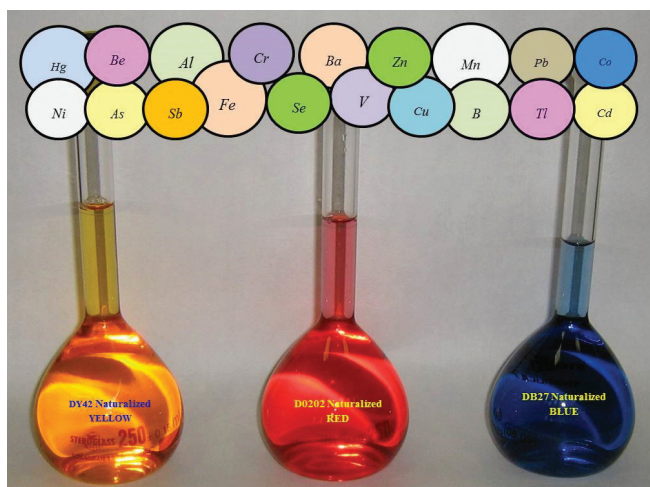


LACTOSE CUTS DOWN HEAVY METAL CONTAMINATION FROM COMMERCIAL DYES

Inductively Coupled Plasma Optical Emission and a Direct Analyzer were used to detect metals in dyes of the Life+ Bionad project. Blue Teratop GLF and Foron Brown Yellow showed a high amount of Hg. Chromophores in the wet press-cake form had low levels of metals that did not increase, when lactose was employed to obtain the corresponding naturalized dyes.



Pollution of water and soil by metals is an issue of great concern, because metals can interfere with the physiology of the ecosystems, animals and plants, impacting eventually on the environment and human health [1]. Metals occur in the Earth's geological structures and can therefore enter water resources through natural processes, but many human activities can generate metal pollution, as well as some industries (*e.g.*, those of the mining and extraction sectors) are likely to pollute more than others (*e.g.*, the food industry). Metal based colorants are well established in the dyeing industry, to add value to textiles, leather, wood or paper [2] and usually, the metal content is only a fraction of the whole formulation. However, undesired contamination by heavy metals may occur as a result of the poor quality of the dye or additives used to prepare blended products, even those supposed to be metal free. Despite some elements (*e.g.*, As, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se, Sn, V, Zn) are essential for metabolic processes at trace level [3, 4], some of these (*e.g.*, Cd, Cr, Hg, Pb) are very toxic and their presence in the finished goods and dyeing wastewaters is highly undesirable [5, 6].

In the recent decade naturalized dyes have emerged as a new class of water soluble colorants, based on the chemical bond between a chromophore and a sugar, preferentially lactose [7, 8]. They can be regarded as glyconjugated chromophores, placing the organic dye in an apparently more inert position than the anomeric one of the sugar (*e.g.*, the 6' position of lactose) for higher stability of the whole structure. The lactose derived colorants showed their potential to dye materials of different nature [9] becoming also the subject of the EU funded project Life+12 ENV/IT/000352 under the acronym "BioNaD" (www.lifebionad.com) to demonstrate their capacity to enhance the quality features of leather [10]. In addition, the compliance of their synthetic process to the European regulation of REACH (Registration, Evaluation, Authorization and Restrictions of Chemicals) makes them attractive for market development. In this paper, we report the results about the determination of metals in some commercial and naturalized dyes of the "BioNaD" project, expecting the products under scrutiny to possess low levels of metal contamination *a priori*. Quantitative analysis was carried out using the Inductively Coupled Plasma Optical Emission (ICP-OES) technique for all metals, except for mercury, which was analyzed *via* a Direct Analysis method.

Material and Methods

Sample preparation and metal element determination

The reagents employed were of analytical grade (TraceSELECT®, Fluka) and were used without purification. Deionized water was obtained from an ion resin exchange process, using the Purelab Classic UV (ELGA) system. All dyes were digested prior the quantification of metals, except Hg. Digestion was carried out on 0.2 g of dye, kept in a mixture of 69% HNO₃ (6 mL) and 30% H₂O₂ (2 mL) in a loosely capped PTFE pressurized bomb at 20 °C for 60 min. Then, the bomb was tightly closed, placed in a microwave (Ethos, Milestone, Italy), heated up to 200 °C within 10 min., kept at 200 °C for 20 min. and cooled to 20 °C. The digest was transferred in volumetric flask and diluted up to 50 mL with deionized water. Blank samples were run in parallel for the quantification of con-



tamination, due to the reagents used. Quantification of metals was performed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) using an Optima 8000 (Perkin Elmer) spectrometer, which was calibrated with a multi element standard solution (Multielement Standard solution Fluka 4 for ICP - TraceCERT®) plus Sb (Antimony Atomic Absorption Standard Fluka) (5 mg/L); Y (2 mg/L) was employed as internal standard for monitoring the plasma stability.

