

MICROBIAL DETERIORATION STUDIES OF MILITARY COTTON AND LEATHER BASED UNIFORMS

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ABSTRACT

Microbial deterioration studies on selected cotton based uniforms were carried out using test-fungi earlier isolated from the uniforms stored in the Military Warehouses. These fungi includes, *Aspergillus nigar*, *A. fumigatus*, *C. globosum*, *C. thermophile*, *Var. coprophile*, *B. stolonifer* and *S. pulverulentum*. The deteriorative assessment carried out include Visual Assessment, Microscopic and Agar Plate Assessment, Soil Burial Assessment and Decay Studies under which the tensile strength, weight loss measurement swelling test, Brilliant blue test and Methylne blue tests were carried out. The results showed that the materials were in different stages of deterioration, colourations, stains, reduction in tensile strength and loss of weight. The implications of the findings are discussed.

INTRODUCTION

Microbial deterioration is the study of any undesirable change in the properties of a material caused by the activities of microorganisms. Any investigation on materials deterioration would inevitably involve the estimation or precise measurement of the extent of the materials loss.

Hueck-Vah plas (1965) and Sharp (1978) reported on certain reliable techniques in the measurement of fungal growth and decay of materials. In addition, a number of national and international standard methods for the determination of fungal decomposition of cotton based textiles and leather based materials exist. These include standard methods ASTM D 1456T (American Society for Testing and Materials 1956) and BS 6085 (British Standards Institution).

Loss in both weight and tensile strength of materials are widely employed as convenient measurement of decay, although as it is well known, their relationships to other physiological and biochemical changes may be inconsistent (Pechman and Shaile, 1950; Richards, 1954). There are very few materials known which are resistant to decay by microorganisms. Some materials like food, papers, wood and cotton decay relatively quickly and this requires preservation treatments. Others like Plastics, rubber, oil, stone, bitumen and leather decay slowly and only need occasional treatment in order to prolong its service life while some like treated metals, fluorinated plastic polymers are recalcitrant and persist even at disposal sites (Sharp, 1978).

The manufacturer of biocides is interested, among other things, in an economic reproducible laboratory method for screening and the buyer or user is also interested mainly in the rot resistance in practice. The person who finished the textiles so as to protect it against rot will take into consideration the results from laboratory tests and the flow of results from practical usage. Hausam (1967) has dealt critically with the effectiveness of laboratory tests in forecasting practical experience. However, there are objections on the reliability of this method because of the high degree of variability of results from different soils (Siu, 1951) and lack of agreement experience at different occasions in the same laboratory (Llyoyed, 1969). This according to Turner (1971) was due to the influence of many factors in rot resistance tests by soil burial methods as the various results obtained depend on the operator, re-use of soil and degree of compaction of the soil among other things. Therefore, it was postulated that a good laboratory soil-burial method should optimally decay any material which is not protected. It should also identify as good during a defined testing time, any treated homogenous material which has been proved in practice under wet conditions.

Also, National Standards for specific laboratory methods for checks on taking delivery, for Military and Private use, exist in various countries. They apply the laboratory soil burial test. Examples include; SIS 251237 (1977), SNM 564280 (1977), EMPA 223/11 (1980), AATC test method 30 (1981)k, BS 6085 (1981) and NF X 41 - 514 (1981).

Despite the lack of record of decay of some material, it has been stated regardless of complexity, all material are ultimately degradable. Fungi therefore cause extensive deterioration of materials of economic importance such as cotton and leather based materials. Measured in economic terms, the ravages are enormous. Much progress has already been made in combating decay but further advances are still necessary.

It is on the basis of this fact that Military personnel, due to the nature of their duty/operations which expose them to constant contact with the soil and soil being reservoir of biodeteriogens, that this study was designed. Its major objectives were to carry out decay and soil burial test which could provide good forecasts on the behaviour of the test materials in practice. The tests could act as simple screening methods for manufacturers of these materials or could serve as methods for quality control based on user's specific requirements. This objective is very good especially now that Military Uniforms are manufactured locally.

MATERIAL AND METHODS

TEST ORGANISMS

The following fungi, *A. niger*, *A. fumigatus*, *C. globsum*, *C. thermophile* var. *Coprophile*, *R. stolonifer* and *S. pulverulentums* were chosen for detailed investigation. The choice of these organisms was based on the fact that they were found to have high range of frequency of occurrence during the isolation experiments, and also most of them were reported to have a wide distribution in Nigeria having been isolated from cellulosic materials, soil and aerial environment of Nigeria (Ogbonna, 1980). The same worker reported them as important decay biodeteriogens, having been screened on decayed organic materials.

