Chemosphere 286 (2022) 131809

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Exposure to metals and metalloids among pregnant women from Spain: Levels and associated factors

Manuel Lozano[a,b,*], Mario Murcia[b,c,d], Raquel Soler-Blasco[b], Maribel Casas[c,e,f], Begotxu Zubero[g,h], Gabriel Riutort-Mayol[i], Fernando Gil[j], Pablo Olmedo[j], Joan O. Grimalt[k], Rubén Amorós[b], Aitana Lertxundi[c,g,h], Martine Vrijheid[c,e,f], Ferran Ballester[b,c,l], Sabrina Llop[b,c]

[a] Preventive Medicine and Public Health, Food Sciences, Toxicology and Forensic Medicine Department, Universitat de València, Valencia, Spain
[b] Epidemiology and Environmental Health Joint Research Unit, FISABIO–Universitat Jaume I–Universitat de València, Valencia, Spain
[c] Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Madrid, Spain
[d] Servicio de Análisis de Sistemas de Información Sanitaria, Conselleria de Sanitat, Generalitat Valenciana, Valencia, Spain
[e] ISGlobal, Barcelona, Spain
[f] Universitat Pompeu Fabra, Barcelona, Spain
[g] Preventive Medicine and Public Health Department, University of Basque Country, UPV/EHU, Leioa, Bizkaia, Spain
[h] Health Research Institute, Biodonostia, San Sebastian, Gipuzkoa, Spain
[i] Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO-Public Health, Spain
[j] Department of Legal Medicine and Toxicology, School of Medicine, University of Granada, Spain
[k] Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Catalonia, Spain
[l] Department of Nursing, Universitat de València, Valencia, Spain

HIGHLIGHTS

- Zn was the element detected in the highest concentrations and Tl in the lowest levels.
- Associations of As, Cd, Cu, Sb, Tl, Zn with working situation, social class, age, were found.
- Seafood, meat, fruits, nuts, vegetables and alcohol intake affected all metals levels but Cd, and Cu.
- Proximity to industrial areas, fields and air pollution, were related to all metals except Cd, Sb and Se.

ARTICLE INFO

Handling Editor: Lena Q. Ma

Keywords:
Metals
Metalloids
Prenatal exposure
Environmental exposure
Diet

ABSTRACT

Background: Humans are regularly exposed to metals and metalloids present in air, water, food, soil and domestic materials. Most of them can cross the placental barrier and cause adverse impacts on the developing foetus.
Objectives: To describe the prenatal concentrations of metals and metalloids and to study the associated sociodemographic, environmental and dietary factors in pregnant Spanish women.
Methods: Subjects were 1346 pregnant women of the INMA Project, for whom the following metals arsenic (As), cadmium (Cd), cobalt (Co), copper (Cu), molybdenum (Mo), nickel (Ni), lead (Pb), antimony (Sb), selenium (Se), thallium (Tl) and zinc (Zn) were determined in urine, at both the first and the third trimesters of gestation. Sociodemographic, dietary and environmental information was collected through questionnaires during pregnancy. Multiple linear mixed models were built in order to study the association between each metal and metalloid concentrations and the sociodemographic, environmental and dietary factors.
Results: The most detected compounds were As, Co, Mo, Sb, Se and Zn at both trimesters. Zn was the element found in the highest concentrations at both trimesters and Tl was detected in the lowest concentrations. We observed significant associations between As, Cd, Cu, Sb, Tl and Zn concentrations and working situation, social class and age. Seafood, meat, fruits, nuts, vegetables and alcohol intake affected the levels of all the metals but Cd and Cu. Proximity to industrial areas, fields and air pollution were related to all metals except Cd, Sb and Se.

---

[*] Corresponding author. Preventive Medicine and Public Health, Food Sciences, Toxicology and Forensic Medicine Department, Universitat de València, Valencia, Spain.
E-mail address: manuel.lozano@uv.es (M. Lozano).

https://doi.org/10.1016/j.chemosphere.2021.131809
Received 26 April 2021; Received in revised form 2 August 2021; Accepted 3 August 2021

Conclusions: This is the first large prospective longitudinal study on the exposure to metals and metalloids during pregnancy and associated factors to include several cohorts in Spain. The present study shows that some modifiable lifestyles, food intakes and environmental factors could be associated with prenatal exposure to metal (loid)s, which may be considered in further studies to assess their relationship with neonatal health outcomes.

1. Introduction

Metals and metalloids can be released into the environment from both natural and anthropogenic sources, mainly mining and industrial activity. Specifically, for lead (Pb) the most significant sources are energy-related sources associated with fuel combustion, and heat-generating and industrial facilities (Briffa et al., 2020). Elevated levels of cobalt (Co) in the soil may result from anthropogenic activities such as the application of Co-containing sludge or phosphate fertilizers, the disposal of Co-containing waste and atmospheric deposition from activities such as mining, smelting, refining or combustion. Sources of nickel (Ni) emissions into the air include burning coal and oil for power and heat, waste and sewage sludge incineration, mining and steel production industries, as well as electroplating. Smoking is a relevant source of cadmium (Cd). Selenium (Se) is present in the soil and humans are exposed to different Se compounds depending on the food source; since, plants accumulates the inorganic form and animals transform Se into the organic form, mostly selenocysteine and selenomethionine (Peters et al., 2016). The most important sources of thallium (Tl) exposure in the general population are the air emissions from coal-burning power plants and copper (Cu), Pb and zinc (Zn) smelters (Nordberg et al., 2014).

Once emitted from their sources, metals have the property of accumulating in the environment for many years. Humans are regularly exposed to metals and metalloids present in air, water, food, soil and domestic materials. Whereas certain metals and trace minerals are essential for life, e.g. Zn, Cu, Se and iron (Fe), others such as Pb, arsenic (As), Tl, chromium (Cr) or Cd can be toxic, even at low concentrations (Fort et al., 2014). Additionally, human exposure rarely occurs in isolation (Sanders et al., 2015). Regarding their distribution, environmental levels of trace elements and heavy metals clearly differ from one country or region to another around the world (Wang et al., 2019a). Each country with data available has its own report based on large-scale population-based studies, but the results are heterogeneous with respect to each other. This fact is based on their different socioeconomic status and demographic indicators, which affect the relevance of each anthropogenic source (Callan et al., 2013; Lewin et al., 2017). Metals enter the human body through inhalation, ingestion and skin contact. In fact, the body burden of metal elements in humans is known to be influenced by dietary habits, lifestyles and environmental conditions. In Spain, most of the metals measured in urine samples are similar to those reported in previous studies worldwide, especially from non-contaminated sites (Forns et al., 2014; Pérez et al., 2018).

Most of the metals can cross the placental barrier and be transferred to the foetus, so both pregnant women and foetus are considered a vulnerable group in terms of exposure (Iyengar and Rapp, 2001). This group of metals may have adverse impacts on the developing foetus, as they have an affinity for the same ligands and transporters used by essential metals (Nazzareno, 2002). Extensive research has been conducted on the effect of prenatal exposure to metals on children's health and development, but most of the studies were based on specific metals in maternal blood samples (Inadera et al., 2020; Goto et al., 2021; Kobayashi et al., 2019). Some of this research was carried out within the framework of the INMA – INfancia y Medio Ambiente (Environment and Childhood) – Project (http://www.proyectoinma.org/), which is a network of seven birth cohorts in Spain that aims to study the role that exposure to environmental pollutants in air, water and diet during pregnancy and childhood plays in child growth and development (Guxens et al., 2012; Lozano et al., 2020, 2021; Soler-Blasco et al., 2020, 2021).

Therefore, the objective of the present work is to describe the prenatal concentrations of metals and metalloids and to study the associated sociodemographic, environmental and dietary factors in pregnant Spanish women participant in three of the INMA cohorts.

2. Methods

2.1. Study population

Subjects were participants in the Valencia, Gipuzkoa and Sabadell cohorts of the INMA Project (Supplemental Figure S1). The study protocol has been reported elsewhere (Guxens et al., 2012). Briefly, during the first trimester of pregnancy, 2270 pregnant women were recruited during their first antenatal visit (2003–2008) in 3 regions of Spain: Valencia (east, $n = 855$), Gipuzkoa (north, $n = 638$) and Sabadell (north-east, $n = 777$). Recruitment took place by consecutive sampling of those women who met the inclusion criteria: ≥16 years, singleton pregnancy, non-assisted conception, delivery scheduled at the reference hospital, and no impairment hindering communication. The study sample comprised mothers for whom the following metals As, Cd, Co, Cu, Mo, Ni, Pb, Sb, Se, Tl and Zn had been determined in urine ($n = 1346$, 59.3 %), at both the first and the third trimesters of gestation.

Analyses were performed to determine any differences between the population included in the study (considering mothers with double urinary measurements for at least one metal during pregnancy, $n = 1346$, 59.3 %) and those who were not included (because they had no double urinary measurements for any metal, $n = 924$, 40.7 %). Among the participants, there was a higher percentage of slightly older mothers with a higher level of education and social class than in the case of non-participants. Sociodemographic, dietary and environmental characteristics of both study participants with double urine measures and the population not included in the study are shown in Supplemental Table S1.

Informed consent was obtained from all participants in each phase, and the study was approved by the hospital ethics committees in the participating regions.

2.2. Measurements of metals

Concentrations of metals were determined in spot urine samples (Gaitens et al., 2021) taken at the first (mean [SD] = 13.13 [1.47] weeks of gestation) and third (mean [SD] = 33.08 [2.13] weeks of gestation) trimesters of pregnancy.

In the Gipuzkoa and Valencia cohorts, the urine samples were analysed at the University of Granada as follows: the samples were kept frozen at −20 °C until the analysis. A calibration curve was prepared in ultrapure water (Milli-Q) with 2 % HNO_3 (Merck) and 1 % HCl (Merck) using appropriate standard metal solutions (Agilent Technologies). Urine samples were diluted 1:10 in ultrapure water (Milli-Q) with 2 % HNO_3 (Merck) and 1 % HCl (Merck). Appropriate blanks were analysed to correct the results. The multi-element analyses were performed on an Agilent 8900 triple quadrupole ICP-MS-MS (Agilent Technologies, Santa Clara, CA, USA). The instrument was tuned and performance parameters were checked prior to analysis. To ensure the quality of the results, a multi-element 400 μg/L internal standard solution with Sc, Ge, Ir and Rh was added to the samples online. Furthermore, suitable certified reference materials [National Institute of Standards and Technology NIST (USA) Trace Elements in Natural Water Standard Reference Material SRM 1640a and Seronorm (Sero, Billingstad, Norway) Trace Elements Urine L1 and L2 (references 210,605 and 210,705 respectively)] were reanalysed together with a blank and an intermediate calibration standard every 12 samples. Additionally, one in every 12 samples was reanalysed at the end of each session.

On the other hand, in the Sabadell cohort, urine samples were analysed at the Institute of Environmental Assessment and Water Research (Barcelona) by inductively coupled plasma quadruple mass spectrometry (Q-ICP-MS). Prior to instrumental analysis, urine samples were digested and diluted as follows: 3 mL of urine were introduced in Teflon vessels together with 3 mL of Instra-Analysed 65 % HNO3 (J.T. Baker, Germany) and 1.5 mL of Instra-Analysed 30 % H_2O_2 (Baker) and they were left in an oven at 90 °C overnight. Once all liquid had been evaporated off, the resulting solid samples were dissolved with 3 mL of 4 % HNO3 dilution, placed in 7 mL glass bottles and subsequently stored in a refrigerator until instrumental analysis. An internal standard of indium (10 ppb) was introduced and, depending on the density, samples were diluted with MilliQ water to 30 mL or 60 mL to prevent spectral interferences. Q-ICP-MS analysis was performed by an X-SERIES II device from Thermo Fisher Scientific. One MilliQ water blank was processed in each batch of samples to control for possible contamination. A Bio-Rad Level 1 (Lyphochek Urine Metals Control 1–69131; Marnes-la-Coquette, France) urine reference was extensively used to evaluate analytical performance, as it contains metal concentrations close to those in the urine samples from the study cohort. This reference material provided certified values for As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Sb, Tl, Zn and Se. Prior to digestion, the lyophilized reference urine samples were reconstituted with 25 mL of MilliQ water as recommended by the manufacturer. One aliquot of this standard was analysed within each batch of samples as a control. The instrumental limit of detection (LOD) for most metals was 0.2 ng/mL except in the case of Cu, Mo and Zn, in which it was 1.0, 2.0 and 2.5 ng/mL.

