



## ARAŞTIRMA MAKALESİ/RESEARCH ARTICLE

### SOIL MICROFUNGI OF AGRICULTURAL FIELDS POLLUTED BY LEAD (Pb<sup>2+</sup>) IN SATILMIŞOĞLU VILLAGE (ESKİŞEHİR-TURKEY)

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#### ABSTRACT

The microfungial flora of the agricultural fields exposed to lead pollution due to a nearby lead recovery factory in the vicinity of Satılmışoğlu Village, Eskişehir, was investigated both qualitatively and quantitatively. Thirty six soil samples from nine selected areas were taken seasonally for a whole year (1997). Probable effects of lead pollution aside, seasonal variations and some ecological parameters on soil microfungi were investigated.

The results obtained showed the number of microfungi in one-gram oven dried soil to range between 40 000 and 310 000 CFU. Apart from determining the number of microfungi, we also isolated and defined 45 species of 13 genera (*Aspergillus*, *Cladosporium*, *Fusarium*, *Geosmithia*, *Helminthosporium*, *Macrosporium*, *Paecilomyces*, *Penicillium*, *Rhizoctonia*, *Rhizopus*, *Stachybotrys*, *Thielaviopsis* and *Trichoderma*). The most commonly distributed species in the research area were determined to be *Penicillium aurantiogriseum*, *Trichoderma* sp., *P. puberulum*, *Rhizopus* sp., *P. simplicissimum* and *T. koningii*. It was observed a variance in the diversity of *Penicillium* species in the station with the highest levels (380 ppm) of contamination. It was thought that these species, *P. albidum*, *P. aurantio-virens*, *P. canescens*, *P. cyclopium* and *P. lanoso-coeruleum* may be resistant and/or tolerant to lead ions.

**Key words:** Soil microfungi, Lead pollution, Agricultural fields, Wheat fields, Eskişehir.

### SATILMIŞOĞLU KÖYÜ (ESKİŞEHİR/TÜRKİYE) KURŞUN (Pb<sup>2+</sup>) POLLUSYONLU TARIM ALANLARININ TOPRAK MİKROFUNGUSLARI

#### ÖZ

Bu araştırmada, Eskişehir iline bağlı Satılmışoğlu Köyü yakınında faaliyet gösteren kurşun geri kazanım fabrikası çevresindeki kurşun bulaşmış tarım topraklarının mikrofungus florası kalitatif ve kantitatif olarak araştırılmıştır. Seçilen 9 farklı alandan mevsimsel olarak bir yıl (1997) boyunca 36 toprak örneği alınmıştır. Mevsimsel değişimler ve bazı ekolojik parametreler ile birlikte kurşun kirliliğinin toprak mikrofungusları üzerine olası etkileri araştırılmıştır.

Bu araştırmadan elde edilen bulgular, bir gram fırın kuru topraktaki mikrofungus sayısının 40 000- 310 000 CFU arasında olduğunu göstermiştir. Toplam 13 cins (*Aspergillus*, *Cladosporium*, *Fusarium*, *Geosmithia*, *Helminthosporium*, *Macrosporium*, *Paecilomyces*, *Penicillium*, *Rhizoctonia*, *Rhizopus*, *Stachybotrys*, *Thielaviopsis* and *Trichoderma*), 45 tür izole edilerek tanımlanmıştır. Araştırma bölgesinde *Penicillium aurantiogriseum*, *Trichoderma* sp., *P. puberulum*, *Rhizopus* sp., *P. simplicissimum* and *T. koningii*'nin en yaygın olan türler olduğu bulunmuştur. En yüksek kurşun konsantrasyonuna (380 ppm) sahip olan istasyonda *Penicillium* tür çeşitliliğinde farklılık gözlenmiştir. Bu türler *P. albidum*, *P. aurantio-virens*, *P. canescens*, *P. cyclopium* and *P. lanoso-coeruleum* olup kurşun iyonlarına dirençli ya da hoşgörülü olabilecekleri düşünülmüştür.