SURVEY ON DETERIORATIVE CONDITIONS OF THE EXPERIMENTAL MATERIALS

a. VISUAL ASSESSMENT

The extent of fungal growth on the stored uniforms from the five experimental sites was assessed visually and recorded in accordance with the scheme specified in the standard (BS 6085: 1981). The aim of this subjective assessment of spoilage of test materials was to observe the growth of microorganism in situ, which make the test materials nasty when looked at. Sharp (1978) reported that unsightly appearance of textile and leather material is another form of deterioration which reduce the economic value of these materials.

This was carried out during the two marked season (dry and raining season) by simple observation of the physical condition of the test materials. Records of the various assessments were kept.

b. MICROSCOPIC AND AGAR PLATE ASSESSMENT

These methods which were described by Martin and Milton (1950), Warcup (1964) were most ideal for both qualitative and quantitative assessment of microbial decay of materials.

Scrapings were taken from the fungal colonised areas of the research samples into sterile petri dishes with aid of sterile blades. The scraping was carried out aseptically and plated out on Czapek Dox Agar (CZA) (Raper and Thorn 1949), Malt Extract Agar (MEA) (Hesseltine, 1954), Cellulose Agar Medium (CAM) (Eggins and Pugh 1962), Yeast Starch Agar (YSA) (Cooney and Emmerson, 1964) using the soil plate method described by Warcup (1950). In this method, 0.03g of these scrapings from the desurfaced materials was placed in sterile Petri dish. 15ml of freshly prepared agar medium, cooled to 44°C, was poured to cover the scrapings in each plate and the plate swirled in order to allow for even mixing of the medium and scrapings. The plates were allowed to solidify and eventually divided into batches and incubated at different temperatures of 25°C, 37°C, 45°C and 50°C. These were examined with the aid of microscope after 7 days and then re-examined after a week for the appearance of additional fungal species that are slow growers. The results were recorded accordingly.

c. SOIL BURIAL EXPERIMENTS

Samples of the cotton textiles and leather materials were used for this experiment. A modification of the methods described by Manowitz (1952) National International Standards; AATC test method 30 (1981), BS 6085 (1981) EMPA 223 (1980), NFX 41-514 (1981), SIS 2512337 (177), SNV 564280 (1977) and Ogbonna (1980) were employed for the tests.

In the Modified methods, rectangular shaped strips of cotton textiles and leather surfaces sterilized materials measuring 10cm x 3cm were obtained from the test materials and divided into groups. These were buried in the soil samples inside glass jars. The glass jars were filled with highly packed soil samples to just cover half of their capacities in order to allow enough aeration. The soil moisture content of each of the jars was raised to 80% of its water holding capacity prior to the introduction of the test materials.

The soil used was the top soil which is normally richer because of the high organic content as described by Ogbonna (1980). The jars were then fitted with cotton plugs and incubated at 30°C for six months, after which the test materials were removed and the tensile strength and weight losses determined. The jars were kept under observation during the incubation period with sterile water added at intervals to the burial soil samples in order to avoid the drying out of the soil samples.

- i) Twelve (6 strips each of cotton and leather) were buried in non sterile soil samples.
- ii) Another 6 strips each of cotton and leather sterile were buried in sterile soil samples.
- iii) Another 6 sterile test textile/leather strips were buried in non-sterile soil samples.
- iv) Then sterile test materials (cotton and leather) each were buried in sterile soil samples and inoculated with selected fungi.
- v) Six sterilized test materials each of cotton and leather were buried in sterilized soil sample and these served as control.

Here the spores of the selected fungi were introduced into the sterile soil sample. The spores were mixed thoroughly aseptically in sterile flask prior to their introduction into the sterile soil in the jar. Spore suspensions from 2 weeks old fungal culture were used for the inoculation. Sterile distilled water (10ml) was added to each of the culture dishes and the surface of the fungal culture was carefully scrapped and squeezed with a sterile glass rod. The resultant spore suspension was transferred with pipette under sterile conditions to flask containing 100ml. of distilled water to give the final spore suspensions of each of the selected fungi. Fungal re-isolations were made at the end of the experiment in order to make sure that the test fungi were still present in the soil and that there has not been other fungal contaminations.

