Journal of Environmental Radioactivity 149 (2015) 73–80

Contents lists available at ScienceDirect

Journal of Environmental Radioactivity

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

Modification of ^{137}Cs transfer to rape (*Brassica napus* L.) phytomass under the influence of soil microorganisms

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ARTICLE INFO

Article history:
Received 16 January 2015
Received in revised form
25 June 2015
Accepted 1 July 2015
Available online 24 July 2015

Keywords:
Chernobyl
Soil bacteria
Plants
^{137}Cs accumulation

ABSTRACT

After nuclear accidents, such as those experienced in Chernobyl and Fukushima, microorganisms may help purify contaminated soils by changing the mobility of radionuclides and their availability for plants by altering the physical and chemical properties of the substrate. Here, using model experiments with quartz sand as a substrate we investigate the influence of microorganisms on ^{137}Cs transfer from substrate to plants. The highest transition of ^{137}Cs from substrate to plants (50% increase compared to the control) was observed after *Brassica napus* L. seeds were inoculated by *Azotobacter chroococcum*. The best results for reducing the accumulation of ^{137}Cs radionuclides (30% less) were noted after the inoculation by *Burkholderia* sp.. Furthermore, *Bacillus megaterium* demonstrated an increased ability to accumulate ^{137}Cs. This research improves our prediction of the behavior of radionuclides in soil and may contribute towards new, microbiological countermeasures for soil remediation following nuclear accidents.

1. Introduction

Microorganisms play an important role in converting the physicochemical state of substances in soil (Pepper et al., 2008). Depending on the type of soil and microorganism community, these processes may be accelerated or decelerated (Anderson et al., 2003; Simonoff et al., 2007; White et al., 1995). Microorganisms are fundamental to decomposition and related life-cycle processes. To perform these functions, microorganisms may alter the physical and chemical properties of soils, including modifications to the acidity and electrical conductivity of the substrate (Kazy et al., 2006; Wang et al., 2007). These functions can affect the mobility of radionuclides in the soil. For example, the availability of cesium increases with decreased pH (Dumat and Staunton, 1999; Kruyts and Delvaux, 2002). Soil microorganisms, such as bacteria communities, may therefore affect the physical-chemical radionuclide environment (Niedrée et al., 2012) and importantly, radionuclide availability to plants. The challenge is to predict the effect of soil microflora on radionuclide migration processes, including their influence on plant uptake. This is necessary to understand the contamination of plants within radionuclide contaminated areas.

Data on the impact of contamination on soil microbial communities is very limited. Tomioka et al. (1992) found that different species have significant differences in their ability to accumulate radionuclides. For example *Rhodococcus* sp. accumulated significantly more cesium-137 (^{137}Cs) when grown in a medium, than *Pseudomonas* sp., that demonstrated no tendency towards accumulation. Johnson et al. (1991) reported differences in the absorption of ^{137}Cs by bacteria, isolated from soil contaminated with radioactive cesium.

The different abilities to accumulate cesium ions can be explained by different levels of their affinity with various cations, and the specific transport system in cells. Nevertheless, some physical and chemical factors, including pH, organic matter content and the soil water regime may have a significant impact on the

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http://dx.doi.org/10.1016/j.jenvrad.2015.07.003
0265-931X/

ionic bonding processes (Anderson et al., 2003; McCabe, 1990; Simonoff et al., 2007; White et al., 1995).

Microorganisms play an important role in cesium remobilization from water deposits (Zhdanova et al., 2000), storing cesium in the upper layers of the soil profile (Johnson et al., 1991; Yerusalimskaya et al., 1999) and extracting cesium from plant residues in terrestrial ecosystems (Clint et al., 1992). In addition, the presence of ^{137}Cs in higher level organisms may be due to the transfer in trophic chains originating from microorganisms, rather than direct absorption from the environment (Avery and Tobin, 1992; King, 1964). Although some bacteria may stimulate the transfer of ^{137}Cs into the plant biomass (Song et al., 2012), the relationship between the ability of bacteria to accumulate radioactive elements and their possibility to influence this transfer into plants has been poorly studied.

In this study we investigate the influence of microorganisms on ^{137}Cs transfer from soil to plants and the effect of ^{137}Cs and microorganisms on the morphological characteristics of plants. Our objective is to determine the composition of microflora in soil from the Chernobyl exclusion zone, evaluate the affinity for the key representatives this soil microflora community to cesium, and investigate the effect of soil microflora on ^{137}Cs transfer from soil to plants. This information is necessary for the prediction of radionuclide behavior in soil and may lead towards new microbiological countermeasures for soil remediation following nuclear accidents.

