Human exposure to thallium through tap water: a study from Valdicastello Carducci and Pietrasanta (northern Tuscany, Italy)

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Abstract

A geological study evidenced the presence of thallium (Tl) at concentrations of concern in groundwaters near Valdicastello Carducci (Tuscany, Italy). The source of contamination has been identified in the Tl-bearing pyrite ores occurring in the abandoned mining sites of the area. The strongly acidic internal waters flowing in the mining tunnels can reach exceptional Tl concentrations, up to 9000 µg/L. In September 2014 Tl contamination was also found in the tap water distributed in the same area (from 2 to 10 µg/L). In the same month the local authorities imposed a Do Not Drink order to the population.

Here we report the results of an exposure study carried out from October 2014 to October 2015, and aimed at quantifying Tl levels in 150 urine and 318 hair samples from the population of Valdicastello Carducci and Pietrasanta. Thallium was quantified by inductively coupled plasma – mass spectrometry (ICP-MS). Urine and hair were chosen as model matrices representing different time periods of exposure (short-term and long-term, respectively).

Thallium values found in biological samples were compared with Tl concentrations found in tap water in the living area of each citizen, and with his/her habits. Thallium concentration range found in hair and urine was 1 – 498 ng/g (reference values in unexposed subjects 0.1 –6 ng/g) and 0.046 – 5.44 µg/L (reference value for the European population 0.006 µg/L), respectively. Results show that Tl levels in biological samples were significantly associated with residency in zones containing elevated water Tl levels. The kinetics of decay of Tl concentration in urine samples was also investigated. At the best of our knowledge, this is the first study on human contamination by Tl through water involving a such high number of samples.

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Keywords

1. Introduction

Thallium (Tl) is a non-essential, extremely toxic metalloid. Its poisonousness to mammals is considered comparable to that of mercury, cadmium or lead (Rodriguez-Mercado and Altamirano-Lozano 2013).

Thallium occurs naturally in soil (ranging from 0.1 to 1 mg/kg), marine water (10–20 ng/L), freshwater (5–10 ng/L), and air (less than 1 ng/m^3) (Peter and Viraraghavan 2005; Rodriguez-Mercado and Altamirano-Lozano 2013). It is employed by several industries (cement plants ???, electronics, glass and pharmaceutics), and it is released in the environment with the combustion of fossil fuels, refinement of oil fractions, smelting of ferrous and non-ferrous ores and mining of sulfide ores (Peter and Viraraghavan 2005).

A recent geological study (Petrini and others 2015) showed the presence of Tl at high concentrations (up to 4500 times higher than the limit fixed by U.S. EPA) in groundwater near Valdicastello Carducci and Pietrasanta. The source of Tl has been identified in the Tl-bearing pyrite ores still residing in the tunnels of the abandoned mining sites occurring in this area. Pyrite ores containing Tl at concentration levels of 100-600 mg/kg (Biagioni and others 2013) are subjected to both biotic and abiotic oxidation processes releasing Tl and other metals (e.g. Fe, As, Sb, Pb, Zn, Cd etc.) into water. Acid drainages from the abandoned mining tunnels typically contain 200-1000 µg/L of Tl, while some very acidic (pH = 1.5-2.0) water ponds inside the tunnels can reach even more extreme Tl levels, up to 9000 µg/L. In September 2014 a significant Tl contamination was also found in tap water distributed in the same area. The Tl-contaminated water that fed the aqueduct of Valdicastello Carducci and part of that of Pietrasanta originates indeed from a natural water spring (10-80 L/s) located very close to the abandoned mining sites. Studies are currently in progress in order to assess the origin of this Tl contamination.

The goal of our work was to assess the impact of Tl exposure as a result of tap water contamination in Valdicastello Carducci and Pietrasanta, quantifying Tl in hair and urine and correlating these concentrations with Tl levels in the tap water used by inhabitants.

Commented [MD2]: il tallio non è usato nei cementifici !!

Our study was performed in an "emergency" condition. After the researchers communicated the information on Tl contamination to the local authorities, these imposed to the population a Do Not Drink order. In order to evaluate the contamination level of the inhabitants of this region, urines and hair sampling was performed immediately after the ordinance. Urines are, indeed, a reliable matrix to evaluate the short-term exposure to metals (Gil and Hernández 2015). Urines were also collected in the following months in order to evaluate Tl clearance from the body as result to the Do Not Drink order imposed to the population. Hair samples were collected in order to evaluate the long-term exposure.

2. Materials and methods

2.1 Studied area

The research included 4 km^2 of urban and suburban area. From the end of the World War II to 1990 an intense mining activity characterized the mountainous area immediately north of Valdicastello Carducci. The materials extracted from these mines were barite and iron oxides (both hematite and magnetite) used as weighting agents in petroleum well drilling mud. The abandoned mining tunnels, mine dumps and plants for the mineral treatment are still present, posing a series of environmental threats. The mining sites are distributed in the catchment area of the Baccatoio stream and very close to the watercourse itself. The Baccatoio stream (about 10 km in length) originates from the drainage tunnels of the abandoned mines, crosses Valdicastello Carducci and the coastal plain due south of Pietrasanta, and flows into the Ligurian Sea.

Pietrasanta is a municipality located in northwestern of Tuscany with approximately 25000 residents. Valdicastello Carducci, a small village about 3 km due east of Pietrasanta, has about 1000 inhabitants.

