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Study of fungal Diversity on different types of Finished Leather and Leather Articles

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Abstract

Finished leather and leather articles, manufactured from the animal skin are highly Susceptible for fungal attack. Theanimal skin contains large number of microbes, derived from air, water, soil and extraneous filth, when the animal is alive, most of these microbes have little effect on the skin. But after the removal of the skin from the dead animals, during finishing operation, all microbes find themselves in a perfect medium for the growth and immediately start multiplying at an enormous rate. The observations of many types of leather samples will give a large number of fungi which grow and infest the various types of finished leather and cause deterioration The present study deals with the collection of various types of finished leather samples and infested leather articles from different places. Isolations were made from such samples to know about the qualitative and quantitative spectrum of fungi to deteriorate the collected samples. In all leather samples and leather articles total 52 fungal forms belonging to 19 different genera were isolated. The present investigation provides impetus to develop certain preventive measures to make these finished leather free from infestation of fungi under the conditions of high R.H. and optimum temperature, the knowledge of specific microflora and their relative capacity to infest leather is necessary for solving this problem.

Keywords: Finished leather, leather articles, fungi, infestation, relative humidity.

Introduction

Leather is utilized in making a large number of commercial commodities and it has gained a status symbol as one of the topmost foreign exchange earner and belongs to the elite of society. India is fortunate in having a good raw hide base but biodeterioration of leather in industries by fungi is fairly common during leather manufacture, finishing, storage and in use. Leather, organisms and environment are intimately concerned in biodeterioration.

The number and kinds of microorganisms on leather vary according to the storage conditions and presence of spores in the air of storage places. The results clearly indicated that a high relative humidity of storage not only helped in the multiplication of fungal species but it also favoured the establishment and development of the fungi during storage. Many fungal species which were isolated initially showed decreased frequency and some fungal forms appeared in due course of time.

Thus, certain fungal forms showed preferential requirements of relative humidity and storage time to colonize the finished leather, at suitable temperature.

Material and Methods

Collection of leather samples: The survey of the different leather factories and tanneries were made during in Gwalior, Kanpur and Madras followed by the collection of different qualities of finished leather belonging to different animals, tanning also the leather from different stages of tanning process.

The various types of finished leather i.e. vegetable tanned sole leather (buff), semi-chrome tanned leather (buff), chrome tanned softy leather (cow), Zuggrain chrome tanned leather (cow), chrome retan leather (cow), vegetable tanned leather (goat), chrome tanned leather (goat), oil tanned chamois leather (goat), vegetable tanned leather (sheep), and chrome tanned leather (sheep).

Further, various types of naturally deteriorated finished leather's articles like gent's footwear, ladies footwear, belts, leather cases, bags or purses and other articles were also collected from different places of Gwalior.

Method of Sampling: Collection of leather samples was made following IS:5868-1969 methods[1]. Samples were placed into sterilized polythene bags and brought to the laboratory.

Tropical Chamber: The tropical chamber fabricated for these studies measured 54 X 25.5 X 26.5 inches with double walls (wooden). The inner surface was coated with white enamel paint with 0.2-0.3 per cent w/v B- naphthol and P- nitrophenol to check the fungal growth on inner side of chamber. A layer of good sandy loam soil (4"thick) was spread over the floor. The soil surface was covered with jute cloth to avoid the displacement of soil during spraying process. The humidity within the cabinet was maintained by sand saturation of alternative days and a carbon filament bulb (25w) was used for

maintaining temperature inside the chamber during winter season. The observations of R.H. inside the chamber were made regularly each day at 18.0 and 6.0 hour. The values of R.H. and the temperature were maintained throughout the experimental period between 85-90 per cent and 28-30^{0}C, respectively. The assessment of R.H. was made using dial hygrometer.

Fungal growth on leather: The samples were exposed to air and dust for 7 days to get them charged with microorganisms in open air. The exposed leather were then hanged on wire strings in tropical chamber at 28 ± 1^{0}C temperature and approximately 90 ± 5 per cent R.H. The observations were taken after 7 days interval regularly for fungal growth on the leather samples.

Isolation of Microflora: Fungi were isolated from various deteriorated finished leather samples, footwear and other articles following method outlined by CMI, Kew, England (1960) as followed by Orlita[2].

