

# Italian network for human biomonitoring of metals: preliminary results from two Regions

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**Summary.** The Italian program for human biomonitoring (HBM) of chemical elements, PROgram for Biomonitoring of the Exposure (PROBE), started in 2008 with the aim to provide the knowledge about risk assessment of the Italian population following the environmental exposure to metals. The project is implemented through a HBM campaign for the production of data on 19 metals in the blood and serum of subjects living in different Italian Regions. The metals studied are: antimony, beryllium, cadmium, chromium, cobalt, iridium, lead, manganese, mercury, molybdenum, nickel, palladium, platinum, rhodium, thallium, tin, tungsten, uranium and vanadium. The first phase of the project has included the development and validation of laboratory protocols for the collection of fluids and quantification of metals. The second phase provides the HBM data expressed as the reference values (RVs) for the Italian population, *i.e.*, as the level of metals in the general population not occupationally exposed. In this paper, the experimental protocols used for the maintenance of high standards of analysis and the RVs for metals in serum of inhabitants of two Italian Regions (Calabria and Umbria) are described.

*Key words:* biological monitoring, reference values, metals, serum, population.

**Riassunto** (*La rete italiana per il biomonitoraggio umano dei metalli: risultati preliminari ottenuti in due regioni*). Il programma italiano per il biomonitoraggio umano degli elementi chimici, PROgramma per il Biomonitoraggio dell'Esposizione (PROBE), è stato avviato nel 2008 con l'obiettivo di fornire le conoscenze necessarie per la stima del rischio della popolazione italiana in seguito all'esposizione ambientale a metalli. Il progetto è realizzato attraverso una campagna di biomonitoraggio per ottenere dati su 19 metalli nel sangue e nel siero di soggetti distribuiti in varie regioni italiane. I metalli studiati sono: antimonio, berillio, cadmio, cobalto, cromo, iridio, manganese, mercurio, molibdeno, nichel, palladio, piombo, platino, rodio, stagno, tallio, tungsteno, uranio e vanadio. La prima fase del progetto ha previsto lo sviluppo e la validazione di protocolli di laboratorio per la raccolta dei fluidi e la quantificazione dei metalli. Nella seconda fase si forniscono i dati di biomonitoraggio, espressi come i valori di riferimento (VR) per la popolazione italiana, ovvero come i livelli dei metalli nella popolazione generale non professionalmente esposta. In questo lavoro si presentano i protocolli sperimentali utilizzati per il mantenimento di elevati standard analitici e i VR per i metalli nel siero di residenti in due regioni italiane (Calabria e Umbria).

*Parole chiave:* monitoraggio biologico, valori di riferimento, metalli, siero, popolazione.

## INTRODUCTION

Human biomonitoring (HBM), *i.e.* the measurement of people's exposure to toxic chemicals in the environment by measuring them in human specimens (blood, serum, urine, hair, etc.), emerges as an indispensable tool for combining health and environment. The evaluation of the internal dose of contaminants as metals in the general population is currently available for only a few geographical areas in Italy. In 1990, data for 20 metals in the Region of Lombardia have been published [1], and, after a gap of many years (in 2005), a survey on 26 metals in the Region of Lazio was produced [2]. It should also

be noted that for some metals (for example, iridium, palladium, platinum, rhodium and uranium) data are still missing for the Italian general population. The meta-analysis approach has been exploited as a possible alternative to produce reference values (RVs) for metals and data for 18 elements were obtained by pooling published statistics and individual data [3].

The main reason for the paucity of data on this topic is that the production of RVs requires the rigorous application of a methodology aimed at controlling the pre-analytical, analytical and biological factors that could alter the final data [4]. Many difficulties

are often encountered in performing quality control programs of trace metal analysis in biological fluids. For example, critical aspects are those related to the risk of contamination during fluid sampling – e.g., due to the use of anticoagulants – or arising from the laboratory environment, or the risk of losses during storage [5, 6]. Moreover, other crucial factors come from biological variability that could affect the concentrations to be determined, such as the route of absorption, the presence of sources of environmental pollution in certain residential areas, the physiological variables and life styles. These aspects are frequently disregarded or partially explored.

