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Anthropogenic Effects on Soil Micromycetes

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Abstract: *This paper is a synthesis of long-term investigations based on the effect of different (mineral and organic fertilisers, heavy metals, contaminated irrigation water, nitrification inhibitor and detergents) on the dynamics of soil fungi number.*
The investigations were performed at the Microbiology Department and trial fields of the Faculty of Agronomy in Cacak on smonitza and alluvium soils in field and greenhouse conditions. Maize, wheat, barley and red clover were used as test plants in these studies. The quantitative composition of the fungi in the soils investigated was determined by the Czapek selective agar dilution method.
The study results show that the number of soil fungi was dependent on the type and rate of agrochemicals used, on the growing season and the soil zone the samples were taken from for the analysis. Lower nitrogen fertiliser rates (80 and 120 kg·ha-1) and organic fertilisers stimulated the development of soil fungi, unlike the rate of 150 kg·ha-1. Heavy metals, mercury and cadmium in particular, as well as high rates of the N-serve nitrification inhibitor inhibited the development of this group of soil microorganisms. Generally, the adverse effect of contaminated irrigation water on the soil fungi was recorded in both soil types, and particularly in the smonitza under red clover. Low detergent (Meril) concentrations did not have any significant effect on this group of microorganisms.
In this respect, it can be concluded that the soil fungi number dynamics can be used in monitoring soils polluted by different toxinogenic substances.
Keywords: *contaminated irrigation water, detergents, fungi, heavy metals, mineral fertilisers, nitrification inhibitor, organic fertilisers*

1. INTRODUCTION

One of the greatest scientific contributions by *Evgenii Nikolaevich Mishustin* is study and description of environmental-geographic distribution of soil microorganisms (Mishustin, 1966; Mishustin, Yemtsev, 1978). The results of these studies have been cited ever since in all textbooks of soil and applied microbiology throughout the world and are considered basal (*Atlas, Bartha*, 1992). The environmental-geographic distribution of microscopic soil fungi was investigated most detailedly in soils of different natural zones of former USSR and described in works by *Mircink* (*Mircink* et al., 1981; *Mircink*, 1988).

As showed by these investigations, irrespective of the fact that the nominal composition of isolated fungi species in different soils can be fairly similar, fungal groups with a specific species structure (ratio of predominant, typical, rare and accidental species) are nevertheless created in concrete non-disturbed zonal soils. This thesis has been confirmed in numerous works by other authors (***Babjev, Sizov***, 1983; ***Yegorov***, 1986; ***Velikanov***, 1997 and others).

The overall development of biocenological researches in the seventies of the last century discovered the specificity of the soil fungi complex not only from the biogeographic point of view, but also for specific zonal biotopes. It was determined that soil fungi formed typical complexes in zonal soils under specific plant communities geographically very distant – the European part of Russia, Canada, the north of the USA (*Mircink*, 1988; *Chrystensen*, 1969, 1989).

The last decades of the XX century were characterised by increased diversity and intensity of anthropogenic factors in the biosphere, due to which there occurred a change in the structure and function of terrestrial and aquatic communities of organisms (*Miller*, 1990). Whereas these disturbances related to animals and plants were convincingly described as early as in the 60s-70s of the last century (*Odum*, 1986), the communities of soil microorganisms, on the other hand, were long considered to be more resistant to anthropogenic factors. These issues related to the complexes of soil microorganisms became more intensified due to methodical problems with isolating microorganisms from soils and difficulties over identifying them at the species level.

The aim of our long-term investigations (1990-2006) was to determine whether the effect of different anthropogenic factors (mineral and organic fertilisers, heavy metals, nitrification inhibitors, detergents, contaminated irrigation water) could bring about a change in the quantitative composition of fungi and whether there was a possibility of mycological indication of the effect of any specific anthropogenic factor.

