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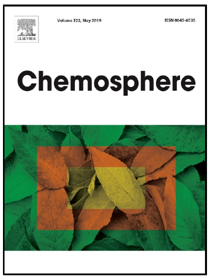

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# Human biomonitoring health surveillance for metals near a waste-to-energy incinerator: the 1-year *post-operam* study

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## Abstract

This human biomonitoring (HBM) follow-up survey, within the SPoTT project, assessed the temporal and spatial trends of exposure to 18 metals in a cohort living around the waste-to-energy (WTE) incinerator of Turin (Italy) before (T0, 2013) and after 1-year of plant activity (T1, 2014). Urine of 380 adult individuals (186 exposed and 194 unexposed subjects, classified on fallout maps) were analyzed by sector field inductively coupled mass spectrometry. A decrease trend of the majority of metals in all subjects indicates that the overall air quality of the studied sites was not significantly compromised, also in proximity of the WTE plant, as corroborated also by air monitoring data of the regional agency. The only relevant exception was the higher Cr levels found at T1 than T0 in exposed subjects, suggesting a possible contribution from the WTE plant. Chromium, Mn and Pt urine levels were also higher in the site far from the WTE, in relation to other sources as vehicular traffic, industrial and civil activities. Whilst, As and Cd were influenced by fish intake and tobacco smoke. A very small number of individuals at T1, equally distributed in both areas, exceeded the health-based guidance values and so, at current knowledge, living near the Turin incineration did not significantly influence the exposure status of the population.

***Keywords***: waste-to-energy (WTE) incinerator, follow-up human biomonitoring (HBM), metals, exposure assessment, Health Based Guidance Values (HBGVs)

## 1. Introduction

A recent study commissioned by the European Environment Agency (EEA) showed that the incineration capacity in the EU-28 countries increased by 6% to 81 Mt/year between 2010 and 2014 (Wilts et al., 2017). In Europe, the Municipal Solid Waste (MSW) average production was 480 kg per person in 2016, and in Italy the production lied above the EU average value (497 kg/pp/year) (http://ec.europa.eu/eurostat/statistics-explained/index.php/Municipal_waste_statistics#Municipal_waste_generated_by_country). The waste-to-energy (WTE) facilities offer effective solution to convert a MSW from a pollution source to a renewable energy resource; even though in 2013 only close to 2.5 Mt of waste was shipped for energy recovery. Thus, in the last years, great efforts towards a better exploitation of technical potential of WTE plants have been prompted by the EC (Saveyn et al., 2016). To date, more than 200 WTE plants are active in 14 European countries; in Italy there are 41 incinerators including the newly built Gerbido WTE plant in the Turin area which is one of the biggest facility in Europe (European Commission, 2017). The plant treats both household and special waste (421 Mt/year with an electricity generation capacity of 350,000 MWh/year), providing to meet the needs of 175,000 homes (Bena et al., 2016a; Bena et al., 2016b).

The Waste Incineration Directive (WID) 2000/76/EC and the newer Industrial Emissions Directive (IED) 2010/75/EU looks to achieve significant levels of environmental and human health protection by setting very strict operational and technical limit values for various parameters, including metals (European Commission, 2000; European Commission, 2010). The IED was transposed in Italy by the Legislative Decree 46/2014 and, regardless of the waste incineration technology, compliance with the emission standards for metals like arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), antimony (Sb), thallium (Tl), and vanadium (V) is required (Decree-Law 46/2014).

Although WTE plants are monitored by governments, the local residents show strong opposing public opinion, especially in cities with high population densities (Ren et al., 2016). Nevertheless, this adverse incineration risk perception, the WTE technology can be a climate- and environmental-friendly solution as compared to conventional incinerators (Seltenrich et al., 2016). In the SPoTT project, the concern arising from population has encouraged local health authorities and the scientific community to study the real exposure arising from WTE plants, including also the assessment of risk perception of the population and the maintenance of a constant dialogue through a local control committee (Bena et al., 2016a; Bena et al., 2016b). Regarding local risk perception, a questionnaire was submitted to SPoTT participants with specific questions mainly designed to investigate the degree of concerns of the population about environmental and health risks. Residents living closer to the incinerator showed greater concern than more distant ones, especially with regard to anthropogenic and natural air and water pollution, but also to waste management. Moreover, population believed that environmental pollution definitely causes acute and chronic diseases and the exposed people considered themselves to be much more at risk than unexposed (Bena et al., 2019).

Among different approaches for exposure assessment, the human biomonitoring (HBM) allows to evaluate the internal dose of metals in people living around WTE incinerators, laying the basis for a major awareness on health risk/benefit of this strategy.

In this context, the SPoTT (Italian acronym for *Population health Surveillance in the Turin incinerator's area*) surveillance program used the HBM approach to detect 18 metals and their temporal trends in urine of a cohort of individuals living around the WTE incinerator of Turin. The rationale of the SPoTT program as the population recruitment criteria, questionnaires, and fall-out maps has been previously described (Bena et al., 2016).

The SPoTT cohort was prospectively followed by two measurements over time. The first time measurement (T0, 2013) corresponded to the baseline survey before the cohort was exposed to WTE plant emissions and represented the reference values (RVs) for 18 metals in urine and Pb in blood (Bocca et al., 2016). The second time measurement (T1, 2014) represented the potentially exposed SPoTT cohort after 1-year of the WTE plant activity. The same metals previously investigated in T0 survey were analyzed in this follow-up study using the same analytical method validated at T0; collection of samples was limited to urine, avoiding the invasive and less practical blood collection; and, a questionnaire was given to participants with the aim to update personal information including residence, occupation, diet and lifestyles. Data of the cohort at T1 were compared to the baseline (T0) to highlight differences in metal levels according to exposure to the WTE incinerator, considering also the influence of individuals' habits. Health assessment of the cohort before and after 1-year of WTE activity was also evaluated by comparison of results with the available health-based guidance values (HBGVs).

## 2. Methods

### 2.1 Population and methodology

The population recruited at T0 (2013) was invited to participate at T1 survey (2014). In Figure 1 is shown a flow-chart of the SPoTT enrollment activity, including the time of recruitment and sampling, percentage of refusals, and when the WTE plant started to operate. Individual exposures were recorded by the classification used in T0 survey and based on previously described fall-out maps- namely, the unexposed area (Area 1) with people residing in the range 0-0.007 mg/m$^2$/year of annual deposition of metals and exposed area (Area 2) with residing in the range 0.014-0.11 mg/m$^2$/year (Bena et al. 2016a, Bocca et al., 2016). A total of 380 individuals - 186 individuals in Area 1 and 194 in Area 2 - agreed to participate in the second round.

The used methodology was the same as that of the baseline study (Bocca et al., 2016). As regards number of participants involved, the sample size was selected so as to obtain differences in metal content below 20% with an α error of 0.05. Potential variability of the population was appropriately characterized by the use of questionnaires tracking any resultant inter- and intra-individual variability (like age, gender, education level, social class, residence, occupation, alcohol consumption, smoking, food consumption, etc.).

