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Microfungal communities in soil polluted with fluoride

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ABSTRACT

There have been identified three zones according to the degree of soil pollution with fluoride in the impact area of air emissions of the Kandalaksha Aluminium Smelter (Russia): zone of maximum pollution up to 2.5 km from the emission source with the content of fluoride from 5000 to 1200 mg/kg, zone of strong pollution up to 13 km from the plant with the content of fluoride between 1200-400 mg/kg and zone of moderate pollution up to 20 km from the source with content of fluoride between 400-200 mg/kg. Emissions of the aluminium plant have reduced the number and the diversity of fungi and have caused an increase in fungal communities that are potentially pathogenic fungi. The biomass of fungi has decreased in the organic horizon of the maximum polluted soil from 5.4 to 3.6 mg/g. As a whole, emissions from the aluminium plant in the Murmansk region are less toxic for the environment, than emissions of copper-nickel enterprises.

Keywords: Pollution Gradient; Fluoride; Soil Fungal Communities; Number; Diversity; Structure

1. INTRODUCTION

The combination of high level of industrial development and extreme nature condition in the Murmansk region of Russia affect the condition of ecosystems. Traditionally in the Kola Peninsula researchers pay the most attention to the environmental pollution by heavy metals and sulphur dioxide [1-5]. At the same time, the enterprises of non-ferrous metallurgy and superphosphate factories emit into the air of Murmansk region more than 800 tons of fluoride hydrogen (HF) per year. Most part from this volume (700 tons) is from Kandalaksha Aluminium Smelter (KAS). The influence on ecosystems from companies like Kandalaksha Aluminium Smelter has been insufficiently studied. The first recognized research has shown that macro- and microelements found in air emissions from KAS are accumulated considerably in the soil, mainly in its organic horizon, as well as in plants [6,7].

The main compounds in the air emissions from KAS are fluoride and aluminium (Al_2O_3—5000 t/year). In spite of the fact, that more aluminium gets to the environment with air emissions, than fluorine, the toxicity of the latter one for biota is more considerable. Fluorine and its compounds belong to the 1st class of hazard of substances and are found in emissions basically in the form of water-soluble compounds, accessible for biota compounds (up to 90%). Aluminium belongs to the third class of hazard and is part of, basically, a solid phase of atmospheric emissions poorly accessible to live organisms (up to 98%) [8]. Besides, the studied Al-Fe-humus soils dominating in the Kola Peninsula, contain significant amounts of aluminium (Al_2O_3 up to 15% on anneal soil), and we can speak about the adaptation of soil microbiota to the high content of this element.

It is known, that high concentrations of fluorine and its compounds are dangerous for living organisms, including humans being [9-13]. Higher content of fluorine-ions lead to the inhibition of some enzyme reactions, to the linking of biogenous elements (P, Ca, Mg etc.) and the disturbance of their balance in the organism. The increased concentrations of compounds of aluminium and fluorine are the reason of diseases of respiratory organs and ossa.

Microscopic fungi are essential components of the forest ecosystem. Fungi take part in the soil-forming processes mainly by destructing organic compounds (vegetable waste, humus, xenobiotic). Fungi reduce toxicity of soils by accumulation, chelatization, and detoxication of toxic elements. They perform biogenic migration of elements in soil and also are bioindicators of pollution. Change in the structure and functions of fungal communities, which in the modern biosphere, primarily relate to the anthropogenic effects may lead to abnormalities in substance transformation in soils and the biosphere as a whole.

The goal of work is to study the number and diversity of soil microfungi, structural changes of fungal communities in soils under influence of polluted air emissions from an aluminium plant, herein KAS as an example.

2. MATERIAL AND METHODS

2.1. Stationary Plots

The studies are carried out at stationary plots located along the KAS air emissions gradient (67°00´N, 32°00´E) at 2, 5, 10, 20 and 50 (the background plot) km northwards from the plant. All the plots (100 m^2) were established in pine forests with dwarf shrubs (predominantly, crowberry *Empetrum hermaphroditum*) and green mosses in the ground cover. The soil cover of territories in which the stationary plots are located, is presented by Al-Fe-humus podzol on sandy moraine. The thickness of organic horizon is about 3-5 sm. The sampling was carried out in spring, summer, autumn periods in 2003-2007 years in three replications (162 samples). The mass of each sample made 300-400 g. In 2005 year soil samples were taken from organic horizon following the pollution gradient of 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0, 50.0 km from the emission source, three replications at each point (42 samples).

