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ON THE USE OF TOLUENE AS INHIBITOR IN ENZYMOLOGICAL SURVEYS OF FRESHWATER BOTTOM DEPOSITS

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The measurement of biological activity in bottom deposit samples collected from Lake Balaton and from other water bodies was performed according to the methods by HOFMANN and co-workers (1951—1955) in our laboratory (SZABÓ 1959, 1960). The criticism by CLAUS and MECHSNER (1960) in relation to HOFMANN's methods necessitates to check against the inhibitory effect of toluene on the microbial growth in experimental samples. The experiments were started on the basis of two considerations.

The first consideration was that in the enzymological literature toluene is generally regarded as the best antiseptic to be given to enzym-preparata. By its presence the enzyme is not destroyed by microorganisms, or else the result of the test is not obscured by action of microorganisms on the substrate (SUMNER and SOMERS 1953, 57).

On the other hand it is well known, as regards soil metabolism, that a compound which normally does not affect a given organism grown in pure culture, might be highly inhibitory to the development of the same organism in soil. This may be explained by the stimulating effect of the compound in question on other organisms present in soil, which are highly inhibiting to the organism under investigation. Nevertheless, it has been also demonstrated, that a substance highly inhibitory to an organism grown in pure culture has but little effect on the organism developing in soil. This may be due to rapid decomposition of the substance in soil or to the development of resistant or adapted strains of the organism under study (QUASTEL and SCHOLEFIELD in COLOWICK and KAPLAN 1957, IV. 341).

Material and methods

The experiments were carried out with two bottom deposit samples collected in the last days of October 1960 partly from under off shore water, partly from a detritus deposit near the shore line in the bay "Kis-öböl" in the neighbourhood of the Biological Research Institute. The samples were air-dried at room temperature and homogenized in porcelain mortar.

Invertase (saccharase) activity was measured according to the instructions given by HOFMANN and SEEGERER (1951). 10 g homogenized dry matter was weighted and, after addition of 2,5 ml toluene, the mixture was allowed to stand for 15 minutes. 10 ml phosphate buffer (pH 5,5) and 10 ml 20%

sucrose solution were added successively. After 24 hours long incubation at 37°C in thermostate the suspension was filled up to 100 ml, and the sucrose concentration was measured in its aliquots by a modified FEHLING method, *i. e.* by the iodometric titration of cupric ions remaining in Fehling-solution, after the reduction. The results obtained are expressed as titration differences existing between the samples and the blank ones. Control experiments, without toluene, were also made.

The microbiological test of samples was carried out by the usual agar pour plate method. Two different media were examined for suitability in these investigations. On bouillon agar certain microorganisms grew more rapidly than others, therefore the results based on the number of colonies are unreliable. The mud extract agar, prepared from the lake bottom deposit proved more suitable for our purposes. Equal quantity of mud and lake water was autoclaved for half an hour. Thereafter the extract was filtered and 2 g KH_2PO_4 and 1.5 g washed agar was added per litre. The pH of this medium was adjusted to 7 with a few drops of 2N NaOH. Aliquots of suspensions used in activity measurements were mixed with this agar medium and were poured into PETRI dishes. Incubation time was 24 hours at 37°C. The bacterial and fungous colonies were counted under a low magnifying stereomicroscope. All numbers are averages of at least five parallels.

Results and discussion

The results of countings of microorganisms and of the biological activity measurements are summarized in *Table 1*. The data of this table are very remarkable. The inhibiting effect of toluene on the microbial growth is undoubtful. The significant differences between the results obtained by CLAUS and MECHSNER and those, exposed in our *Table 1* are explainable only by different physico-chemical nature of bottom deposits and soils used by them. It may not be disregarded that the activities in samples treated with toluene are slightly greater than in untreated ones. It is presumed that this increase is due to the well known modifying effect of toluene, chloroform or similar solvents on the permeability of cell membranes. It may be concluded on basis of these findings that, working with the method of HOFMANN and co-workers, suitable results can be obtained only by sticking closely to the original prescriptions, and that the numerals can be used only for comparisons.

Summary

The effectiveness of toluene in blocking microbial growth and activity was estimated in two bottom deposit samples originating from Lake Balaton. According to the agar pour plate countings the toluene stops the growth of microorganisms not only in mud samples but also in agar plates. The saccharase activity measurements show that, due to the effect of ow toluene concentration, the activity will be slightly greater.

