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Heavy Metals in Tattoo Inks: Developing an Analytical Methodology for Accessing Customer Safety

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Tattoo inks contains metal salts or different types of coloured organic molecules. To the best of our knowledge, there are few data on the concentration of hazardous metals in tattoo inks sold online or by makeshift hawkers. The aim of this work was to organize an analytical methodology to ensure the simultaneous quantitative determination of 18 elements in a complex matrix, like inks for tattooing, by Inductively Coupled Plasma Mass Spectrometry. The total concentrations of metals in the 16 analysed tattoo ink samples ranged from 0.060 to 16.9 g kg^{-1}. Zinc is the most present metal in the samples, in fact it is in the range 3.4–13882 mg kg^{-1}. In three of the 16 samples the zinc concentrations exceed those required by legislation (2000 μg g^{-1}). Cr concentration in all cases is higher than allowed (0.5 μg g^{-1}). The weight loss by heating the ink samples to 105 °C and, subsequently, to 550 °C showed that in all cases the volatile component (ethanol, solvents, etc.) varies from 48 (Wh1) to 95 %, while the percentages of the residue at 550 °C ranged from 0 % (BK0) to 47 % (Wh1). Considering values limit, four of analysed samples should not be used by tattoo artists.

Introduction

Tattooing practices are as old as humans. Tattoos are a widespread form of body art to which at least 12 % of the European population has undergone.[1] In the 18 to 35 age group, the likelihood of getting a tattoo is double that of the rest of the population. Tattoos are applied to the skin by injecting coloured inks containing pigments to obtain a permanent design and are meant to remain for life. Generally, tattoo inks contain pigments consisting of inorganic metal salts or different types of coloured organic molecules.[1,2] People, every day, are exposed to the hazardous substances (metals, PAHs, phthalates, pesticides, etc.) through a wide range of routes.[3–6] Although atmospheric emissions tend to be the greatest cause for concern in terms of human exposure and health,[7] however, other less obvious sources of exposure[8,9] must be considered, including the use of cosmetic, pharmaceutical and tattoo products. In the last half century, numerous cases of malignant melanomas[10–14] attributable to the presence of tattoos in the human body have been highlighted while, in other cases of rare types of skin cancers it was not possible to associate the pathology with tattoos.

Metals and their compounds, which can be classified as ink contaminants or the pigments themselves, have been associated with cutaneous pathologies.[1,15–17] In the past, red tattoo inks, containing mercury and those yellow, containing cadmium were considered to be the most hazardous.[1]

Until now, the international legislation concerning the products used for the execution of tattoos is quite lacking and often difficult to interpret. Council of Europe Resolution (2008)[18] take account a list of 13 elements that should not exceed recommended concentrations as impurities in tattoos (European Commission (EC), 2008a).

The Commission Regulation n° 2020/2081 of the European Union of 14 December 2020[19] modified Regulation no. 1907/2006 of the European Parliament and of Council regarding the registration, evaluation, authorization and restriction of chemicals (REACH)[20] relating to substances contained in tattoo inks or in the permanent makeup, limiting the use or percentages of many heavy metals and hazardous chemicals. In relation to the most recent legislation, Table 1 shows the maximum concentrations of certain metals allowed in tattoo inks.

The control concerning the inks used in the tattoo operations are very difficult because most of the products are purchased online, often from small companies located in areas where the legislation is more permissive than the European

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Table 1. - Maximum Allowed Concentrations of Metals and Metalloids recommended by the Council of Europe and the European Union.[19,20]

Maximum Allowed Conc. ($\mu g\,g^{-1}$)	As	Ba	Cd	Co	Cr	Cu	Hg
ResAP (2008)1 MAC	2	50	0.2	25	0.2	25[a]	0.2
Commission Regulation 2020/2081 MAC	0.5	500[a]	0.5	0.5	0.5	250[a]	0.5
Maximum Allowed Conc. ($\mu g\,g^{-1}$)	Ni	Pb	Sb	Sn	Sr	V	Zn
ResAP (2008)1 MAC	[b]	2	2	50	34	0.3	50
Commission Regulation 2020/2081 MAC	5	0.7	0.5	0.5[c]	1.8	3.5	2000[a]

[a] soluble; [b] as low as technically achievable, also, the presence of trace amounts of nickel in tattoo products should be indicated on the packaging along with a warning (for example, *Contains nickel. May cause allergic reactions*);; [c] organometallic.

one. To the best of our knowledge, there are a small number of data on the concentration of hazardous metals in tattoo inks,[1,21–23] in particular for those sold online or by makeshift hawkers.