(i) Direct- agar inoculation method: The samples were observed under binocular microscope and spores were picked up by inoculating needle from distinct colonies developing on leather surface and were directly inoculated into agar plates under aseptic conditions. The plates were incubated at 28 ± 1^{0}C for fungal growth upto 7 days. The pure fungal isolates were maintained on Czapek's dox agar slants.

(ii) Serial dilution method: The fungi from deteriorated surface were isolated by swabbing the sample with sterilized moist cotton which was shaken in 10.0 ml sterilized distilled water in a culture tube. Dilutions (1:10, 1:100, 1:1000, 1:10000) were made and 1.0 ml of each of these dilutions was asceptically transferred to sterilized petridishes. 20.0 ml of sterilized medium was added to each dish. The media used were potato-dextrose agar and Czapek's dox agar. The petridishes were incubated for 7 days at 28 ± 1^{0}C temperature. The isolates were transferred into Czapek's dox agar slants.

Purifications, maintenance and identification of cultures: The fungal isolates thus obtained in various isolations were maintained on Czapek's dox agar and Potato-dextrose-agar slants and were kept 5 ± 1^{0}C in refrigerator.

Identification of fungi were made following Raper and Thom (1949), Gilman (1957), Barnett (1960), Raper and Fennell (1965), Smith (1969), Subramanian (1971), Mukerjee and Juneja (1974), Ellis (1971) and Singh *et al*. (1991). Finally, the Identification was confirmed through the curtsey of Director , Commonwealth Mycological Institute, Kew, England .

Results: In the present investigation a number of fungal growth were observed and isolated from different types of leather samples, leather articles. Generally, maximum fungi appeared on the 4th day on various samples in tropical chamber. In all leather samples and leather articles total 52 fungal forms belonging to different genera were isolated. The number of fungi isolated from finished leather samples exposed in tropical chamber were 47 (table I). Out of these fungal species, 26 from vegetable tanned leather (sheep), 25 each from chrome tanned leather (sheep) and vegetable tanned leather (goat) 24 and 23, from vegetable tanned sole leather (buff) and Zuggrain chrome tanned leather (cow) respectively, 20 each from chrome retan (cow) and oil tanned chamois leather (goat) and minimum 18 each from semi-chrome leather (buff) and chrome tanned leather (goat) as shown in figure-I.

In all, 50 fungal species (Table-II) were isolated from naturally deteriorated leather articles. Maximum number of fungal species i.e. 38 were found on gents footwear in comparison to other articles. Similarly, 30, 27, 25, 25 and 25, were isolated from ladies footwear, leather case, bag or purses, belts and other articles (figure-2).

In all the cases the luxuriant growth of mycelium was observed towards grain side of the leather samples and comparatively less growth was recorded on the flesh side. Vegetable sole leather samples showed uniform profuse pattern of growth.

The first indication of deteriogenesis, appears with the darkening effect, which soon turned into blackish, was mainly due to degradation of nutrient followed the appearance of vegetative structure of deteriogens at the edges of leather species. Its appearance differs from age to age. The first effect resembles a strain but later on a sub-lanose appearance which subsequently turns in characteristic colour, exudation and texture.

A defect appeared on the grain side of coloured skin in the form of light spots, varying in size from 0.1 to 10 mm in diameter. This defect was not confined to any particular coloured skin nor to any one part of the skin. In every case the colour of the spots were decidedly lighter than the base colour. The defect appeared on the leather after the colouring and drying operations. The most unusual conditions, we encountered was at the finishing stage. Study showed that the vegetable tanned leather is much susceptible than chrome tanned leather. The effect of fungi attack on different types of leather were as follows. i. Finished chrome tanned leather did not readily become mouldy. ii. Semi chrome tanned leather more easily became mouldy. iii. Vegetable tanned leather of the three most readily supported the mould growth.

Finished leather was considered much susceptible for fungal attack, if stored under hot and humid conditions.