Against this background, a national HBM survey on the environmental exposure of the general population to metals – PROgram for biomonitoring of the exposure (PROBE) – was started by the Italian National Institute for Health in 2008, in cooperation with the Italian Blood Volunteer Association (AVIS) and the National Italian Association against Microcythemia (ANMI).

Using PROBE, it will become possible, for the first time, to make available representative data on the body burden of 19 environmental metals in the Italian population (both adults and adolescents) over a 2-years period. The metals measured are: antimony (Sb), beryllium (Be), cadmium (Cd), cobalt (Co), chromium (Cr), iridium (Ir), lead (Pb), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), palladium (Pd), platinum (Pt), rhodium (Rh), thallium (Tl), tin (Sn), tungsten (W), uranium (U) and vanadium (V).

Main objectives of PROBE are: *i*) to determine which and what levels of metals are found in biological fluids of the Italians due to the background environmental exposure; *ii*) to establish RVs that can be used to determine persons or groups with an unusually high exposure to metals; *iii*) to determine whether different exposure levels occur in adolescents or in other potentially vulnerable groups; and *iv*) to track time-trends and Regional differences in the levels of exposure to metals. The results provided by PROBE can be finally used by national and international authorities both to assess the effectiveness of public health efforts to reduce exposure to specific metals and to set priorities for research on human health effects.

The operative phases of PROBE are the following: *i*) critical evaluation of previous literature data; *ii*) standardization of procedures to select the population and collect human fluids; *iii*) development and validation of analytical methods; *iv*) assessment of the RVs for metals in the Italian population, and their stratification according to age, sex, geographic area, etc.; *v*) identification of susceptible population groups; and *vi*) dissemination of the obtained knowledge by means of an easily accessible web database.

The following data are here presented: *i*) the interview scheme for the selection of individuals; *ii*) the *modus operandi* for the blood collection; *iii*) the quality assurance programme (validation and un-

certainty estimation of measurements) for the analysis of metals; and *iv*) the RVs for metals in serum of inhabitants of two Regions of Italy (*i.e.*, Umbria and Calabria).

## SELECTION OF SUBJECTS

An informed written consent was obtained from each subject included in PROBE, and an inquiry by means of a questionnaire was carried out in parallel. More specifically, the questionnaire key points were: *i*) identification of the subject in terms of sex, age, height, weight, body mass index, place of residence and of business; *ii*) anamnesis in terms of acute or chronic diseases (age at diagnosis), recent drug intake (60 days), dental fillings or metal implants (type, number, how long), change in body weight over the last two years; *iii*) life style in terms of alcohol consumption (type, quantity, frequency), smoke (type, quantity, frequency), exercise (type, frequency), traffic at home (type, intensity), distance of the home from industrial areas and type of industrial area; *iv*) diet in terms of type (normal, vegetarian, etc.), consumption of fish (weekly amount), consumption of milk and dairy products (weekly amount); and *v*) physiological state in terms of pregnancy, hormonal supplementation therapy, use of contraceptive drugs, stress.

The exclusion criteria adopted in PROBE were the following: cardiological, respiratory, kidney or liver disorders; intestinal absorption abnormalities; active infections; assumption of thyroid hormones or lithium; psychoactive drug intake; assumption of vitamins or mineral integrators; iatrogenic exposure from metallic implants such prostheses, surgical screws or intrauterine inserts. Subjects resulting out of the physiological ranges about chemical-clinical parameters were also excluded.

