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Assessment of the Ecological Risk of Technogenic Soil Pollution on the Basis of the Statistical Distribution of the Occurrence of Micromycete Species

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Abstract—A methodology of substantiating the ecological risk of technogenic soil pollution has been described according to the results of bioindication using microfungal communities. At the first state, the values of the factors under which the frequency of each species is maximal were estimated using ordination methods. The statistical distribution of the species sensitivity was simulated according to these data. The methodology was illustrated using the results of analysis of the toxicity of soil samples from uranium mine dumps (the village of Kadzhi-Sai, Kyrgyzstan). The preliminary estimates of the critical values of six soil pollution indices that provide the defined allowable probability of the ecological risk have been given.

Keywords: bioindication, fungi, ecological risk, technogenic soil pollution, multidimensional nonmetric scaling, species occurrence distribution

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The current methodology of substantiating ecological risks is to reveal the critical levels of effects that are treated "as the beginning of the most rapid transformation of an ecosystem or as a point after which the main components of biocenosis begin to drop out or the systemic relationships begin to break down [1].

Soil micromycetes are one of the most extensive and various ecological groups of organisms that are used for the biodiagnosis of the ecological state of biotopes as well as for regulating effects and assessing ecological risks [2–6]. The effect of technogenic pollution on fungal communities is expressed in different forms. Thus, heavy metals in large concentrations inhibit processes of mineralization in soils, suppress respiration, cause a mycostatic effect, and can act as a mutagenic agent. They significantly influence the abundance and species composition of fungi; in particular, they increase the share of species that are pathogenic for plants, animals, and human beings. As in the case of heavy metals, radioactive contamination leads to a growth in the share of melanized fungi [7, 8].

An effective method of regulating technogenic pollutions in the area under study is to analyze the occurrence of indicator species. The decrease in the abundance of individual taxa and the species wealth of the biocenosis under study when the concentration of toxic ingredients or their mixture increase is usually described by statistical models of species sensitivity distribution (SSD).

Classically, the dependence of SSD [9] determines the sequence of threshold (critical) concentrations (HC_p) of a toxic agent that will be hazardous for *p* % of the total number of the most sensitive species in the biotope and ineffective for the other ones. The SSD curve is treated as an integral function of a theoretical distribution of probability density, the parameters of which are estimated according to the sampling of taxonomic groups that are provided with toxicometric indices (the semieffective LC_{50} or the no observed effect concentration (NOEC)), which were established, e.g., in the course of the laboratory biotesting of the spectrum of cultivated fungal species.

The statistical distribution of effective xenobiotic concentrations, *EC*s, for the probabilistic estimate of the ecological risk of soil pollution in an ecosystem under study is then simulated according to the results of field bioindication observations. In this case, the risk is quantitatively treated as the probability, $p\,(EC > HC)$, of the excess of harmful effect under real conditions over the analogical effect determined by SSD for any arbitrary species that was randomly chosen from the biocenosis being studied [10].

The method requires the performance of intensive laboratory studies, and sampling for SSD simulation should be ecologically and statistically representative for each toxicant being considered. In particular, the acceptable risk assessment is possible when the number of species being analyzed is not less than 30 [11]. Therefore, it is relevant to search for approaches that make it possible to perform a quick assessment of the ecological soil state only on the basis of the data of field observations without toxicometric experiments.

The objective of this study was to substantiate the method of the approximate estimate of ecological risks using ordination procedures and multidimensional smoothing models. The applicability of the methods being proposed is considered based on the example of the response of soil microbiota to technogenic pollution in the course of the open-cut mining of uranium-containing ore minerals.

MATERIAL AND METHODS

The integrated studies on soil pollution were carried out in the vicinity of the village of Kadzhi-Sai (Kyrgyzstan), where ore deposits with a low uranium content were developed from 1947 to 1965. All sample plots that are located under similar natural conditions in the southern shore of Lake Issyk Kul and in the Boom Gorge (42°08′48″ N, 77°11′10″ E) are represented by light-brown soils with a homogeneous vegetation cover. The biotopes of the control sites (conventionally clean areas that are located at the closest distance to the shore of the lake) and polluted sites of the uranium tailing are dominated by tarragon (*Artemisia dracunculus*) and Fedchenko wormwood (*Artemisia fedtschenkoana*); ephedra (*Ephedra intermedia*) also occurs here. Soil samples were selected in May 2014 using the envelope method in sample plots with an area of 100 m^2 from the same horizon with a depth of 0–20 cm (GOST 17.4.4.02-84). Point-based samples (with a weight of approximately 1 kg) from the same plot were intensively mixed and the combined sample was then reduced using quartering. The air-dry samples (0.3 kg) were stored at a temperature of +4°C. The soils under study contained 0.5–1.5% of humus and 0.1–0.2% of total nitrogen and their pH was 8.0–8.5.