2. Materials and methods

2.1. Methods overview

Multiple approaches were used to investigate the modification of ^{137}Cs concentrations ratio by soil microorganisms. Soil samples were obtained from sites with different densities of ^{137}Cs contamination. Bacterial diversity was examined, followed by an identification of the dominant bacteria species in each soil sample. Four species, which inhabited the plant rhizosphere, were chosen and aggregated to form group A (Fig. 3). Transmission electron microscopy (TEM) was used to determine sizes and morphology of the bacteria species. To create a control group of non-irradiated cells, four species-agents of bacterial fertilizers were obtained from the Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine (IMV NAS) – these bacteria strains formed group B. Key mechanisms impacting the soil-to-plant concentration ratio were examined by incubating bacteria cells in ^{137}Cs contaminated media. Afterwards, the ability of bacteria to modify the soil-to-plant concentration ratio was investigated by inoculating rape seeds with sterilized surfaces, with the selected species of bacteria and growing these plants in artificially contaminated substrate. Specific details follow below (Fig. 2).

2.2. Strain source, isolation, cultivation and identification

Bacteria were isolated from soils within the Chernobyl exclusion zone and the unconditional (mandatory) resettlement zone with different densities of radionuclide contamination. Five sampling sites, listed in the Ukrainian Institute of Agricultural Radiology database (Fig. 1), were selected with the goal of maximizing the similarity of environmental conditions between sites. Soil was sampled with standard microbiological techniques (Pepper et al., 2008), first in September 2011, and then monthly from June to October 2012.

Bacteria were allocated by serial tenfold dilutions screening of bacterial suspensions on a glucose-peptone agar medium. Microorganisms were grown in an incubator at a temperature of 28 °C for 4 days, after which colonies of the dominant organisms were isolated in pure culture and prepared for PCR analysis. DNA was extracted from the bacteria using UltraClean Microbial DNA Isolation Kits (MoBio, USA) in accordance to manufacturer guidelines. The species identification of the dominant microbial strains was performed by PCR analysis (Innis et al., 1990). Obtained strains were collected and included in group A (Pareniuk et al., 2013).

Strain-agents of bacterial inoculants were obtained from the collection of the IMV NAS to compare to the ability of sampled soil microorganisms to accumulate radionuclides. The strain-agents (group B) were: *Azotobacter chroococcum* UCM B-6003, *A. chroococcum* UCM B-6082, *Bacillus megaterium* UCM B-5724, and *Agrobacterium radiobacter* IMV B-7246 (Tytova et al., 2010).

2.3. Transmission electron microscopy (TEM)

Samples were prepared with the modified floating-drop method (Boretska et al., 2013). A 1 mL aliquot of the culture of interest was centrifuged at 10,000 rpm for 5 s and a 100 μL drop of the re-suspended cell pellet was placed on the surface of a piece of Parafilm (Bemis, USA). Cu-coated grids (Formvar, Russia) were placed onto the drop and cells were allowed to adhere for 5–10 min before the grids were air-dried without staining. Images were obtained using a Jeol 1400 electron microscope (Jeol, Japan) at 80 kV, with 20 fields of view imaged for each sample.

2.4. Biosorption experiments

Four species inhabiting the plant rhizosphere at each sampling point in the Chernobyl exclusion zone were selected for analysis: *Bacillus mycoides* BCHMAC12, *Burkholderia glathei*, *Burkholderia* sp., *Pseudomonas frederiksbergensis*, the latter being the only rhizospheric from the isolated dominant species. The ^{137}Cs biosorption of these species (group A) were compared to the four species-bioagents of group B of microbial fertilizers were analyzed: *A. chroococcum* UCM B-6003, *A. chroococcum* UCM B-6082 *and B. megaterium* UCM B-5724 *and Agrobacterium radiobacter* IMV B-7246.

Biosorption experiments were performed using a potassium-free glucose-peptone broth medium (pH 7). Except when noted, 2 mL of 12-h bacterial cultures (10^7 cells/mL) were added to 10 mL of a fresh culture medium that was coated with 2600 Bq/mL of ^{137}Cs solution. The mixture was incubated for 12, 24, 48, 72 h while shaking (RS 24, Biosan Multibio, Latvia) at 28 °C. After harvesting, the medium containing the cells was centrifuged at 8000 rpm for 5 min. The pellet was separated from the culture broth, and, to avoid the contamination of the biomass by radionuclide from the nutrient media, it was rinsed three times in 0.6% NaCl, and centrifuged afterwards at 8000 rpm for 5 min. The resulting biomass was dissolved in 1.4 mL of 65% HNO_3 and prepared for gamma-spectrometry. Experiments were conducted in triplicate. The statistical significance between the samples was examined by ANOVA with Tukey HSD amendment to meet multiple comparisons. The difference of sample from all others considered only if differences between the test sample and every other sample, listed in comparison matrix, were greater confidence level ($p < 0.05$).

2.5. Bioavailability experiments

To understand the bioavailability of ^{137}Cs, multiple experiments were conducted with *Brassica napus* L. To eliminate the influence of external factors, including the decrease of ^{137}Cs bioavailability due to its absorbance in clay minerals (He and Walling, 1997), plants were grown hydroponically with grassroots watering. The 0.8–1.2 mm fraction of quartz sand (Kd < 1 L/kg ([Cs] = 10^{-8} M, (Lieser and Steinkopff, 1989)), was autoclaved for 20 min (140 °C, 0.2 MPa)