Figure 1 shows the map of Italy, the enlargement of the investigated region and the public fountains (F1-F5) chosen for water sampling.
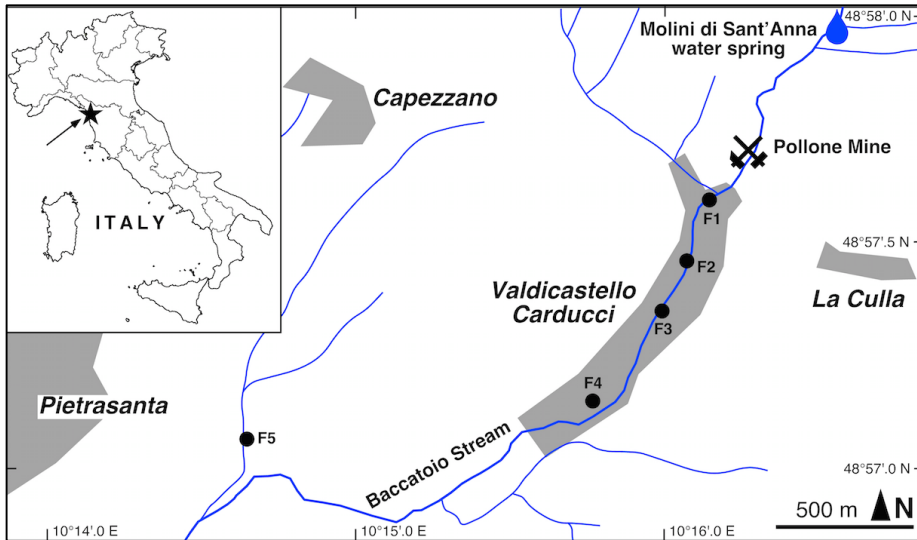
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Fig. 1. Sketch map showing the localities cited in this work. F1 to F5 are the public fountains whose water was analyzed for this study (fountain F6 is located close to the Pietrasanta cemetery, outside the map).

2.2 Volunteers' recruitment

Residents were invited to participate to the study through public meetings. Participation rates exceeded 95% of those approached. In each household, we recruited participants of both sex, children (defined here as ≤14 years) and adults (defined here as >14 years). All participants gave a written informed consent, and for each participant we obtained – via a questionnaire administered face-to-face – demographic data, gender, a brief residential history, use of tap water to cook, and type of water usually drunk (tap or bottled water).

A total number of 150 subjects (114 adults and 36 children) provided a urine sample. The participants were aged between 2 and 84 years (mean 38 years). The number of households and individuals was 66 and 108, respectively. The male/female ratios were 53/61 in adults and 21/15 in children.

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The first urine sampling was carried out during October 2014, within a month after the Do Not Drink order imposed to the population. A second urine sampling was carried out on 140 subjects during November 2014, aiming to evaluate the contamination level at a distance of 60 days after the Do Not Drink order imposition. For N = 38 volunteers (14 males, 24 females, age 4 – 77 years, mean age 38 years) living in the selected areas (26 from Valdicastello and 12 from Pietrasanta), urine sampling was carried out also for several months up to 1 year.

A total number of 318 subjects (189 adults and 129 children) provided a hair sample. The participants were aged between 1 and 84 years (mean 31 years). The number of households and individuals was 63 and 108, respectively. The male/female ratios were 90/99 in adults and 54/75 in children. Hairs were sampled during October 2014, within a month after the Do Not Drink order imposed to the population.

The demographic characteristics of the study population are summarized in Table 1.

Table 1. Demographic characteristics of the study population.

Total	318 (100)	150 (100)
Gender		
Males	144 (45)	74 (49)
Females	174 (55)	76 (51)
Age group (years)		
≤14	189 (59)	36 (24)
> 14	129 (41)	114 (76)
Residence area		
Upper Valdicastello	70 (22)	41 (27)
Medium Valdicastello	113 (36)	76 (51)
Lower Valdicastello	37 (12)	7 (5)
Pietrasanta	98 (31)	26 (17)

We classified the population into four groups according to the extent of water pollution in the places of residence (upper, medium and lower Valdicastello, and Pietrasanta), established by the

analysis of Tl concentration found in the water from the public fountains. Each inhabitant was assigned to a specific group on the basis of the proximity of his/her residence house to the fountain.

2.3 Sampling and sample preparation

Tap water. In the study area, the drinking water was supplied by a water spring located very close to an abandoned mining site. Water samples were collected from 6 public tap fountains, which provided the same tap water delivered in residents' houses. Water samples were collected, without filtering, in 50 mL polypropylene tubes (Falcon® Centrifuge Tubes, Polypropylene, Sterile, Corning), previously conditioned with 2% HNO_3. Before sampling, water was left flowing from the tap for at least three minutes. A suitable volume of ultrapure concentrated HNO_3 was added to all samples in order to obtain a final value of pH < 2. Samples were stored at 4°C.

Hair and urine. Because of the difficult of obtaining 24-h urine samples from non-hospitalized volunteers, we decided to collect spot urine samples. Each volunteer provided the first urine after the night into a 50 mL Eppendorf tube. Urine samples were frozen at −20 °C until analysis.

Before the analysis, urine samples were defrosted at room temperature, centrifuged (5000 rpm for 10 min) and diluted 1: 10 with 2% nitric acid.

Hair samples were collected from the nape of the neck and as close to the scalp as possible using stainless steel scissors (thinning type). Exclusively untreated hair with a length ranging between 3-15 cm were collected, cut in pieces, mixed and 200 – 300 mg considered for the analysis. Hairs shorter than 3 cm were not sampled. The hair part longer than 15 cm was discarded. Considering that the hair growth is about 1-1.5 cm/month, Tl quantitation in hair evidenced of an average exposure of 2-7 months.

Samples were stored in zip lock polyethylene bags. About 200 – 300 mg of hair from each sample were weighed into Teflon vessels, washed three times with 1:1 acetone/ultrapure water and pre-digested for 1 h at room temperature with HNO_3 (6 mL, 69 % w/w) and H_2O_2 (2 mL, 30 % w/w). Then, the vessels were hermetically closed and placed in the microwave oven for the digestion

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(from room temperature up to 200°C in 10 min, 20 min at 200°C and cooling). After cooling at room temperature, samples were quantitatively transferred into 50-mL flasks and diluted with ultrapure water up to 50 mL. Samples were stored at room temperature.

2.4 Chemicals

All the reagents were of analytical grade and used without further purification. Concentrated HNO_3 (69 % w/w) and hydrogen peroxide (30 % w/w) for trace analysis from Sigma-Aldrich (Milan, Italy) were used for the sample digestion and preparation. Nitric acid (2%) for the analysis was prepared by diluting a suitable volume of concentrated HNO_3 with ultrapure water. Thallium standard solutions were prepared in 2% HNO_3 from a multielemental standard (5183-4688, Agilent Certified Environmental Calibration Standard). The final calibration range was 0.1 – 50 µg/L.