Discussion: A perusal of results reveal that variable number of fungal species were reported from various types of finished leather collected from different places belonging to different and leather articles. Susceptibility of leather depends on the animal, sex of animals[3], breed[4], hair[5], dye[6], age of animal [7], castration and season[8], hence the difference of the number in fungal species in all samples. The number of both fungi and bacteria in

the air and deposited on the surface varied substantially from month to month, hour to hour, place to place, and especially with difference in height above ground[9]. These results are significant inspite of variation between parallel samples. During tanning process first step is soaking which inoculates large number of fungi into skin. Therefore, after only 4-5 hours these microorganisms can grow[10]. Fungi present in the air settle down on the finished leather and causes changes in the fat and oil contents of vegetable tanned leather[11].

The phialide production phase in *Fusarium graminearum* may also be triggered by a rise in intracellular Ca^{2+} [12,13]. During liming after soaking process, Ca^{2+} are provided to the hide and certain activities of fungi may also be regulated by the cation The fungi preffered acidic environment, fungi are usually encountered in the tannery after the pickling[14].

During the process of fat liquoring, tanned skin is treated with oils or greases or oil emulsions leave the leather very liable to mould growth in a damp atmosphere. It has been observed in the present study that the leather treated with a moderate amount of oil may form a suitable substrate for mould growth.

It was observed during tanneries and factories survey that the time before the skins are coloured may vary from three to five days. In this state the mould colonies have sufficient time to grow, especially when temperature and humidity, range above normal.

The defect appeared on the leather after colouring and drying out operations. The most unusual conditions was encountered at the finishing stage and showed that the vegetable tanned leather were very susceptible to microorganisms. This may be because of the fact that various oils i.e. turkey red oil, coconut oil, fish oil and castor oil; water soluble, vegetable tanning materials (tannins or non-tannins) and extracts of barks e.g. *Acacia Arabica* (babul) , *Cassia fistula* (amaltas), *Terminalia chebula* (mycroblans) amd *Mimosa* bark (wattle bark) are absorbed in higher quantities by the pelt fibres during vegetable tanning and fat liquoring which provide an enriched medium for fungal growth.

These vegetable tanning materials are tannin and catechin, which were degraded and utilized by fungi. The spoiled *Areca* nuts containing tannin were *Aspergillus niger, Aspergillus sp., Cladosporium herbarum*, P*aecilomyces sp; Tricoderma spp., Mucor* spp. and other unidentified fungi[15]. They also found that as a result of fungal spoilage, the tannins were degraded , the reduction in tannins result in changes of flavour and consequently reducing the overall quality[16].

Leather is a biological product and very suitable for the growth of microorganisms due to presence of protein and lipids in the form of glycerides. The proteins and fats in the hide present an ideal source of nutrients with a pH of about 4, for fungal growth[17].

In all type of leather it was also observed that grain side was very much susceptible for fungal growth than flesh side. This may be due to the incorporation of leather finishes i.e. pigments, protein binders, casein, gelatin, egg and blood albumins, waxes and mucilaginous substance on this side. All these substances serve as the best source in primary colonization of the mycoorganisms.

The chrome tanned leather are most resistant for fungal attack than the tanned ones. However, their high content of fat somewhat decreases their resistant to mould growth[18]. The present results were supported by Sharma and Sharma (1978) who recorded 14 species on chrome tanned leather[19]. Further the chrome retan and oil tanned chamois leather are usually heavily oiled and consequently difficult to wet, hence moulding were reported less rapidly.

A good growth of *A. niger* at 95 per cent R.H. on leather samples[20]. He found that vegetable tanned sole leather during drying were covered with a heavy mat of moulds and pink to deep red spots were produced by the fungus. This was reported as due to increased atmospheric humidity and delay in the drying of the leather. The leather was with wattle extract and myrobalan and oiled with admixture of groundnut and pungan oil. The fungus was isolated and identified as *p. purpurogenum*. It was also reproduceable on similar vegetable tanned leather. *A. nidulans* and *A. niger* were isolated from violet spots on E.I. kips.

The results obtained from the leather articles indicated that the maximum fungi were isolated from leather articles in comparison to those of finished leather. The maximum fungal forms were noted on gents footwear, leather covers and bags or purses, in comparison to those of other articles. This variation in number of microorganisms may depend on the material used for manufacture of footwear and environmental factors in which the footwear were used. Results showed that footwear contained species of fungi that were also recorded on tan liquor, on finished leather, atmosphere, and in soil. These fungi might have come from any of these source to the footwear but the main role is played by the contamination from air and the surroundings, particularly, in closed type of footwear and articles like shoes, R.H. and moisture of leather surface is considerably raised by the perspiration from foot skin, the temperature is also increased and these factors support the fungal growth to great extent in closed type of footwear.

