INTRODUCTION

The majority of abiotic stress studies performed under controlled laboratory conditions does not reflect the actual situations that occur in the field. Therefore, to understand effects of contaminant exposure on natural populations properly we must pay attention to what is actually going on in natural conditions. Field studies are particularly useful for assessing long-term biological effects induced by chronic low dose-rate and multi-pollutant exposure at contaminated sites. On the other hand, radiation effects are not always easy to part from modifications caused by other factors. Radiation interacts with other sources of stress, which reduces considerably our ability to predict effects on natural systems [1]. Although radionuclides and heavy metals cause primary damage at the molecular level, there are emergent effects at the level of populations that are not predictable solely from knowledge of elementary mechanisms of the pollutants’ influence. Up to now we have known little about responses of plant and animal populations to environmental pollutants in their natural environments. These data gaps imply that the protection of the environment from ionizing radiation will require more experimental data related to effects of chronic low-level exposure to radionuclides at the population level.

The knowledge gained from field research would be valuable at the most fundamental level for increasing our understanding of microevolution and organism response to stress. Previously completed and ongoing field studies that have been carried out in Laboratory of Plant Ecotoxicology, RIARAE in different species of wild and agricultural plants [2-8] are briefly summarized in Table 1. A wide range of radioecological situations and climatic zones have been covered in frames of this work. To illustrate the main findings, two field studies are discussed here.

MATERIALS AND METHODS

In 2005-2008 seeds of crested hairgrass (Koeleria gracilis Pers.) were collected from six sites of the Semipalatinsk Test Site (Kazakhstan). Radiation background at the sites and specific activity of the main dose-forming radionuclides in soil samples were measured. Doses to generative organs of crested hairgrass were calculated. Squashed slides for cytogenetic analysis were prepared of coleoptiles (2-5 mm in length) of germinated seeds. In every slide, all ana-telophase cells (4800-11900 ana-telo-phases in 30-90 slides) were scored to calculate frequency of aberrant cells. Detailed description of methods used is given in [8].

To study biological effects in Scots pine populations (Pinus sylvestris L.) experiencing chronic radiation impact, six test-sites were chosen in the Bryansk region radioactively contaminated as a result of the Chernobyl accident. Pine cones were collected in autumns of 2003-2007. Specific
activities of radionuclides in soil samples were measured, and doses to the pine trees’ generative organs were estimated. Aberrant cells were scored in root meristem of germinated seeds in ana-telophases of the first mitosis. The method of isozymic analysis of megagametophytes was used for an estimation of genetic variability in populations of Scots pine. Five enzymatic loci (GDH, LAP, MDH, DIA, 6-PGD) were studied in endosperms of the seeds collected in 2005. Detailed description of materials and methods used can be found in [6, 7].

**RESULTS AND DISCUSSION**

Cytogenetic effects in crested hairgrass populations from the Semipalatinsk Test Site (Kazakhstan). In the Semipalatinsk Test Site (STS), 116 atmospheric and ground-surface explosions for nuclear and hydrogen bomb testing were carried out between 1949 and 1963. A study of crested hairgrass (*Koeleria gracilis* Pers.) populations, a typical wild crop for Kazakhstan, showed that the frequency of cytogenetic disturbances in coleoptiles of germinated seeds increases proportionally to the dose absorbed by plants during vegetation period (Figure 1). The agreement between findings from four successive years of study (2005-2008), different in weather conditions, suggests the leading role

**Table 1. Field studies on wild and agricultural plants**

<table>
<thead>
<tr>
<th>Species</th>
<th>Site &amp; Time</th>
<th>Assay and/or endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter rye and wheat, spring barley and oats</td>
<td>10-km ChNPP zone (11.7-454 MBq/m²), 1986-1989</td>
<td>Morphological indices of seeds viability, cytogenetic disturbances in intercalary/seedling root meristem [2]</td>
</tr>
<tr>
<td>Scots pine, couch-grass</td>
<td>30-km ChNPP zone (2.5-27 μGy/h), 1995</td>
<td>Cytogenetic disturbances in seedling root meristem [3]</td>
</tr>
<tr>
<td>Wild vetch</td>
<td>Radium production industry storage cell, Komi Republic, Russia (0.7-33 μGy/h), 2003-2007</td>
<td>Embryonic lethals, cytogenetic disturbances in seedling root meristem [5]</td>
</tr>
<tr>
<td>Scots pine</td>
<td>Bryansk region radioactively contaminated in the Chernobyl accident (0.6-3.5 μGy/h), 2003-2008</td>
<td>Cytogenetic disturbances in seedling root meristem, enzymatic loci polymorphism analyses, abortive seeds portion [6, 7]</td>
</tr>
<tr>
<td>Crested hairgrass</td>
<td>Semipalatinsk Test Site, Kazakhstan (0.7-36 μGy/h), 2005-2008</td>
<td>Cytogenetic disturbances in coleoptiles of germinated seeds [8]</td>
</tr>
</tbody>
</table>