Morning urine spot samples of participants were collected between June and September 2014 and stored at -20° until analysis. Eighteen metals including As, Be, Cd, Co, Cr, Cu, Hg, Mn, Ni, Sb, Sn, Tl, V, Zn and the Platinum Group Elements (PGEs: Ir, Pd, Pt, Rh) were measured in urine samples by the sector field inductively coupled mass spectrometry (SF-ICP-MS, Element2, ThermoFisher, Bremen, Germany). The LoDs in urine were the following: 0.01 µg/L for Cd, Cr, Sb, V; 0.02 µg/L for Co, Mn; 0.03 µg/L for Tl; 0.04 µg/L for Be, Sn; 0.12 µg/L for Ni; 0.25 µg/L for Hg; 0.30 mg/L for Cu; 0.92 µg/L for As; 2.0 µg/L for Zn. As regards the PGEs, limits were as follows: 0.50 ng/L for Ir; 1.34 ng/L for Pt; 7.7 ng/L for Pd; 6.7 ng/L for Rh.

Regarding the quality assurance (QA) scheme, it included the analysis of internal and external quality control (IQC and EQC) samples (Ruggieri et al., 2016). In particular, IQC samples consisted of Certified Reference Materials (CRMs), namely the Lyophilized Human Urine at Level 1 and Level 2 (Sero AS, Billingstadt, Norway) or in-house spiked urine samples for the analysis of PGEs that were not certified in the above-mentioned CRMs. These IQC samples were analyzed concurrently with test samples at a frequency of one per 20 samples during the sequence to monitor daily recovery and repeatability. Recoveries were between 90-110% and repeatability was better than 20%. Moreover, the method was controlled over-time by analyzing EQC samples provided by the Italian External Quality Assessment Scheme (EQAS) in the field of occupational and environmental medicine (OELM). Regarding uncertainty of measurements, it was calculated for each urinary metal using QA/QC data and applied to each SPoTT urine sample (Ruggieri et al., 2016). The method used obtained the accreditation according to the ISO/IEC 17025 standard by the Italian accreditation body (Accredia).

### 2.2 Statistical analysis

Data treatment, already utilized in T0 study, was applied: i) urinary metals were normalized by the specific gravity (SG); ii) for values below the limit of detection (LoD), the LoD/2 values were used; and iii) outliers, defined as samples higher than [Q3+3×(Q3-Q1)], where Q3-Q1 was the interquartile distance - were excluded from the statistical analysis (Bocca et al., 2016). Due to not-normality of the distribution of metal concentrations, median and 95th percentile values were used to describe the data and non-parametric tests were applied. The Mann-Whitney U test was used to compare the two areas of exposure at T1 and the Wilcoxon signed-rank test to compare observations over time (survey T0 - 2013 and T1 - 2014). The role of fish consumption (the day before and the week before sampling) and smoking habit (non-smoker: cotinine <14.0 ng/mL; smoker: cotinine ≥ 14 ng/mL) were also examined. A result was considered statistically significant if associated with a p value <0.05. Analyses were conducted using the statistical package IBM SPSS Statistic 24.

## 3. Results and discussion

### 3.1 Rating of the follow-up study

Of the population re-contacted at T1 (Figure 1), the respondents were 186 in Area 1 on a total of 196 at T0, so only the 5.1% declined to adhere to the new study. In the Area 2, the exposed one, the rate of participation at T1 was even higher (194 respondents on 198 at T0) with very few cases of refusals (2.02%). The time of recruitment was between June and September both at T0 and T1.

Comparison of characteristics of SPoTT population enrolled in T0 and T1 phases is reported in Table 1. On a total of 394 recalled T0 subjects, only 14 individuals (3.55%) in both areas declined to take part in T1 phase for different reasons (i.e., untraceability of the subjects or loss of interest towards the study). Usually, unbiased results are minimized if the overall follow-up rate reaches 80% or more of subjects whose exposure is measured at baseline (Padula et al., 2017); in this study more than 95% of eligible persons contributed to the second round program, therefore there was an appropriate representativeness of the sample and not-respondents and/or refusals were indeed not substituted. Those subjects which changed area of residence were not enrolled. Sex balance was achieved (191 women and 189 men) though a slightly higher amount of women rather than men (4.02% *vs* 3.08%) declined to participate at T1 survey. A higher percentage of older people (51-69 ys: 4.13%) than younger (36-50 ys: 2.84%) did not take part to T1 campaign. Regarding areas of exposure, the more active participation in the second survey was obtained for the exposed people (Area 2) of both sexes and age classes, being probably more motivated than the unexposed population (Area 1).

With concern to tobacco smoking, the number of smokers were lower in T1 respect to T0 (18% *vs* 30%). People who consumed fish during the day before urine collection were fewer in T1 respect to T0 survey (11% *vs* 53%), as well those who consumed fish during the last week (67% *vs* 87%). The decrease of fish consumers might reflect a major awareness of participants towards the study and/or a great capability of the SPoTT team of motivating the enrolled subjects to follow the advice of avoiding to eat fish the last day before sampling.

Table 2 shows that more than 90% of samples in both T0 and T1 studies were above LoD values, thus reaching a sufficient number of cases to address and compare the exposure assessment at the two monitoring periods (LaKind et al., 2014). In addition, the number of samples below LoDs increased in T1 respect to T0 (0-5.3% vs 0-9.2%), and the major number of values <LoDs was obtained for PGEs found in urine at ultra-trace levels (order of ng/L or fractions).

### 3.2 Spatial and temporal exposure to metals

Table 2 reports the median and 95th percentiles of urine content in the SPoTT cohort at T0 and T1 monitoring periods by exposure areas (Area 1 and 2).

Comparing spatial distribution of metals at T1, significantly higher Cr levels in people living in Area 2 ($p<0.0001$) and higher Mn levels in those living in Area 1 ($p=0.0017$) were observed.

Comparing the cohort at the two time measurements, the analysis showed a general significant reduction after 1-year of WTE plant activity for As, Be, Cd, Cu, Hg, Ir, Mn, Pd, Rh, Sb, Sn, Tl, V and Zn; also Co and Ni decreased but not significantly. Chromium was significantly higher in T1 respect to T0 (with an increment more marked in Area 2) and Pt showed levels 1.3-times higher in T1 respect to T0, but only in Area 1.

The percentage variation on median values ($Var\% = \frac{(T1 - T0)}{T0} \times 100$) between the two measurement times (Figure 2) clearly demonstrated the declining trend of metals in the whole group and in both exposure areas, with a reduction of 40-60% for some of them (as As, Cd and Sn). Only Cr showed an increment of 25% in the total population, 23.7% in the exposed subjects (Area 2) and 14.3% in those unexposed (Area 1), while, the increment for Pt was equal to 26.6% in the far area (Area 1).

The HBM data showed urinary levels of metals not significantly associated with the activity of the WTE plant because similar concentration for the majority of metals were detected in both areas; to the opposite, quite all metals were found decreased after the plant started to operate. The only potentially associated metal with the incinerator was the urinary Cr whose levels was significantly higher in exposed subjects (Area 2) compared to unexposed ones (Area 1); moreover, Cr showed an increase over time between T0 and T1 study principally in the Area 2 (Figure 2). The urinary Pt showed a significant increment during T1 monitoring period only in the unexposed area (Area 1) (Figure 2). A significant increment of Mn and a slight increase for other metals like As, Cd, and Rh were found in the unexposed area respect to that exposed, even if, all these metals showed a decreasing trend at 1-year distance.