These materials in (v) above acted as the control experiment and helped to correct for changes in the tensile strength and weight losses that were not due to fungal attack. The soil in each jar was moistened to 80% of its water holding capacity (w.h.c) prior to the introduction of the materials. The jars were incubated at 27°C and 30°C, and observed periodically with sterile distilled water added to avoid drying out of the soil. The experiments were repeated using strips of leather materials.

d. **DECAY CRITERIA**

The criteria used to assess the decay of the test materials were loss in tensile strength, loss in weight and subjective visual assessment of spoilage.

e. **TENSILE STRENGTH DETERMINATION**

The methods used for the measurement of tensile strength loss of the test materials was that described by Sharp (1978) and Ogbonna (1980). A tensiometer was employed for the tensile strength determination, the test materials were conditioned to the room environmental condition where the tensiometer was installed for a period of 24 hours, after which the known weight of material was fixed at the jaws of the machine and electrically operated. The jaws moved apart until the test strip was fractured. The reading of the force exerted was indicated and known as the load which is expressed in

Newton. The load (Newton) divided by the area of cross-section of the material gave the tensile strength.

f. PERCENTAGE LOSS OF TENSILE STRENGTH

This was calculated by finding the width and thickness of the test material in (mm). Then area of cross-section which was the width multiplied by the thickness (mm^2), when this was known, the load (Newton) was determined using the tensiometer machine. The initial strengths of all the test materials were taken before any experiment was carried out. So the difference between the initial tensile strength of the non decayed materials and the deteriorated materials was regarded as due to microbial decay of the test materials.

CALCULATION

Width	=	(mm)
Thickness	=	(mm)
Area of Cross-section	=	(width x thickness) - (mm^2)
Load	=	(Newton)
Tensile strength	=	Load + Area of Cross-section)
Percentage Tensile Strength	=	The difference between (I) initial Tensile strength (N/mm^2) and (F) Final Tensile strength divided by the initial tensile strength (N/mm^2) multiplied by 100.
General Formula	%Tensile Strength	= $\frac{I - F}{I} \times \frac{100}{1}$

Source: (Sharp, 1978)

g. WEIGHT LOSS DETERMINATION

PERCENTAGE WEIGHT LOSS: The test materials used in the tensile strength determination were dried to constant weight and their mean dry weight taken. Weight loss of the buried or non-buried test materials due to fungal or microbial decay was regarded as the difference between this initial mean dry weight of each batch of the test materials.

CALCULATION:

If the original mean dry weight of the test materials = X and the mean dry weight of the decayed material = Y

$$\begin{aligned} \text{Weight loss due to fungal decay} &= X - Y \\ \% \text{wt loss due to fungal decay} &= \frac{X - Y}{X} \times \frac{100}{1} \end{aligned}$$

Source: (Ogbonna, 1980)

h. THE BURIED TEST SAMPLES

Test samples were removed from the experimental jar monthly and the effect of Microorganism or fungi on the strength tested. The samples were then dried to constant

weight of the test material caused by microorganism. The adherent mycelium and soil particles were carefully scrapped off before drying.

i. **SOIL STERILISATION**

The jars containing the soil samples to be sterilized were plugged with non-absorbent cotton wool and then covered with aluminium foil. The soil samples were then sterilised by autoclaving them for one hour at 121°C as described by Ogbonna (1980). The jars were then left until the next day to allow any living heat resistant spores in the soil to germinate. The soil samples were then autoclaved for a second time for one hour at 121°C. This second autoclaving sterilised the soil samples and killed any heat resistant spores remaining in the soil. After cooling the test materials (Sterilised or unsterilised depending on the soil burial investigation) were introduced into the sterilised soil vertically. The soil covered the entire test materials. The test strips were adequately labelled. The whole process was carried out aseptically to avoid the possible introduction of an external agent.

j. **STERILISATION OF THE MATERIALS**

The test materials (cotton textiles and leather uniforms) to be sterilised were heated to 80°C as described by Ogbonna (1980). The procedure was repeated several times. This was done in order to kill off the initial microbial flora of the test materials.

k. **SWELLING TEST**

Swelling test was employed in order to carry out microscopic examination of the decayed fibres of the cotton based textiles samples. This is another method of detecting decay in polymeric materials like cotton. The test was carried out as described by Trotman (1970), Hawker *et al.*, (1971) and Sadov *et al.*, (1973). In this method, about 1g of the cotton materials were boiled in 15% solution of NaOH which was later neutralised with acetic acid and then washed in distilled water. The materials were then steeped in 15ml of 15% NaOH solution to which 1.5ml of carbon disulphide had been added. The fibres resulting from this process described above were then mounted on a slide in water and examined under a microscope after a period of 45 minutes.