We consider the level of technogenic impact on the area according to two groups of indices: the indices of radioactive concentration and the content of heavy metals in the soil. Slags in tailing dumps that are periodically denudated by mudflows and rains on mountain slopes are the source of pollution of the sites under study. The activity on the basis of the nuclear decomposition of two radionuclides (^{238}U and ^{226}Ra) was measured using a GX4019 gammaspectrometer (Canberra). The chemical soil composition with respect to 16 elements was analyzed using a DELTA Classic X-ray fluorescent spectrometer.

The total soil pollution with heavy metals (Z_c) was estimated as the geometrical mean value of concentration excess factors:

$$Z_c = n(K_1\,K_2 \ldots K_n)^{1/n} - (n - 1),$$

where n is the number of ingredients that were taken into account, $K_i = C_i/C_{ib}$, where C_{ib} and C_i are the background and actual contents of the ith element in the soil [12, 13]. The toxicity of the elements was recorded on the basis of the separate calculation of particular indices $Z_{c(i)}$ for three classes of hazard according to GOST 17.4.1.02-83: $Z_{c(1)}$ with a high hazard for As, Cr, Pb, and Zn; $Z_{c(2)}$ with a moderate hazard for Co, Mo, and Cu; and $Z_{c(3)}$ (which included background and rare-earth elements, namely, Ba, Ti, Fe, Mn, Sr, K, Ca, Rb, and Zr) with a low hazard. The total pollution index was calculated taking into account toxicity correction factors [14, pp. 9–11]:

$$Z_c = 1.5\,Z_{c(1)} + 1.0\,Z_{c(2)} + 0.5\,Z_{c(3)}.$$

The response of the ecosystem was assessed using the results of bioindication studies of micromycete communities in soil samples that had been collected from four sample plots in disturbed habitats and three sample plots in conventionally clean areas (control) (Table 1). Cultivated fungi were isolated on the basis of the standard method [15] of seeding soil water suspension from 10^2 dilutions (1 g of soil per 100 mL of water) on the agarized Chapik medium with streptomycin (100 mg/L) in three replications. The species were identified on the basis of cultural and morphological features using the contemporary identification guides and molecular properties of sterile isolates during the sequenation of their DNA. The number of identified micromycete species is 41.

The statistical processing for estimating the critical levels of effect and ecological risk was performed in two stages: (1) the calculation of the values of pollution factors that correspond to the maximum abundance of fungi of each species using ordination methods and (2) the approximation of the data of the curve of the theoretical probability of species occurrence.

Micromycete communities were ordinated using the nonmetric multidimensional scaling (NMS) algorithm [16]. We used the matrix of Manhattan distances between each pair of soil samples, which provided the maximum value of Spearman correlation coefficients ($p = 0.56$) between the found ordination and environmental factors compared to other distance metrics (Jaccard, Kul'chitskii, Bray–Curtis, and Euclid metrics). In the resulting ordination diagram (Fig. 1a), the mutual distances between the points S_1 and S_2 of the sample plots on the plane with latent axes were selected so as to introduce minimum distortions compared to the initial configuration of objects in the multidimensional space of species.

Table 1. Species composition of micromycetes that were identified in soil samples; IndVal—indices of the indicator value [25] that are statistically insignificant for background species and, therefore, are not given for them