Some pathogenic fungi which lodge in the leather or lining of shoes may, when suitably exposed, cause infection foot fungi, *Trichophyton interdegitale* and *T. rosaccum*, may lodge in the interstices of the leather or linings of the shoes and cause repeated reinfection of the foot.

Table-1
Fungi isolated from various finished leather in tropical chamber test
Temp. 28 ± 1^{0}C R.H. 90 ± 5%

S. No.	*Fungi*	Leather Samples									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1.	*Aspergillus niger*	+	+	+	+	+	+	+	+	+	+
2.	*A. chevalieri*	+	+	-	+	-	+	-	-	+	+
3.	*A. nidulans*	-	-	-	-	-	+	-	-	-	+
4.	*A. fumigatus*	+	+	+	+	+	+	+	+	+	+
5.	*A. conicus*	-	-	+	-	-	-	-	-	-	-
6.	*A. humicola*	-	-	-	-	-	-	+	-	+	+
7.	*A. flavus*	+	+	+	+	+	+	+	+	+	+
8.	*A. terreus*	+	-	-	+	-	+	-	+	-	-
9.	*A. repens*	-	-	-	+	-	-	-	-	-	-
10.	*A. sulphureus*	-	-	-	+	-	+	-	-	+	-
11.	*A. candidus*	-	-	-	-	-	-	+	-	+	+
12.	*A. tamari*	+	-	-	-	+	+	+	-	-	-
13.	*A. luchuensis*	-	+	+	-	+	+	+	+	+	-
14.	*A. ochraceous*	-	-	-	-	-	-	+	-	+	+
15.	*A. amstelodami*	+	+	+	+	+	+	+	+	+	+
16.	*A. sydowii*	+	+	+	+	+	+	+	+	+	+
17.	*Penicillium stipitatum*	-	-	+	-	-	-	-	-	-	+
18.	*p. camemberti*	-	-	+	-	-	-	-	-	-	-
19.	*p. purpurogenum*	+	-	-	+	+	+	+	-	+	-
20.	*p. asperum*	-	-	-	+	-	-	-	-	-	-
21.	*p. oxalicum*	+	+	+	+	+	+	+	+	+	+
22.	*p. funiculosum*	+	+	+	+	+	+	+	+	+	+
23.	*p. citrinum*	+	+	+	+	+	+	+	+	+	+
24.	*Alternaria geophila*	+	-	+	-	-	-	+	-	-	-
25.	*A. humicola*	-	-	-	-	-	+	+	-	-	-
26.	*A. alternata*	+	-	-	-	-	-	-	-	-	-
27.	*Fusarium neoceras*	+	+	+	-	+	+	+	+	+	-
28.	*F. solani*	+	-	-	-	-	-	-	-	-	-
29.	*Fusarium sp.*	-	+	-	+	-	+	-	+	-	+
30.	*Curvularia lunata*	+	-	-	+	+	-	-	-	-	-
31.	*c. pallescens*	-	+	-	-	-	-	-	+	+	-
32.	*Rhizopus nigricans*	-	-	+	-	+	+	+	-	+	-
33.	*R. oryzae*	-	-	+	-	+	-	+	-	-	-
34.	*Mucor ambiguus*	+	+	-	-	+	+	-	-	+	-
35.	*M. mucedo*	-	-	-	-	-	-	-	-	+	-
36.	*Trichoderma koningi*	-	-	-	-	-	-	+	-	+	-
37.	*T. lignorum*	-	+	+	+	-	-	+	+	+	-
38.	*Botrytis cinarea*	-	+	+	+	+	-	+	+	-	-
39.	*Botryoderma sp.*	+	-	-	-	-	+	-	-	-	-
40.	*Cunninghamella sp.*	+	-	-	-	-	+	-	-	-	-
41.	*Cladosporium herbarum*	+	-	+	+	+	+	-	-	-	+
42.	*Chaetomium globosum*	+	+	-	+	+	+	+	+	+	+
43.	*Drechslera papendorfii*	+	+	+	+	-	+	+	+	+	+
44.	*Helminthosporium sp.*	-	-	-	+	-	-	-	-	-	-
45.	*Mycelia sterila*	-	-	-	-	-	-	-	-	-	+
46.	*Paecilomyces varioti*	+	+	+	+	+	+	+	+	+	+
47.	*Torula lucifuga*	-	-	+	-	-	-	-	-	-	+
Total	**Species**	**24**	**18**	**22**	**23**	**20**	**26**	**25**	**18**	**25**	**20**

