Effect of copper sulphate on assimilation of various substrata by soil fungi

L. Levinskaitė

Institute of Botany,
49 Žaliųjų ežerų,
LT-2021 Vilnius, Lithuania

Influence of 10 mM copper sulphate incorporated in the soil (under laboratory conditions) on the count of fungi and their ability to grow on different substrata was investigated. The obtained results showed that copper sulphate reduced the total amount of fungi from $11.2 \cdot 10^3$ to $8.2 \cdot 10^3$/g. The count of micromycetes growing on specific substrata allowing to detect fungi producing proteases, amylases, chitinases, cellulases and phenoloxidases also decreased. When copper sulphate was added into the media (the direct effect), the total number of fungi and their capability of assimilating different substrata was reduced much more. The most significant decrease of the fungal count was noticed for cellulase-producing fungi and the lowest for protease-synthesizing micromycetes. The number of fungi – possible producers of phenoloxidases increase under the influence of this factor.

Key words: mycromycetes, copper sulphate, substrata, count reduction

INTRODUCTION

Micromycetes are ubiquitous organisms in terrestrial ecosystems and take part actively in the degradation of plant remnants, animal debris and other organic materials, and thus through direct synthesis of high molecular weight polymers such as humic-acidlike substances and polysaccharides participate in soil structure formation [3, 12, 24]. Pollution of the soil by various harmful ingredients including heavy metals change not only the composition of microbial communities, but also affect general physiological processes of microorganisms and inhibit their development [1, 5, 15].

Some authors have indicated that heavy metals can reduce activity of microorganisms [17, 19, 20]. A significant reduction in litter decomposition has been observed in sites polluted with zinc, cadmium and other metals [2, 6]. It was shown that cadmium inhibited one or several steps in protein biosynthesis and thus lead to enzyme deficiency [4, 22]. Copper plays an essential role in many enzymes, but its high concentrations can exert a harmful effect [14, 16, 18]. Copper sulphate is widely used as an effective antifungal chemical for various purposes. Copper in the sulphate form enters the soil from various industrial sources and agriculture activities. Therefore, influence of copper sulphate on alterations of physiological activity and viability of soil fungi is understood insufficiently and remains as a problem to be studied.

In a natural environment, however, it is often difficult to find out if differences in the microorganism count and activity in polluted and non-polluted sites resulted from a particular harmful factor or from other environmental influences. Therefore, the aim of this work was to determine, under controlled conditions, the effect of $CuSO_4$ on the number of fungi and their activity towards some substrata.

MATERIALS AND METHODS

Soil samples (a sandy loam soil) were taken from a forest (Verkiai regional park) at a depth of 10–20 cm. Air-dried soil samples were put into sterile vessels with light ventilation. The samples were divided into two parts: the soil of part A was wetted with sterile distilled water of the same volume, and the part B was treated with $CuSO_4 \cdot 5H_2O$ solution. Sterile $CuSO_4$ water solution was added in the amount enough for the final concentration in the soil to reach 10 mM. Two months later, isolation of fungi was performed by the dilution plate technique. Effect of $CuSO_4$ on fungal viability and their enzyme systems enabling to use different substrata was evaluated according to the change of a number of colony-forming units on various substrata. Diluted soil

samples were plated on the Czapek medium where carbon source was sucrose (a control), and media of the same composition without sucrose but amended with other substrata: 1% skim milk, starch, chitin or cellulose (paper) for testing the growth of protease, amylase, chitinase and cellulase producing fungi [10]. The samples also were plated on the Czapek medium with 0.005% γ-naphthol, and according to the dark-blue color released under colonies, fungi producing phenoloxidases were determined [25].

In order to reveal a direct $CuSO_4$ effect on fungal ability to use these substrata, additionally one series of samples was plated on the same media but supplemented with 5 mM $CuSO_4$. All the platings were performed in triplicates.

RESULTS AND DISCUSSION

A 2-month exposition of soil samples to the effect of $CuSO_4$ revealed that this factor not only changed the total number of micromycetes, but also affected assimilation of different substrates (table).

The total fungal count from untreated samples (A) was $11.2 \cdot 10^3$ /g, while the total amount of fungi was reduced by 10 mM $CuSO_4$ in the soil (B samples) to $8.2 \cdot 10^3$ /g or 73.2% (Fig. 1, Table). It should be noted that the pH of the untreated soil was 5.5, while in the soil with $CuSO_4$ added the pH reached 6. Changes of pH could also have influenced the count and activity of micromycetes.