The Regional Agency for the Protection of the Environment (ARPA Piemonte) collected $PM_{10}$ samples in air monitoring stations located in the area of the WTE plant (Area 2) and inside urban settings (Area 1) in the same two periods (2013 and 2014) of the SPoTT urine campaigns (ARPA, 2013; ARPA, 2014). Reports by ARPA described amounts of Cd, Co, Cu, Ni and Zn lover in $PM_{10}$ samples collected during the WTE plant activity (2014) than 1-year before (2013), in both areas. On the contrary, the ARPA air monitoring showed higher Cr levels in $PM_{10}$ sampled during the second follow-up study rather than 1-year earlier (ARPA, 2013; ARPA, 2014). In Figure 3, metals measured in air and urine were compared; the Figure shows, in both Areas 1 and 2, a decreasing trend at T1 respect to T0 for all metals in $PM_{10}$ samples consistent with the metal profile in urine, except for Cr. The ARPA also monitored the levels of As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, Sb, V, Zn in water intended for human consumption in the municipalities within the Areas 1 and 2 (data are not shown). None difference was detected between the two monitoring periods (2013 and 2014) in drinking water and the decreased urine metal levels observed in the SPoTT cohort could not be linked with water (http://www.arpa.piemonte.it/dati-1).

In previous literature, the concentrations of metals in biomonitoring samples collected in people living near newer incinerators are so low that they were not different from control people. In Italy, none of the metals determined in urine (As, Cd, Cr, Cu, Hg, Ni, Pb Sn, Tl, V and Zn,) showed a clear relationship with the MSW incinerator exposure (Gatti et al., 2017). In Spain, blood levels of Cd and urinary Hg and Cr were comparable both before and after 2-years of MSW incinerator and between people living near and far from the plant (Gonzalez et al., 2000). Similarly, in 10-years follow-up studies in Spain, decreasing levels of many metals (As, Be, Cd, Hg, Mn, Ni, Pb, Sn, Tl and V) were found in hair and blood of subjects living near a hazardous waste incinerator from 1998 to 2007 (Ferré-Huguet et al., 2009; Nadal et al., 2005). Another study revealed a significant reduction of blood Cd, Hg and Pb concentrations in residents near two different MSW plants in Portugal in succeeding observational periods (Reis et al., 2007a and 2007b). In Bilbao, the over-time levels of blood Pb and urinary Cd, Cr and Hg were similar between 2006 and 2008 and between areas close and far from the MSW incinerator (Zubero et la., 2010).

Regarding Cr, the increment of this metal in airborne particles around MSW incinerators - covering different abatement system technologies - was observed in the last decades in several countries as Denmark, Portugal, China and UK (Astrup et al., 2005; Quina et al., 2008; Hu et al. 2003; Tian et al., 2012; Font et al., 2015). In hair of children, a significant increase of Cr was found during the 10 years follow-up study conducted in Spain near a hazardous waste incinerator (Ferré-Huguet et al., 2009).

Metals urinary profile of the SPoTT population were comparable to those found in a cohort of 2000 people living in a city of central Italy (Civitavecchia) with urban and industrial environment (Ancona et al., 2016); the only exception was the 2-fold higher level of Cr in SPoTT subjects at T1 (0.20 μg/L) than those in Civitavecchia (0.13 μg/L) suggesting the WTE plant emission might be an additional source of Cr.

At the same time, the increment observed for urine Cr and Pt in the area far from the WTE (Area 1; Figure 2), means that these metals are also emitted from a mixture of environmental sources, as industrial effluents, civil activities and traffic vehicular.

As concern Pt, although no environmental data on its content in $PM_{10}$ samples are available, a greater amount of $PM_{10}$ was observed in the Area 1 (urban area) respect to Area 2 (incinerator area) (ARPA, 2014). Both in Italy and many other European cities the abundance of Pt was found significantly associated to the size of the population and number of vehicles rather than industrial emissions (Jackson et al., 2010; Bocca et al., 2003; Gómez et al., 2002). In an Italian study, a significantly higher concentration of urinary Pt was found in subjects living in the metropolitan area than those living nearby a plant that recycles and refines precious metals including Pt, defining the urban traffic pollution as the main source of this metal (Chellini et al., 2017).

This study also found a significantly higher urine Mn in T1 subjects living in the far area (Area 1) with respect to Area 2. The Mn values were therefore not likely the result of higher WTE plant emissions, but higher background Mn concentrations from natural and/or anthropogenic sources. The general population may be exposed to Mn through consumption of food and water, inhalation of air, and dermal contact with air (ATSDR, 2012). In 2014, the ARPA evaluated the Mn content in $PM_{10}$ highlighting a greater Mn content in samples collected in urban settings (Area 1) than in those collected near the WTE plant (ARPA, 2014).

Regarding the other metals, all were found lower in urine in the second sampling campaign respect to the first one, and two of them (namely Co and Ni) remained comparable. These metals are present as a mixture in the atmosphere and in foods and water, or they can came from personal habits. For example, urinary Co was found to be significantly associated with the use of jewellery; Zn with alcohol consumption; Ni to the wearing of piercing; Pd with dental restorations (Bocca et al., 2016). However, based on the findings achieved so far, they were not actually associated with 1-year of incinerator activity.

### 3.3 Exposure to other variables

Controlling the factors of variability is a critical issue in HBM studies; among them, diet, smoking and habits in general are those that can mainly affect and modify the internal dose of metals (Skelly et al., 2012). Differences in exposure by several variables were previously assessed at the baseline scenario of the SPoTT population (Bocca et al., 2016); for this reason, a new questionnaires collection was made in T1 survey to account for these variables. Furthermore, the study period was the same in the two surveys (i.e., summer season), as other external exposure sources might vary by seasonal period and weather conditions (mainly traffic and heating). Diet, educational level, occupation, drug/supplement use, domicile, presence of implants or amalgam fillings, alcohol intake and other variables were not changed over time; this was expected because a very little variation on personal lifestyles generally happens in 1-year. Some differences (Table 1) were found indeed for smoking and fish consumption (1-day or 1-week before sampling) and a stratified analysis for controlling the contribution of these variables was performed.

Regarding fish consumption only As showed significantly differences at T1 sampling; As urinary excretion was higher in subjects who ingested fish the day before urine collection in both areas of exposure (Figure 4a); it was also significantly higher in those who ate fish 1-week before urine sampling (Figure 4b). The lack of difference for urinary Hg at T1 with fish intake - neither for last-day nor for last-week consumption in both areas – supported the knowledge of urinary Hg level as indicator of exposure to inorganic form rather than that organic assumed from fish (Ruggieri et al., 2017).