Results were reported in mg/kg (part per million) and they were compared to the threshold values of the analytical methods, *i.e.* the Limit Of Quantification of the metal (LOQ_m) according to:

$$LOQ_m = (LOQ_s \times V) / m \quad (1)$$

where, V is the volume of digestion, m is the mass of the solid dye involved in the analysis and LOQ_s is the Limit of Quantification in the digested solution, which could be calculated from the specific Limit of Detection (LOD) parameter of each element, given by the manufacturer ($LOQ_s = LOD_s \times 10/3$).

Determination of mercury was carried out in a direct mode on 0.2 g of dye sample without any pretreatment step, using a Direct Mercury Analyzer (DMA 80, Milestone, Italy). Calibration of the instrument was carried out with the internal calibration curve of the instrument, which is periodically checked with a Hg standard solution (Mercury Atomic Absorption Standard - Fluka). Absolute LOD of the DMA80 is better than 5 pg of Hg, giving a $LOQ_m = 8.5 \times 10^{-5}$ mg/kg of mercury, using a 0.2 g of solid dye sample.

The precision on concentrations reported in Tab. 1 and 2 are typically better than 3% for mercury (RSD on five replicates) and 5% for the other elements (RSD on three replicates).

Commercial and naturalized dyes

Commercial disperse dyes Teratop Brown GWL, Foron Brown Yellow S-2RFL 150 and Foron Brilliant Red S-RGL 200 were purchased from Biokimica SpA (S. Croce sull'Arno, Pisa, Italy). The single chromophore species contained in those products was purchased in the form of crude wet press-cake material from Cromatos Srl (Forlì, Italy) in the absence

Element	Commercial Dyes				Crude Chromophores			LOQ_m (mg/kg)
	Teratop Brown GWL (mg/kg)	Foron Brown Yellow S-2RFL150 (mg/kg)	Foron Brilliant Red S-RGL200 (mg/kg)	Teratop Blue GLF (mg/kg)	Disperse Yellow 42 (mg/kg)	Disperse Orange 30 (mg/kg)	Disperse Red 202 (mg/kg)	
Al	32.7	49.8	47.6	26.1	64.3	-	-	0.83
As	8.1	- ^a	7.5	-	0.94	-	-	0.83
B	-	-	-	64.1	3.6	-	29.0	0.83
Ba	0.84	0.63	4.8	3.0	1.7	-	-	0.02
Be	-	-	-	-	-	0.30	-	0.07
Cd	0.10	0.13	-	-	0.15	-	-	0.08
Co	-	-	-	-	-	-	-	0.17
Cr	-	3.8	3.3	2.8	0.59	-	0.60	0.17
Cu	-	-	-	11.4	0.40	-	1.4	0.33
Fe	21.5	49.9	44.5	112.4	29.2	1.8	33.7	0.08
Hg	0.0070	0.46	0.0017	0.66	0.060	0.010	0.090	8.5×10^{-5}
Mn	11.2	18.3	12.9	59.1	0.91	0.25	0.34	0.08
Ni	-	2.4	2.4	2.1	0.42	-	-	0.42
Pb	-	-	-	3.5	2.0	1.5	1.3	0.83
Sb	2.1	1.9	-	-	-	-	-	1.70
Se	3.9	2.2	2.0	-	2.4	-	-	1.70
Tl	-	-	-	-	-	-	-	1.70
V	9.8	14.3	22.1	1.8	0.73	1.1	-	0.42
Zn	2.9	-	-	10.6	4.6	0.62	1.6	0.17
Total ^b	97.7	148.7	153.0	303.8	115.6	14.4	76.0	[10.4] ^c

^aConcentrations that are not reported are below LOQ_m
^bSum of concentrations includes LOD_m whenever the determined values cannot be quantified
^cSum of LOQ_m

Tab. 1 - Comparison of the concentrations of selected trace elements in commercial dyes and crude chromophores