When the soil samples were plated on the medium with addition of copper sulphate, fungal growth was more suppressed. The total amount of fungi from A samples on the Czapek medium with incorporated 5 mM $CuSO_4$ decreased to $2.6 \cdot 10^3$ colony forming units and the number of micromycetes from soil samples affected with $CuSO_4$ decreased even to $2.2 \cdot 10^3$ or by 23.2% and 19.6%, respectively (Fig.1).

When the soil suspensions were plated on the medium amended with skim milk for fungi producing proteases, it was found that $2.1 \cdot 10^3$ fungi from the untreated soil were able to grow on this substrate and only $1.4 \cdot 10^3$ micromycetes developed from $CuSO_4$ treated soil, or 18.8% and 12.5%, respectively, in comparison to the total amount of fungi isolated from the untreated soil samples A on the Czapek medium (control). Protease-producing fungi when plated on the medium with addition of copper sulphate were decreased to $1.6 \cdot 10^3$ from A samples and $0.9 \cdot 10^3$ from B samples (14.35% and 8.0%). (Fig.1).

Quite high amounts of fungi able to produce amylases were found both from the control samples and those affected by $CuSO_4$ ($8.3 \cdot 10^3$ and $7.1 \cdot 10^3$, respectively). (Fig.1). However, a significant decrease of fungal counts was noticed on the medium containing copper sulphate. The number of amylase-producing fungi on this medium from A samples was reduced to $1.9 \cdot 10^3$ and not differed much from the amount of fungi from B samples on the same medium ($1.7 \cdot 10^3$, what corresponded to 20.5% and 15.2%, respectively, in comparison to the total count of fungi).

Chitin assimilation ability was also noticed to be reduced by the treatment with copper sulphate: on the chitin-amended medium fungal count from the control samples was $3.2 \cdot 10^3$ and from the samples treated with copper sulphate slightly decreased to $2.3 \cdot 10^3$, or 28.6% and 20.5%. The amount of chitinase-producing fungi on the medium supplemented with copper sulphate was reduced almost three times – to $1.2 \cdot 10^3$ (from the untreated soil) and to $0.7 \cdot 10^3$ (from the soil treated with copper sulphate). (Fig. 2).

Cellulase-producing micromycetes made up quite a low amount ($2.0 \cdot 10^3$) from the untreated soil and

Table. **Effect of $CuSO_4$ on the count of fungi growing on various substrata, %. 10 mM $CuSO_4$ was added to the soil samples and 5 mM $CuSO_4$ to the media**

Samples	Control (Czapek medium)	Substrata				
		skim milk	starch	chitin	cellulose	sacharose + 0.005% α-naphtol
A samples plated on the medium without $CuSO_4$	100.0	18.8	74.1	28.6	17.9	3.6
B samples plated on the medium without $CuSO_4$	73.2	12.5	63.4	20.5	16.1	0.9
A samples plated on the medium amended with 5 mM $CuSO_4$	23.2	14.3	20.5	17.0	1.8	5.4
B samples plated on the medium amended with $CuSO_4$	19.6	8.0	15.2	6.3	1.3	6.5