2.2. Micological Analyses

For the micological analysis soil samples from organic horizon were taken. Totally, 204 soil samples were analysed. All analyses are carried out the next day after sampling that means fresh samples was analysed. The total the length of fungal mycelium is determined by the method of fluorescent microscopy using polycarbone membrane filters Cyclopore Blask with pores diameter of 0.8 microns and FITC dye (fluorescein-5-isothiocyanate) [14]. The biomass of fungi was determined, by assuming the weight of 1 m of mycelium equal to $1.1 \cdot 10^{-6}$ g. The number of viable fungi was found by the plating method on wort agar with streptomycin. Separation of fungi was realized from this same medium. The identification of microscopic fungi was done based on keys [15-18]. Species names of fungi were specified according to lists in the database "Species fungorum" (www.speciesfungorum.org). For characterization of structure of fungal communities are used indices of spatial and temporal occurrence frequency of species [19] (see the bottom).

The fungus ***Aspergillus niger*** was used as a test culture**,** capable of changing pigmentation of spores, depending on the nutrient medium composition [20]. The fungus was grown on liquid wort with addition of NaF. Concentrations of fluorine ion were, mg/l: 50, 100, 500, 1000, 1500. The control wort did not have F^-. Each variant comprised three replications. Incubation was carried out within 7 days at +27℃.

2.3. Chemical Analyses of Soil

The content of fluorine in organic horizon was found by ionselective method using the pH/ION ANALYSER after burning soil at temperature of 950℃ and fusion of ashes with borax. The total composition of organic horizons was determined after burning soil of the sample (950℃) and fusion of ashes with soda. Si was found using the weight method, while P and Ti were determined by colorimetri, K and Na—using a flame photometer, other elements using the AAS-method in accredited laboratoty INEP.

2.4. Statistics

Quantitative ecological indices (Shannon, Pielu, Simpson), characterizing the structure of the fungal community, have been calculated by Odum [21]. The obtained data have been processed by means of Excel 2003 statistical programs.

3. RESULTS AND DISCUSSION

3.1. The Content of Macro- and Microelements in Soil

A considerable part of components contained in air emissions from KAS, gets into the soil surface in the form of solid falls [22]. They cause essential accumulation of mineral substances in the organic horizon of soil, as a result of the ash content in the heavily polluted organic horizon reaches 60% while the background area does not exceed 10%, and the carbon and nitrogen content decreases 2 times (**Table 1**). The increase of the ash content in organic horizons took place as a result of an increase in the content of most of the chemical elements. The most evident dependence of the total content on the intensity of pollution is observed concerning Si, Al and Ti. The content of heavy metals Zn, Cr, Mn and Cu also increases. So the quantity of Cr here exceeds 7 times while Zn almost 3 times, compared to the content in the

$$\text{Spatial frequency, \%} = \frac{\text{Number of samples in which the species was found}}{\text{Total number of samples analysed}} \bullet 100$$

$$\text{Temporal frequency, \%} = \frac{\text{Number of sampling period in which the species was found}}{\text{Total number of sampling period}} \bullet 100$$

Table 1. Chemical composition of organic horizon (n = 162).

Distance, km	SiO_2	Al_2O_3	Fe_2O_3	TiO_2	CaO	MgO	P_2O_5	K_2O
	% of dry matter							
2	28.0 ± 4.19	17.80 ± 0.28	3.67 ± 0.06	0.60 ± 0.01	0.38 ± 0.03	0.88 ± 0.00	0.29 ± 0.02	0.88 ± 0.01
5	11.71 ± 0.86	6.21 ± 0.39	1.59 ± 0.08	0.19 ± 0.01	0.36 ± 0.02	0.33 ± 0.04	0.24 ± 0.01	0.32 ± 0.05
10	8.74 ± 0.08	4.6 ± 0.12	2.26 ± 0.02	0.16 ± 0.00	0.28 ± 0.01	0.29 ± 0.00	0.23 ± 0.01	0.27 ± 0.01
20	4.66 ± 1.31	1.51 ± 0.29	0.68 ± 0.10	0.06 ± 0.01	0.45 ± 0.02	0.20 ± 0.01	0.24 ± 0.01	0.19 ± 0.02

