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RESEARCH ARTICLE

CHROMIUM TOXICITYAGAINST MYCOLOGICAL DETERIORATION IN CHROME TANNED COMPARED WITH VEGETABLE TANNED LEATHER ARTIFACTS

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ABSTRACT

Biological degradation affected different types of organic materials that located in museums, storages and libraries. According to their hygroscopic and sensitive properties towards the environment conditions, the microorganisms are activated resulted in different deterioration aspects on the organic materials. Leather artifacts represent most of these organic materials in museums. It is known that the tanned leather is more resistant to the bio-deterioration than untannedleather. But it was noticed thatchrome tanned leathers are more resistant than the vegetable tanned leathers when both exposed to bio-deterioration. Chromiumleathers were being used primarily in the form of leather artifacts e.g.sandals, luggage and other such items.This study aims tomake a comparison between both types of tanned leather;explain what is discriminatethe chrome-tanned leathers resistance towards microbiological deterioration according to chrome toxicity; also measuring the mechanical properties (tensile strength – elongation)of the samples under microbiological accelerated ageing cycle; and finallyaddress a surveyof most common microorganisms that infected the archaeological vegetable/chrome tanned leathers in museums.

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INTRODUCTION

It is known as a fact that collagen is the major structural component of leather which composed of coiled-coil structures; called chains. Tanning procedure can be considered as chemical processin which the covalent bonds are formed between reactive amino acids, carboxyl groups and the tanning agent. This process resulted in a stable collagen complexthat improved the leather properties of resistance towards heat, water, and biological degradation.As the stability of collagen is mainly due to inter chain hydrogen bonds, involving the occurrence of glycine. Stability also results from restricted rotation about the bonds along the polypeptide backbone, due to the high amino acids content(Valentin, 2003; Brown *et al.,* 1997b). Through the tanning treatments,the alkali makes the collagen structure opened up, accessible and permeable to tanning agents. Also this step loosens the hair follicles from the skin that allowing their removal after that. so soakingthe raw hides with sodium chloride then removal of unwanted fleshresulted in the cysteine cross-links in the hair (keratin) are hydrolyzed with alkali (generally lime), and Sodium sulphide is added as a reducing agent to prevent new disulphide bridges forming. Also the number of free carboxyl groups is increased and nitrogen is lost as ammonia and keto-amide groups in the backbone of collagen chains. This results in a swelling of the fibrils rupturing some of the covalent bonds between the protein chains. The hides are then pickled using sulphuric acid, prior to tanning with chromium sulphate (Gustavson, 1949; Mann, 1971). Certainly, raw hides containing high levels of nitrogen. So proteins are considered high quality resources for micro-organisms which capable of the degrading. Besides animal skin are highly susceptible for microbiological attack. The animal skin contains large number of microbes 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.So leather which manufactured from the animal skinin all its forms of artifacts is highly susceptible for microbiological attack resulted in aggressive deterioration aspects during leather manufacture, finishing process, storage cases and in use as archaeological covers of manuscripts or leather artifacts(Rathore, et al., 2013; 2015). This type of degradation depends on a number of factors: time, temperature, moisture content, and the state of the hide. But tanning process reduced microbial decomposition and produce more durable product especially chromium agents in chrome tanned leather. Unfortunately, the microbial decomposition of

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chromium tanned is poorly studied. This study aims to compare between the chrome tanned and vegetable tanned leather in resistance against the microbial degradation according to the toxicity of chrome and explain how chrome is characterizes with this properties by measuring the mechanical properties of the prepared samples.

MATERIALS AND METHODS

Method of Sampling and preparation of new samples: According to the survey of the different finished leather artifacts belonging to different animals, tanning with vegetable/chrome deteriorated with micro-organisms from different places on museums, storages, tannery places and libraries mentioned before, identified microorganisms were selected (Aspergillus flavus fungal contamination) and new leather samples were prepared. Samples preparations of leather were applied according to the ancient recipes and the references in this field (Reed, 1972; EL-Moselhy, 2012, 2016); then the samples were cut into specific dimensions according to mechanical properties (tensile strength – elongation) analysis requirements.

