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Human biomonitoring of polycyclic aromatic hydrocarbonsand metals in the general population residing near the municipal solid waste incinerator of Modena, Italy

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HIGHLIGHTS

- 488 subjects residing near a solid waste incinerator were studied by biomonitoring.
- 10 polycyclic aromatic hydrocarbons (PAHs) and 12 metals were quantified in urine.
- A wide range of confounders were collected by questionnaires and measurements.
- No association between urinary metals and exposure was found.
- Significant associations between urinary PAHs and exposure were found.

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ABSTRACT

Background and objectives: A cross-sectional biomonitoring study was carried out to investigate exposure to incinerator emission in relation to the body burden of selected biomarkers in the population living around the plant.
Methods: Approximately 500 people, aged 18–69 yrs, living within 4 km from the incinerator were randomly selected form the population register. Exposure was measured through fall-out maps of particulate matter (PM), used as tracer for incinerator emissions. Ten metabolized polycyclic aromatic hydrocarbons (PAHs), from naphthalene to chrysene, 1-hydroxypyrene and twelve metals (Cd, Cr, Cu, Hg, Ni, Pb, Ni, Zn, V, Tl, As, Sn) were measured in spot urine samples. Confounders, such as diet, smoking, traffic, occupation and personal characteristics were assessed by questionnaires and objective measurements, and included into multivariate linear regression models.
Results: Metal concentrations in urine were in line with or higher than Italian reference limits, besides Cr and V with more than twofold concentrations. Metal levels did not show clear association to exposure categories. Most abundant PAHs were naphthalene (median 26.2 ng/L) and phenanthrene (7.4 ng/L). All PAHs, but benz[*a*]anthracene and 1-hydroxypyrene, were found in more than 52% of samples, and included in regression models. Significant associations between urinary PAHs and exposure were found, strong for fluorene, and weaker for naphthalene, fluoranthene and pyrene. Results were confirmed by sensitivity analyses. Correlation with variables reported in literature were observed.
Conclusions: The study indicates that the emissions were very low and highlights that specific urinary PAHs provided useful information about the internal dose arising from incinerator emission.

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1. Introduction

Both inorganic and organic substances have been identified in emissions of waste incinerators, including volatile organic compounds, polycyclic aromatic hydrocarbons (PAHs), particulate matter (PM), dioxins, furans, and metals (WHO, 2007).

Exposure assessment is one of the most critical issue in environmental epidemiology studies. Among the vast array of exposure assessment approaches, biological monitoring allows to assess the absorbed (internal) dose of xenobiotic chemicals taking into account individual characteristics such as age, gender and physiological conditions (Albertini et al., 2006). Several studies assessed the impact of solid waste incinerators (SWI) by biomarkers of exposure and effect, mainly dioxins, furans and heavy metals (Agramunt et al., 2005; Fierens et al., 2007; Kurttio et al., 1998; Reis et al., 2007a, 2007b; Schuhmacher et al., 2002; Zubero et al., 2010, 2011). A minor number investigated PAHs in the general population (Ranzi et al., 2013; Schroijen et al., 2008; Staessen et al., 2001). Notwithstanding the significant emission reduction of modern plants implementing technology to comply with emission standards, there is still concern about the impact on human health (Caserini et al., 2004; Schroijen et al., 2008).

Eight municipal SWIs are operating in the Emilia-Romagna region of Italy. One of these, in the municipality of Modena, has been operating since 1980. In 2007, the operator requested the approval for the expansion and upgrade of the plant. The authorization body (Province of Modena) commissioned to the Local Health Unit an epidemiological assessment regarding the health impact on the exposed population living near the plant according to the Integrated Pollution Prevention and Control (IPPC) directive, in force at the time of request, which imposed the study of industrial pollution consequences (European Commission, 2000). Along this line, a pilot biomonitoring study was carried out in 2010 (Ranzi et al., 2013). This study investigated the relationships between exposure variables and the levels of some biomarkers of PAHs, benzene, toluene, ethylbenzene and xylenes and metals. A major novelty was the use of urinary PAHs for the investigation of environmental exposure to SWI emissions. Once absorbed in the body, PAHs are metabolized to phenols, while a very small percentage is excreted unmetabolized (Rossella et al., 2009; Waidyanatha et al., 2003). The analysis of parent PAHs, from naphthalene to chrysene, allows obtaining a direct association between excretion and exposure due to their minor intra-individual variability and their higher specificity in comparison to hydroxylated metabolites. The multiple linear regression analysis showed that blood cadmium and mercury, and urinary manganese, fluorene, phenanthrene, anthracene and pyrene were inversely correlated to the distance of subject's residence from the SWI, while urinary manganese, fluorene and phenanthrene were directly correlated to particulate matter emitted from the SWI. Moreover, the study provided sound information regarding technical - logistical constraints as well as the suitability of operative procedures. Overall, the implementation of this pilot study demonstrated the feasibility of a human biomonitoring investigation among the population living in the proximity of the SWI of Modena.

For this reason a new study was implemented, including a larger group of subjects, selected to represent the town population. Moreover, the population recruitment criteria was based on SWI exposure estimation by means of fall-out modelling (Ashworth et al., 2013; Cordioli et al., 2013; Zandbergen and Chakraborty, 2006). The choice of biomarkers was driven by the results of the pilot study; given the good performance of the urinary biomarkers, and to facilitate the participation of subjects, the collection of specimens was limited to urinary samples, while invasive and less practical blood collection was not performed.

Thus, among the previously investigated biomarkers, urinary PAHs and metals were included also in the new study, while urinary benzene, toluene, ethylbenzene and xylenes were abandoned, as they resulted predominantly associated with vehicular traffic (Ranzi et al., 2013). Moreover, 1-hydroxypyrene (1OHPYR), a biomarker of PAH exposure, was added together with an expanded list of urinary metals, based on the monitoring data on incinerator emissions in the study area (Candela et al., 2015; Ranzi et al., 2013, 2011).

At last, considering the large number of confounding factors, an extensive pool of complementary information including diet, environmental sources of exposure, occupational exposure, characteristic of the residence, atmospheric parameters, and personal habits, were collected.

The main aim of the new study was to further investigate exposure to municipal SWI emissions in a representative group of adults living in Modena through biological monitoring, highlighting differences in selected biomarker levels according to the SWI exposure levels.

2. Methods

2.1. Study population and area

A cross-sectional biomonitoring study was carried out in the winter season 2012/2013 (November to March) in Modena, Italy, a medium-sized town of 180,000 residents in the Po Valley characterized by a flat topography and meteorological conditions which favor pollutant accumulation.

A municipal solid waste incinerator has been operating since 1980 in the industrial/rural area of Modena, north-west of the town center. The plant underwent several substantial modifications, the last time in 2010. The incinerator emission monitoring complies with the emission laws (ARPA, 2013). The plant is considered a modern facility with one combustion line (stack height of 80 m), and an annual capacity of around 180,000 tons. Besides, the area of the incinerator is characterized by industrial pressure factors (medium-sized metalworking and processing industry, logistics and transport services).

According to previous human and environmental monitoring studies carried out in this zone (Candela et al., 2015; Ranzi et al., 2013, 2011), the study area was defined as a circular area (radius of 4 km), centered on the SWI. Records of the population living in the study area were extracted from the population register. Participants' addresses were geocoded in ArcGIS10 (ESRI, Redlands, CA, USA) using the street number database provided by the regional cartographic service. Coordinates were expressed by the World Geodetic System of 1984 “WGS84_UTM32N”. Around one third of the municipal population resides in the study area, and the population does not differ from the municipal population composition in terms of age, gender and citizenship.

