CHAPTER 6

EFFECTS OF MULTIPOLLUTANT EXPOSURES ON PLANT POPULATIONS

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Abstract: Results of laboratory, "green-house" and long-term field experiments carried out on different plant species to study ecotoxical effects of low doses and concentrations of most common environmental pollutants are presented. Special attention is paid to ecotoxic effects of chronic low dose exposures, synergistic and antagonistic effects of multipollutant exposure. Plant populations growing in areas with relatively low levels of pollution are characterized by the increased level of both cytogenetic disturbances and genetic diversity. The chronic low dose exposure appears to be an ecological factor creating preconditions for possible changes in the genetic structure of a population. A long-term existence of some factors (either of natural origin or man-made) in the plants environment activates genetic mechanisms, changing a population's resistance to exposure. However, in different radioecological situations, genetic adaptation of plant populations to extreme edaphic conditions could be achieved at different rates. The findings presented indicate clearly that an adequate environment quality assessment cannot rely only on information about pollutant concentrations. This conclusion emphasizes the need to update some current principles of ecological standardization, which are still in use today.

Keywords: radioactive and chemical contamination; multipollutant exposure; bioindication; plant populations; environment quality assessment

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Introduction to Bioindication Approach

Contamination of the environment has become a worldwide problem. Therefore, a clear understanding of all the dangers posed by environmental pollutants to both human health and ecologic systems is needed. Knowledge of the existence of an environmental stress situation is the prerequisite for its solution or amelioration. With this in mind, considerable efforts have been undertaken to develop effective methods for assessing the quality of the environment.

Generally, two approaches are used. The first is based on chemical-physical techniques for laboratory analysis of air, water and soil samples. At this, an evaluation of true exposure characteristics is complicated; however, since most quantification techniques are capable of recognizing just a specific compound or its metabolites. So, this approach gives only a part of the knowledge necessary to evaluate the harmful potential of pollutants.

The other approach is to score biological effects in animals or plants that could be exposed at contaminated sites. An obvious advantage of this method is a demonstration of the direct results from the pollutants' action on living nature. This limits the usefulness of this approach as an analytical method to investigate the total pollutants' burden, but enhances it ability to measure environmental quality. The use of biomarkers could remove much of the uncertainty associated with current ecological risk assessments and provide meaningful indicators of biological damage. In contrast to the specific nature of assessments on exposure, studies of biological effects integrate the impacts of all the harmful agents, including synergistic and antagonistic effects. The biomarkers may also illuminate previously unsuspected chemical or natural stressors in the study area or reveal damage caused by a pollutant that has since degraded and is no longer detectable by residue analysis. Therefore, this approach is particularly useful for assessing unknown contaminants, complex mixtures, or hazardous wastes.

Certainly, it will never be possible to replace direct physical-chemical measurements of pollutant concentrations entirely by a detection of effects in bioindicators. An increased understanding of fate and exposure pathways of harmful substances in various test-systems is also needed for a better prediction of what has happened in the field. It is obvious that a correct estimation of the environment pollution risk needs to be derived from biological tests and pollutant chemical control in ecosystem compartments. Chemical and biological control methods need to be used simultaneously, which allows an identification of the relationships between the pollutant concentrations and the biological effects that they cause. In turn, such relationships may help in an identification of the contribution from specific pollutants to the overall biological effect observed. The knowledge generated makes it possible to limit an effect of unfavorable factors on biota and predict the further ecological alterations in regions submitted to intensive industrial impact.

To assess the quality of the environment, we mostly used plants as test-objects, for several reasons. Plants are higher eukaryotes and essential components of any ecosystem. Plant species are the most important primary producers and most relevant in the food web of the ecosystem. Plants seem to be especially well suited for an environmental assessment since they have fast growth rates and provide a large number of offspring. Owing to their settled nature, plants are constantly exposed to pollution and, therefore, can characterize the local environment in the best way. Plants do not have a predetermined germline (Walbot, 1985); the germ cells are produced during plant development from somatic cells, and, consequently, mutations occurring during somatic development can be inherited. Furthermore, in many cases, plant bioassays are the simplest and most cost effective among test-systems for an environmental assessment.