The ICP-MS operative conditions were optimized with a tuning solution of 1 µg/L Ce – Co – Li – Mg – Tl – Y (Agilent Technologies, Tokyo, Japan) in 2% HNO_3. Iridium purchased from Sigma Aldrich (207209, Milan Italy) was used as internal standard. Ultrapure water prepared with an Elga Purelab-UV system (Veolia Environment, Paris, France) was used throughout. Ultrapure grade carrier (99.9995%) argon (Ar) and helium (He) were supplied by Rivoira (Italy).

Certified reference materials SRM 2670a (lyophilized urine) and SRM 1640e (water) were purchased from the National Institute of Standard and Technology (NIST).

2.5 Instrumentations

Sample digestions were carried out with a microwave oven (Milestone Ethos Start D, FKV, Bergamo, Italy). An Eppendorf Centrifuge model 5804R was used throughout for sample preparation.

A quadrupole ICP-MS Agilent model 7700 (Agilent Technologies, Tokyo, Japan) equipped with a collision cell system was used for water and biological sample analysis. Samples were introduced into the plasma by an autosampler Agilent model ASX-520 (Agilent Technologies, Tokyo, Japan).

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The instrument was fitted with a MicroMist nebulizer (Agilent nebulizer standard for 7700) with a Scott-type double-pass glass spray chamber cooled down to 4°C. A solution of 10 µg/L iridium in 2% HNO_3 was used as internal standard. Table 2 summarizes plasma operating conditions and acquisition parameters.

Table 2. Instrumental parameters for the Agilent 7700 ICP-MS.

Radiofrequency power	1550 W
Plasma flow rate	15.0 L min^{-1}
Carrier flow rate	1.05 L min^{-1}
Helium	4.5 mL min^{-1}
Octapole bias	-18.0 V
Octapole RF	200 V
KED	3.5 V
Sampling depth	8 mm
Measured isotopes	^{203}Tl, ^{205}Tl, ^{193}Ir
Dwell time	300 ms
Number of sweeps	300

2.6 Method Validation

Samples and standards were analyzed in triplicate. The limit of detection (LOD) for this study, based on the mean of the blank samples from all runs plus three times its standard deviation, was 2 ng/L for aqueous samples. Sample blanks and standard solutions were run with each batch of samples as quality control.

The precision of ICP-MS analysis was determined on N=3 replicates of urine and hair samples. The average recovery of the internal standard (iridium) was 100 ± 5 %, indicating the absence of a significative matrix effect in the adopted procedure.

Since Tl concentrations in human matrices are generally near the detection limits of ICP-MS, a validation of the analytical technique was mandatory. To evaluate the accuracy of the instrument itself, a water sample certified for Tl (NIST1640A, purchased from the National Institute of

Standard Technology, USA) was analyzed. The certified concentration of Tl reported for SRM 1640e was 1.619 ± 0.016 µg/L, and the concentration that we found was 1.61 ± 0.08 µg/L.

Urine. ICP-MS method was validated analyzing a certificate sample of lyophilized urine (HIGH content NIST 2670). The certificate Tl concentration was 5.417 ± 0.064 µg/L, and we found a concentration of 5.34 ± 0.11 µg/L (n=6, RSD% 2.06).

The limit of detection for urine, based on the mean of the blank samples from all runs plus three times its standard deviation, was 20 ng/L.

Hair. Currently, hair reference materials certified for Tl are not commercially available. Thus, the European Reference Material ERM-DB001 (purchased from Sigma-Aldrich, Milano) certified for seven heavy metals (As, Cd, Cu, Hg, Pb, Se, Zn) represents the only way to evaluate the matrix effect for hair samples. We spiked the certified material, before the digestion procedure, with 250 µL of Tl standard solution, to obtain a final concentration of 81.0 ng/g. We found a Tl concentration of 79.6 ± 4 ng/g, corresponding to a recovery of 98.3% (6 replicates, RSD% 5.5%).

The limit of detection for hair, based on the mean of the blank samples from all runs plus three times its standard deviation, was 0.6 ng/g.

Hair washing, digestion, and ICP-MS analysis were repeated three times for 7 samples, to estimate the reproducibility of the entire procedure, and the maximum relative standard deviation obtained was 11%.

2.7 Statistical methods

Collected data were subjected to the following statistical treatments: (i) calculation of description statistics (mean, geometric mean, 5% and 95% percentile, median and relative standard deviations); (ii) testing for normality and log-normality (Shapiro-Wilk, Lilliefors and Kolmogorov-Smirnov tests) in order to determine the best distributions to describe the data set; (iii) correlation analysis between Tl values in urine, hair and different variables (such as age, sex, and habits), performed by

estimation of the correlation coefficient (r) with a significance level of P < 0.05 (Pearson, Spearman and Kendall tests). The non-parametric Mann-Whitney U test was used to determine statistical significance (two-tailed p-value ≤ 0.05). The limited number in some age groups did not allow reliable age stratified statistical analysis.

Statistical treatments were performed with OriginLab 8.6 software package.

3. Results

3.1 Thallium in tap water

Five public fountains in Valdicastello and one in Pietrasanta were selected for water sampling. Fountains F1 – F5 are located along Valdicastello, thus covering the entire study area. The fountain F6 in Pietrasanta was fed by the same spring of fountains in Valdicastello.

