM Bokashi	26.5 ± 0.1*	84.7 ± 0.1*	16.9 ± 0.5*
EM + KCl	28.0 ± 0.5*	94.4 ± 0.5*	18.9 ± 0.3*

*Significantly different from the control (non-parametric *t*-test, $p < 0.05$).

3. Results and discussion

3.1. Crop productivity

The effects of KCl, bokashi, EM, and EM in combination with bokashi or KCl on the productivity of lettuce were evaluated (Table 2). The application of EM alone significantly increased plant height, plant weight, and crop yield compared with those for the control. EM increased crop yield by 38% relative to the control (Table 2). The application of bokashi alone significantly increased plant height and plant weight; bokashi increased crop yield by 10% compared to the control, although this difference was not significant (Table 2). The application of KCl alone significantly increased plant height and crop yield, with crop yield increasing by 13% compared with that of the control (Table 2). EM combined with bokashi significant increased plant height, plant weight, and crop yield compared with those for the control, with crop yield increasing by 67%. Thus, EM in combination with bokashi produced a greater increase in yield than the application of EM or bokashi alone (Table 2).

The combination of EM and KCL resulted in the greatest increases in plant height, plant weight, and crop yield. Crop yield increased by 87% compared to the control (Table 2).

For barley, the application of EM alone significantly increased plant height along with the crop yields of biomass and grain; the crop yields of biomass and grain were increased by 193% and 22% compared to the control, respectively (Table 3). The application of bokashi alone significantly increased the crop yields of biomass and grain by 100% and 14% compared to the control, respectively. The combination of EM and bokashi increased the crop yields of biomass and grain by 342% and 20% compared to the control, respectively (Table 3). The application of KCl alone significantly increased the crop yield of biomass by 175% compared to the control. The crop yield of grain was increased by 2% compared to the control, although this difference was not significant. The combination of EM and KCl resulted in the greatest increases in the

Table 1
Procedure of sequential extraction of the physico-chemical forms of ^{137}Cs from soil.

Fraction	Extractant	Procedure
I. Water-soluble	Distilled water, pH = 5–6	24 h at 23 °C with periodic mixing
II. Exchangeable	1 mol/L acetous ammonium, pH = 7	2 h at 23 °C
III. Bound with iron and manganese oxides	0.04 mol/L hydroxylamine chloride in 25% acetic acid	6 h at 80 °C
IV. Bound with iron and aluminum sesquilateral oxides	0.02 mol/L ammonium oxalate +0.1 mol/L oxalic acid, pH = 3–2	1 h at 23 °C
V. Bound with the soil organic matter	30% hydrogen peroxide + nitric acid (2 drops), pH = 2	6 h at 80 °C
VI. Steadily fixed on soil minerals	7 mol/L nitric acid	6 h at 80 °C
VII. Non-extractable	Soil sediment after all the extractions	

Table 3
Effects of KCl, bokashi, and EM application on the productivity of barley.

Treatments	Plant height, Cm	Crop yield of the above-ground biomass (dry state), t/ha	Crop yield of the grain (natural moisture), t/ha
Control	63 ± 1.0	0.45 ± 0.09	2.47 ± 0.03
KCl	65 ± 1.0	1.24 ± 0.08*	2.52 ± 0.05
EM	68 ± 0.5*	1.32 ± 0.13*	3.02 ± 0.03*
Bokashi	65 ± 1.1	0.90 ± 0.06*	2.82 ± 0.03*
EM + Bokashi	67 ± 0.6*	1.99 ± 0.02*	2.96 ± 0.01*
EM + KCl	70 ± 0.6*	2.50 ± 0.12*	3.08 ± 0.01*

*Significantly different from the control (non-parametric *t*-test, $p < 0.05$).

crop yields of biomass and grain compared to the control (455% and 25%, respectively; Table 3).

The effects of EM and EM-fermented organic fertilizer on crop and vegetable production have been reported by several researchers. For example, the application of EM was shown to increase onion yield by 29%, pea yield by 31%, and sweetcorn cob weight by 23% (Daly and Stewart, 1999). EM positively affected cabbage yield by improving photosynthesis (Chantal et al., 2010). The foliar application of EM in combination with a soil amendment increased nodulation and yield in peas (Javaid and Mahmood, 2010), and the soil application of EM-bokashi improved radish yield (Suthamathy et al., 2013). In addition, the long-term (11 years) application of EM compost significantly increased wheat straw biomass, grain yield, and straw and grain nutrition compared to the use of traditional compost and the control (Hu et al., 2013). Thus, the results of this study, in which the application of EM and bokashi enhanced the yields of lettuce and barley, support the results of previous studies.