l. **BRILLIANT BLUE TEST**

The brilliant blue dye was described by Sadov *et al.*, (1973) as a very good reagent for the determination of deteriorated fabrics by microorganism. The test was carried out as described by the same author. In this test, the test materials were simply immersed in a solution of the dye after which the materials were examined visually.

m. **METHYLENE BLUE TEST**

Pure cellulose has no affinity for Methylene blue but the presence of carboxyl groups associated with hydrocellulose or residual mineral acid associated with hydro cellulose formation do cause cellulosic fibres to absorb dye. Both qualitative and quantitative test are based on these facts (Trotman, 1970).

This test was carried out as described by Trotman (1970). The cotton textiles samples were cut into known sizes 5cm x 3cm and then immersed in a cold solution of methylene blue. This was then rinsed with boiling water. The product was observed and recorded visually.

RESULTS

a. VISUAL ASSESSMENT

The results of the visual assessment showed that the test materials were in different stages of deterioration and were most colonized during the raining season when the relative humidity is high and the temperature is also high. This pattern was observed in the five experimental sites. The details results are presented in Table 1a.

b. MICROSCOPIC ASSESSMENT

The microscopic assessment of the decayed/desurfaced test materials from the five experimental sites showed a similar pattern of fungal distribution as recorded in the soil aerial mycoflora survey respectively. The materials showed a higher fungal load in the months of May to August in Jos, Kaduna and Makurdi samples while the peak in Port Harcourt and Lagos was between February to May.

TABLE 1a: VISUAL ASSESSMENTS OF THE TEST MATERIALS FROM THE FIVE SELECTED NAF WAREHOUSE

TEST MATERIALS	STORAGE PERIOD	VISUAL ASSESSMENTS OF THE EXPERIMENTAL MATERIALS FROM THE FIVE NAF WARE HOUSE				
		JOS	KAD	MAK	P/H	LAG
C1 . . Tent	6 months	4	4	3	4	4
C2 . . Haver Sack	6 months	4	3	2	3	3
C3 . . No 5 Belt	3 months	2	2	1	2	2
C4 . . No 4 Shirt	3 months	1	1	1	1	1
C5 . . Beret	2 months	4	4	3	3	4
C6 . . No 5 "OG Green"	4 months	3	3	2	3	2
L1 . . DMS Boot	6 months	3	2	2	3	2
L2 . . Flying Boot	6 months	4	4	3	4	4
L3 . . Air Force Shoe	3 months	4	3	3	4	4
L4 . . Georges Boot	3 months	4	4	2	3	4
L5 . . Rank Badge	2 months	4	3	1	2	2
L6 . . Pistol Holdster	4 months	3	2	1	3	3

KEY:
 NO GROWTH (NG) = 0
 LIGHT GROWTH (LG) = 1
 MEDIUM GROWTH (MG) = 2
 GROWTH (G) = 3
 HEAVY GROWTH (HG) = 4

c. SOIL BURIAL STUDIES

The soil decayed leather and cotton textile samples were completely discoloured with fungal and other microbial pigments. The loss in weight and strength could easily be felt by mere observation of the decayed materials. Plate 1 shows the detailed appearance of the decayed strips of military uniform. The decayed materials looked very different from the non-decayed ones.

d. TENSILE STRENGTH AND WEIGHT REDUCTIONS OF THE TEST SAMPLES

The tensile strength and weight losses of the decayed test materials are presented in (Fig 1 and 2). The non-sterile test materials buried in non sterile soil sample were found to have had the highest strength and weight reductions. They were followed by the sterilized test materials buried in non-sterile soils and the non-sterile test materials buried in sterile soil. Comparatively, the least reductions were observed in the sterile test materials buried in sterile soil and inoculated with the test fungi.

e. **TENSILE STRENGTH AND WEIGHT LOSSES OF WAREHOUSE EXPOSED TEST MATERIALS**

The results obtained are presented in Fig. 1 for cotton based samples and Fig 2 for leather based samples which revealed that there was a decrease in both the weight and tensile strength of the warehouse decayed test samples. The effect was more pronounced on the cotton textiles uniforms than the leather based uniforms and during the rainy season than dry season.

f. **SWELLING BRILLIANT BLUE AND METHYLENE BLUE TESTS**

The results of the of the swelling test showed that some fibres of cotton based samples had even swelling and lacked globular formation while others showed different swelling and globular formation.