## BLOOD COLLECTION

In general, the entire experimental scheme was designed taking strict precautions to avoid alterations in the analytical information from samples. Briefly, the safety measures included the use of the following set of devices and reagents: *i*) powder-free latex gloves; *ii*) Teflon endovenous catheters or needles for metal analysis; *iii*) decontaminated polystyrene disposable tubes; *iv*) hydrogen peroxide of suprapur grade (Merck, Darmstadt, Germany); and *v*) high purity deionised water (EASY Pure system, Barnstead, Dubuque, USA). Blood drawings were executed within 8 and 10 a.m. on subjects fasted overnight, and the venipuncture area was disinfected by means of hydrogen peroxide and then rinsed with deionized water. At the end of blood donation, a 10 mL aliquot was used for the project purposes. This procedure allowed to rinse catheter and pipes and further minimize contamination of metals which can be transferred to blood. Blood and serum were collected in special containers, *i.e.* the S-

Monovette® for Trace Metal Analysis that were used in conjunction with a S-Monovette® needle; these products were suitable for trace metal determination because at low level of metal impurities (Sarstedt, Nümbrecht, Germany). The specimens were stored at -20 °C and transported to the laboratories in a deep-frozen state.

### QUANTIFICATION

Serum samples were diluted 1+4 (v/v) with deionized high purity water. The subtraction of signal blanks, the internal standardization with <sup>133</sup>Cs and <sup>69</sup>Ga at the concentration of 1 µg/L and the addition-calibration approach were chosen as tools to control for possible contaminations due to ancillary equipments, instrumental drifts and matrix effects.

The sector field inductively coupled plasma mass spectrometry (SF-ICP-MS) – Element2, Thermo Scientific, Bremen, Germany – equipped with a Meinhard nebulizer, a water cooled spray chamber (Scott-type), and a guard electrode device, was used for the quantification of metals. The instrumental parameters (torch position, lenses, argon flow rates, and radio frequency power) were optimized by measuring the signals of the <sup>115</sup>In, and counts per second (cps) > 1 000 000 in low resolution (LR, m/Δm = 300) and > 100 000 in medium resolution (m/Δm = 4000) were established as good enough to start the

analysis. Metal oxides and double charged ions were daily minimized on the BaO<sup>+</sup>/Ba<sup>+</sup> and Ba<sup>2+</sup>/Ba<sup>+</sup> ratios, respectively. Also instrumental stability was every day checked and an instability below 2% on <sup>7</sup>Li, <sup>115</sup>In and <sup>238</sup>U masses was considered acceptable.

The operative conditions that met these criteria were as follows: radiofrequency power, 1250 kW; plasma flow rate, 15 L/min; auxiliary and sample gas flows ranging from 0.8 to 1.1 L/min; peristaltic pump speed, 1.0 mL/min; temperature of the spray chamber, ca. 10 °C. The analytical masses <sup>9</sup>Be, <sup>114</sup>Cd, <sup>202</sup>Hg, <sup>193</sup>Ir, <sup>100</sup>Mo, <sup>208</sup>Pb, <sup>195</sup>Pt, <sup>121</sup>Sb, <sup>120</sup>Sn, <sup>205</sup>Tl, <sup>238</sup>U, <sup>51</sup>V and <sup>184</sup>W were selected for quantification. For interfered elements as <sup>59</sup>Co, <sup>52</sup>Cr, <sup>55</sup>Mn, <sup>60</sup>Ni, <sup>108</sup>Pd and <sup>103</sup>Rh the MR setting was used so as to unequivocally separate the analyte from interferences. Calibration solutions for metals were prepared by appropriate dilution of 1000 mg/L mono-element stock standard solutions (Spex, Edison, NJ, USA).

### METHOD PERFORMANCE AND UNCERTAINTY

The method validation consisted of the assessment of the following performance characteristics: *i*) limit of detection (LoD); *ii*) trueness and recovery; and *iii*) precision (inter-day repeatability and within-laboratory reproducibility), as previously reported [7].