Potassium is the third most important plant macronutrient and is required for plant growth, metabolism, and development. Soil microorganisms play an important role in the natural potassium cycle. Considerable populations of potassium-solubilizing microorganisms are present in the soil and plant rhizosphere (Sugumaran et al., 2007; Meena et al., 2014). The application of EM significantly increased the concentrations of nutrients (nitrogen and potassium) in the rhizosphere, which was attributed to the rapid breakdown of the added organic material (Sangakara, 1993). In combination with potassium fertilizer, EM improved plant growth and yield by enhancing soil fertility (Arafa et al., 2011). In this study, the combination of EM and KCl improved the yields of lettuce and barley compared to the application of EM or KCl alone.

3.2. ^{137}Cs transfer into crops

The effects of EM, bokashi, KCl, and EM in combination with bokashi or KCl on the transfer of ^{137}Cs in lettuce and barley were evaluated (Tables 4 and 5). The application of EM and KCl alone significantly decreased the transfer of ^{137}Cs in the above-ground biomass of lettuce by 11% and 14% compared to the control. EM in combination with bokashi or KCl significantly decreased the specific activity of ^{137}Cs in lettuce biomass by 23% or 21%, respectively. These decreases were larger in magnitude than those observed when EM, bokashi, or KCl were applied alone (Table 4).

Table 4
Uptake of ^{137}Cs into the above-ground biomass of lettuce.

Treatments	^{137}Cs specific activity in plants, Bq·kg^{-1}	Density of soil contamination by ^{137}Cs, kBq·m^{-2}	T_{ag}, m^2·kg^{-1}·10^{-3}	F_v
Control	30.15 ± 2.45	155.92 ± 4.85	0.19 ± 0.03	0.051
KCl	25.93 ± 1.02*	156.22 ± 5.96	0.17 ± 0.05	0.046
EM	26.73 ± 1.21*	146.82 ± 3.46	0.18 ± 0.02	0.049
Bokashi	26.87 ± 1.61	149.54 ± 9.01	0.18 ± 0.02	0.049
EM Bokashi	23.35 ± 1.63*	138.89 ± 6.46	0.17 ± 0.01*	0.046*
EM + KCl	23.95 ± 1.33*	145.80 ± 6.58	0.16 ± 0.02*	0.043*

*Significantly different from the control (non-parametric *t*-test, $p < 0.05$).

Table 5
Uptake of ^{137}Cs into the above-ground biomass[a] of barley.

Treatments	^{137}Cs specific activity in plants, Bq·kg^{-1}	Density of soil contamination by ^{137}Cs, kBq·m^{-2}	T_{ag}, m^2·kg^{-1}·10^{-3}	F_v
Control	19.93 ± 2.76	126.16 ± 6.51	0.16 ± 0.01	0.042
KCl	14.07 ± 3.87**	145.38 ± 3.52	0.10 ± 0.04	0.027
EM	17.30 ± 1.40	167.32 ± 5.50	0.10 ± 0.03**	0.027
Bokashi	15.87 ± 1.90**	169.73 ± 5.16	0.09 ± 0.04**	0.024*
EM Bokashi	13.20 ± 2.30**	170.64 ± 1.73	0.08 ± 0.02**	0.021**
EM + KCl	10.30 ± 1.16**	171.06 ± 3.28	0.06 ± 0.02**	0.016*

*&**Significantly different from the control at $p < 0.05$ and $p < 0.01$, respectively (non-parametric *t*-test).
[a] Specific activity of ^{137}Cs in grain was less the minimum detectable level.

In barley, the application of KCl or bokashi alone significantly decreased the transfer of ^{137}Cs in above-ground biomass by 29% or 20% compared to the control, respectively. EM in combination with bokashi or KCl decreased the specific activity of ^{137}Cs in the above-ground biomass of barley by 34% or 48%, respectively. These decreases were greater than those obtained when applying EM, bokashi, or KCl alone (Table 5).

Regarding T_{ag}, EM in combination with bokashi or KCl significantly decreased the transfer of ^{137}Cs in lettuce by 11% or 16% compared to the control, respectively (Table 4). In barley, the application of EM alone, bokashi alone, EM in combination with bokashi, and EM in combination with KCl significantly decreased the transfer of ^{137}Cs by 38%, 44%, 50%, and 63% compared to the control, respectively (Table 5). In both crops, the application of EM in combination with bokashi or KCl resulted in lower above-ground part T_{ag} values compared to treatment with EM, bokashi, or KCl alone. In both crops, the largest reductions in ^{137}Cs transfer were obtained for the application of EM in combination with KCl. Thus, EM significantly strengthened the ability of potassium fertilizer to decrease ^{137}Cs transfer in plants. Finally, the reduction effects of all the microbial treatments in this study on the ^{137}Cs transfer in biomass were greater for barley as compared to those observed in case of lettuce (Tables 4 and 5). The final observation is confirmed by the two-way ANOVA results that are as follows: $F_{crop} = 42.5$ ($p < 0.01$; DF = 1), $F_{treatment} = 5.6$ ($p < 0.05$; DF = 5), and $F_{crop \times treatment} = 556.3$ ($p < 0.01$).

