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Results of the first national human biomonitoring in Slovenia: Trace elements in men and lactating women, predictors of exposure and reference values

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ABSTRACT

The first national human biomonitoring in Slovenia surveyed cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As), manganese (Mn), selenium (Se), copper (Cu) and zinc (Zn) in a childbearing population (18–49 years) selected from lactating *primiparous* women and men (N = 1084). The overall aim was to estimate trace elements' levels and geographical variations in order to identify sources of possible exposures and set the national reference values. The study population was selected evenly from 12 study areas across Slovenia, including rural, urban and known or potentially contaminated environments. Within 6–8 weeks after delivery, venous blood, spot urine, scalp hair and breast milk samples were collected to determine the selected elements. The data analysis included descriptive statistics and multiple linear regression using elemental concentrations in biological matrices, questionnaire data and environmental datasets. Essential elements showed no significant deficiencies or excessive levels in the study population and were largely determined by sex and/or the participating women's physiological status (*postpartum*, lactation), as well as by certain dietary sources. Toxic elements' levels were mainly below the levels considered to present increased health risk. Lifestyle and nutritional habits appeared as significant determinants of exposure to Cd (smoking and game meat consumption), Hg (seafood and amalgam fillings), As (seafood) and Pb (alcohol consumption, smoking, game meat consumption and type of water supply). A distinctive geographical pattern was confirmed, due to past mining activities combined with naturally elevated background levels in the cases of Pb (Mežica Valley), Hg (Idrija and Posočje) and As exposure (Zasavje). Increased seafood consumption in the coastal study area contributed to higher Hg and As (arsenobetaine) levels. Extensive sample size database accompanied with life-style and environmental data improved the prediction of exposure patterns, set the reference values for the childbearing population living in Slovenia, and provided a strong basis for evaluating spatial and temporal trends in exposure. To our best knowledge, this is the first study to establish reference values for lactating *primiparous* women.

1. Introduction

Based on the legislation for the implementation of human biomonitoring (HBM) in Slovenia (Act for Chemicals, No. 110/03), a protocol to start the first national HBM survey was established in 2007, mainly triggered by the absence of an overall exposure data about chemicals originating from the environment or food intake. The methodology to asses exposure to chemicals in the national survey was selected based on the initiatives and the recommendations of the World Health Organization (WHO) - the International Programme on Chemical Safety (IPCS, 2004), and the European Environment and Health Action Plan 2004–2010 (CEC, 2004). The selection criteria for the chemicals, study areas and the study population were based on national air and soil monitoring results; the chemicals' toxicological hazards, persistence

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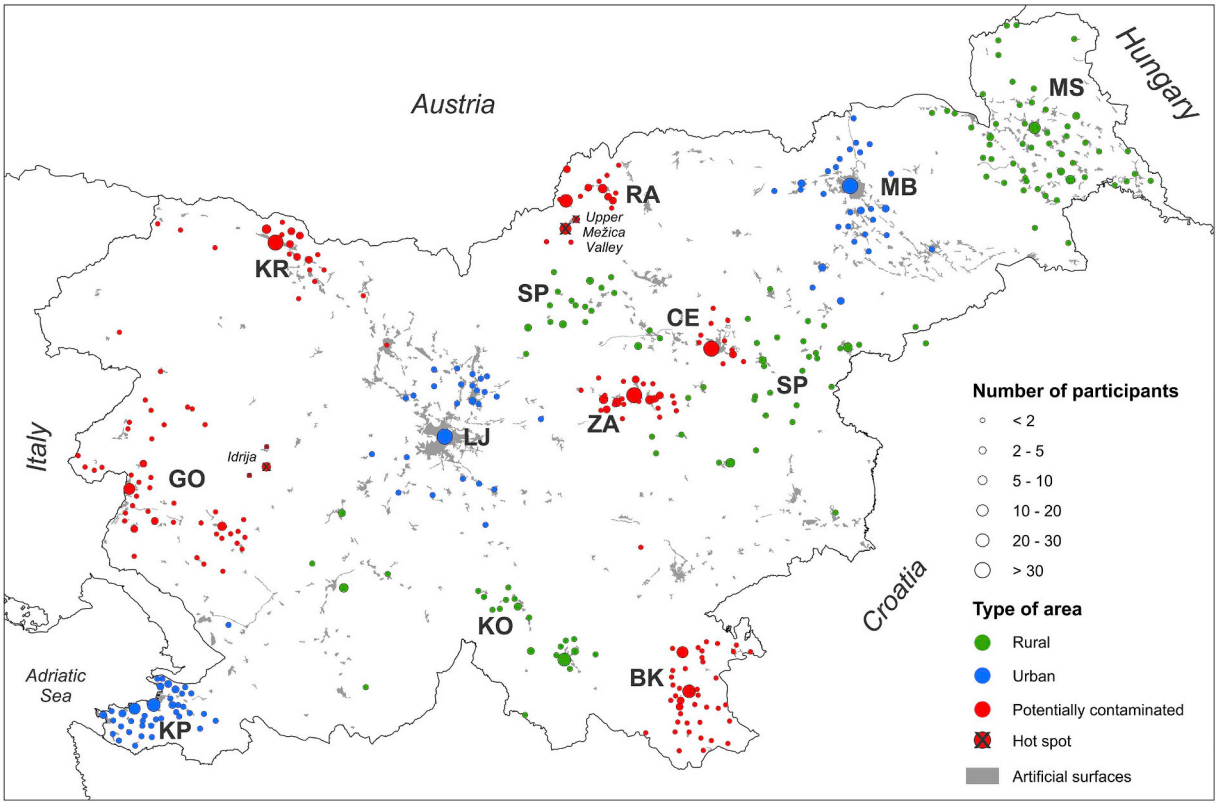