Species abbreviation and names		IndVal
Species that are characteristic of control sites		
AltCh	*Alternaria chlamydospora* Mouch.	0.67
MyrVe	*Myrothecium verrucaria* (Alb. & Schwein.) Ditmar	0.67
PenCi	*Penicillium citrinum* Thom	0.57
PenJn	*P. janthinellum* Biourge	0.48
AcrCh	*Acremonium harticola* (Lindau) W. Gams	0.33
AltAl	*Alternaria alternata* (Fr.) Keissl.	0.33
AspSc	*Aspergillus sclerotiorum* G.A. Huber	0.33
MorIs	*Mortierella isabellina* Oudem	0.33
PenCa	*Penicillium canescens* Sopp	0.33
PenJa	*P. janczewskii* K.M. Zalessky	0.33
Species that are characteristic of polluted sites		
AspFu	*Aspergillus fumigatiaffinis* S.B. Hong, Frisvad & Samson	0.75
TriHa	*Trichoderma harzianum* Rifai	0.75
UloCo	*Ulocladium consortiale* (Thüm.) E.G. Simmons	0.75
AcrSt	*Acremonium strictum* W. Gams	0.50
CloRo	*Clonostachys rosea* (Link) Schroers, Samuels, Seifert & W. Gams	0.50
PsePa	*Pseudogymnoascus pannorum* (Link) Minnis & D.L. Lindner	0.50
SeiSp	*Seimatosporium* sp. Corda	0.50
FusOx	*Fusarium oxysporum* Schlecht	0.33
Background species		
AcrMu	*Acremonium murorum* (Corda) W. Gams	
ActEl	*Actinomucor elegans* (Eidam) C.R. Benj. & Hesselt	
AspNi	*Aspergillus niger* Tiegh.	
AurPu	*Aureobasidium pullulans* (de Bary & Löwenthal) G. Arnaud	
BotSp	*Botryotrichum* sp. Sacc. & Marchal	
CadSp	*Cadophora* sp. Lagerb. & Melin	
EpiNi	*Epicoccum nigrum* Link	
GeoLu	*Geomyces luteus* Kwasna, H & Bateman	
GonCa	*Gonytrichum caesium* Nees	
TriSp	*Trichoderma* sp. Pers.	
PenLi	*Penicillium lividum* Westling	
PhoMe	*Phoma medicaginis* Malbr. & Roum	
PhoPo	*Phoma pomorum* Thüm	
PurLi	*Purpureocillium lilacinum* (Thom) Luangsa-ard, Hywel-Jones & Samson	
RhiAr	*Rhizopus arrhizus* A. Fisch	
SarKi	*Sarocladium kiliense* (Grütz) Summerb.	
BasPe	*Basidioascus persicus* S. Nasr, M.R. Soudi, S.M. Zamanzadeh Nasrabadi, M. Moshtaghi Nikou	
StaCh	*Stachybotrys chartarum* (Ehrenb.) S. Hughes	
ApiMo	*Apiospora montagnei* Sacc.	
TriSp	*Trichurus spirales* Hasselbr.	
VerTe	*Verticillium tenerum* Nees	
EmbSp	*Embelissia* sp. E.G. Simmons	
AcrAl	*Acremonium alternatum* Link	

The positions of the maxima of the occurrence of fungi of each species on the influencing factor scale was calculated as follows:

(1) the weighted average coordinates s_1 and s_2 of individual micromycete taxa on the NMS-projection were estimated to determine their position with respect to the sample plots and the ordination diagram of species was built (Fig. 1b);

(2) a three-dimensional generalized additive model (GAM) was calculated for each pollution index Y according to its empirical values for each plot [17]:

$$Y = \alpha + f_1(s_1) + f_2(s_2) + f_3(s_1, s_2) + \varepsilon,$$

where f_1, f_2, and f_3 are functions in the form of smoothing polynomials with $k = 2$ degrees of freedom from NMS-coordinates s_1 and s_2;

(3) according to the constructed models, the predicted $\hat{Y}_j$ values that corresponded to the coordinates of the most probable position of each jth fungal species ($j = 1, 2, ..., 41$) on the NMS-projection were found;

(4) as presumably dangerous values of the factor for the jth species, we assumed Y values that are so high that they were hardly probable within the smoothing GAM model; i.e., the upper limits of confidence intervals $\hat{Y}_{E_j} = \hat{Y}_j + t_{\alpha/2}S_{\hat{Y}_j}$, where $t_{\alpha/2}$ is the inverse Student's distribution at $\alpha = 95\%$ and $S_{\hat{Y}_j}$ is the error of the predicted value $\hat{Y}_j$.