Soil microorganisms have important effects on the physicochemical properties of various substances in soil and their distributions. Microorganisms affect the physicochemical forms of radioisotopes in the soil, thereby influencing their mobility and bioavailability (Francis, 1990; Roussel-Debet, 2005). Many microorganisms living in the soil accumulate radioactive Cs via absorption and adsorption, including fungi (Sugiyama et al., 2000; Zhdanova et al., 2003; Mahmoud, 2004; Dighton et al., 2008), mycorrhizal fungi (Declerck et al., 2003), cyanobacteria (Sasaki et al., 2013; Avery et al., 1991), yeasts (Hoptroff et al., 1997; Ohnuki et al., 2015), bacteria (Tomioka et al., 1992; Perkins et al., 1995), actinomycetes (Kato et al., 2000). In addition, soil microorganisms affect the behavior and bioavailability of radioactive Cs in the soil-plant system (Ehlken et al., 2002). When rape seeds (*Brassica napus* L.) were inoculated with different species of bacteria and cultivated in a substrate contaminated with ^{137}Cs, the transfer of ^{137}Cs to plants was greatly increased when *Azotobacter chococcum* was inoculated; conversely, ^{137}Cs transfer was strongly inhibited when *Burkholderia* sp. was inoculated (Pareniuk et al., 2015). When komatsuna (four cultivars), Indian mustard, and buckwheat inoculated with *Bacillus* or *Azospirillum* were cultivated in ^{137}Cs-contaminated soil, the transfer of ^{137}Cs was enhanced in komatsuna Nikko inoculated with *Bacillus*. However, some combinations of plants and bacteria species resulted in the suppression of ^{137}Cs transfer (Djedidi et al., 2015).

Importantly, the experimental results indicate that the application of EM and bokashi has the potential to reduce the transfer of radioactive Cs into plants. The application of EM to soil has been reported to increase soil microbial activity (Valarini et al., 2002), and the bioaccumulation of ^{137}Cs by these activated soil microorganisms is thought to be one of the mechanisms by which EM reduces the transfer of radioactive Cs. The results of this study confirm that the suppressive effect of EM on Cs transfer was enhanced by combining the EM with bokashi to further increase soil microbial activity. In addition, one of the main types of bacteria in EM is lactic-acid bacteria, which have been reported to show biosorption activity towards Cs ion (Kinoshita et al., 2015).

In this study, when EM was combined with KCl, the suppression of radioactive Cs transfer was enhanced compared to the use of KCl alone. Potassium fertilizers are commonly used to prevent the uptake of radioactive Cs into plants because the presence of sufficient potassium in the soil hinders Cs transfer into plants. Potassium fertilizers are also known to enhance crop productivity, especially in potassium-poor sod-podzolic sandy soils and sandy-loam soils (Lembrechts, 1993; Zhu et al., 2000; Bogdevich et al., 2002; Ageyets, 2001). On the other hand, EM promotes plant growth by increasing the efficiency of both organic and mineral nutrient sources, including potassium in soil (Khaliq et al., 2006; El-Shafei et al., 2008; Hussain et al., 1999). Therefore, the finding shows that the application of potassium fertilizers, as a method for reducing the transfer of ^{137}Cs into crops, could be further improved by combining with EM.

3.3. Distribution of physicochemical forms of ^{137}Cs

In the field experiments, EM and Bokashi suppressed the transfer of radioactive Cs from soil into plants. To elucidate the mechanism of this effect, the physicochemical forms of radioactive ^{137}Cs present in the soil were evaluated.

^{137}Cs in the soil was primarily fixed on clay minerals (43.74%–45.29%) in all samples (Table 6). The considerable radionuclide content in the control soil samples was mainly bound with organic matter (19.04%) or found in the non-extractable fraction (20.61%). Smaller amounts of radionuclides in the control soil samples were bound with Al and Fe sesquilateral oxides (1.69%) or with Fe and Mn oxides (7.40%). Soluble and exchangeable fractions of Cs, which can transfer from soil into plant biomass, accounted for 0.62% and 6.90% of ^{137}Cs in the control soil, respectively.

The addition of EM to the soil decreased the percentage of soluble ^{137}Cs by almost two times compared to the control. This effect was more obvious in the soil enriched with bokashi; the portion of the soluble ^{137}Cs was reduced by 3.5 times compared to the control. The share of exchangeable fraction of ^{137}Cs did not prominently decrease in the EM-treated soil and was reduced significantly by 21% in the soil enriched by bokashi only. In the EM-treated soil, the percentage of ^{137}Cs bound with soil organic matter was increased significantly compared to the control, whereas the percentage of non-extractable ^{137}Cs was decreased.

