Pol. J. Environ. Stud. Vol. 31, No. 3 (2022), 2256-2272
DOI: 10.15244/pjoes/143509 ONLINE PUBLICATION DATE: 2022-02-28

Original Research

Ecological Assessment of Technogenically Disturbed Soils of the Mountain Ecosystems of Kyrgyz Republic Based on the TRIAD Method

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Received: 12 July 2021
Accepted: 31 October 2021

Abstract

Comprehensive studies of soil contamination were performed in the area of settlement Kichi-Kemin (Kyrgyz Republic). Soils of the Kyrgyz Republic are exposed to waste (tailings) of many industrial enterprises of the mining industry not far from the village of Ak-Tuz, located 145 km from the capital of Kyrgyz Republic, Bishkek, at an altitude of 2300 m above sea level. It is noted that 4.17 million tons of radioactive waste from one of the enterprises were once disposed of at four tailings in the Kichi-Kemin Valley. The main waste elements are radioactive thorium, heavy metals such as cadmium, molybdenum, lead, zinc, beryllium, and oxides of hafnium and zirconium. The impact of these wastes on soil ecotoxicity and microbial communities is not yet well understood. In this work, soil environmental assessment near waste deposits were investigated using an integrated method known as the Triad approach. The integral index of soil disturbance was calculated from the data of ecological observations of soil microbial communities (bioindication), data of ecotoxicological index by phytotesting approach (bioassay), and chemical index reflecting the results of a quantitative chemical analysis of the content of pollutants. The ecological index, calculated from the bioindication parameters of the soil microbiota communities, has become a reputable indicator of the state of soils from vulnerable mountain ecosystems. Studies of soil microorganisms have shown a decrease in species diversity in contaminated soils. The most resistant species of fungi were *Aspergillus* and *Penicillium*. Among the actinomycetes of the genus Streptomyces, the *Albus* and *Cinereus* sections proved to be stable. Estimation of anthropogenic impacts on mountain ecosystems in the Kyrgyz Republic using the Triad approach has shown that the most sensitive toxicity index in the soils of the Ak-Tuz is the ecotoxicological

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indicator EtoxRI; determined by the phytotesting method. The integrated index calculated on the basis of the Triad method gave a more complete picture of the influence of pollutants on the soils ecosystem of the Ak-Tuz, characterizing its severe deterioration.

