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Determination of Heavy Metal Toxicity of Finished Leather Solid Waste

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Abstract This paper investigates the toxicity in leather products of heavy metals known to be detrimental to the ecosystem. Heavy metal concentrations in leather samples were identified with ICP-OES, and toxicity was determined using a MetPLATE bioassay. Chromium and aluminium were found to constitute 98% of the total concentration of heavy metals in finished leather tanned with chromium and aluminium salts, while in some vegetable-tanned leather, zirconium was the only heavy metal identified. The average inhibition values for chromium, aluminium and vegetable tanned leather were 98.08%, 97.04% and 62.36%, respectively.

Keywords Heavy metals · Tanning · Leather · MetPLATE

It is reported that 90% of all global production of tanned leathers is tanned using chromium sulfates (Aslan et al. 2007). The remainder are tanned using other metal sulfates, mostly aluminium, vegetable tannins or a combination of both. However, the tanning process alone cannot provide the characteristics and quality expected of finished leather. Therefore, tanned hides are tanned a second time with either the same metal sulfate as used in the tanning process or a different one (Wachsmann 1999). Except in some special situations, a lower ratio of metal salts is used in the secondary tanning process. During subsequent coloring and finishing processes, the leathers are treated with pigments and dyes containing heavy metals (Basaran et al. 2006). The finished leathers are put through mechanical processes such as trimming before being passed on to the garment industry. These mechanical processes result in the generation of unusable solid waste. Further cutting takes place at the garment workshops, depending on the quality of the leather and the requirements of the end product. This generates additional finished leather solid waste. Furthermore, like all ingredients and materials, leather goods themselves become waste at the end of their life span.

In the event of leather being released to the environment as waste, the heavy metals within the leathers may harm the ecosystem and threaten human health by transferring indirectly into the food chain (Aslan et al. 2006). Chemical and instrumental analyses have been carried out to determine the environmental effects of various inorganic substances within the leather waste. These analyses provide the necessary information to some extent, but can not determine the effects of heavy metals on biological systems. For that purpose, the MetPLATE method was used. This recent development allows quick evaluation and shows only heavy metal toxicity (Kong et al. 1998). This test is sensitive to several metals and has been used successfully in determining the metal toxicity of waste in various industrial sectors (Stook et al. 2004). In this study, the heavy metal concentrations of some of the most commonly used types of leather, tanned using various methods, were determined using ICP-OES; The toxicity of samples was investigated using the MetPLATE bioassay.

Materials and Methods

Five different types of tanned leather were chosen and identified as groups 1–5 in this study. These were

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chromium-tanned leathers (garment, upper and vachetta leathers) in group 1, chromium and vegetable-tanned leathers (insole leathers) in group 2, aluminium-tanned leathers (garment and upper leathers) in group 3, aluminium and vegetable-tanned leathers (garment and upper leathers) in group 4 and vegetable-tanned leathers (sole leathers) in group 5. Samples used in the study were taken according to the random sampling method from finished leathers produced in the Menemen Leather Free Zone and sold to leather garment manufacturers and shoe factories. Fifty different samples were obtained consisting of ten pieces of each of the five groups, each of which had been produced with different properties depending on the type of product and field of utilization.

The test samples were cut into small pieces and these pieces were ground with a Restch SKI mill in order to make analyses according to SLC 2. The mill was thoroughly cleaned before processing each sample (SLTC 2008).

The pH of ground samples was measured according to SLC 13. Ground samples of 5 g (±0.1 g) were transferred into wide-necked flasks and 100 mL of distilled water was added. The caps were secured and the flasks were shaken by hand for about 30 s to wet the leather powder. Then the flasks were shaken continuously for 24 h in a Nüve ST 402 shaker at 20 ± 2°C, after which the pH was measured with a Mettler Toledo pH-meter (SLTC 2008).