Effects of contaminants on biota first appear in the cellular level making cellular responses not only the first manifestation of harmful impact, but also suitable tools for an early and reliable detection of exposure. Cellular changes would initially be less obvious than the direct visible effects of pollutants, but in the long run they could be more significant. At ecological risk assessment, basic subjects for protection are populations, communities, and ecosystems, and thus, the biomarker response should be tightly linked to effects in these biological systems. It is becoming increasingly clear (Theodorakis et al., 1997) that cellular alterations may afterwards influence biological parameters important for populations such as health and reproduction. These types of effects are of special concern because they can manifest themselves long after the source of contamination has been eliminated. Therefore, it is genetic test-systems that should be used for an early and reliable demonstration of the alterations resulting from human industrial activity.

Nonlinearity of Dose-Response Relationship

Effects detected in field observations are usually difficult or impossible to relate to specific contaminants or their sources in the environment because of the influence of noncontaminant-mediated factors. In such cases, laboratory studies may sometimes be important stage for establishing a chain of causality. An exposure to low doses and dose-rates of ionizing radiation is one of the inevitable factors in the current environment, and biota, including man, is chronically subjected to low-level radiation. Understanding the risks of low doses of radiation is also important with regard to various issues such as cancer screening, occupational exposure, frequent-flyer risks, and the future of nuclear power. For example, most radiological examinations produce doses in the range from 3 to 30 mSv (Brenner et al., 2003). Since the concept of radiation protection for humans and biota should be based on a clear comprehension of the consequences of low-level exposure, a correct estimation of the effects of low doses is an important topic.

A review of published and own data showed (Geras'kin, 1995) that the regularities of cytogenetic disturbances at low-level radiation are often characterized by a sound nonlinearity and have universal character. To verify this conclusion, a study was undertaken on cytogenetical damage occurrence in meristem cells of irradiated barley seedlings (Geras'kin et al., 2007). Aberrant cells frequency obtained is presented in Fig. 1 in dependence on dose and shows a deviation of linearity. Cytogenetic damage increases the control level at a dose of 50 mGy and above, but stays at the same level in the dose range of 50–500 mGy.

How important is the deviation of linearity observed in this study? It could be answered from a comparison of linear and non-linear models on their potential to fit the data obtained. At looking for the best model for data approximation, it is important, however, to get an improvement in the goodness-of-fit not merely by means of a model complicating through additional terms, but achieving a mutual conformity between a biological phenomenon and its mathematical model. Figure 1 illustrates results of the data approximation with three of six examined mathematical models (Geras'kin et al., 2007). The piecewise linear (PL) model (2 in Fig. 1) supposes a non-linearity of a dose dependency through including a dose-independent plateau. For the given data set, the plateau limits are calculated as 83.4 and

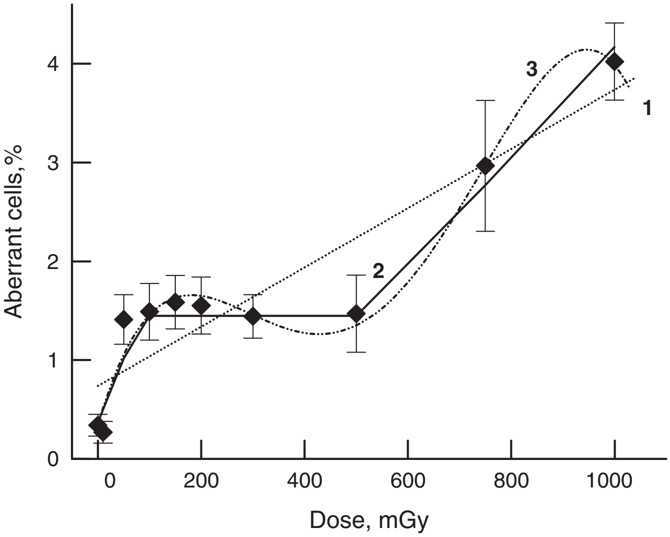

Fig. 1. Frequency of aberrant cells in barley seedlings (mean ± se) exposed to low-radiation doses and approximation of the data with linear (1), piecewise linear (2) and 4th degree polynomial (3) models.

513.7 mGy. The comparison of approximation quality by the most common quantitative criteria (Geras'kin et al., 2007) shows that the PL model statistically surpasses all the other tested variants. In particular, it fits the data significantly better than the linear model, according to the Hayek criteria ($p<5\%$). And this improvement is reached not through model complicating. Indeed, the criterion of structural minimization penalizing a model for any additional free parameter (Geras'kin and Sarapul'tzev, 1993) shows that, despite of five free parameters, the PL model is more advantageous than the linear model with only two parameters.