**Table 1** | Performances of the SF-ICP-MS method

| Element | LoD<br>µg/L | Trueness/Recovery   |               | Repeatability<br>RSD (%) | Reproducibility<br>RSD (%) |
|---------|-------------|---------------------|---------------|--------------------------|----------------------------|
|         |             | µg/L                |               |                          |                            |
|         |             | Certified or spiked | Found         |                          |                            |
| Be      | 0.022       | 0.10**              | 0.10 ± 0.01   | 8.9                      | 8.8                        |
| Cd      | 0.015       | 0.50 ± 0.04         | 0.51 ± 0.06   | 4.8                      | 8.7                        |
| Co      | 0.035       | 0.23 ± 0.04         | 0.25 ± 0.02   | 5.7                      | 8.5                        |
| Cr      | 0.015       | 0.54 ± 0.07         | 0.50 ± 0.09   | 7.4                      | 12.0                       |
| Hg      | 0.079       | 1.10 ± 0.10         | 1.12 ± 0.05   | 6.9                      | 8.4                        |
| Ir*     | 0.500       | 10.0**              | 10.2 ± 1.70   | 6.7                      | 11.9                       |
| Mn      | 0.015       | 8.90 ± 0.90         | 8.86 ± 0.83   | 7.1                      | 6.8                        |
| Mo      | 0.050       | 0.52 ± 0.04         | 0.55 ± 0.07   | 7.3                      | 9.0                        |
| Ni      | 0.030       | 4.00 ± 0.40         | 4.31 ± 0.26   | 9.3                      | 6.3                        |
| Pb      | 0.038       | 2.90 ± 0.20         | 2.89 ± 0.16   | 4.7                      | 11.0                       |
| Pd*     | 2.850       | 20.0**              | 20.6 ± 1.8    | 7.4                      | 10.3                       |
| Pt*     | 0.742       | 12.00 ± 2.00        | 12.24 ± 0.54  | 9.7                      | 12.3                       |
| Rh*     | 2.050       | 20.0**              | 19.8 ± 2.9    | 10.5                     | 7.9                        |
| Sb      | 0.012       | 51.60 ± 3.70        | 49.29 ± 1.23  | 6.6                      | 8.7                        |
| Sn      | 0.060       | 1.24 ± 0.17         | 1.23 ± 0.11   | 9.9                      | 9.8                        |
| Tl      | 0.005       | 0.029 ± 0.005       | 0.031 ± 0.003 | 4.2                      | 8.4                        |
| U       | 0.025       | 0.21 ± 0.02         | 0.21 ± 0.04   | 7.5                      | 8.0                        |
| V       | 0.015       | 0.71 ± 0.10         | 0.78 ± 0.05   | 4.4                      | 6.9                        |
| W       | 0.019       | 0.15 ± 0.08         | 0.15 ± 0.02   | 8.0                      | 7.0                        |

\*: ng/L; \*\*: spiked; RSD: relative standard deviation.

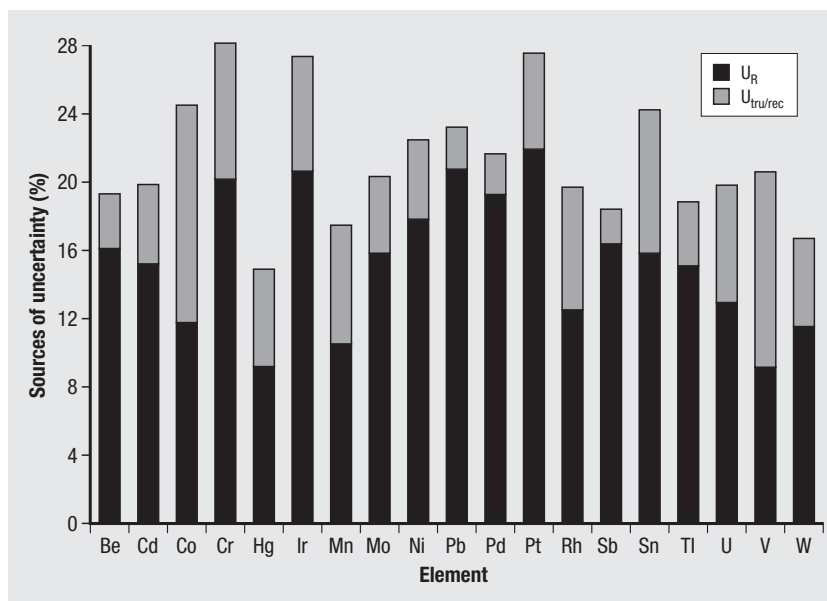
Results of the validation study are given in *Table 1*. The LoD was expressed as the analyte concentration corresponding to the value of 3-times the SD of measurements on serum samples. The trueness was checked against a certified reference material (CRM) (Sero AS, Billingstätt, Norway) certified for Cd, Co, Cr, Hg, Mn, Mo, Ni, Pb, Pt, Sb, Sn, Tl, U, V and W. A recovery test was adopted for Be, Ir, Pd and Rh as they were not certified in the CRM, and, serum was spiked with known amounts of metals (*i.e.*, 1-time the native serum concentration). The trueness ranged from 92.6% (Cr) to 108.7% (Co), and the recovery was between 99.0% and 103.0%. According to the Commission Decision 2002/657/EC, the method passed the test for trueness/recovery because the distance between the found values and the certified/spiked ones was contained in the  $\pm 10\%$  interval [8]. To check the inter-day repeatability of the method, serum samples were analyzed in three different times by the same analyst using the same equipment. The within-laboratory reproducibility was assessed by analyzing serum samples by two different analysts in two different days. The repeatability resulted to be  $\leq 10.5\%$  and the reproducibility was  $\leq 12.3\%$  (*see Table 1*), both lower than the limits of tolerance recommended by the Commission Decision 2002/657/EC [8].