Smoking habits was associated to urinary content of Cd, Cu, Sb, Sn, and Tl at T1 survey. Regarding Cd (Figure 5), smokers had levels higher than non-smokers in both areas, as previously obtained in the baseline survey, and confirming the tobacco smoke as a key determinant in the Cd body burden (Bocca et al., 2016). Smokers living in the exposed area (Area 2) had higher Cu ($p<0.018$), Sb ($p<0.004$), Sn ($p< 0.024$), and Tl ($p<0.009$) levels than non-smokers. All of these elements have been recognized in tobacco smoke (Stohs et al., 1997; Richter et al., 2009),

### 3.4 Comparison with health-based guidance values (HBGVs)

The HBM data obtained in the SPoTT population at the baseline and after 1-year of the plant operation were compared with health-based guidance values (HBGVs). These values correspond to biomarker concentrations consistent with exposure levels and are derived by population surveys and/or epidemiological and toxicological studies (Ruggieri et al., 2017). For some metals (As, Cd, Hg and Tl) do exist different HBGVs: 1) the German HBM-I and HBM-II values for adults based on epidemiological data on human toxicity (Apel et al., 2017; Schulz et al., 2011); 2) the Biomonitoring Equivalent (BE) values based on the acceptable level of exposure calculated by the chronic ATSDR minimal risk level (MRL) or US EPA reference dose (RfD) (Hays et al., 2008; Hays et al., 2009). The German HBM-I value is used as primary HBGV and exceeding this value implied that an investigation of potential sources of exposure should be undertaken and exposure to such sources should be minimized. The HBM-II value is used as secondary HBGV in order to establish the exposure level potentially associated to an increased risk of harmful effects for which immediate actions should be considered (Apel et al., 2017). In Figures 6-9, urine data (median and dot plot values) of the SPoTT population benchmarked against the HBGVs at the two monitoring periods are reported.

Regarding As, the total content in urine accounted for the sum of the organic As (less toxic) assumed mainly by fish and inorganic As much more toxic and assumed generally by other routes of exposure (e.g., drinking water, diet, environmental pollution). In the absence of fish consumption, levels of total As >50 µg/L are at slight risk as reported by the ATSDR; values >100 µg/L are considered abnormal (ATSDR, 2007). Studies on general populations suggested that health risks, like peripheral vascular disease and skin lesions, may be associated with total As urinary levels >50 µg/L (Valenzuela et al., 2005; Tseng et al., 2005; Caldwell et al., 2009). Among subjects (no. 120) (Figure 6) that avoided fish 1-week and 1-day before urine collection in T1 survey, only 1 subject had total As values >50 µg/L, and none of them >100 µg/L. In fish consumers, 1.5% of last-week consumers and 7.3% of last-day consumers had urine As >50 µg/L, confirming fish as a major contributing source of organic and non-toxic As in this subgroup. The higher As excretion in recent fish consumers reflected the short biologic half-life of this metal (less than 20 hours) and its rapid urinary clearance (≤ 48 hours) after ingestion (ATSDR, 2007).

The application of the HBM-I value (1.0 µg/L) to urinary Cd concentrations, 10% of the population at T1 survey (12% in Area 1 and 9.3% in Area 2) exceeded this value (Figure 7). In the cohort stratified by smoke, 7.8% of non-smokers and 15.5% of smokers exceeded the HBM-I threshold value, indicating that smoking habits contributed two-fold more than other sources to the internal dose of Cd. On the contrary, Hg and Tl were lower than their HBM-I values (7.0 µg/L and 5.0 µg/L, respectively) (Figures 8 and 9). The use of HBM-II values (much more higher than HBM-I values) for Cd and Hg showed that none of the participants did exceed these thresholds (Figures 7 and 8) and so the exposure was far from potential harmful health effects.

According to BE values, acceptable levels for urinary Cd were estimated equal to 1.2 µg/L and 1.5 µg/L corresponding to the ATSDR MRL and US EPA RfD. The first threshold indicated low to medium priority for exposure assessment follow-up; while the second threshold correlated to the critical Cd concentration in the renal cortex. This last issue because the concentration of Cd in the renal cortex was believed to be the critical dose metric associated with Cd-induced proteinuria, and urinary Cd levels were highly correlated with renal cortex Cd concentrations (Hays et al., 2008; Hays et al., 2009). The BE of 1.2 μg/L revealed that 4.2% of the population (4.8% in Area 1 and 3.6% in Area 2) exceeded this value (Figure 7) and so additional studies on pathways and aspects that might affect the exposure should be carried out. The more critical Cd-BE of 1.5 μg/L was exceeded by 2.9% of the population (3.2% in Area 1 and 2.6% in Area 2) indicating that exposure - in this part of population - did not guarantee protection against an increased risk of Cd-proteinuria. Stratifying for smoking habits, 1% of non-smokers and 5.6% of smokers exceeded the Cd-BE of 1.5 μg/L, again confirming smoke as a relevant influencing factor.

Moreover, a lower frequency of metals' exceedances respect to the HBGVs was found in T1 respect to T0 survey (Figures 6-9), indicative of an improvement of the exposure status of the Turin population. In the case of As, the frequency of exceedances from the cut-off value for not-fish consumers was 10-folds lower in T1 compared to T0 (0.8% *vs.* 10%); for Cd the exceeding of HBM-I value were 3-fold lower than in the previous campaign (10% *vs* 30%); and in the case of Hg a percentage (3.0%) of exceedance respect to the HBM-I was observed only in the first measurement survey and not in the second one.

At last, because the extent of exceedance values for As and Cd were very comparable between subjects living in the area near and far from the WTE plant (Figures 6-7), the residing in the vicinity of the Turin incineration did not significantly impact the exposure to these metals.

## 4. Conclusions

In this follow-up study the exposure to 18 metals was evaluated in the adult SPoTT cohort living around a WTE plant before (2013) and after 1-year of operation (2014). The high follow-up participation and the ICP-MS method sensitivity used for sample analyses allowed to reach quality and reliability of the HBM surveillance program. It was also beneficial to have a not-exposed area in order to evaluate not only temporal but spatial trends as well. Findings revealed that the majority of urinary metals were not associated neither with 1-year of plant emissions nor with the distance from the WTE incinerator; to the opposite a declining trend between 2013 and 2014 was observed in both exposed and unexposed areas. These data indicated that total exposure to metals decreased over two consecutive years also supported by the general decrease in metals' levels in the air compartment, as measured by the regional agency. A marked increment in urine concentration of Cr was observed in the exposed area before and after 1-year of WTE activity. There were also increments in Cr, Mn, and Pt urine levels in the area far from the WTE. These variations observed might be due to the multi-pathway (industrial, traffic, civil) exposure to these metals to the local residences. Comparing data with the available health-based guidance values (HBGVs), only few individuals reached urine levels of As and Cd of attention, but in this subgroup the exposure was much more related to fish consumption and smoke. In general, a lower frequency of metals' exceedances respect to the HBGVs was found in 2014 than 2013 in both exposed and not-exposed areas, indicative of an improvement of the exposure status of the Turin population.

Notwithstanding this study represents the first one performed in Italy with a longitudinal design, several open issues should be considered. Firstly, it could be supposed that the prolonged activity of the Turin's WTE plant will lead to deterioration and, eventually, to potential increments of the population exposure to facility emissions. Secondly, evaluating the exposure by a single time point and a single biomarker concentration (especially for not persistent metals) - which was a compromise between validity and facility of participants' recruitment - might not completely underline the metals' behavior over time. Basing on these issues, another observation period has been performed after 3-years from incinerator activity to update spatial and temporal trends of metals exposure and data are under evaluation.