Table-2
Fungi isolated from natural deteriorated leather articles

S. No.	Fungi	Gents footwear	Ladies footwear	Leather belts	Leather cases	Leather bags(purses)	Other leather articles
1.	*Aspergillus niger*	+	+	+	+	+	+
2.	*A. chevalieri*	-	-	+	+	-	+
3.	*A. nidulans*	-	-	-	+	-	-
4.	*A. fumigatus*	+	+	+	+	+	+
5.	*A. conicus*	-	-	-	+	-	+
6.	*A. humicola*	+	+	-	-	-	-
7.	*A. flavus*	+	+	+	+	+	+
8.	*A. terreus*	+	-	-	+	+	-
9.	*A. repens*	-	-	+	-	+	-
10.	*A. sulphureus*	-	-	-	+	-	-
11.	*A. candidus*	-	-	-	-	+	+
12.	*A. tamarii*	-	-	-	+	-	-
13.	*A. luchuensis*	+	+	-	-	+	-
14.	*A. ochraceous*	-	-	-	+	-	+
15.	*A. amstelodami*	+	+	+	+	+	+
16.	*A. sydowii*	+	+	+	+	+	+
17.	*A.japonicus*	+	+	-	+	+	+
18.	*Penicillium stipitatum*	+	-	+	+	+	-
19.	*p. camemberti*	+	-	+	+	+	-
20.	*p. purpurogenum*	+	-	+	+	+	-
21.	*p. asperum*	-	-	+	-	+	-
22.	*p. oxalicum*	+	+	+	+	-	+
23.	*p. funiculosum*	+	+	+	+	+	+
24.	*p. citrinum*	+	+	+	+	-	+
25.	*p. variable*	+	+	-	+	+	-
26.	*p.expansum*	+	-	+	-	+	+
27.	*Alternaria geophila*	+	+	-	-	-	+
28.	*A. humicola*	+	-	-	-	-	-
29.	*A. alternata*	-	-	-	-	+	-
30.	*Fusarium neoceras*	+	+	+	-	-	-
31.	*F. solani*	+	-	-	-	-	-
32.	*Curvularia lunata*	+	-	-	+	-	+
33.	*C.pallescens*	+	+	-	-	-	-
34.	*Rhizopus nigricans*	+	+	+	+	+	+
35.	*R. oryzae*	+	+	-	-	+	-
36.	*Mucor ambiguus*	+	+	+	+	+	+
37.	*Trichoderma koningi*	+	-	-	+	-	-
38.	*T. lignorum*	+	+	+	+	+	+
39.	*T.harzianum*	+	-	-	-	-	-
40.	*Botrytis cinarea*	+	+	+	+	+	+
41.	*Botryoderma sp.*	+	-	+	-	-	-
42.	*Cunninghamella sp.*	-	-	+	-	-	-
43.	*Cladosporium herbarum*	+	+	+	+	-	+
44.	*Chaetomium globosum*	+	+	+	-	+	+
45.	*Drechslera papendorfii*	-	-	-	+	+	-
46.	*Helminthosporium sp.*	+	-	-	+	+	-
47.	*Torula lucifuga*	+	+	-	-	-	+
48.	*Paecilomyces varioti*	+	+	+	+	+	+
49.	*Mycelia sterile*	+	+	-	-	-	-
50.	Unidentified	+	-	-	-	-	-
	Total	38	25	25	30	27	25

+ = present, - = Absent.

Orlita (1971) made similar observations regarding the mould growth in footwear during user conditions and stated that the inner space of shoe become a tropical chamber in which there is an optimum temperature, humidity and sufficient amount of nutrients for the development of not only the leather deteriorating fungi, but parasitic skin mildew also, which are dangerous for the health of foot skin[21]. According to American statistical data 53.2 per cent of men and 15.3 per cent of women were found suffering from darmatomycosis, foot skin diseases caused by the fungi growing in shoes. Similarly, in Nigeria, athelete's foot in boot-wearing policeman were reported[22].