Concentrations below the LOD obtained from both laboratories were replaced with the LOD divided by 2 for analysis. Limits of detection (LOD) are reported in Supplemental Table S2 for each element.

2.3. *Measure of creatinine*

Creatinine concentrations were also measured in the same urine samples at the first and third trimesters of pregnancy by DRI® Creatinine-Detected® Test using AV680 from Beckman Coulter. Urinary metal concentrations were expressed for descriptive purposes in μg/g of creatinine to control for differences in urine dilution.

2.4. *Sociodemographic covariates*

Women filled in two questionnaires during their pregnancy, at the first trimester (mean [SD] = 13.21 [1.58] weeks of gestation) and at the third trimester (mean [SD] = 32.3 [2.21] weeks of gestation). The questionnaires were administered by trained interviewers and focused on sociodemographic, environmental and lifestyle information during pregnancy. The maternal covariates used in this study were age (years), body mass index (BMI) (kg/m^2), country of birth (Spain, Latin America, other), education level (up to primary, secondary, university), parity (0, 1, ≥2), type of zone of residence (urban, semi-urban, rural), working during pregnancy (yes/no), smoking before pregnancy (yes/no), smoking during pregnancy (yes/no) and passive exposure to tobacco smoke (yes/no).

We defined parental social class from the parental occupation during pregnancy according to a widely used Spanish adaptation of the International Standard Classification of Occupations coding system (ISCO88). Class I + II included managerial jobs, technical staff and commercial managers; Class III included skilled non-manual workers; and class IV + V included manual and unskilled workers (Domingo-Salvany et al., 2013).

2.5. *Dietary covariates*

Information on diet during pregnancy was obtained from a semi-quantitative food frequency questionnaire (FFQ) at the first and third trimester. This FFQ was validated in the Valencia cohort with good reproducibility for nutrient and food intake (Vioque et al., 2013). We obtained data (expressed as the mean of the daily servings registered in both trimesters) on the intake of dairy products, eggs, meat (including red and white meat categories), seafood (including lean fish, oily fish and others), fruit, vegetables, legumes, nuts, potatoes, cereals and pasta, bread, sweets, beverages with and without alcohol, coffee and other infusions (such as tea), animal and vegetal fats, processed food and dressings (see Supplemental Table S1). Estimates on daily caloric intake were derived from the FFQ.

2.6. *Environmental covariates*

Exposure to environmental pollutants was assessed by analysing outdoor exposures based on the home address for the period of pregnancy. Some of these variables were collected through questionnaires at the third trimester of pregnancy and include the proximity to a street with traffic (metres), the frequency with which cars pass near the house (constantly, frequently, rarely, never), the frequency with which heavy traffic passes near the house (constantly, frequently, rarely, never), and the proximity to a greenhouse (yes/no), to a crop field (yes/no) or to industrial activity (yes/no).

Percentages of agricultural areas, semi-natural areas and wetlands in use, urban fabric land use and green urban areas, and sports and outdoor leisure facilities near the house were obtained within a buffer area of 300 m for each geocode. Land use information was obtained from the Urban Atlas database for the period 2006–2012, except for INMA Gipuzkoa, where local data from the European Nature Information System (EUNIS) was used in 2009.

Prenatal exposure to air pollutants including particulate matter (<2.5 μm in diameter, $PM_{2.5,}$ $\mu g/m^3$) and nitrogen dioxide (NO_2) was also measured following the methodology explained in previous INMA studies (Lertxundi et al., 2019; Iñiguez et al., 2009). Land-use regression (LUR) was employed to predict NO_2 and $PM_{2.5}$ levels at the women's residential addresses using the information from empirical measures and Geographical Information Systems data (altitude, distance to roads, traffic and land uses). Estimations of the women's $PM_{2.5}$ exposures during pregnancy followed different procedures in each cohort. In the case of Gipuzkoa, daily $PM_{2.5}$ levels were measured at seven sites in the study area throughout the whole period of the pregnancies. In the case of Sabadell, $PM_{2.5}$ measurements were taken 3 times a day for 14 days in different seasons in 2009. In the case of Valencia, $PM_{2.5}$ measurements were taken five times in five different months (twice in the summer and once in each of the other seasons) at four monitoring sites between April 2004 and June 2005, coinciding with the pregnancy of the participating women.

2.7. *Statistical analysis*

Basic descriptive statistics were calculated for each metal overall, each weekly measure and by participant characteristics. Metal concentrations in linear regressions were adjusted for creatinine, week of measure and cohort in all cases. The distributions of urinary metal concentrations were skewed and, for further analyses, they were log-transformed to approach normality (Supplemental Figure S2). Paired t-tests were used to assess differences between concentrations adjusted for creatinine measured at the first and third trimesters of pregnancy. The ANOVA F-test was applied to compare the mean of the metal concentrations across categories of the study population's categorical characteristics. Correlations between the log2-logarithms and continuous population characteristics, as well as among metals, were assessed by the Pearson test, adjusting for creatinine (average of both measures), week of measure and cohort. Differences between included and non-included participants in terms of sociodemographic, dietary and environmental characteristics were assessed by means of the Fisher exact test for categorical covariates and Mann-Whitney *U* test for continuous covariates.

Principal component analyses (PCA) with varimax normalized rotation (Kaiser, 1958), using only the urinary metal concentrations (as the first- and third-trimester average concentrations) adjusted for creatinine, were used in order to assess correlation patterns and identify clusters among metals. These clusters may indicate which urinary metal (oid)s are associated with each other based on sources of exposure and the influence of associated sociodemographic, environmental and dietary factors (Bommarito et al., 2019).

Simple and multiple linear mixed models were built, including a random intercept for each participant, to study the relationship between each metal concentration at the two trimesters of pregnancy and the sociodemographic, dietary and environmental factors. The covariates creatinine, week of measurement and cohort were included in all models in order to adjust for metal concentrations. Models with dietary covariates were also adjusted for energy intake. Each multiple model was built following three steps: 1) Obtaining a sociodemographic multiple basal mixed model by using all the sociodemographic covariates previously associated with a p-value <0.2 in the simple analyses. Following a backward elimination procedure, all the sociodemographic covariates associated with the metal concentrations at a p-value level <0.1 in the likelihood ratio test were retained in the model; 2) Dietary covariates were added to this sociodemographic basal model individually and those with a p-value <0.2 were candidates to enter in the model. Following a backward elimination procedure, all the dietary candidate covariates associated with the metal concentrations at a p-value level <0.1 were retained in the model. Although food intake variables were mutually correlated, we found no collinearity problems among them; 3) The same procedure was repeated on this new sociodemographic and dietary basal model using environmental covariates in order to obtain the final multivariate model. Statistical analysis was carried out using R statistical package version 3.5.1 (R Core Team, 2017).

3. Results

3.1. Metal levels in urine

The Geometric Means (GM) (95%CI) of measured maternal urinary As, Cd, Co, Cu, Mo, Ni, Pb, Sb, Se, Tl and Zn concentrations are shown in Table 1 (limits of detection and frequencies by trimester of measure are detailed in Supplemental Table S2). All metals except arsenic showed significant differences between the first and third trimesters (p-value <0.05). Concentrations were higher in the first trimester of pregnancy for As, Cd, Mo, Sb, Se and Tl. However, concentrations were higher in the third trimester for Co, Cu, Ni, Pb and Zn. Supplemental Table S3 shows the maternal urinary As, Cd, Co, Cu, Mo, Ni, Pb, Sb, Se, Tl and Zn concentrations according to participants' characteristics. Regional differences were found for nearly all the metals, except for Mo. Women born in Spain had the highest As and Se concentrations, and Latin American women had the highest Cd concentrations. Women with university studies presented higher concentrations of As, Cu, Se and Tl, but lower Sb and Zn. Women who lived in urban zones had the highest levels of Cd, Co, Ni, Pb and Sb, and women who lived in rural areas presented the highest concentrations of Se, As and Zn. Smokers had higher concentrations of Cd, Pb, Sb and Zn, but lower concentrations of Cu and Mo.

3.2. Correlations, principal components and cluster analysis

Correlations among log2-transfomed urinary metal concentrations calculated by the mean of the two measures (first and third trimesters) are shown in Fig. 1. Correlations were higher throughout pregnancy among the following groups of metals: (1) Co-Ni; (2) Cd-Cu-Pb; (3) Mo-Zn; and (4) As-Se. Supplemental Figure S3 shows correlations between

Table 1

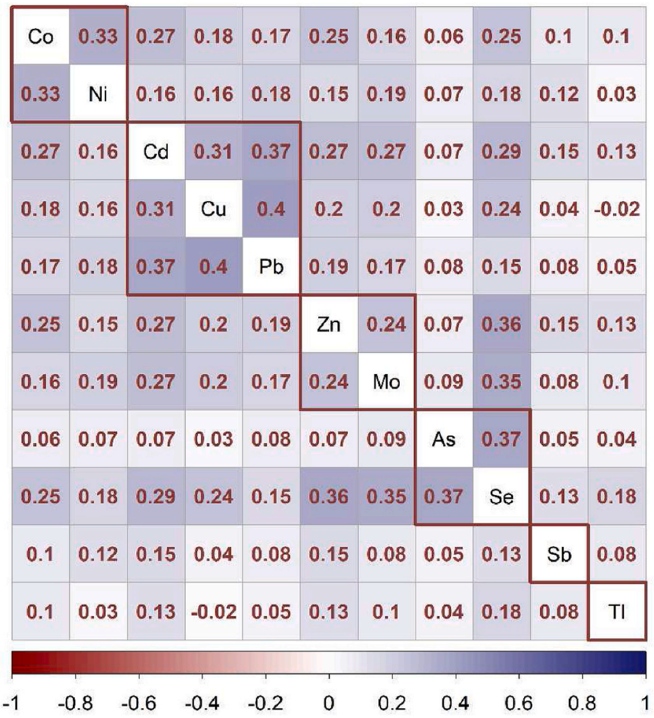
Geometric means and 95 % confidence intervals of measured urinary metal concentrations. INMA Project (Gipuzkoa, Sabadell and Valencia. Spain. 2003–2008).