Results obtained from the pilot study (Ranzi et al., 2013) were considered to determine the sample size. The number of subjects to be included was calculated to detect a significant difference of at least 20% in biomarker levels comparing the most exposed to the least exposed subject; this results in about 500 individuals.

Eligible adults, i.e. aged 18–69 years with a minimum residence duration of at least three years, were randomly selected from the population base. The sampling procedure included four sampling strata based on PM_{10} fall-out maps (see below), gender and age class (18–34, 35–49, 50–69 years). Sampling comprised the selection of three replacements for each subject, belonging to the same sampling stratum.

Invitations to participate in the study were sent by post, together with a first questionnaire, a disposable polyethylene

bottle, and the instruction to collect a spot urine sample from the first void of the day. Subjects were contacted by telephone about one week after the dispatch of the invitation letter and those answering positively were invited to the Local Health Unit to provide an urine sample, to complete other questionnaires, and to be submitted to anthropometric and physiological measurements (weight, height, waist circumference and blood pressure). Not-respondents and refusals were substituted in an appropriate manner to guarantee the stratification homogeneity and to reach the appropriate study size.

The Ethical Committee of Modena approved the study. All subjects were informed about the goal and the protocol of the study and signed an informed consent. On occasion of the interview, we asked participants whether they wanted to receive their own results, and accordingly we reported the results back to the study participants.

2.2. Questionnaire: collection of individual characteristics and habits

Health operators specifically trained in recruitment, specimen and data collection carried out the field study.

All study participants were administered three different questionnaires. The first questionnaire was finalized to collect data on diet and smoking during the week prior to the sample collection; it was mailed home and self-filled by the subjects. A second, interview-guided questionnaire was administered by trained operators to collect information on personal and socio-demographic data (age, gender, nationality, and education level), lifestyle, occupation, residential characteristics (including the presence of fireplaces, stove, and presence of mold on the wall) and health status. Smoking (passive and active) was based on both self-report and assessment of urinary cotinine, a widely used metabolic by-product of nicotine. Traffic exposure was assessed through a set of questions on average daily time spent outdoor, kind of places most frequently attended when outdoor (high traffic areas, urban parks or green areas, extra-urban green areas), average daily time spent in vehicular traffic and means of transport used. A third self-administered validated food-frequency questionnaire (EPIC), was used to collect diet during the previous 12 months (Pisani et al., 1997).

2.3. Exposure to SWI pollutants

Exposure assessment was performed at address level, using fall-out maps of PM_{10} emitted by the plant. This represents a suitable exposure assessment approach (Ashworth et al., 2013; Cordioli et al., 2013), because it allows the investigation of pollutants emitted by the plant, taking into account their diffusion, mainly driven by local meteorological conditions. PM_{10} was used as the tracer of air pollution from the incinerator as data on this pollutant are routinely monitored at the stack every 30 min, while data on PAHs and metals are collected less regularly. Furthermore, simulation studies indicated similar distributions of PM_{10} and metal fall-out (Candela et al., 2015). Concentration fall-out maps of PM_{10} were provided by the local section of the Regional Agency for Prevention, Environment and Energy of Emilia-Romagna, using the quasi-Gaussian dispersion model ADMS-Urban (Cambridge Environmental Research Consultants, Cambridge, UK).

Four sampling strata, based on quartiles of the 2010 annual median PM_{10} concentration fall-out map of SWI emissions, were defined (Fig. 1) and used for subject recruitment. This map was the latest available at the beginning of the study; however, former annual fall-out maps did not differ substantially from each other in terms of their distribution shape.

For each study subject the actual exposure level was calculated on a monthly base. Monthly fall-out PM_{10} concentration was assigned to each subject for a period of 30 days prior to the sampling date; this time window was chosen as the study biomarkers reflect a short-time exposure. According to the subjects' time-location pattern, as collected by questionnaire, exposure was calculated considering both the PM_{10} concentration at the place of residence (PM_{home}) and at the place of work or school (PM_{work}). A total exposure time of 20 h/d was included, leaving a 4-h span for further microenvironments not distinct by the study. Exposure was calculated as follows:

- for subjects studying/working in the study area an exposure duration of 12 h at home and 8 h at work/school was considered, according to the formula: $PM = 12/20 \times PM_{home} + 8/20 \times PM_{work}$
- for non-working subjects (pensioners, unemployed), spending time mostly inside home, the exposure was corresponding to the level of PM at home, according to the formula: $PM = PM_{home}$
- for remaining subjects (studying/working outside study area or spending time mostly outside home) a 12 h residential exposure was considered, according to the formula: $PM = 12/20$ X PM_{home}

2.4. Other exposure sources and meteorological variables

The presence of environmental exposure sources at residence, other than the SWI, was assessed with particular reference to: zone classification, outdoor traffic exposure and residential traffic exposure, and residential heating.

The prevalent land use within a 300-m buffer of the subject's home was derived from the Regional Geographic Information Systems Service which is based on the Corine Land Cover classification system; on this base the residential zone was classified as rural, industrial, urban or mixed (Bossard et al., 2000).

Outdoor traffic exposure was based on the places mostly frequented when outdoors (extra-urban green areas, urban parks or green areas, and high traffic areas), using questionnaire data. Accordingly, subjects were classified as belonging to low, medium and high traffic exposure groups.

Residential traffic exposure was estimated using questionnaire data (windows fronting onto busy roads or traffic jams nearby the house). Moreover, the distance of the residence from the nearest major road (10,000 vehicles/d) was calculated to obtain an objective measure of traffic exposure at home. In order to better correct the effects of environmental exposure, we also used daily outdoor temperature and precipitation data of the week prior to sample collection, obtained from the regional meteorology network. Especially, temperature allowed to account for the effect of low temperature reported to reduce chemical vapor pressure, and thus resulting in enhanced adsorption of low-weight PAHs on particulate matter (Sofuoglu, 2001; Yamasaki et al., 1982).

In order to account for residential heating exposure, Nitrogen oxides (NOx μg/m3) fall-out maps at street number level were used as a tracer (Candela et al., 2015).

2.5. Collection of urine for biological monitoring

Study subjects were duly instructed regarding the appropriate sample collection procedure. Urine spot samples (about 50 mL) were collected at the first morning void in disposable polyurethane test tubes; the choice of sampling tubes has been driven by previous laboratory check to insure the absence of contaminants. Urine samples were refrigerated at 4 °C for up to 5 days and then frozen at - 20 °C until analysis. Samples were analyzed within 6 months, according to biomarker stability.