Figure 2 shows the levels of Tl measured in tap water collected from fountains F1 – F5 during September 2014. Table 3 reports Tl concentrations found in tap water collect from fountain F6 from September 2014 to January 2015.
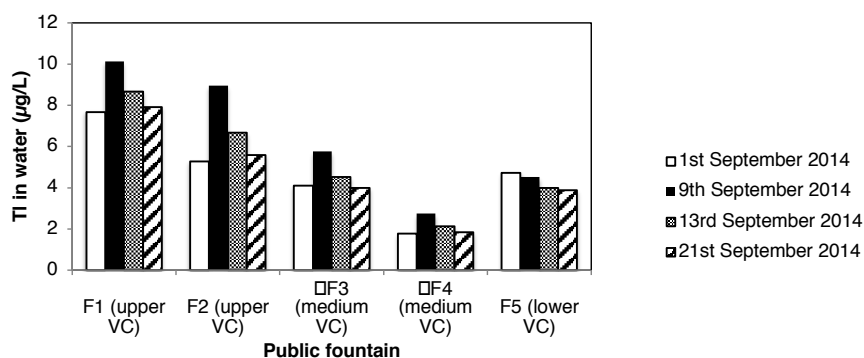

Fig. 2 Levels of Tl measured in tap water collected from 5 public fountains in Valdicastello (F1-F2: upper Valdicastello; F3-F4: medium Valdicastello; F5: lower Valdicastello) sampled during September 2014.

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Table 3. Levels of Tl (as µg/L) found in a public fountain located in the south part of Pietrasanta, and fed by the same water spring of Valdicastello.

13rd September 2014	0.12
28th September 2014	0.37
1st October 2014	0.07
3rd October 2014	0.04
9th November 2014	0.12
8th January 2015	1.04

Until September 2014, the drinkable-water distribution system of Valdicastello Carducci (and, thus, also the public fountains F1 – F5) was fed by a single water spring (located at an altitude of 318 m a.s.l. about 1000 m due NNE of the higher part of Valdicastello). This water also fed about 10% of the drinkable water distribution system of Pietrasanta (public fountain F6). The water spring (named Molini di Sant'Anna) lies within the abandoned mining area, and it was found to be heavily contaminated by Tl (from 5 to 37 µg/L). During the period of this study, we observed a progressive decrease of the Tl concentration in the water of the fountains moving downstream along Valdicastello. Very likely this decrease was related to the progressive adsorption of Tl on the surfaces of the Fe oxy-hydroxide scales lining the internal surface of the steel pipes of the aqueduct. Studies to confirm this hypothesis as currently in progress.

On October 3rd, 2014 local authorities imposed a Do Not Use order both for drinking and cooking, and excluded the contaminated spring from the distribution system.

3.2 Hair analysis

Figure 3 shows the frequency distribution (a) and the box plot (b) relative to Tl concentrations found in the hair of the examined population, sampled within a month after the Do Not Drink order (3rd October – 3rd November). Thallium content in hair is usually described as ppb or ng/g (Tobin 2005).
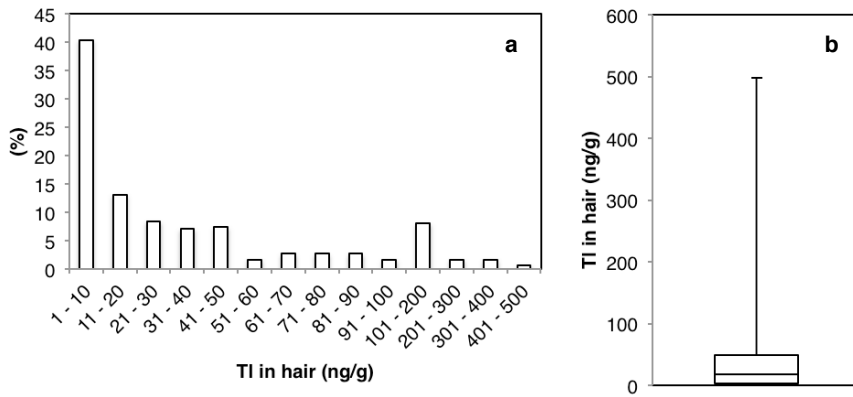
Fig. 3 a) Frequency histograms of the raw data set; b) Box plot of Tl concentration (ng/g) found in the hair of 318 subjects. Middle band, box and whiskers represent the median, 25th and 75th percentile, and the most extreme values, respectively.

Thallium levels in hair (N = 318) ranged from 1 to 498 ng/g, with arithmetic mean 41 ng/g (standard deviation 68 ng/g), geometric mean 15 ng/g and 95th percentile 157 ng/g.

Thallium concentrations in hair and urine samples have to be discussed in relation to the environmental background levels representing Tl exposure. Figure 4a shows the arithmetic and geometric mean of Tl levels in the hair of subjects divided according to their residence area. Comparing the results from hair analysis with the residence of the subjects, the upper area of Valdicastello emerged as the residence area of the most contaminated people. This trend is analogous to that found in tap water reported in Figure 2. The analogy between the two trends shown in figures 2 and 3a suggests a correlation between Tl levels found in hair and Tl concentration in tap water.
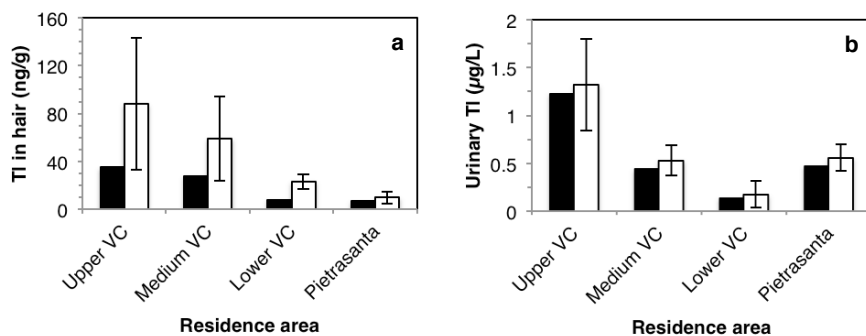
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Fig. 4 Arithmetic mean (white bars) with standard deviation and geometric mean (black bars) of Tl levels found in hair (a) and urine (b) classified according to the residence of the subjects.

The Spearman correlation coefficient for Tl concentration in hair versus sex (analysis stratified for Valdicastello and Pietrasanta) is near zero, suggesting no statistically significant difference of Tl concentrations in hair of male (N =139) and female (N=179).

Stratified statistical analysis according to residence area (Valdicastello or Pietrasanta) and age (adults or children) revealed that adults (N=189, arithmetic mean 58 ng/g, geometric mean 23 ng/g) accumulated more Tl respect to children (N=129, arithmetic mean 20 ng/g, geometric mean 8 ng/g). Due to the limited number in some age groups, a reliable age stratified statistical analysis was not allowed.