Fig. 1. Geographical distribution of participants in the Slovenian HBM survey. Circles depict total number of people recruited in an individual settlement. Study areas: BK = Bela Krajina, CE = Celje, GO = Idrija and Posočje, KO = Kočevje and Cerknica, KP = Koper, KR = Jesenice, LJ = Ljubljana, MB = Maribor, MS = Pomurje, RA = Mežica Valley, SP = Savinjsko-Posavska, ZA = Zasavje.

and bioaccumulation potential; the estimated size of exposed populations, analytical capacity, certain public concerns, and trends in other countries. Slovenia has three well-known heavily polluted areas ('hotspots'), where increased exposure of non-occupationally exposed inhabitants has been reported. These sites include the Upper Mežica Valley in the north, with residual pollution from a closed lead (Pb) and zinc (Zn) mine with a smelting plant (Eržen and Janet, 2005; Finžgar and Leštan, 2008; Miler and Gosar, 2012); the former mercury (Hg) mine town of Idrija in the west (Kobal et al., 2017; Kocman et al., 2011; Kotnik et al., 2005); and the area of Krupa River (southwest), contaminated with polychlorinated biphenyls (PCBs) from improper waste disposal from a former transformer and capacitor factory (Jan and Tratnik, 1988). In this paper, we do not deal with exposure to PCBs and other organic pollutants.

Along with the Upper Mežica Valley, other potentially Pb-contaminated areas in Slovenia include Šaleška Valley with its thermo-power plant Šoštanj and the industrial area of Celje (Eržen, 2011; Eržen and Janet, 2005). Other potentially polluted areas from past and current industrial activities are more or less spread across the country, including Lower Mežica Valley and Jesenice (northwest) with an ironworks industry; Central Sava Valley (central) with its coal-mining activities, thermo-power plant, cement plant, chemical company and glassware; and Celje (east) with its metallurgy, chemical-processing company and ironworks. Moderate arsenic (As) contamination due to other industrial/mining activities was reported in Mežica Valley, Sava Valley (Zagorje, Zasavje area), Idrija, Podljubelj and Litija, Celje and Jesenice (Gosar and Šajn, 2005; Perharič, 2013). However, a recent study on 3–12-year-old children in Sava Valley, specifically in the vicinity of the former Znojile antimony mine, did not show elevated As levels in their urine samples (Perharič et al., 2017).

For all these areas with various or even mixed industrial activities (past and/or current), complete population datasets on exposure to potentially toxic elements, accompanied by essential elements, are scarce. Therefore, several elements – Pb, Hg, cadmium (Cd), As, Zn, copper (Cu), manganese (Mn) and selenium (Se) – were selected for the first national HBM survey. Along with the potentially toxic elements, monitoring of endogenous essential elements enables a more realistic estimate of the health risks from low to moderate exposures. The elements Zn, Cu and Se can interact with toxic metals (and vice versa), influencing their absorption, distribution, metabolism and elimination in the human body. For example, Zn and Cu deficiency can increase the manifestation of the toxic effect of non-essential metals (Fitzgerald et al., 2008; Goyer, 1995), while the micronutrient Se can protect against the harmful effects of Hg by preventing damage from free radicals or forming biologically inactive Se-Hg complexes (Goyer, 1995; Suzuki et al., 1998) and similarly protect against As (Zeng et al., 2005) and also other non-essential elements (Cd, Pb) toxicity (Rahman et al., 2019). Various other mechanisms are proposed as well (Rahman et al., 2019). Of course, it should be noted that essential metals can also be toxic in cases of excessive exposure. The latter is well recognised for Mn, an essential element, which may act as a neurotoxicant in case of excessive exposure, reported mainly in adults' occupational exposures (Dobson et al., 2004; Horning et al., 2015).