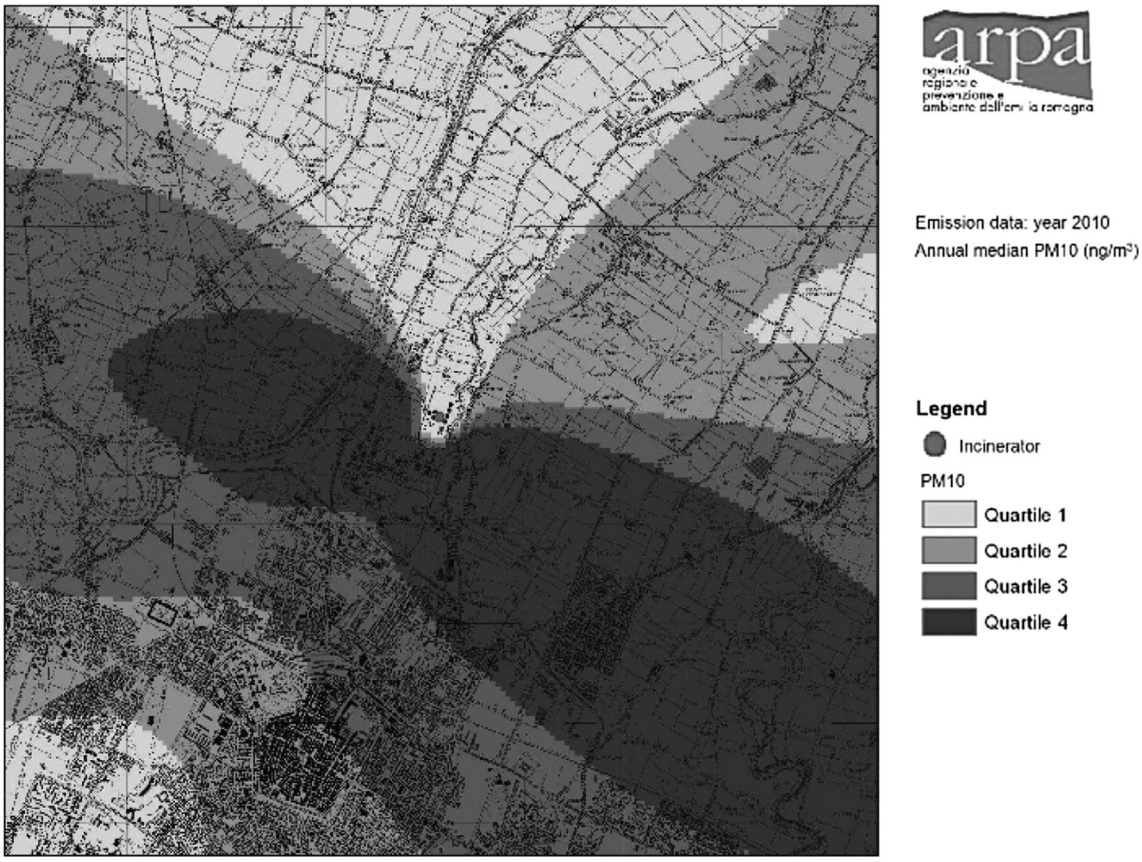

Fig. 1. 2010 Annual median PM_{10} concentration fall-out map of SWI emissions, stratified by quartiles.

2.6. Laboratory analysis

Urinary PAHs [naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), benz[*a*]anthracene (BaA), and chrysene (Chr)] were determined by solid-phase microextraction (SPME) followed by gas chromatography coupled to mass spectrometry with triple quadrupole (GC-MS/MS). Prior to analysis, samples were spiked with a mixture containing 10 deuterated analogues as internal standards (Campo et al., 2011). Analyte quantification was performed using a calibration curve for each specific analyte. Overall, quantification limits (LOQ) where in the 0.2–5.4 ng/L range, within-run and between-run precision, expressed as the coefficient of variation (CV%), were both <20%, and accuracy varied from 91 to 120%.

1OHPYR was determined by liquid chromatography coupled to mass spectrometry with triple quadrupole, after enzymatic hydrolysis and solid phase extraction. Analysis was performed in the presence of deuterated internal standard. LOQ was 0.05 μg/L, precision and accuracy, calculated on internal quality standards (0.5 and 2 μg/L) were <10% and in the 96 to 99% range, respectively. Accuracy, calculated on low (NIST SRM 3673 Organic contaminants in non-smokers' urine, 0.03 μg/L) and high (NIST SRM 3672 Organic contaminants in smokers' urine, 0.17 μg/L) standard reference materials was 79 and 107%, respectively.

Urinary metals [arsenic (As), cadmium (Cd), lead (Pb), chromium (Cr) nickel (Ni), manganese (Mn), vanadium (V), thallium (Tl), zinc (Zn), mercury (Hg), copper (Cu) and tin (Sn)] were determined by collision cell technology inductively coupled plasma mass spectrometry (CCT- ICP-MS), in the presence of suitable internal standards (Sc, In, Y, Tb), working with collision cell for interference elimination. Before analysis, urine samples were thawed for 5 min at 60 °C and then diluted 1:5 with 0.069% nitric acid (Suprapur nitric acid for trace analysis 69%, Merck, Italy). LOQs of urinary metals were in the 0.01–2 μg/L range, within-run and between-run precision were both <9%, and accuracy varied from 86 to 113%.

Urinary cotinine, as metric of tobacco smoking, was determined by liquid chromatography coupled with triple quadrupole mass spectrometry, in the presence of cotinine-d3 as an internal standard (Fustinoni et al., 2013). LOQ was 0.1 μg/L, within-run and between-run precision were both <10%, and accuracy was <10% of the theoretical value. Subjects with cotinine level >30 μg/L were classified as active smokers (Fustinoni et al., 2013). Urinary creatinine was determined using Jaffe's colorimetric method (Kroll et al., 1986).

For each analyte, accuracy and precision were assessed by internal quality controls and using certified reference materials when available.

2.7. Statistical analysis

For each biomarker, a multivariate linear regression model was estimated. The chosen covariates (as shown in appendix A, table A.1) for modelling were both based on the marginal analysis and the pertinent literature. Some covariates were included in the model as fixed variables, regardless of the statistical significance. These were: gender, age (analyzed as a continuous variable, and in addition grouped as 18–34, 35–49, and 50–69 years), education level (low/high), citizenship (Italian/others), body mass index

(BMI), cotinine, creatinine, residence zone (rural, industrial, urban, mixed), daily temperature, precipitation, outdoor traffic exposure (low, medium, high), heating exposure (NO_x), time spent at home (h/d), residential distance from major roads and occupational exposure to metals or PAHs (yes/no). Sampling day was introduced as a spline variable (with up to 3 knots) to control for exposure variability not captured by the monthly maps.

To account for the impact of a potential selection bias, the Inverse Probability Weighting (IPW) was calculated (Narduzzi et al., 2014); the weighting allows to correct for the likelihood of adherence estimated by socio-demographic variables (gender, age, citizenship), which were available both for respondents and non-respondents.

Biomarkers were not normally distributed, and thus transformed according to the distribution characteristics by box-cox transformation. For biomarker values below the LOQ the actual values were used; this approach is justified by the need to obtain the best distributions from a statistical standpoint (Lu et al., 2014; Whitehead et al., 2013). Outliers, defined as samples with biomarker levels above the 99th percentile, were excluded from statistical analysis. Samples with creatinine concentrations above 3 g/L or below 0.3 g/L were excluded as well (WHO, 1996). Analysis was carried out for each biomarker with a detection rate above 52%.

For each model, an analysis on the residuals was carried out and the results were compared with those obtained from a quantile regression model estimated on various percentiles.

In order to verify the stability of the overall model additional sensitivity analyses were performed evaluating, among the others, the influence of the sampling day, the citizenship, living in industrial zone, and the distribution of biomarker data (values below the LOQ and above the 95th percentile). Statistical analyses was performed using STATA 11 (Stata Corp LP, College Station, TX).