The resulting empirical distribution of ecological maxima of species, $\hat{Y}_j$, along the axis of the pollution index, $\hat{Y}_{E_j}$, was approximated for each type of pollution using the theoretical distribution of a continuous random variable. The choice of the best distribution from the set of possible pretenders (normal distribution, lognormal distribution, Weibull distribution, Cauchy distribution, etc.) and the estimate of its parameters were made on the basis of the maximum logarithm of the likelihood function. The confidence intervals were found for the cumulative distribution curves using a parametric bootstrap [18].

The calculations were made using the R v. 3.02 statistical environment and its software packages, vegan and mgcv [19].

RESULTS

Construction of Ordination Diagrams

Analysis of the species diversity of microfungi in the area under study showed a rather clear differentiation of the sample plots. According to the ordination diagram in Fig. 1a, the soil samples from the uranium mine dumps (2 and 3) and the Boom Gorge (14) occupied the extreme positions on the main axis S_1 of the nonmetric projection. The second ordination axis S_2 determined the variability in the structure of microbiota in the other habitats with the intermediate pollution level.

If we calculate the coefficients of correlation between soil pollution indices and projective coordinates, s_1 and s_2, we can draw additional axes of physical gradients that reflect the pattern and extent of the influence of each factor. Since the arrows of factor loadings that are given in Fig. 1a have approximately the same direction and length, it can be said that the components of technogenic pollution in the region under study form an interrelated multicollinear complex. The highest correlation, ($R^2 = 0.83$, $p = 0.022$), was recorded between the variation in the structure of micromycete communities (with respect to the frequency of occurrence of isolated species) and the content of cobalt in the soil (mg/kg) (see the isolations of the smoothing surface on the basis of the GAM model in Fig. 1a).

The ordination of habitats is closely correlated with the ordination of micromycete species groups (see Fig. 1b), where the location of each species was determined on the basis of the weighted average coordinates of some of its possible habitats. If we construct a three-dimensional smoothing surface for any of the soil pollution indices being analyzed, we can easily calculate the value of the factor for each species that is most likely to provide conditions for the emergence of the species. For instance, in Fig. 1b, the coordinates of the species *Trichoderma harzianum* (*TriHa*) are located close to the isoline with the Saet index $Z_c = 12$, which can be further used for simulating the probable distribution of species abundance.

Statistical Distribution of the Probability of Species Occurrence

The further calculations were performed for six indices of technogenic soil pollution that are given in Fig. 1a. The statistical distributions of maxima of occurrence, $F(\hat{Y})$, and effective concentrations, $F(\hat{Y}_E)$, for 41 micromycete species according to the scale of all factors being analyzed were subject to the lognormal law.

The examples of cumulative distribution curves are given in Fig. 2; they show the differences between the patterns of the response of microfungal communities under the effect of different factors. The general pattern is a significant decrease in the species wealth and diversity of the complex of soil micromycetes under the effect of heavy metals. The occurrence distribution with respect to the scale of radionuclide activity and cobalt content is rather uniform, while the species tolerance to the other heavy metals and integral index Z_c is more contrasting. At the same time, one can easily isolate groups of sensitive species and species with increased resistance to separate pollution forms.

If we set arbitrary critical properties, e.g., $p = \{5, 10, 16, 20, \text{and } 50\%\}$, the use of cumulative distribution curves, $F(\hat{Y})$ and $F(\hat{Y}_E)$, will allow us to esti-

