ROCZNIKI GLEBOZNAWCZE T. XXV, DODATEK, WARSZAWA 1974

BOLESŁAW SMYK

BIOLOGICALLY ACTIVE TOXIC SUBSTANCES OF FUNGAL ORIGIN AND THEIR INFLUENCE UPON THE SOIL HABITAT

Institute of Soil Science, Agricultural Chemistry and Microbiology,
Agriculture University of Cracow

In world literature we often meet works in the field of biochemistry of soils and ecological microbiology, informing of the appearance of the pathological phenomenon in the soils agriculturally exploited, decreasing the fertility of soils known in the agricultural practice under the name of *soil-sickness* [3-9, 11, 12].

The results of FAO data show that soil-sickness causes heavy losses in the vegetable production of the globe (Table 1). It has been agreed that on more than 4400 million standard ha utilised agriculturally (arable land, meadows and pastures), there is a yearly loss of yields in an amount of about 25% (in relation to general biological losses) caused just by soil-sickness this is so large a loss that it seriously threatens the perspective plans of supplying the worlds 3,6 million people with food (according to FAO, 1970).

The above mentioned fact of soil-sickness which is a result of breaking the biological balance in nature by man is potentielly the largest danger threatening contemporary world agriculture [11, 12, 14].

At the present time of chemicalisation of agriculture (the wholesale applying of pesticides and so on, of the facilities of plant protection as well as controllers of plant growth and many other phytochemitherapeutical preparations) and at the intensification of agriculture (among other things, using large amounts of nitrogen fertilizers, especially at non-observing biological foundations of the succession of plants in rotational structures; the increase of the level of mechanization in agriculture and so on) the necessity arises for recognizing the influence of the above mentioned factors upon the biology of soils and the biological productivity of agrocenose and other ecosystems, found in the immediate range of the economic activity of man.

Table 1

Losses in vegetable production in agriculture caused by the soil-sickness of arable lands*
/expressed proportionally in relation to general biological damage/

	%	
EUROPE		
Belgium	20	
Czechoslovakia	18	
Denmark	24	
France	22	
Holland	26	
German Democratic Republic	24	
German Federal Republic	32	
Poland	16	mainly in the growing of tobacco, the family Papilionaceae vegetable and in cultivation of meadows
Switzerland	20	
Sweden	12	
AFRICA		
Kenya	30	mainly in the cultivation of cotton, coffe, vegetables and other subtropical and tropical plants
Tanzania	25	
Uganda	24	
Egypt	33	
NORTH AMERICA		
The United States of America	28	
Canada	21	
SOUTH AMERICA		
Argentina	18	
Brazil	30	

* According FAO data for 1970

Numerous examples of the annihilation of enormous cultivations accomplished by man in such agricultural countries as Africa, Europe, South America (Brazil), North America (The United States of America and Canada) and also Poland-indicate the necessity of closer cognition of these facts threatening the natural soil habitat.

The aim of this work was the recognition of the prevalence of Fungi with toxin-creative capabilities in the soils of chosen sinusion and plant communities coming into the groups (association) of agroecosystems and also grassy and forest ecosystems as well as in the soil habitat found in the reach of intensive economic activity of man (with special regard to soil-sickness, as, for instance, clover and alfa-alfa soil-sickness, soil-sickness in berseem and Persian clover, tobacco-soil-sickness and others) together determining the influence of the products of their metabolism (mycotoxins) upon the chosen soil microorganisms and soil habitat.

METHODOLOGY OF WORK

The research work connected with the subject of the present study was carried out in the Department of Agricultural Microbiology at the University of Agriculture in Cracow (Poland) now known as the Institute of Soil Science, Agricultural Chemistry and Microbiology of the University of Agriculture in Cracow, in the years 1960-1972. Some taxonomic designations of fungi, as well as analytic examination in the range of toxic substances (mycotoxins) were performed at the Microbiological Institute of the Federal Technical University in Zürich and at the Institute of Applied Microbiology in the Agricultural University in Vienna.

The isolation of toxin-producing fungi were selected from different soils of chosen plant communities, entering into the composition of nature and changed by the economic activity of man (agrocenoses), grassy and forest ecosystems situated in a specified area of Southern Poland. This isolation was done by the Koch's plate cultural method using modified medium of Czapek [2, 3, 10, 13].