Keywords: Triad approach, phytotesting, heavy metals, soil, tailings

Introduction

The territory of Kyrgyz Republic is subject to the impact of waste from various enterprises–primarily the mining industry. Bulk waste that contains radioactive elements and heavy metals, as a rule, are displaced on the soil. The ability of heavy metals to move in soil and to bioaccumulate by living organisms, thereby increasing their toxicity, makes them one of the greatest global environmental problems [1]. Heavy metal pollution can inevitably affect the environmental quality of soil or water over time [2, 3]. The awareness of risks assessment shall be a priority considering constant increase in heavy metal and general environmental pollution globally in soil, water and air [4]. Kyrgyz Republic is a mountainous country with an average elevation of 2750 m above sea level. Comprehensive studies of soil contamination were performed in the area of the settlement Kichi-Kemin Valley. Tailings of various industrial enterprises of the mining industry were laid on the territory of the republic on mountain ranges. One of those mountain ranges is the Ak-Tuz, located 145 km from the capital of Kyrgyz Republic at an altitude of 2300 m above sea level. As a result of the mining industry's activities of the Ak-Tuz, radioactive waste of 4.17 million m^3 was deposited in four tailing dumps in the Kichi-Kemin Valley. At the moment, three tailings are conserved and tailing №4 is active [5]. The main types of waste are radioactive thorium, heavy metals such as cadmium, molybdenum, lead, zinc, beryllium, and oxides of hafnium and zirconium. Chemical composition of waste: Th – 0.038%, Pb – up to 0.12%, Zn – up to 0.07%, Th_2O_3 – (0.11-0.15)%, Y_2O_3 – up to 0.02%, ZrO_2 – (0,2-0.5)%, Mo – up to 0.007%, F – up to 0.1%, Cu – up to 0.015% [6]. The ecological situation in the region is unfavorable. In December 1964, there was a breakthrough of the holding dam which caused more than 600,000 m^3 of radioactive and toxic waste to flow out of the tailing dump site and into the river and valley of the Kichi-Kemin [7]. Thereby endangering the safety of the entire ecosystem of the Kichi-Kemin Valley. Many scientists of Kyrgyz Republic studied the impact of the Ak-Tuz ore mining on the environment [8]. According to their research, the activities of the rare-earth metal enrichment factory Ak-Tuz REMEF (Ak-Tuz REMEF) – its waste and especially the accident that occurred in 1964 – has had a negative impact on the health of Kichi-Kemin Valley's residents. According to the Scientific Research Institute of Oncology and Radiology of the Kyrgyz Republic, the incidence of neoplasms in the Kemin District in 2005-2010 are about 145 per 100,000 people; these indicators for the neighboring Issyk-Ata district are about 105 and in the whole country about 90. Assessment of the ecological status of ecosystems in the region was mainly conducted by analytical methods. In other words, the content in test samples of heavy metals by chemical methods and their comparison with the established MPC does not provide a reliable and objective assessment of the ecological state of ecosystems. To fully assess the impact of anthropogenic factors on the environment it is important to study the influence of pollutants on biogenic and abiogenic components [9]. The most urgent problem remains to find the best resolution to integrate environmental monitoring data [10, 11]. Among them is the Triad approach which is based on the methodology of interdisciplinary level considering the data of chemical, ecological and ecotoxicological studies [12, 13]. Comprehensive studies of soil contamination were performed in the area of the settlement Kichi-Kemin Valley. The purpose of our studies was to assess the toxicity of the Ak-Tuz soil ecosystem using the Triad approach.

Material and Methods

Study Area and Sampling Procedures

The object of the research was Ak-Tuz REMEF located in Kemin district, Chui region (Fig. 1).

The object of our study, the Kemin Valley, is located in the north-eastern part of Kyrgyz Republic and consists of two geographic separate intermontane depressions of Chon-Kemin and Kichi-Kemin. The climate of the Kemin Valley is continental with dry, hot summers, with moderately cold winters. The average annual air temperature ranges from 4.7 to 7.50ºC, and the amount of precipitation ranges from 300 mm in the valley to 600-700 mm in the mountains. The mid-June temperature is 17-190ºC and the January temperature is from 5-70ºC to 10.50ºC frost. The vegetation is represented by wormwood-fescue-feather grass steppes, tall grass steppes, meadow steppes and cobresia wastelands. In terms of soil, the Kemin Valley is part of the North Kyrgyz soil province. Mountain dark chestnut soils have up to 6% humus, horizon A is 40-60 cm thick, and has a good granular crumbly or finely crumbly structure. Belt of mountain chernozems form under highly developed diverse meadow steppes at altitudes of 1700-2600 meters.