3. Results

3.1. Study population

Overall, 497 subjects agreed to participate in the study, 9 of them were excluded from statistical analysis due to residence out of the study area, resulting in 488 study subjects. The response rate was 54% (non-availability rate 19%, refusal rate 27%).

The mean residential PM_{10} exposure from SWI was 0.25 ± 0.3 ng/m^3 (maximum 2.51 ng/m^3). Four SWI exposure levels were obtained based on the quartile distribution: level 1: <0.09 ng/m^3; level 2: 0.09–0.19 ng/m^3; level 3: 0.20–0.29 ng/m^3; level 4: >0.29 ng/m^3.

Incinerator emissions varied during the study period and subjects may have experienced different exposure even though residing in close area.

The main characteristics of study subjects, stratified by SWI exposure levels, are shown in Table 1. No significant differences were found in terms of gender, age and BMI. Some differences were related to education level and citizenship, with a lower percentage of highly educated subjects in the highest SWI exposure levels, and higher presence of foreign citizens in the lowest SWI exposure level. No differences were observed among SWI exposure levels and smoking status neither for urinary cotinine nor for self-reported smoking status.

The percentage of subjects reporting presence of mold on the residence wall was higher in the lowest SWI exposure levels.

Regarding residential traffic exposure, in the SWI exposure level 3, a higher percentage of subjects reporting the presence of windows fronting onto busy roads or traffic jams nearby the house was found, whereas the residential distance from major roads was similar across SWI exposure levels.

Concentrations of NO_x were the lowest in the SWI exposure level 4.

No significant difference was found for: use of fireplaces or stoves, average time spent at home, occupational exposure, drug or supplement use, presence of prostheses or amalgam fillings, and exposure to toxic substances (solvents, paint, stain removers, gasoline, glue, cleansers, disinfectants and toners) during the week before sample collection.

Dietary habits were similar in all exposure levels, besides some differences in the consumption of game, mushrooms, nuts, chargrilled food, and beer (Data not shown).

3.2. Biological monitoring

The summary statistics of urinary biomarkers are reported in Table 2. Urinary PAHs were detected at least in 68% of samples, beside BaA, that was detected only in 20% of samples. Nap, Phe, Ant were detected in all samples. The observed values of most PAHs were lower than the values obtained in the pilot study (Ranzi et al., 2013). 1OHPYR was quantified only in 52% of subjects, with levels well below the reference values for the Italian population.

Urinary metals were detected at least in 77% of samples. Levels were in line with or higher than (up to 40%) the references values proposed for the Italian population, beside for Cr (95th percentile 0.86 vs. 0.35 μg/L) and V (95th percentile 0.81 vs. 0.2 μg/L), Median urinary cotinine was 0.8 μg/L while the mean value was 312 μg/L; the large difference is due to the presence of a majority of non-smokers (69%) in the investigated subjects. Dividing subjects according to actual smoking habit, the median levels were 0.48 and 883 μg/L in non-smokers and smokers, respectively.

3.3. Multivariate linear regression analyses

3.3.1. Polycyclic aromatic hydrocarbons

Multivariate regression models were performed for each analyte, except for BaA and 1OHPYR, whose values resulted below the LOQ in over 48% of samples. Fig. 2 illustrates the adjusted ß-coefficients and 95% confidence intervals of the relationship between selected PAH biomarkers and SWI exposure levels. Whereas Table 3 shows the results of the adjusted linear regression models in detail.

SWI exposure positively affected Nap, Flu, Flt, and Pyr, but not Ace, Acy, Phe, Ant and Chr. In particular Flu was significantly increased for subjects belonging to exposure level 3 and 4 (ß = 0.124 and ß = 0.125, respectively). An increment of 0.047 ng/L was observed for Flu passing from exposure level 1 to 2, 0.185 ng/L from level 2 to 3, and 0.0002 ng/L from level 3 to 4; the mean increment was 0.0775 ng/L.

Flt, and also Nap and Pyr, although with a weaker evidence, were increased only for subjects in exposure level 3 (ß = 0.099; ß = 0.038, and ß = 0.091, respectively).

Female gender increased Phe (ß = 0.012) and Chr (ß = 0.182). Age weakly increased Flt (ß = 0.003) and Pyr (ß = 0.002), while a negative influence was found for Ace (ß = −0.006). Education level and BMI showed no significant relationship with urinary PAHs, while foreign citizenship was positively associated with all PAHs, but Ace and Ant.

Tobacco smoking positively influenced all PAHs, but Nap and Phe. While the relationship between cotinine and PAHs was highly significant, the percent increment (beta) was very small, counting from less than 0.0023% (for Pyr and Chr) to 0.11% (for Flu) for each 10-fold increase in cotinine excretion. With regard to the residence zone, Nap and Ace showed a positive significant relationship with residence in industrial area (reference category: rural area, ß = 0.112 and ß = 0.431, respectively), while Flu had a significant negative relationship with residence in industrial, urban and mixed

Table 1
Summary of study subject characteristics divided according to SWI exposure levels.

	SWI exposure levels				
Quartiles	1	2	3	4	Total
PM_{10} (ng/m^3)	<0.09	0.09–0.19	0.20–0.29	>0.29	
Enrolled individuals, n	122	122	122	122	488
Male, n (%)	56 (46)	54 (44)	67 (55)	60 (49)	237 (49)
Age class, n (%)					
18-34 yrs.	37 (30)	33 (27)	34 (28)	48 (39)	152 (31)
35-49 yrs.	41 (34)	45 (37)	39 (32)	33 (27)	158 (32)
50-69 yrs.	44 (36)	44 (36)	49 (40)	41 (34)	178 (37)
BMI kg/cm^2 (mean ± SD)	26.2 ± 5.2	25.8 ± 5.0	26.1 ± 5.2	26.5 ± 5.6	26.1 ± 5.6
Education, n (%)					
Primary school/lower	5 (4)	5 (4)	3 (3)	6 (5)	19 (4)
Secondary school	29 (24)	16 (13)	39 (32)	34 (28)	118 (24)
High school	50 (41)	69 (57)	42 (35)	57 (48)	218 (45)
Degree/higher	38 (31)	32 (26)	37 (31)	23 (19)	130 (27)
Missing	0	0	1	2	3
Smoking, n (%)					
Non smokers	87 (71)	80 (66)	83 (69)	86 (72)	336 (69)
Smokers	35 (29)	42 (34)	38 (31)	34 (28)	149 (31)
Missing	0	0	1	2	3
Citizenship, n (%)					
Italian	105 (86)	117 (96)	111 (91)	116 (95)	449 (92)
Foreign/double	17 (14)	5 (4)	11 (9)	6 (5)	39 (8)
Mold on residence wall, n (%)	26 (21)	28 (23)	12 (10)	19 (16)	85 (17)
Residence zone, n (%)					
Rural zone	10 (8)	6 (5)	4 (3)	10 (8)	30 (6)
Industrial zone	9 (7)	1 (1)	4 (3)	3 (2)	17 (4)
Urban zone	83 (68)	97 (79)	92 (75)	69 (57)	341 (70)
Mixed zone	20 (16)	18 (15)	22 (18)	40 (33)	100 (20)
Outdoor traffic exposure, n (%)					
Low	10 (8)	13 (11)	10 (8)	24 (12)	57 (12)
Medium	87 (72)	80 (66)	79 (67)	72 (66)	318 (66)
High	24 (20)	29 (23)	29 (25)	23 (22)	105 (22)
Missing	1	0	4	2	8
Windows over busy roads, n (%)	20 (17)	26 (21)	38 (32)	22 (19)	106 (22)
Residential distance from major roads, m (mean ± SD)	179 ± 129	174 ± 147	159 ± 124	178 ± 122	173 ± 131
Heating exposure, NO_x, $\mu g/m^3$ (mean ± SD)	13.1 ± 8.7	15.4 ± 9.5	15.6 ± 7.5	10.2 ± 5.5	13.6 ± 8.2

area (ß = −0.234, ß = −0.284 and ß = −0.146, respectively).