**Anahtar Sözcükler:** Toprak mikrofungusları, Kurşun kirliliği, Tarım alanları, Eskişehir.

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## 1. INTRODUCTION

Fungi are ubiquitous in natural environments and important in industrial processes. Their most important roles are being decomposers of organic material, with concomitant nutrient cycling, being pathogens and symbionts of animals and plants and as spoilage organisms of natural and synthetic materials. They are also utilized as producers of substances which hold economic importance, such as ethanol, citric acid, antibiotics, polysaccharides, enzymes and vitamins (Gadd, 1993).

While several studies have been made into microfungi in agricultural soils (Hasenekoğlu, 1985; Hasenekoğlu, 1987; Haliki and Dizbay, 1997; Asan, 1997; Ilhan and Asan, 2001) in Turkey so far, the number of studies into microfungi of soils polluted and /or disturbed is rather limited (Hasenekoğlu, 1982; Hasenekoğlu and Sülün, 1990; Hasenekoğlu and Azaz, 1991). Interestingly enough, no study into the variation of microbiological properties of agricultural soils polluted by heavy metals has been reported in the literature up to date. Babich and Stotzky (1979) studied abiotic factors having an effect upon the toxicity levels of lead in relation to fungi. The microfungi species composition in coniferous forest soil surrounding a brass mill at Gusum in southeast Sweden was studied by Nordgren et al. (1985). Konopka et al. (1999) determined microbial community diversity, potential microbial activity, and metal resistance in three soils whose lead contents ranged from 0.00039 to 48 mmol of Pb/kg soil.

Lead is regarded as a strategic metal belonging to the "three big" toxic heavy metals and is therefore being widely used for so many important industrial applications. According to local directives the permissible limit values of lead in soil are 50 mg/kg oven dried soil when pH of soil is smaller than 6 and 300 mg/kg oven dried soil when pH of soil is greater than 6 (Anonymous, 2001). The permissible limit of lead in drinking water is 5 µg/L. The presence of lead in drinking water above the permissible limit causes such diseases as anaemia, encephalopathy, hepatitis and nephritic syndrome (Giller et al., 1998).

The lead recovery factory near Satılmışoğlu Village, Eskişehir-Turkey, seemed to contribute to lead pollution in the soil occurring in this area, to the soil in the fields occurring in the direction of the dominant wind in particular. Since wheat was cultivated in these fields, determination of the microbial population activities in the soil became of high interest in our research. This paper focuses on determining microfungi essential for productivity of the soil in a quantitative manner as well as on determining the variation of natural microfungi flora of cultivated fields in the vicinity of the lead recovery factory.

## 2. MATERIAL AND METHODS

### 2.1 Description of the Research Area

The research area surrounding the TEK Lead Industry Limited Firm in Satılmışoğlu Village is on the road connecting Eskişehir and İstanbul, at a distance of 10 km from Eskişehir province (Figure 1). Some agricultural fields cultivated wheat occur in the surroundings of the research area. The average temperatures, the amount of rainfall and soil temperature values in the study area taken from meteorological reports have been presented in Table 1.



Figure 1. The map showing Eskişehir (Turkey) province and research area.

Table 1. The average temperature, rainfall and soil temperature values in the study area.

	Mean temperature <sup>a</sup> (°C)	Total rainfall <sup>a</sup> (mm)	Soil temperature <sup>b</sup> (°C)
February 1997	- 0.7	10.0	1.2
May 1997	15.5	40.5	17.5
August 1997	18.6	23.7	20.8
November 1997	5.8	17.6	7.2

<sup>a</sup> Obtained from Eskişehir meteorological station.

<sup>b</sup> Average, in 100 mm depth.

The lead factory near Satılmışoğlu Village, Eskişehir that we chose as our research area is a pollutant source attributable to the waste released from its chimney. The factory consists of such units as separation of plastics from batteries, clearing, breaking into pieces, preparation of row material, melting, spilling to crucible and getting rigid. Burning and melting lead take about 6-7 hours, with the temperature of the oven being about 1000-2000 °C and through the chimney of the melting oven are the lead compounds released into the atmosphere (Kara et

al. 1995). The gasses and lead particles released from the factory chimney have been filtered for several years. However, before the authorities could adopt necessary measures by supplying the factory with filters according to laws, high levels of lead had already accumulated in the agricultural soil occurring around the factory. We, therefore, thought that biological nature was highly vulnerable to the resulting effects of lead accumulation in agricultural soil.