Stratified analysis according to the residence area (Valdicastello or Pietrasanta) allowed us to detect significant differences in male who used or not to drink tap water, while for women no significant correlations were observed (see Table 4). This result can be due to the fact that hair contains information about the exposure window. As about 80% of hair samples from men were about 3 cm long, the type of sample is more homogenous and it reflects a shorter exposure period just before the Do Not Drinking order. Instead, more than 90% of women had long hair. Thus, the absence of a significant difference between drinkers/ not drinkers women reflects a longer exposure period in which, likely, the Tl contamination level was not constant.

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Table 4. Thallium levels in hair in relation to residence area, sex and habits. Number of persons, arithmetic means, standard deviations (SD), geometric means and 95% confidence limits of the means are given.

Valdicastello				
Male	Not drinker (N=56)	39 (67)	11	21.4 - 56.5
	Drinker (N=83)	62 (87)	25	43.3 - 80.7
Female	Not drinker (N=75)	58 (87)	17	38.3 - 77.7
	Drinker (N=104)	63 (79)	24	47.8 - 78.2
Pietrasanta				
Male	Not drinker (N=56)	7.2 (7.7)	7.2	5.1 - 9.3
	Drinker (N=22)	11 (8.8)	9.9	7.3 -14.7
Female	Not drinker (N=75)	9.6 (10)	6.9	7.3 - 11.9
	Drinker (N=31)	11 (11)	6.1	7.1 - 14.9

Figure 5 shows the trend of Tl in a 30 cm hair lock sampled in November 2014 in a 13 years old girl. The hair lock was cutted in pieces of 3 cm (except for the last 8 cm piece). The x-axis report the length of the hair lock from the scalp to the tip; the y-axis reports the Tl concentration in ng/g. Considering that in the specific subject hair grew up around 1.3 cm/month, the maximum exposure to Tl was around December 2013 – January 2014 and in the investigated period the concentration was always above 25 – 50 times the value of non exposed subjects.
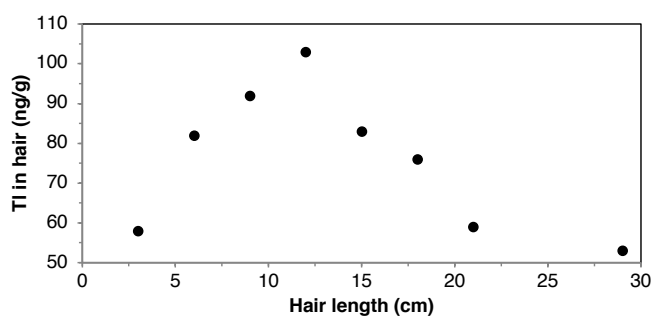
Fig. 5 Trend of Tl (ng/g) in sequential segments of a hair lock sampled in November 2014 in a 13 years old girl.

3.3 Urine analysis

Figure 6 shows the frequency distribution (a) and the box plot (b) relative to urinary Tl levels found in the examined population, sampled within a month after the Do Not Drink order (3rd October – 3rd November).
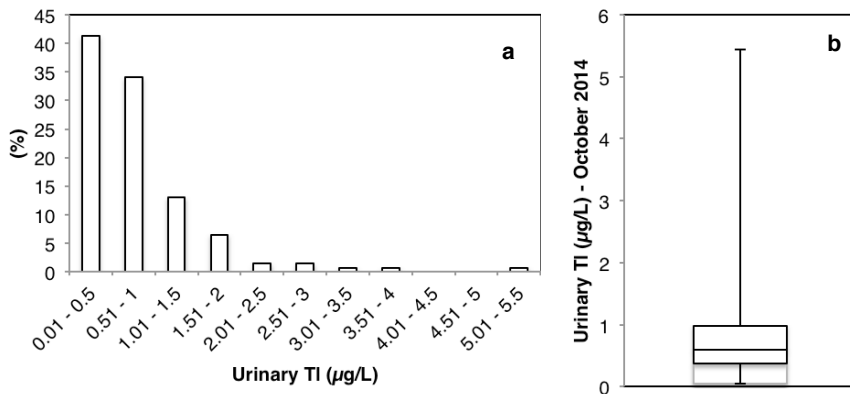

Fig. 6 a) Frequency histograms of the raw data set; b) Box plot of Tl concentration (μg/L) found in the urine of 150 subjects. Middle band, box and whiskers represent the median, 25th and 75th percentile, and the most extreme values, respectively.

Urinary thallium levels (N = 150) ranged from 0.046 to 5.44 μg/L, with arithmetic mean 0.74 μg/L (standard deviation 0.67 μg/L), geometric mean 0.55 μg/L and 95th percentile 1.88 μg/L.

Table 4 summarizes the analytical results of Tl levels determined in 140 urine samples collected during October and November 2014, thus within 30 and 60 days after the Do Not Drink order, respectively. The two groups were significantly different ($p < 0.05$) based upon the non-parametric Mann-Whitney U statistic test (two-tailed p-value).

Table 4. Comparison of Tl concentrations in 140 urine samples collected within 30 and 60 days after the Do Not Drink order.

Range	0.046 – 5.4	0.057 – 2.3
Mean (SD)	0.74 (0.67)	0.47 (0.39)
Geometric mean	0.55	0.37
Median	0.54	0.35
Mode	1.1	0.13
P5[b]	0.15	0.11
P95[c]	1.88	1.16