Distance, km	F	Al	Mn	Zn	Cr	Ni	Cu
	Mg/kg						
2	2587 ± 136	256000 ± 2800	384 ± 58	201 ± 7	85 ± 12	80 ± 9	104 ± 9
5	711 ± 57	781000 ± 3900	273 ± 34	109 ± 10	35 ± 4	86 ± 6	59 ± 1
10	360 ± 26	108000 ± 1200	207 ± 25	111 ± 14	28 ± 3	128 ± 1	84 ± 5
20	114 ± 13	9000 ± 2900	161 ± 35	73 ± 3	12 ± 3	117 ± 7	53 ± 9

organic horizon of the background plot.

If we estimate the content of heavy metals and compare it to the indicators of maximum permissible concentrations (MPCs) it can be noted, that in the most polluted organic horizon the content of Cu had reached the MPC for Cu, which according to Kloke [23] is 100 mg/kg for soil. The contents of Zn and Cr are below the MPCs (300 and 100 mg/kg, respectively). The amount of Ni in all soil samples exceeded 2 and more times the MPC for Ni (50 mg/kg), possibly additional Ni comes from the Severonikel plant located 100 km to the north of the studied area. As a whole we can note that the long-term impact on soils of industrial deposition from the aluminium plant the pollution level with heavy metals has not reached the critical state.

The distribution of total fluoride in organic horizon along the pollution gradient is presented in **Figure 1**. A clear dependence of the content of fluoride in soil on the distance from the source of emissions can be seen. The most abrupt drop of the content of total fluoride is observed throughout the first 2 km from the emission source, the farther from the plant the lower is the content of fluoride.

There were identified three zones, differing by the intensity of the pollution of organic horizon of the forest podzol soil with fluoride (**Table 2**). In the zone of maximum pollution the fluoride content in organic horizon exceeds the MPC of F (200 mg/kg) 25 times. In the zone of strong pollution the total content of fluoride in soil exceeds the MPC almost ten times, and in the zone of moderate pollution—two times. The shares of water-soluble fluorides are on the average 10.3% of the total content of fluoride in organic horizon.

Near the plant an abrupt decrease of soil acidity (pH water suspension from 4.05 to 5.75) is observed. This is connected with the neutralization of the bases Mg and Ca contained in the emissions.

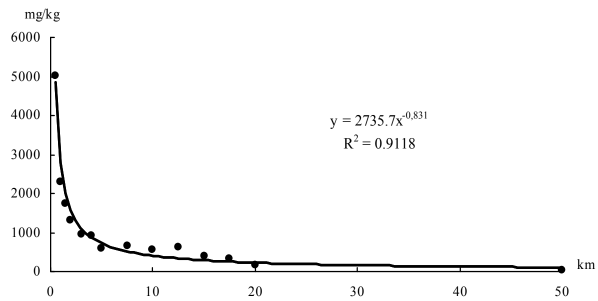

Figure 1. Content of total fluorine in soil (mg/kg) along the pollution gradient from KAS (mean data, n = 42).

Table 2. Zones of soil pollution (organic horizon) with fluorine.

Pollution level	Distance, km	F, mg/kg
Maximum	0-2	> 1200
Strong	2-13	1200-400
Moderate	13-20	400-200
Background	> 20	< 200

Thus, the influence of emissions from the KAS has affect on not only the fluoride content in soil but has also led to considerable accumulation in the organic horizon of mineral substances, including many macro-and microelements. The influence of solid emissions was so high that it caused a basic change of the ratio of organic and mineral parts of the organic horizon. In the zone of maximum pollution the mineral part became a main component of the upper soil horizon.