In this paper, we summarise the results of the first national HBM survey that monitored Cd, Pb, Hg, As, Mn, Se, Cu and Zn levels in adults of childbearing age, selected from lactating *primiparous* women and men of the same age range. Participants from 12 study areas across Slovenia were recruited, including rural, urban and presumably metal-contaminated areas, and biological matrices were used to assess internal exposure. The main aims were to (1) determine trace elements levels and their geographical variations, (2) identify sources of possible exposures, (3) estimate babies' exposure via maternal milk and (4) establish the reference values for selected elements in the selected population. The latter is particularly important in relation to the lactating

primiparous women, for whom the reference levels are not available. *Postpartum* period is considered as the most critical period in the lives of mother and babies; whereat the drop of oestrogen after birth occurs between second and fifth month (Romano et al., 2010). The sources of potential exposure, tested in relation to the concentrations in the biological matrices, were selected from available data, starting from the general knowledge that exposure routes in non-occupationally exposed humans may be direct, through environmental compartments (air, soil and water) or food and lifestyle habits.

2. Methods

2.1. Study population and study areas

The study population included lactating *primiparous* mothers (mainly 6–8 weeks after delivery) and male participants of the same age range recruited from 12 geographically separate areas of Slovenia. The areas were classified into rural (n = 3), urban (n = 3) and potentially contaminated environments (n = 6) (Fig. 1). Kočevje and Cerknica (KO), Pomurje (MS) and Savinjsko-Posavska (SP) were selected as rural study areas; Ljubljana (LJ), Koper (KP) and Maribor (MB) as urban areas; and Bela Krajina (BK), Celje (CE), Jesenice (KR), Mežica Valley (RA), Posočje and Idrija (GO) and Zasavje (ZA) as potentially contaminated due to geological presence of metals, past and/or recent industrial activities (smelting plants, cement factory, power plant, glass factory, former Hg and Pb mines, ironworks, and former transformer and capacitor factory). The known 'hotspots' are within the GO (former Hg mine) and RA (former Pb mine) areas (Fig. 1). The selected rural areas have mainly agricultural activities, while the urban areas have multiple sources of current pollution, mainly from traffic. Potentially contaminated areas of CE and KR are at the same time also urban environments.

2.2. Recruitment and sampling

Recruitment and sampling spanned six years in two separate periods. The first period (2008–2009) covered KO, LJ and BK; the second (2011–2014) covered the rest of the study areas. Women were recruited in the third trimester of their pregnancies via gynaecologists, maternity hospitals or maternity schools in the selected study areas. Their partners were invited to participate in the study at any point. In each study area, 50 women and 50 men were planned for inclusion. In case a mother's partner was unwilling or ineligible to participate, another male participant was selected among family members or friends. The inclusion criteria for the participant were as follows: (a) residency in one of the selected areas in the same place for at least five years; (b) childbearing age; (c) mother's first and singleton pregnancy; (d) both mother and child should be healthy, without any problems in pregnancy; (e) the mother was breastfeeding; and (f) the mother was available for sampling 2–8 weeks after birth. Occupational exposure to chemicals was excluded from the study. For the participants in urban and rural areas, an additional exclusion criteria was (g) living within a 100–200-m radius from active waste dumps, mines, industrial objects or crematories.