Chr and Flt were positively related with the temperature on the day of urine sampling (ß = 0.035, ß = 0.009, respectively); conversely, a negative relationship was found for Ace (ß = −0.026) and, with a weaker significance, for Flu (ß = −0.012). Mean daily precipitations of the week prior to urine sampling weakly influenced Flu (ß = 0.012), Nap and Acy (ß = 0.004, and ß = 0.021).

Outdoor traffic exposure increased Acy, in subjects frequently attending high traffic areas (ß = 0.199) and it increased Phe and Ant in those attending medium traffic areas (ß = 0.015 and ß = 0.129, respectively).

Exposure to heating system emissions, expressed as NO_x, slightly influenced Flu (ß = 0.005).

Considering the distance of the residence from the nearest road, an inverse relationship with most PAHs was found, although without statistical significance.

Among the other investigated variables the presence of mold on the residence wall increased the levels of Acy, Phe, Ant, Flt, and Pyr (ß = 0.163, ß = 0.015, ß = 0.135, ß = 0.099, and ß = 0.080, respectively).

Minor role was played by food, drugs, dietary supplements, and use of chemicals in domestic environments (see Table 3).

3.3.2. Metals

Table 4 shows the results of the adjusted linear regression models. None of the metals showed clear relationship with SWI exposure; however, some indications that exposure level 3 marginally increased Hg and Tl (ß = 0.232 and ß = 0.211) were found; on the contrary exposure level 4 was negatively correlated to As (ß = - 0.483).

Female gender resulted in an increased level of Cu, Pb, Cd, Ni, and As, and a decreased level of Zn. Age positively increased Pb, Cd, As and V (ß = 0.019, ß = 0.008, ß = 0.014, ß = 0.004), and decreased Cu (ß = - 0.008). Education level was inversely associated with Cu, Pb and V (ß = - 0.333; ß = -0.152; ß = −0.155), and positively associated with Hg (ß = 0.186). Foreign citizenship showed positive associations with Sn and Tl (ß = 0.325; ß = 0.308). BMI was inversely associated with Zn (ß = −0.050).

Tobacco smoking, measured as urinary cotinine, increased Pb and Cd.

Major associations were observed with diet. Among the variables investigating exposure to toxic substances or compounds known as metal sources, significant positive associations between dental fillings and Pb, toothpaste in metal tubes and Hg, presence of prostheses and Mn, sprays/hairsprays and Cr were observed.

3.4. Sensitivity analysis

The sensitivity analyses indicated that the investigated parameters made little difference on the PAHs results, and in particular, Flu never showed relevant variation in the ß-coefficient or significance level. Conversely, metals appeared more variable in terms of ß-coefficient and significance level.

4. Discussion

This biomonitoring study aimed to evaluate the exposure to PAHs and metals in a representative group of adults living near a

Table 2
Summary statistics and reference values for biomarker of exposure to PAHs, metals and tobacco smoke (cotinine).

Analyte	Results of the present study					Reference values/previous experience		
	LOQ	% > LOQ[a]	Mean	Median	5th - 95th percentiles	All	Non smokers	Smokers
Urinary PAHs								
Nap (ng/L)	5.4	100	35.2	26.2	18.8–81.6	122.3[b]		
Acy (ng/L)	0.3	94	0.7	0.6	<0.3–1.5	3.7[b]		
Ace (ng/L)	0.6	81	1.6	0.9	<0.6–3.5	11.1[b]		
Flu (ng/L)	0.9	97	2.1	1.6	0.9–5.3	10.4[b]		
Phe (ng/L)	0.5	100	10.0	7.4	4.8–16.6	20.8[b]		
Ant (ng/L)	0.4	100	2.2	2.1	0.9–3.5	2.3[b]		
Flt (ng/L)	0.6	76	0.8	0.7	<0.6–1.6	2.2[b]		
Pyr (ng/L)	0.4	93	0.7	0.6	<0.4–1.2	2.8[b]		
BaA (ng/L)	0.3	20	0.2	<0.3	<0.3–0.8	2.3[b]		
Chr (ng/L)	0.2	68	0.3	0.2	<0.2–0.7	1.1[b]		
Hydroxylated PAH								
1OHPYR (μg/L)	0.05	52	0.11	0.05	<0.05–0.4		<0.5[c]	<1.0[c]
Urinary metals								
Cu (μg/L)	0.01	99	6.9	5.3	1.1–19.0	4 - 15[c]		
Zn (μg/L)	2.0	100	261	206	41–688	250-650[c]		
Mn (μg/L)	0.01	77	0.35	0.06	<0.01–1.16	0.2–4.0[c]		
Pb (μg/L)	0.1	92	0.9	0.6	<0.1–2.5	0.01–2.0[c]		
Cd (μg/L)	0.01	97	0.26	0.18	0.04–0.73		0.1–1.0[c]	0.1–1.5[c]
Ni (μg/L)	0.1	94	2.0	1.1	<0.1–6.6	0.1–5.0[c]		
Hg (μg/L)	0.1	87	0.72	0.45	<0.1–2.58	0.1–5.0[c]		
Cr (μg/L)	0.01	93	0.17	0.09	<0.01–0.86	0.05–0.35[c]		
As (μg/L)	0.1	93	28	11	<0.1–102	<100[d]		
Sn (μg/L)	0.011	83	0.68	0.4	<0.011–2.37	0.05–2.28[f]		
V (μg/L)	0.01	95	0.30	0.21	0.01–0.81	0.05–0.2[c]		
Tl (μg/L)	0.01	100	0.21	0.15	0.03–0.65	0.05–0.5[c]		
Metric of tobacco smoking								
Cot (μg/L)	0.1	88	312.4	0.8	<0.1–1957	0.1[e]	≤30[e]	>30[e]

[a] % > LOQ: percentage of samples above LOQ.
[b] 95th percentile based on previous experience in the Modena pilot study (Ranzi et al., 2013).
[c] 5th - 95th percentiles based on SIRV (SIVR, 2011).
[d] 95th percentile based on ASTDR (ATSDR, 2007).
[e] Based on study results (Campo et al., 2016).
[f] 5th- 95th percentiles based on (Goulle et al., 2005).

municipal SWI, and to investigate the variation of the urinary biomarkers in association with incineration emissions, evaluated by PM_{10}. Results showed a low exposure, with biomarkers in the range of values observed in the general population. Significant associations between urinary PAHs and SWI exposure were found, strong for Flu, and weaker for Nap, Flt, and Pyr. For metals, weak associations were found only for Hg and Tl.

As for PAHs, only few studies investigated this exposure in the general population around SWI plants, and measured only 1OHPYR (Schroijen et al., 2008; Staessen et al., 2001). Given that these compounds are present in the atmosphere as a mixture with relative proportion varying accordingly to the source and the temperature of emission, a multiple-analyte approach was used by measuring 1OHPYR and 10 urinary PAHs. 1OHPYR was quantifiable only in 52% of subjects, with median levels well below reference values for the Italian population (<0.5 μg/L in non-smokers and <1.0 μg/L in smokers) (SIVR, 2011).