The estimation of the toxicity of isolated fungi in relation to the chosen soil microorganisms was examined with the use to modern microbiological and biological methods [1, 2, 5, 6, 8, 11, 13]. Taxonomic studies of isolated fungi were carried out on the grounds of realized diagnostic-taxonomic examination and basic taxonomic keys. (*The Penicillia* by Raper, Thom and Fennel, 1949; *The Genus Aspergillus* by Raper, 1965; *The Fungi* by Wolf and Wolf, 1969; *Pilze aus Agrarböden* by Domsch and Gams, 1970).

The estimation of the contents of mycotoxins in the cultures of isolated fungi were determined with the following methods: spectrophotometric (Nabney and Nesbit, 1965) and the chromatographic method [1, 5, 7, 8].

I received from dr A. Campbell of the Food and Drug Institute in Washington (USA) standards of aflatoxins and some mycotoxins as ochratoxins A and B,F-2 (zearalenone), penicillic acid, mycophenolic acid, citrinin and citreoviridin—for which, at this time—I want to express my warmest words of acknowledgement.

Other mycotoxins were received from the following chemical firms: Calbioch., Los Angeles, Calif.; Sigma Chemical Co., St. Louis, Mo.; K. & K. Laboratories Plainview, N.Y.—USA.

A part of the mycotoxins was determined in foreign laboratories (Inst. of Applied Microbiology at the Agricultural University in Vienna).

THE GENERAL RESULTS OF THE STUDIES

The results from studies carried out in the years 1960-1972, disclose that mycological examinations (ecological and synecological studies) of

chosen, differing soil habitats from which toxin-producing fungi from the class *Fungi imperfecti* were separated out, posses a large capacity in the range of mycotoxin synthesis, substances which are biologically active (Tables 2, 3).

The metabolites of examined soil fungi are characterized by a direct toxic influence upon soil microorganisms (active in nitrogen and carbohydrate metabolism in soil) and some cultivated plants. Moreover it appears from the obtained results of examinations and chemical designations that the toxic products of metabolism, examined *in vitro Fungi*, are represented by the following groups of chemical compounds:

—aliphatic metabolites: epoxides and epoxialcanes (epoxysuccinic acid

Table 2

Isolation of mycotoxin-producing fungi from agricultural soils in Poland

Fungus	Mycotoxin	Occurence in ecosystems:		
		grassy	forest	agrocenose
Aspergillus chevalieri, Thom and Church	Gliotoxin and other Epipolythiadioxopiperazines*	+	+	+
Aspergillus flavus, Link	Aflatoxin	+	–	
Aspergillus ochraceus, Wilh.	Ochratoxin	–	–	+
Aspergillus terreus, Thom.	Citrinin	+	+	+
Aspergillus versicolor, /Vuill./ Tirab.	Sterigmatocystin	–	–	+
Fusarium nivale, /Fr./Ces.	Nivalenol	+	–	+
Fusarium graminearum, Schwabe	F-2 /zearalenone/**	+	–	–
Fusarium scirpi, Lamb. and Fautr.	Scirpenol	+	+	+
Fusarium sporotrichioides, Sherb.	Sporofusarin	–	–	+
Penicillium brunneum, Biourge	Rugulosin	+	–	+
Penicillium citreoviride, Biourge	Citreoviridin	+	–	+
Penicillium citrinum, Th.	Citrinin	+	–	+
Penicillium cyclopium, Westling.	Cyclopiazonic acid and penicillic acid	+	+	+
Penicillium islandicum, Sopp.	Luteoskyrin	+	+	+
Penicillium puberulum, Bein.	Penicillic acid and other carcinogenic lactones***	+	–	+
Penicillium rubrum, Stol.	Rubratoxin	+	–	+
Penicillium rugulosum, T.	Rugulosin	–	–	+
Penicillium tardum, Thom	Rugulosin	–	+	+
Penicillium variabile, Sopp.	Aflatoxin	–	–	+
Alternaria longipes, T. a W.	Alternariol	+	+	+
Thielaviopsis basicola, Berk.et Br. /Ferr./	Thielaviotin	–	–	+