3.2. Number, Biomass, Diversity of Fungi and Structure of Fungal Communities in Soils Polluted with Fluoride

The emissions from the KAS have in the most considerable way affected the number and diversity of fungi. There have been observed a decrease of the number and biomass of fungi as the degree of soil pollution with emissions of aluminium plant is increasing using both the methods: of plating and the method of fluorescent microscopy.

The quantity of fungi colony-forming units (CFU) in the organic horizon of the plot located 2 km from the plant is 5 times less than the CFU number in the soil of plots at the distance of 10 and 20 km from the plant, and 9 times in comparison with the background plot (**Figure 2**). The length of fungal mycelium was reduced from 5000 m/g in the soil of the background to 3000 m/g in the most polluted soil plot ($t = 12.4$, $p < 0.001$), and the biomass from 5.4 mg/g to 3.6 mg/g.

There were isolated 44 species of microscopic fungi from the soils of stationary plots. They related to 18 genera, 6 orders, 4 classes and 3 divisions (**Table 3**). There have been revealed distinctions in taxonomic diversity of soil fungi in the background and polluted soils. In the polluted soil a tendency of reduction of species diversity of fungal communities, a change of their composition and structure compared to the background soil is observed. In the most polluted soil 26 species of microscopic fungi have been identified, 29 species in the strong polluted soil and 35 species in the background soil.

The dominating position in podzols of the Kola Peninsula is occupied by fungi of *Penicillium* genus [24], which is characteristic also for the podzols polluted with fluoride. In the maximum polluted soil this genus is presented by 10 species, in the strong polluted one by 14, and in the background by 13 species. The representatives of *Mucorales* order, sensitive to emissions of the aluminium enterprise, are more widely spread in the background soil.

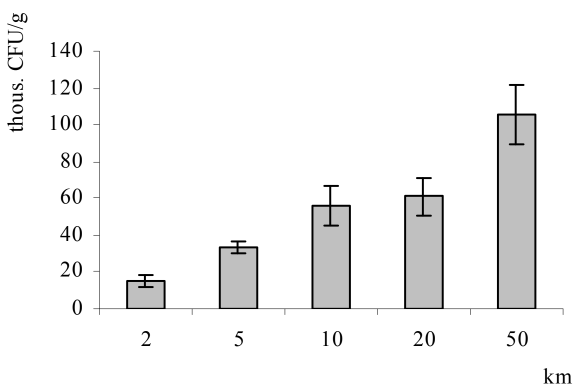

Figure 2. Changes of fungal number (thous. CFU/g) in soil along the pollution gradient from KAS.

Under the influence of emissions from the aluminium plant essential changes in the structure of the fungal communities have taken place. Indices of spatial and temporal occurrence frequency of species are calculated. Based on these indices the dominating species in the fungal communities as defined as (spatial and temporal occurrence frequency > 60%), frequent (spatial and temporal occurrence frequency > 30%), rare (spatial occurrence frequency < 30%, and the temporal one > 30%, and casual species (both indices < 30%).

In the polluted soil the following species dominates: *Penicillium canescens, P. decumbens* and *P. raistrickii*, in the background: *P. implicatum, P. decumbens, Umbelopsis isabellina* and *Mortierella longicollis.* The frequent species in the polluted soil includes: *Aspergillus fumigatus, P. glabrum, P. ochraceum, P. spinulosum, P. thomii* and *Trichoderma viride,* and in the background: *Acremonium rutilum, Aspergillus fumigatus, Aureobasidium pullulans* var. *pullulans, Cladosporium herbarum, P. adametzii, P. restrictum, P. glabrum* and *P. canescens.*

Only in the polluted soil there are revealed rare or quite atypical species for zonal soils: *Aspergillus niger* var. *niger, Paecilomyces variotii*, *P. chermesinum*, *P. variabile*, *Phoma medicaginis*, *Thielaviopsis basicola, Torula allii, Myxotrichum cancellatum* and *Trichocladium asperum.* Only in the background soil was found *Mortierella alpina, Mucor plumbeus, M. racemosus, P. lividum, P. citrinum, Sordaria macrospora* and *Trichoderma polysporum.*

Under the influence of industrial emissions from the aluminium plant the part of pathogenic and potentially pathogenic species causing various kinds of mycoses of both endogenous and exogenous character increases in the fungal community. At the background plot, the part of potentially pathogenic fungi is 25%, and at the polluted one—40% of the total population of species. This include first of all opportunistic mycoses pathogens from genera *Aspergillus* (*A. fumigatus, A. niger*), *Cladosporium* (*C. herbarum*) and *Paecilomyces* (*P. variotii*) [25,26]. The ones that possessing pathogenic properties are *Alternaria alternata, Aureobasidium pullulans, P. aurantiogriseum, P. glabrum, P. simplicissimum, Trichoderma viride* and *T. koningii,* these are causing diseases of respiratory and digestive systems.