**Fig. 1. Frequency of aberrant cells in coleoptiles of germinated seeds of crested hairgrass collected in the Semipalatinsk Test Site, Kazakhstan in 2005-2008 in dependence on annual dose absorbed. Ref 1 and Ref 2 are the reference sites in 2005-2007 and 2008, accordingly. Significant difference from the corresponding reference site: * - p < 0.10; ** - p < 0.05**
of radioactive contamination in an occurrence of cytogenetic effects. Severe disturbances of single and double bridges and laggard chromosomes contribute mainly to the observed cytogenetic effect [8].

Dose rate in the epicenter of nuclear tests amounts to 36 μGy/h, which is more than 3 fold of the predicted no-effect dose rate of 10 μGy/h derived in the EC ERICA project [9]. It is, however, well below the threshold for statistically significant effects (100 μGy/h) derived at the FASSET Radiation Effects Database analysis [10]. It is not surprising, than, that in the STS study there are found significant cytogenetic effects in crested hairgrass populations but no morphological alterations have been registered. Thus, the findings obtained are in agreement with the benchmark values proposed in the FASSET and ERICA EC projects to restrict radiation impact on biota.

**Biological effects in Scots pine populations inhabiting radioactively contaminated sites.** Forest trees have gained much attention in recent years as nonclassical model eukaryotes for population, evolutionary and ecological studies [11]. Because of their potential to affect many other species, any responses to selection pressures that are exerted on such keystone species as forest trees are especially important to quantify. The low domestication, large open-pollinated native populations and high sensitivity to environmental exposure make conifers almost an ideal species for the study of environmental effects of radioactive contamination.

In Figure 2, the results of long-term (2003-2007) study of cytogenetic effects in Scots pine populations growing in the Bryansk region radioactively contaminated as a result of the Chernobyl accident are presented. Populations under investigation did not show a significant difference between years, so our results are robust and replicable over time. Aberrant cell frequency in root meristem of germinated seeds collected from these populations significantly exceeded reference level and shows statistically significant correlation to specific activity of 137Cs, the main dose-forming radionuclide, in pine cones during all five years of study. Although there is a tendency for aberrant cells occurrence to increase with the dose absorbed by the pine trees’ generative organs, it is not always significant. Compiled with data from other our studies [2-5, 8], these findings indicate that an increased level of cytogenetic disturbances is a typical phenomenon for plant populations growing in areas with relatively low levels of pollution.

The registered cytogenetic effects are observed at dose rates of 0.5 to 3.5 μGy/h that are below the ERICA predicted no-effect dose rate of 10 μGy/h [9]. It should be noted that, in the STS study, a wide range of doses from 4 to 265 mGy absorbed by the plant generative organs was studied, and dependence of cytogenetic effects on dose was revealed. On the contrary, in the Bryansk region, the range of doses absorbed by the pine trees’ generative organs at the study sites is much narrower (from 7.4 to 37.8 mGy), which could be a reason for an absence of statistically significant increase of biological effect with the dose absorbed in some years of observations.

With each passing year since the Chernobyl accident of 1986, more questions arise [12] about the potential for organisms to adapt to radiation exposure. It is becoming increasingly clear that cytogenetic disturbances detected in our studies might only be tip of an iceberg, reflecting global structural and
Functional rearrangements induced by radiation in exposed populations. An increase in mutation rate can affect the population-genetic structure by producing new alleles or genotypes, and thereby has ecologically relevant effect. Alterations in the genetic make-up of populations are of primary concern because somatic changes, even if they lead to a loss of individuals, will not be critical in populations with a large reproductive surplus.

To analyze whether an exposure to radionuclides causes changes in population genetic structure, we evaluated frequencies of three different types of mutations (null allele, duplication and changing in electrophoretic mobility) of enzymatic loci in endosperm and embryos of pine trees from the studied populations. It is found that chronic radiation exposure results in the significant increase of total occurrence of enzymatic loci mutations. In particular, frequencies of mutations for loss of enzymatic activity increase with a dose absorbed by critical organs of pine trees (Figure 3).

There are plenty of theoretical treatments of evolution, but what is important is to see what happens in practice. Mutations in plants or animals are not necessarily bad events when they do not adversely affect fitness characteristics of the population. Mutation is one of the mechanisms that maintains genetic variation within natural population and thus enables that population to cope with an adversely changing environment. This may speed up adaptation and microevolution under adverse environmental conditions. Indeed, phenotypic variability in the exposed pine trees populations, estimated via the Zhivotovsky index [11], significantly exceeds the reference level and increases with dose absorbed by generative organs of pine trees (Figure 4).