In the literature Kisza[1] noted a discrepancy between metals and metalloids associated with ink hues and their actual concentrations reported by recent studies.

By semi-quantitative Inductively Coupled Plasma Mass Spectrometry and other analytical techniques, Battistini[21] simultaneously determine both 18 metal content and the size of nanoparticles in twenty inks of different brands and colours sold in Italy and in United States in 2019.

Manso[22] in 2019 used synchrotron radiation X-ray fluorescence spectroscopy and atomic absorption spectroscopy to quantify Cr, Ni, Cu, Cd, Hg and Pb in tattoo inks samples. The concentrations were above the maximum allowed by the Council of Europe through the resolution Res AP (2008).[18]

The aim of our work was to organize an analytical method to ensure the simultaneous quantitative determination of 18 elements (As, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Mo, Pb, Sb, Sn, Se, Sr, V and Zn) in a complex matrix, like inks for tattooing, by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Results and Discussion

The weight loss by heating the ink samples to 105 °C and, subsequently, to 550 °C showed that in all cases the volatile component (ethanol, solvents, etc.) varies from 48 (Wh1) to 95 %, while the percentages of the residue at 550 °C ranged from 0 % (BK0) to 47 % (Wh1). It is important to consider that the residues of all analyzed samples consisted of white solids, except for Blba ink (bleu). This leads to the conclusion that the colored component of the investigates inks, except for white ink Wh1, consists of organic colorants. This conclusion is in good agreement to the observation of Kisza[1] that evidences the industrial replacement of inorganic pigments with organic and organometallic colorants in the manufacture of tattoo inks. However, whether originating as pigments or contaminants, hazardous elements continue to be included at concentrations considered unsafe.

Regarding metals, quantification limits (LOQ) (Table 2) ranged from 0.3 $\mu g\,kg^{-1}$ for vanadium to 100 $\mu g\,kg^{-1}$ for zinc, referred to dry samples.

The precision (repeatability) (Table 2) of the whole method, calculated as the relative standard deviation (RSD %) ranged from 0.5 to 40 %. Apart the selenium whose high value is justified by the low concentrations (0.004–0.26 $\mu g\,kg^{-1}$) in the analysed samples, all the other RSD values are always less than 4 %, quite satisfactory for the purpose of this research. The regression factor values (r^2) of calibration curves ranged from 0.992 to 0.999 indicating linearity between ICP-MS signal and concentrations. For all the elements investigated these values were considered satisfactory. The total concentrations of metals in the 16 tattoo ink samples ranged from 0.060 to 16.9 $g\,kg^{-1}$. Meanly, Zn, Ba, Cu and Sr were the main elements (from few $mg\,Kg^{-1}$ to thousands of $mg\,kg^{-1}$) (Table 3).

Zinc is the element most present in the samples (Figure 1), in fact, it is in the range 3.4–13882 $mg\,kg^{-1}$, probably this is due to the fact that ZnO, a white pigment, is often used not only to obtain white materials, but also to produce pastel colours. As it can be seen from Figure 1, zinc predominates in green ink (sample Gr0) and is also present in gold sample (Go) and in the brown (BR) which also contains high amount of barium (6537 $mg\,kg^{-1}$), often used in the form of sulphate to obtain pastel shades from darker pigments. In the analysed white inks (Wh0 and Wh1) zinc appears only at trace levels, probably, in this case, TiO_2 was used to obtain the desired colour (titanium was not being determined), certainly more opaque and stable than zinc oxide. In three of the 16 samples the zinc concentrations exceed those required by legislation (2000 $mg\,kg^{-1}$).[19]

Blue inks, generally, are obtained using Cu(II) compounds because are more stable and less toxic than cobalt based pigments, in fact, the sample Blba contained 296 $mg\,kg^{-1}$ of

Table 2. Quantification limits (LOQ $\mu g\,kg^{-1}$) and precision (RSD %).