Media used: PDA medium consists of 200gm. aqueous extract of potato; 20gm. Dextrose; 20gm. Agar and 1 liter distilled water (Kamal, 1976). Sucrose (source of Carbone) was removed from the cultural of fungi was melt extract agar and autoc1aved at 121°C for 20 minutes. Media pH was 5.5 (Raper and Fennell, 1965; Barnett and Hunter, 1972; Domsch*et al*., 1980; Stevens, 1981; and Carlile*et al*., 2001).

Accelerated ageing technique used (micro ageing technique): Microbiological accelerated ageing will be used in short time period applied on the new leather samples infected by micro-organisms using spreading spores' technique (Pangallo, 2007). The plates were incubated for two months of 42˚C. The plates were observed and the infected leather samples measured by mechanical properties analysis.

Mechanical properties (tensile strength – elongation): Mechanical properties of the aged leather and blank samples were tested using tensile testing machine of model H5KT, Tinius Olsen Co. SDL-UK of capacity 5kN (1,000 lbf). In the Textile Testing Lab., Division of Chemical Metrology, National Institute for Standards, Egypt. The maximum tensile strength and elongation of control and infected leather samples were measured to assess the loss of resistance of the leather fibers due to fungal attack.

RESULTS AND DISCUSSION

Mechanical properties (tensile strength – elongation): The bio-deterioration of fungi caused clear damage on the leather surface which affects the historical manuscripts leather covers in museums. Table (1) exhibits the results of leather mechanical properties test (tensile strength and elongation) evaluation of chrome and vegetable-tanned contaminated samples. The tensile strength and elongation values for the control samples (not attacked by the fungus) were higher than those presented by contaminated samples.

The values of elongation were 60.51 and turned into 50.18 after two months of ageing cycle in the chrome tanned leather samples. On contrary, the values were 45.31 and turned to 30.15 in the vegetable tanned leather samples. Also the results of the tensile strength were 11.39 and 7.16 in the control samples after the first month of microbiological ageing cycle, but become 11.15 and 6.68 for the same samples. All the results explained in table 1. This proves that microbiological contamination in leathers leads to structural changes and loss in their physical mechanical properties.

Table 1 Tensile strength and elongation values of the microbiological infected vegetable/chrome tanned leather samples after two months of ageing cycle

Month	Microbiological aged tanned leather samples	Samples No.	Elongation (%)	Tensile strength (Maximum force (N/mm^2))
M.1	Chrome tanned leather samples	Control sample	70.74	11.39
		Aged sample	60.51	10.05
	Vegetable tanned leather samples	Control sample	55.50	7.16
		Aged sample	45.31	6.18
M.2	Chrome tanned leather samples	Control sample	68.35	11.15
		Aged sample	50.18	7.25
	Vegetable tanned leather samples	Control sample	53.48	6.68
		Aged sample	30.15	5.10

On contrary, it was noticed that Chrome tanned leather samples had higher tensile strength and elongation values compared to the vegetable-tanned leather. Which emphasizes that the chrome tanned leather is more resistant against microbiological deterioration than the vegetable tanned leather samples in losing in physical – mechanical properties. This important characteristic in chrome tanned leather is due to chrome toxicity.