While determining the stations, gas mixture and some particles released the factory chimney were deemed as the most important pollutant factors. What was also taken into consideration was that the annual average wind velocity is 2.8 m/sec. The highest wind velocity measured during the observation time was 27.8 m/sec, blowing from the north and the northwest (N and NW) in July. The measurements recorded for this area for 31 years seemed to indicate that the primary dominant wind direction is the west (W), the second being the east (E) and the third being the northwest (NW) and the number of blows was 8405, 4418 and 3646, respectively, in Eskişehir (Bayar, 1991).

## 2.2 Soil characteristics

Soil moisture, pH, organic matter content and lead ion quantity of each soil sample were measured in the laboratory of Ministry of Environment, Forest, Soil and Ecology Research Institute, Eskişehir/Turkey. Soil moisture level of each sample was calculated as percentage moisture. The organic matter analysis was made by the Walkley and Black method (1934). Lead ion quantity was measured with a Perkin Elmer 3110 Atomic Absorption Spectrometer.

## 2.3 Collection of Soil Samples and Isolation of Microfungi

The soil samples were collected according to the Brown's technique (1958) in the middle of February (1997), May (1997), August (1997) and November (1997). For each station, the soil samples were taken with a sterilized trowel from 5 points, in 10 cm depth and were then mixed (total approx. 300-500 g). The samples were transferred into sterile polyethylene bags. On arrival at the laboratory, the soil samples were stored at 4°C until they were ready for applications. The soil samples were taken to be examined for the enumeration and isolation processes of microfungi within 24 hours.

Microfungi were isolated by the soil dilution plate method (Waksman, 1922; Warcup, 1955). Twenty five grams of each soil samples (dry weight) were initially diluted in sterile distilled water and this dilution ( $10^{-1}$ ) was shaken for about half an hour in a shaker to break up the soil particles. In sterile distilled water, dilutions of  $10^{-4}$  of each sample were used for inoculation. For the purpose of isolating fungi and

estimating the fungal number, peptone-dextrose agar containing Rose-Bengal and streptomycin was used. Ten petri dishes were inoculated with one ml portion of the ultimate dilution at a time. The dishes were incubated at 25-27 °C for 7-14 days. Following the calculation of the number of microfungi per gram of oven dried soil, the microfungi were transferred into malt extract agar slants to isolate pure fungal cultures.

## 2.4 Identification of Microfungi

Characteristics of fungal species were determined based on micro and macro morphology, reverse and surface coloration of colonies grown on relevant media. Fungi were identified at the genus levels using Barnett and Hunter's work (1999). "The Genus *Aspergillus*" (Raper and Fennell, 1965), "A Manual of the *Penicillia*" (Raper and Thom, 1949), "The Genus *Penicillium* and its Teleomorphic States *Eupenicillium* and *Talaromyces*" (Pitt, 1979) were used for the identification of *Aspergillus* and *Penicillium* species. Various mycological references were used for identification of other fungi at the species levels (Gilman, 1957; Booth, 1971; Joffe, 1974; Domsch et al., 1980; Samson et al., 1981; Hasenekoğlu, 1991). Microscopic examinations and measurements of the fungal structures were performed in lactophenol developed by AMMANN. All names of the identified species and authors were cited according to Kirk and Ansell (1992).

Statistical analyses were made by SPSS 10.0 packet program (Özdamar, 1999). A correlation analysis was achieved between the number of microfungi and environmental factors.

## 3. RESULTS AND DISCUSSION

The average temperature, rainfall and soil temperature values in the study areas have been presented in Table 1, while the sites of soil samples and their mechanical composition have been presented Table 2. Table 3 shows the mean number of the fungi with soil properties of each station and Table 4 presents the seasonal variation and distribution of fungi isolated in the research stations.