The brilliant blue test results showed spots on some parts of the cotton textile samples which were detected because of the characteristic intensive colouring.

The Methylene blue test also showed some spots on the test fabric. However, a higher degree of staining of the affected test materials were observed.

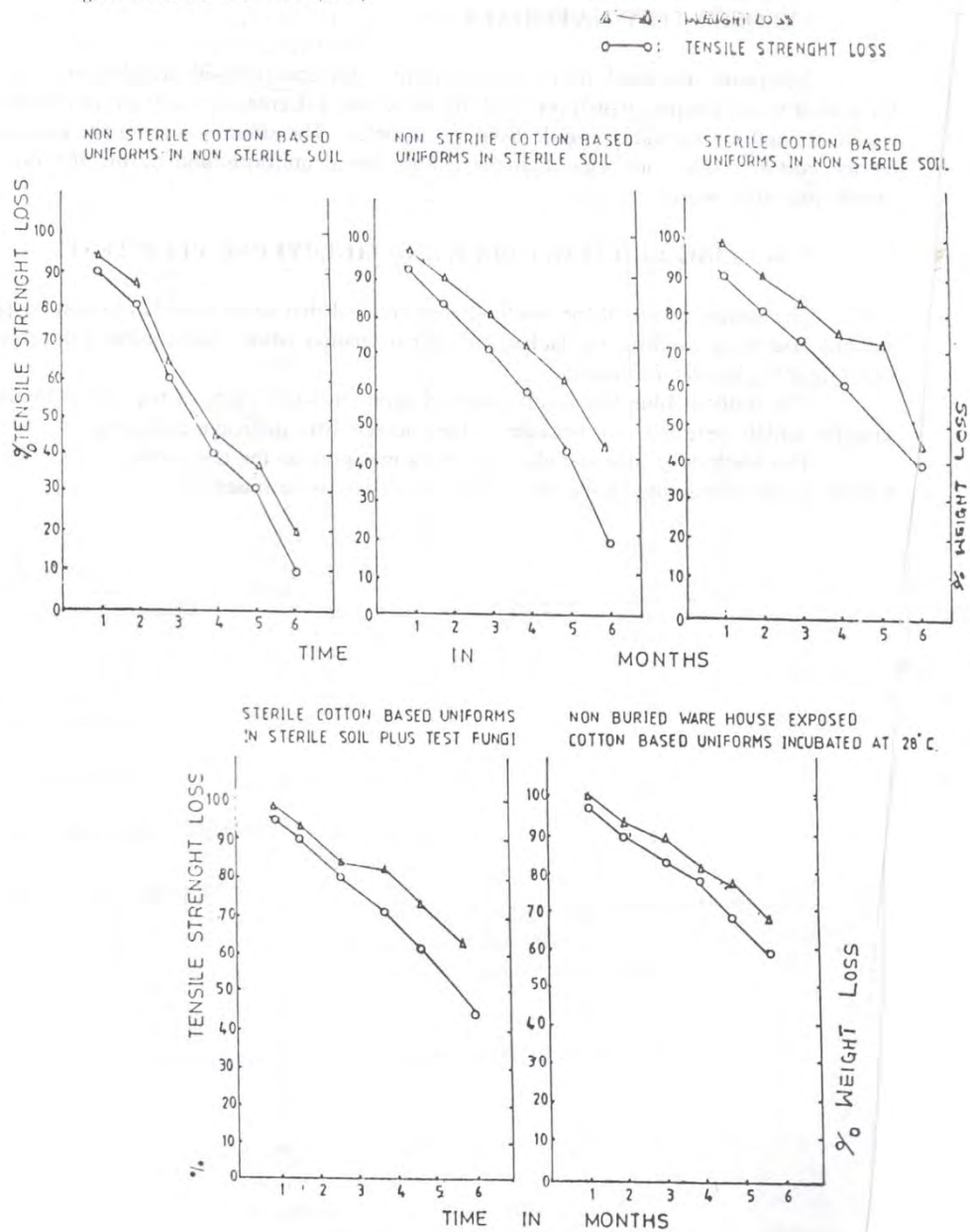


FIG 1A PERCENTAGE WEIGHT & TENSILE STRENGTH LOSSES OF THE TEST MATERIALS RECORDED AFTER THE SOIL BURIAL AND WAREHOUSE EXPOSURE EXPERIMENTS.