Metal	N[b]	Geometric mean (CI 95 %)						
		Both measures		First trimester		Third trimester		
		µg/L	µg/g creat	µg/L	µg/g creat	µg/L	µg/g creat	P[a]
Arsenic (As)	1346	34.43 (32.93,36.00)	42.69 (40.91,44.55)	35.05 (32.88,37.37)	43.85 (41.20,46,67)	33.82 (31.79,35.98)	41.55 (39.20,44.04)	**0.386**
Cadmium (Cd)	1121	0.23 (0.22,0.23)	0.28 (0.27,0.29)	0.24 (0.23,0.25)	0.30 (0.29,0.32)	0.21 (0.20,0.22)	0.26 (0.25,0.27)	**<0.001**
Cobalt (Co)	1246	0.52 (0.50,0.54)	0.64 (0.62,0.67)	0.31 (0.30,0.32)	0.39 (0.37,0.40)	0.87 (0.83,0.92)	1.07 (1.03,1.12)	**<0.001**
Copper (Cu)	1192	7.18 (6.88,7.49)	8.93 (8.59,9.28)	4.69 (4.39,5.02)	5.90 (5.54,6.28)	10.97 (10.55,11.41)	13.58 (13.14,14.03)	**<0.001**
Molybdenum (Mo)	1346	38.80 (37.81,39.81)	48.11 (47.12,49.13)	41.77 (40.29,43.30)	52.27 (50.68,53.92)	36.04 (34.75,37.37)	44.24 (43.06,45.47)	**<0.001**
Nickel (Ni)	1305	1.27 (1.22,1.32)	1.58 (1.52,1.64)	1.21 (1.14,1.28)	1.51 (1.43,1.59)	1.35 (1.27,1.42)	1.66 (1.57,1.75)	**0.001**
Lead (Pb)	1217	1.14 (1.09,1.20)	1.48 (1.36,1.49)	0.97 (0.91,1.05)	1.22 (1.14,1.31)	1.34 (1.27,1.42)	1.65 (1.57,1.74)	**<0.001**
Antimony (Sb)	1274	0.35 (0.32,0.37)	0.43 (0.41,0.46)	0.38 (0.34,0.42)	0.48 (0.43,0.53)	0.32 (0.29,0.35)	0.39 (0.36,0.43)	**<0.001**
Selenium (Se)	1318	17.09 (16.55,17.66)	21.21 (20.62,21.82)	19.20 (18.37,20.07)	24.12 (23.20,25.08)	15.22 (14.52,15.95)	18.63 (17.90,19.39)	**<0.001**
Thallium (Tl)	1346	0.17 (0.17,0.18)	0.22 (0.21,0.22)	0.19 (0.18,0.20)	0.24 (0.23,0.25)	0.16 (0.15,0.17)	0.20 (0.19,0.21)	**<0.001**
Zinc (Zn)	1337	305.18 (296.42,314,19)	378.21 (368.64,388.03)	288.48 (277.18,300.24)	361.53 (348.74,374.78)	322.84 (309.50,336.75)	395.87 (381.74,410.54)	**<0.001**

Creat: creatinine; CI: confident interval; LOD: limit of detection; µg/L: micrograms per litre; µg/g creat: micrograms per gram of creatinine.

[a] p-value from paired *t*-test between concentrations adjusted for creatinine measured at the first and third trimester of pregnancy.

[b] Metal measures available for the same participants in both the first and third trimester.



Co	0.33	0.27	0.18	0.17	0.25	0.16	0.06	0.25	0.1	0.1
0.33	Ni	0.16	0.16	0.18	0.15	0.19	0.07	0.18	0.12	0.03
0.27	0.16	Cd	0.31	0.37	0.27	0.27	0.07	0.29	0.15	0.13
0.18	0.16	0.31	Cu	0.4	0.2	0.2	0.03	0.24	0.04	-0.02
0.17	0.18	0.37	0.4	Pb	0.19	0.17	0.08	0.15	0.08	0.05
0.25	0.15	0.27	0.2	0.19	Zn	0.24	0.07	0.36	0.15	0.13
0.16	0.19	0.27	0.2	0.17	0.24	Mo	0.09	0.35	0.08	0.1
0.06	0.07	0.07	0.03	0.08	0.07	0.09	As	0.37	0.05	0.04
0.25	0.18	0.29	0.24	0.15	0.36	0.35	0.37	Se	0.13	0.18
0.1	0.12	0.15	0.04	0.08	0.15	0.08	0.05	0.13	Sb	0.08
0.1	0.03	0.13	-0.02	0.05	0.13	0.1	0.04	0.18	0.08	Tl

Fig. 1. Correlations among log2-transfomed urinary metal concentrations measured during pregnancy, calculated by the mean of the first and third trimester measures. INMA Project (Spain, 2003–2008).

metals at the first and third trimesters of gestation separately. At the first trimester of gestation the most correlated groups of metals were: (1) Cd-Cu-Ni-Pb; and (2) Se-Tl-Zn. At the third trimester, correlations were higher among: (1) Cd-Ni-Pb; and (2) Cu-Mo-Se-Zn (Pearson correlation coefficients >0.20 and $p < 0.05$ in all cases). Correlations between the two measures were also calculated (Supplemental Figure S4). Overall, correlations between the two measures of metals were low, except for Cd (Pearson coefficient = 0.41).

PCA with varimax normalized rotation pointed to 4 optimal principal rotated components (RC) by using non-graphical solutions for Cattell's scree test (Raîche et al., 2013), which explained 57 % of the total variance (Supplemental Figure S5). PCA clustered the metals to each RC as follows (Briffa et al., 2020): RC1: Co-Cu-Ni (16 %) (Peters et al., 2016); RC2: Sb-Tl-Zn (14 %) (Nordberg et al., 2014); RC3: As-Se (13 %); and (Fort et al., 2014) RC4: Cd-Mo-Pb (14 %) (see Supplemental Table S4).

Independent multivariate linear regressions between levels of metals measured in maternal urine and sociodemographic, dietary and environmental factors were plotted grouped on the basis of this PCA clustering (Figs. 2–5) in order to assess similarities among them. Results according to the clustering are presented as follows (only significant covariates are indicated in the text):

3.3. Multivariate analyses

3.3.1. Cobalt-copper-nickel

Results for the Co, Cu and Ni multivariate models can be seen in Fig. 2. Co concentrations were significantly lower in Latin American mothers and higher in those from the Valencia and Sabadell cohorts. However, Ni showed lower levels in the Valencia cohort but higher in Sabadell, compared to Gipuzkoa. Women from Sabadell presented the lowest concentrations for Cu but the highest for Co and Ni (p-value <0.001 in all cases). Increased parity was positively associated with Co and Ni concentrations (β [95%CI], p-value: 0.34 [0.15, 0.54] and 0.19 [0.02, 0.37], <0.001, respectively). Smoking during pregnancy increased Ni levels (0.14 [0.05, 0.24], 0.003) but decreased Co concentrations (−0.12 [-0.23, −0.02], 0.022). Regarding dietary habits, the intake of nuts increased Ni concentrations (0.59 [0.05, 1.13], 0.033) but the consumption of alcoholic drinks significantly decreased them (−0.52 [-0.94, −0.09], 0.018). White meat was associated with lower Co levels (−0.30 [-0.54, −0.05], 0.016). On the other hand, the proximity of the house to a greenhouse decreased Ni concentrations (−0.33 [-0.51, −0.16], <0.001), proximity to both crop fields and industrial activity reduced Co levels (−0.12 [-0.22, −0.02], 0.022 and −0.13 [-0.24, −0.03], 0.015, respectively) and proximity to agricultural areas lowered the levels of Cu (−0.10 [-0.19, −0.01], 0.030).

3.3.2. Antimony-thallium-zinc

Fig. 3 shows the multivariate models for Sb, Tl and Zn. Latin American women showed higher levels of Tl and Valencian mothers had lower concentrations. Zn was directly related to the Valencia cohort but inversely related to the Sabadell cohort (p-value <0.001 in all cases). Zn was also lower in non-rural zones (β [95%CI], p-value: −0.21 [-0.39, −0.03], 0.019, and −0.18 [-0.35, −0.01], 0.036, for urban and semi-urban areas, respectively). Smokers and women belonging to the

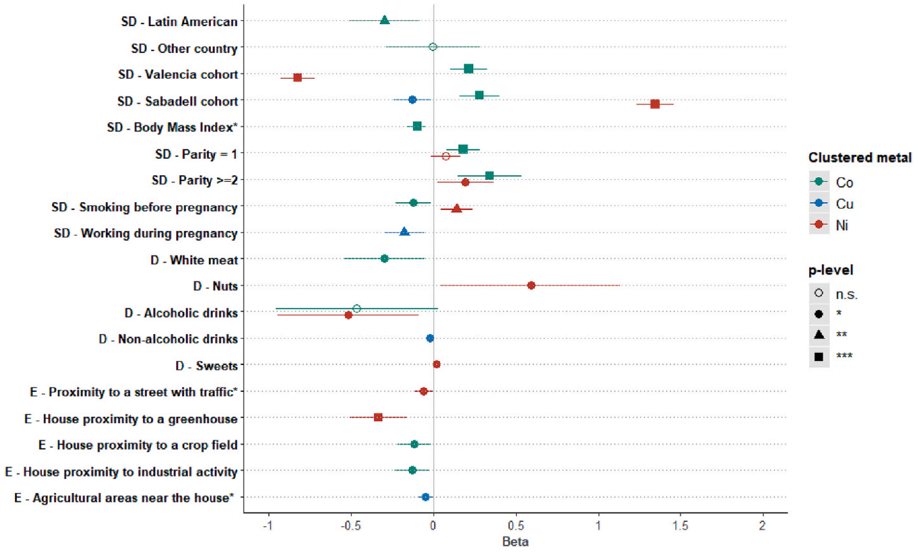


Fig. 2. Estimated effects of sociodemographic, dietary and environmental factors on cobalt, copper and nickel concentrations, in the linear regression analyses. INMA Project (Spain, 2003–2008).

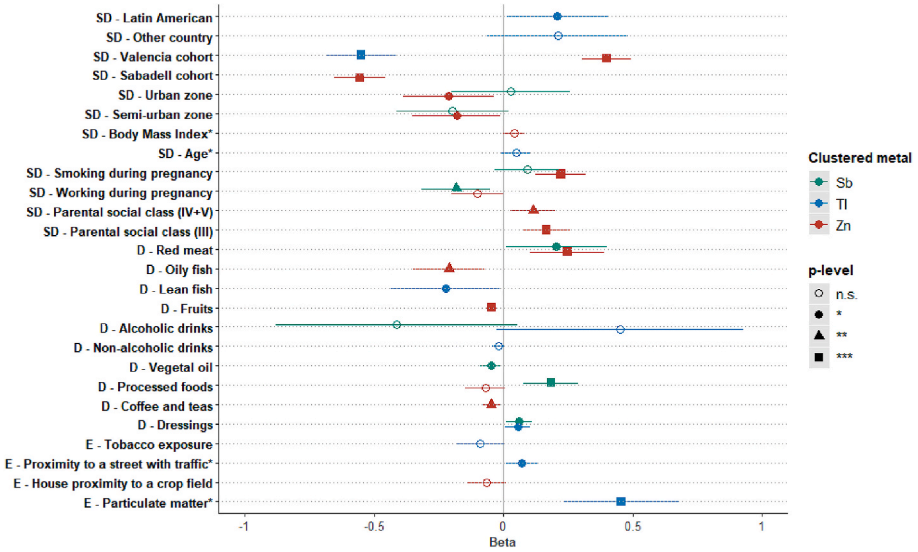


Fig. 3. Estimated effects of sociodemographic, dietary and environmental factors on antimony, thallium and zinc concentrations, in the linear regression analyses. INMA Project (Spain. 2003–2008).