The eligible participants donated venous blood (women 16 mL, men 23 mL), spot urine (50 mL), 3 cm of scalp hair close to the scalp from the occipital area (1 g) and women a maximum of 80 mL of breast milk (collected from multiple feeds in max 6 days). Sampling was carried out by regional health centres mainly 6–8 weeks after each mother's delivery (the actual range was 2 weeks–7 months, but 73% of women were within 6–8 weeks). The samples were transported to the central laboratory within 4–6 h after the sampling, where they were aliquoted into subsamples for specific analysis. In case some samples were transported to the central laboratory the next day, partial aliquoting was done in the regional laboratory. Sample cooling at 2–8 °C during transport and storage before aliquoting was guaranteed. Blood, urine and milk samples were stored long-term at −20 °C. Prior to the sampling, urine collection containers, as well as sample storage material (cryo-vials), were checked for trace elements content. Blood was collected in specialty tubes for trace element testing (Becton Dickinson Vacutainer® No. 368381). Standard needles to withdraw venous blood were used, pre-screening was not performed. Individual hair samples were stored in clean zip-lock polypropylene bags and an additional paper envelope. They were transported away from any Hg sources and stored in a clean room (< 10 ng Hg/m^3). Hair samples were not washed prior to analysis. All materials used in the laboratory analysis were checked for contamination through blank sample measurements. The study was approved by the Republic of Slovenia National Medical Ethics Committee, with numbers of accordance 42/12/07 and 53/07/09. Informed written consent was obtained from all participants. Participation was voluntary, and the participants could withdraw from the study at any stage.

2.3. Questionnaire data

The mothers and the male participants filled in questionnaires composed of (a) general information (age, body weight - for women pre-pregnancy weight, body height, education level and occupation), (b) basic home characteristics (residential environment - rural, urban, suburban; type of heating; type of water supply - public, private, bottled; vicinity of industry; age of residence), (c) health conditions (medicine, dietary supplements and number of dental amalgam fillings) and (d) lifestyle (smoking and hobbies) and nutritional habits (food consumption frequency, daily water intake, alcohol and coffee/tea intake and use of glazed pot). The participants reported their intake separately for fresh, canned and frozen seafood, which was set as relevant for Hg exposure. Due to the relatively high uncertainty of the self-reported consumption data, the intake of specific types of seafood was not summed up for the purpose of determining exposure sources in the cases of other elements. An additional set of questions regarding the pregnancy and lactation period was included in the maternal questionnaire (birth weight, birth length, intake of alcohol and smoking during pregnancy, and feeding).

2.4. Chemical analysis

2.4.1. Trace elements

Trace element analysis were performed in blood, milk, urine and hair samples using validated analytical methods. Both analytical and quality control procedures of element analysis used are described in detail in Supplemental material.

Briefly, measurements of Cd, Pb, As, Mn, Se, Cu and Zn in previously digested or diluted samples were performed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) equipped with an Octapole Reaction System (ORS) (Jagodic et al., 2017; Potočnik et al., 2016; Stajnko et al., 2017). Total mercury was determined by cold vapour atomic absorption and fluorescence spectrometry (CVAAS and CVAFS) using three different analytical procedures (Akagi, 1997; Horvat et al., 1991; Miklavčič et al., 2013a, 2011), depending on the matrix type and the sampling period. All measurements were made under strict quality control procedures. Blank samples, control samples and reference materials were tested together with the samples on a daily basis. The limits of detection (LOD) were 0.2, 0.4, 0.1, 0,4, 8, 7, 30, 0.1 ng/mL for Cd, Pb, As, Mn, Se, Cu, Zn, and Hg in blood, respectively. In urine the respective LODs were 0.03, 0.3, 0.1, 2, 3, 3, 0.1 ng/mL and in milk 0.1, 0.2, 0.05, 2, 6, 35, 0.5 ng/mL. The LOD of the procedure to determine Hg in hair was 1 ng/g of hair.

Concentrations of elements in urine were normalised for differences in diuresis among samples using (1) creatinine (crt) concentration and (2) specific gravity (SG). Although it is known that crt-normalised levels predict the daily excretion of trace elements better than non-normalised levels for most of the elements due to systematic variation in urinary

flow rates (Hays et al., 2015), there is the risk of underestimating the exposure due to creatinine over-compensation, particularly for Cd and As (Hoet et al., 2016). Besides, creatinine varies depending on sex, age, body mass index, fat-free mass and race/ethnicity (Barr et al., 2005). Specific gravity appears to be a more reliable alternative in the context of environmental exposures, without the risk of over-adjustment and with fewer uncertainties associated with its use (Hoet et al., 2016). This finding has been confirmed in our recent study (Stajnko et al., 2017); moreover, SG normalisation has been used in As methylation studies due to the common nutritional factors influencing both creatinine and As levels (Balakrishnan et al., 2018; Drobná et al., 2016; Skröder et al., 2018). However, to enable comparability, we have decided to present urine concentrations expressed per volume, as well as crt- and SG-normalised.