The current radiological protection practice is based on the linear non-threshold extrapolation of effects into the range of low-level exposures, which has been justified by a lack of reliable data on the effects of low doses. This approach, however, has been widely questioned recently because of many doubts in its consistency with knowledge and last data available. The findings presented here give further evidence that biological response to low dose exposures could essentially deviate of the linear non-threshold dependency. Consequently, it is necessary either scientifically justify the use of the LNT, or, having proved it is not scientifically robust, to develop a new approach that would be fair at different levels of radiation exposure.

Deviations of Additivity at Multipollutant Exposures

Ecosystems will often be polluted with a mixture of pollutants rather than a single pollution. Despite this, and as a result of time and financial constraints, toxicity testing has generally been restricted to studying the effects of single pollutants on a target organism under controlled conditions. Recent research has shown (Howard, 1997) that the synergistic effects among pollutants are much more dramatic than was previously thought. In most environmental situations, potentially harmful substances present at low doses and concentrations; nevertheless, a risk of such impact should not be underestimated as synergetic effects are most often registered at a combination of pollutants at low levels. This was, in particular, demonstrated in our combined-effect studies with a number of common stressors like acute and chronic γ-radiation, heavy metals, pesticides, artificial and heavy natural radionuclides; there were used different plant species such as spring barley, bulb onion, spiderwort and others. Moreover, at certain conditions, nonlinear effects were found to contribute significantly to a plant response (Evseeva et al., 2003a; Geras'kin et al., 2005a).

A study of cytogenetic disturbances induction in intercalary meristem cells of spring barley grown on soil contaminated with radioactive caesium (^{137}Cs) and one of heavy metals, Cd or Pb, can be an example illustrating an importance of non-linear interaction of damage caused by different factors (Geras'kin et al., 2005a). Table 1 presents interaction coefficients calculated

TABLE 1. Interaction coefficient for an endpoint of aberrant cell frequency in root meristem of barley at combined ^{137}Cs + Cd and ^{137}Cs + Pb soil pollution

	Cd, mg/kg			Pb, mg/kg		
^{137}Cs, kBq/kg	2	10	50	30	150	300
4.92	1.84*	1.74**	1.46**	0.82	0.74	0.75
24.6	0.76*	0.63**	0.65	0.61	0.54*	0.53*
49.2	0.36**	0.32**	0.50*	0.52*	0.54**	0.61*

Note: Interaction coefficient differs from 1.
*$p < 5\%$;
**$p < 1\%$.

as a ratio of increments in cytogenetic effect observed at combined pollution, and expected from an additive hypothesis. In a case of ^{137}Cs + Cd soil contamination, the interaction coefficient differs of 1 for eight of nine tested mixtures, so, there are significant deviations of additivity (Table 1). When the lowest ^{137}Cs specific activity of 4.92 kBq/kg was combined with any of the cadmium concentrations, significant synergistic effect was observed. It is of special importance as such ^{137}Cs specific activity occurs in the territories contaminated by the Chernobyl accident. At the ^{137}Cs–Pb combined exposure, the interaction effects are also essential; when these contaminants are applied at high concentrations, the confident antagonisms are registered. These findings show that a forecast of cytogenetic consequences for combined exposures based on an additive model would be incorrect and cause essential deviations from experimentally observed data.

Short-Term Laboratory Studies

The earlier presented examples emphasize that assessments of environmental risks based solely on physical-chemical control methods are often inadequate to an actual situation, and an integrating of bioindication assays could improve the system of ecological monitoring. In most bioindication studies, standard indicator plant species such as *Tradescantia, Allium cepa* or *Vicia faba* were used. Among the test-systems suitable for toxicity monitoring, the *Allium*-test is well known and commonly used in many laboratories. Results from the *Allium*-test have shown a good agreement (Fiskesjo, 1985) with results from other test-systems, eukaryotic as well as prokaryotic. As a genotoxicity test, the *Allium*-based assay of chromosome aberration in anaphase-telophase is for many reasons especially useful for the rapid screening of pollutants posing environmental hazards. In addition, a good toxicity