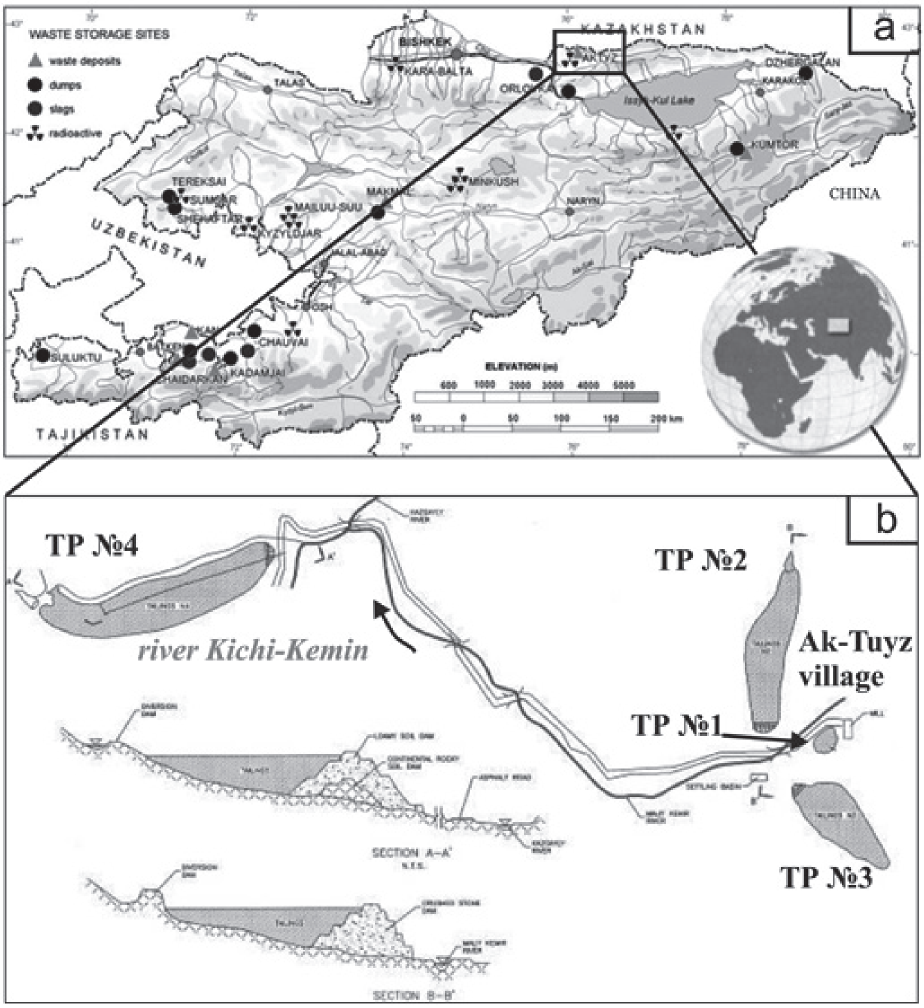

Fig. 1. Map of tailing dumps in the territory of Kyrgyz Republic: a) location of the Ak-Tuz village; b) the tails of Ak-Tuz № 1-4 in the valley of the river Kichi-Kemin.

These soils are highly fertile. The humus content in the upper horizon reaches 12-15%. Medium humus (up to 10%) mountain chernozems in meadow steppes and fat (up to 15%) beneath steppe tall grass meadows are distinguished. The structure of the humus horizons is lumpy-granular. Despite the high potential fertility, chernozem soils are not intensively used for agriculture since they are formed at significant heights, on the steep slopes of the mountains. Instead, these soils are used for hayfields and pastures, and where the terrain allows plowing, for growing potatoes.

The soil samples were collected directly in four tailings located in the territory of the factory, in spring and autumn 2011-2014. Control sample was taken at a distance of 10 km in Kichi-Kemin village. We delineated two separate sampling depths: 0-5 cm and 5-20 cm. From each depth, five subsamples were taken within one plot and joined in a single mixed sample. 250 soil samples were collected from 25 plots. Soil samples were homogenized, air dried at 20ºC for 3 days, and passed through a 2 mm mesh.

Chemical Properties - Chemical Risk Index (ChemRI)

For trace element analysis in soils, samples were dried, sieved and homogenized. The amounts of heavy metals (Zn and Pb) were extracted with a mixture of HNO_3, HF and $HClO_4$ and their concentration was measured with an ICP-MS instrument [14]. The pH was measured using a Horiba B-213 Twin pH Meter; each soil sample directly placed on the sensor and added a drop or two of deionized water.

Bioassay - Ecotoxicological Risk Index (EtoxRI)

Phytotoxicity tests were conducted according to the Russian Federal Register FR 1.31.2012.11560 [15] using monocotyledonous plant – oat (*Avena sativa* L.) and dicotyledonous plants – radish (*Raphanus sativus* L.) and cress (*Lepidium sativa* L). Phytotoxicity test was carried out in modification as described in detail in [16].