The uncertainty calculation was that based on the use of information from the validation process [9-11] and the following sources were considered in its quantification: *i*) the uncertainty ( $u_R$ ) given by the within-laboratory reproducibility study; *ii*) the uncertainty ( $u_{tru/rec}$ ) given by the trueness/recovery study; and *iii*) the identification and evaluation of other uncertainty ( $u_{other}$ ) contributions not adequately covered by the trueness/recovery and precision studies as the effects of operator, calibration, laboratory equipment, etc. Because these effects were

already included in the reproducibility uncertainty, the  $u_{other}$  can be considered unnecessary. The way of calculation of each contribution to the uncertainty is reported elsewhere [7, 12, 13]. According to the law of error propagation, the combined uncertainty ( $u_{comb}$ ) is given by this formula  $u_{comb} = \sqrt{u_R^2 + u_{tru/rec}^2}$  and the expanded uncertainty estimate (U) is the  $u_{comb}$  multiplied for a coverage factor of 2, which gives a level of confidence of approximately 95%. As shown in *Figure 1*, the expanded uncertainty budget, calculated at the level corresponding to 1-time the baseline serum concentration, ranged between ca. 15.0% (Hg) and 28.0% (Cr, Ir and Pt). The overall uncertainty was found to be dominated by the precision term for all the elements analyzed, and, in some cases (Be, Pb, Pd, Sb) the  $u_R^2$  is much bigger than  $u_{tru/rec}^2$  (from 5-fold to 9-fold).

### THE RVs PRODUCTION FOR TWO ITALIAN REGIONS (UMBRIA AND CALABRIA)

*Tables 2* and *3* give the basic statistical treatment of data for serum of two urban population groups living in Umbria and Calabria. To describe the distributions of levels of metals in serum, the following statistical parameters are stated in the *Tables*: number of cases (valid, outliers and values below the respective analytical LoD), arithmetic mean and standard error (AM  $\pm$  SE), geometric mean (GM), minimum and maximum value, percentiles (5°, 25°, 50°, 75°, 95°). From the data set were excluded as outliers those values out of 3-fold the interquartile range. Because the percentage of measured values below the LoD were low, they were not used for calculations. All metal values felt within the previously reported literature ranges [6, 13]. Higher concentrations were revealed for W and U and the most prob-



**Fig. 1** | Contribution of uncertainty sources ( $u_R$  and  $u_{tru/rec}$ ) to the expanded uncertainty of the measurements.