Element	Purified Chromophores				Crude Chromophores			LOQ _m	(mg/kg)
	Disperse Yellow 42 (mg/kg)	Disperse Orange 30 (mg/kg)	Disperse Red 202 (mg/kg)	Disperse Blue 27 (mg/kg)	DY42 Nat (mg/kg)	D030 Nat (mg/kg)	DR202 Nat (mg/kg)	DB27 Nat (mg/kg)	
Al	-	-	-	-	-	0.90	6.6	-	0.83
As	8.2	-	3.9	-	-	-	-	-	0.83
B	-	-	-	-	-	-	-	-	0.83
Ba	0.37	-	-	-	-	0.03	0.17	0.19	0.02
Be	-	-	-	-	0.31	0.29	0.27	-	0.07
Cd	0.13	-	-	-	-	-	-	-	0.08
Co	-	-	-	-	-	-	-	-	0.17
Cr	-	-	-	1.1	0.85	0.45	4.5	2.1	0.17
Cu	-	-	-	1.0	-	-	-	-	0.33
Fe	-	-	-	2.0	1.7	1.0	49.7	2.1	0.08
Hg	0.00015	0.0012	0.0001	0.0030	0.010	0.020	0.010	0.020	8.5x10 ⁻⁵
Mn	-	-	-	-	-	0.19	0.57	0.25	0.08
Ni	-	-	-	0.50	-	-	2.1	1.2	0.42
Pb	-	-	-	3.7	2.2	-	-	-	0.83
Sb	3.7	1.7	-	-	-	-	-	-	1.70
Se	6.8	-	-	-	-	-	-	-	1.70
Tl	-	-	-	-	-	-	-	-	1.70
V	-	-	-	-	1.2	1.3	1.2	1.0	0.42
Zn	-	-	-	1.7	17.7	2.8	6.4	15.5	0.17
Total ^b	25.3	10.4	12.6	17.6	31.9	15.6	79.7	31.4	[10.4] ^c

^aConcentrations that are not reported are below LOQ_m.
^bSum of concentrations includes LOD_m whenever the determined values cannot be quantified
^cSum of LOQ_m

Tab. 2 - Comparison of the concentrations of selected trace elements in different classes of dyes

of any additive for commercial formulation, as follows: Disperse Yellow 42 (C.I.10338, CAS [5124-25-4]) from Teratop Brown GWL, Disperse Orange 30 (C.I.11119, CAS [12223-23-3]) from Foron Brown Yellow S-2RFL 150 and Disperse Red 202 (C.I. 113373, CAS [6371-23-9]) from Foron Brilliant Red S-RGL 200. The naturalized dyes DY42nat, D030-nat and DR202-nat were synthesized from the crude press-cake chromophores, according to the naturalization process reported elsewhere [9]. The synthesis of the blue dye DB27-nat was accomplished only after the isolation of chromophore Disperse Blue 27 (C.I.60767, CAS [15791-78-3]) from commercial Teratop Blue GLF purchased from Chimica Tessile (Prato, Italy) through extraction with a Soxhlet apparatus and dichloromethane [11]. This purification procedure was also applied to Teratop Brown GWL, Foron Brown Yellow S-2RFL 150 and Foron Brilliant Red S-RGL 200, to isolate the chromophore and compare the metal content analyses with those obtained from the wet crude press-cake materials.

Results and discussion

The concentrations of metal elements for commercial disperse dyes and crude chromophores were reported in Tab. 1 and for purified chromophores from commercial dyes and naturalized dyes in Tab. 2. In many

cases the concentration of the metal could not be quantified, because it was below the limit of quantification, LOD_m. Therefore the total amount of metal content could be calculated taking into account the determined concentrations (for values \geq LOD_m) whereas for undetermined concentrations (values $<$ LOD_m) the values were taken equal to LOD_m. This was necessary in order to take into account the limit case in which the concentration values were below LOD_m and thus, the total concentration could be any value below the sum of individual LOD_m [\sum (LOD_m) = 10.4 mg/kg, last column of Tab. 1 and 2] for each of the nineteen elements. It arises that the total concentration of trace element for each of the dyes and chromophores could not be less than 10.4 mg/kg. Comparison of total element concentration is reported in Fig. 1.

The analysis of collected data highlighted that the total trace element content decreased in the order commercial dyes > crude chromophores \geq naturalized dyes > purified chromophores (from commercial dyes). The higher level of trace elements in commercial dyes could be associated to the poor quality of the chemical additives used to formulate the commercial products. This finding emphasized the relevance of the new naturalized dyes developed from lactose, since these new chemical species are unique and with their own features, possessing low level of



contaminants. Examining the concentration of the single element, other consideration could be drawn up. Co and Tl were not quantified (below LOQm), in any of the samples whereas eight elements (As, B, Cd, Co, Cu, Sb, Se and Tl) could not be quantified in naturalized dyes. Al and Fe were the most abundant elements in the commercial dyes, except for the pure Disperse Orange 30 (Tab. 1).