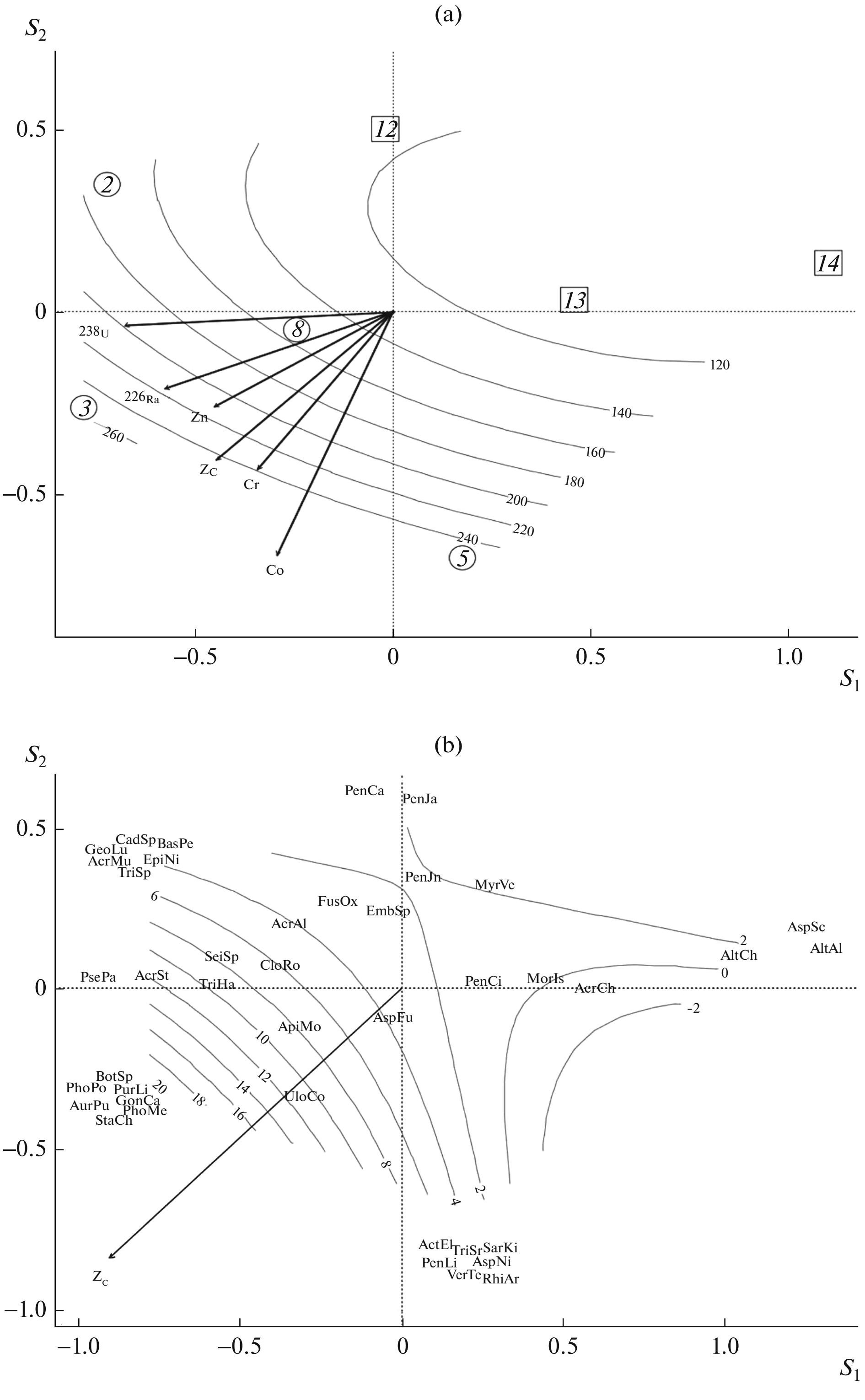

Fig. 1. Ordination of the obtained data using the method of multidimensional nonmetric scaling: (a) sample plots (polluted areas; the numbers of plots are given in circles): (2), (3), and (5) the dump area, (8) the residential area of the village of Kadzhi-Sai; conventionally clean areas (the numbers of plots are given in squares): (12) and (13) the shore of Lake Issyk Kul, (14) the Boom Gorge); (b) microfungal species (see the codes in Table 1). The arrows show the additional axes of physical gradients: the Saet index Z_c, the activity of radionuclides ^{238}U and ^{226}Ra in the soil, and the content of Co, Cr, and Zn. The isolines of the content of cobalt (a) and Saet index Z_c (b) that were calculated according to the additive model are shown.