TABLE 2. *Allium*-test application for the environment quality assessment

Site	Contamination	Results
Radium production industry storage cell territory, Komi Republic, Russia	Heavy natural radionuclides & chemical pollution	All water samples caused a significant increase in the chromosome aberration frequency. Genotoxic effect was a result of chemical toxicity mainly (Evseeva et al., 2003b)
Underground nuclear explosion site, Perm region, Russia	Radionuclides	^{90}Sr significantly contributes to the induction of cytogenetic disturbances (Evseeva et al., 2005)
Radioactive waste storage facility, Obninsk, Russia	Radionuclides & chemicals	All water samples caused a significant increase in the chromosome aberration frequency. Genotoxic effect was a result of chemical toxicity mainly (Oudalova et al., 2006)
Upper Silesian Coal Basin, Poland	Heavy natural radionuclides & chemical pollution	All water and sediment samples caused a significant increase in the chromosome aberration frequency. Ra, Ba, Sr, and Cu contribute significantly to the induction of cytogenetic disturbances
Semipalatinsk Test Site, Kazakhstan	Radionuclides	Data analysis in progress

indicator is given by the mitotic index. In our laboratories, several studies on the environment quality assessment have involved the *Allium*-test, and some results are briefly summarized in Table 2. In all these studies, chemical and biological control methods were applied simultaneously. This helps in identifying relationships between the pollutant concentrations and biological effects they cause.

As an illustration of the *Allium*-test application, the frequency of aberrant cells in root meristem of sprouted bulbs, grown on sediment sampled from the post-mining areas of the Upper Silesia, is presented in Fig. 2. Cytogenetic damage to meristem cells of *Allium cepa* is evident in all non-control variants (1–4) which are significantly higher than the control value of variant 5. So, the clear genotoxic effect of sampled sediment is shown. Also, an important contribution of such severe types of cell damage as chromosome bridges and laggings is demonstrated.

Concentrations of 12 heavy metals and 4 radionuclides are measured in the sediments. All samples contain extremely high concentrations of Ba, from 5 to 50.5 times over the maximum permissible level. In variants 3 and 4, concentrations of Ba, Cu, Mn, and Zn are above the permissible limits. Specific activities of radium nuclides in the samples from the underground gallery (variant 1) and the bank of the Rontok pond (variant 2) are

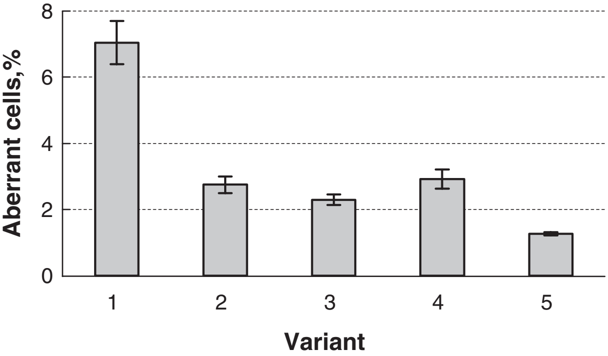

Fig. 2. Aberrant cells frequency in root meristem cells of *Allium cepa*, grown in sediment sampled from the post-mining areas in the Upper Silesia, Poland. Sampling variants: (1) underground galleries, (2–4) settling ponds, (5) control.

over the corresponding values of the minimum significant specific activity. Key pollutants governing biological effect observed are revealed, through an adaptation of mathematical and statistical techniques of multivariate analysis, including correlation analysis, step-by-step inclusion/exclusion of essential predictors, and multivariable linear regression. From preliminary assessments, the best model to describe an aberrant cell's occurrence in root meristem cells of *Allium cepa* germinated on the sediment sampled is

$$Y\,(\%) = (3.32 \pm 0.28) - (0.104 \pm 0.009)[\mathrm{Cu}] + (2.10 \pm 0.18)10^{-2}\,[\mathrm{Ba}] \\ + (4.94 \pm 0.46)10^{-4}\,[\mathrm{Ra}_{\Sigma}],$$

where [Cu] and [Ba] are concentrations of chemical elements in soil, in mg/kg; $[\mathrm{Ra}_{\Sigma}]$ is total specific activity of Ra isotopes analysed in soil, in Bq/kg; the determination coefficient $R^2 = 60.2\%$; Fisher statistics $F = 49.5$.