Quantitative ecological indices confirm the conclusions about the decrease of species diversity and the change of the structure of fungal communities in the soils

Table 3. Species composition and occurrence frequency of fungi were separated from soil polluted with fluorine.

Species	Occurrence frequency, %		
	2 km	10 km	50 km
	Division *Zygomycota*		
	Class *Zygomycetes*		
	Order *Mucorales*		
Gongronella butleri (Lendn.) Peyronel et Dal Vesco	–	28/20	20/20
Mortierella alpina Peyronel	–	–	25/20
M. longicollis Dixon-Stew.	37/40	65/80	62/60
Mucor plumbeus Bonord.	–	–	+
M. racemosus Fresen.	–	–	15/20
Mucor sp.	–	40/40	10/40
*Umbelopsis isabellina (*Oudem.)W.Gams	–	27/30	70/60
U. ramanniana (A.Møller)W.Gams	24/20	–	32/20
	Division *Ascomycota*		
	Class *Pyrenomycetes*		
	Order *Sphaeriales*		
Sordaria macrospora Auersw.	–	–	10/20
	Order *Eurotiales*		
Myxotrichum cancellatum W.Phillips	+	–	–
	Mitosporic fungi		
	Class *Hyphomycetes*		
	Order *Hyphomycetales*		
Acremonium rutilum W.Gams	21/20	38/40	31/60
Alternaria alternata (Fr.:Fr.)Keissl.	–	20/40	40/40
Aspergillus fumigatus Fresen.	31/40	30/40	46/40
A. niger var. *niger* Tiegh.	+	–	–
Aureobasidium pullulans var. *pullulans* (de Bary) G.Arnaud	25/40	29/20	50/40
Cladosporium herbarum (Pers.)Link	–	15/20	35/40
Paecilomyces variotii Bainier	+	–	–
Penicillium adametzii K.M.Zalessky	–	32/40	45/60
P. aurantiogriseum Dierckx	–	+	+
P. canescens Sopp	79/100	22/40	38/40
P. citrinum Thom	–	–	+
P. chermesinum Biourge	+	–	–
P. corylophilum Dierckx	+	+	–
P. decumbens Thom	75/80	72/100	61/100
P. dierckxii Biourge	–	18/20	6/40
P. implicatum Biourge	–	60/60	71/60
P. glabrum (Wehmer) Westling	70/40	35/40	40/60

P. lividum Westling	–	–	+
P. ochraceum Bainier:Thom	35/40	15/20	–
P. raistrickii G.Sm.	72/60	63/80	28/80
P. restrictum J.C.Gilman et E.V. Abbott	–	40/40	38/60
P. simplicissimum (Oudem.)Thom	–	+	–
P. spinulosum Thom	32/40	32/40	13/20
P. thomii Maire	43/40	42/80	7/20
P. variabile Sopp	25/40	–	–
Thielaviopsis basicola (Berk. et Broome) Ferraris	+	–	–
Torula herbarum (Pers.)Link	18/20	15/20	–
T. allii (Harz) Sacc.	+	–	–
Trichocladium asperum Harz	+	–	–
Trichoderma koningii Oudem.	30/20	20/20	12/40
T. polysporum (Link)Rifai	–	–	24/20
T. viride Pers.	50/60	13/40	17/40
	Order *Agonomycetales*		
Mycelia sterilia white	84/100	60/100	62/100
	Class *Coelomycetes*		
	Order *Sphaeropsidales*		
Phoma eupyrena Sacc.	–	22/20	10/40
P. medicaginis Malbr. et Roum.	21/20	–	–

polluted with fluoride (**Table 4**). The Shannon index, characterising the general species diversity, in the background, heavily polluted and strong polluted soil is 3.05, 2.35 and 2.08 bits/copies, respectively.