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## Conflict of interest

The authors disclose no actual or potential conflict of interest.

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Figure 1. Flow-chart of the SpoTT follow-up study

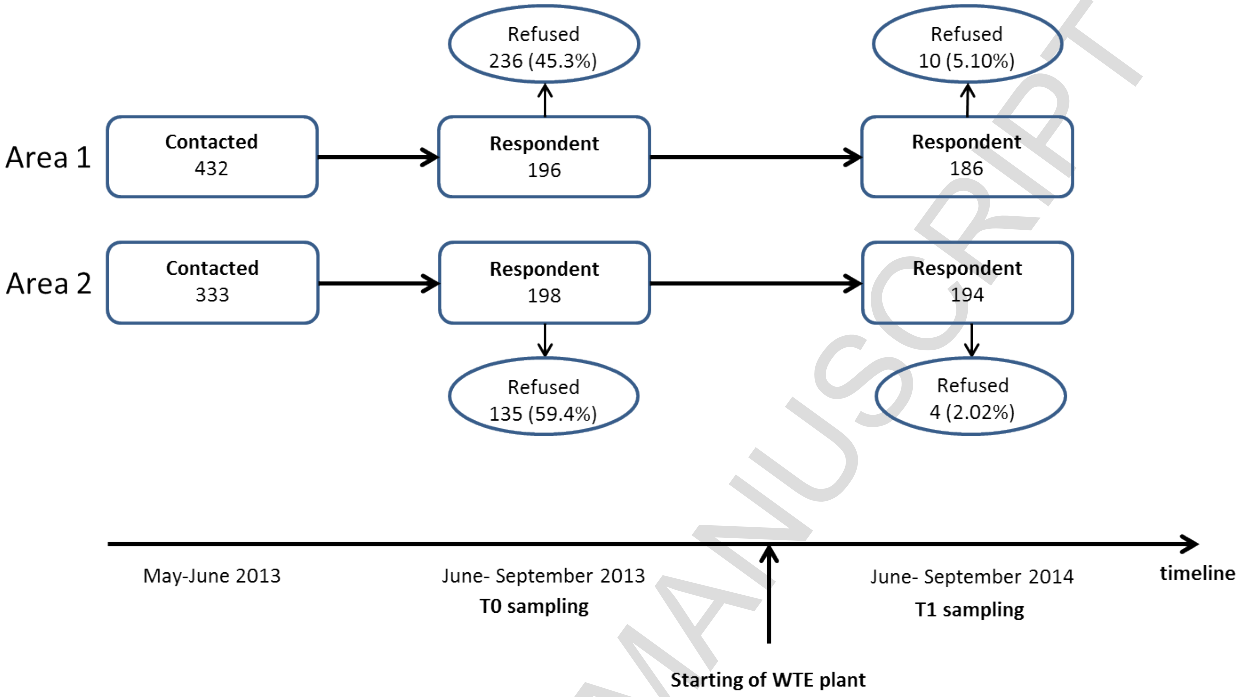


**Figure 2. Percentage variation (Var%) of median values from T0 to T1 by exposure area**

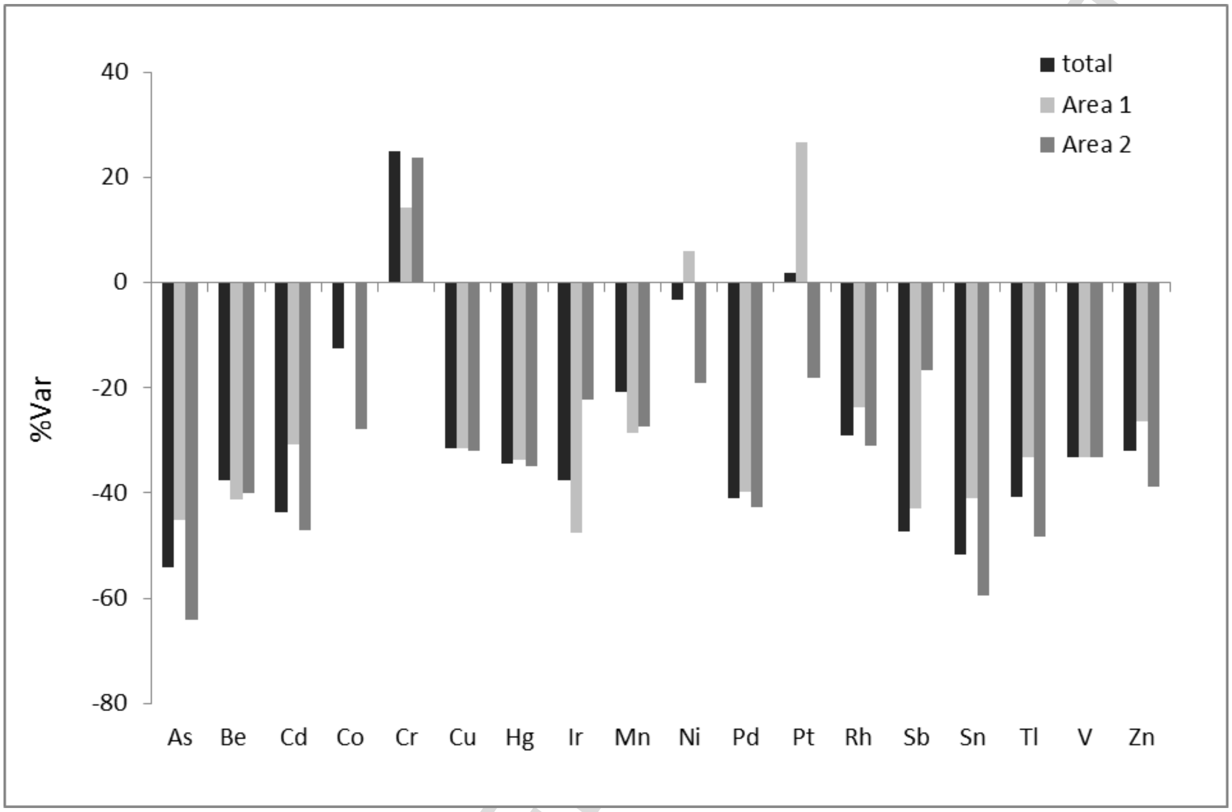


**Figure 3. Comparison of percentage variation ( Var%) of As, Cd, Co, Cr, Cu, Ni, and Zn in urine and in $PM_{10}$ (ref. ARPA, 2013 and 2014) from T0 to T1 by exposure area.**