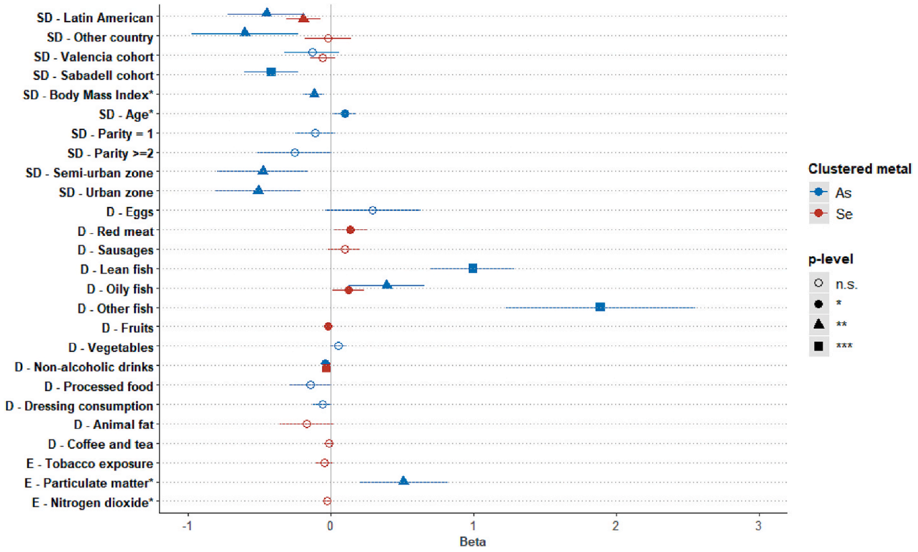


Fig. 4. Estimated effects of sociodemographic, dietary and environmental factors on arsenic and selenium concentrations, in the linear regression analyses. INMA Project (Gipuzkoa, Sabadell and Valencia. Spain. 2003–2008).

lowest social class presented higher Zn concentrations (0.22 [0.13, 0.32], <0.001 and 0.11 [0.03, 0.2], 0.008, respectively). Dietary habits affected these metals in several ways. Red meat consumption was associated with higher Zn concentrations (0.25 [0.1, 0.39], 0.001) but this metal showed an inverse relationship with the intake of oily fish (−0.21 [-0.35, −0.07], 0.003) and to a lesser extent with the consumption of fruits and tea and coffee. Lean fish intake decreased Tl levels but the consumption of dressings increased them. On the other hand, Sb levels increased with the consumption of processed foods (0.18 [0.08, 0.29], 0.001) but exhibited a slight decrease with vegetal oil intake. Finally, particulate matter levels were related to Tl concentrations (0.45 [0.23, 0.68], <0.001).

3.3.3. *Arsenic-selenium*

The results of the As and Se multivariate models are shown in Fig. 4. Lower As and Se concentrations were found in foreign women (p-values = 0.001). As levels were also lower in mothers from the Sabadell cohort (β [95%CI], p-value: −0.42 [-0.61, −0.23], <0.001) and in those who live in non-rural areas (−0.48 [-0.79, −0.16], 0.003, and −0.51 [-0.81, −0.21], 0.001, for urban and semi-urban zones, respectively), and inversely associated with their BMI (−0.12 [-0.19, −0.04], 0.002). In contrast, As levels slightly increased with age (0.10 [0.01, 0.18], 0.021). Consumption of all types of seafood was associated with higher As concentrations (0.99 [0.7, 1.29], <0.001, for lean fish; 0.39 [0.13, 0.66], 0.004, for oily fish; and 1.89 [1.23, 2.55], <0.001, for other fish) but only oily fish was related to Se (0.12 [0.01, 0.23], 0.032). Se levels were also increased with red meat consumption (0.14 [0.02, 0.25], 0.019). Both metals showed a slight decrease in their concentrations with increasing intake of non-alcoholic drinks. Regarding environmental factors, the levels of particulate matter were related to As concentrations (0.51 [0.2, 0.82], 0.001).

3.3.4. *Cadmium-molybdenum-lead*

Fig. 5 presents the results for the multivariate models for the cluster Cd-Mo-Pb. Higher levels of Pb were found in women from the Sabadell cohort (β [95%CI], p-value: 1.43 [1.3, 1.57], <0.001), smokers (0.48

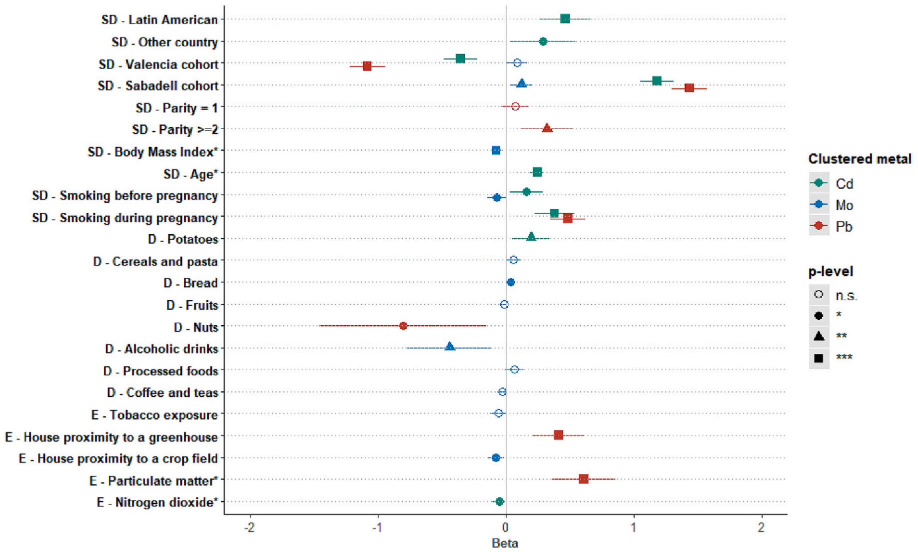


Fig. 5. Estimated effects of sociodemographic, dietary and environmental factors on cadmium, molybdenum and lead concentrations, in the linear regression analyses. INMA Project (Gipuzkoa, Sabadell and Valencia. Spain. 2003–2008).

[0.35, 0.62], <0.001) and who gave birth ≥2 times (0.32 [0.11, 0.52], 0.002). Moreover, Latin American women and those who participated in the Sabadell cohort had significantly higher levels of Cd (0.46 [0.27, 0.66], <0.001, and 1.18 [1.05, 1.31], <0.001), respectively). Smoking habit was also significantly associated with increasing Cd concentrations (0.38 [0.22, 0.54], <0.001). In addition, women from Sabadell also had higher levels of Mo (0.12 [0.04, 0.2], 0.005). Regarding dietary habits, the intake of potatoes was associated with increasing Cd concentrations (0.19 [0.05, 0.34)] 0.008) and the intake of bread was associated with increasing Mo. In addition, the consumption of nuts displayed an inverse relationship with Pb levels (−0.80 [-1.45, −0.15], 0.015), as did alcoholic drinks with Mo (−0.44 [-0.77, −0.11], 0.009). Furthermore, proximity to a greenhouse and prenatal exposure to particulate matter were related to increasing levels of Pb (0.41 [0.21, 0.61], <0.001 and 0.61 [0.36, 0.85], <0.001, respectively).

4. Discussion

In this Spanish birth cohort study, we have analysed different metal and metalloid concentrations at two different time points during pregnancy, namely, the first and the third trimester of gestation. All the metals and metalloids were detected being the most detected compounds As, Co, Mo, Sb, Se and Zn at both trimesters. Zn was the element found in the highest concentrations at both trimesters and Tl was detected in the lowest concentrations. We also studied the factors associated with the metal and metalloid concentrations and observed

Table 2

Urinary metal(loid) concentrations in pregnant women in the present and previously published studies.

	Country	Sample size	Years	Descriptive[a]	As	Cd	Co	Cu	Mo	Ni	Pb	Sb	Se	Tl	Zn
Present study	Spain	1346	2004–2008	GM	34.43	0.23	0.52	7.18	38.80	1.27	1.14	0.35	17.09	0.17	305.18
Callan et al., 2013	Australia	157	2008–2011	Median	13.20		1.20	10.40		2.30					396.00
Hinwood et al., 2015	Australia	173	2008–2011	Median		0.66					0.55	<0.25		0.29	
Wai et al., 2018	Burma	409	2016	Median	55.30	0.60					1.30				
Wang et al., 2019	China	7359	2012–2014	GM	15.01	0.31					1.81		8.98	0.27	248.78
Wang et al., 2020	China	389	2014–2015	Median	17.86	0.44							12.75		
Wu et al., 2020	China	244	2014–2015	Median	26.38	0.85	0.58	18.35					8.14	0.44	
Dereumeaux et al., 2016	France	990	2011	GM	11.04	0.12	0.85			1.38					
Shirai et al., 2010	Japan	78	2007–2008	GM	76.90	0.77		12.80	79.00		0.48				393.00
Lewis et al., 2018	Mexico	212	1997–2004	GM	13.80	0.18	1.20		17.30	9.50	2.90				288.00
Birgisdottir et al., 2013	Norway	174	2003	Median	79.60	0.16									
Ashrap et al., 2020	Puerto Rico	1285	2011–2017	GM	10.90	0.12	1.00	14.00	58.90	5.40	0.25	0.09			266.00
Bocca et al., 2020	Spain	49	2016–2017	Median	20.00	0.70	0.90	16.00		1.70	1.20		21.00		383.00
Bommarito et al., 2019	USA	390	2006–2008	Median	17.90	0.08		8.96	51.30	2.84	0.35		37.00	0.13	242.00
Cowell et al., 2020	USA	100	2011	Mean	11.45	0.16				1.46	0.45				
Jain et al., 2013	USA	1565	2003–2010	GM			0.45		43.84		0.43	0.07		0.16	
Kalloo et al., 2018	USA	389	2003–2006	GM	5.30	0.20					0.70				
Kim et al., 2020	USA	130	2006–2008	Median	18.00	0.08		9.08	51.30	2.84	0.35		37.20	0.13	367.50
Wang et al., 2019	USA	1335	1999–2000	GM	14.20	0.43	0.61	9.74	40.70	3.70	0.84	0.09	1.07	0.12	270.00
Watson et al., 2020	USA	404	1999–2016	GM	8.77	0.15	0.60		49.30		0.52	0.08		0.17	

GM: geometric mean.

[a] Unadjusted values. Concentrations from studies in other units were transformed to µg/L.

significant associations with some sociodemographic (working situation, social class, age), lifestyle (smoking and alcohol consumption), dietary (intake of seafood, meat, fruits, nuts, vegetables) and environmental (proximity to industrial areas, fields and greenhouses, traffic density and air pollution) variables.

For most of the metals measured in urine, there are no established reference values for pregnant women. However, the urine concentrations of metals and metalloids in the present study and in previous studies conducted on pregnant women have been compared (Table 2).

4.1. Arsenic

The concentrations of total As observed in our study population were higher than those found in pregnant women from Australia (Callan et al., 2013), China (Wang et al., 2019a, 2020; Wu et al., 2020), France (Dereumeaux et al., 2016), Mexico (Lewis et al., 2018), Puerto Rico (Ashrap et al., 2020), USA (Bommarito et al., 2019; Cowell et al., 2020; Hoover et al., 2020; Watson et al., 2020; Jain, 2013; Kalloo et al., 2018; Kim et al., 2020) and those from the HEALS-EXHES Spanish cohort (Bocca et al., 2020). Among the studies reviewed, only pregnant women from Burma (Wai et al., 2018), Japan (Shirai et al., 2010) and Norway (Birgisdottir et al., 2013) presented higher levels of As than our study population (Table 2).

As it is excreted relatively rapidly via urine, in fact, urinary concentration of As is usually used as an indicator of recent exposure (Soler-Blasco et al., 2021). Reference levels of arsenic in the urine from the general population appear to be in the range of 5–50 μg/L (Nordberg et al., 2014). Among the studies reviewed, only those conducted on populations from Burma (Wai et al., 2018), Japan (Shirai et al., 2010) and Norway (Birgisdottir et al., 2013) showed a median or GM of As above this range.