Total protein was measured according to SLC 7. This method is the Kjeldahl determination of total nitrogen. Ground samples of 3 g (±0.001 g) were digested with 98% (m/m) concentrated sulphuric acid in the presence of copper sulfate as a catalyst. The solution was made alkaline with 35% concentrated (m/m) sodium hydroxide solution in the presence of a few drops of phenolphthalein indicator. The ammonia was steam-distilled, absorbed in a saturated solution of borate-free boric acid, and the amount was measured by titration with 0.5 N hydrochloric acid to pH 4.6. Total nitrogen content determined was multiplied by 5.62 to find total protein value (SLTC 2008).

The total amount of sulphate as was measured according to SLC 6. Ground samples of 2 g (±0.001 g) were carefully carbonized with 2 M of sulphuric acid over a low flame in porcelain crucibles. Samples were then ashed in a Protherm furnace at 750°C for 2 h. After the crucibles had been cooled in a desiccator, the ashes were measured (SLTC 2008).

Substances soluble in dichloromethane were measured according to SLC 4. Ground samples of 10 g (±0.1 g) were dried in an oven for 4 h at 102 ± 2°C and then cooled in a desiccator. Continuous extraction with dichloromethane was carried out in Velp Ser 148 Soxhlet apparatus. The extracts were dried, cooled and weighed again, and the amount of ash was calculated (SLTC 2008).

To detect the total heavy metal contents of finished leathers and to reveal whether there were any differences between amounts in these five groups, the leathers were digested according to modified EPA 3050B (EPA 2008). Ten milliliter of concentrated nitric acid was added to a 0.5 ± 0.001 g dry weight ground sample and placed in a digestion vessel and covered with a ribbed watch glass. Then, the sample was placed on the Elektro-mag hot plate and the solution was allowed to evaporate at 95 ± 5°C without boiling to ~5 mL. After this step was completed and the sample was cooled, 2 mL of distilled water and 5 mL of 30% H_2O_2 were added. The sample was covered with a ribbed watch glass and heating of the acid-peroxide digestate was continued at 95 ± 5°C without boiling until the volume had been reduced to ~5 mL. After cooling, the digestate was diluted to 100 mL with distilled water. The heavy metal contents of the sample were determined using Perkin Elmer Optima 2100 DV ICP-OES. Tests were carried out to detect the metals most used in the tanning industry, and some of those for which limits have been set by the EU (Basaran et al. 2006).

Toxicity of leathers was determined using a slightly modified MetPLATE enzymatic test. A toxicity test was then run according to Rossel et al. (1997). The six tubes used for the bioassay process were divided into two groups of three tubes each. The first group of tubes was called sample *S* and the controls were called *C* and *C**. The second group of tubes were called sample blank *B*s and the control blanks *B*c and *B*c*. The initial step of the assay was that 0.1 ± 0.001 g of ground leather was weighed and transferred to each of the test tubes *S*, *C*, *B*s and *B*c. After that, 0.9 mL of MiliQ of distilled water was added to tubes *S* and *C**, and 1.0 mL of MiliQ distilled water was added to *B*s and *B*c. In the second step of the assay, 0.1 mL of *Escherichia coli* bacteria was added to tubes *S* and *C**. The tubes were vortexed for 10 s and then incubated at 35°C while shaking for 1 h. Following a 1-h contact period, 0.5 mL of 125 ppm chlorophenol-red-β-D-galactopyranoside was added as an enzyme substrate in a 0.15 M PO_4 buffer (pH 7.0) to tubes *S*, *C**, *B*s and *B*c*, and incubated at 35°C until a reddish color developed. Tube *C** was added to tube *C* and tube *B*c* was added to *B*c; they were then vortexed for 15 s, and 2 min was allowed for contact. Next, suspensions *S*, *C*, *B*s and *B*c were centrifuged at 10,000 rpm for 5 min. One milliliter of each filtrate was pipetted on to quartz cell and absorbance at 575 nm was read using a Varian Cary 300 Bio UV–Visible spectrophotometer. Percent inhibition was estimated as follows:

$$\% \text{ Inhibition} = \left[\frac{(C - Bc) - (S - Bs)}{(C - Bc)}\right] \times 100$$

All analyses for each sample were carried out in triplicate and all reagents in this study except for

chlorophenol-red-β-D-galactopyranoside (Sigma) were obtained from Merck.