**Table 2** | Reference values ( $\mu\text{g/L}$ ) for metals in serum of subjects from the Region of Umbria

| Element | Cases |          |       | AM $\pm$ SE      | GM   | Min  | Max  | Percentiles |      |      |      |      |
|---------|-------|----------|-------|------------------|------|------|------|-------------|------|------|------|------|
|         | Valid | Outliers | < LoD |                  |      |      |      | 5°          | 25°  | 50°  | 75°  | 95°  |
| Be      | 104   | 1        | 5     | 0.06 $\pm$ 0.002 | 0.06 | 0.02 | 0.13 | 0.03        | 0.05 | 0.06 | 0.07 | 0.09 |
| Cd      | 255   | 3        | 33    | 0.16 $\pm$ 0.005 | 0.14 | 0.07 | 0.45 | 0.07        | 0.10 | 0.13 | 0.18 | 0.32 |
| Co      | 289   | 1        | 0     | 0.32 $\pm$ 0.007 | 0.30 | 0.10 | 0.73 | 0.17        | 0.24 | 0.29 | 0.38 | 0.59 |
| Cr      | 280   | 10       | 0     | 0.14 $\pm$ 0.004 | 0.13 | 0.02 | 0.43 | 0.06        | 0.09 | 0.13 | 0.17 | 0.26 |
| Hg      | 288   | 1        | 2     | 0.78 $\pm$ 0.02  | 0.70 | 0.19 | 1.85 | 0.29        | 0.51 | 0.75 | 1.04 | 1.43 |
| Ir*     | 283   | 0        | 8     | 3.20 $\pm$ 0.14  | 2.46 | 0.50 | 11.7 | 0.66        | 1.34 | 2.54 | 4.41 | 7.62 |
| Mn      | 289   | 1        | 0     | 0.92 $\pm$ 0.02  | 0.88 | 0.39 | 1.93 | 0.55        | 0.71 | 0.88 | 1.08 | 1.40 |
| Mo      | 290   | 1        | 0     | 1.03 $\pm$ 0.02  | 0.95 | 0.20 | 2.75 | 0.45        | 0.75 | 0.95 | 1.26 | 1.86 |
| Ni      | 285   | 3        | 2     | 0.47 $\pm$ 0.01  | 0.43 | 0.14 | 1.07 | 0.19        | 0.33 | 0.43 | 0.57 | 0.86 |
| Pb      | 278   | 13       | 0     | 0.21 $\pm$ 0.006 | 0.18 | 0.04 | 0.61 | 0.09        | 0.13 | 0.17 | 0.24 | 0.44 |
| Pd*     | 283   | 1        | 6     | 12.0 $\pm$ 0.31  | 11.0 | 5.00 | 36.0 | 5.51        | 7.80 | 11.4 | 15.4 | 21.0 |
| Pt*     | 288   | 3        | 0     | 7.76 $\pm$ 0.23  | 6.87 | 0.96 | 23.9 | 3.28        | 4.82 | 6.98 | 9.41 | 15.8 |
| Rh*     | 274   | 5        | 11    | 10.6 $\pm$ 0.33  | 9.40 | 3.35 | 30.4 | 4.22        | 6.55 | 9.80 | 12.9 | 21.8 |
| Sb      | 286   | 5        | 0     | 0.13 $\pm$ 0.007 | 0.09 | 0.02 | 0.57 | 0.03        | 0.05 | 0.08 | 0.18 | 0.38 |
| Sn      | 286   | 5        | 0     | 0.38 $\pm$ 0.014 | 0.32 | 0.08 | 1.40 | 0.12        | 0.20 | 0.33 | 0.51 | 0.86 |
| Tl      | 281   | 10       | 0     | 0.03 $\pm$ 0.001 | 0.03 | 0.01 | 0.08 | 0.01        | 0.02 | 0.02 | 0.03 | 0.05 |
| U       | 288   | 3        | 0     | 0.26 $\pm$ 0.004 | 0.26 | 0.15 | 0.47 | 0.18        | 0.22 | 0.25 | 0.29 | 0.38 |
| V       | 283   | 7        | 0     | 0.06 $\pm$ 0.001 | 0.05 | 0.02 | 0.14 | 0.03        | 0.04 | 0.05 | 0.06 | 0.09 |
| W       | 290   | 1        | 0     | 0.15 $\pm$ 0.004 | 0.14 | 0.05 | 0.40 | 0.08        | 0.10 | 0.14 | 0.18 | 0.26 |

\*: ng/L; AM: arithmetic mean; SE: standard error; GM: geometric mean; Min: minimum value; Max: maximum value.