2. MATERIAL AND METHODS

The investigations were conducted at the Microbiology Department and trial fields of the Faculty of Agronomy in Cacak. Field, laboratory and mathematical-statistical research methods were used in the study. Indication of the anthropogenic effects of mineral fertilisers, heavy metals, contaminated irrigation waters, nitrification inhibitors and detergents on the number of soil fungi was performed on smonitza and alluvium soils under field and greenhouse conditions. Maize, wheat, barley and red clover were used as test plants in these investigations. The quantitative fungi composition in the soils investigated was determined by the dilution method on the *Czapek* selective substratum by culturing 10^{-5} soil dilution. Following a seven-day incubation, their average number was determined and calculated per gram of absolutely dry soil.

The results obtained were assessed by the analysis of variance method, and the statistical significance of individual and interactive media was determined by the Lsd test.

3. RESULTS AND DISCUSSION

It was determined that a decrease in the qualitative diversity of natural ecosystems could be one of the most important consequences of the anthropogenic effect on them, which was confirmed several times via different plant and animal communities (*Bigon* et al., 1989). The results of our investigations showed that the anthropogenic factors affected the quantitative fungi composition in different soil types and under different crops (*Djukic, Mandic*, 1997; *Mandic* et al., 2004; *Mandic* et al., 2005a, b; *Djukic* et al., 2006).

For most microscopic soil fungi the distribution range is wide, hence the isolation of stenotopic species is made very difficult. The most illustrative example of such species is ***Mortierella ramanniana***, known as a species typical for non-disturbed podzolic zonal soils (*Mircink*, 1988). Under the effect of a number of anthropogenic factors (pollution with heavy metals, recreation and pasture plant cover degression etc.) the number of this species is decreased (*Marfenina*, 1985, 1997; *Marfenina, Popova*, 1989; *Jemtsev, Djukic*, 2000), the fungal complex “loses” shape, characteristic of a specific zonal soil type and the frequency of its distribution is reduced (tab. 1). Special experiments have determined that in conditions of anthropogenic disturbances the maturation of sporangia and spores in them does not occur (*Marfenina, Lukina*, 1989).

Tab. 1 The change in the distribution frequence of the Mortierella ramanniana species in different soils under anthropogenic effects

Soil	Type of effect	Distribution frequency, %	
		Control	Trial
Podzol	Recreation degression	65*	10
Turfy-podzolic cultivated	Long-term NPK fertilisation	70*	40
	Liming	70*	20
	Pollution with Cd, 100 mg/kg	100*	-
Turfy-podzolic uncultivated	Recreation degression	60*	10
	Transport-associated pollution	40*	-
Brown forest soil	Pasture degression	80*	20
	Recreation degression	40*	-

* Reliable differences with probability >0.95

In anthropogenically disrupted soils far more transparent are resistant fungi species which are often simultaneously resistant to several anthropogenic effects, which are preserved in the soils and which begin to predominate in them (tab. 2). They are, generally, eurytopic species with a wide distribution range (*Domsh* et al, 1992) and a high sporogenesis level.

It is a known fact that one of the aspects of the natural zonal diversity loss is “trivialisation” of the flora and fauna, i.e. an increase in the density of the so-called “weed” species in communities (*Bigon* et al., 1989). These tendencies can be manifested not only for higher organisms, but also for soil fungi. Table 2 lists soil fungi, the qualitative composition of which in the soil is increased under the effect of certain anthropogenic factors. It should be particularly emphasised that all these species are eurytopic.

Bearing in mind that **fertilisation** is one of the most important meliorative measures in modern production of agricultural crops, it can be also considered to be a serious anthropogenic attack on soil microorganisms.

Soil fungi, as an important indicator of soil biogenity, can be used, among others, as indicators of the economic justification of using different types of fertilisers, particularly their higher rates. An increase in the number of this group of microorganisms, under the effect of nitrogen fertilisers, can be considered positive within certain limits. However, their excessive activation can be also harmful, because the processes focused on establishing the disturbed equilibrium lead to a mineral fertiliser loss, degradation of the soil physico-chemical and biological characteristics and other serious environmental consequences (*Knowles*, 1982).