Table 6
Percentages of ^{137}Cs physicochemical forms with different bioavailabilities in the soil treated with EM and bokashi.

Form	Control	EM	Bokashi
Soluble	0.62 ± 0.08	0.32 ± 0.07*	0.17 ± 0.05*
Exchangeable	6.90 ± 0.62	6.14 ± 0.52	5.44 ± 0.48*
Adsorbed on Fe and Mn oxides	7.40 ± 0.68	7.96 ± 0.67	6.29 ± 0.50
Adsorbed on Al and Fe sesquilateral oxides	1.69 ± 0.20	1.96 ± 0.17	1.67 ± 0.17
Adsorbed on soil organic matter	19.04 ± 1.73	23.46 ± 2.05*	17.5 ± 1.37
Adsorbed on clay minerals	43.74 ± 3.46	44.55 ± 3.19	45.29 ± 3.24
Non-extractable	20.61 ± 1.59	15.62 ± 1.08*	23.64 ± 1.75

*Significantly different from the control (non-parametric *t*-test, $p < 0.05$).

In complex and multicomponent systems such as soil, many physical and chemical processes occur simultaneously. Thus, it is difficult to identify the specific mechanisms through which the bioavailabilities of pollutants are altered. (Ledin, 2000). However, soil microorganisms have the potential to increase or decrease the transfer of ^{137}Cs into plants by interacting with the plant roots and/or converting radionuclides into different physicochemical forms with different biological availability (Pareniuk et al., 2015). Microorganisms may convert radioactive metals and metalloids in less-bioavailable forms via direct enzymatic biotransformation (Lloid and Renshaw, 2005).

The metabolites released by microorganisms can react with bioavailable forms of radionuclides, leading to complex formation, precipitation, and coprecipitation with iron and manganese oxides along with mineral formation (Francis, 1990). Heterotrophic microorganisms may either increase or decrease radionuclide bioavailability as a result of changes in soil pH, changes in redox potential, and the excretion of chelating agents, organic and inorganic acids, and other metabolites (Gadd, 2007). Spraying EM on the soil has been reported to increase soil biological activity and improve the physical and chemical properties of the soil (Valarini et al., 2003).

In this study, the application of EM decreased the fraction of soluble ^{137}Cs, and the application of bokashi decreased the soluble and exchangeable fractions. Thus, the reduction in ^{137}Cs transfer into crops observed in the field experiments can be explained by the decreased bioavailability of ^{137}Cs in the soil treated with EM or bokashi. Few studies have investigated the effects of microorganisms on the transfer of radioactive ^{137}Cs into agricultural crops. Furthermore, until now, no studies have reported that bacteria reduce ^{137}Cs transfer into plants by decreasing the bioavailability of ^{137}Cs.

EM, a commercially available organic material certified by the Japanese Agricultural Standard, has received safety approval and has been used as a soil-conditioning agent for over 25 years. Bokashi has been used for a long time in Japan as a traditional soil amendment. This study demonstrated that EM is effective in enhancing agricultural production and as a countermeasure to suppress the transfer of radioactive Cs into plants. EM showed promise as an alternative to potassium fertilizers and demonstrated the ability to enhance the suppressive effect of potassium on radioactive Cs transfer. However, further research is needed to validate the use of EM using other soil types and plant species.

4. Conclusion

1 The application of EM, which consists of lactic-acid bacteria, photosynthetic bacteria, and yeast, along with the application of EM combined with bokashi or potassium fertilizer to the soil surface and above-ground parts of plants increased the crop yields of barley and lettuce.

2 Enriching sod-podzolic sandy-loam soil by EM and bokashi significantly decreased the biological availability of ^{137}Cs by 38% and reduced the transfer of Cs into the biomass of barley by 44%. The greatest reductions in ^{137}Cs transfer into barley biomass were obtained when EM was combined with bokashi or potassium fertilizer, which reduced ^{137}Cs transfer by 50% or 60%, respectively.

3 The suppressive effect of EM on ^{137}Cs transfer varied depending on the plant species; the effect of EM on ^{137}Cs transfer was greater in barley than in lettuce.

4 The decrease in ^{137}Cs uptake into plants caused by EM can be attributed to the reduction in the fraction of bioavailable forms of ^{137}Cs (soluble and exchangeable forms). After four months of treatment with EM and bokashi, the soluble form of ^{137}Cs in the soil decreased by 48% and 72% compared to the control, respectively.

The fraction of exchangeable ^{137}Cs was significantly reduced by 21% compared to the control in the soil treated with bokashi only.

Declaration of interest statement

The possible conflict of interest lies in the fact that some authors of the article (Shuichi Okumoto and Shintani Masaki) are employees of a company (EM Research Organization) engaged in the development and marketing of microbiological preparation for agriculture (EM-1) used in these investigations.

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