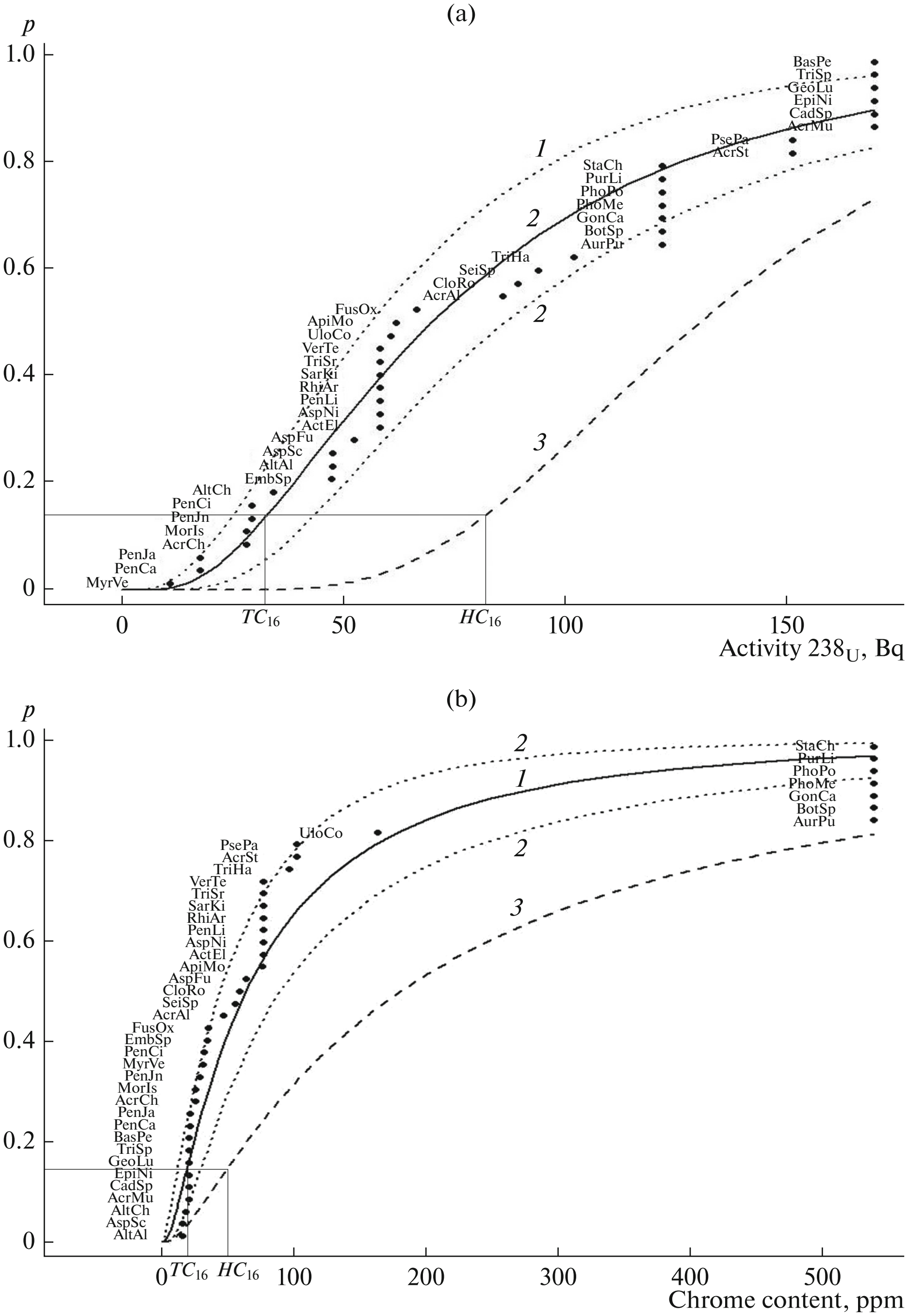

Fig. 2. Lognormal distribution of the probability p of the maximum occurrence $F(\hat{Y})$ of micromycete species (*1*) under different levels of effect of soil pollution factors: (a) the activity of radionuclide ^{238}U, Bq/kg, (b) the content of chrome cations, mg/kg; (*2*) the upper and lower envelopes of the 95% confidence region; (*3*) the species sensitivity distribution $F(\hat{Y}_E)$ if dangerous effects are reached.

mate the set of isoeffective values of tolerant (TC_p) and hazardous (HC_p) concentrations of the influencing factor. Their essence is that the exposure level that exceeds TC_p makes the ecological maximum of occurrence unattainable for p % of the total number of species; in addition, HC_p makes their presence in the community hardly possible (see examples in Fig. 2).

DISCUSSION

Undesirable events that cause an ecological risk may occur at the level of an ecosystem, a community, or separate species, populations, or individuals. The species sensitivity distribution (SSD) is associated only with one, narrowly determined segment of risk assessment, namely, a decrease in species diversity.