After:
* A.Taylor – 1971
** C.J. Mirocha et al. – 1971
*** A.Ciegler et al. – 1971

Table 3

The influence of extracts of Thielaviopsis basicola Berk. Br. upon the chosen soil microorganisms

	Soil bacteria	Chlorophormic extract	
		of 14 days old mycelium	of 20 days old culture
	Microbiological method	Inhibitionzone - mm	
1	Arthrobacter globiformis, Conn a. Dimmick	10	8
2	Arthrobacter terregens, Lochhead a. Burton	12	10
3	Arthrobacter sp.? strain "SB"	10	10
4	Azotobacter chroococcum, Beijerinck	12	10
5	Bacillus subtilis, Cohn	10	9
6	Bacillus cereus, Frankl.	15	11
7	Rhizobium leguminosarum, Frank.	10	8
8	Rhizobium meliloti, Dangeard	10	10
9	Rhizobium trifolii, Dangeard.	12	10
10	Rhizobium lupini, Eckl.	10	8

* Cultures of Bacteria mentioned in the position 1, 2, 5, 6 come from the National Coll. of Industr. Bacteria - Aberdeen; whereas cultures mentioned in position 4, 7, 8, 9, 10 - come from Rothamsted Expt. Agricult. Station, Harpenden /Herts./.

= 1-trans-oxidoethylene-α-β-dicarboxylic acid: tetronic acids); compounds of the furan rings as: sterigmatocystin and 2-hydroxymethyl-furan-5--carboxylic acid; penicillic acid; out of the group of compounds of α--pyrone ring: kojic acid, patulin, citrinin and citreoviridin;

—aromatic metabolites: aflatoxins, ochratoxins, mycophenolic acid; cyclopolic acid; tropolones (as puberulic and puberulonic acid); dianthraquinones (for instance: skyrin and iridoskyrin, rubroskyrin, luteoskyrin, rugulosin); antraquinones (for instance: emodic acid); hemiquinones (for instance: fuscin and purpurogenone);

—metabolites containing nitrogen (for instance: β-nitropropionic acid);

—metabolites containing sulphur (for instance: gliotoxin);

—metabolites containing chlorine (for instance: nidulin, griseofulvin) and also

—lactones (for instance: curvularin, zearalenone).

The above mentioned metabolites of fungi (mycotoxins) are characterized by large biological activity. Some of them, as for instance alfatoxins, belong to the strongest carcinogens of the animal world. Alfatoxins and the other above mentioned mycotoxins operate also as teratogens (teratogenetically). All of the above mentioned metabolites of the examined soil fungi are distinguished by strong phytotoxic activity. The

majority of examined mycotoxins (especially from the group of aromatic metabolites) belongs to the group of strong inhibitors of the synthesis of RNA and DNA [5, 6]. According to Fishbein, Flamm and Falk [6], some mycotoxins (alfatoxins, ochratoxins, patulin, skyrins, rubroskyrins, and luetoskyrins) produced by the soil fungi, are marked also by specific mutagens (mutagenal) activity both in regard to microorganisms and also to higher organisms. The attendance of the induced mutation with the help of chemical mutagens at procaryotic and eucaryotic microorganisms is much higher than when using physical mutagens [6].

To a group of these compounds belong, among other, alkanes, epoxialcanes, lactones and also alkylating agents (for instance: epoxides, epichlorohydrin, aldehydes). Moreover to chemical mutagens are included also Nitrosamines, Aziridines and related derivates and the majority of pesticides (within it also some herbicides) and miscellaneous mutagens and thus chemical compounds or products of their biodegradation—put commonly into practice in agriculture and forestry.

CONCLUSIONS

On the basis of obtained results from the studies carried out in the years 1960-1972 of mycological examinations of soils of chosen field ecosystems (agrocenose), of forest and grassy ecosystems and chemical investigations at toxic substances biologically active and their influence at the microflora of soil habitat—we may draw the following very general conclusions:

1. Out of different soil habitats of chosen vegetable communities entering into the composition of examined agro-ecosystems, forest and grassy ecosystems and also the soils situated within the direct reach of the intensive economic activity of man 21 species of fungi, mycotoxin-creating being dominate were separated out from the soils of examined vegetable sinusion. It was observed that in examined arable lands—especially the so called *soil-sickness* of known chemical activity (pesticides) and biological activity (mycotoxins and other biotic factors) of the casual factors (of definite vegetable sinusion) toxin producing fungi were found to be dominant, creating *in vivo* as well as *in vitro* biologically toxic substances (mycotoxins).