	As	Ba	Cd	Co	Cr	Cu	Fe	Hg	Mn
LOQ ($\mu g\,kg^{-1}$)	5.6	10	1.5	2.2	3.6	27	91	4.0	6.8
RSD (%)	3.5	1.5	1.7	3.2	1.3	1.6	1.5	3.8	1.5
	Mo	Ni	Pb	Sb	Se	Sn	Sr	V	Zn
LOQ ($\mu g\,kg^{-1}$)	2.9	33	6.0	1.7	0.7	1.6	34	0.3	100
RSD (%)	2.2	1.7	0.5	2.2	40	2.7	1.8	3.5	2.1

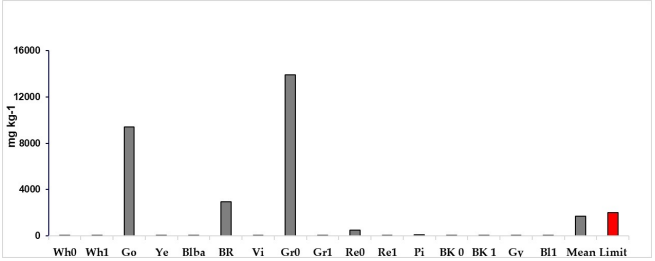

Figure 1. Zinc concentration ($mg\,kg^{-1}$) in the analysed samples.

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Table 3. Metal concentration (mg kg^{-1}) in the analyzed ink samples.

Samples	Wh0 mg kg^{-1}	Wh1 mg kg^{-1}	Go mg kg^{-1}	Ye mg kg^{-1}	Blba mg kg^{-1}	Bl1 mg kg^{1}	Br mg kg^{-1}	Vi mg kg^{-1}	Gr0 mg kg^{-1}	Gr1 mg kg^{-1}	Re0 mg kg^{-1}	Re1 mg kg^{-1}	Pi mg kg^{-1}			Gy mg kg^{-1}	Mean mg kg^{-1}
As	0.70	1.1	0.02	0.11	1.4	0.11	0.18	0.24	0.76	0.47	0.18	0.07	< 0.035	0.03	0.11	0.22	0.36
Ba	0.03	22	652	33	6.6	29	6537	77	1253	76	1009	33	15	6.7	31	8.0	612
Cd	2.6	0.71	1.8	0.04	0.04	0.02	0.08	0.03	2.6	0.23	0.11	0.03	0.01	1.3	0.02	0.46	0.63
Co	0.27	0.02	0.24	0.15	0.42	0.02	0.44	0.08	0.21	0.24	0.29	0.28	0.04	0.57	0.02	0.45	0.2
Cr	0.97	0.57	2.2	1.2	4.6	1.4	2.4	1.8	5.6	2.0	4.5	4.1	1.0	1.9	1.5	2.1	2.2
Cu	24	9.9	0.01	4.2	296	2.8	0.48	184	1505	99	251	6	114	15	4.0	14	158
Fe	19	47	157	84	42	14	81	55	160	48	96	129	45	76	14	44	67
Hg	0.06	0.04	< 0.004	0.05	0.04	0.07	0.04	0.01	0.02	0.04	0.02	0.12	0.05	0.06	0.07	0.07	0.05
Mn	0.77	0.54	1.9	0.88	0.98	0.16	1.8	1.1	4.1	1.8	2.2	0.00	0.99	1.6	0.17	0.84	1.2
Mo	0.01	0.09	0.11	0.12	0.28	0.16	0.04	0.42	0.70	0.44	0.49	0.41	0.16	0.07	0.17	0.14	0.21
Ni	0.64	0.74	15	2.0	4.4	2.4	4.4	6.5	7.9	6.4	2.9	44	0.44	19	2.6	2.9	7.0
Pb	1.6	0.12	0.17	0.48	0.44	0.11	0.69	0.44	0.81	0.47	0.92	1.0	0.24	0.71	0.12	0.46	0.54
Sb	4.0	0.12	0.04	0.40	0.46	0.04	0.16	0.04	0.04	0.65	0.25	0.01	0.01	2.6	0.04	1.1	0.60
Se	0.26	0.01	0.15	0.01	0.01	0.04	0.07	0.00	0.14	0.02	0.04	0.01	0.01	0.06	0.04	0.01	0.05
Sn	0.40	0.45	0.15	0.94	0.60	0.07	0.46	0.19	0.52	0.56	0.41	1.5	0.00	1.1	0.07	1.16	0.52
Sr	0.77	0.51	14	1.1	1.1	0.77	44	1.44	45	1.4	20.1	2.8	0.46	1.2	0.84	0.67	7.14
V	0.95	0.12	0.02	0.44	0.45	0.04	0.09	0.14	0.14	0.18	0.05	0.18	0.20	0.01	0.04	0.44	0.21
Zn	4.1	4.4	9499	5.0	61	4.2	2954	67	14882	5.0	507	14	94	7.9	4.6	12	1689
Total metals	g Kg^{-1}	g Kg^{-1}	g Kg^{-1}	g Kg^{-1}	g Kg^{-1}	g Kg^{-1}	g Kg^{-1}	g Kg^{-1}	g Kg^{-1}	g Kg^{-1}	g Kg^{-1}	g Kg^{-1}	g Kg^{-1}	g Kg^{-1}	g Kg^{-1}	g Kg^{-1}	g Kg^{-1}
	0.059	0.077	10	0.14	0.41	0.054	9.6	0.49	17	0.24	1.9	0.24	0.27	0.14	0.06	0.077	2.6
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	
Solvent	95	48	80	88	84	93	87	88	86	77	80	75	74	94	68	87	
Organic matter	1.1	4.0	6.0	7.2	3.0	4.0	8.2	9.3	10	14	17	18	25	6.0	31	1.5	
Residue	3.7	47	14	4.7	13	2.7	4.1	3.1	4.0	8.8	2.3	7	1.1	0	0.2	11	