Data are presented as mean ± SD. Statistical differences between groups were analyzed using one way analysis of variance (ANOVA) followed by posthoc analysis with Bonferoni test (SPSS Ver. 11.0.0, SPSS Inc.). Linear regression analysis was performed as a measure of linear association between parameters.

Results and Discussion

Characteristics of leathers are dependent on their physical structure and chemical composition, and the mechanical operations during the manufacture of the leather (Bienkiewicz 1983). Some characteristics of leather samples are shown in Table 1.

The pH values of the finished leathers need to be within certain limits in order not to have adverse effects during use. The pH values for liquid extracts at a ratio of 1:20 should not be below 3.5 (BASF 1996). The pH values for all in this study groups varied from 4.28 to 5.46. These values are in agreement with the recommended standard. There were no statistically significant differences in pH values between groups ($p > 0.05$).

Table 1 Some characteristics of the leather samples collected

	pH	Ash (%)	Fat (%)	Protein (%)
Group 1				
Min. value	4.11	3.14	8.29	70.91
Max. value	4.46	9.85	19.24	87.57
Mean value	4.28	6.92	12.98	79.79
SD	0.18	3.44	5.64	8.38
Group 2				
Min. value	4.06	5.86	3.48	80.12
Max. value	5.50	11.09	6.78	88.74
Mean value	4.77	8.17	4.83	85.69
SD	0.72	2.67	1.73	4.83
Group 3				
Min. value	4.54	3.67	3.47	78.45
Max. value	5.31	3.92	11.65	83.45
Mean value	4.90	3.78	7.33	81.63
SD	0.39	0.13	4.10	2.76
Group 4				
Min. value	5.21	3.14	12.35	76.89
Max. value	5.62	4.45	19.26	81.68
Mean value	5.46	3.71	15.54*	79.54
SD	0.22	0.67	3.49	2.44
Group 5				
Min. value	5.12	1.54	5.31	80.32
Max. value	5.45	2.75	5.99	88.10
Mean value	5.26	1.95*	5.63	84.85
SD	0.17	1.08	0.32	4.04

* $p < 0.05$, when compared to group 2

Total ash is an indicator of the inorganic content of leathers, including the inorganic materials used during the production stages. Mineral substances can be found in the natural constitution of raw hide at a rate of 0.5% (BASF 1996). The concentration of ash in the leathers in this study varied from a low of 1.95% in the vegetable-tanned group to a high of 8.17% in the chromium + vegetable tanned group and there was a significant difference between these groups ($p < 0.05$). Cantera et al. (1994) and Taylor et al. (1997) found 6.7% and 8.5% total ash values, respectively in chromium-tanned leathers. In our study, it was seen that the values of group 1 resembled those obtained in the above studies. Rajamaran et al. (1978) stated that chromium-vegetable tanned leathers have 6.45% total ash content. The amount of ash for group 2 leathers were found to be higher than in the other research mentioned. According to Mahdi et al. (2008), ash content of aluminum-tanned leathers varied from 2% to 6%. In group 3, ash contents of the leathers were found match the study of Mahdi et al. (2008). Slabbert (1981) reported 2.4% ash value in leathers tanned with aluminum-mimosa. In this research, the amount of ash in group 4 was higher than the values of aluminum-mimosa tanning study. Group 5 ash results (1.95%) are comparable with those previously reported by Rajamaran et al. (1978) for vegetable-tanned leathers (7.42%), where a lower value was detected.

Leathers undergo different techniques of fat-liquoring according to the required softness of the finished leathers and depending on the intended place and purpose of use. The fat content of the samples ranged from 4.83% in group 2% to 15.54% in group 4, showing a significant difference between these groups ($p < 0.05$). According to the results obtained, the fat contents of the leather samples also agreed with aforementioned previous reports. UNIDO (1996) recommended that a 4%–10% fat content was enough for chromium-tanned garment leathers. The fat contents of leathers may differ depending on the type and amount of tanning and the type of raw hides used in processing, and these can vary by 4%–10% (Sharphause 1989; BASF 1996; Bitlisli et al. 2004).