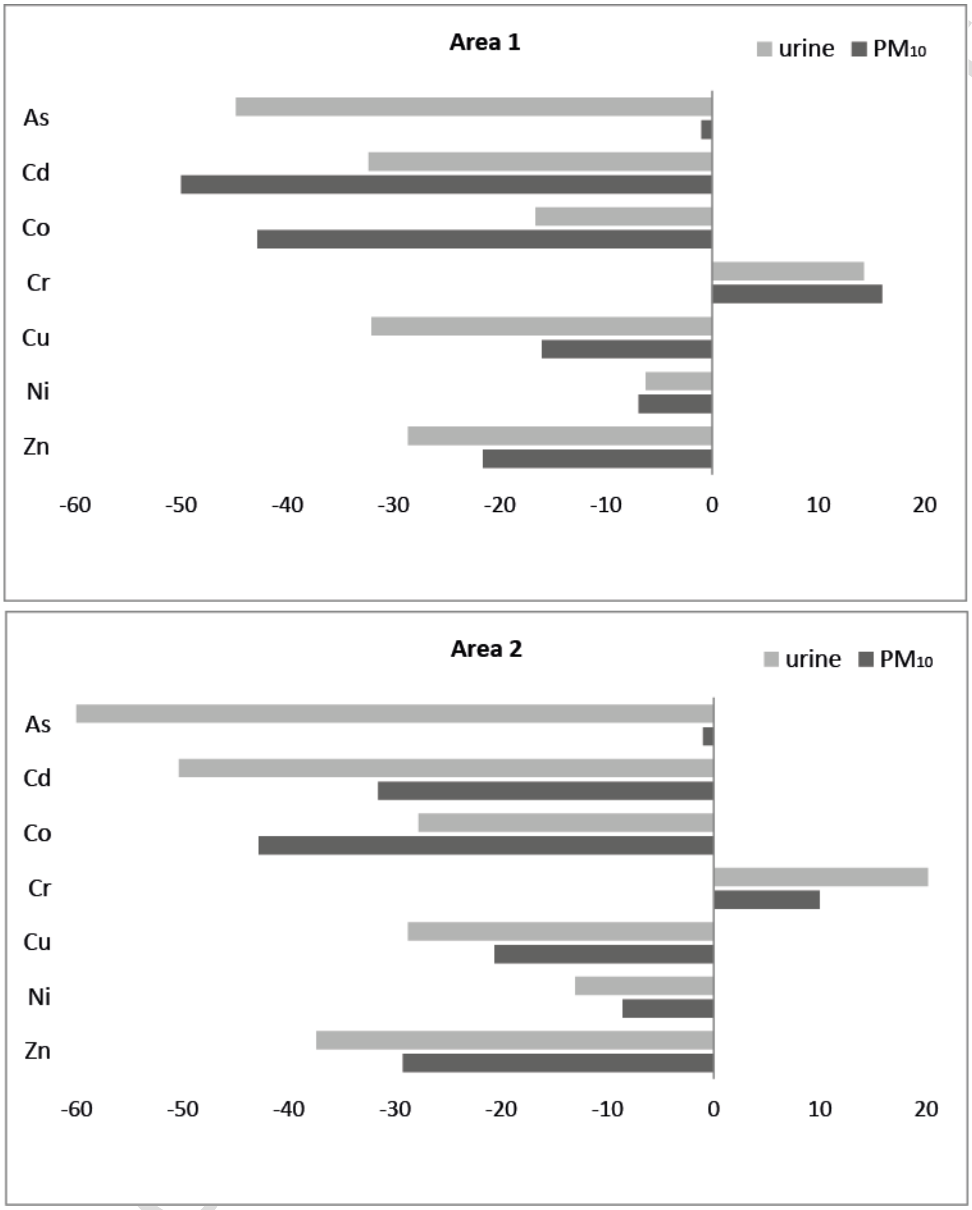


**Figure 4. Urinary levels of As (μg/L) at T1 by fish consumption of the day (a) and week (b); p-value by Mann-Whitney test.**

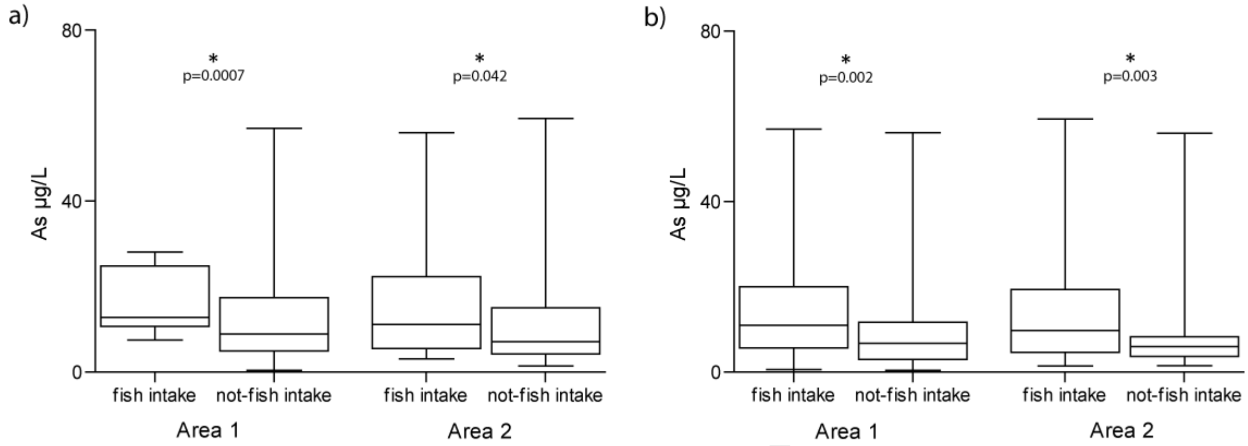


**Figure 5. Urinary levels of Cd (µg/L) at T1 by smoking habit; p-value by Mann-Whitney test.**

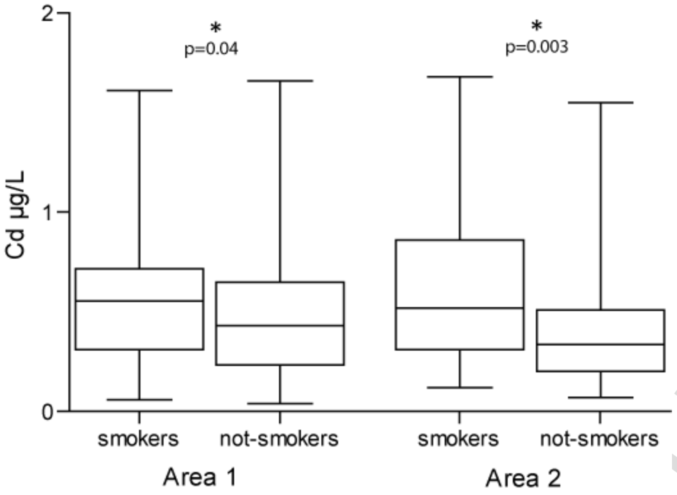
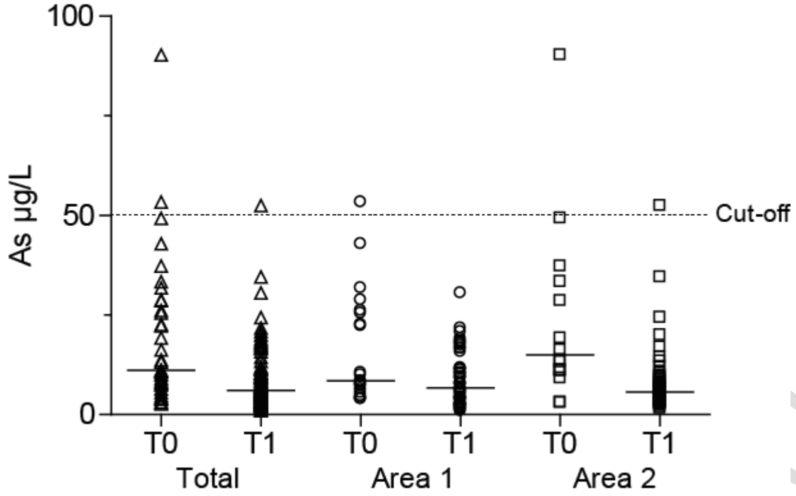
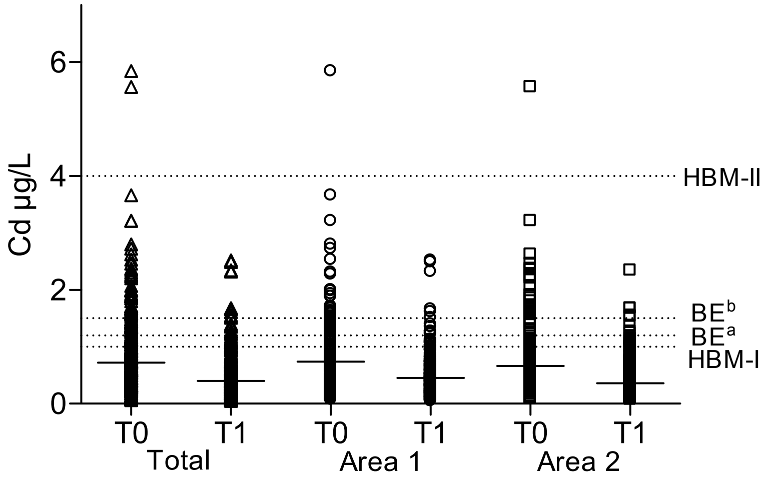