In the study population, in the months following the end of the exposure, urinary Tl levels decreased in a non-linear pattern fitted by an exponential model, showed in Figure 7. Figure 7 reports indeed the mean value and the standard deviation found in 38 volunteers whose urine was sampled over 12 months after the end of Tl exposure. Thallium content in the urine decreased approximately to 30% in the first 2 months, suggesting a fast release of Tl in the first 1 – 2 months following the end of the exposure and a very slow release in the following months. After one year, mean urinary Tl was still twice with respect to the reference values of the Italian population (Alimonti and others 2005).
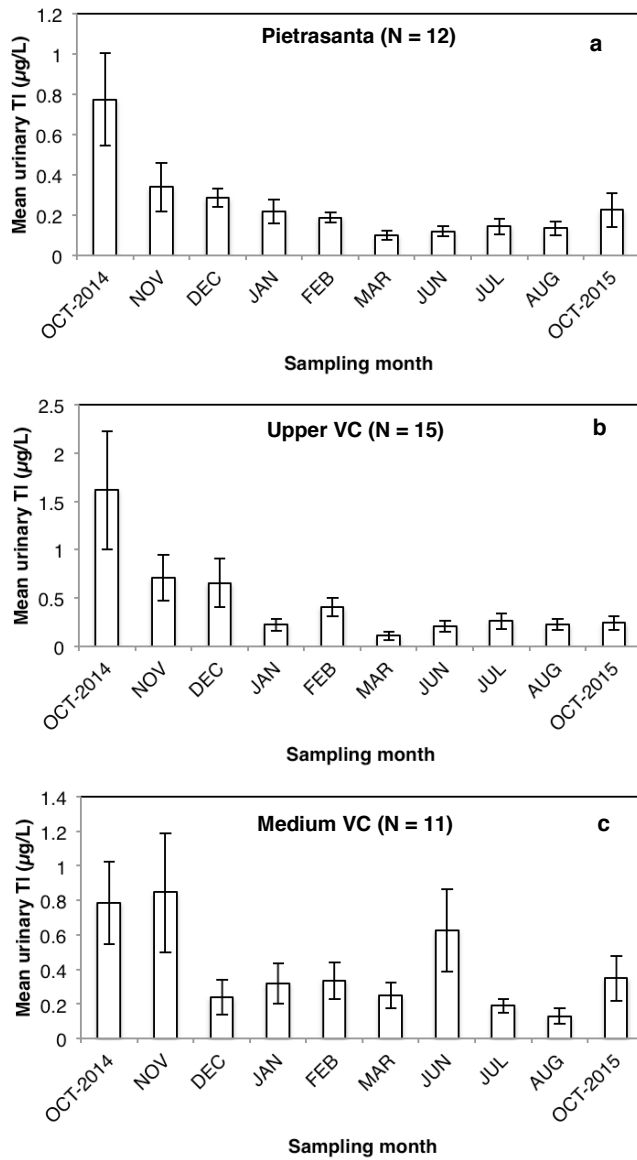
Fig. 7 Pattern of urinary Tl decay from October 2014 to October 2015 in (a) 12 inhabitants from Pietrasanta; (b) 15 inhabitants from upper Valdicastello Carducci; (c) 11 inhabitants from medium

Valdicastello Carducci.

Figure 4b shows the arithmetic and geometric mean of urinary Tl levels of subjects divided according to their area of residence. As observed for Figure 4a, also in the case of urine the upper area of Valdicastello emerged as the major area of exposure. The contamination level in medium, lower Valdicastello and Pietrasanta was not significantly different.

No significant correlation was observed in the simple descriptive statistics between Tl levels in the urine and age or sex. Considering the different extent of Tl pollution in tap water, additional statistical analyses were performed stratifying the population according to the residence area. To detect any differences according to sex and drinking/not drinking habit, the population was divided only into two groups (Valdicastello and Pietrasanta), as population size did not allow stratified analysis according to upper, medium, and lower Valdicastello.

For both females and males results of testing for normality and log-normality (Shapiro-Wilk, Lilliefors, Kolmogorov-Smirnov, for a 95% confidence level) gave evidence that Tl in hair and urine is described by a multinomial distribution.

Several statistical tests (Pearson, Spearman and Kendall) were used to evaluate the strength of the association between Tl levels in urine and hair. Spearman and Kendall tests are necessary for non-normally distributed data. We found a significant positive correlations at 0.05 level between hair and urine (Figure 8, N = 75 available data).
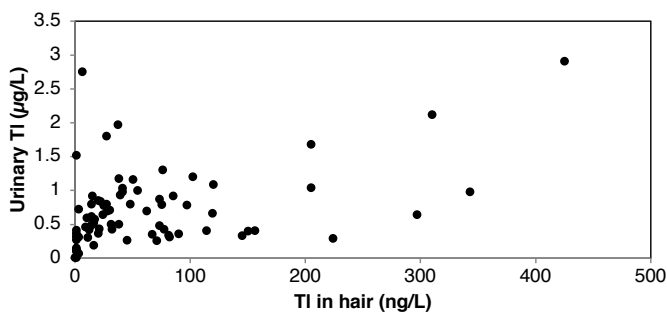
Fig. 8 Correlation plot of Tl concentration levels in urine and hair samples from 75 subjects of the examined population.

4. Discussion

The U.S. Environmental Protection Agency (U.S. EPA) included Tl in the list of the 13 priority toxic pollutants (United States Environmental Protection Agency 2009). Thallium is easily absorbed into the human body by skin contact or ingestion, and it is known to have mutagenic, carcinogenic and teratogenic effects (Léonard and Gerber 1997).

Despite its limited use by industry, Tl was involved in some accidental acute poisonings, reported in several papers (Atsmon and others 2000; Hirata and others 1998; Peter and Viraraghavan 2005; Rusyniak and others 2002; Saddique and Peterson 1983; Schaller and others 1980; Shabalina and Spiridonova 1978). Episodes of contamination as results of natural release of this metal in the environment are instead less documented (Cheam 2001; Staff and others 2014; Xiao and others 2007).

U.S. EPA fixed the maximum contaminant level of Tl in drinking water at 2 μg/L, with the goal of lowering it at 0.5 μg/L (United States Environmental Protection Agency 2009). The maximum concentration level of Tl in tap water is regulated by several guidelines also in other countries, such as China (China National Standards 2006), Canada (Canadian Council of Ministers of the Environment 1999), Mexico and Latin America (Organización Panamericana de la Salud 2001), the European Union (Jørgensen and others 2008) and Russia (Pohanish 2011). In Italy, Tl concentration is regulated only in waste waters (MINISTERO DELL'AMBIENTE E DELLA TUTELA DEL TERRITORIO 2003) and groundwaters (maximum contaminants level fixed at 1 μg/L and 2 μg/L, respectively) (MINISTERO DELL'AMBIENTE E DELLA TUTELA DEL TERRITORIO E DEL MARE 2006). Recently, the European Union financed a COST activity to improve the understanding of the environmental processes and potential health threats of this type of 'exotic' elements, including Tl (Cobelo-García and others 2015).

