Journal of Environmental Radioactivity 104 (2012) 55–63

Contents lists available at SciVerse ScienceDirect

Journal of Environmental Radioactivity

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

Effects of chronic exposure in populations of *Koeleria gracilis* Pers. from the Semipalatinsk nuclear test site, Kazakhstan

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A R T I C L E I N F O

Article history:
Received 15 April 2011
Received in revised form
20 September 2011
Accepted 30 September 2011
Available online 1 November 2011

Keywords:
Semipalatinsk nuclear test site
Radioactive contamination
Absorbed dose
Crested hairgrass
Cytogenetic effects
Radio-adaptation

A B S T R A C T

Morphological and cytogenetic abnormalities were examined in crested hairgrass (*Koeleria gracilis* Pers.) populations inhabiting the Semipalatinsk nuclear test site (STS), Kazakhstan. Sampling of biological material and soil was carried out during 3 years (2005–2007) at 4 sites within the STS. Activity concentrations of 10 radionuclides and 8 heavy metals content in soils were measured. Doses absorbed by plants were estimated and varied, depending on the plot, from 4 up to 265 mGy/y. The frequency of cytogenetic alterations in apical meristem of germinated seeds from the highly contaminated plot significantly exceeded the level observed at other plots with lower levels of radioactive contamination during all three years of the study. A significant excess of chromosome aberrations, typical for radiation exposure, as well as a dependence of the frequency of these types of mutations on dose absorbed by plants were revealed. The results indicate the role radioactive contamination plays in the occurrence of cytogenetic effects. However, no radiation-dependent morphological alterations were detected in the progeny of the exposed populations. Given that the crested hairgrass populations have occupied the radioactively contaminated plots for some 50 years, adaptation to the radiation stress was not evident. The findings obtained were in agreement with the benchmark values proposed in the FASSET and ERICA projects to restrict radiation impacts on biota.

1. Introduction

The Semipalatinsk nuclear test site (STS) in Kazakhstan was the main site for testing nuclear weapons in the former Soviet Union. Between 1949 and 1989, 459 nuclear tests were conducted at the STS (Logachev et al., 2002). Among them, 116 explosions were aboveground. The approximate cumulative explosive yield of these tests, 6.4 MT, is about 6 times greater than the explosive yield of the aboveground tests at the Nevada Test Site (Simon et al., 2003). As a result, large-scale, heterogeneous radioactive contamination occurred on the STS and its adjacent territories. High levels of radioactive contamination still exist within some areas of the STS (Yamamoto et al., 1996). The STS is interesting because the primary dose-contributing radionuclides are different from those studied by radioecologists in the area of the South Ural radioactive trace, territories contaminated after the Chernobyl accident, and areas with enhanced background radiation (Table 1). Thus, research conducted at the STS represents a unique class of radioecological studies that offers additional opportunities to understand the effects of radiation exposure.

The STS is located within a steppe geographical zone, where it experiences a sharply continental climate with low humidity and rare rainfall. The climatic conditions of the Kazakh steppe result in a community of biological species that are different to those more often studied by radioecologists. This also emphasizes the importance of radiobiological studies at the STS.

Previous investigations conducted at the STS have focused on assessing the radioactive contamination (Carlsen et al., 2001; Vintro et al., 2009; Yamamoto et al., 1996), radionuclide behavior (Howard et al., 2004), estimating doses (Simon et al., 2003) and the health effects to humans living in nearby settlements (Chenal et al., 2006; Dubrova et al., 2002). However, there are few data available on the effects of radioactive contamination on non-human biota, although many plants and animals currently inhabiting the STS are

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doi:10.1016/j.jenvrad.2011.09.015

Table 1
Radioecological and climatic differences between the Semipalatinsk nuclear test site and areas affected by the Kyshtym and Chernobyl accidents.

	STS	South Urals radioactive trace	Areas affected by Chernobyl accident
Climate	Sharply continental	Continental	Temperate
Main soil type	Chestnut	Chernozem	Podzolic
Typical ecosystem	Steppe	Forest-steppe	Forest
Current primary dose-forming radionuclides	^{137}Cs, ^{152}Eu, ^{90}Sr, ^{154}Eu, ^{60}Co, ^{239}Pu, ^{241}Am	^{90}Sr, ^{137}Cs	^{137}Cs, ^{90}Sr, ^{239}Pu, ^{241}Am

from a lineage that experienced acute radiation impacts during nuclear weapons tests, and have then been exposed to chronic irradiation over subsequent generations. Since wild organisms pass on genes to future generations, deleterious mutations may accumulate in the populations and augment damage caused by ongoing genotoxic exposure. Therefore, studies of plant and animal populations inhabiting the STS should enhance our understanding of the biological consequences from long-term radiation exposure over many generations.