**Figure 6. Urinary levels of As (µg/L) in not-fish consumers benchmarked against the Health-Based Guidance Values (HBGVs) by exposure area and monitoring period**



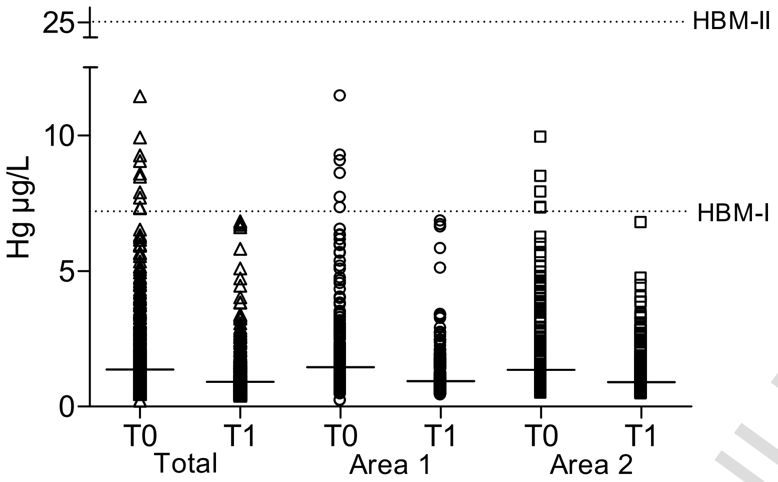
Cut off value established by Valenzuela et al., 2005; Tseng et al., 2005; Caldwell et al., 2009: 50 µg/L for total As

**Figure 7. Urinary levels of Cd (µg/L) benchmarked against the Health-Based Guidance Values (HBGVs) by exposure area and monitoring period.**



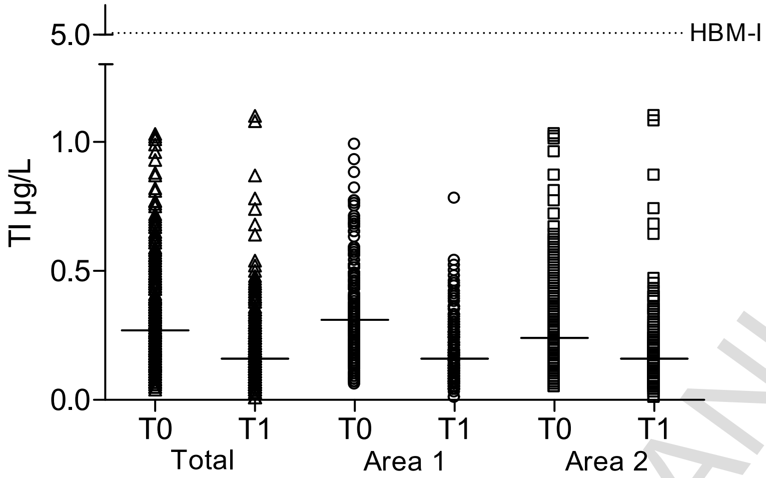
HBM-I and HBM-II: Human Biomonitoring values set by the German Commission: HBM-I: 1 µg/L; HBM-II: 4 µg/L
BE[a]: Biomonitoring Equivalent calculated using the ATSDR Minimal Risk Level (MRL): 1.2 µg/L
BE[b]: biomonitoring equivalent calculated using the US EPA Reference Dose (RfD): 1.5 µg/L

**Figure 8. Urinary levels of Hg (µg/L) benchmarked against the Health-Based Guidance Values (HBGVs) by exposure area and monitoring period.**



HBM-I and HBM-II: Human Biomonitoring values set by the German Commission: HBM-I: 7 µg/L; HBM-II: 25 µg/L

**Figure 9. Urinary levels of Tl (µg/L) benchmarked against the Health-Based Guidance Values (HBGVs) by exposure area and monitoring period.**



HBM-I: Human Biomonitoring values set by the German Commission: HBM-I: 5 µg/L

***Highlights***

- Human biomonitoring can be used to monitor the impact of a Waste-to-Energy (WTE) incinerator
- After 1-year of the WTE plant activity, internal dose of metals in the local community was very low
- Currently, metals' exposure from the WTE plant was not associated with health risk in local subjects

**Table 1. Population characteristics by exposure area and monitoring period and percentage variation from T0 to T1**

| Variable | Total | Area 1 | Area 2 |
|---|---|---|---|
| **Monitoring period** | | | |
| T0 | 394 | 196 | 198 |
| T1 | 380 | 186 | 194 |
| Not-attending at T1 survey, % | 3.55 | 5.10 | 2.02 |
| **Sex** | | | |
| T0 ♀ | 199 | 98 | 101 |
| ♂ | 195 | 98 | 97 |
| T1 ♀ | 191 | 91 | 100 |
| ♂ | 189 | 95 | 94 |
| Not-attending at T1 survey, % ♀ | 4.02 | 7.14 | 0.99 |
| Not-attending at T1 survey, % ♂ | 3.08 | 3.06 | 3.09 |
| **Age** | | | |
| T0 36-50 ys | 176 | 87 | 89 |
| 51-69 ys | 218 | 109 | 109 |
| T1 36-50 ys | 171 | 84 | 87 |
| 51-69 ys | 209 | 102 | 107 |
| Not-attending at T1 survey, % 36-50 ys | 2.84 | 3.45 | 2.25 |
| Not-attending at T1 survey, % 51-69 ys | 4.13 | 6.42 | 1.83 |
| **Smoking habit** | | | |
| T0 Non-smokers | 275 | 126 | 149 |
| smokers | 119 | 69 | 50 |
| T1 Non-smokers | 309 | 151 | 158 |
| smokers | 71 | 35 | 36 |
| Decrement of smokers at T1 survey, % | 40.3 | 49.3 | 28.0 |
| **Fish consumption 1-day before sampling** | | | |
| T0 yes | 211 | 98 | 113 |
| no | 169 | 88 | 81 |
| T1 yes | 41 | 17 | 24 |
| no | 339 | 169 | 170 |
| Decrement of consumers at T1 survey, % | 80.1 | 82.7 | 78.8 |
| **Fish consumption 1-week before sampling** | | | |
| T0 yes | 343 | 169 | 174 |
| no | 51 | 27 | 24 |
| T1 yes | 257 | 135 | 122 |
| no | 123 | 51 | 72 |
| Decrement of consumers at T1 survey, % | 25.1 | 20.1 | 29.9 |