To perform the analysis of urinary PAHs, the analytical method used in the pilot study was improved introducing the use of GC/MS/MS instead of GC/MS (Ranzi et al., 2013). Consequently, a higher number of quantifiable samples was achieved (for example, Acy: 94% vs. 6.5%; Flt: 76% vs. 29%; Chr: 68% vs. 13%, in the present vs. the pilot study, respectively). Median urinary PAHs were in the ng/L levels and similar to those obtained in the pilot study, and in a small subpopulation of the reference adolescent population participating to the Flemish Environment and Health Study (De Craemer et al., 2016), but lower than in adults living in an industrial polluted area in Poland (Campo et al., 2014).

As for metals, in comparison with the pilot study, the panel of investigated urinary metals has been expanded and the analytical assays have been improved with the use of CCT-ICP-MS for all analytes, leading to lower quantification limits and better specificity. The percentage of quantified samples ranged from 77% (for Mn) up to 100% (for Zn and Tl). Levels were generally lower than those of the pilot study (for example, median Mn 0.06 vs 0.18 μg/L, Cu 5.3 vs 11 μg/L, Ni 1.1 vs. 6.98 μg/L, Zn 205 vs. 352 μg/L, in the present vs. the pilot study, respectively); these may be ascribed, at least in part, to the different analytical methods.

The levels were comparable with Reference Value of the Italian population (SIVR, 2011) and with those reported in a general adult population survey in Canada (Health Canada, 2010) (95th percentile 0.166; 0.4; 26.7; 4.5; 1088; 70.6; 1.65; 2.11 μg/L for V, Mn, Cu, Ni, Zn, As, Cd, and Pb, respectively in 5492 adults); and by a French study (95th percentile 2.28, 0.84 and 2.14 μg/L for Sn, Tl and Pb, respectively, in 100 adults) (Goulle et al., 2005). Moreover, the levels were comparable with those reported in a recent Italian study, investigating 394 adults living near a waste-to-energy incinerator prior to operation (Bocca et al., 2016).

Few studies reported urinary metals in the general population potentially exposed to waste incinerator emissions and the panel of investigated metals is limited to Cr, Cd and Hg. Among the available studies, for urinary Cd, a geometric mean level of 0.14 nmoL/mmol creatinine (about 0.19 μg/L) was reported in adolescents living in an industrial area including two waste incinerators in the Flanders (Staessen et al., 2001), while in adults of Bilbao, Spain, a geometric mean values of 0.18, 0.37 and 0.38 μg/g creatinine was reported for Cr, Cd and Hg, respectively (Zubero et al., 2010). Our data are comparable to or lower than those reported in these studies (Staessen et al. 2001; Zubero et al., 2010).

Overall, based on the comparison with previous reported data

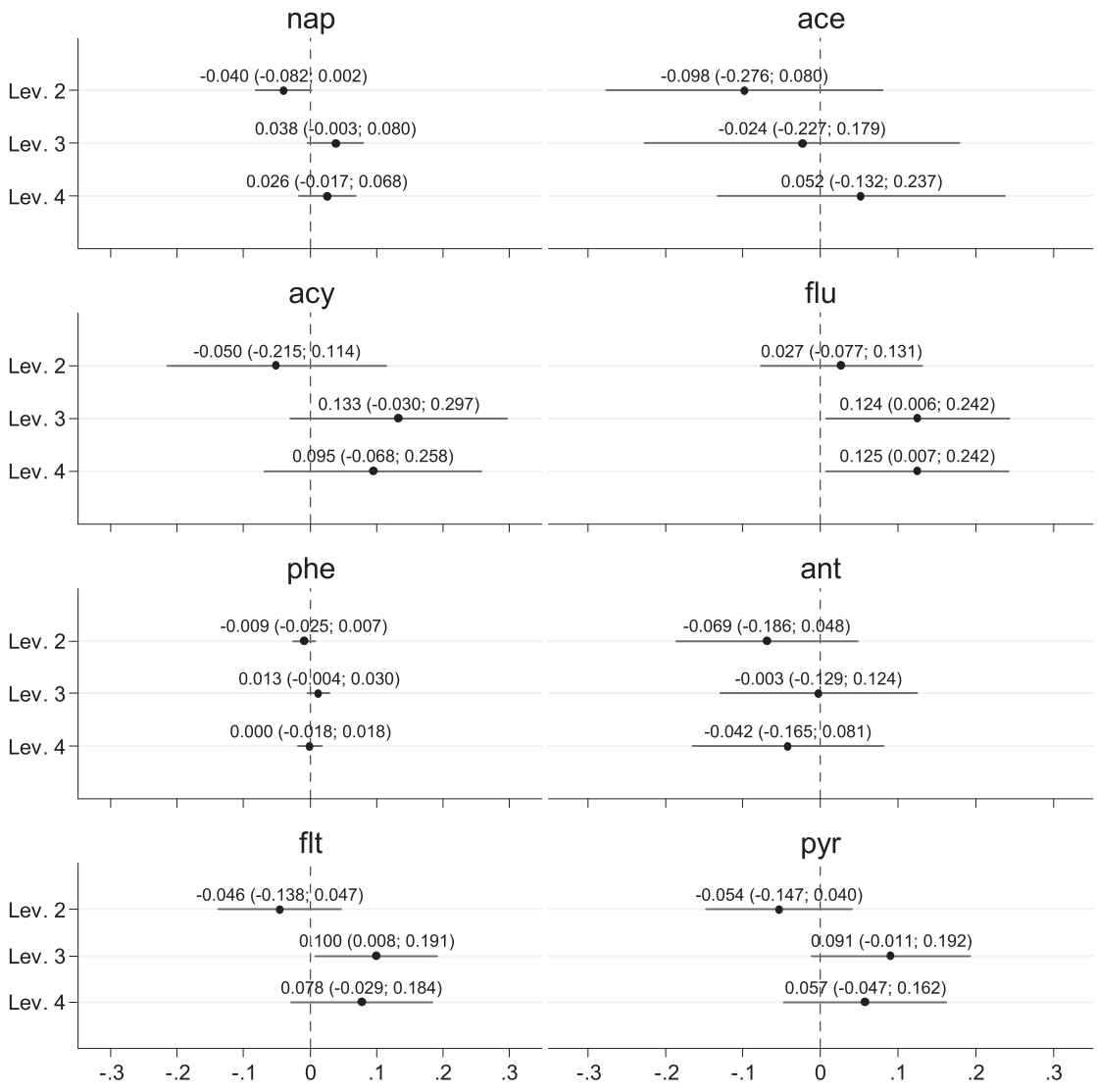

Fig. 2. Relation of Nap, Ace, Acy, and Flu (upper panel) and Phe, Ant, Flu, and Pyr (lower panel) with SWI exposure levels. Forest plots of the adjusted regression coefficients and the 95% confidence intervals, exposure level 1 was considered the reference category.

on urinary PAHs, 1OHPYR, and metals, albeit scarce for some of them, our results do not highlight any critical exposure in the study population.

In order to optimize the exposure assessment, a dispersion model was applied to investigate subjects, allowing us to estimated exposure to PM_{10} down to individuals' street number. We used PM_{10} as proxy for incinerator emissions, assuming that pollutants are adsorbed onto particulate matter. This assumption is valid for several metals and heavier PAHs (in our study Flt, Pyr, BaA, and Chr) (Lollar, 2005).