As concentrations in our study were directly associated with maternal seafood consumption as a main dietary predictor factor. The main form of As present in seafood is the organic one, arsenobetaine (Hackethal et al., 2021). Maternal urinary As has been also associated with seafood intake in previous studies (Callan et al., 2013; Ashrap et al., 2020). Another factor related to maternal As concentrations in our study was the particulate matter levels, which according to previous studies carried out on particulate matter speciation in outdoor and indoor environments is mainly due to mineral matter resuspension (Rivas et al., 2014; Mao et al., 2020). Other maternal characteristics associated with the total As concentrations were country of birth, location, BMI before pregnancy, age, parity and zone of residence. Non-Spanish women, those participating in the Sabadell cohort, women with lower BMI, younger, with higher parity and living in the semi-urban and urban zones had lower urinary As concentrations. Other studies also showed some of these relationships. Thus, in a study from the USA, an inverse association between As and maternal BMI and a direct association with seafood and low-fat yogurt intake were observed (Osorio-Yáñez et al., 2018). In China, a direct association was also found between maternal urinary As concentrations and increasing age and BMI, passive smoking during pregnancy and doing exercise (Wang et al., 2019a).

Arsenobetaine has been found to be the arsenic form in the highest concentrations in our population (Soler-Blasco et al., 2021). Arsenobetaine and other organic As forms, such as arsenosugars and arsenolipids, are generally considered less toxic than inorganic As (Soler-Blasco et al., 2021), although *in vitro* studies have revealed cytotoxic effects of certain arsenic-containing hydrocarbons (Bornhorst et al., 2020).

4.2. Cadmium

Cadmium concentrations in our population were higher than in pregnant women from France (Dereumeaux et al., 2016), Mexico (Lewis et al., 2018), Norway (Birgisdottir et al., 2013), Puerto Rico (Ashrap et al., 2020) and most of the USA studies (Bommarito et al., 2019; Cowell et al., 2020; Watson et al., 2020; Kalloo et al., 2018; Kim et al., 2020). Nevertheless, they were lower than in pregnant women from Australia (Hinwood et al., 2015), Burma (Wai et al., 2018), China (Wang et al., 2019a, 2020; Wu et al., 2020), Japan (Shirai et al., 2010), the Spanish study (Bocca et al., 2020) and one of the studies from the USA (Wang et al., 2019b) (Table 2).

Both urine and blood are useful matrices for detecting exposures, as blood Cd primarily reflects recent exposure and urine Cd represents long-term exposure (Sanders et al., 2015). In urine, reference levels would vary with age, area and smoking habits, but they are generally <1 μg/g creatinine. Cd concentrations observed in our population were all below this level (Nordberg et al., 2014).

In our study, urinary Cd concentrations were higher in women who were smokers or ex-smokers with respect to non-smokers. This association with ex-smokers could indicate the long half-life of this compound in the body; Cd can be accumulated for around 10–30 years in muscle, bone, kidney and liver (Nordberg et al., 2014). Other factors associated with Cd concentrations were increasing maternal age, the intake of potatoes, and country of birth (non-Spanish women had higher Cd concentrations). The positive association between urinary Cd and age has been supported by previous studies (Ashrap et al., 2020; Wang et al., 2019b; Gil and Hernández, 2015). Additionally, the European Food Safety Agency (EFSA) analysed the occurrence of Cd in different foodstuffs and found that potatoes were one of the highest dietary contributors to the Cd body burden (European Food Safety Authority, 2009).

Maternal Cd accumulates mainly in the placenta with partially direct transfer to the foetus; however, maternal blood Cd concentrations have been inversely associated with children's IQ assessed at 60 months of age in a birth cohort in Korea (Cheng et al., 2017). Additionally, in a mother-child cohort study in rural Bangladesh, maternal urinary Cd was associated with hyperactivity assessed at 10 years of age (Gustin et al., 2018). Foetus can also be affected indirectly; the placental cadmium accumulation could interfere with placental transport of key micronutrients and disrupt foetal growth and development (Zhao et al., 2020; Cheng et al., 2017).

4.3. Lead

Pb concentrations in our population were found to be lower than those observed in the studies conducted on pregnant Burmese (Wai et al., 2018), Chinese (Wang et al., 2019a) and Japanese (Shirai et al., 2010) women (Table 2). This fact could be explained by the ban on Pb in petrol in Spain as of 2001 (Llop et al., 2013).

Urinary Pb has been used in the biological monitoring of lead, but only to a limited extent (Tsaih et al., 2001). Although there is an association between Pb concentrations in urine and blood, the variation is too large to allow prediction of individual blood Pb from a urinary Pb concentration. Pb concentration in blood is considered more suitable for biomonitoring than in urine because it reflects the combination of exposure during the previous months and exposure over the past few years (Nordberg et al., 2014). Due to negative feedback regulation, Pb exposure causes an increase in aminolevulinic acid (ALA) in various tissues and in plasma, and consequently excretion of ALA in urine is elevated, and has been proposed as a suitable alternative to detect Pb exposure (Sakai, 2000).

Urinary Pb concentrations in our study were associated with the proximity of the dwelling to a greenhouse and the particulate matter concentrations. Similarly to As, Pb has also been detected in particulate matter measured in outdoor and indoor environments, associated with mineral matter resuspension (Rivas et al., 2014; Li et al., 2009). Other factors associated with urinary Pb concentrations were smoking during pregnancy and increasing parity. These two factors were also found to be related to maternal lead levels in Puerto Rico (Ashrap et al., 2020) and USA (Jain, 2013).

Although the Pb levels in populations around the world have decreased due to the phasing out of leaded petrol, there is still a debate about the lead concentrations considered to be safe for humans (Shefa and Héroux, 2017). Early exposure to Pb has been related to adverse effects on children's cognitive and behavioural development (Grandjean and Landrigan, 2006).

4.4. Other non-essential metals and metalloids: nickel, antimony, thallium

Our population presented the lowest concentrations of Ni of those reviewed. Urinary Ni concentrations were positively associated with the intake of sweets and nuts. Similarly, urinary Ni was positively associated with the intake of nuts, hazelnut spread and chocolate among 3- to 14-year-old German children (Wilhelm et al., 2013). Sweet cakes, pastries and nuts were the foodstuffs that were found to have the highest Ni concentrations in a total diet study conducted in Spain (González-Weller et al., 2012). Moreover, smoking before pregnancy has been associated with urinary Ni concentrations in the present study. This relationship is well known, since tobacco has been shown to be a considerable source of Ni (Stojanović et al., 2004). The adverse effects of early exposure to Ni have not been explored in depth. Nevertheless, some evidence of a negative association between prenatal exposure to Ni and birth weight has been observed (Sun et al., 2018; McDermott et al., 2015), although further studies are needed to confirm it. Additionally, Ni has been seen to interfere with TLR4 receptors resulting in toxic effects in broilers that could affect their mucosal innate immunity (Wu et al., 2014).

The Sb concentrations observed in our study were higher than in the other studies reviewed. Urine is considered the most reliable biological medium for testing Sb, as it is indicative of recent exposure due to its elimination half-life of 95 h; this characteristic appears to be greater for pentavalent Sb than for trivalent compounds (Centre for Disease Control and Prevention (CDC), 2013). Regarding the associated factors, we observed a positive association between urinary Sb concentrations and the intake of processed food, dressings and red meat. Sb is usually found in the composition of polyethylene terephthalate (PET) food packaging (Jiang et al., 2010) and migration of this compound from this type of plastic to food has been reported (Haldimann et al., 2013).

Levels of Tl in the present study were similar to those found in the USA studies (Bommarito et al., 2019; Jain, 2013; Kim et al., 2020; Wang et al., 2019b) and lower than the levels observed in China and Australia (Wu et al., 2020; Hinwood et al., 2015; Wang et al., 2019b) (Table 2). The WHO Task Force (WHO task force, 1996) considered that exposures leading to urinary Tl concentrations of <5 μg/L are unlikely to cause adverse health effects. Urinary samples have shown reliable biomarkers of Tl status (Hinwood et al., 2015).

In the present study, urinary Tl concentrations were positively associated with particulate matter concentrations and the proximity of the residence to a street with traffic. Tl is widely, but sparingly, distributed around the world, although the most important sources of Tl exposure in the general population are air emissions from coal-burning power plants and smelters. Tl is also found in fossil fuels (Nordberg et al., 2014). It is excreted in both animals and humans by the kidneys and intestine, and to a small extent through the hair and in milk. It can also cross the placental barrier. The number of epidemiological studies that assess the health effects associated to Tl exposure during childhood is still limited. However, prenatal Tl has recently been linked to preterm delivery (Karakis et al., 2021), ADHD symptoms (Tong et al., 2020), a delay in postnatal growth (Qi et al., 2019), shortened neonatal telomere length (Wu et al., 2021) and reduced mitochondrial DNA copy number (Wu et al., 2019). Although the literature is still scarce, the study of the early health effects of thallium exposure seems to be an emerging topic in Environmental Epidemiology.

4.5. Essential metals or metalloids: cobalt, copper, molybdenum, selenium and zinc

Urinary Co concentrations in our population were low and only slightly higher than in one of the USA studies (Jain, 2013). Increased parity and decreased BMI before pregnancy were associated with higher Co concentrations. This inverse association between Co concentrations and the participant's BMI has also been reported in a prospective study of US women (Niehoff et al., 2020) and among pregnant women from Puerto Rico (Ashrap et al., 2020). Regarding Cu, concentrations observed in our population were the lowest among those observed in pregnant women in all the studies reviewed. In the present study, Cu levels were lower among mothers who worked during pregnancy, those from the Sabadell cohort, those who drank alcohol during pregnancy, and those who lived near an agricultural area. Other studies observing these specific relationships have not been found. The main contributor to the amount of Cu in the body is the diet; however, we did not observe any significant association with any of the foodstuffs. Urinary Co and Cu levels have been shown to reflect recent exposure (Agency for Toxic Substances and Disease Registry, 2004). Despite the fact that only 2 % of Cu is excreted in urine (Wijmenga and Klomp, 2004), the amount excreted is directly related to the internal dose and can therefore be a useful exposure biomarker (Nordberg et al., 2014).

Urinary Mo levels in our study were similar to those observed in pregnant women from the USA (Watson et al., 2020; Jain, 2013; Kim et al., 2020; Wang et al., 2019b), lower than in pregnant women from Japan (Shirai et al., 2010) and Puerto Rico (Ashrap et al., 2020), and higher than in pregnant women from Mexico (Lewis et al., 2018). Urinary Mo is mostly an indicator of long-term exposure, with urine being the primary route of excretion (Turnlund et al., 1995). Mo concentrations in our study were associated with decreased BMI and with bread consumption. The main route of exposure to Mo in the general population is the diet, especially the intake of cereals and dairy products (Nordberg et al., 2014). Positive relationships between Mo concentrations and foodstuff intakes have previously been reported with seafood and rice (Wang et al., 2019a), as well as insoluble fibre and low-fat yogurt in women who also displayed an inverse association between BMI and Mo levels (Osorio-Yáñez et al., 2018).

Few studies have reported urinary Se concentrations among pregnant women. Levels observed in our study were quite similar to those found in Spain (Bocca et al., 2020) but higher than in all the Chinese studies (Wang et al., 2019a, 2019b; Wu et al., 2020) and lower than most of the USA studies that reported Se levels (Bommarito et al., 2019; Kim et al., 2020). Urinary Se is an indicator of short-term exposure and studies have shown a correlation between dietary Se intake, the primary route of human exposure, and daily urinary excretion (Hays et al., 2014; Sanz Alaejos and Díaz Romero, 1993).

The factors associated with urinary Se in our study were the levels of particulate matter, the intake of seafood, living in a semi-urban and urban zone, increased age, decreased BMI before pregnancy, and being born in Latin America. Up to 90 % of the Se content in ambient air is emitted during the burning of fossil fuels and bound to fly ash and to suspended particles (Nordberg et al., 2014). However, the main source of Se exposure in the general population is diet, with cereals, meat, seafood, eggs and milk/dairy products being the main dietary sources (Rayman, 2012). Urinary Se concentrations were also associated with seafood consumption in the previous Spanish study (Bocca et al., 2020), but the rest of the studies with Se data reviewed only associated the metal with passive smoking and the intake of multivitamins (Wang et al., 2019a).