A decrease in heterozygosity within individuals has been associated [14] with decreased resistance to diseases, decreased growth rates, and decreased fertility. This would suggest that variations in individual heterozygosity may affect population growth and recruitment. The observed heterozygosity in pine trees populations at the radioactively contaminated sites is essentially higher than the expected one and increases with dose absorbed by critical organs of pine trees (Figure 5).

Information on specific functions connected with isoenzymes is scarce. We can, nevertheless, conclude that the relationship between radioactive contamination and genetic variability provides evidence of adaptation which optimizes the physiological response of a population to environmental changes. Keeping in mind all the data mentioned, it could be concluded that a high level of mutation occurrence is intrinsic for descendants of pine trees.

![Figure 3](https://via.placeholder.com/150)

**Fig. 3.** Frequency of null alleles in enzymatic loci of endosperms (2005) in dependence on annual dose absorbed by generative organs of pine trees. Significant difference from the Ref 1 population: ** - p < 0.01, *** - p < 0.001

![Figure 4](https://via.placeholder.com/150)

**Fig. 4.** Phenotypic variability estimated via the Zhivotovskiy index in dependence on annual dose absorbed by generative organs of pine trees. Significant difference from the Ref 1 population: * - p < 0.05, *** - p < 0.001

![Figure 5](https://via.placeholder.com/150)

**Fig. 5.** Heterozygosity in endosperms of Scots pines in dependence on annual dose absorbed by generative organs of pine trees. * - significant difference from the Ref 1 population, p < 0.01
in the investigated populations, and genetic diversity in the populations is essentially conditioned by radiation exposure. So, in spite of their low values, dose rates observed can be considered a factor able to modify the genetic structure of population. Furthermore, an increased genetic diversity within the population of keystone species should be positively correlated with increased species diversity of the dependent community [15].

Although great progress is been made in understanding the nature of mutations, too little is yet known about the way in which mutations can lead to effects at the level of the organism and population. The effect of severe conditions on an organism is often considered to eliminate individuals. However, the alternative effect is to change the number of offspring produced by individuals without killing them. The plasticity of plants, and the fact that their reproductive organs are usually the terminal points of a branching structure, means that they tend to respond to environmental stresses by variation in reproductive rate without death. It is true that a much larger number of seeds are produced than developed into adult plants, and that the changes in frequency of the different genotypes are due to a greater death of some genotypes than others. This is a form of response to selection, and a very powerful one [16]. From the results gathered a question arises: what could be an effect of high mutation rates revealed in our study on a reproductive potential of pine seeds? Figure 6 shows that under chronic exposure pine tree populations developed seed descendants with significantly lower reproductive potential than the reference population.

**CONCLUSIONS**

A basic level of concern within a newly developing system for radiological protection of the environment is a population. Of special importance in this context are studies of plant and animal populations inhabiting sites with contrasting levels and spectra of radioactive contamination. Special attention should be paid to population-level effects such as radioadaptation, changes in sexual, age and genetic structure of populations, since knowledge of elementary mechanisms of the radionuclides’ impact is insufficient to predict them. Corresponding studies are likely to increase in importance as the rate at which we change the environment worldwide continues to accelerate. The findings presented here clearly indicate that plant populations growing in areas with relatively low levels of pollution are characterized by an increased level of both cytogenetic disturbances and genetic diversity. Concordant responses between changes in population genetic structure and elevated levels of cytogenetic damage provide evidence that the population genetic changes are influenced by exposure to radionuclides. Effects of contaminants on genetic diversity within a population are important because the level of genetic biodiversity affects a population’s ability to adapt or the rate of adaptation to changes in the environment. Therefore, the amount of genetic variation within a population can influence its relative susceptibility to extinction. Indeed, under chronic exposure conditions in pine tree populations there were developed seed descendants with significantly lower reproductive ability than in the reference population. Our findings also suggest that effects detected in short-term studies with plant populations are of limited value in understanding and predicting biotic changes in polluted ecosystems, because such studies may easily overestimate the adverse effect, which will be much lower under chronic exposure due to development of pollution tolerance and adaptation to altered environmental conditions. Finally, in spite of the wealth of information collected so far, much more still remains to be explained in order to fully understand the basis of plant populations’ adaptation to a harmful environment.

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References

1. Geras’kin SA, Kim JK, Dikarev VG, Oudalova AA, Dikareva NS, Spirin YV. Cytogenetic effects of combined radioactive (137Cs) and chemical (Cd, Pb, and 2,4-D herbicide) contamination on spring barley intercalary meristem cells. Mutation Research. 2005;586:147-59.