The contribution of Al and Fe to the total metal content was about 60% on average, indicating that contamination may be due to the release of the metals from the production plants. In the case of Blue Teratop GLF metal contamination was even more extensive, since relevant amounts of B and Mn were determined. We were rather surprised to find unexpected high levels of mercury in Blue Teratop GLF (0.66 mg/kg) and Foron Brown Yellow (0.46 mg/kg), that may represent an issue of great concerns for dyers, both for the quality of the finished products and the dyeing effluents. For instance, according to the Italian law, the threshold limit for mercury is 1 ppb in water for industrial and human purposes. In particular, this aspect would alert the leather industry, since accumulation of Hg could become a growing issue: both for the quality of the finished products affecting their trading and the dyeing baths, since these use a higher concentration of dyes than those of the textile industry. The high level of contamination for the Blue Teratop GLF may well attributed to the poor quality of the chemical additives used to formulate the commercial product, as the amount of the anthraquinone chromophore Disperse Blue 27 is just about 12% by weight of the commercial product. In the case of the purified chromophores (Tab. 2 and Fig. 1) the total trace element content was considerably reduced: especially for dyes Disperse Orange 30 and Disperse Red 202, that could be considered almost metal free. The total trace element content in the naturalized products (Tab. 2 and Fig. 1) was higher with respect to the purified chromophores. In particular, relatively high contents of Zn and Fe were found, compared to those of the purified chromophores and it is probably due to metal contamination during their synthesis. However, the metal content was by far significantly lower than that found in the commercial products.

Conclusions

The quality of the dyes employed in the LIFE+ ENV/IT/00352 "Bionad" project of the European Union, was satisfactory on average, although significant differences were found among the different classes of dyes. The total trace element content decreased in the order commercial dyes>crude chromophores>naturalized dyes>purified chromophores (from commercial dyes). In the case of commercial Blue Teratop GLF and Foron Brown Yellow S-2RFL150, we found a significant concentration of Hg (0.66 and 0.46 mg/kg) that may represent an issue in the leather dyeing process. The purification of the commercial dyes by simple solvent extraction demonstrated the effectiveness of the method to furnish almost metal free chromophore. The trace element content in the naturalized products confirmed that quite often metal contamination is due to the production process and relatively high level of Zn, Fe and Al may focus the attention on the quality of the equipment used. The evidence reported in this work indicates that the insertion of lactose into the synthesis of new chromophoric species allows the creation of a new class of dyes with much lower metal content with respect to commercial dyes and thus, with a high purity profile. This makes the naturalized dyes interesting for future applications devoted toward the improvement of the eco

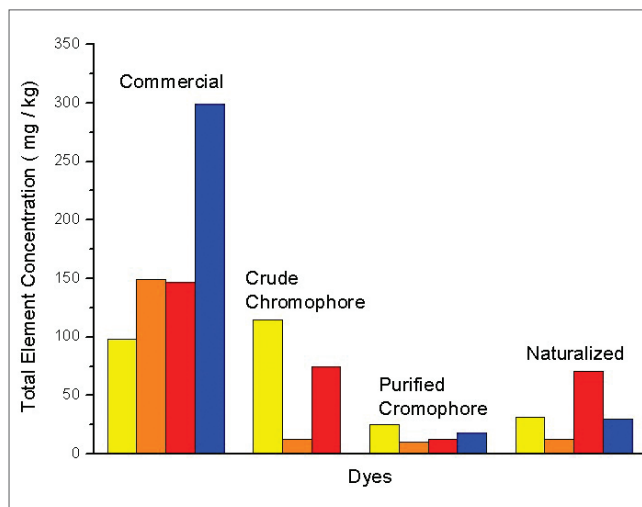


Fig. 1 - Comparison of total element concentrations for the investigated of yellow, orange, red and blue dyes and chromophores

sustainability of the dyeing process both for leather and textile materials, in a view to remove chemical additives employed in large amount to formulate commercial disperse dyes.

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Il lattosio abbate la contaminazione da metalli pesanti nei coloranti commerciali

La spettroscopia ottica ad emissione per induzione al plasma e un analizzatore diretto sono stati usati per determinare i metalli in coloranti del progetto Life+ Bionad. Il Blue Teratop GLF e il Foron Brown Yellow hanno presentato alti livelli di Hg. I cromofori crudi hanno mostrato bassi livelli di metalli, rimasti tali quando il lattosio è stato impiegato per ottenere i coloranti naturalizzati.