Chromium toxicity: Chrome is a special element used in leather tanning as an important factor. There are different forms of chromium. The elementary, trivalent forms and the hexavalent (Tegtmeyer and Kleban, 2013). It is a necessary metal that is involved in the metabolism of glucose in every biological organism, but its hexavalent form is very toxic and carcinogenic; Hexavalent chromium is the main chromium species used in different industrial processes also chrome leather tanning (Rahmaty, 2011). It is extremely venomous and carcinogenic to humans, animals and environment (Harshita, *et al.,* 2015). Naturally chromium occurs in various oxidation states, but Cr (III) and Cr (VI) are remarkable components biologically (Cr in the hexavalent phase (Cr-Vl) is more toxic then in trivalent state (Cr-III)). Chromium in the trivalent form Cr (III) has a lower biological toxicity than Cr (VI) due to its impermeable nature through cell membranes. Cr (V) and other intermediates have a very short life time within the cellular membrane (Rutland, 1991). Chrome has been found to play a role in glucose and cholesterol metabolism in mammals and bio-organisms (Wackett*et al.,* 1989).

Under normal mammalian physiological conditions, Cr (VI), can easily taken up into cells through nonspecific ion channels, in a similar manner to phosphate and sulphate (Stearns *et al*., 1995). Although Cr (III) compounds can rarely cross the cellular membranes (Sanderson, 1982). It is noticed that Cr (V) regenerates Cr (VI) by undergoing one electron redox cycle and transferring electron oxygen. This leads to the production of one reactive oxygen species (ROS) that can easily combine with DNA protein complexes. Cr (IV) may determine the normal physiological functions by binding to cellular materials (Cheung, 2007; Manimita*et al*., 2015). In contrast, other heavy metals, including cadmium, mercury, and zinc, made the cellular toxicity by binding directly to protein sulphydryl groups (Katz and Salem, 1994). Studies by Kortenkamp*et al.* (1996) showed that chromium toxicity was due to single-strand breaks in DNA, or by modification of the purine and pyrimidine bases. However, toxicity caused by DNA-DNA cross-linking by Cr(lII) (Okada *et al*., 1983; Kortenkamp*et al*., 1996). In fungi, both Cr (Ill) and Cr (VI) are resulting in toxicity (McGrath, 1982), with the prevailing view that Cr (VI) is more toxic than Cr (Ill) (Peterson and Girling, 1981, Sharma *et al*., 1995).

Biological degradation of chrome by microorganisms: Even if the chrome tanned leathers proved its durability and resistance against the microbiological deterioration more than the vegetable tanned leathers, but these chrome tanned leathers are damaged also with continuous ageing time due to microorganisms which can be explained as the following. Unlike organic contaminants, the metals cannot be ruined, but can be converted to a stable form or removed.

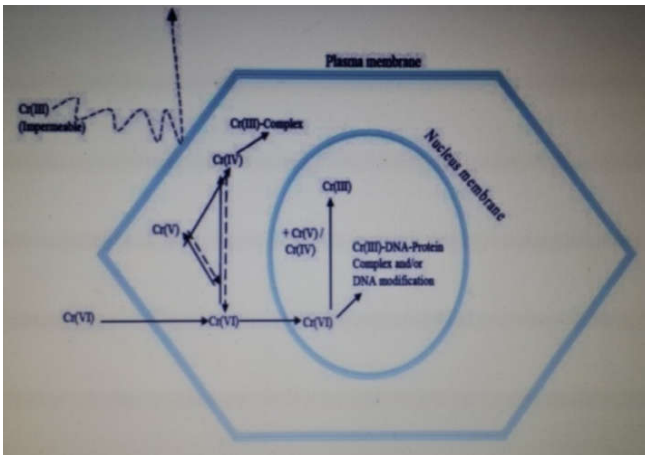

Fig.1. Schematic diagram of the toxicity of Cr (VI) (Cheng *et al.,* 2010)

This process of using microorganisms is known as biodegradation or bioremediation. The term "bioremediation" has been used to describe the process of using microorganisms to degrade or remove hazardous pollutants from the environment (Glazer and Nikaido, 1995). Also it should be noticed that the rate of microbiological degradation process of chrome depends on microbes (biomass concentration, population diversity and enzyme activities), substrate (physicochemical characteristics, molecular structure and concentration), and a range of environmental factors (pH, temperature, moisture content, availability of electron acceptors, and carbon and energy sources) (Vijayanand*et al*., 2012).