Tab. 2. Microscopic soil fungi species resistant to different types of the anthropogenic effect

Type of effect	**Resistant species**
Acid precipitations	*Penicillium spinulosum*
Heavy metals: Lead	*Mucor hiemalis* *Penicillium funiculosum* *Aspergillus niger* *Paecilomyces lilacinus*
Cadmium	*Penicillium funiculosum*
Transport-associated pollution	*Aspergillus fumigatus* *Aspergillus niger* Dark-coloured fungi: *Aureobasidium pullulans* *Cladosporium cladosporioides* *Alternaria alternata*
Urbanisation	*Aspergillus fumigatus* *Aspergillus flavus* *Aspergillus niger* *Fusarium oxysporum* *Fusarium moniliforme* *Paecilomyces variotii* *Penicillium vulpinum*
Recreation soil degression	Dark-coloured fungi: *Ulocladium botrytis* *Cladosporium cladosporioides*
Livestock grazing	*Aspergillus flavus*
Long-term nitrogen fertilisation	*Penicillium funiculosum*
Liming	Dark-coloured fungi: *Alternaria alternata* *Cladosporium cladosporioides*

For instance, according to the data by *Djukic* (1992) and *Milosevic* et al. (1993), the long-term use of nitrogen fertilisers leads to a change in the structure of soil microorganism complexes and to an increase in the number of phytopathogenic microorganisms, particularly when the monoculture cropping system is used.

According to our long-term investigations (*Djukic, Mandic*, 1997; *Djukic, Mandic*, 2001; *Mandic, Djukic*, 2004), the number of soil fungi depended not only on the species and fertilisation concentrations used, but also on the soil zones the samples for analysis were taken from and on the crop growing period, as well as on their interactive effects (Tab. 3.).

In general, the most pronounced stimulatory effect on soil fungi is produced by organic fertililsers, solid manure in particular, which may result from an increase in the organic matter amount in the soil, as well as from an improvement in the soil water-air relationship and nutrient regime (*Jarak* et al., 1991).

High nitrogen rates (N_3-150 kg·ha^{-1}) have a destimulatory effect on this group of microorganisms, particularly in initial vegetative phases (tab. 3), whereas lower rates (N_1-80 kg·ha^{-1} and N_2 -120 kg·ha^{-1}) statistically highly significantly stimulate the development of soil fungi.

Given that metabolites of plant and microbiological origin are important regulators of biological value of the soil (*Govedarica* and *Jarak*, 1995), the significantly higher number of soil fungi in the rhizosphere soil compared to that in the edaphosphere soil is a completely expected occurrence.

A decline in the number of soil fungi in final phases of maize development is a consequence of an increase in the amount of precipitations in these phases, which in the soil with a high clay amount creates unfavourable conditions for the development of all aerobic groups of microorganisms (*Drkacev* and *Balog*, 1979)

Tab.3. Numbers of fungi (10^5 g^{-1} dry soil) in the soil under maize as affected by the fertilisers applied (A), sampling zone (B) and vegetation period (C) – Mandic et al., 2004

A		Control		N_1		N_2		N_3		Solid manure		Liquid manure		$\overline{X}$
B		Ed.	Rh.	Ed..	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	Ed.	Rh.	
Periods (C)	I	18.0	27.0	38.3	39.0	30.0	40.3	12.6	15.3	25.0	34.0	24.3	30.6	28.28
	II	31.3	45.3	33.3	39.3	41.3	40.0	21.0	30.3	50.3	91.3	37.0	44.6	42.19
	III	7.3	8.6	19.3	21.7	27.0	29.6	11.3	21.0	11.3	15.3	7.0	8.6	15.14
$\overline{X}$		22.94		31.83		34.72		18.61		37.88		25.38		
$\overline{X}$	Edaphosphere	25.19												
	Rhizosphere	31.88												