The following species of *Aspergillus* (7), *Cladosporium* (1), *Fusarium* (2), *Geosmithia* (1), *Helminthosporium* (1), *Macrosporium* (1), *Paecilomyces* (1), *Penicillium* (30), *Rhizoctania* (1), *Rhizopus* (2), *Stachybotrys* (1), *Thielaviopsis* (1) and *Trichoderma* (2) were determined as 45 species and 13 genera in the nine selected research stations. Some microfungi that could not be identified due to non-reproductive structures were also observed in each station (Table 4).

The species determined to occur most were as follows: *P. aurantiogriseum*, *Trichoderma* sp., *P. puberulum*, *Rhizopus* sp., *P. simplicissimum* and *T. koningi*. Their total frequency of occurrences were determined to be 50%, 30%, 25%, 25%, 19.4%,

19.4%, respectively. All these were present in at least seven of total 32 the soil samples. However, 21 species could be recorded in one soil sample only, which seems to be suggestive of the scarcity of these species in this area (Table 4). Our findings confirmed the generally accepted view, which states that the most common soil fungi are the members of *Aspergillus*, *Penicillium*, *Rhizopus*, *Fusarium* and *Cladosporium* genera.

Studies into the soil fungi show that a wide spectrum of microfungi is present in the soil. They also point out that the quantity and variability of *Aspergillus* and *Penicillium* species are higher than those of the other microfungi in soil (Asan, 1997; İlhan and Asan, 2001). While *Aspergillus* species prefer the warm areas mostly, the preference of *Penicillium* species is a temperate climate (Domsch et al., 1980; Hasenekoğlu, 1985). The climate of Eskişehir is convenient for distribution of the *Penicillium* species (Tables 1 and 4). In view of that, the species belonging to this genus were most frequently determined in the research area. According to our results, 50% of the *Penicillium* species were isolated in the winter time. For spring, autumn and summer, these rates were 45 %, 35% and 23 %, respectively. Most of the species determined in our research had also been determined in some other studies carried out in Turkey (Asan, 2004). In one of our previous studies, it was reported that *A. parvulus*, *P. ademetzii*, *P. citrinum*, *P. restrictum*, and *P. velutinum* were isolated from wheat fields in Eskişehir (İlhan and Asan, 2001). Another study isolated the following species from the cultivated fields in Eskişehir; *A. terricola* var. *americanus*, *A. versicolor*, *P. aurantiogriseum*, *P. brevicompactum*, *P. granulatum*, *P. griseoroseum*, *P. implicatum*, *P. purpurogenum*, *P. viridicatum* and *Paecilomyces lilacinus*. (Demirel, 2003). We can, therefore, conclude that studies carried out in Eskişehir and in Turkey have determined the species of *Penicillium* to be the most widely-distributed species of fungi.

The generally accepted number of microfungi in one gram of the fertile soil in agriculture fields is about 400.000 (Asan, 1997). The mean numbers of the isolated microfungi determined in some investigations have shown the differences (Rama Rao, 1970; Haliki and Dizbay, 1997; Hasenekoğlu, 1985). Some numbers of microfungi determined in our research is in agreement with the results of some other studies (Hasenekoğlu and Sülün, 1990; İlhan and Asan, 2001). The highest number of fungi was obtained from station III (310 000 cfu/g dry soil) in autumn, while the lowest number of microfungi was recorded during the summer at station I (40 000 cfu/g dry soil). The differences in the variances occurring in the stations could be attributed to some factors exclusive to each type of soil. The order of the stations in terms of the mean number of microfungi was determined as station III, IX, VIII, IV, II (Table 1).

It is a well-documented fact that several factors may affect the number of varieties and the quantitative distribution of microfungi in the soil in a both positive

and negative way. These factors are physical and chemical properties of the soil as well as seasonal changes (Rama Rao, 1970). But, in this study no significant correlation could be achieved between the environment factors (moisture, pH, organic matter) and the number of fungi. Nevertheless, it was determined that the correlation between the lead available and the number of fungi in soil was significant ( $r=0.370$ ,  $p<0.05$ ). The correlation was positive. Our soil samples had low  $Pb^{2+}$  concentrations. In addition, pH and clay content of our soil samples may have an effect on availability and toxicity of lead. Much of the lead may have been unavailable due to reactions with clay minerals, soil organic matter, or inorganic anions. Therefore, when growth of very susceptible species were inhibited, growth of resistant and/or tolerant species were not affected. Nonetheless, the microfungal species composition in the soil samples may vary due to different  $Pb^{2+}$  concentrations and its bioavailable degrees in the soil.