Cu. While, in green inks the colour is obtained by using copper and iron salts, in fact, as can be seen in the Table 2 and Figure 2 this sample (Gr0) contains 1505 mg kg^{-1} of Cu and 160 mg kg^{-1} of Fe.

In all other ink samples, copper is present at lower concentrations (Figure 2). On average, the concentration of copper is equal to 158 mg kg^{-1} and in 3 of the analysed inks, it exceeds the legal limits (250 mg kg^{-1}).[19] Referring to elements which are mostly looked as those allergologically relevant, even if, generally, chromium is the constituents of red, orange, and yellow inorganic pigments, in our case it is present in all the inks taken into account in this research. Its concentration ranged from 0.57 to 5.6 mg kg^{-1} and in all cases it is higher than allowed (0.5 mg kg^{-1}).[19] This evidence is in good agreement to recent[21] that note the prevalence of chromium in tattoo inks of all hues. We can hypothesize that chromium contained in the inks of all hues, likely originates as a contaminant of raw materials and/or leaching from the protective metal coatings manufacturing equipment.

Cobalt and nickel are contained in all the ink samples in the range 0.02–0.57 mg kg^{-1} and 0.43–43 mg kg^{-1} respectively, and in most cases they are higher than allowed (0.5 and 5 mg kg^{-1} respectively).[19] The Co, Cr (Figure 3) and Ni (Figure 4) concentrations of the samples taken in consideration in this study can be compared with the published maximum values considered as allergologically safe for consumers exposed to materials containing the metals.

In this context, researchers[24] has shown that contact with irritants and/or following repeated exposures to Co, Cr and Ni such individuals rarely react to levels below 10 mg kg^{-1}. Considering this value limit, four of analysed samples should not be used by tattoo artists. Basketter[24] suggests that consumer products should not contain more than 5 mg kg^{-1} of Co, Cr and Ni or for a better health protection the concentrations should be less 1 mg Kg^{-1}. Cadmium is present in all samples. The highest concentrations were found in green (Gr0) (2.6 mg kg^{-1}), white (2.6 mg kg^{-1}) and gold (1.8 mg kg^{-1}) inks. In four samples (Wh0, Go, Gr0, Bk0) the concentrations exceed the legal limit (0.5 mg kg^{-1}).[19] Antimony, although present in the samples, exceeds the legal limit (0.5 mg kg^{-1})[19] in four inks samples (Wh0, Gr1, Bk0, Gy).