Urinary PAHs, BaA, could not be studied owing to the high number of samples below the LOQ; also Chr, although investigated, did not show an association with SWI exposure, maybe due to the high percentage of undetected samples (32% < LOQ). On the other hand, Flt and Pyr showed a positive association with SWI exposure level 3. The lighter PAHs are, to a certain extent, emitted in gaseous form (Yuan et al., 2005); however, they showed better results than heavier PAHs; this is the case of Flu, which was positively associated with SWI exposure level 3 and 4, and Nap, weakly associated with SWI exposure level 3. These results could be explained by the study period, it is documented that low temperatures of the winter season potentially increase the adsorption of low-medium weight PAHs on suspended particles (Sofuoglu, 2001; Yamasaki et al., 1982). The results for Flu and Pyr are comparable to those of the pilot study, strengthening the present outcome. Conversely, a significant relationship for Phe and Ant, observed in the pilot study, was not detected (Ranzi et al., 2013).

Regarding 1OHPYR, the low percentage of measureable samples prevented us from studying the association with the SWI exposure, underlining that 1OHPYR is not a suitable biomarker of low-level PAH exposure.

As regards metals, the lack of the association with SWI exposure for Cu and Zn is not surprising, given the low concentration of PM_{10} emitted by the SWI and the high physiological levels (in the order of some μg/L for Cu and hundreds of μg/L for Zn) in the human body. A higher specificity toward SWI exposure was expected for other investigated urinary metals, given their exogenous origin, however our results do not show clear relations with SWI emissions. Only Hg and Tl showed weak positive relationships with SWI exposure level 3, while, differently from the pilot study, no relationship with

Table 3
Estimates of regression models using urinary PAHs as dependent variables. Beta-coefficients (β) of SWI exposure are shown regardless of the significance level, whereas only β values below the 0.10 significance level are illustrated for the other covariates.

	PAH	Nap	Acy	Ace	Flu	Phe	Ant	Flt	Pyr	Chr
	Box & Cox λ transformation[a]	−0.4	−0.1	0	0	−1	0.6	0.1	0	−0.1
	R^2	0.30	0.12	0.13	0.52	0.20	0.46	0.24	0.12	0.22
		β	**β**	**β**	**β**	**β**	**β**	**β**	**β**	**β**
Fixed variates	**SWI exposure (ref. level 1)**									
	level 2	−0.04*	−0.050	−0.098	0.027	−0.009	−0.069	−0.046	−0.054	−0.191*
	level 3	0.038*	0.133	−0.024	0.124**	0.013	−0.003	0.099**	0.091*	−0.128
	level 4 (highest)	0.026	0.095	0.052	0.125**	0.0001	−0.042	0.078	0.057	−0.086
	Gender (ref. male)	ns	ns	ns	ns	0.012**	ns	ns	ns	0.182**
	Age, years	ns	ns	−0.006**	ns	ns	ns	0.003*	0.002*	ns
	Education level (ref. low)	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Citizenship (ref. Italian)	0.070**	0.265*	ns	0.168**	0.024**	ns	0.162**	0.174**	0.279**
	BMI, kg/cm^2	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Cotinine, μg/L	ns	0.0001**	0.0001**	0.0005**	ns	0.0002**	0.0002**	0.0000*	0.0000**
	Creatinine, g/L	0.05**	ns	0.09*	ns	0.024**	0.127**	0.090**	0.090**	0.123*
	Residence zone (ref. rural)									
	industrial	0.112**	ns	0.431**	−0.234**	ns	ns	ns	ns	ns
	urban	0.035	ns	0.180	−0.284**	ns	ns	ns	ns	ns
	mixed	0.033	ns	0.286*	−0.146**	ns	ns	ns	ns	ns
	Temperature, °C	ns	ns	−0.026**	−0.012*	ns	ns	0.009**	ns	0.035**
	Precipitation, mm	0.004*	0.021*	ns	0.012*	ns	ns	ns	ns	ns
	Outdoor traffic exposure (ref. low)									
	medium	ns	0.084	ns	ns	0.015**	0.129**	ns	ns	ns
	high	ns	0.199**	ns	ns	0.007	0.142	ns	ns	ns
	Heating exposure as NOx μg/m3	ns	ns	ns	0.005*	ns	ns	ns	ns	ns
	Time spent at home, h	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Residential distance from major road, m	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Occupational exposure to PAH	ns	ns	ns	ns	ns	ns	ns	ns	ns
PAH-specific variates	**Mold on the residence wall**	ns	0.163**	ns	ns	0.015*	0.135**	0.099**	0.080*	ns
	Medication[b]	ns	ns	0.133*		0.011*	ns	0.094**	ns	ns
	Char-grilled food[b]	ns	ns	0.153*						
	Tuna[b]	ns	ns	0.248**						
	Paint use[b]					ns	0.252*			
	Coffee[b]							ns	ns	0.180*
	Whole cereals[b]							ns	−0.068*	ns

ns = not significant.
** p-value <0.05.
*p-value <0.1.
[a] Logarithmic transformation if λ of the Box-Cox transformation equals to zero.
[b] Previous week.

urinary Mn was observed. The pilot study indicated an inverse, although weak, correlation of blood Hg with the distance of a subject's residence from the SWI. However, it should be taken into account that Hg, owing to its emission in the vapor state appears to be poorly represented by fall-out maps used in our study (Yuan et al., 2005). Furthermore, sensitivity analyses quite influenced the results of the studied metals. Increased Hg in hair was found in a study in Finland in individuals who have lived for 10 years near a hazardous waste incinerator (Kurttio et al., 1998). Whereas no trend appeared in other European studies (Fierens et al., 2007; Zubero et al., 2010).

Controlling the sources of variability is a critical issue in biomonitoring studies (Nordberg, 2007).

Substantial effort was undertaken to adjust for this variability through a detailed data collection, which comprised information on potential confounding factors such as smoking, traffic exposure, and diet. Furthermore, the period of the study is crucial, as exposure sources might vary by season (mainly traffic and heating). In order to reach the required sample size, a certain operating period was necessary. We started the study in late fall and finished by end of the heating period as we were able to correct for further sources, which guaranteed homogeneity of exposure conditions and facilitated subject recruitment.

Exposure to heating was taken into account by means of dispersion models of NOx due to non-industrial combustion (namely methane, LPG, fuel, diesel). Residual confounding related to the recent increase in the use of biomass for domestic heating is possible, but there is no suggestion of a geographic distribution of this confounding factor similar to the incinerator exposure.

Traffic data (related to annual average information on traffic density in the area) could present higher values during the study period, related to the average value used for correction, with the same spatial distribution.

A strong positive relationships between Cd and smoking was observed, as expected (Nordberg, 2007), while weaker positive relationships were found with Pb and with the large majority of urinary PAHs.

Residence zone showed interesting relationships with some PAHs; in particular, Flu was higher in those living in rural area, possibly due to the burning of brushwood, on the other hand Nap and Ace showed increased levels in the industrial area.

Heating exposure as NOx, showed significant relationship with Flu, but not with the other PAHs and with metals. NOx failed to show significant relations with most analytes; probably because it did not sufficiently reflect the actual activity (switch on/off periods) of the heating plants, and provided only a general spatial exposure indication.