2.4.2. Arsenic speciation

Arsenite (AsIII), arsenate (AsV), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) were determined in 1:1 diluted and thawed urine samples using high-performance liquid chromatography (HPLC) separation on a Hamilton PRP-X100 column, followed online by hydride generation and CVAFS, as described earlier (Šlejkovec et al., 2008). The LODs ranged from 0.1 (AsIII, MMA) to 0.2 µg/L of urine (AsV, DMA). Speciation was performed in a subset of samples (n = 161) containing elevated total As (> 15 µg/L of urine). In selected samples, arsenobetaine (AsB) was determined using the same method with the Zorbax SCX chromatographic column and with additional online UV digestion before the hydride generation step.

2.5. Biochemical analysis

Haematocrit (%) in blood samples was determined as part of haemogram obtained using standard routine method at the University Medical Centre Ljubljana.

Creatinine in urine was determined by a standardised routine method (compensated Jaffé reaction) on the biochemical analyser Dimension (Dade Behring). Repeatability was assured by the reference material supplied by the analyser/reagent producer. The method's comparability and accuracy were verified by the inter-laboratory comparison certificate.

The SG was determined using a PAL-10S refractometer (Atago®, Japan), with a measurement range of 1.000–1.060. As the standards in SG normalisation calculations (Suwazono et al., 2005), SG means of 1.013 for females and 1.019 for males were used.

2.6. Acquisition of environmental and other ancillary data

The database on the drinking water comprised the concentration levels of the selected elements measured at 108 locations, evenly distributed across Slovenia. The available data was part of the radon (Rn) survey results about tap water in Slovenia in 2014 (Vaupotič and Gregorič, 2014). Information on actual concentrations in tap water for the individual subjects was not available, therefore drinking water concentrations from the closest location were assigned to each subject.

The database on the bio-indicators of air quality and deposition comprised the results of measurements of As, Cd, Cu, Hg, Pb, Zn, Mn and Se performed in moss *Hypnum cupressiforme* at 102 locations, evenly distributed across Slovenia, in 2010 (Harmens, 2010). The measured values from the closest location were assigned to each subject.

The geochemical composition of soil (Cd, Pb, Hg, Mn, Cu and Zn) at the address of each subject was obtained from an interpolated geochemical map of Slovenia (Geological Survey of Slovenia, 2006), available in raster format with a 500-m resolution.

Concentrations of selected elements in drinking water, moss and soil are presented by specific study areas (Tables S39–46 in the Supplementary data).

2.7. Statistical analysis

The descriptive statistics included the determination of median and mean values; standard deviation; 95% CI (CI_{95}) of the mean; 5^{th}, 10^{th}, 50^{th}, 90^{th} and 95^{th} percentiles (P5, P10, P50, P90 and P95). To derive reference values, the CI_{95} of P95 was determined for toxic elements as defined by the German HBM Commission and P2.5 and P97.5 for essential elements according to the International Federation of Clinical Chemistry (IFCC). The corresponding CI_{95} were calculated using a parametric method on ln-transformed data. The data below the LOD was assigned values of LOD/2. In cases > 10% samples were below the LOD (Cd in blood and milk, Pb in urine and milk, Cu in urine), the actual measured levels below the LOD were used in data analysis. In cases > 50% of the samples were below the LOD, only the percentile distribution was provided. Elemental concentrations were further stratified by sex and separate study areas (provided in the Supplementary tables). To compare concentrations of elements among the study areas or defined population groups, a one-way ANOVA was performed. Multiple linear regression models were used to evaluate the association between the elemental levels, separately in blood and urine (dependent variables), and potential predictor variables (independent variables) which were obtained through (a) questionnaires and (b) environmental databases. The models were omitted for all elements in breast milk and essential elements in urine. The first because the levels in this matrix could vary largely depending on the water/fat content (Gidrewicz and Fenton, 2014), and no normalisation method was used; and the second because levels in urine do not reliably reflect the status of essential elements. Confounding variables (sex and age) were fixed into the models. The sampling period (first versus second) was additionally fixed into the model if a significant difference was found between the two periods. In the blood models, haematocrit level was used as a confounder as well. In the urine models, Se concentration in blood was used as a confounder to account for a possible increased excretion of toxic elements. Because of the explained risk of misinterpretation using crt-normalised levels (Barr et al., 2005; Hoet et al., 2016), urinary models for specific elements were constructed based on SG normalised levels. In the sex-specific models for women, time between delivery and sampling (days) was included as a confounder due to gradual return to pre-pregnancy state. A separate set of variables was used for each model, they were selected from the bivariate correlations (ANOVA, Pearson's correlation) with probability values (p-values) < 0.25 and based on the predictions from the literature or previous studies. Highly correlated variables were not included in the same model to avoid the multi-collinearity effect. Log-normally distributed data was normalised using a natural logarithm. P-values < 0.05 were considered statistically significant, and p-values < 0.1 were marginally significant. Statistical analyses were performed using STATA 12 (USA).