On the other hand, in the soil habitat of examined forest ecosystems (*Fagetum carpaticum* and *Tilio-Carpinetum*) and grassy ecosystems (*Arrhenatheretum elatioris* and *Gladiolo-Agrostidetum*)—the above mentioned mycotoxin-producing fungi, occur sporadically.

2. Mycotoxins—as products of metabolism of examined soil fungi out of the class *Fungi imperfecti* (Table 2) are characterized by large

biological activity in relation to soil microorganisms (Table 3) and cultivated plants. Some of the determined mycotoxins, produced by the eliminated soil fungi are characterized also by distinct mutagens (mutagenal) activity in relation to soil michroorganisms as well as higher organisms [5, 6, 9, 1].

3. From the above mentioned data it results that mycotoxins—as metabolites of examined toxin-producing fungi are characterized by large biological activity (among others they are distinguished by mutagenal and carcinogenal activity, bacteri- and fungicidal and also phytoxic activity) they can influence, in definite ecological conditions, a destructive effect upon the biological balance of a definite soil habitat—biocenose.

Consedering that the soil habitat is influenced by—besides the above mentioned mycotoxins—pesticides (herbicides, fungicides, insecticides and so on-chemical compounds for the protection of plants) fill the soil from several to more than ten months or several to more than ten years. The final effect may lead to essential troubles in the biological balance of the soil community of agroecosystems. It refers to troubles in the metabolic processes as well as the biocenotic soil microorganisms and also physiopathological changes of succesion crops within definite —crop rotations systems.

REFERENCES

[1] Ajl S. J., Ciegler A., Kadis S., Montie T. C., Weinbaum G.: Microbial Toxins — A Comprehensive Treatise, New York — London 1970-1972, Academic Press, vol. 1-8.

[2] Ainsworth G. C., Sussman A. S.: The Fungi — An Advanced Treatise, New York — London 1965, 1966, 1968, Academic Press, vol. 1-3.

[3] Aleksandrowicz J., Smyk B.: Mycotoxins and their role in oncogenesis with special references to blood diseases, Polish Medical Science Bull. 14, 1971, 25-30.

[4] Domsch K. H.: Microbial Stimulation and Inhibition of Plant Growth, 9th Intern. Congress of Soil Sci. 2, 1968, 455-464.

[5] Fishbein L.: Chromatography of Environmental Hazards. I. Carcinogens, Mutagens and Teratogens. Amsterdam — London — New York 1972, Elsevier Publishing Company, pp. 499.

[6] Fishbein L., Flamm G. W., Falk H. L.: Chemical Mutagenes — Environmental Effects on Biological Systems. New York — London 1970, Academic Press, pp. 306.

[7] Isvaran V., Harris J. R.: Microbiological Aspects of Phytotoxicity in Soil. The Madras Agri. Journal 55 (9), 1968, 401-405.

[8] Maggon K. K., Viswanthan L., Venkitausbramanian T. A., Mukerji H. G., Aflatoxin Production by Some Indian Strains of *Aspergillus flavus* Link ex Fries. J. Gen. Microb. 59, 1969, pp. 119-124.

[9] McCalla T. M., Haskins F. A.: Phytotoxic Substances from Soil Microorganisms and Crop Residues. Bact. Rev. 28, 1964, 181-207.

[10] Norris J. R., Ribbons D. W.: Methods in Microbiology. New York and London 1969-1972, Academic Press, vol. 1-7B.
[11] Smyk B.: Zmęczenie gleb uprawnych w świetle badań mikrobiologicznych i agrobiologicznych. Post. Mikrob. 8, 1969/70, 2, 205-224.
[12] Smyk B.: Trendy w podnoszeniu żyzności gleb, efekty gospodarcze i konsekwencje eutrofizacji środowiska glebowego w rolnictwie i w leśnictwie. PAN Zesz. probl. Post. Nauk rol. 155, 1974.
[13] Turner W. B.: Fungal Metabolites. London 1971, Academic Press, p. 456.
[14] Virtanen A. I.: Antimikrobiologische Substanzen in unseren Kulturpflanzen und ihre Bedeutung für die Pflanzen und für die Ernährung des Menschen und des Tiere. Path. et Microb. 21, 1963, 970-979.