In general terms, toxic metals are believed to affect fungal populations by reducing abundance and species diversity and selecting a resistant/tolerant population. However, the effects of toxic metals on microbial abundance in natural habitat vary with the metal species and organisms present and with environmental factors (Gadd, 1993, Gadd and Griffiths, 1978). Several studies have indicated to be susceptible and tolerant/resistant of *Penicillium* spp. to heavy metals (Duxbury, 1985, Stokes and Lindsay, 1979). In this study, it has been emphasized that diversity of *Penicillium* spp. decreased at station III with the highest lead concentrations (mean 380 ppm  $Pb^{2+}$ ). In the other stations, the diversity of *Penicillium* determined varied between 9-18 species while station III had 5 *Penicillium* species, *P. albidum*, *P. aurantio-virens*, *P. canescens*, *P. cyclopium* and *P. lanosocoeruleum*. These species may be resistant and/or tolerant to lead ions.

#### 4. CONCLUSIONS

It may generally be expressed that the differences of microbial species composition in the soil samples may be attributed to the different amounts of  $Pb^{2+}$  concentrations and some other effective environmental factors in the soil itself. We can, therefore, suggest that elevated concentrations of heavy metals may affect the qualitative and quantitative compositions of fungal populations. However, we emphasize that it may be difficult to separate metal effects from those of other environmental components.

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Table 2. Some characteristics and the number of microfungus of examined soils.

Station No.	Seasons	pH	Organic matter (%)	Moisture (%)	Pb <sup>2+</sup> (ppm)	The number of microfungus (cfu/g dry soil)
1	Wi	7.60	2.34	21	28.18	65 000
	Sp	7.70	2.45	13	14.80	67 000
	Sm	7.60	2.52	16	6.68	40 000
	Au	7.75	2.61	21	4.83	49 000
	Average	<b>7.66</b>	<b>2.48</b>	<b>17.75</b>	<b>13.62</b>	<b>55 000</b>
2	Wi	7.60	2.57	23	6.15	159 000
	Sp	7.60	2.88	14	18.59	86 000
	Sm	7.70	2.43	16	7.42	163 000
	Au	7.70	2.61	22	6.93	86 000
	Average	<b>7.65</b>	<b>2.62</b>	<b>18.75</b>	<b>9.77</b>	<b>123 000</b>
3	Wi	7.50	2.41	23	404.93	162 000
	Sp	7.55	3.61	13	405.57	110 000
	Sm	7.55	2.70	18	294.89	144 000
	Au	7.65	2.99	23	417.90	310 000
	Average	<b>7.6</b>	<b>2.93</b>	<b>19.25</b>	<b>380.82</b>	<b>181 500</b>
4	Wi	7.60	2.75	23	13.78	232 000
	Sp	7.70	3.59	14	7.50	53 000
	Sm	7.60	2.36	4	14.55	143 000
	Au	7.70	2.66	20	5.14	103 000
	Average	<b>7.65</b>	<b>2.84</b>	<b>15.25</b>	<b>10.24</b>	<b>132 750</b>
5	Wi	7.77	2.59	19	9.40	142 000
	Sp	7.75	2.72	16	5.66	41 000
	Sm	7.80	1.88	12	4.28	61 000
	Au	7.75	2.31	17	2.41	162 000
	Average	<b>7.77</b>	<b>2.38</b>	<b>16</b>	<b>5.44</b>	<b>101 550</b>
6	Wi	7.75	2.76	18	4.77	95 000
	Sp	7.65	2.59	18	5.67	76 000
	Sm	7.70	2.32	14	2.96	64 000
	Au	7.70	2.60	17	0.31	105 000
	Average	<b>7.7</b>	<b>2.57</b>	<b>16.75</b>	<b>3.43</b>	<b>85 175</b>
7	Wi	7.70	2.64	22	91.31	120 000
	Sp	7.60	2.10	15	25.58	71 000
	Sm	7.65	2.36	17	70.07	50 000
	Au	7.80	2.08	23	46.51	126 000
	Average	<b>7.69</b>	<b>2.30</b>	<b>19</b>	<b>58.37</b>	<b>91 750</b>
8	Wi	7.70	2.93	22	12.88	100 000
	Sp	7.65	2.06	16	3.64	93 700
	Sm	7.65	2.69	12	6.89	54 000
	Au	7.70	2.60	23	22.99	306 000
	Average	<b>7.68</b>	<b>2.57</b>	<b>18</b>	<b>11.60</b>	<b>138 425</b>
9	Wi	7.70	2.28	23	34.81	74 000
	Sp	7.70	2.45	14	150.57	105 000
	Sm	7.60	2.28	20	97.85	115 000
	Au	7.70	2.69	23	86.62	278 000
	Average	<b>7.68</b>	<b>2.43</b>	<b>20</b>	<b>92.46</b>	<b>143 000</b>