Field Studies on Wild Plant Populations and Crops

The other possible way to assess the quality of the environment is the use of plants directly growing in contaminated sites. This approach is particularly useful for assessing long-term ecotoxical effects induced by chronic low dose-rate and multi-pollutant exposure at contaminated sites. Up to now we have known little about responses of plant and animal populations to environmental pollutants in their natural environments. Although radionuclides and heavy metals cause primary damage at the molecular level, there are emergent effects at the level of populations, non-predictable solely from the knowledge of elementary mechanisms of the pollutants' influence. The usefulness of data gathered both in laboratory-based studies and field-based monitoring observations may, therefore, be significantly affected by our present lack of knowledge in this area of environmental research. Previously completed and ongoing field studies on biological effects in different species of wild and agricultural plants are briefly summarized in Table 3.

With each passing year since the Chernobyl accident of 1986, more questions arise about the potential for organisms to adapt to radiation exposure. It is often thought to be attributed to somatic and germline mutation rates in various organisms. Studies into the mechanisms of plant

TABLE 3. Field studies on wild and agricultural plants

Species	Site & Time	Contamination	Assay and/or Endpoints
Winter rye and wheat, spring barley and oats	10 km ChNPP zone (11.7–454 MBq/m^2),1986–1989	Radionuclides	Morphological indices of seeds viability, cytogenetic disturbances in intercalar and seedling root meristems (Geras'kin et al., 2003a)
Scots pine, couch-grass	30 km ChNPP zone, (250–2690 µR/h), 1995	Radionuclides	Cytogenetic disturbances in seedling root meristem (Geras'kin et al., 2003b)
Scots pine	Radioactive waste storage facility, Leningrad Region, Russia, 1997–2002	Mixture	Cytogenetic disturbances in needles intercalar and seedling root meristems (Geras'kin et al., 2005b)
Wild vetch	Radium production industry storage cell, Komi Republic, Russia, (73–3300 µR/h), 2003–2006	Heavy natural radionuclides & chemical pollution	Embryonic lethals, cytogenetic disturbances in seedling root meristem (Evseeva et al., 2007)
Scots pine	Sites in the Bryansk Region radioactively contaminated in the Chernobyl accident (451–2344 kBq/m^2), 2003–2006	Radionuclides	Cytogenetic disturbances in seedling root meristem, enzymatic loci polymorphism analyses
Scots pine	10 km ChNPP zone (1100 µR/h), 2004	Radionuclides	Morphological modifications in pine needles, cytogenetic disturbances in seedling root meristem
Koeleria gracilis Pers., Agropyron pectini forme Roem. et Schult.	Semipalatinsk Test Site, Kazakhstan, (74–3160 µR/h), 2005–2006	Radionuclides	Cytogenetic disturbances in seedling root meristem

adaptation to environmental stresses and increased radiation levels still lag far behind many other areas of plant molecular biology. Adaptation is a complex process (Kovalchuk et al., 2004) by which populations of organisms respond to long-term environmental stresses by permanent genetic change. Studies of multiple generations exposed to radiation have rarely been undertaken due to the difficulties of creating a suitable model to study the effects of chronic exposure. While limited in number, these radioecological studies have attracted a great deal of interest in the question of how organisms adapt to ionizing radiation. In 1987–1989, an experimental study on the cytogenetic variability in three successive generations of winter rye and wheat, grown at four plots with different levels of radioactive contamination, was carried out within the 10 km ChNPP zone (Geras'kin et al., 2003a). In the autumn of 1989, aberrant cell frequencies in intercalary meristem of winter rye and wheat of the second and third generations significantly exceeded these parameters for the first generations (Table 4). In 1989, plants of all three generations were maintained in the identical conditions and accumulated the same doses, which is why the most probable explanation of the registered phenomenon relates to a genome destabilization in plants grown from radiation-affected seeds. Such detailed analysis of several generations of plants exposed to radiation provides some insight into possible mechanisms of plant adaptation to chronic radiation exposure. It relates to higher-order ecologic effects, as well as to contaminant-induced selection of resistant phenotypes. From these viewpoints, the results observed in (Geras'kin et al., 2003a) and indicating a threshold character of the genetic instability induction may be a sign of an adaptation processes beginning. That is, the chronic low dose irradiation appears to be an ecological factor creating preconditions for possible changes in the genetic structure of a population.

The adaptation processes in impacted wild plant populations were also investigated within the framework of other studies. The results of these experiments indicate (as an example, see Table 5) that an increased level of cytogenetic disturbance is a typical phenomenon for plant populations growing in areas with relatively low levels of pollution.