Many aerobic and anaerobic microorganisms are capable of reducing Cr (VI) to Cr (III), bioremediation may play an important role for the detoxification from Cr (VI) even at very low (ppm or ppb) level. It has already been reported that because of the presence of some enzymes called chromium reductases (Gu and Cheung, 2001), completely different microorganisms belonging particularly to the genus, Pseudomonas can reduce Cr (VI) to Cr (III). The reduction of transformation capacity of Cr (VI) by microorganisms at higher initial concentration of Cr (VI) has been observed by other researchers (Biddut, 2013; Arellano *et al*., 2004; Middleton, 2003) and the phenomenon has been explained by the presence of inhibitory effect of Cr (VI) at high concentration level (Turick and Apel, 1997). Even though Cr (VI) can be reduced by algae or plants (fungi), microorganism has been confirmed to be most effective (Ganguli and Tripathi, 2002; Francisco *et al*., 2002; Basu*et al.,* 1997).

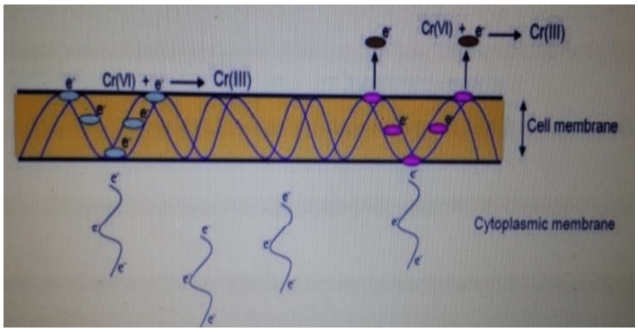

Fig. 2. Cr (VI) reduction in respiratory chain involving trans membrane protein (Myers, *et al.*, 2000).

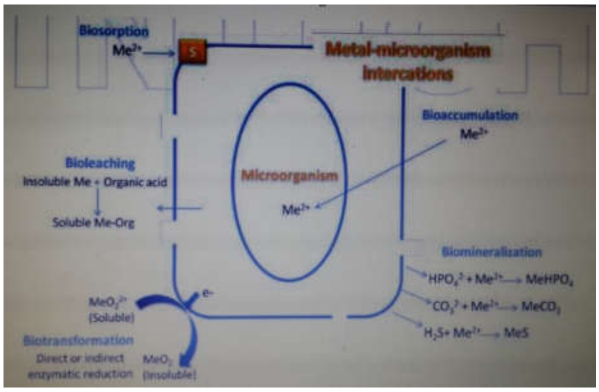

Fig. 3. Microbial processes used in bioremediation technologies modified from Lloyd and Lovley (Lloyd, and Lovley, 2001)

Survey of the most common microorganisms that infected the vegetable/chrome tanned leathers:

- Gibbons and MacDonald, 1961; Waldvogel and Swartz, 1969 found Collagenase activity by bacteria of *Clostridium spp., Bacteriodes spp.* And *Staphylococcus aereus* in the chrome tanned leather.
- Welton and Woods, 1973 reported case of an aerobically produced collagenase was from a strain of *Achromobacteriophagus* isolated from chromium leather samples.
- Pettit and Abbott, 1975 reported that the conditions of 32 ^{o}C, 80-90 % relative humidity were ideal for the proliferation of microorganisms of the leather. Microbial growth, especially fungal growth, has been observed on finished leathers after storage in warm humid conditions that increasing susceptibility of degradation.
- Datta and Chandra, 1982 isolated a single bacterial strain possessing gelatinolytic activity that was able to hydrolyse chromium tanned and vegetable tanned leathers. After incubation for 72 hours at 28-37 °C and pH 7-8, 20 % of the chromium tanned substrate had been hydrolysed.
- Aislabie and Loutit, 1986 isolated *Coryneform* bacteria from a chromium contaminated marine samples which were able to tolerate or resistance Cr (III).
- Sivaparvathi*et al.,* 1986b reported that a strain of *Pseudomonas aeruginosa* was able to hydrolyse chrome tanned leather samples after 16 hours incubation.
- Birbir and llgaz, 1996 reported that the bacterial flora of leather changed during the manufacture of finished leather from raw hide. *Bacillus spp.* were the most