Letters indicate: Sm: Summer; Au: Autumn; Wi: Winter; Sp: Spring



Table 4 (Continued)

<i>P. chrysogenum</i> Thom	Wi	Wi		Wi				Sp	
<i>P. citrinum</i> Thom							Sp		
<i>P. claviforme</i> Bain.								Wi	
<i>P. corylophilum</i> Dierckx	Au			Sp, Au	Au				Au
<i>P. daleae</i> K.M. Zalessky	Wi	Wi	Wi						
<i>P. echinulatum</i> Fassatiöva	Sp							Au	
<i>P. funiculosum</i> Thom				Au				Wi	
<i>P. granulatum</i> Bain.					Sp				
<i>P. griseoroseum</i> Dierckx									Au
<i>P. implicatum</i> Biourge								Sm	
<i>P. janczewskii</i> K.M. Zalessky				Au					
<i>P. janthinellum</i> Biourge		Wi			Wi		Wi	Wi	
<i>P. jensenii</i> K.M. Zalessky				Wi				Sp	
<i>P. miczynskii</i> K.M. Zalessky						Sm			
<i>P. oxalicum</i> Currie & Thom						Sp			
<i>P. puberulum</i> Bain.	Wi, Sm	Wi, Sm		Wi	Au		Sp, Sm		Sm
<i>P. purpurogenum</i> Stoll									Wi
<i>P. restrictum</i> J.C. Gilman & E.V. Abbott									Wi
<i>P. roseopurpureum</i> Dierckx		Au		Sm				Sp	
<i>P. simplicissimum</i> (Oudem.) Thom		Sp, Sm			Sp		Sp	Wi	Wi, Sm
<i>P. spinulosum</i> Thom	Wi								
<i>P. velutinum</i> J.F.H. Beyma	Wi	Sp, Wi			AS	Au			
<i>P. viridicatum</i> Westling		Au		Wi	Sp	Sp	Au		
<i>Rhizoctonia sp.</i> D.C. ex Fr.	Au			Au					
<i>Rhizopus oryzae</i> Went & Prins. Geerl.		Sp, Sm	Au		Wi	Sp		Wi	
<i>Rhizopus sp.</i> Ehrenberger		Wi	Wi, Au	Wi	Sm, Wi		Sp	Au	Sm
<i>Stachybotrys chartarum</i> (Ehrenb.) S. Hughes	Au								
<i>Thielaviopsis basicola</i> (Berk. & Broome) Ferraris			Sm				Sm		
<i>Trichoderma koningii</i> Oudem.		Au	Wi	Au			Sp, Sm	Sp	Au
<i>Trichoderma sp.</i> Pers. ex Fr.	Sp	Sm		Wi	Sp, Sm	Sp, Wi	Sp, Wi	Wi	Wi
Sterile	AS	AS	AS	AS	AS	AS	AS	AS	AS

Letters indicate: Sm: Summer; Au: Autumn; Wi: Winter; Sp: Spring; AS: All Seasons

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