SUB-SECTION IX c

PHYSICO-CHEMICAL METHODS.

IX c 1

THE MICROBIAL DECOMPOSITION OF RESINOUS STABILISING AGENTS IN SOIL

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SUMMARY

The microbial decomposition of resinous substances by the microflora of soils has been but little studied, although some attention has been paid to substances such as phenol, cresols and naphtalene which have been used as soil fumigents. It was found that in low concentration these substances are readily decomposed. Again, the decomposition of Guayule resins has been studied and several organisms capable of decomposing them have been isolated, notably pseudomonads.

In connexion with the stabilisation of soils with resinous materials a study has been made of the resinolytic organisms present in natural soils. Techniques of isolating and counting these organisms have been developed, using enrichment cultures and agar media in which the appropriate resin is colloidally dispersed after saponification into a buffer solution and provide an opaque plating medium on which resin decomposition may be detected by the "halation" produced by resin digesters.

Using such media, a number of resin-attacking bacteria and fungi (notably Aspergilli) have been isolated from all the soils studies and the more active isolates shown to attack resins both in vitro and in via. These organisms have been diverse and have included proactinomycetes, pseudomonades and other eubacteria, Penicillia and Aspergilli.

Resin stabilised soil specimens were inoculated with resin-attacking organisms, and the relation between the relative increase in the numbers of the latter and the subsequent breakdown of the specimens was observed.

A search for remedial antiseptics was then made and it was found that mercuric chloride and sodium pentachlorophenate were particularly effective. The organisms studied are acrobic and it seems that the combination of induced anaerobic conditions and an antiseptic that does not inhibit the stabiliser and is not itself decomposed in a short time would effectively delay or prevent the breakdown of resinous stabilisation agents.

INTRODUCTION.

The incorporation of resinous stabilising agents in soils to reduce water absorption has met with success, but failures have been observed which may have been due to breakdown of the resin by microbial attack. The soil is the natural storehouse for all species of microbes other than those that are actively pathogenic (although these are capable of living in soil for short periods). It is known that micro-organism of varied food requirements can be found in soils and it seems that, in general, whatever the food provided, there will be present in the soil some micro-organisms capable of decomposing it, otherwise various types of organic material would accumulate in the soil. Since the resins used for stabilising soils are, at least initially, natural products, they would seem a priori readily available to soil microbes.

The investigation described in this paper was therefore made to establish whether in fact such microbial attack did occur, to determine which organisms were concerned, and to find out what steps (including the use of antiseptics) could be taken to minimise it.

Very little previous work has been done on the microbial attack on natural resins, apart from the Guayule group 1), but it has been established for instance, that phenols, cresols and naphtalene 2) which are used as soil fumigants, are decomposed if present in low enough concentrations.

PRELIMINARY INVESTIGATION.

Preliminary investigations were made to determine whether "Vinsol" resin or gum rosin were attacked by soil microbes, and to find out whether these resins had a toxic effect on microbes, when present in the quantities used in soil stabilisation. It was found by means of plate counts and enrichment culture techniques that many organisms present in soil attacked the resins, and also that resin contents up to 4 per cent had no toxic effect.

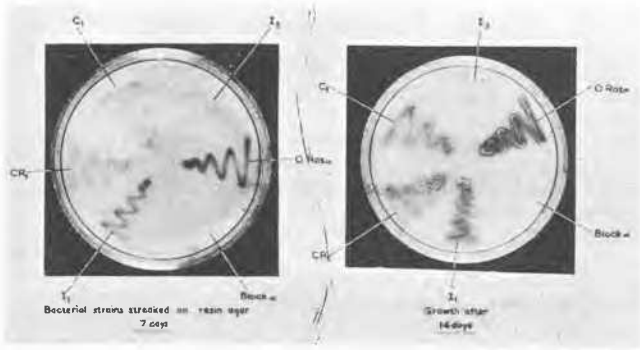
ISOLATION AND COUNTING OF RESIN-ATTACKING ORGANISMS.

The next stage was to develop a method of isolating and counting resin-attacking organisms. This was done by preparing an opaque colloidally dispersed resin agar medium on which the decomposition of the resins could be observed by the development of a translucent zone around a colony of bacteria or of a fungus.