prevalent, and were found in nearly all steps of the tanning process especially *Bacillus cereus* and *Bacillus subtilis* have been found with the majority of cases of deterioration of hides and skins.

- Baird, 1998 studied the short term decomposition of the autoclaved chrome tanned leather for 30 and 60-day incubations and found pH increased from 7.5 to 5 between 20 and 60 days, and a great deal of decomposition occurred also observed extensive microbial growth on leather substrates decomposed for 9 months (Fungal hyphae, actinomycetes were seen on most substrates, and *Cocci* bacteria).
- Valentín, 2003studied the biodeterioration of proteinaceous materials: which is detected by stained spots, loss in tensile strength, and hydrolysis of the proteinic compounds. Among the microorganisms that commonly growth on proteinaceous materials, Collagen can be hydrolysed by collagenase produced by bacteria such as *Clostridium*. Strains of *Bacillus, Pseudomonas, Sarcina*, *Bacillus subtilis* exhibits very high activity in hydrolyzing collagen at 95% RH approximately.
- Megharaj*et al.,* 2003 and Piñón‑Castillo *et al.,* 2010 studied species of bacteria *Pseudomonas, Bacillus and Arthrobacter* for reducing the level of chromium which depend on microbial activities. But, exposure to chromium for a long time can reduce microbial diversity and activity.
- Rai *et al.,* 2005 did a study on seasonal variation of algal growth in tannery leather samples and metal accumulation potential for Chromium removal scheme. It has been noticed that different algal species found with accumulated Chromium in their tissue, which could be used in bioremediation process for chrome degradation.
- Rathore*et al.,* 2013 studied the effects of fungi on 47 samples of vegetable/ chrome tanned leather and fungal species were recorded were *Aspergillus niger, A. flavus, A. fumigatus, A. amstelodami, A.sydowii and P.citrinum etc.*
- Rathore, 2015 studied 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). Also various types of naturally deteriorated finished leather's articles like gent's footwear, ladies footwear, belts, leather cases, and bags or purses. The fungus was isolated and identified were*p. purpurogenum, A. nidulans, Aspergillus niger, Aspergillus sp.,Cladosporiumherbarum*, P*aecilomycessp; Tricodermaspp., Mucor*spp., *Trichophyton interdegitale* and *T. rosaccum* .

According to Orlita 1968; 2004 leather is a biological product and very suitable medium for the growth of microorganism due to presence of protein and lipids in the form of glycerides (Orlita, 1968; 2004). Leather is manufactured from the raw hides of cattle and sheep in a multistep process to produce vegetable/chromium tanned leather. Chrome tanning is one of the best inventions in leather history (Tegtmeyer and Kleban, 2013). It is noticed that mold growth does not attack the hide-tannin complex itself. The components of leather which support mold growth are the lubricants, the conditioning materials and the finish (Skooglund, 1924). Fungi play an important role by means of their hyphal systems they are also able to colonize and penetrate substrates rapidly and to transport and redistribute nutrients (Joutey *et al*., 2013). Also fungi behave as bio-absorptive material to eliminate hexavalent chromium. Bio-sorption mechanism (metal sorption to cell surface by physicochemical mechanisms) is done by two methods- metabolism dependent and non-metabolism dependent. The other processes happened are bioleaching (heavy metal mobilization through the excretion of organic acids or methylation reactions), bio-mineralization (heavy metal immobilization through the formation of insoluble sulfides, etc), accumulation inside cell, and enzyme-catalyzed reactions (redox reactions).The chemicals get attach to the functional groups on the surface and get absorbed. Several fungi can be treated as a natural bio-sorbents to absorb hexavalent chromium in environment. Bio-sorption of the chromium ion Cr (VI) from the cell surface are Trichoderma fungal species.