The red inks are generally obtained using mercury sulphide, cadmium sulphide and cadmium selenide as well as iron oxide and ferric hydrate. In this study, the red sample (Re0) contains Ba, Zn, Ni and Fe as the highest elements, fortunately, mercury, selenium and other hazardous heavy metals are also present in all samples only at very low concentrations, in particular Hg (0.01–0.07 mg kg^{-1}), fortunately, do not exceed the legal limits (0.5 mg kg^{-1}).[19] The red colour in the sample Re1 is obtained by organic colorant because the characterizing elements (Fe, Hg, etc.) are absent. The higher lead content (1.6 mg kg^{-1}) was found, as shown in Figure 5, in one of the white inks (Wh0) in which, given the low percentage does not constitute the pigment, which could be constituted of titanium oxide, but could be due, as in many other cases, to impurities present in the materials used. In five of the samples (Wh0, Gr0, Re0, Re1, Bk0) analysed, the lead content is higher than that required by current legislation (0.7 mg kg^{-1}).[19]

In no case selenium exceeds the limits established by legislation (2.0 mg kg^{-1}),[19] in fact the concentration in the samples of analysed inks is between 0.01 and 0.26 mg kg^{-1}).

Generally, the black inks for different uses are obtained from iron oxides. This element is present in all analysed samples in a large concentrations range (14–160 mg kg^{-1}). There are no legal limits for this element in products that come into contact with the skin. Only one of black inks analysed

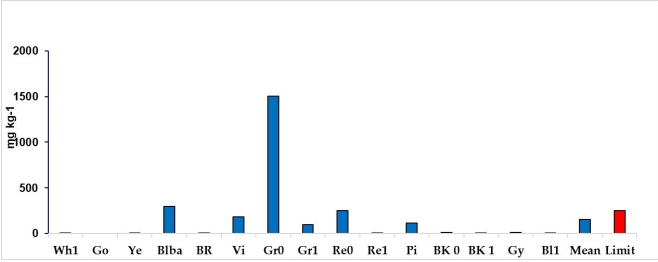

Figure 2. Copper concentration (mg Kg^{-1}) in the analysed samples.

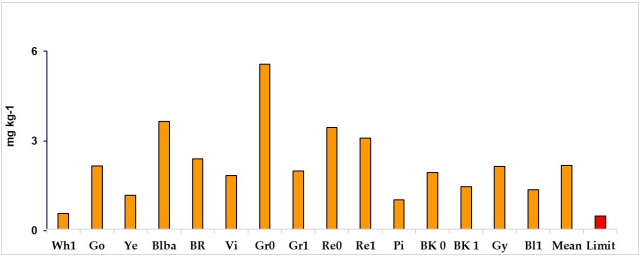

Figure 3. Chromium concentration (mg kg^{-1}) in the analysed samples.

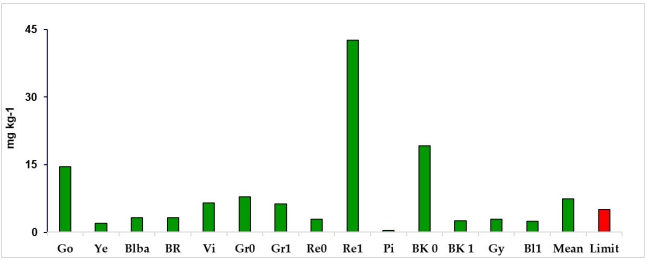

Figure 4. Nickel concentration (mg kg^{-1}) in the analysed samples.