Table 2. Critical values of indices of soil pollution (HC_p) that were calculated according to the curve of distribution of micromycete species occurrence for different levels of risk (p %); TC_{16} are concentrations that make the maximum of occurrence unattainable for 16% of species (see Fig. 2)

Soil pollution indices	Range of observations	Tolerant TC_{16} values	Dangerous values		
			HC_5	HC_{10}	HC_{16}
Cobalt Co, mg/kg	110–261	130.3	141.3	160.0	176.5
Chrome Cr, mg/kg	15–362	20.6	24.0	37.5	53.4
Zinc Zn, mg/kg	11–382	11.3	12.1	22.31	36.2
Saet index	1.12–20	1.94	3.5	5.2	7.1
Activity of ^{238}U, Bq/kg	24–145	34.8	64.5	75.3	85.1
Activity of ^{226}Ra, Bq/kg	24–134	34.4	81.2	91.9	101.4

However, the methodological solution of this problem is far from being completely achieved. It is necessary and desirable to use the toxicometric indices (NOEC and EC_{50}); however, their common determination is almost unrealistic for different ecotoxicants and numerous soil biota species. Another important argument against the traditional use of biotests is the mechanical transfer of chemical exposure conditions during laboratory tests to the conditions of a specific natural ecosystem. Analysis of SSD does not use information on the ecology of communities (interspecific interactions, trophic chains, habitat conditions, or the relative significance of key species and functional groups); therefore, a relevant alternative is to estimate the thresholds of toxicity under the conditions of a passive procedure of sampling from a natural ecosystem.

The purpose of regulation on the basis of the collective response of soil communities is to select the value of the HC_p concentration of a toxicant for the predefined risk level, taking into account the required safety coefficient, that is treated as the level of loss of tolerance for p % of species in the community that is used for bioindication. In this study, we associated HC_p with the confidence regions of points on the smoothing surface. Table 2 provides the critical values of soil pollution indices that were calculated taking into account this assumption for different levels of ecological risk (5, 10, and 16%).

Two conditions make us consider the threshold values in Table 2 only as preliminary ones. First, the estimate of the tolerant ranges of species occurrence is more correct when generalized regression models are used for each of them [20]; however, this requires several dozens of observations under the conditions of an ecosystem being studied. Second, it is not clear what share of p losses from the total number of species should be considered critically dangerous for the ecosystem. For micromycete communities, we propose to use the values of the 16% level in the first approximation that were calculated with respect to the $F(\hat{Y}_E)$ curve.

Another uncertainty during the regulation of technogenic effects is associated with a high spatial heterogeneity of technogenic soil pollution, which, undoubtedly, affects the number, abundance, and distribution of fungal species [21–23]. The results of bioindication significantly depend on the redistribution of the content of pollutants under the influence of precipitation within the microrelief as well as on the position of sample plots, the variability of the assimilation capacity and biological soil activity, etc. All of this requires the arrangement of a large number of observations.

Point-based observations in geographical coordinates for compensating random fluctuations can be performed using Kriging models [24]. We propose to construct a smoothing surface after projecting the source data to the plane with abstract axes that are directly associated with the species structure of the indicating community (thereby, actually refusing from natural spatial coordinates). In this case, the use of the multidimensional nonmetric scaling method allows the construction of smooth and stable smoothing surfaces.

CONCLUSIONS

On the basis of the analysis of the species structure of microbiota in soil samples that were collected from the sample plots of the uranium Kadzhi-Sai province (Kyrgyzstan) with different degrees of heavy metal and radionuclide pollution, we showed the possibility of a quick assessment of ecological risks using the species sensitivity distribution. The proposed algorithm makes it possible to approximately estimate the ecological optimum and range of tolerance to an influencing factor for each species under specific field conditions on the basis of the generalized statistical model. The approximation of these results of the cumulative lognormal distribution curve makes it possible to predict the percentage p % of the total number of species that will disappear from the composition of the community under a specific value of the HC_p index of soil pollution (toxicant concentration). As an example, we calculated the critical values of six polluting soil elements for the predefined levels of the ecological risk (5, 10, and 16%).

The main idea of our study was not so much to determine the specific values of concentrations of the identified toxicants but to experimentally substantiate the methodology of assessing the quality of the medium with respect to a decrease in the diversity and changes in the species structure of the entire community in response to the effect of a regulated pollutant dose. In our opinion, this approach successfully combines in situ bioindication studies themselves and toxicological laboratory experiments in which effective (threshold) toxicant concentrations are determined.

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