This study investigated two questions: (i) does chronic exposure to ionizing radiation cause dose-dependent morphological or cytogenetic effects in plants that inhabit the STS; and (ii) does chronic irradiation of plants over many generations lead to the development of adaptation and radioresistance in progeny?

2. Material and methods

2.1. Study area

Observations were carried out at the “Experimental Field” technical area located in the northwest of the STS (Fig. 1). According to Logachev et al. (2002), 86 air and 30 ground nuclear tests were conducted at the Experimental Field by the end of 1962. Grassland cover over much of the site is comparatively sparse and is punctuated by low bushes and scrub. The area is dominated by chestnut soils with localized sites of solonetz and solonchaks. In general, the soil is alkaline and low in organic matter content. The cation exchange capacity value is rather low but the exchangeable Ca content is within the reported range for many soil types (Howard et al., 2004). The climate is sharply continental and arid with a low rate of precipitation of 250–300 mm/year.

Four plots with different levels of radioactive contamination were chosen in 2005 (Geras'kin et al., 2009) to study the biological effects in populations of wild grasses (Table 2). Latitude and longitude were recorded for each location using a geographical positioning system (GPS). The γ-dose rate in air at 1 m and the ground surface, as well as α- and β-particle flux density were measured using a radiometer RKS 01 SOLO (Solo Ltd, Kazakhstan) at each plot.

2.2. Determination of radionuclides and heavy metals concentrations in soil samples

To measure the radionuclide composition and estimate the doses absorbed by plants, samples of soil were collected in July 2006. According to Dubasov (1997) 95–99% of the total amount of artificial radionuclides was located in the upper 10 cm soil layer at the epicenter of aboveground nuclear tests in the Experimental Field. Based on this, soil samples were taken from two soil layers at a depth of 0–5 and 5–10 cm. There were five sampling points at every plot, located at the four corners and the center of 10 m × 10 m square (an ‘envelope’ technique). Samples were bulked within each soil layer so that eventually two soil samples were analyzed from each plot.

Soils collected were air-dried and then sieved through a 1.0 mm filtering screen. Activity concentrations of ^{40}K, ^{60}Co, ^{137}Cs, ^{152}Eu, ^{154}Eu, ^{226}Ra, ^{232}Th, ^{238}U, and ^{241}Am in soil samples were determined by γ-ray spectrometric analysis (CD Canberra GC-0515R, USA). ^{90}Sr activity concentrations were determined by radiochemical isotope ^{90}Sr/^{90}Y separation with spectral measurements of ^{90}Y activity (Assessment, 1999) using a computer-assisted program “PROGRESS” (VNIIFTRI, Russia). All measurements were made in the National Nuclear Center of the Republic of Kazakhstan, Kurchatov.

Total concentrations as well as water-soluble (extraction by distilled water), mobile (extraction by CH_3COONH_4, pH 4.8) and acid-soluble (extraction by 1 N solution of HCl) forms of heavy metals (Mn, Cu, Zn, Cd, Pb, Cr, Ni, and Co) in soil samples were determined by atomic absorption in the Semipalatinsk state teacher's training college, Kazakhstan.

2.3. Test organism

Crested hairgrass (*Koeleria gracilis* Pers.) is a perennial wild cereal typical of Kazakhstan and an important component of steppe ecosystems. The height of this grass is usually between 20 and

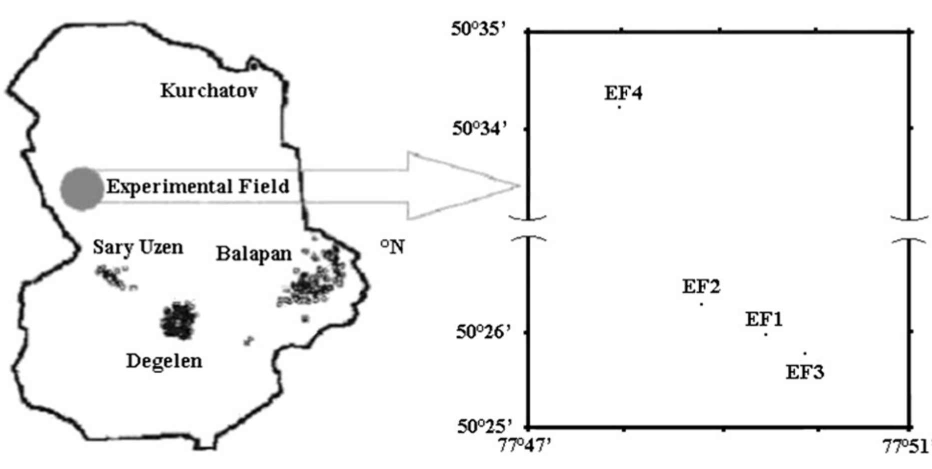

Fig. 1. Location of plots within the STS.