This resin agar medium was prepared from a standard salt solution 1) consisting of:-

K_2HPO_4	0.35	g./litre
KH_2PO_4	0.15	"
NaCl	0.10	"
$MgCl_2$	0.10	"
$CaSO_4$	0.05	"
NH_4NO_3	0.1	"

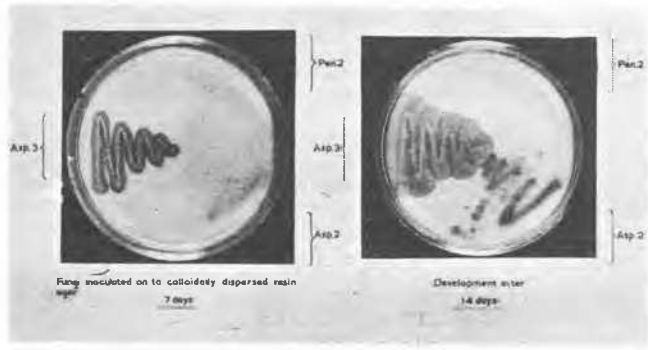
to which was added 1.5 per cent agar.



Growth of bacteria on resin

Attack on resin is shown by the halation of the resin medium used. Strains O Ros α, Cl, CR1 and I, are seen to halate the media in the Petri dishes photographed. However, Strain I_3 shows some slight surface growth (not visible in the photographs) and is concluded to attack resin; whereas Strain Block α is found to show little or no growth.

FIG.1



Growth of fungi on resin

Halation produced by Asp. 1 and Asp. 3, slight surface growth with Pen. 2, hence Asp. 1 and Asp. 3 attack resin.

FIG.2

atmosphere for periods up to 28 days.

THE INVESTIGATION OF SAMPLES FROM A RESIN - STABILISED ROAD.

Bacterial counts were made from samples from an experimental resin-stabilised road at Abingdon, Berks. These were made with five identical plates at a 1:250,000 dilution of the fresh samples of soil, on nutrient and on a "Vinsol"-resin agar medium. Counts were made four days after collection of the samples and the results are shown in Table 1.

TABLE 1

Bacterial counts on samples from an experimental resin-stabilised road at Abingdon, Berks

Sample	4-day count (per ml.)		
	Nutrient medium	"Vinsol" medium	V/N
Subsoil, near "Vinsol" section	52	1	0.02
Subsoil with 1 per cent "Vinsol"-resin	33	1	0.03
Subsoil with 1 per cent "Vinsol"-resin x)	448	35	0.08
Subsoil with 1 per cent "Vinsol"-resin and 0.5 per cent $Al_2(SO_4)_3$	36	7	0.19
Subsoil with 2.2 per cent "Vinsol"-resin and 1 per cent $Al_2(SO_4)_3$	346	81	0.23

V/N = ratio of count on "Vinsol" to count on nutrient media.
x) Sample from area of road that had failed.

Examples of this technique are shown in Figs. 1 and 2 which illustrate respectively isolated and purified resin-attacking bacteria and resin-attacking fungi.

It was found that a large number of soil microbes and fungi were capable of attacking resins; these included proactinomycetes, pseudomonads and fungi. When 1 per cent of resin was incorporated in most of the soils tested a crop of fruiting bodies of a resin-attacking fungus quickly resulted, which was not the case when no resin was added. This is a common experience in the Capillary Water Absorption test, which is used to determine whether a resin has any water-proofing action in a soil, and in which cylinders of a soil-resin mixture are allowed to stand in a humid

It will be seen that the samples containing "Vinsol"-resin in general gave bacterial counts on the "Vinsol" medium that were both absolutely higher than that for the adjoining subsoil, and also show higher ratios of "Vinsol"-attacking organisms to general bacteria, i.e. higher V/N ratios. This supports the previous evidence that the incorporation of resins causes a differential increase in organisms that will attack these substances.

Some of the road samples contained alu-

minium sulphate added to make the soil more acid. These samples showed higher ratios of "Vinsol" medium to nutrient medium counts than did samples containing the resin alone and this suggests that the addition of the aluminium salt increases the proportion of "Vinsol"-attacking organisms compared to the general microflora.

It will be noted that the sample from the failed area of the stabilised road gave higher counts on both media than the samples from the rest of the road.