Urinary Zn concentrations varied from one study to another. We found levels that were higher than China (Wang et al., 2019a), Mexico (Lewis et al., 2018) and Puerto Rico (Ashrap et al., 2020), and lower than those in Australia (Callan et al., 2013), Japan (Shirai et al., 2010) and the previous Spanish study (Bocca et al., 2020). Urinary samples have been shown to be reliable biomarkers of Zn status (Lowe et al., 2009). The determinants of higher Zn concentrations in our study population were the intake of red meat, lower social class, smoking during pregnancy, living in a rural zone and belonging to the Valencia cohort. The intake of meat is considered the main dietary contributor to Zn concentrations in developed countries (Nordberg et al., 2014). Only the relationship with the smoking habit has been found previously (Wang et al., 2019a).

Most of these essential elements play an important role in human health and metabolism. For instance, Se plays a key role in several major metabolic pathways such as thyroid hormone metabolism, antioxidant defence systems and immune functions (Rayman, 2012). It is incorporated into selenoproteins in the form of selenocysteine and is fundamental for their functioning (Bellinger et al., 2009). Cu is a transition metal involved in numerous biological processes such as cellular respiration, antioxidant defence, connective tissue formation, neurotransmitter biosynthesis, peptide hormone maturation, pigmentation, keratinization and iron homeostasis (Uriu-Adams et al., 2010). Co is essential to mammals in the form of cobalamin (vitamin B12). Zn is an essential component of proteins including antioxidant enzymes, metalloenzymes, zinc-binding factors and transporters. These are required for biological processes including carbohydrate and protein metabolism, DNA and RNA synthesis, cellular replication and differentiation, and hormone regulation (Nordberg et al., 2014). However, some studies have shown an association between them and negative aspects of children's health and development. For instance, increasing prenatal levels of Se have been associated with lower head circumference and lower gestational age (Lozano et al., 2020) in our study population. Additionally, we observed that the relationship between prenatal Se and children's cognitive development at 14 months (Amorós et al., 2018) and 5 years of age (Amorós et al., 2019) was inverted U-shaped, so both low and high Se concentrations seem to have deleterious effects on neuropsychological development. We also observed a negative association between prenatal serum levels of Cu and children's neuropsychological development (Amorós et al., 2019). Zn deficiency during pregnancy has been associated with an increment in the risk of maternal and neonate morbidity and mortality (Wilson et al., 2016). However, an excessive intake of zinc can have the adverse effect of suppressing Cu absorption (Fassier et al., 2019). Very few epidemiological studies have evaluated the association between maternal levels of Co and child development. A case control study conducted in China suggested that the occurrence of congenital heart defects at birth may be associated with cobalt exposure during pregnancy (Zhang et al., 2020). Another study conducted in the USA observed that placental levels of Co were associated with an increase in newborns' weight, but with a non-linear shape, a turning point for the association being observed at about 0.011 μg/L (Mikelson et al., 2019).

4.6. *Metal relationships*

We calculated both correlations and principal components with the metals and metalloids in order to study the possible relationship between them. Some of the results coincided between the two analyses. Firstly, a high correlation between Co and Ni was observed, both elements being clustered in the first component. Also, a high correlation between Cd and Pb was seen, both of them being clustered in the fourth component. And finally, As and Se were highly correlated and clustered in the fourth component.

Cd and Pb have been found to be correlated or clustered in previous studies (Ashrap et al., 2020; Wang et al., 2019b; Kim et al., 2019). Cd and Pb are found together in ores in relatively high concentrations. In fact, Cd is obtained as a by-product of the refining of zinc and other metals, particularly Cu and Pb (Nordberg et al., 2014). In our study population, women who smoked and those who belonged to the Sabadell cohort had higher levels of both elements. Co-exposure to both metals has been associated with renal dysfunction (Chen et al., 2019).

Urinary Co and Ni was also found to be correlated in the study conducted with midlife women in USA (Wang et al., 2019b). Both metals have been found together in ores and in industrial sources, such as metallurgy or battery production (Nordberg et al., 2014). The most important associated factor shared by these two elements in our study population is the cohort of origin, women from Sabadell being the ones who presented higher co-exposure.

Finally, As and Se have been found to be correlated and clustered in our population, with oily fish consumption as the main shared source. The organic form of As is the most present in seafood (Hackethal et al., 2021), although it is considered the less toxic one. Due to the high seafood consumption in this population (mean of 5.04 servings per week), we can assume that most of the total As observed is the organic one, arsenobetaine (Soler-Blasco et al., 2021). Another study conducted on a Mediterranean population observed a moderate but significant correlation between As and Se measured in cord blood, and both biomarkers were also correlated with maternal seafood consumption (Miklavčič et al., 2013). Antagonistic effects or mutual detoxification between As and Se have also been confirmed in many animal species, including humans (Levander, 1977; Zeng et al., 2005).

4.7. *Strengths and limitations*

This is the first large prospective study that includes several Spanish birth cohorts, which provides a unique opportunity to characterize exposure to metal(loid)s in this population. Few studies have assessed exposure to multiple metal(loid)s among pregnant women. The study design allows for repeated collection of urine samples and questionnaire data to account for the varying levels of exposure during pregnancy. We measured a large panel of metal(loid)s, which helps to inform about their relationships in future epidemiological analyses. Nevertheless, the study does have some limitations: 1) the metal(oid) concentrations were obtained from two laboratories using different analytical methods and materials, which limited the metal(loid) availability in all three cohorts and could affect the reproducibility of our results regarding low concentrations; and 2) the lack of confounding for multivitamin supplements could have caused a bias in some results. Some studies have shown associations between multivitamin supplements intake and urinary metal(iod)s levels. Thus, Mo and Sb concentrations have been positively related to folic acid and iron supplements (Ashrap et al., 2020), and lower urinary Cd, Se, Tl and Zn levels with folic acid supplementation (34, 41, 50,99), which is likely to be caused by decreased intestinal absorption (99). On the other hand, urinary Cd and Se concentrations have been shown to be increased in pregnant women taking multivitamin supplementation (Bocca et al., 2020). The possible explanation may be the fact that the bioavailability of Se increases with a diet rich in protein and vitamins (mainly A, C and E); it also stimulates the immune system and acts antagonistically to certain heavy metals (100).

5. Conclusions

This is the first large prospective birth cohort study on the exposure to metals and metalloids and associated factors among pregnant women from several regions of Spain. We analysed different metals and metalloids at two different time points during pregnancy, namely, at the first and the third trimester of gestation. We observed that the levels for most of them were not correlated between measures. This fact could indicate differences in metabolism throughout pregnancy, and thus only a spot urine measure of metals may not be a reliable biomarker of exposure to metals and metalloids for the whole pregnancy. We observed significant associations between As, Cd, Co, Cu, Mo, Ni, Pb, Sb, Se, Tl and Zn urine concentrations and some sociodemographic (working situation, social class, age), lifestyle (smoking and alcohol consumption), dietary (seafood, meat, fruits, nuts, vegetables) and environmental (proximity to industrial areas, fields and greenhouses, traffic density, and air pollution) variables. The present study shows that some modifiable lifestyles, food intakes and environmental factors could be associated with prenatal exposure to metal(loid)s, which may be considered in further studies to assess their relationship with neonatal health outcomes.

Author statement

Manuel Lozano, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Visualization. Mario Murcia, Methodology, Formal analysis, Supervision, Writing – review & editing. Raquel Soler-Blasco, Writing – review & editing. Maribel Casas, Writing – review & editing, Funding acquisition. Begotxu Zubero, Writing – review & editing. Gabriel Riutort-Mayol, Writing – review & editing. Fernando Gil, Investigation, Writing – review & editing. Pablo Olmedo, Investigation, Writing – review & editing. Joan Grimalt, Investigation, Writing – review & editing. Rubén Amorós, Formal analysis, Writing – review & editing. Aitana Lertxundi, Writing – review & editing, Funding acquisition. Martine Vrijheid, Writing – review & editing, Funding acquisition. Ferran Ballester, Conceptualization, Writing – review & editing, Funding acquisition. Sabrina Llop, Conceptualization, Writing – original draft, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding and Acknowledgements

This study was funded by Grants from EU (FP7-ENV-2011 cod 282957 and HEALTH.2010.2.4.5–1); Spain: ISCIII (Red INMA G03/176, CB06/02/0041; FIS-FEDER: PI03/1615, PI04/1509, PI04/1112, PI04/1931, PI05/1079, PI05/1052, PI06/0867, PI06/1213, PI07/0314, PI09/00090, PI09/02647, PI11/01007, PI11/02591, PI11/02038, PI13/1944, PI13/2032, PI13/02187, PI14/00891, PI14/01687, PI16/1288, PI17/00663, PI18/01142 and PI19/1338; Miguel Servet FEDER MS15/00025, MS20/0006, CPII16/00051, and FIS-FSE: 17/ 00260); CIBERESP; Generalitat Valenciana: FISABIO (UGP 15–230, UGP-15-244, and UGP-15-249), AICO/2020/285, and Alicia Koplowitz Foundation 2017; Generalitat de Catalunya-CIRIT 1999SGR 00241; Department of Health of the Basque Government (2005111093, 2009111069, 2013111089 and 2015111065); Provincial Government of Gipuzkoa (DFG06/002, DFG08/001 and DFG15/221).

We acknowledge support from the Spanish Ministry of Science, Innovation and Universities through the "Centro de Excelencia Severo Ochoa 2019–2023" Programme (CEX2018000806-S), and from the Generalitat de Catalunya through the CERCA Programme, as well as the municipalities in the study area.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.131809.

References

Agency for Toxic Substances and Disease Registry, 2004. Toxicological Profile for Cobalt. U.S Department of Health and Human Services, Agency for Toxic Substances and Disease Registry (ATSDR) [Internet]. Available from: https://www.atsdr.cdc.gov/toxprofiles/tp33.pdf.

Amorós, R., Murcia, M., González, L., Rebagliato, M., Iñiguez, C., Lopez-Espinosa, M.-J., et al., 2018. Maternal selenium status and neuropsychological development in Spanish preschool children. Environ. Res. 166, 215–222. Oct 1.

Amorós, R., Murcia, M., González, L., Soler-Blasco, R., Rebagliato, M., Iñiguez, C., et al., 2019. Maternal copper status and neuropsychological development in infants and preschool children. Int. J. Hyg Environ. Health 222 (3), 503–512.

Ashrap, P., Watkins, D.J., Mukherjee, B., Boss, J., Richards, M.J., Rosario, Z., et al., 2020. Predictors of urinary and blood Metal(loid) concentrations among pregnant women in Northern Puerto Rico. Environ. Res. 183, 109178. Apr.

Bellinger, F.P., Raman, A.V., Reeves, M.A., Berry, M.J., 2009. Regulation and function of selenoproteins in human disease. Biochem. J. 422 (1), 11–22. Jul 29.

Birgisdottir, B.E., Knutsen, H.K., Haugen, M., Gjelstad, I.M., Jenssen, M.T.S., Ellingsen, D. G., et al., 2013. Essential and toxic element concentrations in blood and urine and their associations with diet: results from a Norwegian population study including high-consumers of seafood and game. Sci. Total Environ. 463–464, 836–844. Oct 1.

Bocca, B., Ruggieri, F., Pino, A., Rovira, J., Calamandrei, G., Mirabella, F., et al., 2020. Human biomonitoring to evaluate exposure to toxic and essential trace elements during pregnancy. Part B: predictors of exposure. Environ. Res. 182, 109108. Mar.