Lsd	A	B	C	A´B	A´C	B´C	A´B´C
0.05	2.18	1.17	1.44	3.11	3.80	2.04	5.38
0.01	2.88	1.55	1.91	4.11	5.03	2.69	7.13

Ed.- Edaphosphere; Rh. - Rhizosphere

Heavy metals also significantly affect the number, species composition and viability of soil fungi (*Duxbury*, 1985, *Djukic* et al., 1999; *Djukic, Mandic*, 2000 a,b). They inhibit the processes of mineralisation and synthesis of different substances in the soil, suppress soil fungi respiration, cause a fungistatic effect and the like (*Skvorcova* et al., 1980).

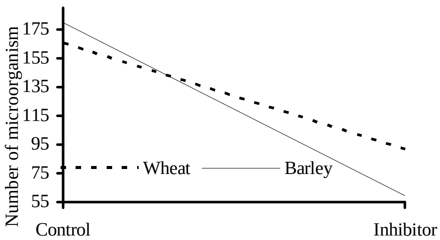
At increased concentrations most heavy metals reduce the number of soil fungi (tab. 4). In laboratory examination conditions, in this respect, a particularly inhibitory effect is exhibited by lead and cadmium (*Djukic, Mandic*, 2006). However, it should be pointed out that concentrations of heavy metals, which undoubtedly inhibit the activity of soil fungi, differ significantly in laboratory and field conditions, so that a minimum reliable reaction in field conditions is evident at metal concentrations 10-50 times higher than the basal ones (Duxbury, 1985).

Tab. 4. Effect of diverse concentrations of Pb, Cu, Cd, Hg on the number of fungi (10^5/1g absolutely dry soil) - Djukic et al., 1996

Heavy metals	*Concentrations*	*Number of fungi*
	Control	*18.0*
Pb	*6.25 mg·l^{-1}*	*12.0*
	0.625 mg·l^{-1}	*13.3*
	0.125 mg·l^{-1}	*13.6*
Cu	*6.00 mg·l^{-1}*	*10.0*
	0.60 mg·l^{-1}	*16.3*
	0.160 mg·l^{-1}	*16.6*
Cd	*2.70 mg·l^{-1}*	*6.3*
	0.27 mg·l^{-1}	*11.6*
	0.027 mg·l^{-1}	*12.0*
Hg	*2.220 mg·l^{-1}*	*1.1*
	0.220 mg·l^{-1}	*6.6*
	0.022 mg·l^{-1}	*11.0*

The **nitrification inhibitor** effect on soil fungi depends on the inhibitor species and concentration, time of application, soil type and its characteristics, as well as on environmental factors determining the direction, rate, speed and products of microbiological transformation (*Kanivec* and *Kiselj,* 1978; *Smirnov* et al., 1981; *Graceva*, 1982; *Muravin* et al. 1985; *Mandic, Djukic*, 1997).

Our investigations (*Djukic*, *Mandic*, 1999) indicated that the number of soil fungi depended not only on the inhibitor type used (N-serve) but also on the soil type and the crop cultivated. A continual decline in their number, under the effect of the inhibitor used, was more pronounced in the soil under barley than in that under wheat (graph 1.).



Lsd 5% = 7.98
1% = 11.04

Graph 1. The interaction between nitrification inhibitor and crop effects on the fungi number in the soil (10^5/1 g absolutely dry soil) - Mandic, Djukic (1999)

Table 5. An overview of physico-chemical characteristics of the water used for irrigating agricultural crops (Djukic et al., 1999)