**Table 2. Median and 95th percentile of urinary metals (µg/L) by exposure area and monitoring period**

| | Site | N. T0 *(% <LoD)* | T0 *Median (95th percentile)* | T1[b] *Median (95th percentile)* | N. T1 *(% <LoD)* |
|---|---|---|---|---|---|
| **As** | **Total** | 0 (0) | 18.2 (88.9) | 8.34 (40.6) | 3 (0.8) |
| | **Area 1** | | 17.5 (93.1) | 9.58 (45.4) | |
| | **Area 2** | | 20.3 (87.5) | 7.30 (34.5) | |
| **Be** | **Total** | 7 (1.8) | 0.16 (0.34) | 0.10 (0.22) | 8 (2.1) |
| | **Area 1** | | 0.17 (0.34) | 0.10 (0.23) | |
| | **Area 2** | | 0.15 (0.35) | 0.09 (0.20) | |
| **Cd** | **Total** | 0 (0) | 0.71 (1.93) | 0.40 (1.13) | 0 (0) |
| | **Area 1** | | 0.65 (1.91) | 0.45 (1.13) | |
| | **Area 2** | | 0.78 (1.98) | 0.36 (1.12) | |
| **Co** | **Total** | 0 (0) | 0.16 (0.56) | 0.14 (0.53) [c] | 0 (0) |
| | **Area 1** | | 0.15 (0.46) | 0.15 (0.61) [c] | |
| | **Area 2** | | 0.18 (0.59) | 0.13 (0.40) | |
| **Cr** | **Total** | 0 (0) | 0.16 (0.44) | 0.20 (0.86) | 0 (0) |
| | **Area 1** | | 0.14 (0.43) | 0.16 (0.81) | |
| | **Area 2** [a] | | 0.19 (0.46) | 0.24 (0.88) | |
| **Cu** | **Total** | 1 (0.2) | 10.8 (26.6) | 7.39 (16.6) | 0 (0) |
| | **Area 1** | | 10.8 (27.7) | 7.40 (16.4) | |
| | **Area 2** | | 10.8 (24.1) | 7.35 (16.9) | |
| **Hg** | **Total** | 3 (0.8) | 1.35 (5.16) | 0.89 (2.66) | 0 (0) |
| | **Area 1** | | 1.36 (4.94) | 0.90 (2.44) | |
| | **Area 2** | | 1.32 (5.67) | 0.86 (2.81) | |
| **Ir*** | **Total** | 3 (0.8) | 1.70 (3.80) | 1.06 (2.47) | 35 (9.2) |
| | **Area 1** | | 1.98 (4.37) | 1.04 (2.45) | |
| | **Area 2** | | 1.42 (3.39) | 1.11 (2.48) | |
| **Mn** | **Total** | 0 (0) | 0.12 (0.25) | 0.10 (0.28) | 0 (0) |
| | **Area 1** [a] | | 0.14 (0.25) | 0.10 (0.30) | |
| | **Area 2** | | 0.11 (0.29) | 0.08 (0.26) | |
| **Ni** | **Total** | 0 (0) | 0.89 (3.04) | 0.86 (2.48) [c] | 0 (0) |
| | **Area 1** | | 0.85 (3.04) | 0.90 (2.48) [c] | |
| | **Area 2** | | 0.99 (3.23) | 0.80 (2.48) [c] | |
| **Pd*** | **Total** | 10 (2.5) | 23.5 (63.2) | 13.9 (40.4) | 19 (5.0) |
| | **Area 1** | | 23.4 (63.7) | 14.1 (55.8) | |
| | **Area 2** | | 23.6 (60.5) | 13.5 (39.4) | |
| **Pt*** | **Total** | 21 (5.3) | 2.97 (9.98) | 3.03 (6.90) [c] | 5 (1.3) |
| | **Area 1** | | 2.29 (8.15) | 2.90 (7.76) | |
| | **Area 2** | | 3.93 (11.1) | 3.22 (6.68) [c] | |
| **Rh*** | **Total** | 7 (1.8) | 17.8 (51.3) | 12.6 (36.9) | 24 (6.3) |
| | **Area 1** | | 18.2 (57.5) | 13.9 (39.9) | |
| | **Area 2** | | 17.3 (34.3) | 11.9 (27.5) | |
| **Sb** | **Total** | 9 (2.3) | 0.06 (0.18) | 0.04 (0.13) | 6 (1.6) |
| | **Area 1** | | 0.07 (0.20) | 0.04 (0.14) | |
| | **Area 2** | | 0.06 (0.15) | 0.05 (0.13) | |

| | | | | | |
|---|---|---|---|---|---|
| **Sn** | **Total** | 1 (0.2) | 0.62 (1.99) | 0.30 (1.10) | 0 (0) |
| | **Area 1** | | 0.56 (1.86) | 0.33 (1.15) | |
| | **Area 2** | | 0.69 (2.32) | 0.28 (1.01) | |
| **Tl** | **Total** | 1 (0.2) | 0.27 (0.68) | 0.16 (0.40) | 7 (1.8) |
| | **Area 1** | | 0.24 (0.66) | 0.16 (0.41) | |
| | **Area 2** | | 0.31 (0.70) | 0.16 (0.39) | |
| **V** | **Total** | 1 (0.2) | 0.03 (0.11) | 0.02 (0.06) | 9 (2.4) |
| | **Area 1** | | 0.03 (0.11) | 0.02 (0.07) | |
| | **Area 2** | | 0.03 (0.11) | 0.02 (0.06) | |
| **Zn** | **Total** | 0 (0) | 389 (1146) | 265 (771) | 0 (0) |
| | **Area 1** | | 368 (1023) | 271 (806) | |
| | **Area 2** | | 402 (1298) | 246 (729) | |

***** ng/L;

[a] Area 1 vs. Area 2 at T1 (Mann-Whitney U test): Cr higher in Area 2 ($p<0.0001$); Mn higher in Area 1 ($p=0.0017$)

[b] T1 vs. T0 (Wilcoxon signed-rank test): metals significantly different (p-values <0.0001-0.020)

[c] T1 vs. T0 (Wilcoxon signed-rank test): metals not significantly different ($p>0.05$).