Previous studies (Biagioni and others 2013) demonstrated that in the dismissed mining areas of Valdicastello Carducci in the Versilia area (Tuscany, Italy) Tl is hosted in base metal sulfides, mainly pyrite, in concentrations up to hundreds of mg/kg. From these sources Tl is readily mobilized to waters during oxidation of the sulfide minerals, reaching up to 1000 μg/L in mine drainages. Thallium is dispersed into the ecosystem via water transport pathways with low attenuation when dissolved Fe(II) starts oxidizing and precipitating as insoluble oxide-hydroxides (Petrini and others 2015).

In the study area Tl-contaminated groundwater was used as supply in the potable water distribution network of Valdicastello Carducci and part of Pietrasanta. Studies are currently in progress to demonstrate that Tl is retained in scale encrustation within the pipeline at wt% concentration and then released to drinking water.

The population was classified into four groups by the extent of water pollution in the places of residence, and the water from the public fountains F1-F6, fed by the same spring water, was considered representative of the tap water distributed in each single house. Water sampling from public fountains F1-F5 was carried out four times on the same days during September 2014.

The analysis of water sampled revealed Tl concentrations ranging from 1.77 to 10.1 μg/L. These values exceed by far the highest level of contamination fixed by several countries.

On October 3rd, 2014 local authorities imposed to not use tap water for drinking and cooking. One month later Tl was found > 1 μg/L also in the tap water in the center of Pietrasanta (3 km far from Valdicastello).

A fundamental step in the evaluation of the negative and cumulative effects of a water pollutant for humans is the measurement of its content in biological samples. Urine and hair represent convenient matrices, due to their straightforward sample collection, sufficient quantities for analysis, lower costs of storage and shipping, and ethical approval for sampling more easy to

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obtain. The exposure to heavy metals and metalloids is reflected in elevated concentrations in the hair, which provides informations about long-term exposure (Gil and Hernández 2015). The distribution profile of trace elements along the length of the hair strand can be analyzed to trace the intake/exposure history of trace elements in individuals. The Agency for Toxic Substances and Disease Registry (ATSDR) has explored human hair analysis as a potential additional tool to assess exposure, and the US-EPA has indicated hair as one of the most important biological materials for worldwide environmental monitoring of elements (Baran and Wieczorek 2013; Harkins and Susten 2003). Hair has also been used by the International Atomic Energy Agency (IAEA) to monitor trends in element levels (Chatt and others 1985). However, although there is reasonable agreement that the qualitative results from hair analysis are valid, the interpretation of the results is still under debate owing to unresolved questions such as the influences of external contamination or cosmetic treatment, and possible genetic differences (Tobin 2005).

In almost 40% of the examined population Tl levels exceeded 30 ng/g. Batista et al. report concentrations ranging from 0.1 to 6 ng/g as reference values for Tl in hair of unexposed population (Batista and others 2009). In their work Violante et al. found 1 ng/g as mean Tl concentration in the hair of 92 children (9 – 10 years old) living nearby a thermoelectric power plant (Violante and others 2000). For people exposed every day to Tl in their workplace, concentrations found in hair could be greater than 900 ng/g (Ciszewski and others 1997).

Young subjects are considered to be more vulnerable than adults to health effects associated with metal contamination, so there is an increasing attention on risks to which they are exposed (Järup 2003). Forty four % of subjects with less than 14 years (59% of the examined population) were highly contaminated, with values ranging from 5 to 150 times above the values of unexposed subjects (0.1 –1 ng/g, see references above).

Trace elements in hair do not always reflect the levels arising only from endogenous uptake, due to the possible contamination by exogenous materials such as soil, dust, and water that might be

adhering to it. Thus, we carried out an optimized washing procedure before analysis to prevent over or underestimation of trace element exposure. To further validate our results, N = 7 samples were analyzed before and after the washing procedure, finding not significant differences in Tl content.

Depending on the length of the hair, it is also possible to map out changes over time. The study of contamination along a 30 cm hair lock (Figure 5) allowed us to hypothesize a chronic exposure to Tl for about 15-20 months before the discover of tap water contamination. Considering that the habits and the living location of the subject involved in the experiment of Figure 5 did not change in the last two years, it is likely to hypothesize that the trend of Tl concentration shows changes in the water thallium levels in the tap water. Recently, the water management institute of Tuscany and the authorities recognized, indeed, the presence of Tl in tap water since June 2011.

Despite hair is not considered a reliable matrix to study metal contaminants, here hair analyses are supported by results from urine. Our work contributes also to establish references ranges for Tl in hair, giving a picture of Tl accumulation in hair when a population is exposed through drinking water.

Whereas hair is advantageous for the assessment of chronic or continuous exposure, urinary excretion of metals represents the amount removed from the body, related to the recent exposure. Urine is indeed not useful for determining whether chronic exposure has occurred to metallic elements except for those with long half-lives (e.g. Cd) (Gil and Hernández 2015). Urinary Tl in human is generally excreted after 10 – 30 days (World Health Organization/International Program on Chemical Safety 1996), though some authors reported a half-life of 3 – 8 days (Talas and others 1983) or 12.5 days (Krahwinkel and others 1988).

We collected urine samples within one month after the Do Not Drink order. Thus, considering the medium half-life of urinary Tl, it is reasonable to state that in this case urine is a reliable matrix to assess the contamination immediately before the end of exposure.