Indicators	**Value registered**	**Indicators**	**Value registered**
pH	7.34	Ca	96.18 mg·dm^{-3}
NH_3	0.156 mg·dm^{-3}	Mg	14.59 mg·dm^{-3}
NO_3	66.00 mg·dm^{-3}	Mn	0.00 mg·dm^{-3}
NO_2	0.775 mg·dm^{-3}	Cu	0.0008 mg·dm^{-3}
$KMnO_4$	340.00 mg·dm^{-3}	Zn	0.03 mg·dm^{-3}
O_2	1.42 mg·dm^{-3}	Pb	0.031 mg·dm^{-3}
BPK_5	3.57 mg·dm^{-3}	As	0.008 mg·dm^{-3}
HPK	2.99 mg·dm^{-3}	Ni	0.028 mg·dm^{-3}
Suspended matter	556.00 mg·dm^{-3}	Hg	0.0018 mg·dm^{-3}
Total hardness	18.05 mg·dm^{-3}	Cr^{6+}	0.030 mg·dm^{-3}
Chlorides	1.37 mg·dm^{-3}	Cr (total)	0.042 mg·dm^{-3}
Sulfates	71.70 mg·dm^{-3}	Phenols	0.002 mg·dm^{-3}
Phosphates	0.314 mg·dm^{-3}	Detergents	0.090 mg·dm^{-3}
Fe	0.525 mg·dm^{-3}	Mineral oils	0.170 ml·dm^{-3}

The use of **polluted waters** for irrigation and waste waters for soil fertilisation as well, due to the range of pollution of most watercourses, has a number of advantages but also shortcomings. The former include soil amendment and increase in the activities of microorganisms, increase in soil capacity to bind water and biological soil activation. The shortcomings of using polluted waters are as follows: the risk of the presence of heavy metals, toxins and pathogenic bacteria, and of the unproportional percentage of nutritive substances (as a rule, K supplements are necessary), certain nutrients can be found in unsoluble form, heavy metal ions and sulphuric compounds lead to a change in soil microbial cenosis (*Yevdokimova, Mozgova*, 1976; *Mandic* et al., 1994; *Djukic, Mandic*, 1996) etc. Waste water irrigation has an adverse effect on the organic matter evolution in surface soil horizons, being exhibited in a low organic matter humification degree and a proportionally lower content of highly polymerised humic acids (*Konecka-Betley and Zebrowski*, 1978) and therefore its biological value.

The investigations by *Djukic* et al. (1999) indicate that soil fungi can be used as a parameter for evaluating the quality of waters to be used for irrigating agricultural crops.

The water used for irrigating the agricultural crops selected (tab. 5) was loaded with different pollutants including primarily different agents entering it through industrial and municipal waste waters. Low O_2 concentration (1.42%) and values of BPK_5 (3.57 $mg{\cdot}dm^{-3}$), NO_2 (0.775 $mg{\cdot}dm^{-3}$), NO_3 (65 $mg{\cdot}dm^{-3}$), NH_3 (0.156 $mg{\cdot}dm^{-3}$), PO_4 (0.314 $mg{\cdot}dm^{-3}$), $KMnO_4$ consumption (340 $mg{\cdot}dm^{-3}$) and pH above 7 (7.34) indicated that intensive organic matter decay processes were underway. Of the toxic substances, the following were registered: phenols (0.002 $mg{\cdot}dm^{-3}$), mineral oils (0.17 $ml{\cdot}dm^{-3}$), detergents (0.09 $ml{\cdot}dm^{-3}$), Pb (0.031 $mg{\cdot}dm^{-3}$), As (0.008 $mg{\cdot}dm^{-3}$), Ni (0.028 $mg{\cdot}dm^{-3}$) etc.

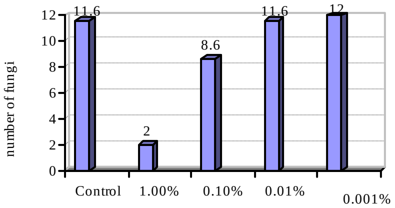
Generally, the irrigation water had an adverse effect on soil fungi in both soil types, and particularly in the smonitza under red clover (tab. 6). During the growing season, a rise in the fungi number was observed, which was especially pronounced towards the end of the growing season, which was due to microbiological inactivation of toxicants from the irrigation water, improvement of soil structure and balancing of the water-air relationship (*Uhrecky, Zvanovec*, 1956). Absolute fungi number values were higher in the smonitza under all three crops.