It was noticed that the chromium binding sites on the fungal cell surface were most likely carboxyl and amine groups (Vankar, 2008; Lloyd, and Lovley, 2001). Also many bacterial species are surviving in presence of chromium for years in contaminated sites which considered important factors for removal of chromium (e.g. Bacillus coagulans, Corynebacterium, Mycobacterium, and Aeromonas) (Viti, 2001). However, some bacteria within the presence or absence of oxygen will reduce the toxic form of Cr (VI) to its trivalent form (Francisco *et al*., 2002). These are identified as chromium reducing bacteria (CRB). For example gram-positive CRB are significantly tolerant to Cr (VI) toxicity at relatively high concentration, whereas gram-negative CRB are more sensitive to Cr (VI) (Sarker*et al*., 2013; QuiIntana, 2001). Microbial reduction of chromate can occur both aerobically (Bopp, 1988) and aerobically (Komori *et al*., 1990) which can be summarized of the most common microorganisms as follows:

Table 2. Most common micro-organisms' functions in reduction of chrome

Micro-organism	Function	Reference(s)
*Pseudomonas fluorescens*LB300	Uptake of $CrO_4$2- by the strain with plasmid	Ohtake, *et al.*, 1987
Schizosacc haromycespombe	Lysine and leucine auxotrophic and heterothallic strains of this microbe were used to obtain Cr-sensitive and tolerant mutants by UV radiation-induced and nitrosoguanidine induced mutagenesis	Czakó-Vér, *et al.,* 1999
Pseudomonas ambigua G-1	Bioreduction of the Cr-concentration from 150-35mgL-1 in 36hr in liquid media	Losi, 1994
Bacillus firmus	Capable of absorbing Cr6+ efficiently into their biomass	Bennett, *et al.,* 2013
Klebsiella pneumoniae	Capable of absorbing Cr6+ efficiently into their biomass	Bennett, *et al.,* 2013
Mycobacterium sp.	Capable of absorbing Cr6+ efficiently into their biomass	Bennett, *et al.,* 2013
Bacillus cereus IST105	Absorption of chromate on the bacterial cell wall takes place through surface functional groups like carboxyl, amide, phosphoryl and hydroxyl	Naik, *et al.,* 2012
Bacillus megatarium TKW3	Hexavalent chromium reduction associated with membrane cell fraction	Cheung, 2006
Bacillus circulans	Removal of chromium by bioabsorption	Khanafari, 2008
Bacillus subtilis	Able to reduce chromate at concentrations ranging from 0.1 to 1	Garbisu, *et al.,* 1998
Bacillus methylotrophicus	Chromate reduction activity was found to be 91.3% at 48hrs	Mala, 2015

Conclusion

Historical organic materials spread in museums, when museums lacks to standers of preservation and the suitable conditions if found, historical manuscripts and other organic collections exposed to aggressive damage from different microorganisms. These manuscripts covered with historical leathers besides different leather artifacts. This study compare between both kinds of tanned leathers to explain the durability of the chrome tanned leathers against microbiological deterioration according to chrome toxicity that not found in the vegetable tanned leathers also how this damage affected the mechanical properties of the leathers through analysis of elongation and tensile strength of the samples. The results proved that chrome tanned leathers is more resistant than vegetable tanned leathers against microorganisms degradation. According to the results values of chrome tanned leather were higher in tensile strength and elongation values compared to the vegetable-tanned leather. Due to microbiological degradation in leathers which leads to structural changes and loss in the mechanical properties. Also the study explains the mechanism of microbiological degradation of chrome through a historical survey of the most common microorganisms that infected the leather artifacts in museums.

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