Specially designed boxes were used. Each box had two compartments with dimensions of 13.5 x 8.5 x 0.8 cm each (length x width x height, respectively); i.e., the volume of each compartment was 92 cm^3. The lower compartment was for root growth (and was filled in with soil in the whole soil protocol), whereas the upper one was for shoot growth.

Soil samples were riddled through a sieve d = 2 mm and placed in lower part of phyto-plate, distilled water was injected by syringe and ten healthy looking seeds were planted. The assays were performed in three replicates per treatment (N dilutions) and one control. Exposure was carried out in thermostat at 25ºC±2ºC for 3-5 days.

The following endpoints were measured to evaluate phytotoxicity: seed germination (%), shoot lengths (mm), root lengths (mm). Plants that did not germinate were not considered in the subsequent analysis of growth. Phytotoxic effect PE (%) is calculated by the formula:

$$\mathrm{PE}(\%) = \left(\frac{\mathrm{R_{control}} - \mathrm{R_{sample}}}{\mathrm{R_{control}}}\right) \times 100\% \quad (1)$$

where R_{sample} – response in samples, $R_{control}$ – response in control. When evaluating the results of the tests, the following toxicity criteria were accepted: non-toxic samples PE<10%; low-toxic samples 10%<PE<50%; toxic samples 50%<PE<100%; highly-toxic samples PE = 100%.

We used PE as Ecotoxicological Risk Index (EtoxRI) in Further Calculations

Bioindication - Ecological Risk Index (EcoRI)

Isolation of cultured fungi was carried out by a standard method of inoculating an aqueous soil suspension from the dilutions 1: 100 on the Czapek medium with streptomycin (100 mg/l) in 3-fold replication. Identification of species was carried out on the basis of cultural and morphological features using modern determinants. The number of microorganisms was determined by the plate method. Ten grams of each soil sample were added to 90 mL of distilled water. The solution was diluted (10^{-1} to 10^{-6}) and aliquots of the resulting solutions plated on appropriate culture media. Czapek media was used for fungal growth, meat-peptone agar (MPA) for bacteria growth and starch-ammonia agar (SAA) for actinomycetes growth. All experiments were performed in triplicate. After incubation at 25 or 30ºC, for up to 10 days, the colony forming units (CFU) were counted. In addition, the cultures were determined according to macromorphological types. The identification of microorganisms was carried out in accordance with the most common and taxonomic changes reflected and new guidelines were considered. In some cases, electronic interactive "keys" and information sites of Internet resources were used (http://www.indexfungorum.org; http://biodiversity.bio.uno.edu/~fungi/; www. Cbs.knaw. Nl)

Data Analysis

A chemical risk index (ChemRI), ecotoxicological risk index (EtoxRI), and ecological risk index (EcoRI) in the range 0–1, were calculated according to Dagnino [17]. The data were analyzed by comparing the results from investigated sites with those from the low-contaminated control site (10 m) and by computing the risk indices for each parameter. Finally, the integral risk index (RI) was calculated applying weighting factors based on the ecological relevance of the different data (Eq. 2): 1, 1.5 and 2, respectively for ChemRI, EtoxRi and EcoRI as suggested and justified in the article [22].

$$\mathrm{RI} = \left(\frac{\mathrm{ChemRI} \times 1 + \mathrm{EtoxRI} \times 1.5 + \mathrm{EcoRI} \times 2.0}{1 + 1.5 + 2.0}\right) \quad (2)$$

All data analyses were performed using Statistica 13.0 (USA). All figures created in the Excel program from the Microsoft Office program package.