3. Results and discussion

3.1. General characteristics of the study population

The study population included the adult childbearing population living in Slovenia, which was diverse in terms of the type of living environment, sex, education (although skewed towards higher levels) and occupation (Table 1). Among the women invited to participate in the study through gynaecologists, 17% responded positively. The number of women recruited through maternity schools and maternity hospitals was small and their response could not be estimated, as well could not be the response rate of the men. Sex was equally distributed among the study areas; so was the participants' mean age. Less than half (42%) of the male participants were female's partners. The majority of the participants had finished secondary or high school; more women (49%) were university degree holders compared with men (30%). A university degree (or higher) was the most frequent in urban areas; the primary level or apprenticeship was the least represented in most study

Table 1
General characteristics of the study population of men and lactating women from Slovenia.

Population group	n	Living environment (%) *			Age (years)	Level of education (%)			BMI (kg/m^2)	Smoking (%)**		Occupation (%)		
		R	U	Sub	Mean (min-max)	I	II	III	Mean (min-max)	YES	NO	I	II	III
Total	*1084*	*38*	*29*	*33*	*30 (18–49)*	*12.7*	*47.8*	*39.5*	*24.6 (16.6–44.8)*	*11.0*	*89.0*	*27*	*51*	*13*
Female	536	39	28	33	29 (19–39)	7.2	43.4	49.4	23.2 16.6–44.8)	13.3	86.7	34	51	2
Male	548	37	29	34	31 (18–49)	18.1	52.1	29.8	26.0 (17.5–37.6)	8.8	91.2	20	50	24
Study area														
Celje (CE)[c]	79	14	49	37	31 (20–40)	11.5	43.6	44.9	24.5 (18.3–34.9)	6.3	93.7	34	50	6
Posočje and Idrija (GO)[c]	96	44	28	28	30 (21–49)	12.2	51.1	36.7	24.3 (16.6–35.5)	11.7	88.3	30	39	20
Koper (KP)[b]	102	40	22	38	30 (20–42)	12.4	37.1	50.5	24.2 (17.2–36.8)	6.0	94.0	27	58	8
Jesenice (KR)[c]	83	30	38	32	29 (19–49)	12.2	60.8	27.0	24.4 (17.5–35.6)	14.5	85.5	20	62	12
Maribor (MB)[b]	98	19	35	46	31 (23–39)	3.0	50.0	47.0	24.7 (17.7–37.6)	14.0	86.0	29	56	3
Pomurje (MS)[a]	90	74	15	11	29 (20–48)	15.3	54.1	30.6	24.7 (17.7–35.1)	12.6	87.4	26	51	12
Mežica Valley (RA)[c]	79	42	30	28	33 (18–42)	16.0	48.0	36.0	24.7 (17.6–37.9)	3.8	96.2	30	44	11
Savinjsko-Posavska (SP)[a]	81	67	7	26	29 (21–40)	16.7	55.1	28.2	25.0 (18.0–36.9)	8.5	91.5	23	53	14
Zasavje (ZA)[c]	104	20	40	40	29 (21–44)	7.1	55.1	37.8	25.3 (18.2–35.9)	4.9	95.1	45	84	16
Ljubljana (LJ)[b]	100	16	40	43	30 (23–44)	4.1	25.8	70.1	23.7 (16.6–40.4)	5.7	94.3	26	63	6
Bela Krajina (BK)[c]	101	60	11	29	29 (21–41)	19.4	52.0	28.6	25.3 (18.8–44.8)	32.3	67.7	16	53	23
Kočevje and Cerknica (KO)[a]	71	37	25	37	28 (19–38)	30.3	43.9	25.8	24.4 (18.6–37.1)	10.5	89.5	25	34	31