Bommarito, P.A., Kim, S.S., Meeker, J.D., Fry, R.C., Cantonwine, D.E., McElrath, T.F., et al., 2019. Urinary trace metals, maternal circulating angiogenic biomarkers, and preeclampsia: a single-contaminant and mixture-based approach. Environ. Health 18 (1), 63. Jul 12.

Bornhorst, J., Ebert, F., Meyer, S., Ziemann, V., Xiong, C., Guttenberger, N., et al., 2020. Toxicity of three types of arsenolipids: species-specific effects in Caenorhabditis elegans. Metallomics 12 (5), 794–798. May 27.

Briffa, J., Sinagra, E., Blundell, R., 2020. Heavy metal pollution in the environment and their toxicological effects on humans. Heliyon 6 (9), e04691. Sep 1.

Callan, A.C., Hinwood, A.L., Ramalingam, M., Boyce, M., Heyworth, J., McCafferty, P., et al., 2013. Maternal exposure to metals—concentrations and predictors of exposure. Environ. Res. 126, 111–117. Oct 1.

Centre for Disease Control and Prevention (CDC), 2013. National Biomonitoring Program, Biomonitoring Summary Antimony [Internet]. Available from: http://www.cdc.gov/biomonitoring/Antimony_BiomonitoringSummary.html.

Chen, X., Zhu, G., Wang, Z., Zhou, H., He, P., Liu, Y., et al., 2019. The association between lead and cadmium co-exposure and renal dysfunction. Ecotoxicol. Environ. Saf. 173, 429–435. May 30.

Cheng, L., Zhang, B., Zheng, T., Hu, J., Zhou, A., Bassig, B.A., et al., 2017. Critical windows of prenatal exposure to cadmium and size at birth. Int. J. Environ. Res. Publ. Health 14 (1).

Cowell, W., Colicino, E., Tanner, E., Amarasiriwardena, C., Andra, S.S., Bollati, V., et al., 2020. Prenatal toxic metal mixture exposure and newborn telomere length: modification by maternal antioxidant intake. Environ. Res. 190, 110009. Nov.

Dereumeaux, C., Saoudi, A., Pecheux, M., Berat, B., de Crouy-Chanel, P., Zaros, C., et al., 2016. Biomarkers of exposure to environmental contaminants in French pregnant women from the Elfe cohort in 2011. Environ. Int. 97, 56–67. Dec.

Domingo-Salvany, A., Bacigalupe, A., Carrasco, J.M., Espelt, A., Ferrando, J., Borrell, C., 2013. Proposals for social class classification based on the Spanish National Classification of Occupations 2011 using neo-Weberian and neo-Marxist approaches. Gac. Sanit. 27 (3), 263–272.

European Food Safety Authority, 2009. Cadmium in Food - Scientific Opinion of the Panel on Contaminants in the Food Chain [Internet]. European Food Safety Authority. Available from: https://www.efsa.europa.eu/en/efsajournal/pub/980. (Accessed 25 January 2021).

Fassier, P., Egnell, M., Pouchieu, C., Vasson, M.-P., Cohen, P., Galan, P., et al., 2019. Quantitative assessment of dietary supplement intake in 77,000 French adults: impact on nutritional intake inadequacy and excessive intake. Eur. J. Nutr. 58 (7), 2679–2692. Oct.

Forns, J., Fort, M., Casas, M., Cáceres, A., Guxens, M., Gascon, M., et al., 2014. Exposure to metals during pregnancy and neuropsychological development at the age of 4 years. Neurotoxicology 40, 16–22. Jan 1.

Fort, M., Cosín-Tomás, M., Grimalt, J.O., Querol, X., Casas, M., Sunyer, J., 2014. Assessment of exposure to trace metals in a cohort of pregnant women from an urban center by urine analysis in the first and third trimesters of pregnancy. Environ. Sci. Pollut. Control Ser. 21 (15), 9234–9241.

Gaitens, J.M., Brown, C.H., Strathmann, F.G., Xu, H., Lewin-Smith, M.R., Velez-Quinones, M.A., et al., 2021. The utility of spot vs 24-hour urine samples for metal determination in veterans with retained fragments. Am. J. Clin. Pathol. 155 (3), 428–434.

Gil, F., Hernández, A.F., 2015. Toxicological importance of human biomonitoring of metallic and metalloid elements in different biological samples. Food Chem. Toxicol. 80, 287–297. Jun.

González-Weller, D., Gutiérrez, Á.J., Rubio, C., Revert, C., Hardisson, A., 2012. A total diet study of nickel intake in a Spanish population (Canary Islands). Int. J. Food Sci. Nutr. 63 (8), 902–912. Dec.

Goto, Y., Mandai, M., Nakayama, T., Yamazaki, S., Nakayama, S.F., Isobe, T., et al., 2021. Association of prenatal maternal blood lead levels with birth outcomes in the Japan Environment and Children's Study (JECS): a nationwide birth cohort study. Int. J. Epidemiol. 50 (1), 156–164. Feb 1.

Grandjean, P., Landrigan, P.J., 2006 Dec 16. Developmental neurotoxicity of industrial chemicals. Lancet 368 (9553), 2167–2178.

Gustin, K., Tofail, F., Vahter, M., Kippler, M., 2018. Cadmium exposure and cognitive abilities and behavior at 10 years of age: a prospective cohort study. Environ. Int. 113, 259–268.

Guxens, M., Ballester, F., Espada, M., Fernández, M.F., Grimalt, J.O., Ibarluzea, J., et al., 2012. Cohort profile: the INMA—INfancia y Medio ambiente—(environment and childhood) Project. Int. J. Epidemiol. 41 (4), 930–940. Aug.

Hackethal, C., Kopp, J.F., Sarvan, I., Schwerdtle, T., Lindtner, O., 2021. Total arsenic and water-soluble arsenic species in foods of the first German total diet study (BfR MEAL Study). Food Chem. 346, 128913. Jan 5.

Haldimann, M., Alt, A., Blanc, A., Brunner, K., Sager, F., Dudler, V., 2013. Migration of antimony from PET trays into food simulant and food: determination of Arrhenius parameters and comparison of predicted and measured migration data. Food Addit. Contam. Part A Chem Anal Control Expo Risk Assess 30 (3), 587–598.

Hays, S.M., Macey, K., Nong, A., Aylward, L.L., 2014. Biomonitoring equivalents for selenium. Regul. Toxicol. Pharmacol. 70 (1), 333–339. Oct.

Hinwood, A.L., Stasinska, A., Callan, A.C., Heyworth, J., Ramalingam, M., Boyce, M., et al., 2015. Maternal exposure to alkali, alkali earth, transition and other metals: concentrations and predictors of exposure. Environ. Pollut. 204, 256–263. Sep.

Hoover, J.H., Erdei, E., Begay, D., Gonzales, M., NBCS Study Team, Jarrett, J.M., et al., 2020. Exposure to uranium and co-occurring metals among pregnant Navajo women. Environ. Res. 190, 109943. Nov.

Inadera, H., Takamori, A., Matsumura, K., Tsuchida, A., Cui, Z.-G., Hamazaki, K., et al., 2020. Association of blood cadmium levels in pregnant women with infant birth size and small for gestational age infants: the Japan Environment and Children's study. Environ. Res. 191, 110007. Dec 1.

Iñiguez, C., Ballester, F., Estarlich, M., Llop, S., Fernandez-Patier, R., Aguirre-Alfaro, A., et al., 2009. Estimation of personal NO2 exposure in a cohort of pregnant women. Sci. Total Environ. 407 (23), 6093–6099.

Iyengar, G.V., Rapp, A., 2001. Human placenta as a 'dual' biomarker for monitoring fetal and maternal environment with special reference to potentially toxic trace elements. Part 3: toxic trace elements in placenta and placenta as a biomarker for these elements. Sci. Total Environ. 280 (1), 221–238. Dec 3.

Jain, R.B., 2013. Effect of pregnancy on the levels of urinary metals for females aged 17-39 years old: data from National Health and Nutrition Examination Survey 2003-2010. J. Toxicol. Environ. Health 76 (2), 86–97.

Jiang, X., Wen, S., Xiang, G., 2010. Cloud point extraction combined with electrothermal atomic absorption spectrometry for the speciation of antimony(III) and antimony(V) in food packaging materials. J. Hazard. Mater. 175 (1–3), 146–150. Mar 15.

Kaiser, H.F., 1958. The varimax criterion for analytic rotation in factor analysis. Psychometrika 23 (3), 187–200.

Kalloo, G., Wellenius, G.A., McCandless, L., Calafat, A.M., Sjodin, A., Karagas, M., et al., 2018. Profiles and predictors of environmental chemical mixture exposure among pregnant women: the health outcomes and measures of the environment study. Environ. Sci. Technol. 52 (17), 10104–10113. Sep 4.

Karakis, I., Landau, D., Gat, R., Shemesh, N., Tirosh, O., Yitshak-Sade, M., et al., 2021. Maternal metal concentration during gestation and pediatric morbidity in children: an exploratory analysis. Environ. Health Prev. Med. 26 (1), 40. Mar 25.

Kim, S.S., Meeker, J.D., Keil, A.P., Aung, M.T., Bommarito, P.A., Cantonwine, D.E., et al., 2019. Exposure to 17 trace metals in pregnancy and associations with urinary oxidative stress biomarkers. Environ. Res. 179 (Pt B), 108854. Dec.

Kim, S.S., Meeker, J.D., Aung, M.T., Yu, Y., Mukherjee, B., Cantonwine, D.E., et al., 2020. Urinary trace metals in association with fetal ultrasound measures during pregnancy. Environ. Epidemiol. 4 (2). Apr.

Kobayashi, S., Kishi, R., Saijo, Y., Ito, Y., Oba, K., Araki, A., et al., 2019. Association of blood mercury levels during pregnancy with infant birth size by blood selenium levels in the Japan Environment and Children's Study: a prospective birth cohort. Environ. Int. 418–429.

Lertxundi, A., Andiarena, A., Martínez, M.D., Ayerdi, M., Murcia, M., Estarlich, M., et al., 2019. Prenatal exposure to PM2.5 and NO2 and sex-dependent infant cognitive and motor development. Environ. Res. 174, 114–121. Jul 1.

Levander, O.A., 1977. Metabolic interrelationships between arsenic and selenium. Environ. Health Perspect. 19, 159–164.

Lewin, A., Arbuckle, T.E., Fisher, M., Liang, C.L., Marro, L., Davis, K., et al., 2017. Univariate predictors of maternal concentrations of environmental chemicals: the MIREC study. Mar 1 Int. J. Hyg Environ. Health 220 (2), 77–85. Part A.

Lewis, R.C., Meeker, J.D., Basu, N., Gauthier, A.M., Cantoral, A., Mercado-García, A., et al., 2018. Urinary metal concentrations among mothers and children in a Mexico City birth cohort study. Int. J. Hyg Environ. Health 221 (4), 609–615. May.

Li, X., Zhang, Y., Tan, M., Liu, J., Bao, L., Zhang, G., et al., 2009. Atmospheric lead pollution in fine particulate matter in Shanghai, China. J. Environ. Sci. 21 (8), 1118–1124. Jan 1.

Llop, S., Porta, M., Martinez, M.D., Aguinagalde, X., Fernández, M.F., Fernández-Somoano, A., et al., 2013. Estudio de la evolución de la exposición a plomo en la población infantil española en los últimos 20 años: ¿un ejemplo no reconocido de «salud en todas las políticas»? Gac. Sanit. 27 (2), 149–155. Apr.

Lowe, N.M., Fekete, K., Decsi, T., 2009. Methods of assessment of zinc status in humans: a systematic review. Am. J. Clin. Nutr. 89 (6), 2040S–2051S. Jun.