Results

Assessment of the Ecological State of Soils by Chemical Parameters

The studied soils have a slightly alkaline reaction of the soil solution (pH 8.5, 7.8, 8.2, 8.1), respectively in the tails № 1,2,3,4. An important criterion affecting the ecological state of soils is contamination with heavy metals. The content of heavy metals in the studied soils are shown in Table 1. The content of zinc on all four tailing plots exceeds the background values. The largest excess of zinc was recorded at tailing site №2, where its content exceeds the background values by 1.38 times. Insignificant elevated lead concentrations were noted at tailings №2 and №3 as compared to the background sample while in tailings № 1 and № 4 its concentrations decreased. On the territory of the Ak-Tuz factory the exposure level of the dose of gamma radiation on the surface of objects is in the range from 40-60 microR/hour. However, at certain anomalous points it reaches 700 microR/hour [18].

Assessment of the Ecological State of Soils by Toxicological Indicators

In environmental-risk assessment (ERA) the use of toxicity bioassays is essential to determine the potential risk of pollution for individual living organisms and the ecosystem as a whole [19, 20]. Test cultures showed high sensitivity to heavy metals and in our study, they were established in the following order: *Raphanus sativus <Lepidium sativa <Avena sativa*. The most sensitive

Table 1. The total content of heavy metals (mg/kg) in soil samples.

Soil samples	Zn±SD	Pb±SD
Tailing № 1	658,90±26.38	469.92±26.81
Tailing № 2	669,70±31.37	387.25±27.87
Tailing № 3	781,90±26.15	391.56±34.52
Tailing №4	698.20±27.54	1847.51± 35.56
Background	104.86±17.19	628.22±17.04

SD standart deviation

were the seeds of the *Raphanus sativus*. The germination of radish seeds was not observed on the samples of tailing dumps №1, №2 and №3 (Table 2). However, at site №4, growth stimulation of all the cultures was manifested. In all soil samples the seed germination energy of *Lepidium sativa* (20-40%) and *Avena sativa* (10-40%) was low compared to background values. The lowest percentage of germination of *Lepidium sativa* (10%) and *Avena sativa* (20%) was noted in the sample of tailing №1. Soil samples of all tailings had a noticeable inhibitory effect on the aboveground and underground parts of salad and oats plants in comparison with the control parameters. Heavy metals are mobile and available to plants which may translocate into edible parts and threaten food safety [21]. The high toxicity caused by the germination of oats and lettuce seeds by more than 50% was demonstrated by all soil samples, confirming the greatest informativeness of biotic control of the toxicity of natural media which was noted by other authors previously [22-24]. Perhaps that is why the incidence rate of the local population with oncological diseases is high.

Assessment of the Ecological State of Soils According to Indicators of Bioindication

The results of bioindication studies have shown that heavy metals have an inhibitory effect on the growth and development of soil microorganisms. The predominance of stable forms of microorganisms in the studied soils was noted. Thus, representatives of the actinomycetes of the genus Streptomyces were

Table 2. Germination, shoot, root lengths for the plant species tested in the different soil samples.

Tested plants	Germination, %	Shoot lengths, mm±SD	Root lengths, mm±SD	Phytotoxic effect of seeds germination, %
Tailing №1				
Lepidium sativa	10	2.55±0.22	2.98±0.23	79
Avena sativa	20	1.05±0.08	3.20±0.24	90
Raphanus sativus	0	0	0	
Tailing №2				
Lepidium sativa	20	1.84± 0.30	2.87±0.39	58
Avena sativa	40	1.53±0.35	4.48±0.55	80
Raphanus sativus	0	0	0	
Tailing №3				
Lepidium sativa	25	2.21±0.44	3.23±0.88	58
Avena sativa	40	2.04±0.43	4.82±0.73	75
Raphanus sativus	20	0	0	
Tailing №4				
Lepidium sativa	40	2.80±0.36	2.47±0.50	68
Avena sativa	30	4.05±0.78	6.30±1.11	50
Raphanus sativus	20	1.65±0.12	0.2	
Background				
Lepidium sativa	100	3.25±0.73	4.06±0.62	
Avena sativa	95	5.96±0.98	7.23±1.15	
Raphanus sativus	10	1	0.3	

SD standard deviation

Table 3. Microbial indicators.