Б. СМЫК

ТОКСИЧЕСКИЕ БИОЛОГИЧЕСКИ АКТИВНЫЕ ВЕЩЕСТВА ГРИБНОГО ПРОИСХОЖДЕНИЯ И ИХ ВЛИЯНИЕ НА ПОЧВЕННУЮ СРЕДУ

Институт почвоведения, агрохимии и микробиологии
Сельскохозяйственной академии в Кракове

Резюме

Среди выбранных культурных почв и почв входящих в состав травяных и лесных экосистем *Arrhenateretum elatioris—Gladiolo-Agrostidetum* и соответственно *Tilio-Carpinetum—Fagetum carpaticum* был выделен 21 вид токсиногенных грибов, м. пр. *Aspergillus, Fusarium, Penicillium, Thielaviopsis,* класса *Fungi imperfecti.*

Экологические исследования показали, что в культурных почвах, а особенно в „утомленных" почвах, преобладают вышеуказанные токсиногенные грибы. В почвенных же средах исследуемых лесных и травяных экосистем упомянутые грибы встречаются лишь в отдельных случаях.

Выделенные токсиногенные грибы образуют как *in vitro* так и *in vivo* (т.е. в почвенной среде) биологически активные вещества — микотоксины, в частности: глиотоксины, афлатоксины, охратоксины, цитронаты, F-2 (zearalenon), стеригматоцистин, ругулозин, цитреовиридин, пеницилловую кислоту, рубратоксин, спорофузарин и др. Микотоксины как метаболиты исследуемых почвенных грибов отличаются высокой биологической активностью (м. пр. их характеризует фитотоксинное и мутагенное, а также бактерио- и фунгицидное и ко-карциногенное воздействие) могут оказывать в определенных экологических условиях почвенной среды деструктивное влияние на биологическое равновесие почвенных биоценозов.

B. SMYK

SUBSTANCES BIOLOGIQUES TOXIQUES ACTIVES PROVENANT DE CHAMPIGNONS ET LEUR INFLUENCE SUR LE MILIEU DE SOL

Institut de Pedologie, de Chimie agricole et de Microbiologie,
Université Agronomique de Cracovie

Résumé

On a isolé des sols cultivables et des sols qui composent des écosystèmes herbeux *Arrhenatheretum elatioris* et *Gladiolo-Agrostidetum* et forestiérs *Tilio-*

-*Carpinetum* et *Fagetum carpaticum* 21 sortes de champignons toxiques de genres: *Aspergillus, Fusarium, Penicillium* et *Thielaviopsis* de la classe *Fungi imperfecti.*

Au cours les examens écologiques on a observe que dans les sols cultivables et surtout dans les sols fatigués des champignons toxiques mentionnés ci-dessus dominent.

Cependant dans les milieux de sols des écosystèmes examinés forestiérs et herbeux les champignons mentionnés sont sporadiques. Les champignons toxiques isolés produisent *in vitro* comme *in vivo* (c'est à dire au milieu du sol) des substances biologiquement actives mycotoxines à savoir: gliotoxines, aflatoxines, ochratoxines, citriniens, F-2 (zearalenone), sterigmaticistine, rugulosine, citreoviridine, acide de pencilline, rubratoxine, sporofusarine etc. Les mycotoxines comme des métabolites des champignons de sols examinés en se distinguant (entre autres) par une grande activité biologique sont caractérisés par l'action phytotoxique l'action mutagène, la réaction bacterio- et fungicide.

Dans des conditions écologiques determinées du milieu du sol peuvent exercer une influence destructive sur l'équilibre biologique des biocenoses du sol.