Lozano, M., Murcia, M., Soler-Blasco, R., Iñiguez, C., Irizar, A., Lertxundi, A., et al., 2020. Prenatal Se concentrations and anthropometry at birth in the INMA study (Spain). Environ. Res. 181.

Lozano, M., Murcia, M., Soler-Blasco, R., González, L., Iriarte, G., Rebagliato, M., et al., 2021. Exposure to mercury among 9-year-old children and neurobehavioural function. Environ. Int. 146.

Mao, X., Hu, X., Wang, Y., Xia, W., Zhao, S., Wan, Y., 2020. Temporal trend of arsenic in outdoor air PM2.5 in Wuhan, China, in 2015-2017 and the personal inhalation of PM-bound arsenic: implications for human exposure. Environ. Sci. Pollut. Res. Int. 27 (17), 21654–21665. Jun.

McDermott, S., Salzberg, D.C., Anderson, A.P., Shaw, T., Lead, J., 2015. Systematic review of chromium and nickel exposure during pregnancy and impact on child outcomes. J. Toxicol. Environ. Health 78 (21–22), 1348–1368.

Mikelson, C.K., Troisi, J., LaLonde, A., Symes, S.J.K., Thurston, S.W., DiRe, L.M., et al., 2019. Placental concentrations of essential, toxic, and understudied metals and relationships with birth outcomes in Chattanooga. TN. Environ Res. 168, 118–129. Jan.

Miklavčič, A., Casetta, A., Snoj Tratnik, J., Mazej, D., Krsnik, M., Mariuz, M., et al., 2013. Mercury, arsenic and selenium exposure levels in relation to fish consumption in the Mediterranean area. Environ. Res. 120, 7–17. Jan.

Nazzareno, Ballatori, 2002. Transport of toxic metals by molecular mimicry. Environ. Health Perspect. 110 (Suppl. 5), 689–694. Oct 1.

Niehoff, N.M., Keil, A.P., O'Brien, K.M., Jackson, B.P., Karagas, M.R., Weinberg, C.R., et al., 2020. Metals and trace elements in relation to body mass index in a prospective study of US women. Environ. Res. 184, 109396. May.

Nordberg, G.F., Fowler, B.A., Nordberg, M., 2014. Handbook on the Toxicology of Metals: Fourth Edition In: Handbook on the Toxicology of Metals, fourth ed., vol. 1, p. 1

Osorio-Yáñez, C., Gelaye, B., Enquobahrie, D.A., Qiu, C., Williams, M.A., 2018. Dietary intake and urinary metals among pregnant women in the Pacific Northwest. Environ. Pollut. 236, 680–688. May.

Pérez, R., Doménech, E., Conchado, A., Sanchez, A., Coscollà, C., Yusà, V., 2018. Influence of diet in urinary levels of metals in a biomonitoring study of a child population of the Valencian region (Spain). Sci. Total Environ. 618, 1647–1657. Mar 15.

Peters, K.M., Galinn, S.E., Tsuji, P.A., 2016. Selenium: dietary sources, human nutritional requirements and intake across populations. In: Hatfield, D.L., Schweizer, U., Tsuji, P.A., Gladyshev, V.N. (Eds.), Selenium: its Molecular Biology and Role in Human Health [Internet]. Springer International Publishing, Cham, pp. 295–305. https://doi.org/10.1007/978-3-319-41283-2_25. Available from: (Accessed 2 November 2018).

Qi, J., Lai, Y., Liang, C., Yan, S., Huang, K., Pan, W., et al., 2019. Prenatal thallium exposure and poor growth in early childhood: a prospective birth cohort study. Environ. Int. 123, 224–230. Feb.

R Core Team, 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Viena.

Raîche, G., Walls, T.A., Magis, D., Riopel, M., Blais, J.-G., 2013. Non-graphical solutions for Cattell's scree test. Methodology 9 (1), 23–29.

Rayman, M.P., 2012. Selenium and human health. Lancet 379 (9822), 1256–1268. Mar 31.

Rivas, I., Viana, M., Moreno, T., Pandolfi, M., Amato, F., Reche, C., et al., 2014. Child exposure to indoor and outdoor air pollutants in schools in Barcelona, Spain. Environ. Int. 69, 200–212. Aug.

Sakai, T., 2000. Biomarkers of lead exposure. Ind. Health 38 (2), 127–142. Apr.

Sanders, A.P., Claus Henn, B., Wright, R.O., 2015. Perinatal and childhood exposure to cadmium, manganese, and metal mixtures and effects on cognition and behavior: a review of recent literature. Curr Envir Health Rpt 2 (3), 284–294. Sep 1.

Sanz Alaejos, M., Díaz Romero, C., 1993. Urinary selenium concentrations. Clin. Chem. 39 (10), 2040–2052. Oct.

Shefa, S.T., Héroux, P., 2017. Both physiology and epidemiology support zero tolerable blood lead levels. Toxicol. Lett. 280, 232–237. Oct 5.

Shirai, S., Suzuki, Y., Yoshinaga, J., Mizumoto, Y., 2010. Maternal exposure to low-level heavy metals during pregnancy and birth size. J Environ Sci Health A Tox Hazard Subst Environ Eng 45 (11), 1468–1474. Sep.

Soler-Blasco, R., Murcia, M., Lozano, M., González-Safont, L., Amorós, R., Ibarluzea, J., et al., 2020. Prenatal manganese exposure and neuropsychological development in early childhood in the INMA cohort. Int. J. Hyg Environ. Health 224.

Soler-Blasco, R., Murcia, M., Lozano, M., Sarzo, B., Esplugues, A., Vioque, J., et al., 2021. Urinary arsenic species and methylation efficiency during pregnancy: concentrations and associated factors in Spanish pregnant women. Environ. Res., 110889. Feb 16.

Stojanović, D., Nikić, D., Lazarević, K., 2004. The level of nickel in smoker's blood and urine. Cent. Eur. J. Publ. Health 12 (4), 187–189. Dec.

Sun, X., Jiang, Y., Xia, W., Jin, S., Liu, W., Lin, X., et al., 2018. Association between prenatal nickel exposure and preterm low birth weight: possible effect of selenium. Environ. Sci. Pollut. Control Ser. 25 (26), 25888–25895. Sep.

Tong, J., Liang, C.-M., Huang, K., Xiang, H.-Y., Qi, J., Feng, L.-L., et al., 2020. Prenatal serum thallium exposure and 36-month-old children's attention-deficit/hyperactivity disorder symptoms: ma'anshan birth cohort study. Chemosphere 244, 125499. Apr.

Tsaih, S.W., Korrick, S., Schwartz, J., Lee, M.L., Amarasiriwardena, C., Aro, A., et al., 2001. Influence of bone resorption on the mobilization of lead from bone among middle-aged and elderly men: the Normative Aging Study. Environ. Health Perspect. 109 (10), 995–999. Oct.

Turnlund, J.R., Keyes, W.R., Peiffer, G.L., 1995. Molybdenum absorption, excretion, and retention studied with stable isotopes in young men at five intakes of dietary molybdenum. Am. J. Clin. Nutr. 62 (4), 790–796. Oct.

Uriu-Adams, J.Y., Scherr, R.E., Lanoue, L., Keen, C.L., 2010. Influence of copper on early development: prenatal and postnatal considerations. Biofactors 36 (2), 136–152. Apr.

Vioque, J., Navarrete-Muñoz, E.-M., Gimenez-Monzó, D., García-de-la-Hera, M., Granado, F., Young, I.S., et al., 2013. Reproducibility and validity of a food frequency questionnaire among pregnant women in a Mediterranean area. Nutr. J. 12 (1), 26. Feb 19.

Wai, K.M., Umezaki, M., Kosaka, S., Mar, O., Umemura, M., Fillman, T., et al., 2018. Impact of prenatal heavy metal exposure on newborn leucocyte telomere length: a birth-cohort study. Environ. Pollut. 243, 1414–1421. Dec 1.

Wang, X., Qi, L., Peng, Y., Xia, W., Xu, S., Li, Y., et al., 2019a. Urinary concentrations of environmental metals and associating factors in pregnant women. Environ. Sci. Pollut. Res. 26 (13), 13464–13475. May 1.

Wang, X., Mukherjee, B., Batterman, S., Harlow, S.D., Park, S.K., 2019b. Urinary metals and metal mixtures in midlife women: the Study of Women's Health across the Nation (SWAN). Int. J. Hyg Environ. Health 222 (5), 778–789. Jun.

Wang, X., Sun, X., Zhang, Y., Chen, M., Dehli Villanger, G., Aase, H., et al., 2020. Identifying a critical window of maternal metal exposure for maternal and neonatal thyroid function in China: a cohort study. Environ. Int. 139, 105696. Jun.

Watson, C.V., Lewin, M., Ragin-Wilson, A., Jones, R., Jarrett, J.M., Wallon, K., et al., 2020. Characterization of trace elements exposure in pregnant women in the United States, NHANES 1999-2016. Environ. Res. 183, 109208. Apr.

WHO task force, 1996. Environmental Health Criteria 182 [Internet]. Available from: http://www.inchem.org/documents/ehc/ehc/ehc182.htm.

Wijmenga, C., Klomp, L.W.J., 2004. Molecular regulation of copper excretion in the liver. Proc. Nutr. Soc. 63 (1), 31–39. Feb.

Wilhelm, M., Wittsiepe, J., Seiwert, M., Hünken, A., Becker, K., Conrad, A., et al., 2013. Levels and predictors of urinary nickel concentrations of children in Germany: results from the German Environmental Survey on children (GerES IV). Int. J. Hyg Environ. Health 216 (2), 163–169. Mar.

Wilson, R.L., Grieger, J.A., Bianco-Miotto, T., Roberts, C.T., 2016. Association between maternal zinc status, dietary zinc intake and pregnancy complications: a systematic review. Nutrients 8 (10). Oct 15.

Wu, B., Cui, H., Peng, X., Fang, J., Zuo, Z., Deng, J., et al., 2014. Analysis of the toll-like receptor 2-2 (TLR2-2) and TLR4 mRNA expression in the intestinal mucosal immunity of broilers fed on diets supplemented with nickel chloride. Int. J. Environ. Res. Publ. Health 11 (1), 657–670.

Wu, M., Shu, Y., Song, L., Liu, B., Zhang, L., Wang, L., et al., 2019. Prenatal exposure to thallium is associated with decreased mitochondrial DNA copy number in newborns: evidence from a birth cohort study. Environ. Int. 129, 470–477. Aug.

Wu, H., Xu, B., Guan, Y., Chen, T., Huang, R., Zhang, T., et al., 2020. A metabolomic study on the association of exposure to heavy metals in the first trimester with primary tooth eruption. Sci. Total Environ. 723, 138107. Jun 25.

Wu, M., Wang, L., Song, L., Liu, B., Liu, Y., Bi, J., et al., 2021. The association between prenatal exposure to thallium and shortened telomere length of newborns. Chemosphere 265, 129025. Feb.

Zeng, H., Uthus, E.O., Combs Jr., G.F., 2005. Mechanistic aspects of the interaction between selenium and arsenic. J. Inorg. Biochem. 99 (6), 1269–1274.

Zhang, N., Yang, S., Yang, J., Deng, Y., Li, S., Li, N., et al., 2020. Association between metal cobalt exposure and the risk of congenital heart defect occurrence in offspring: a multi-hospital case-control study. Environ. Health Prev. Med. 25 (1), 38. Aug 8.

Zhao, H., Tang, J., Zhu, Q., He, H., Li, S., Jin, L., et al., 2020. Associations of prenatal heavy metals exposure with placental characteristics and birth weight in Hangzhou Birth Cohort: multi-pollutant models based on elastic net regression. Sci. Total Environ. 742, 140613. Nov 10.