The uncontrolled discharge of **detergents** into water and arable soil can cause reduced biological production of these ecosystems, leading to adverse consequences, both environmental and economic ones. Soil fungi, in this respect, can affect, up to a certain limit, their biodegradation, converting them into less toxic or often energetically important nutrient sources (*Stojanovic* et al., 1990). Basically, increased detergent concentrations or their accumulation in the soil (*Goncaruk* and *Sidorenko*, 1986) bring about a rapid decline in the number of these microorganisms, and so an analysis of the chemical compounds load limits in the soil would provide a far more realistic picture in the neochemistry-effluents-soil system.

Table 6. The effect of polluted irrigation water on the number of soil fungi (10^{-5}) – Djukic et al., 1999

Sampling phases	ALLUVIUM						SMONITZA					
	Wheat		Barley		Clover		Wheat		Barley		Clover	
	∅	trial	∅	trial	∅	trial	∅	trial	∅	trial	∅	trial
I	14	12	10	6	20	12	14	13	12	13	22	16
II	14	16	16	30	28	38	24	36	40	40	74	42
III	46	38	84	74	136	100	72	51	51	74	150	144
$\bar{X}$	74	66	120	110	184	150	110	100	103	127	242	202
	70.00		115.00		165.00		105.00		115.00		222.00	

Based on our laboratory investigations (*Mandic* et al., 2006) it can be concluded that the number of soil fungi had a high correlation with the concentration of the detergent introduced (graph 2). As a matter of fact, low concentrations of detergents evidently did not have any significant effect on this group of microorganisms. This particularly referred to low (0.001%) and even tenfold detergent rates (0.01%), the effect of which was mildly stimulatory or at the control variant level.



Graph 2. The effect of different "Meril" detergent concentrations on the number of soil fungi ($10^5 g^{-1}$ absolutely dry soil)- Mandic, Djukic, 2006

This is associated with the familiar trait of soil fungi of having a strongly developed enzymic system (*Stojanovic* et al., 1995) securing for them the capacity to degrade and intoxicate different xenobiotics in the soil, as well as a pronounced adaptability to live even in the conditions of increased concentrations of not only detergents, but also of pesticides, heavy metals, mineral fertilisers etc. (*Umarov*, 1980). As opposed to the mentioned, higher concentrations of the detergent (0.1 and 1%), despite the soil fungi features mentioned, caused a significant fungicidal effect.

4.CONCLUSION

Based on the results of the long-term investigations of the effect of different technogenic pollutions (mineral and organic fertilisers, heavy metals, polluted irrigation water, nitrification inhibitor and detergents) on the dynamics of the soil fungi number the following conclusions can be drawn:

- the number of soil fungi depended on the species and rate of agrochemicals used, growing season and soil zone the samples were taken for analysis from;
- lower nitrogen fertiliser rates (80 and 120 kg·ha^{-1}) and organic fertilisers stimulated the development of soil fungi, which was not the case with the 150 kg·ha^{-1} rate;
- heavy metals, particularly mercury and cadmium, inhibited the development of this group of soil microorganisms;
- the use of high N-serve nitrification inhibitor rates gave rise to a more pronounced continual decline in the number of fungi under barley, compared to wheat;
- generally, the polluted irrigation water had exerted an adverse effect on soil fungi in both soil types, particularly in the smonitza under red clover;
- low (Meril) detergent concentrations did not have any significant effect on this group of microorganisms. As opposed to the mentioned, its higher concentrations (0.1 and 1%) caused a significant fungicidal effect;
- generally, the number of soil fungi was significantly higher in the rhizosphere of the crops investigated than in the edaphosphere;
- in terms of the mentioned, we conclude that the soil fungi number dynamics can be used in monitoring soils polluted with different toxinogenic substances.

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