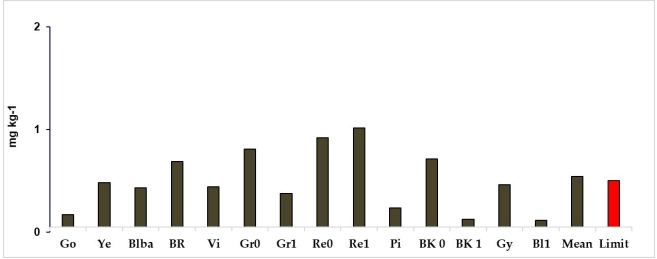

Figure 5. Lead concentration (mg kg^{-1}) in the analysed samples.

(Bk0) contained elevated Fe concentrations (76 mg kg^{-1}), while for the last sample (Bk1) we can assume that coal from plant was used. This is confirmed by the very low ash content. The common metallic salt used for brown coloured tattoos contains Mn. Skin diseases due to this element seem to be quite rare. Nguyen and Allen[25] reported manganese as the possible cause of swelling and itching in the purplish site of the tattoo. Grey ink (Gy) contains iron (44 mg kg^{-1}), probably, as Fe_2O_4, Mn (0.84 mg Kg^{-1}) as MnO_2, zinc (12 mg kg^{-1}), chromium (2.2 mg kg^{-1}) and barium (8.0 mg kg^{-1}). Grey decoration is a most complex recipe, including, probably, several different pigments and several minor components in proportions to obtain the desired hue. In our case, brown ink (Br) contain considerable amounts of barium (6547 mg kg^{-1}), zinc (2954 mg kg^{-1}), iron (81 mg kg^{-1}) and manganese (1.8 mg kg^{-1}) which suggests the use of the pigments Fe_2O_4 and MnO_2. From artistic point of view, when a pastel or clearer colour than the characterizing pigment, as in the case of brown ink sample, the manufacturer adds a white pigment as $BaCO_3$, $BaSO_4$, ZnO, $PbCO_3$ and more recently TiO_2.

Conclusion

The ICP-MS was a particularly suitable technique for our purposes because it has a wide linearity range, in fact, using the same calibration line it is possible to analyze solutions whose concentrations vary from a few ng L^{-1} to about ppm covering a range of several orders of magnitude. This was particularly useful in the analysis of the solutions obtained from the tattoo inks because from color to color and from brand to brand, the samples are very heterogeneous in composition. Considering the standard deviation values on found concentrations we can affirm that the method provides accurate results and therefore suitable for the determination of investigated metals in tattoo inks samples.

Our results showed that the metal contribution to the tattoo ink compositions was highly variable between samples, brands and even among like-colored inks. Zinc, barium and manganese were the main metals. Chromium was above the harmless allergological limit of 0.5 ppm in all the sink samples.

The high chromium percentages in all analyzed ink samples could be the cause of cutaneous sensitization such as chronic dermatitis or dermatitis herpetiform or eczematous and pseudo lymphomatous reactions in the green tattoo areas.[26,27] Toxic elements as Cd, Mn, Pb, Sb and V were over the 1 ppm in few cases, while Hg was in traces.

With regard to the two black inks (BK0 and BK1), from the evaluation of the residue value at 550 °C, respectively zero and 0.2 %, it can be concluded that the color is imparted by carbon or an organic compound.

Our data indicated that the use in the tattooing process of several inks acquired on line at low cost might pose serious risks for the development of dermatological pathologies in tattooed patients. According to our knowledge, there are researches[1,21–23] that quantifies Al, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sb, Sr and V in ink samples purchased from famous manufacturers. Their results indicated that the relative element contribution to the samples compositions was greatly variable between samples, brands and even among like-colored pigments. Al, Ba, Cu, Fe and Sr were the main metals. Allergenic metals as Cr, Ni and Co were lower than the safe allergological limit of 1 ppm in 35, 9 and 1 cases, respectively. Toxic elements as Cd, Mn, Pb, Sb and V were over the 1 ppm in a few cases, while Hg was in traces.

By sectoral field inductively coupled plasma mass spectrometry (SF-ICP-MS), some authors[23] in 2009 quantified different toxic elements (V, Mn, Cd, Sb, and Pb) in 56 tattoo ink samples. The results show that, in some cases, concentrations were greater than 1 ppm. In the same survey, the allergenic metals Cr, Ni and Co had concentrations well above the safe limit in 62.5,16.1 % and 1.8 % of cases, respectively.