Reference Tl values in urine are reported in a wide range. Recent data (2013) from the general European Union population show Tl concentration in urine of 0.066 μg/L (Ćurković and others 2013). The World Health Organization (WHO) reports, in 1996, as mean urinary Tl concentration values between 0.3 to 0.4 μg/L in unexposed populations and between 4.5 – 6 μg/L in exposed subjects with possible health effects (World Health Organization/International Program on Chemical Safety 1996). In their work Staff et al. establish 0.40 μg/L as 95th percentile reference values for Tl in urine from a sample group not occupationally exposed to Tl (Staff and others 2014). Other works report this metal in the range of 0.02 to 1.0 μg/L (International Commission on Radiological Protection. Task and Snyder 1975), 0.4 to 1.8 μg/L (Smith and Carson 1977) or up to 0.8 μg/L (Dolgner and others 1983).

Thallium mean and 95th percentile in U.S. adult population (more than 20 years old), monitered in 2009 – 2010, are 0.142 μg/L and 0.410 μg/L, respectively (United States Environmental Protection Agency 2009). Thallium mean values are slightly higher in males (geometric mean 0.152 μg/L) then in females (geometric mean 0.137 μg/L). Values found in children and adolescents are slightly higher then in adults (6-11 years old, geometric mean 0.161 μg/L; 12-19 years old, geometric mean 0.150 μg/L) (United States Environmental Protection Agency 2009).

The Italian Society for Reference Values (SIVR), in the list of reference values published in 2011, reports 0.05-0.5 μg/L as range for urinary Tl (5°-95° percentile) and 0.1 μg/L as geometric mean for the italian population (Società Italiana Valori di Riferimento 2011).

In the population examined in this work, 40% had Tl urine concentrations below 0.5 μg/L, 35% had Tl levels between 0.5 – 1 μg/L and in 25% Tl exceeds 1 μg/L. The maximum Tl concentration found (5.44 μg/L) is almost 600 times higher than the Tl urinary mean value reported for the European Union.

The Spearman correlation coefficient for urinary Tl concentration versus age/sex is near zero, indicating no correlation. Contrariwise, the Spearman correlation test indicates statistically significant correlation between Tl in water samples and Tl in urine. With respect to geometric

mean Tl values, the coefficient for water Tl versus urinary Tl is r = 0.989.

For 140 subjects, a second urine sampling was performed within 60 days after the Do Not Drink order. The only habit changed after the order was the use of tap water for drinking and cooking. Thus, the significant difference observed between the urine Tl levels sampled within 30 and 60 days following the Do Not Drink order are due to the Tl excretion from the body.

We also found that Tl elimination from urine of the study subjects (N = 38) is characterized by an exponential function. The fast decay of Tl in the first month following the removal of the source of contamination is probably due to the release from soft tissue (i.e. muscle, kidney, liver) (Rodriguez-Mercado and Altamirano-Lozano 2013), while it is released more slowly from bones. The release of Tl from hair is under study.

4. Conclusions

The present study investigates the accumulation of Tl in a population living in a zone of the northwest of Italy where potable water was contaminated by this metal. Hair and urine are suitable matrices for non-invasive exposure assessments in human population. Our findings indicate that people resident in the contaminated area of Valdicastello Carducci and Pietrasanta significantly accumulated Tl in their urine and hair compared to the reference values of Italian population. Furthermore, Tl levels in hair and urine have proven to be positively correlated to each other.

The investigation on saliva matrix is in progress, as well as Tl interaction *in vitro* with thiolic compounds (glutathione, cysteine, proteins) and its effects on cell cultures. Another aspect to be explored is its speciation and the toxicity of Tl species.

Thallium is an emerging pollutant. Its presence with other very toxic, heavy metals (such as berillium) close to mines is proven. The widespread use of Tl, and its subsequent release into the environment, has led to an increase in Tl levels in several ecosystems and trophic chains, increasing

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its exposure to humans and other living organisms. As no regulation exists from EU Authorities, this issue has to be carefully considered.
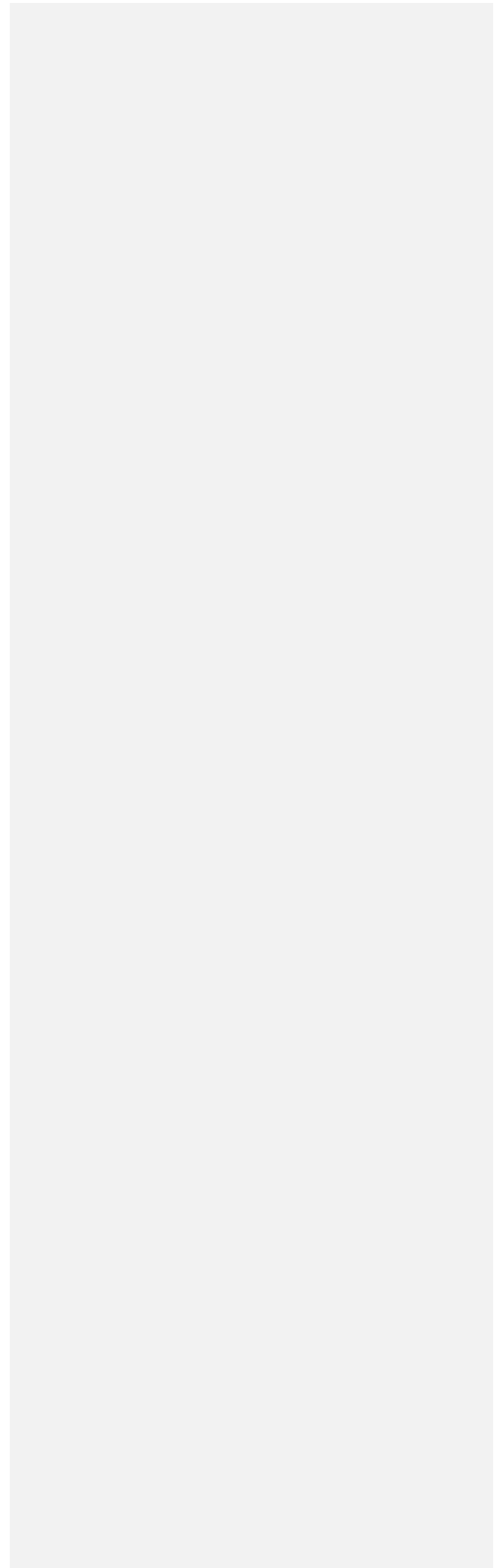
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