Temperature during the week prior to the sample collection showed contrasting relationships with biomarkers. Interpretation of these findings appears difficult, as ambient temperature can modify a variety of situations, related both to the subject and to the deposit of PAHs in particulate matter (Sofuoglu, 2001; Yamasaki

Table 4
Estimates of regression models using urinary metals as dependent variables. Beta-coefficients (β) of SWI exposure are shown regardless of the significance level, whereas only β values below the 0.10 significance level are illustrated for the other covariates.

		Cu	Zn	Mn	Pb	Cd	Ni	Hg	Cr	As	Sn	V	Tl
	Box & Cox λ transformation[a]	0.3	0.2	0	0.3	0.3	0.2	0.3	0.1	0.3	0.2	0.3	0
	R^2	0.42	0.47	0.25	0.43	0.39	0.23	0.16	0.30	0.38	0.12	0.16	0.41
		β	**β**	**β**	**β**	**β**	**β**	**β**	**β**	**β**	**β**	**β**	**β**
Fixed variates	**SWI exposure levels (ref. level 1)**												
	level 2	−0.053	−0.404	−0.000	0.033	0.101	−0.063	0.170	−0.024	−0.188	−0.057	0.082	0.060
	level 3	0.132	−0.444	0.178	−0.033	0.005	−0.035	0.232*	0.061	−0.296	0.137	0.037	0.211*
	level 4	0.044	−0.565	−0.437	−0.146	−0.011	0.011	0.087	0.006	−0.483*	−0.107	0.014	0.109
	Gender (ref. male)	0.270**	−0.761**	ns	0.148**	0.125**	0.280*	ns	ns	0.323**	ns	ns	ns
	Age, years	−0.008*	ns	ns	0.019**	0.008**	ns	ns	ns	0.014**	ns	0.004*	ns
	Education level (ref. low)	−0.333**	ns	ns	−0.152*	ns	ns	0.186*	ns	ns	ns	−0.155**	ns
	Citizenship (ref. Italian)	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.325*	ns	0.308**
	BMI, kg/cm2	ns	−0.050**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Cotinine, μg/L	ns	ns	ns	0.0001**	0.0009**	−0.0001*	ns	ns	ns	ns	ns	ns
	Creatinine, g/L	0.839**	1.963**	ns	0.539**	0.233**	0.479**	0.368**	0.289**	0.910**	0.282**	0.158**	0.559**
	Residence zone	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Temperature, °C	ns	ns	−0.045*	ns	ns	−0.026*	ns	ns	−0.043*	ns	ns	ns
	Precipitation, mm	0.051**	ns	ns	ns	−0.023**	ns	ns	−0.032*	ns	0.048**	ns	ns
	Outdoor traffic exposure (ref. low)												
	medium	ns	ns	ns	ns	ns	ns	ns	ns	−0.439**	ns	ns	ns
	high	ns	−0.802*	ns	ns	ns	ns	ns	ns	−0.508*	ns	ns	ns
	Heating exposure as NOx μg/m3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Time spent at home, h	ns	ns	ns	ns	ns	ns	ns	0.181**	ns	ns	ns	0.140*
	Residential distance from major road, m	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Occupational exposure to metals	−0.238*	ns	ns	ns	ns	−0.241*	ns	ns	ns	ns	ns	ns
Metal-specific variates	**Spray/hairspray**				ns				0.409**				
	Prostheses			0.398*		ns	ns						
	Dental amalgams				0.148*	ns	ns						ns
	Toothpaste in metal tube							0.550*					ns
	Tea			0.305*									ns
	Mozzarella cheese	0.016**											
	Offal (liver)				ns				0.016**				
	White vine		0.003**		ns								
	Processed cheese				0.018**								
	Meat		0.989*		0.009**				0.009*			0.007**	ns
	Bouillon cube								0.008**		ns		
	Spirits				0.009*								0.010**
	Coffee				0.004**							−0.001**	
	Mussels/clams				0.040**					0.078**			0.038**
	Shrimps			0.042*				0.019*					
	Fish	ns				ns		ns		1.049*			0.008*
	Mollusks									0.709**			
	Cabbage							0.023**					
	Egg											0.009*	
	Parsley									ns		ns	0.197**
	Kiwi												0.005**

ns = not significant.
** p-value <0.05.
*p-value <0.1.
[a] Logarithmic transformation if λ of the Box-Cox transformation equals to "0".

et al., 1982). Moreover, it can also represent a proxy of residential heating activity.

In regards to both outdoor and residential traffic exposure, some PAHs, but not metals, showed relationships with attendance in high traffic areas and the distance of dwellings from major roads. This is in line with what it is expected, being urban traffic a major source of PAHs.

Concerning occupational PAHs and metal exposure, the role played by this variable was not relevant, probably because the large majority of subjects reported no occupational exposure.

The citizenship and the presence of mold in the house showed a positive relationship with the majority of PAHs and/or some metals. As this relationship is not obvious, these variables may be proxies of other, not well-identified, confounders. The EPIC food-frequency questionnaire might not be sufficiently accurate in assessing diet habits different from the Italian one; moreover, other factors, such as cooking methods were not assessed. Subjects' residence characteristics and lifestyle factors might have played an impact as well.

As for diet, some interesting relationships, previously reported in the literature, were found for metals: Zn was positively associated with meat consumption (Nordberg, 2007; EPA, 2005), Pb was correlated with coffee, spirits and mollusks (Richter et al., 2013) and As increased with recent consumption of fish and mollusks (Nordberg, 2007; EPA, 2012). Conversely, Hg was not associated with fish consumption but only with shrimps. In fact, methylmercury, present mainly in fish, is excreted primarily via the biliary route (Nordberg, 2007; EPA, 2007), while ethylmercury, excreted in the urine, is found in lower concentrations in fish. Regarding PAHs, recent intake of charcoal grilled food affected only Ace, while coffee intake in the previous week affected only Chr, suggesting that these personal habits do not exert a relevant influence on these biomarkers.

One of the main strength of our study is the study protocol guaranteeing a representative population sample of adequate size, the use of a validated method for the dispersion maps used for exposure assessment and the application of sensitive analytical methods, able to detect biomarkers at low concentration. Moreover, the present results are consistent with those of the pilot study and reinforced by various sensitivity analyses.

Shortcomings are associated with the poor representation of volatile pollutants, possibly not well represented by the fall-out maps of PM_{10}, and with the difficulties still encountered in taking into account the spatial and temporal variation of investigate confounding factors, in particular traffic and heating; also a more accurate lifestyle, diet, and individual behavior characterization might be implemented. Moreover, the choice to investigate urinary metals instead of blood metals, to facilitate subject recruitment, may have limited the possibility to find association between biomarkers and SWI exposure. Toenails may represent a valid alternative, as they are a matrix with a minimal invasive impact during collection and able to represent a longer exposure time (Goulle et al., 2009).

5. Conclusions

The present study suggests that the exposure to SWI emission from the municipal SWI of Modena was very low, with biomarkers ranging within the reference values of general population. However, some associations between SWI emissions and urinary biomarkers were found, in particular urinary PAHs emerged as possible biomarkers of internal exposure to pollution from the SWI. Taking into account also the findings of the previous pilot study (Ranzi et al., 2013); Flu appears to be the best exposure biomarker.

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Declaration of interest

The Authors declare no conflict of interest. The funding body had no involvement in the collection, analysis and interpretation of data; neither in the writing of the article.

Ethics

The study was approved by the Ethics committee of Modena and has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2017.07.122.

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