Soil samples	Bacteria, CFU/g soil	Fungi, CFU/g soil	Actinomycetes, CFU/g soil
Tailing №1	39.67±8.39	1.67±2.89	24.67± 4.73
Tailing №2	141.33±29.5	12.7±2.89	84.67± 28.04
Tailing №3	52.33±31.09	6.33±0.53	2.33± 2.08
Tailing №4	148.67±76.29	11±3.07	87.33± 26.58
Background	88.33±57.47	23±7.81	73.67±7.37

SD standard deviation

represented predominantly by the *Albus* and *Cinereus* sections which indicates their resistance to heavy metals as was noted by other researchers [25]. In tailing №4, in addition to the representatives of the *Albus* and *Cinereus* section, representatives of the *Helvolo Flavus* and *Azureus* section were identified which were absent from other sites. Fungi diversity was represented by species of *Aspergillus, Penicillium, Trichoderma, Fusarium* and *Mucor*. The smallest variety of fungi was noted at the site of tailing №1 (Table 3). The most resistant to high levels of heavy metal contamination in the soil were species of *Aspergillus* and *Penicillium* which was also noted by other researchers [26]. The prevalence of dark-colored fungi was noted in the study area which also confirms the resistance of melanin-colored fungi to increased radiation and heavy metals [27].

Studies of the microbiological diversity of the technogenic ecosystem of the Ak-Tuz by species resistant to the effects of heavy metals and radioactive thorium was noted by an increase in CFU of bacteria and actinomycetes of the genus *Streptomyces* with minimal species diversity which is an indicator of a degrading ecosystem. The more resistant an ecosystem is to the impact of man-made factors, the higher its species diversity is. This applies to the soil microbiota as well.

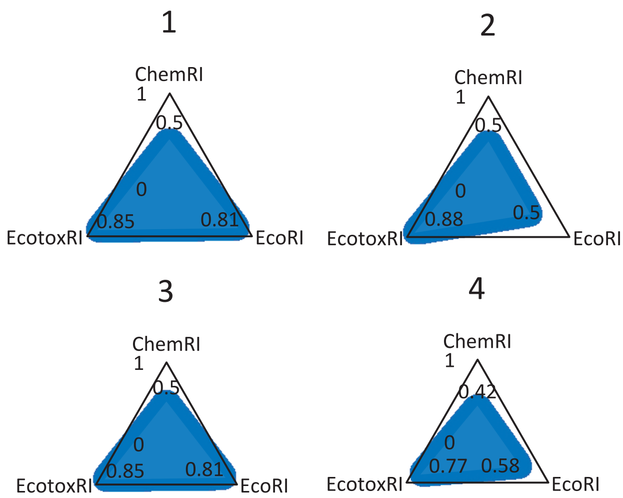

Fig. 2 Calculated risk indices (RI): chemical (ChemRI), ecotoxicological (EcotoxRI), ecological (EcoRI); 1-4 tailings number; the area of the dark triangle reflects the degree of disturbance of the soil.

Complex Ecological Assessment Soil of Conditions Using the TRIAD Method

Fig. 2 presents the results of assessing the ecological quality of soils on tested sites for chemical, toxicological and bioindication data. Ecotoxicological index (EcotoxRI) turned out to be the most sensitive in the assessment of soil contaminated with heavy metals. On the other hand, it proved to be the least sensitive to chemical index (ChemRI).

Thus, the Triad method, which allows one to consider the three risk indexes in assessing the state of contaminated soils, is most informative.

Discussion

The TRIAD approach is based on an interdisciplinary methodology and takes into account toxicological data simultaneously with data from chemical and bioindication studies. Although this methodology was first formulated for bottom sediments [17] (Chapman, Texas A&M University, USA), it is also actively tested on soils [28-30]. Based on Triad approach to integrated environmental assessment of soils, one can obtain the formalized indices of the ecological state of soils, which make it possible to compare areas with varying degrees of the negative influence of pollutants [17, 30-32]. The TRIAD paradigm allows us to assess potential harmful effects on the ecosystem, taking into account the concentration of chemicals, bioavailability of pollutants and environmental parameters of the observed ecosystems.