B. SMYK

BIOLOGISCH AKTIVE TOXISCHE STOFFE VON PILZENHERKUNFT UND DEREN EINFLUSS AUF DAS BODENMILIEU

Institut für Bodenkunde, Agrikulturchemie und Mikrobiologie
der Landwirtschaftlichen Universität in Kraków

Zusammenfassung

Von den erwählten Agrarböden und den Böden, die in die Zusammensetzung der Gras und Waldökosysteme *Arrhenatheretum elatioris* und *Gladiolo-Agrostidetum; Tilio-Carpinetum* und *Fagetum carpaticum* hineingehen, wurden 21 toxinbildende Pilzarten u.a.: *Aspergillus, Fusarium, Penicillium* und *Thielaviopsis,* aus der Klasse von *Fungi imperfecti,* abgesondert.

Die ökologischen Untersuchungen haben gezeigt, das in den untersuchten Agrarböden, und besonders den "ermüdeten" Böden, die obenerwähnten toxinbildenden Pilze vorherrschen. Dagegen in den Bodenmilieus der untersuchten Wald- und Grasökosysteme treten die genannten Pilze vereinzelnd auf.

Von den aggesonderten toxinbildenden Pilzen wurden sowohl *in vitro* als auch *in vivo* (d.h. im Bodenmilieu) biologisch aktive Stoffe — Mykotoxine, und zwar: Gliotoxine, Aflatoxine, Ochratoxine, Citrinin, F-2 (Zearalenon), Sterigmatocystin, Rugulosin, Citreoviridin, Penicillinsäure, Rubratoxin, Sporofusarin u.dgl., abgesondert. Die Mykotoxinen als Metabolite von untersuchten Bodenpilzen zeichnen sich mit hoher biologischen Aktivität aus, indem sie u.a. phytotoxische sowie und stärke mutagene Wirkung auf manche Bodenmikroorganismen sowie auch auf höhere Organismen, ferner eine bakterien- und pilztötende Wirkung aufweisen und können in bestimmten ökologischen Bedingungen des Bodenmilieus einen destruktiven Einfluss auf das biologische Gleichgewicht von Bodenbiozönosen ausüben.

B. SMYK

TOKSYCZNE SUBSTANCJE BIOLOGICZNIE AKTYWNE POCHODZENIA GRZYBOWEGO I ICH WPŁYW NA ŚRODOWISKO GLEBOWE

Instytut Gleboznawstwa, Chemii Rolnej i Mikrobiologii
Akademii Rolniczej w Krakowie

Streszczenie

Z wybranych gleb uprawnych i z gleb wchodzących w skład ekosystemów trawiastych *Arrhenatheretum elatioris* i *Gladiolo-Agrostidetum* oraz leśnych *Tilio-Carpinetum* i *Fagetum carpaticum* wyodrębniono 21 gatunków grzybów toksynotwórczych z rodzajów: *Aspergillus, Fusarium, Penicillium* i *Thielaviopsis* — klasy *Fungi imperfecti.*

W badaniach ekologicznych zaobserwowano, że w glebach uprawnych, a zwłaszcza w zmęczonych, dominują wymienione grzyby toksynotwórcze. Natomiast w środowiskach glebowych badanych ekosystemów leśnych i trawiastych wspomniane grzyby występują sporadycznie.

Wyodrębnione grzyby toksynotwórcze wytwarzają *in vitro,* jak i *in vivo* (tzn. w środowisku glebowym) substancje biologicznie aktywne — mikotoksyny, a mianowicie: gliotoksyny, aflatoksyny, ochratoksyny, cytryniny, F-2 (zearalenon), sterygmatocystynę, regulozynę, citreowirydynę, kwas penicylinowy, rubratoksynę, sporofuzarynę i inne. Mikotoksyny, jako metabolity badanych grzybów glebowych, odznaczając się dużą aktywnością biologiczną (m. in. cechuje je działanie fitotoksyczne i mutagenne, oddziaływanie bakterio- i grzybobójcze oraz ko-karcinogenne) mogą wywierać w określonych warunkach ekologicznych środowiska glebowego destruktywny wpływ na równowagę biologiczną biocenoz glebowych.

Prof. dr hab. Bolesław Smyk
Instytut Gleboznawstwa, Chemii
Rolnej i Mikrobiologii A. R.
Kraków, ul. Mickiewicza 24/28