In 1949–1963, 116 air and ground-surface explosions for nuclear and hydrogen bomb testing was carried out in the Semipalatinsk Test Site. An ongoing study of *Koeleria gracilis* Pers populations, a typical wild crop for Kazakhstan, has shown that not only did the total frequency of cytogenetic disturbances increase significantly with the dose-rate measured in sampling points (Fig. 3A), but the relative contributions of such severe disturbances as double (chromosome) bridges and tripolar mitoses increased as well (Fig. 3B).

Forest trees have gained much attention in recent years as nonclassical model eukaryotes for population, evolutionary and ecological studies

TABLE 4. Aberrant cells in three successive generations of winter rye and wheat, grown on contaminated plots within the 10 km ChNPP zone

Generation	$D_{87–88}$, Gy	$D_{88–89}$, Gy	D, Gy	Aberrant cell frequency, %		$D_{87–88}$, Gy	$D_{88–89}$, Gy	D, Gy	Aberrant cell frequency, %	
				Rye	Wheat				Rye	Wheat
		Plot 1					Plot 2			
X_1			0.01	20.2 ± 1.8	18.0 ± 1.7			0.09	20.6 ± 1.8	20.6 ± 1.8
X_2		0.11	0.01	23.8 ± 1.9	26.0 ± 2.0*		0.95	0.09	31.0 ± 2.1*	29.2 ± 2.0*
X_3	0.18	0.11	0.01	27.2 ± 2.0	26.4 ± 2.0*	1.62	0.95	0.09	35.8 ± 2.1*	30.0 ± 2.0*
		Plot 3					Plot 4			
X_1			0.06	23.4 ± 1.9	21.2 ± 1.8			0.04	22.8 ± 1.9	25.8 ± 2.0
X_2		0.64	0.06	28.8 ± 2.0	27.0 ± 2.0		4.17	0.04	33.0 ± 2.1*	34.0 ± 2.1*
X_3	1.11	0.64	0.06	30.0 ± 2.0	31.4 ± 2.1*	7.17	4.17	0.04	32.6 ± 2.1*	33.4 ± 2.1

Note: D is the dose, accumulated from planting in autumn 1989 to the sampling time; $D_{87–88}$, $D_{88–89}$ are the doses, accumulated by parent plants during the whole vegetative period from planting up to harvesting in 1987–1988 and 1988–1989 years, respectively.

X_1-generation – plants grown from intact seeds and accumulated dose D from planting in autumn 1989 to the sampling time;

X_2-generation – parent plants were sown in 1988, harvested in summer 1989 and planted again on the same plots in autumn 1989. Genetical effects are the result of both the ancestral dose $D_{88–89}$ and current exposure D;

X_3-generation – parent plants grew on the same plots in 1987–1988 and in 1988–1989 and accumulated doses $D_{87–88}$ and $D_{88–89}$, correspondingly. Seeds harvested in 1989 were sown again in autumn 1989 and plants of the X_3 generation got dose D.

*Significance of variation from the level of cytogenetic disturbances in the X_1 generation: $p < 5\%$.

TABLE 5. Aberrant cell frequency in seedling root meristem of Scots pine growing in the Bryansk Region of Russia, radioactively contaminated as a result of the Chernobyl accident

Test site	^{137}Cs contamination density, kBq/m^2	Dose-rate, mGy/year[a]	Aberrant cells (mean ± se), %	
			2003	2004
Reference	–	0.14	0.90 ± 0.09	0.88 ± 0.09
VIUA	451	7.40	1.47 ± 0.15*	1.59 ± 0.14*
Starye Bobovichy	946	15.3	1.32 ± 0.12*	1.37 ± 0.14*
Zaborie 1	1730	28.3	1.69 ± 0.17*	1.67 ± 0.17*
Zaborie 2	2340	37.8	1.63 ± 0.15*	1.68 ± 0.17*

Note: Seeds were collected in 2003 and 2004;