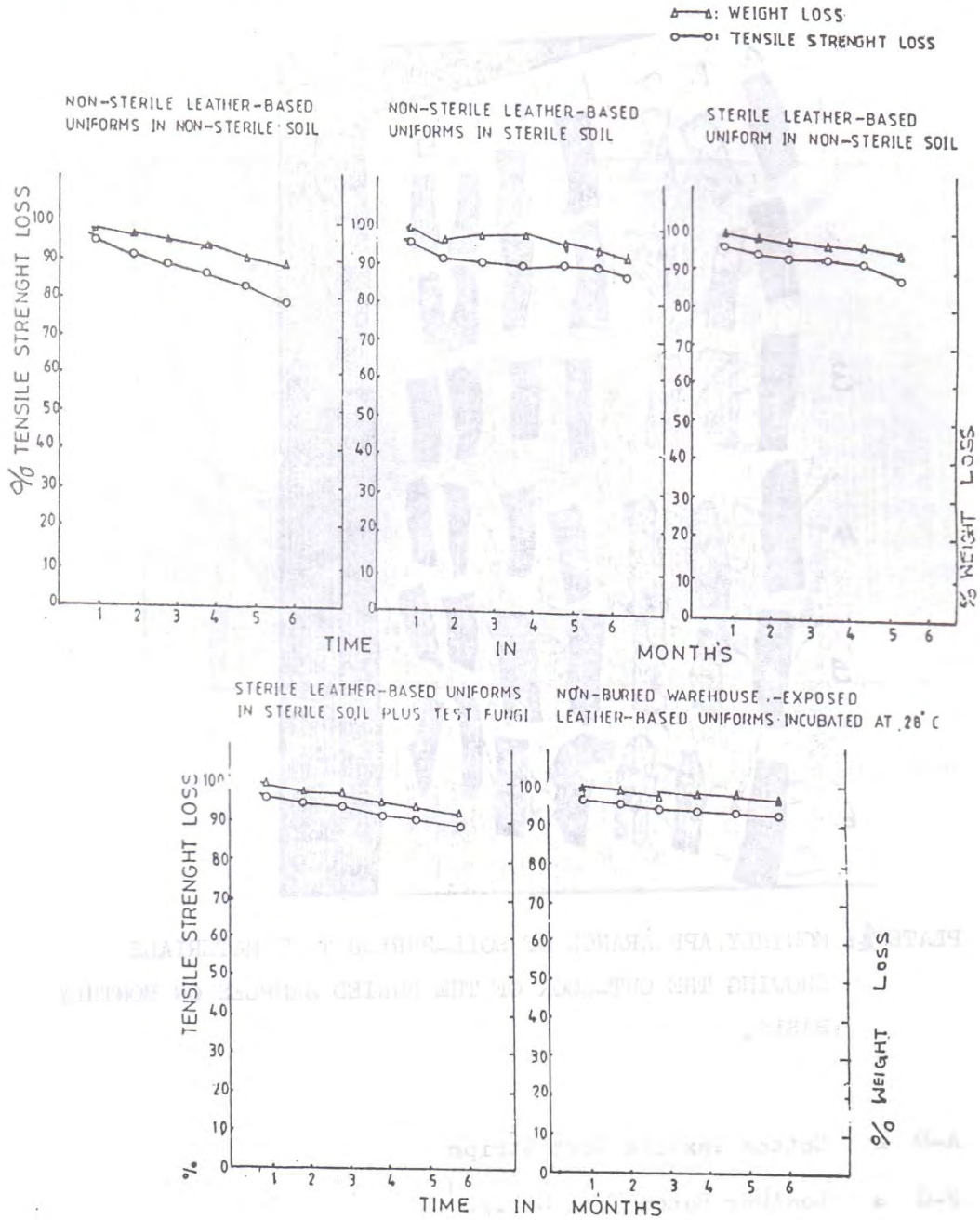


FIG 2D: PERCENTAGE WEIGHT & TENSILE STRENGTH LOSSES RECORDED AFTER SOIL BURIAL EXPERIMENTS & WAREHOUSE EXPOSURE OF THE LEATHER TEST MATERIALS.