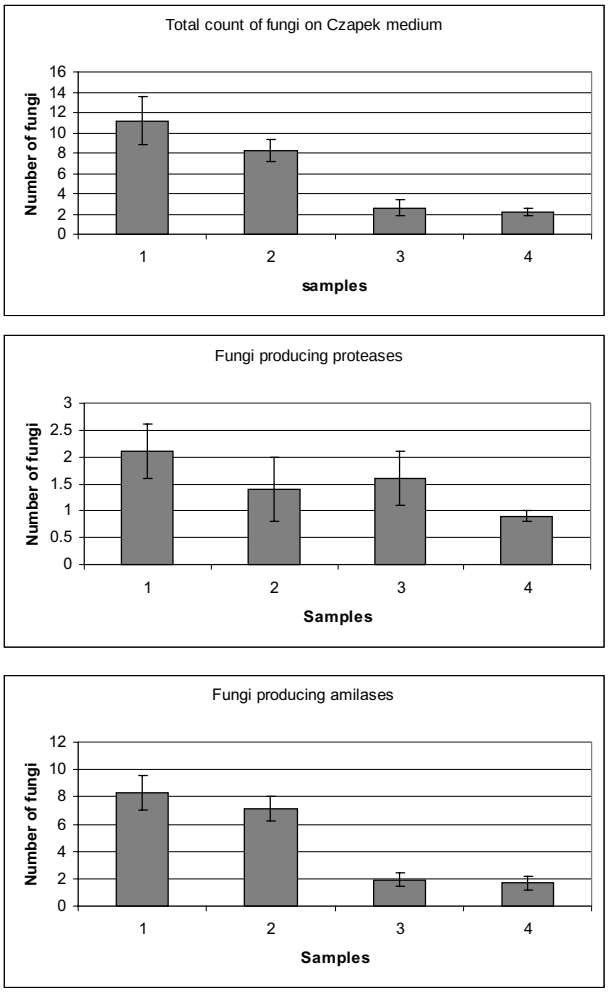

Fig. 1. Effect of copper sulphate on the total count of fungi growing on Czapek medium and counts of micromycetes producing proteases and amylases. 1 – fungi isolated from soil without $CuSO_4$ treatment (A) and plated on media without addition of $CuSO_4$; 2 – fungi isolated from soil treated with 10 mM $CuSO_4$ (B) and plated on media without addition of $CuSO_4$; 3 – fungi isolated from soil without $CuSO_4$ treatment (A) and plated on media supplemented with 5 mM $CuSO_4$; 4 – fungi isolated from soil treated with 10 mM $CuSO_4$ (B) and plated on media supplemented with 5 mM $CuSO_4$

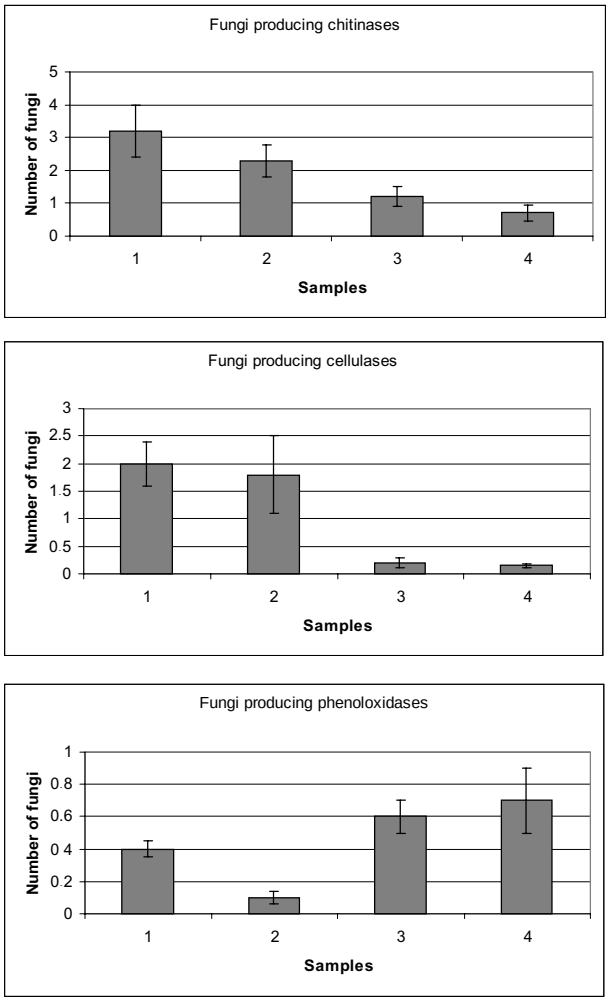

Fig. 2. Effect of copper sulphate on counts of fungi producing chitinases, cellulases and phenoloxidases. Meanings of sample numbers are the same as in Fig. 1

were not so much affected by $CuSO_4$ in the soil ($1.8 \cdot 10^3$). Nevertheless, $CuSO_4$ incorporated in the medium affected greatly the fungal count. In this case, the number of fungi utilising cellulose was found to be very low – $0.2 \cdot 10^3$ (A) and $0.15 \cdot 10^3$ (B), or 1.8% and 1.3% from the total count of fungi (Fig. 2).

A rather different effect of copper sulphate was exerted on fungal ability to produce phenoloxidases. Phenoloxidases are an important factor in soil microbiological activity as they catalyse the breakdown of lignin, humic and fulvic acids or humin [24]. The number of fungi from the untreated soil on the medium with α-naphthol was $0.4 \cdot 10^3$ and from the treated soil only $0.1 \cdot 10^3$ (3.6% and 0.9%). A significantly higher number of micromycetes producing phenoloxidases was noticed on the same medium but additionally supplemented with copper sulphate: $0.6 \cdot 10^3$ from the untreated soil and even $0.73 \cdot 10^3$ from the treated soil (Fig. 2).