* Difference from the reference population is significant, $p < 5\%$

[a]Absorbed doses are estimated for γ- and β-radiation

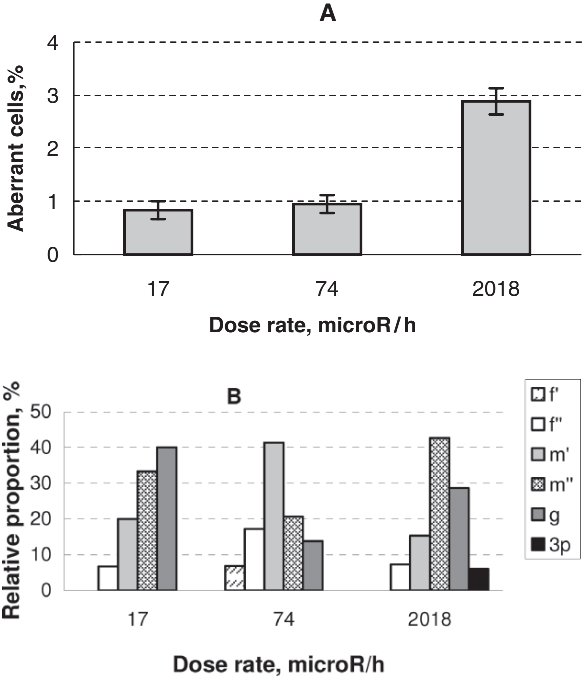

Fig. 3. Frequency of aberrant cells (A) and relative spectrum of aberrations (B) in coleoptiles of *Koeleria* seedlings collected in the Semipalatinsk Test Site, Kazakhstan.

(Gonzalez-Martinez et al., 2006). Because of low domestication, large open-pollinated native populations, and high levels of both genetic and phenotypic variation, they are ideal organisms to unveil the genetic basis of population adaptive divergence in nature. In the field study (Geras’kin et al., 2005b), Scots pine populations were used for an assessment of the genotoxicity originating from an operation of a radioactive waste storage facility. Specifically, frequency and spectrum of cytogenetic disturbances in reproductive (seeds) and vegetative (needles) tissues were studied to examine whether Scots pine trees experienced environmental stress in areas with relatively low levels of pollution. The temporal changes of the cytogenetic disturbances in seedling root meristem from 1997 to 2002 are shown in Fig. 4. There are essential differences between these relationships for the reference and impacted Scots pine populations. Statistical analysis revealed (Geras'kin et al., 2005b) that cytogenetic parameters at the reference site (Bolshaya Izhora) tend to follow cyclic fluctuations in time, whereas in technogenically affected populations ('Radon' LWPE and Sosnovy Bor) this relationship could not be revealed with confidence. Thus, man-made impact in this region is strong enough to destroy natural regularities.

Pollution is a well-documented selective force (Breitholtz et al., 2006), which has been found to induce metal tolerance in plants, pesticide resistance in insects, and pollution tolerance in a variety of aquatic organisms. To study possible adaptation processes in the impacted pine tree populations, a portion of the seeds collected were subjected to an acute γ-ray exposure (Geras'kin et al., 2005b). The seeds from the Scots pine populations experiencing man-made impacts showed (Table 6) a higher resistance than the reference ones. Although the picture of adaptation is far from complete, there is convincing proof (Shevchenko et al., 1992) that the divergence of populations in terms of radioresistance is connected with a selection for changes in the effectiveness of the repair systems.

It is well known (Macnair, 1993; Rajakaruna, 2004) that genetic adaptation in plant populations to extreme environmental conditions can take place quite rapidly, even within a few generations. But this rule does not apply to all the cases. A study of the wild vetch (*Vicia cracca* L.) population for more than 40 years inhabiting a site with an enhanced level of natural radioactivity

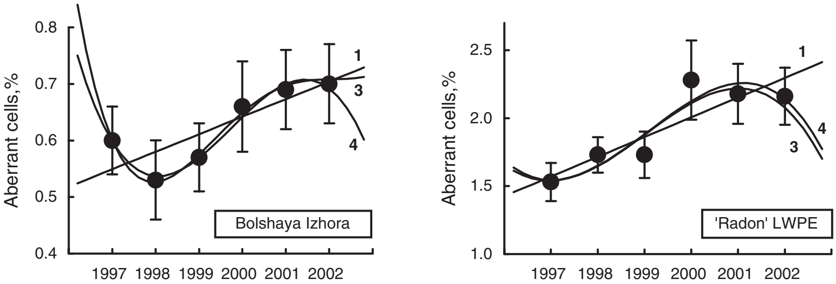

Fig. 4. Aberrant cells percentages in seedling root meristem of Scots pine trees in dependence on year and their approximation by the best models. (1) linear model, (3) and (4) polynomial models of the 3rd and 4th degrees, correspondingly.