Proteins in leather consist of non-fibrillar and fibrillar proteins. Non-fibrillar proteins are removed during the treatment of the hide, leaving the fibrillar proteins, which are known as hide substance. The protein content of the groups ranged from 79.54% in group 4% to 85.69% in group 2. There were no statistically significant differences in protein content between the groups ($p > 0.05$). These values are comparable with those reported by other authors. Nitrogenous organic protein accounts for ~80% of the dry weight of the raw hide (Harkness 1971; BASF 1996).

Cantera et al. (1994) reported 87.60% hide substances value in chromium-tanned leather wastes. In addition, Taylor et al. (1997) emphasized that chromium tanned leather wastes have a proportion of 77.60%–80.44% hide substances. The levels of hide substances of leathers as presented in this study were similar to those found in previous researches.

As presented in Table 2, the total metal concentrations of 11 metals in the various groups were determined in varying concentrations in the range of 0.00–2.71 ppm for Sb, 0.00–0.13 ppm for As, 0.00–0.45 ppm for Cd, 36.61–36000.46 ppm for Cr, 0.00–48.14 for ppm Cu, 0.00–14.37 ppm for Pb, 0.00–0.01 ppm for Hg, 0.00–2.59 ppm for Ni, 0.00–21033.45 ppm for Al, 0.00–70.54 ppm for Zn and 0.00–2667.23 ppm for Zr. The concentrations of some heavy metals in our study were parallel to those determined by Basaran et al. (2006) in his report (0.34–0.55 ppm for Cd, 10,160–19,201 ppm for Cr, 35.37–79.46 ppm for Cu, 4.19–14.42 for Pb, 2.26–3.27 for Ni and 4.19–27.35 for Zn).

Chromium, aluminium and zirconium were found to have the highest concentrations compared to the other metals for groups 1, 3 and 5, exhibiting almost 98%, 98% and 100%, respectively of the total metal contents analyzed. These high chromium and aluminium concentrations are due to main tanning modern process, which is carried out predominantly with basic chrome sulfates or aluminium sulfates (Thorstensen 1983). Although there are no inorganic substances in the vegetable-based tanning process, these leathers may be treated with zirconium sulfate in order to make the leather firmer, make tanning easier and to lighten the characteristic color which the vegetable tannins give the hide (Bienkiewicz 1983). Therefore, due to the fact that they do not undergo any coloring process, the

Table 2 Heavy metal content and percent inhibition of groups

	Heavy metals (ppm)										
	Sb	As	Cd	Cr	Cu	Pb	Hg	Ni	Al	Zn	Zr
Group 1											
Min. value	0.00	0.08	0.23	28000.33	6.89	0.20	0.00	2.25	10.11	27.35	0.00
Max. value	5.12	0.20	0.61	44000.41	35.21	11.06	0.02	3.27	1024.54	28.06	541.11
Mean value	2.71	0.13**	0.41##	36000.46***	18.11	5.15	0.01	2.59$	352.55	27.70	180.37
SD	2.57	0.06	0.19	8000.04	15.04	5.49	0.01	0.59	581.99	0.50	312.41
Group 2											
Min. value	1.89	0.00	0.38	14040.41	12.52	4.07	0.00	0.25	12.5	5.11	0.00
Max. value	3.47	0.00	0.55	18110.21	80.27	18.28	0.00	2.15	486.32	30.35	0.00
Mean value	2.46	0.00	0.45##	16000.23*	48.14	11.15	0.00	1.09	181.37	14.82	0.00
SD	0.88	0.00	0.90	1999.89	34.01	7.08	0.00	0.97	264.60	13.59	0.00
Group 3											
Min. value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17671.28	23.11	0.00
Max. value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25317.23	102.56	974.13
Mean value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	21033.45***	70.54£	461.52
SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3905.40	41.97	488.99
Group 4											
Min. value	0.00	0.00	0.11	21.04	18.56	8.25	0.00	0.21	6651.89	6.71	0.00
Max. value	0.00	0.00	0.30	51.23	50.01	20.35	0.00	1.02	9112.34	23.87	2317.26
Mean value	0.00	0.00	0.20	36.61	30.07	14.37#	0.00	0.57	8215.79**	14.66	772.42
SD	0.00	0.00	0.09	15.12	17.34	6.05	0.00	0.41	1359.22	8.65	1337.87
Group 5											
Min. value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Max. value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4334.46
Mean value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2667.23
SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3357.82

* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ when compared to other groups

# $p < 0.05$ and ## $p < 0.01$ when compared to groups 3 and 5

$ $p < 0.05$ when compared to groups 3, 4 and 5

£ $p < 0.05$ when compared to group 5

only heavy metal identified in the constitution of vegetable-tanned sole leathers is Zr (Wachsmann 1999). However, Sb, As, Cd, Cu, Pb, Hg, Ni and Zn, which are definitely not used during the treatment of hides, have also been identified in the constitution of the leathers. These metals originate from leather dyes, pigments, pesticides, or contaminated equipment used in the tanneries during the treatment of the hides. However, the concentrations of these elements are negligible compare with those of the heavy metals Cr, Al and Zr. The variations in heavy metal concentrations within and between groups relate to the intensity of the tanning and coloring processes. Furthermore, heavy metal levels are also affected by the dye and pigment mixtures used according to the desired color of the leathers (Sharphause 1989).

Table 3 shows the heavy metal toxicity of the five groups under study, according to the MetPLATE assay. The percent inhibition of β-galactosidase enzyme varied from 62.36% to 98.08%. Based upon the percent inhibition, all leather samples appear to display high-level heavy-metal toxicity.

Table 3 Total heavy metal content and toxicity in the leather samples collected

	Total heavy metal content (ppm)	Toxicity (%)
Group 1		
Min. value		94.65
Max. value		99.65
Mean value	36580.96*	98.08
SD	7804.80	1.50
Group 2		
Min. value		87.90
Max. value		96.05
Mean value	12259.71	93.99
SD	1778.63	4.44
Group 3		
Min. value		96.39
Max. value		98.09
Mean value	21565.51	97.04
SD	3743.59	0.63
Group 4		
Min. value		79.88
Max. value		98.78
Mean value	9084.69**	92.26
SD	2401.59	10.73
Group 5		
Min. value		20.55
Max. value		86.03
Mean value	1111.49***	62.36
SD	1925.15	31.11

* $p < 0.05$ when compared to other groups

** $p < 0.05$ and *** $p < 0.01$ when compared to groups 1 and 3

A positive correlation was identified between the total heavy metal concentration of the leathers in the groups and their resulting toxicity ($r^2 = 0.71$, $p < 0.01$). The total heavy metal concentration and toxicity of the groups were identified as 36590.19 ppm/98.08% in group 1; 16259.26 ppm/93.99% in group 2; 21565.52 ppm/97.04% in group 3; 9084.69 ppm/92.26% in group 4 and; 2667.23 ppm/62.36% in group 5. In addition to this, high correlations were found between toxicity in the first and second groups and Cr ($r^2 = 0.99$ and 0.92, respectively, $p < 0.01$), toxicity in the third and fourth groups and Al ($r^2 = 0.99$ and 0.92, respectively, $p < 0.01$), and finally between toxicity in the fourth group and Zr ($r^2 = 0.99$, $p < 0.01$).

Considering the fact that every year million tons of leather goods are discarded and the leather industries generate finished leather solid waste, and that their disposal contributes tons of toxic metals to the environment. This points to the need for recycling instead of disposing to landfill. Also, leather process recipes should be revised to reduce the heavy metal content of leather in order to produce ecological leather products.

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