able contamination source might be the release from the S-Monovette<sup>®</sup> serum gel tubes used for serum collection and storage. Experiments are in progress

to evaluate the actual extent of the systematic error on final W and U concentrations. The percentiles of the upper measuring range could be used for rea-

**Table 3** | Reference values ( $\mu\text{g/L}$ ) for metals in serum of subjects from the Region of Calabria

| Element | Cases |          |       | AM $\pm$ SE      | GM   | Min  | Max  | Percentiles |      |      |      |      |
|---------|-------|----------|-------|------------------|------|------|------|-------------|------|------|------|------|
|         | Valid | Outliers | < LoD |                  |      |      |      | 5°          | 25°  | 50°  | 75°  | 95°  |
| Be      | 106   | 0        | 18    | 0.05 $\pm$ 0.002 | 0.05 | 0.02 | 0.14 | 0.02        | 0.03 | 0.06 | 0.07 | 0.09 |
| Cd      | 209   | 10       | 2     | 0.09 $\pm$ 0.003 | 0.08 | 0.01 | 0.24 | 0.03        | 0.06 | 0.07 | 0.09 | 0.19 |
| Co      | 216   | 5        | 0     | 0.17 $\pm$ 0.006 | 0.16 | 0.06 | 0.48 | 0.07        | 0.11 | 0.15 | 0.21 | 0.38 |
| Cr      | 218   | 3        | 0     | 0.12 $\pm$ 0.004 | 0.11 | 0.03 | 0.37 | 0.05        | 0.08 | 0.10 | 0.15 | 0.25 |
| Hg      | 215   | 5        | 0     | 0.65 $\pm$ 0.02  | 0.57 | 0.14 | 1.95 | 0.24        | 0.41 | 0.58 | 0.78 | 1.37 |
| Ir*     | 218   | 1        | 2     | 3.76 $\pm$ 0.12  | 3.34 | 0.63 | 10.8 | 1.22        | 2.49 | 3.41 | 4.76 | 7.53 |
| Mn      | 218   | 3        | 0     | 0.83 $\pm$ 0.01  | 0.81 | 0.37 | 1.53 | 0.50        | 0.69 | 0.80 | 0.96 | 1.22 |
| Mo      | 218   | 3        | 0     | 0.90 $\pm$ 0.02  | 0.84 | 0.19 | 2.12 | 0.46        | 0.66 | 0.85 | 1.06 | 1.52 |
| Ni      | 216   | 1        | 4     | 0.40 $\pm$ 0.01  | 0.36 | 0.12 | 1.07 | 0.16        | 0.28 | 0.35 | 0.49 | 0.81 |
| Pb      | 213   | 8        | 0     | 0.17 $\pm$ 0.006 | 0.16 | 0.03 | 0.47 | 0.08        | 0.12 | 0.15 | 0.21 | 0.37 |
| Pd*     | 212   | 1        | 8     | 16.0 $\pm$ 0.49  | 14.5 | 5.00 | 38.9 | 6.46        | 11.2 | 14.7 | 19.3 | 31.3 |
| Pt*     | 213   | 5        | 3     | 7.04 $\pm$ 0.21  | 6.39 | 1.10 | 18.6 | 2.78        | 5.22 | 6.44 | 8.59 | 13.7 |
| Rh*     | 220   | 1        | 0     | 12.4 $\pm$ 0.36  | 11.3 | 3.68 | 31.4 | 5.00        | 8.69 | 11.4 | 14.5 | 24.9 |
| Sb      | 217   | 4        | 0     | 0.11 $\pm$ 0.004 | 0.09 | 0.01 | 0.29 | 0.03        | 0.07 | 0.09 | 0.13 | 0.22 |
| Sn      | 219   | 2        | 0     | 0.32 $\pm$ 0.009 | 0.30 | 0.09 | 0.82 | 0.15        | 0.28 | 0.31 | 0.39 | 0.56 |
| Tl      | 210   | 11       | 0     | 0.04 $\pm$ 0.001 | 0.03 | 0.01 | 0.11 | 0.02        | 0.02 | 0.04 | 0.05 | 0.08 |
| U       | 220   | 1        | 0     | 0.26 $\pm$ 0.005 | 0.25 | 0.11 | 0.48 | 0.15        | 0.21 | 0.25 | 0.31 | 0.40 |
| V       | 217   | 4        | 0     | 0.05 $\pm$ 0.001 | 0.04 | 0.02 | 0.10 | 0.02        | 0.03 | 0.04 | 0.05 | 0.08 |
| W       | 218   | 3        | 0     | 0.12 $\pm$ 0.003 | 0.12 | 0.04 | 0.30 | 0.07        | 0.09 | 0.11 | 0.15 | 0.24 |