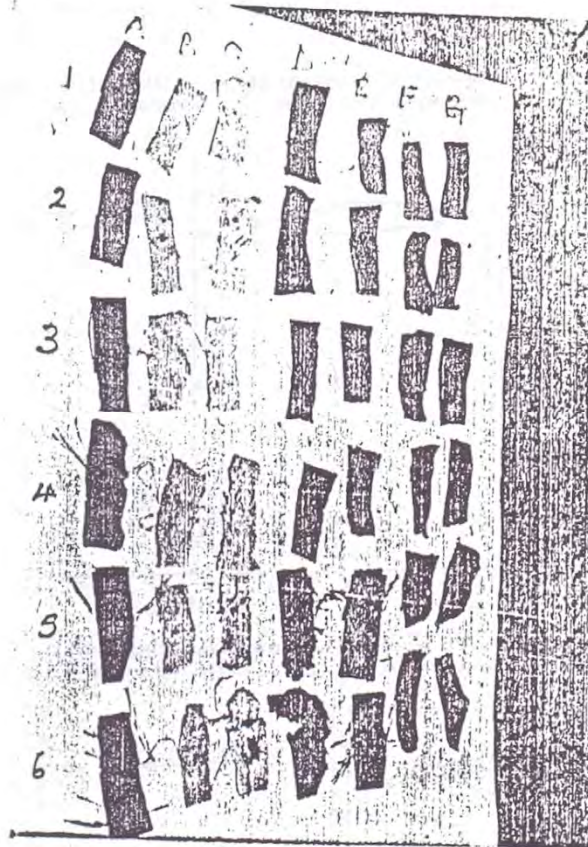


PLATE 1: MONTHLY APPEARANCE OF SOIL-BURIED TEST MATERIALS SHOWING THE OUT-LOOK OF THE BURIED SAMPLES ON MONTHLY BASIS.

A-D = Cotton Textile Test Strips

F-G = Leather Based Test Strips

1-6 = Months of Burial

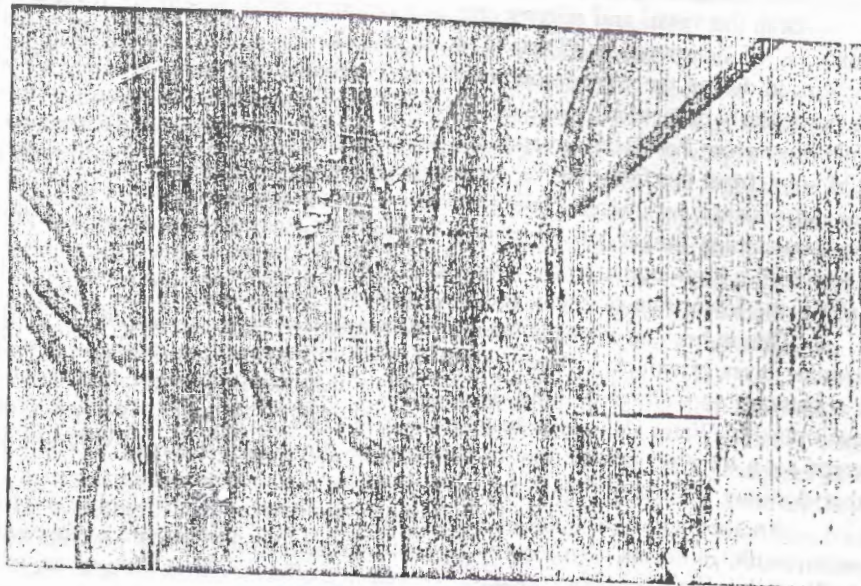


PLATE 3: THE FIBRES OF THE COTTON BASED UNIFORM FROM THE SWELLING TEST.

DISCUSSION

The results obtained from the various experiments have shown that the cotton based textiles and leather based uniforms of the Nigerian Air Force stored in different warehouses in Nigeria are liable to decay by microorganism especially fungi.

Both the visual and microscopic assessment of the test samples revealed various conditions of deterioration (Table 1 & Figs 1 & 2). These conditions were more pronounced during the wet season and the early months of the dry season. This pattern was particularly observed in samples from NAF warehouses in Jos, Makurdi, and Kaduna. While those from Port Harcourt and Lagos showed different pattern of deterioration. This could stem from the fact that, the three sites - Jos, Kaduna and Makurdi have marked seasonal weather variations. There is the wet damp conditions during the wet season which encourage fungal activities and a dry season when the relative humidity might be low with a high temperature especially in Kaduna and Makurdi. This could probably explain the lower rate of decay and microbial load during this period.

This is because water is not only necessary for extracellular metabolism, but an adequate layer of external water is necessary for diffusion of extracellular enzymes and of substrates as well as of toxic products, for the maintenance of turgor for hyphae development. Water also plays an important role in the discharge of spores. Fungi may persist for a long periods of time in the absence of water, usually by virtue of spores or other dormant structures (Ogbonna, 1980).