Notes: Type of study area: **a** – rural, **b** – urban, **c** – potentially contaminated; type of living environment (*independent of the type of study area): **R** – rural, **U** – urban, **Sub** – suburban; Min – minimal value, Max – maximum value; BMI – body mass index; Level of education: **I** – primary or apprenticeship, **II** – secondary or high school, **III** – university or higher. **The women's smoking status refers to the pre-pregnancy period. Occupation type: **I** – managers, professionals; **II** – technicians, clerical support workers, service and sales workers; **III** – agricultural, forestry, fishery, craft and related trade workers, plant and machine operators and assemblers, elementary occupations and armed forces. Unemployed and students are omitted from the classification.

areas. Occupation Group II (see Table 1 for details) was the most represented in all study areas; the urban areas had the lowest proportion of Group III workers. The mean body mass index (BMI) levels were within the range considered normal in all study areas; 10% of the participants (7% women and 12% men) were obese (BMI $>$ 30 kg/m^2). Among the participants, 11% were smokers (the women's smoking status before pregnancy).

3.2. Levels of trace elements

Tables 2 and 3 present the concentrations of selected trace elements in biological samples. The levels of potentially toxic elements in the study population were generally low and, with minor differences, similar to those reported in the general populations studied in Europe (Baeyens et al., 2014; Becker et al., 2003, 2002; Castaño et al., 2012; Černá et al., 2012; Nisse et al., 2017; Puklová et al., 2010) and North America (CDC, 2018a; Haines et al., 2017) over the last two decades. The essential element status in our study population was assessed using the same matrices as those for toxic elements, except in hair. The observed variability was mainly due to the lactating status of the female study group, which was associated with decreased Se and increased Mn, Cu and Zn levels in the blood. Further explanations with national and international comparisons are provided in the specific sections that follow.

The percentages of the study population exceeding the reference levels or not complying with the reference intervals are specified in the Supplementary data (Tables S1–S37). In summary, population percentages exceeding the health- or population-based reference levels for potentially toxic elements (Schulz et al., 2011) or not complying with the essential elements ranges relevant for European populations (Barceloux, 1999; Iyengar, 1998; Wilhelm et al., 2004) were observed to a small extent. However, it should be stressed that for metals which transfer placenta at least to a certain extent (e.g. Cd, Pb, Hg), it is believed that there are no safe limits for maternal blood levels. There is a lack of international levels of concern specifically for pregnancy (or lactating period) and comparisons can only be made with national reference values (Taylor et al., 2014), as was done in the present study.

Table 4 presents predictors of exposure for individual element as obtained by multiple linear regression for the total population and separately for men and lactating women. In summary, life style and/or nutritional habits appeared as significant predictors ($p < 0.05$) of exposure to Cd (smoking and consumption of vegetable and game meat), Hg (consumption of seafood and amalgam fillings), As (consumption of seafood), and also Pb (alcohol intake, smoking, private water supply and consumption of game meat) for the total population. The geochemical data for top soil across Slovenia appeared as a significant environmental predictor in the cases of Hg and Pb exposure. In comparison to soil, Pb concentrations in moss showed stronger correlations with concentrations in biological samples. Blood Hg concentrations were also associated with tap water Hg concentrations. Apart from seafood, As exposure was also associated with tap water As (as indicated by As levels in blood). Exposure patterns for toxic elements noticeably depended on the sex and/or physiological status and the biomarker of exposure. The influence of sex could not be distinguished from the physiological (*postpartum*) status of the women. The models confirmed the sex/physiological difference in Cd concentration (higher in women's blood). Concentrations of Cd and Pb in blood increased with age, but Hg decreased. The most important determinant of all essential elements concentrations was sex/physiological status; men had higher Se levels, while women had higher Mn, Cu and Zn levels, which is consistent with the lactating status of female participants. In general, when comparing the elements according to the type of study area, higher Se and Zn levels were observed in contaminated areas than in rural areas, but did not differ from urban areas. The Cu levels were higher in contaminated areas than in urban areas, and even higher in rural areas. Similarly, Mn and Cd levels (in urine, but not in blood) prevailed in rural areas, presumably as a result of agricultural activities. Nutritional habits showed associations with Mn (consumption of game meat and bottled water), Se and Zn levels (consumption of seafood). Intake of dietary supplements was associated with increased Mn and Se blood levels in men, but decreased Mn and Zn levels in women. The observed predictors are discussed in detail in the specific sections that follow.