Usually the community composition includes some dominating species of high number and many rare species of low number, which was observed in the community of background soil. In the polluted soils the quantity of dominating species decreases. The value of Simpson index reflecting the representativeness of dominant-species decreases when the distance from the plant increases, opposite trend is observed for the Pielu index for uniformity of species diversity the – it increases.

The Sorensen index (S) characterises the similarity (dissimilarity = 100 – S) in species composition of fungal communities at stationary plots depending on the degree of pollution. The similarity in the fungal community between the maximum polluted (2 km from the plant) and the background plot is 50%, *i.e.* 50% of fungi species isolated from the background soil have not been found in soils with high level of pollution with fluorine containing compounds. Between the strong polluted (10 km from the plant) and the background plots the similarity in fungal species composition is considerably above 80%.

Using *Aspergillus niger* as test-culture it was found that fluorine influences the process of spore formation and pigmentation of fungal spores. These fungi can be a good indicator of the fluorine content in soil. As the concentration of F^- increases in the nutrient medium from 0 to 500 mg/l the colouring of *Aspergillus niger* spores provides the whole range of shades from black to white (**Table 5**). At concentration of F^- = 500 mg/l and more the formation of spore stops.

Thus, one of the reasons of a decrease in fungal biomass in the soils that are exposed to emissions from the aluminium plant is the inhibition by fluorine on the spore-formation process. As whole, emissions from the aluminium enterprise have a reducing effect on the development of soil microscopic fungi, causing a decrease

Table 4. Change of quantitative indices are characterized complexes of soil fungi along the pollution gradient (n = 162).

Index	Distance from KAS		
	2 km	10 km	50 km
Shannon index	2.08	2.35	3.05
Pielu index	0.72	0.82	0.95
Simpson index	0.38	0.34	0.18

Table 5. The change of spore pigmentation in *Aspergillus niger* at various concentrations of ions of fluorine in a nutrient medium.

Content of F^- mg/l	Pigmentation of spores
0	Black
50	Dark grey
100	Light grey
300	White
500	There are no spores

in their number, biomass and increase part of pathogenic and potentially pathogenic species in fungal community.

4. CONCLUSIONS

There has been carried out zoning of soils, which are under the impact of air emissions from the aluminium plant (the Kandalaksha plant, Russia). There have been identified three zones based on the degree of soil pollution with fluoride: a zone of maximum pollution up to 2.5 km from the emission source with content of fluoride from 1200 mg/kg and more, a zone of strong pollution up to 13 km from the plant with the content of fluoride between 1200-400 mg/kg and a zone of moderate pollution up to 20 km from the source with the content of fluoride between 400-200 mg/kg. The solid falls, which are a part of the air emissions, have caused a basic change of the ratio of organic and mineral parts in the composition of organic horizon. The mineral part (ashes) has increased up to 60% compared to the background value as a result of accumulation first of all such elements as Si, Al and Ti.

Emissions from the aluminium plant have reduced the number and the diversity of fungi and have caused an increase of fungal communities in the part of potential pathogenic fungi. Only in the polluted soil rare or just atypical species for zonal soils are selected: *Aspergillus niger* var. *niger, Paecilomyces variotii*, *P. chermesinum*, *P. variabile*, *Phoma medicaginis*, *Thielaviopsis basicola, Torula allii, Myxotrichum cancellatum*, and *Trichocladium asperum*. Among these there are activators of opportunistic mycoses. *Aspergillus niger* is a good indicator of soil pollution with fluorine, changing pigmentation of its spores depending on the quantity of fluorine in the medium.

The industrial deposition from the aluminium plant (the Kandalaksha plant) influences to a lesser degree on soil and vegetation cover than emissions from the copper-nickel enterprise ("Severonikel" and "Pechenganikel"). Only an appreciable damage of tree layer at a distance of 5 km from the source is observed for the aluminium plant, compared to the copper-nickel smelter this distance is up to 40 km in the wind rose.

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