\*: ng/L; AM: arithmetic mean; SE: standard error; GM: geometric mean; Min: minimum value; Max: maximum value.

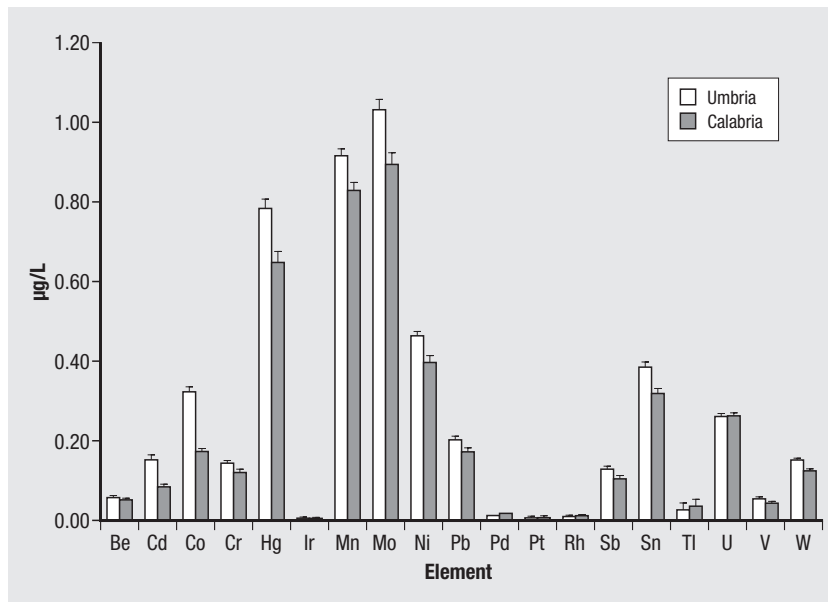


Fig. 2 | Comparisons of metal serum levels ( $\mu\text{g/L}$ ) in subjects from two Regions of Italy.

sions of environmental and health policy because on the one hand, these percentiles can be used to derive the RVs and on the other, populations with a higher exposure (higher body burden) are of particular interest with regard to preventive health care. Figure 2 shows a comparison between the data obtained in the two Regions. A different distribution between the two population groups was observed: the pattern was that the higher values were found in the Region of Umbria for most of the elements, exception for Pd and Rh which were slightly higher in Calabria.

## CONCLUSIONS

The data here presented give a summary of the tools and methodologies used to produce reliable RVs as well the opening results of the PROBE project in terms of the exposure assessment to metals. These data provide a unique information about the serum metal level of two urban population groups living in different Regions of Italy and, due to the number of subjects considered, the results offer a basis to establish the RVs in adults exposed to urban environments. The results fill the gap for some metals (*i.e.*, Ir, Pd, Pt, Rh, U and W) scarcely or never studied before in Italy and they update the pre-existing situation which refers to several years ago and/or to a small number of subjects. The validation process demonstrated the agreement of the method performances with provisions of the

Commission Decision 2002/657/EC for providing accurate and precise analysis. The contributions due to calibration, certified reference material (or spiked samples) and volumetric operations were negligible compared the principal source of uncertainty, which, at the low levels of metals usually expected in human serum, is largely due to reproducibility.

The future activities of the PROBE project will deal with the analysis of subjects living in other Regions of Italy and with the recognition of sub-groups by means of stratification variables (gender, age, place of residence etc.) to help policy makers to find and launch new environment and health measures to reduce the exposure to environmental metals of the Italian population.

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## Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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