On average, the concentrations of some metals (As, Ba, Cd, Fe, Mn and Ni) in the samples analyzed by us are higher (sometimes by an order of magnitude) than those determined by Battistini[21] while regarding the other elements the concentrations are of the same order of magnitude. However, it should be considered that 50 % of the inks used by Battistini[21] came from Italian suppliers or purchased in the United States, while our samples were all of Chinese origin.

Some Italian researchers[28] have recently established that as regards green tattoo ink, European and Asian products use the same pigment (PG7), which is restricted in Europe, although at different percentages. Even if produced in different continents, the inks mentioned above have a similar content of toxicologically dangerous substances (Ni, As, Cd and Sb, siloxanes, including the harmful octamethylcyclotetrasiloxane). European and Asian products differ in terms of physicochemical properties: European inks are more hydrophilic, Asian ink more hydrophobic. In addition, the Asian ink contains teratogenic and chlorinated teratogenic additives and carcinogenic compounds.

Considering that tattoos are permanent, it is important to know the chemical composition and quality of the products used in this practice since, the long contact time between skin and tattoos it may happen that toxic and/or allergenic metals accumulate in the tissues. For this reason, are needed regulations that oblige companies that supply inks and/or other materials for use on the skin to provide detailed information on the chemical composition.

Experimental Section

Quality control and quality assurance

The detection (LOD) and quantification (LOQ) limits of the method, as in other researches[29-31] found in 10 procedural blanks respectively. The choose of procedural blank in trace analytical determination is basic to asses contamination and matrix interferences. To obtain LOD and LOQ, calibration blank or ultrapure water were not used because they could not estimate matrix interferences. Otherwise, to the best of our knowledge a tattoo ink without analita was not available at present. For these reasons, the procedural blank was obtained by subjecting ten different aliquots of procedural blank (2 mL of HNO_3, 2 mL of H_2O_2 and 100 µL of a solution containing Au 100 µg L^{-1}), to the entire mineralization

procedure. The repeatability of the whole method, calculated as the mean relative standard deviation (RSD %) for six independent analysis of portions of the three same samples.

Laboratory equipment

All glassware and sample containers were thoroughly washed with hot HNO_3 5% solution followed by rinsing with purified water and acetone (analytical grade) respectively. These were finally kept in the oven at 110 °C overnight. To avoid contaminations of samples, different glassware and pipettes were used for standards and for solutions obtained from ink samples.

Ink samples

For the purposes of this research, 16 different ink samples produced by two Chinese companies (Drag and Atous) were used. The products were available at very low cost, on the same website, from which we ordered and purchased them online. The chemical composition of inks was not found on the label, probably, to protect manufacturers' proprietary.

Volatiles content

The solvent content was determined by weight loss. About 1 g of an aliquot of sample was completely dried at T of 105 °C for one night in a platinum crucible.

Loss at heating to 550 °C

Ashes were determined by burning at 550 °C the residue of sample previously heated a 105 °C. The organic matter content was determined by weight loss.

Sample mineralization

Tattoo inks are unstable mixtures, in fact, the samples tend to settle on the bottom of the container forming two distinct phases visible to the naked eye, therefore, before weighing the quantity necessary for the analyses, it was necessary to homogenize the sample for a long time. Based on what has been reported in the literature,[23] we decided to weigh about 250 mg of each ink sample. The ink labels indicated the presence of glycerine, which must be oxidized before the subsequent addition of nitric acid to avoid the possible formation of nitro-glycerine (explosive). A microwave oven (Milestone model MLS-1200 Mega, Milestone Laboratory Systems, Italy) with rotor of high pressure (up to 100 bar) was used for sample mineralization. About 250 mg of ink sample, previously homogenized, were weighted, transferred inside Teflon vessels and 1 mL of HCl (47%) (Fluka, Milano), 1 mL of H_2O_2 (40%) (Fluka, Milano) were added. The instrumental conditions used for the microwave digestion were: 1 min at 250 W, 1 min at 0 W, 5 min at 250 W, 5 min at 450 W, 4 min at 600 W and 5 min at 250 W. After allowing to cool, the solutions obtained from the mineralization were transferred to a platinum crucible and dried on a heating plate. The residue, after adding 1 mL of HF (Merck, Darmstadt, Germany) and 4 mL of HNO_3 69% (Fluka, Milano), was again heated on a heating plate until the sample was completely mineralized (few minutes). After digestion, the clear, solution was brought to volume with purified water.