At present, recommendations for using this approach for the integrated environmental assessment of polluted environments have already been developed and implemented at governmental level in a number of countries (Science Advisory Board for Contaminated Sites in British Columbia 2008; ISO 19204). The data obtained from the various branches of TRIAD (bioindication, biotesting and chemical analysis) are heterogeneous and are characterized by different

Table 4. Conformity of the integral index of the ecological state, determined on the basis of the TRIAD approach to the categories of soil quality, the degree of loading and the characterization of the ecological state.

Conformity of index	Soil quality category	Degree of load	Characterization of the ecological state of the soil
CI = 0	I	Permissible	Background
0 ≤ CI ≤ 0.30	II	Low	Slightly disturbed
0.30 <CI ≤ 0.50	III	Average	Disturbed
0.50<CI ≤ 0.79	IV	High	Severely disturbed
0.79 <CI ≤ 1	V	Very high	Irreversibly disturbed

relevance with respect to a full assessment of damage to ecosystem functions (McCarty, Stanford University).

In our work based on the results of assessing the ecological quality of soils from the data of chemical, toxicological and bioindication analysis follows: the most sensitive index in determining the toxicity of contaminated soil is the ecotoxicological index estimated by phytotesting on plates.

The integral index showed the changes in soil biological and ecotoxicity properties. Despite the not very high content of the tested heavy metal, we found changes in the biological parameters of soils. We believe that some other pollutants (expected contamination with radioactive elements) or their combined effect may have such an effect.

The range of values of the integral index of state calculated by the Triad method was divided into five categories as it was in [30]. We compared integral indices with the degree of anthropogenic load on soils and the characteristic of the ecological state (Table 4).

Comprehensive assessment of the ecological state of soils showed that the soils are characterized as severely disturbed and classified as the fourth quality category. Section tailing № 1 is subject to the greatest pressure where the ecological state of soils is characterized as irreversibly disturbed and belongs to the fifth category of quality.

Based on the results of assessing the ecological quality of soils from the data of chemical, toxicological and bioindication analysis follows: the most sensitive index in determining the toxicity of soil contaminated with heavy metals is the ecotoxicological index estimated by phytotesting on plates. In all tailings the ecotoxicological index ranges from 0.77 to 0.99 (Fig. 2). At tailing №1 the chemical index is 0.46 while the ecotoxicological index is 0.99 – the highest may indicate an imperfection of the chemical method for determining contamination. With a chemical integral index of 0.50 in the tailing sites №. 2 and №4, ecological indexes (0.5 and 0.58) report a decrease in species and a quantitative diversity of soil biota from long-term soil contamination of heavy metals. Ecological indices on all sites range from 0.5-0.84 which indicates ecosystem's attempts to restore.

Conclusions

This study aimed at evaluating the ecological risk from the Ak-Tuz factory in Kyrgyz Republic to surrounded soil using an integrated approach. We measured several soil chemical properties, bioassay responses using phytotest and microbial biodiversity. The interdisciplinary approach based on the data of chemistry, ecology and toxicology allows giving an objective picture of environmental problems in the field of operation of the industrial enterprise – the Ak-Tuz REMEF. The high sensitivity of soil ecosystems to contamination with heavy metals, radioactive thorium and a high degree of polluting load adversely affects the ecological state of the Ak-Tuz soil; characterized as severely disturbed and classified as the fourth quality category. Given the vulnerability of high-mountain ecosystems, it is necessary to monitor continuously the state of ecosystems and carry out a set of restoration measures, considering the characteristics of the soil and vegetation.

Acknowledgments

We express our gratitude to the State Agency for Environmental Protection and Forestry of the Kyrgyz Republic for providing the required facilities and for support for this initiative.

Conflict of Interest

The authors declare no conflict of interest.

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