TABLE 6. Aberrant ana-telophases frequency in root meristem of seedlings grown from seeds sampled from the reference and impacted Scotch pine populations in the Leningrad Region of Russia and exposed to acute γ-ray dose of 15 Gy

	1999		2000		2001	
Site	Cells total	Aberrant cells, %	Cells total	Aberrant cells, %	Cells total	Aberrant cells, %
1	3536	7.01 ± 0.43	2350	6.94 ± 0.52	1372	7.07 ± 0.69
2	4913	3.44 ± 0.26*	3383	3.55 ± 0.32*	2661	3.53 ± 0.36*
3	4688	3.56 ± 0.27*	2541	3.74 ± 0.38*	3009	3.69 ± 0.34*

Note: 1 is the reference site (Bolshaya Izhora); 2 is the urban area (Sosnovy Bor settlement); 3 is the territory of a radioactive waste storage facility ('Radon' LWPE).
* Difference from the reference site of Bolshaya Izhora is significant; $p < 5\%$

TABLE 7. Embryonic lethal and chromosome aberration frequency in seedling root meristem of wild vetch inhabiting the site with enhanced level of natural radioactivity (the Komi Republic) (Evseeva et al., 2007)

	Dose-rate, μR/h	Chromosome aberrations frequency, %	Embryonic lethals, %
Reference	9	0.80 ± 0.07	24.62 ± 2.12
Plot 1	3300	1.33 ± 0.12*	33.63 ± 23.32*
Plot 2	2400	1.00 ± 0.09	35.38 ± 2.06*
Plot 3	758	0.87 ± 0.10	25.17 ± 2.24
Plot 4	73	0.98 ± 0.10	24.60 ± 2.08

Note: Seeds were collected in 2003;
*difference from the reference population is significant, $p < 5\%$

showed that transformations of genetic structure in the populations are still ongoing (Evseeva et al., 2007). In this site, both low doses of external exposure and incorporated heavy natural radionuclides have significant effects on the variability in plant populations and their potential for an adaptation. As a selection factor, external exposure results in an increasing of the frequency of embryonic lethal mutations (Table 7). In addition, in contrast with data from the pine populations study (Geras'kin et al., 2005b), the acute γ-irradiation demonstrated rather high radiosensitivity of seeds from the impacted populations of wild vetch. The inherited character of the enhanced radiosensitivity was shown in (Alexakhin et al., 1990). At this point, it is possible to suppose that a probable cause of special features found in this site in the Komi Republic relates to a specific radioecological situation within the uranium–radium anomaly, where a decisive role is played by α-emitters. However, the mechanisms involved in this plant response remain to be studied in more detail.

Conclusions

The increasing degradation and pollution of the environment requires the establishment of biological sentinel systems that provide information on adverse effects on ecosystems and on human health. Findings presented here clearly indicate that an adequate environment quality assessment cannot rely only on information about pollutant concentrations. This conclusion emphasizes the need to update some current principles of ecological standardization, which are still in use today. When assessing potential hazards from radionuclide and chemical pollution for ecosystems, a harmonized approach based on ecotoxicology principles should be applied. At the first stage, it is advisable to use biological testing that result in an integral assessment of effects from all substances presented in the environment. If bioassays give positive responses, a more detailed survey should be undertaken including physical-chemical analysis, geochemical barriers, and study of migration parameters of the contaminants for a certain landscape. An effective linking of bioindication screening assays to well-established environmental pollution monitoring is a way of improving and upgrading an existing system of public and environment protection in order to meet requirements of consistency between current scientific knowledge and decision-making processes.

A better understanding of the genetic aspects of population, community and the whole ecosystem responses to toxic agent's exposure is vital to future environmental management programs. The results described here provide evidence that plant populations growing in areas with relatively low levels of pollution are characterized by the increased level of both cytogenetic disturbances and genetic diversity. Man-made pollution may influence an evolution of exposed populations through a contaminant-induced selection process. The long-term existence of some factors (either of natural origin or man-made) in the plants' environment activates genetic mechanisms, changing a population's resistance to exposure. However, in different radioecological situations, genetic adaptation to extreme edaphic conditions in plant populations could be achieved at different rates. Such evolutionary effects are of special concern because they are able to negatively affect population dynamics and local extinction rates. These processes have a genetic basis; therefore, understanding changes at the genetic level should help in identifying more complex changes at higher levels. Recent studies have shown (Whitham et al., 2006) that heritable traits in a single species have predictable effects on community structure and ecosystem processes. Therefore, we can begin to apply the principles of population and quantitative genetics to place the study of complex communities and ecosystems. 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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