Instrumentation

Eighteen elements (As, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Mo, Pb, Sb, Sn, Se, Sr, V and Zn) were determined in each tattoo ink sample, chosen on the basis of their significance in toxicological fields. An ICP-MS instrument (Thermo Scientific X Series II) equipped with a collision cell, was used for the analyses of all the investigated elements. Considering that the analytical results of the ICP-MS instrument strongly depends on the operating conditions, a 10 $\mu g\,L^{-1}$ solution of ^{7}Li, ^{59}Co, ^{115}In, ^{140}Ce, and ^{248}U (in HNO_3 1%) was used to optimize the instrument in terms of sensitivity, resolution and mass calibration. Also, the $^{140}Ce^{16}O^{+}/^{140}Ce^{+}$ ratio was used to check the level of oxide ions in the plasma that could interfere in the determination of some elements; also, instrumental parameters such as RF power and carrier gas flow were optimized and the level of doubly charged ion was monitored by means of the signal Ba^{2+}/Ba^{+}. The analysis of some elements by ICP-MS is well-known to suffer from polyatomic isobaric interferences. To minimize these, for some elements, we used a collision cell, monitoring the ratios CeO/Ce and Ba^{2+}/Ba^{+} and was maintained less than 4% by setting of the voltages applied to the instrument lenses. The instrumental parameters are shown in Table 4. A solution of HNO_3 1 M was run during the analysis to ensure that the memory effect, due to the more refractory elements, was negligible. Sample blanks were also analysed and subtracted from each determination.

Calibration

Calibration standard solutions for each element were prepared daily by stepwise dilution with HNO_3 2% of a multi-element standard solution containing 29 elements (Perkin Elmer Pure Plus, Atomic Analytical) ($1000 \pm 5\ mg\,L^{-1}$) in 2% HNO_3. The range of concentration of the calibration curves was between 0.05 and 500 $\mu g\,L^{-1}$ except for Hg whose range was from 0.1 to 5 $\mu g\,L^{-1}$. Solutions containing ^{89}Y and ^{187}Re (50 $\mu g\,L^{-1}$) were used as internal standards to compensate any signal instability or sensitivity changes during the analysis. A solution of HNO_3 2% as blank was used. Were prepared three replicate of each point and calculated the linear regression. The analysis of the seven standard solutions was replicated every day. To eliminate memory effects related to the previous analysis, between two subsequent standards analysis, a 25 s washing time was settled.

Table 4. Parameters and operating conditions for the ICP-MS instrument.

Parameter	Value
rf power (W)	1400
Nebulizer (carrier gas) flow rate (l min^{-1})	0.95
Resolution (amu)	0.70
Detector	Dual
Speed of peristaltic pump (rpm)	40
Replicates	4
Dwell time	40 ms
Scan mode	Peak hopping
Collision cell Parameters	
He_2/H_2 reaction gas flow	4.0/0.5 mL min^{-1}
Isotopes monitored in standard mode	^{55}Mn, ^{56}Fe, ^{59}Co, ^{147}Ba, ^{148}Ba, ^{80}Se, ^{88}Sr, ^{118}Sn, ^{200}Hg, ^{202}Hg, ^{208}Pb,
Isotopes monitored in DRC mode	^{59}Co, ^{75}As, ^{52}Cr, ^{111}Cd, ^{51}V, ^{57}Fe, ^{60}Ni, ^{78}Se, ^{64}Cu, ^{65}Cu, ^{66}Zn, ^{68}Zn, ^{95}Mo, ^{121}Sb

Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Keywords: analytical method · heavy metals · ICP-MS · inks · tattoo

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