An interesting additional result (see Table 2) obtained from the investigation of the microflora in the Abingdon experimental road and also from similar road experiments in the grounds of the Road Reasearch Laboratory at Harmondsworth, showed that the addition of 10 per cent of cement considerably reduced the numbers of bacteria in the soil, possibly due to the alkalinity and poor aeration produced by the incorporation of the cement.

TABLE 2

Bacterial counts made from samples from experimental cement stabilised roads at Abingdon, Berks, and at the Road Research Laboratory

Site	Sample	Counts on Nutrient medium (per ml.)
Abingdon, Berks.	Field soil	52
	Field soil 10 per cent cement	5
Road Research Laboratory, Middx.	Field soil	62
	Field soil 10 per cent cement	7

RELATIONSHIP BETWEEN WATER ABSORPTION AND BACTERIAL ACTIVITY.

A series of experiments were made to determine whether the amount of water absorbed by resin-treated soil specimens in a water absorption test was related to the bacterial activity in the specimens. For this purpose, two sets of nine specimens, 2 in. diameter and 1.5 in. high, made with Harmondsworth sandy loam stabilised with 1 per cent of gum rosin were prepared. One set was treated with an inoculum of resin-attacking organisms while a second set remained untreated. Each set was divided into four groups of three specimens, the groups being stored for periods of 2, 4, 6 and 8 weeks respectively. After these periods, the specimens were allowed to absorb water by standing in a shallow dish. The amounts absorbed were determined at 24-hour intervals up to six days, from the increase in weight.

Concurrently, a bacterial count was made using both a nutrient and a selective rosin medium, of the type previously described, first on a dry specimen before absorption and later on a specimen that had been allowed to absorb water in the Water Absorption test. The results of these counts are given in Table 3.

The results of these counts show that:-

1) In both the dry and wet uninoculated specimens a decrease in bacterial activity occurred with increasing time of storage.
2) An increase in bacterial activity with time of storage occurred in the inoculated specimens only after they had been permitted to absorb water.
3) As would be expected, the inoculated specimens showed a greater bacterial activity than the uninoculated series.
4) There was a particularly marked increase in bacterial activity, as recorded by the nutrient medium, in the inoculated specimens that had been stored for 8 weeks and absorbed water.

The numerical results of the water absorption tests have not been reproduced here, as of all the specimens tested, only the one

TABLE 3

Bacterial counts in stored soil specimens (per ml.)

Time of Storage (weeks)	Not inoculated				Inoculated			
	Dry Specimens		Water Absorption Specimens		Dry Specimens		Water Absorption Specimens	
	N	R	N	R	N	R	N	R
2	6	4	74	57	18	20	93	60
4	12	5	50	14	20	9	107	67
6	3	1	68	18	18	12	126	78
8	2	0	59	12	13	7	564	108

N = Nutrient medium. R = Resin medium.

that exhibited the marked bacterial activity referred to in 4) above showed substantially higher values of water absorption than the rest, and in fact it collapsed at the end of the test.

INVESTIGATION OF ANTISEPTICS.

Investigations into the effect of adding crude antiseptics to the soil were made using copper sulphate, zinc chloride, mercuric chloride and sodium pentachlorophenate. A 10 - 20 g. sample of loose soil at a moisture content of 10 per cent and a gum rosin content of 1 per cent were placed in sterile boiling tubes and inoculated with resin-attacking organisms. Blank tests were made with soil containing no rosin. Bacterial counts were made, and in this case the method used was one developed jointly with Dr. J.E. Mollison, in which a direct determination can be made of the number of bacteria associated with unit weight of soil. The results obtained are shown in Table 4 below.

Thus, mercuric chloride and sodium pentachlorophenate in low concentrations are particularly effective in inhibiting resin-attacking organisms, while the copper and zinc salts tested are relatively ineffective even in moderate concentrations. This failure of copper and zinc to check resin-decomposing organisms was confirmed by experiments with pure cultures grown on media containing colloidally dispersed copper and zinc rosinates. Resin-attacking fungi were particularly resistant to copper, an Aspergillus tolerating a concentration of 2 per cent of copper sulphate.

Since it was found that increasing concentrations of sodium pentachlorophenate adversely affected the waterproofing properties of the resinous materials, water absorption experiments were made with low concentrations of this antiseptic and of mercuric chloride. Specimens were also made up with an addition of calcium hypochlorite, which is a readily available crude chemical and is known to have a general antiseptic action.