These data show that $CuSO_4$ influenced the consumption of different substrata. Copper sulphate added to the soil reduced the total count of fungi (grown on standard Czapek medium containing sucrose), and influenced the fungal number on different substrata. The effect of copper sulphate in the soil obviously greatly depended on soil structure and composition, especially on the presence of humus [24]. It is reported that such groups of humic substances as carboxyl, phenolic and alcoholic hydroxyls, carbonyl, metoxyl, amino and azo are very important in fate and toxicity of metals in soil, as these groups are able to coordinate metal ions by binding them [11]. So, soil matter should have significantly affected the toxicity of $CuSO_4$.

Addition of copper sulphate to the growth medium was more effective – fungal count decreased significantly both from the treated and untreated samples. One of the reasons could be that in media, organic complex-forming substances may diminish the free concentration of essential metal ions and thus influence the competition between essential and toxic metals, where metals required for nutrition could be substituted for toxic ones [9]. As in case with some other heavy metals, resistance of fungi tolerating high copper concentrations could be related to metallothioneins, γ-glutamyl peptides and other thiol compounds which may be promising detoxifying agents for copper [14, 23]. The different tolerance to copper may possibly be caused by different ability to induce metallothioneins and γ-glutamyl peptides [7, 14].

The most evident decrease of the fungal number on the medium with $CuSO_4$ was noticed for cellulose-assimilating fungi. Some authors have showed that fungi decomposing the upper litter layer (where cellulases play an important role) were sensitive to copper [15, 17, 21]. In this investigation, copper sulphate in the soil did not affected much the count of cellulase-producing fungi, but a significant reduction of cellulose-degrading micromycetes was established on the medium with incorporated $CuSO_4$. Nevertheless, assimilation of not every substrate was so affected. A comparatively little decrease of the fungal count on the medium containing $CuSO_4$ was noticed for protease-producing fungi.

Inhibition by metals or in some cases an increase in capacity is reported by some other authors. It is suggested that one of the main mechanisms of enzyme inhibition is the high affinity of metals for sulphydryl and thiolate groups [7, 16, 22]. Copper toxicity depends to a large extent on the metal affinity to sulphydryl and other thiolate groups, which are reactive sites on many enzymes, and also on possible binding to amino and imino groups [7, 16, 18].

Pretreatment with copper sulphate in the soil induced neither the total amount of fungi nor producers of particular enzymes which would be more resistant to this chemical. The exception was fungi producing phenoloxidases – their amount was established to be much higher on the medium containing copper sulphate, especially of those pretreated with $CuSO_4$ in the soil, *i.e.*, copper sulphate induced a higher number of possible producers of phenoloxidases. K. Høiland found a positive correlation between resistance of fungi (Basidiomycetes) and synthesis of phenoloxidases (tyrosinases). It is concluded that decomposer fungi living in humus or lignin-rich substrates in polluted environments may have selected ecotypes with tolerance to metals like copper if they produce tyrosinase [8]. On the other hand, fungi living in soil with no or little amount of polyphenols may evolve tolerance to metals.

The obtained results showed that copper sulphate influenced, mostly negatively, the enzymes involved in assimilation of various substrata and the total count of micromycetes. Obviously, natural ecosystems are much more complicated and the effect of copper-based chemicals such as sulphates on soil fungi is quite difficult to determine, but it could be assumed that copper sulphate released into a natural environment could influence fungal ability to degrade particular substrates.

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L. Levinskaitė

VARIO SULFATO POVEIKIS GRYBŲ GEBĖJIMUI ASIMILIUOTI ĮVAIRIUS SUBSTRATUS

S a n t r a u k a

Tirtas 10 mM vario sulfato, įdėto į dirvožemį (laboratorinėmis sąlygomis), poveikis grybų gausai ir jų gebėjimui vystytis ant skirtingų substratų. Gauti rezultatai parodė, kad vario sulfatas sumažino bendrą grybų skaičių nuo 11,2 iki 8,2. Micromicetų, augančių ant specifinių substratų, kurie leidžia nustatyti grybus produkuojančius proteazes, amilazes, chitinazes, celiuliazes ir fenoloksidazes, skaičius taip pat sumažėjo. Kai vario sulfato buvo įdėta į terpę (tiesioginis efektas), bendras grybų skaičius ir gebėjimas asimiliuoti įvairius substratus sumažėjo dar labiau. Ryškiausias grybų skaičiaus sumažėjimas nustatytas celiuliozes produkuojantiems grybams ir mažiausias – proteazes sintetinantiems mikromicetams. Dėl to grybų, galinčių produkuoti fenoloksidazes, padaugėjo.