The findings show that HOFMANN's biological activity measurements give useful results only by adherence to the original instructions and, that the results may be used only for comparisons.

Table 1 — 1. táblázat

Number of microorganisms developed on agar plates, and saccharase activity in bottom deposits of Lake Balaton with and without toluene treatment

Balatoni iszapminták mikroorganizmus-száma és szacharáz aktivitása toluol kezeléssel és anélkül mérve

Sample — Minta	Treatment — Kezelés	Number of microorganisms in one gramm of dry matter Mikroorganizmusok száma egy gramm száraz iszapban		Saccharase activity
		At the start Induláskor	After incubation Inkubálás után	
Detritus 1.	Sterile tap water Steril csapvíz	$13{,}0 \cdot 10^5$	—	—
	Sucrose - Nádcukor	$15{,}0 \cdot 10^5$	$18\,000 \cdot 10^5$	7,3
	Sucrose + 2,5 ml toluene Nádcukor + toluol	$1{,}2 \cdot 10^5$	$0{,}9 \cdot 10^5$	8,0
Detritus 2.	Sucrose	$10{,}6 \cdot 10^5$	$16\,400 \cdot 10^5$	3,6
	Sucrose + 1,25 ml toluene	$3{,}0 \cdot 10^5$	$3{,}7 \cdot 10^5$	5,3
	Sucrose + 5 ml toluene	$0{,}7 \cdot 10^5$	$0{,}6 \cdot 10^5$	3,9
Bottom mud out of the off shore water Nyíltvízi iszap	Sucrose	$0{,}7 \cdot 10^5$	$1\,800 \cdot 10^5$	0,1
	Sucrose + 2,5 ml toluene	$0{,}3 \cdot 10^5$	$0{,}3 \cdot 10^5$	0,1

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A TOLUOL GÁTLÓ HATÁSÁNAK VIZSGÁLATA AZ ÉDESVÍZI FENÉKÜLEDÉKEK BIOLÓGIAI AKTIVITÁSÁNAK MÉRÉSÉNÉL

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Összefoglalás

Édesvízi üledékek mikrobiológiai jellemzésére HOFMANN talaj-enzimológiai módszerét kezdtük el alkalmazni. A múlt évben CLAUS és MECHSNER bírálták ezeket a módszereket, mert kísérleteik szerint a talajmintákhoz adott toluol nem gátolja a mikroorganizmusok továbbszaporodását, tehát a kapott aktivitás a bizonytalanul szaporodó mikrobák teljesítményét tükrözi. Meglepő eredményeik ellenkeznek az enzymológiai irodalom számos adatával, ahol a toluolt mint a leghasználhatóbb antiszeptikumot széles körben alkalmazzák. Meggondolva azt, hogy egyes gátló anyagok másképpen hatnak a természetben vagy a talajmintához keverten, mint in vitro, és hogy emellett a vizsgált talaj- vagy iszapminta tulajdonságai is szerepet játszhatnak, kísérleteket állítottunk be néhány iszapmintával a kérdés tisztázására.

A légszáraz iszapminta 10 g-ját HOFMANN és SEEGERER módszerével 2,5 ml toluollal itattuk át, majd negyed óra múlva 10 ml 5,5 pH-jú foszfát puffert, 10 ml 20%-os nádcukor oldatot adtunk hozzá és jól összerázva 37 fokos termosztátban inkubáltuk 24 óra hosszat. A lebontás fokát a maradék cukor jodometriás mérésével határoztuk meg.

A mikrobiológiai vizsgálatot a baktériumszám meghatározásával végeztük iszapfőzetes ágár lemezen. Minden meghatározás legalább öt számlálás átlaga.

Az *1. táblázat*-ból a toluol szaporodás-gátló hatása biztosan kitűnik. CLAUS és MECHSNER eredményeit az általuk vizsgált talajok pontos ismeretének hiányában hiába is próbálnánk megmagyarázni, de valószínűleg a minták fizikai-kémiai különbsége közt kell az okot keresnünk. Igen feltűnő az a tény, hogy a kis mennyiségű toluollal kezelt minták aktivitása minden esetben nagyobb, mint a kezeletleneké, amit a toluol, kloroform és más hasonló anyagok plazma-permeábilitást megváltoztató hatása okozhat. A HOFMANN-féle módszereknél tehát nagyon kell ügyelni az előírások pontos betartására, az eredmények pedig csak egymásközti összehasonlításra használhatók.