TABLE 4

Effect of the addition of antiseptics on the growth of resin-attacking bacteria

Medium	Thousands of organisms per gram of soil (Averages of three counts)					
	Uninoculated	Inoculated				
	Soil alone	Soil + 1% resin	Soil + 1% resin + 0.5% $CuSO_4$	Soil + 1% resin + o.5% $ZnCl_2$	Soil + 1% resin + 0.25% $HgCl_2$	Soil + 1% resin + 0.25% SPCP
Nutrient	652	∞	∞	∞	9	14
Resin	-	∞	∞	∞	10	28

SPCP = Sodium Pentachlorophenate
∞ = Numbers of bacteria were so high as to be uncountable.

It will be seen that only mercuric chloride and sodium pentachlorophenate (SPCP) were effective. Further tests, the results of which are given in Table 5, were therefore made with increasing percentages of copper sulphate and decreased percentages of mercuric chloride and sodium pentachlorophenate.

In these experiments, specimens were made up with a sandy loam soil containing 1 per cent of either "Vinsol"-resin or of a commercial soil stabilizing agent which is understood to contain 75 per cent rosin and 25 per cent sodium rosinate. All the specimens had initial weights of 150 g., and the

TABLE 5

Effect of the addition of antiseptics on the growth of resin-attacking bacteria

Medium	Thousands of organisms per gram of soil (Averages of four counts)				
	Inoculated				
	Soil alone	Soil + 1% resin	Soil + 1% resin + 1% $CuSO_4$	Soil + 1% resin + 0.12% $HgCl_2$	Soil + 1% resin + 0.02% SPCP
Nutrient	98,000	333,000	116,000	313	375
Resin	24,000	284,000	91,000	188	125

SPCP = Sodium Pentachlorophenate

amounts of water absorbed after one week found by determining the increase in weight. The results are given in Table 6.

TABLE 6

Effect of the addition of antiseptics on the water absorption of soil specimens containing resinous materials

Resinous material	Antiseptic added	Water absorption after 1 week (g.)
None	None	42
1% "Vinsol"-resin	None	13
do.	0.1% Sodium pentachlorophenate	5
do.	1.0% Calcium hypochlorite	36
1% Resin/sodium rosinate mixture	None	6
do.	1.0% Calcium hypochlorite	9
do.	0.016% Mercuric chloride	2

It will be seen that calcium hypochlorite has a deleterious effect on the waterproofing, presumably because of the hygroscopic nature of the calcium chloride constituent. Mercuric chloride, however, appeared to have no deleterious effect when added to the rosin/sodium rosinate mixture, and in fact appeared to improve the waterproofing obtained.

CONCLUSIONS.

To summarise, the following are the more important conclusions drawn from the work described:-

1) Many organisms capable of attacking natural resins are present in soils.
2) These organisms reduce the waterproofing properties of resinous materials admixed with soil.
3) Mercuric chloride and sodium pentachlorophenate are effective antiseptics for combatting the microbial attack of resinous materials in soil.

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IX c 2 INVESTIGATIONS OF DIFFERENT TYPES OF CEMENT FOR GROUTING PURPOSES

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Cement has already been used for a long time as grout for the consolidation and impermeabilization of soil- and rockstrata.
In the usual methods of injection a suspension of cement in water is injected into the soil. The injection material - in this case, the cement - has to fill and seal, as regularly and as tightly as possible, the holes and pores of the soil.

Cement properties have been mainly examined only in view of their utilization for mortars and concretes; very little is known about the behaviour of cement suspensions in water.

Kiel, in his dissertation (see "Ueber das Einpressen von Dichtungsmitteln in waeserundichte Betonteile" Dissert. Hannover-Kiel 1934) has published permeability values of cement sediments. Santarelli has published interesting tests on cement suspensions for grouting.(Il cemento armato - Le industrie del cemento - No 9 - 1942, Sept. Milan). He investigates especially application of adjonctions (puzzolane and diatomaceous earth) to 5 different cements. The author of the present publication - a collaborator of the "Centre d'études et de recherches géotechniques" of Paris as well as of the "Laboratorio Rodio di Meccanica delle Terre" of Milan - has had the opportunity, between 1935 and 1941, to investigate French and Italian cements. The results of his researches are