Journal of Environmental Radioactivity 192 (2018) 491–497

Contents lists available at ScienceDirect

Journal of Environmental Radioactivity

journal homepage: www.elsevier.com/locate/jenvrad

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

Aleksander Nikolaevich Nikitin[a,*], Ihar Anatoljevich Cheshyk[a], Galina Zenonovna Gutseva[a], Elena Aleksandrovna Tankevich[a], Masaki Shintani[b,c], Shuichi Okumoto[b]

[a] Institute of Radiobiology of the National Academy of Sciences of Belarus, Fedjuninskogo str., 4, 246007, Gomel, Belarus
[b] EM Research Organization,Inc., Kishaba1478, Kitanakagusuku-son, Okinawa, 901-2311, Japan
[c] Tokyo Women's Medical University, Tokyo, Japan

ARTICLE INFO

Keywords:
Effective microorganisms
Bokashi
^{137}Cs accumulation
Physicochemical forms
Lettuce
Barley

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

* Corresponding author.
E-mail addresses: nikitinale@gmail.com (A.N. Nikitin), igor.cheshik@gmail.com (I.A. Cheshyk), guzewa@mail.ru (G.Z. Gutseva), elena.karpova1991@mail.ru (E.A. Tankevich), m-shintani@emro.co.jp (M. Shintani), sokumoto@emro.co.jp (S. Okumoto).

https://doi.org/10.1016/j.jenvrad.2018.08.005
Received 1 March 2018; Received in revised form 25 July 2018; Accepted 6 August 2018

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