Thus the results obtained from these studies indicate that the finished leather, used to prepare a number of commodities of daily use are highly susceptible for fungal attack. No leather type is completely resistant. These are subjected to heavy microbial infestation during storage and user conditions. The present investigation provides impetus to develop certain preventive measures to make these articles free from infestation of microfungi under the conditions of high R.H. and optimum temperature, the knowledge of specific microflora and their relative capacity to infest leather is necessary for solving this problem.

Conclusion

It has been concluded by this study that various fungi suspended on the finished leather and find themselves in the suitable medium for their growth. The results obtained from these studies indicate that the fungi at suitable temperature and relative humidity deteriorate the stored leather samples. Subsequently, the vital activities of fungi brought about undesirable changes in the physical and chemical properties of finished leather rendering them unfit for commercial purpose. The present investigation provide impetus to develop certain preventive measures to make this leather free from infestation of fungi. The knowledge of specific fungi is necessary for solving this problem.

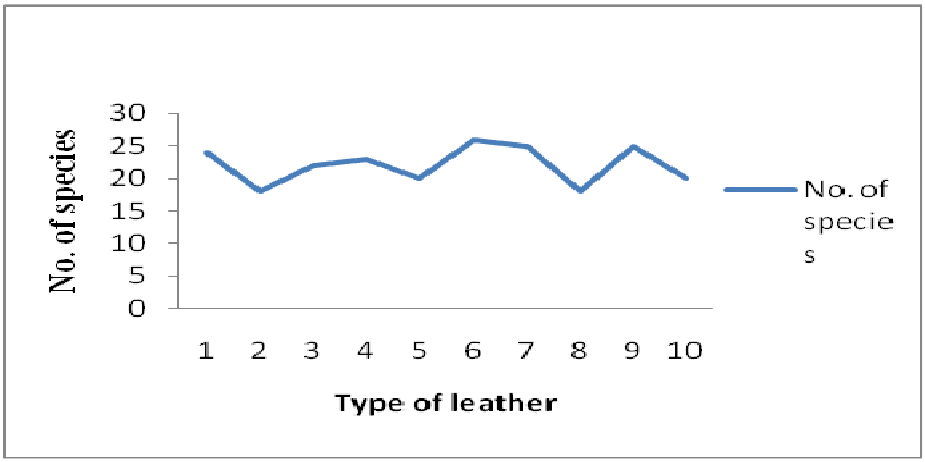

Figure-1
Fungi isolated from various finished leather in tropical chamber test

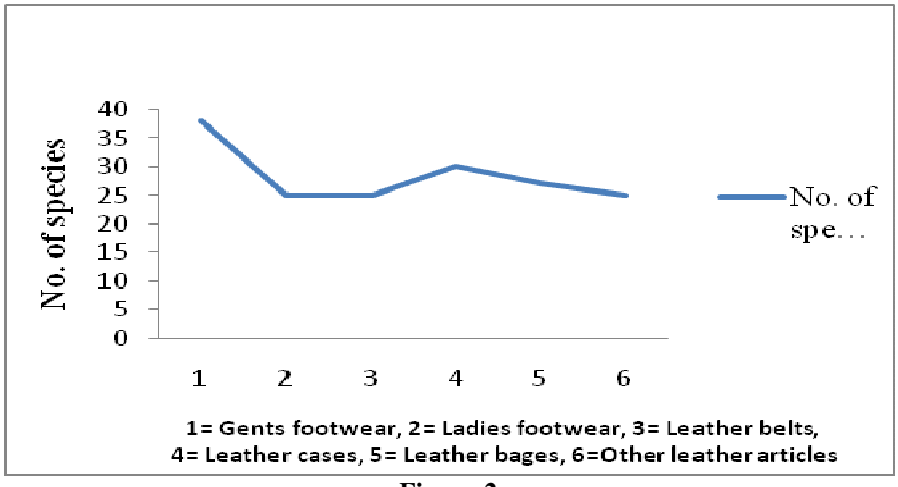

Figure-2
Fungi isolared from natural deteriorated leather articles

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