Another significant visual observation made was the brown line effect on the cotton textile uniforms. This could be due to water absorption and evaporation effect which produces the wet-dry boundary (Brown line effect). The nature of the brown-line substance, the mechanism of its formation, the influence of the ambient environment and the accompanying loss in strength of cotton cellulose alternately wetted and dried have been investigated by Sharp (1978). Water therefore plays an important part in the deterioration of cotton and leather materials subject to wetting and drying and to avoid such losses, we must keep these items dry.

The results of the soil burial studies have shown that the test materials in ground contact under favourable environmental conditions are liable to microbial deterioration. The different stages of deterioration observed for the non-sterile samples buried in the non-sterile soil was a clear indication of the action of soil microorganisms. The sterilised test samples buried in the non-sterile soil showed a greater degree of decay than the non-sterilised test samples buried in sterilised soil. The cotton based textiles samples generally showed a higher degree of attack than the leather based samples. This could probably be ascribed to the complexity of leather structure, the tanning process and finishes of leather materials during manufacturing process which make it more stable than cotton textile materials. The differences in the decay rates observed between the sterile and non-sterile samples probably stemmed from the fact that there were more diverse microorganisms present in the soil than in the test materials. In addition, a combined attack by the varied micro-organisms in the non-sterile soil might have led to the greater decay rate observed in the sterile test materials in non-sterile soil. Also the sterilisation of the soil by heat might have led to the evaporation of some volatile substances in the soil thus leading to the alteration of the soil as reported by Ogbonna (1980).

Lawrence (1956) reported that the amount of each of a number of water-soluble chemical compounds in the soil is made soluble by heat in larger amount than others hence nutrient unbalance commonly ensures. The degree of chemical unbalance ranges

from small to very large, depending on the nature of the soil and the amount of heat applied. Crum structure (tilth) aeration and drainage are all improved. These factors may have contribute to the lower decay rate observed in the non-sterilised test sample in sterile soil as compared to the sterilised test-materials buried in non-sterile soil.

There was a correlation between the tensile strength and weight losses. Greathouse and Wessel (1954) and Hartley (1958) reviewed the scattered literature on strength and weight properties of polymeric materials such as cotton, wood and leather. They reported that the strength and weight of materials decrease with the progressive stages of decay. The present finding is in agreement with this generalisation. Also the close relationship between loss in tensile strength and loss in weight due to fungal decay has been demonstrated by Armstrong and Savory, (1959) and Ogbonna (1980).

The disadvantage in the use of weight loss as a criterion for determining decay is that a long incubation period is needed to attain suitable weight losses and corrections must be made to allow for weight changes caused by other factors, such as uptake of nutrient from the substrate. The introduction of strength determination eliminated the need for weight correction except that expensive testing equipment like the tensiometer must be used.

The decay ability of the micro-organisms in the soil might have depended on the complex interactions of all the soil characteristics, moisture content, water holding capacity, organic content, the mineral status and soil pH. Soil moisture is one of the important ecological factors that affect decay generally (Galloway, 1935 and Snow, 1949). Microbiological degradation of organic materials such as leather and cotton textiles could be initiated if the moisture content exceeds 30% - 40% of their dry-oven weight. This factor was provided in the soil burial experiments due to the fact that the soil was moistened to 80% of its water holding capacity before the test materials were introduced.

The periodic addition of water to the burial jars helped to maintain adequate moisture level in the burial test materials thus enabling them to easy attack by the soil microorganism especially fungi.

The lack of globular formation and uniform swelling on the cotton textile fibres (Plate 2) was a good indication of the microbial alteration of such fibres. Trotman (1970) in a similar experiment reported that if the intermolecular spacing in the crystalline portion is not altered, cotton textiles fibres in solution would show uniform and globular swelling. This further confirmed that the organism associated with the decayed materials were not mere surface contaminants but also contribute to reasonable extent to the decay of these materials.

The result of both the Brilliant and Methylene Blue tests showed dark spot staining on the test materials. This according to Trotman (1970) and Sadov (1973) is an indication of decay. This further confirms the activities of these organisms. The principle underlying the use of these two tests is that pure cellulose has no affinity for the dye and this could explain why the undecayed areas of the test materials were not intensively stained. So the presence of living cells such as the microorganisms associated with other residual mineral acids enables the cellulosic fibres to absorb the dye.

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