3.3. Cadmium

3.3.1. Basic exposure levels

Eight percent of men and two percent of women in our study population exceeded the population-based reference Cd level of 1 μg/L in blood (Schulz et al., 2011), while two and less than one percent,

Table 2
Levels of toxic elements in blood, urine and hair (and breast milk of females) of the study population of men and lactating *primiparious* women from Slovenia. crt – creatinine, SG – specific gravity.

Biomarker	Unit	Population group	N	N < LOD	GM [95% CI]	min-max	P5	P10	P50	P90	P95
Cd in blood	***µg/L***	*Total*	1083	301	**0.28** [0.27–0.30]	< LOD-4.80	< LOD	< LOD	0.29	0.72	1.01
		Men[a]	548	230	**0.23** [0.22–0.25]	< LOD-4.80	< LOD	< LOD	0.23	0.80	1.22
		Women[b]	535	71	**0.35** [0.33–0.37]	< LOD-3.08	< LOD	0.14	0.36	0.71	0.87
Cd in urine	***µg/L***	*Total*	1001	14	**0.19** [0.18–0.20]	< LOD-3.83	0.05	0.07	0.20	0.47	0.67
		Men[a]	506	2	**0.21** [0.19–0.22]	< LOD-1.75	0.07	0.09	0.22	0.49	0.62
		Women[b]	495	12	**0.17** [0.16–0.19]	< LOD-3.83	0.04	0.06	0.17	0.46	0.70
	µg/g crt	*Total*	989	14	**0.20** [0.19–0.21]	< LOD-2.79	0.07	0.09	0.19	0.47	0.61
		Men[a]	500	2	**0.16** [0.15–0.16]	< LOD-1.14	0.06	0.07	0.15	0.32	0.42
		Women[b]	489	12	**0.26** [0.25–0.28]	< LOD-2.79	0.10	0.12	0.25	0.58	0.79
	µg/L SG	*Total*	951	14	**0.21** [0.21–0.22]	< LOD-3.65	0.08	0.10	0.20	0.48	0.57
		Men[a]	479	2	**0.22** [0.21–0.23]	< LOD-1.11	0.08	0.11	0.22	0.46	0.56
		Women[a]	472	12	**0.21** [0.20–0.22]	< LOD-3.65	0.07	0.10	0.19	0.49	0.61
Cd in milk	***µg/L***		470	272	–	< LOD-0.34	< LOD	< LOD	< LOD	0.14	0.18
Pb in blood	***µg/L***	Total	1084	0	**18.0** [17.5–18.5]	3.86–116	9.13	10.3	17.5	32.5	41.5
		Men[a]	548	0	**19.3** [18.5–20.1]	3.86–116	9.16	10.4	18.7	37.4	46.1
		Women[b]	536	0	**16.7** [16.2–17.3]	4.25–71.9	8.82	10.3	16.3	28.0	33.2
Pb in urine	***µg/L***	*Total*	812	247	**0.46** [0.44–0.49]	< LOD-4.41	< LOD	< LOD	0.49	1.43	1.91
		Men[a]	402	65	**0.66** [0.61–0.71]	< LOD-4.40	< LOD	< LOD	0.71	2.31	2.31
		Women[b]	410	182	**0.33** [0.31–0.36]	< LOD-4.41	< LOD	< LOD	0.34	0.97	1.19
	µg/g crt	*Total*	812	247	**0.49** [0.46–0.51]	< LOD-5.31	< LOD	< LOD	0.50	1.19	1.49
		Men[a]	402	65	**0.49** [0.46–0.52]	< LOD-5.31	< LOD	< LOD	0.50	1.12	1.42
		Women[a]	410	182	**0.49** [0.45–0.52]	< LOD-3.44	< LOD	< LOD	0.51	1.27	1.57
	µg/L SG	Total	804	246	**0.53** [0.50–0.55]	< LOD-6.11	< LOD	< LOD	0.55	1.28	1.62
		Men[a]	397	64	**0.70** [0.65–0.75]	< LOD-5.21	< LOD	< LOD	0.75	1.51	2.07
		Women[b]	407	181	**0.40** [0.37–0.43]	< LOD-6.11	< LOD	< LOD	0.39	0.98	1.14
*Pb in milk**	***µg/L***		353	161	**0.23** [0.21–0.25]	< LOD-5.42	< LOD	< LOD	0.21	0.72	0.99
Hg in blood	***µg/L***	Total	1083	2	**1.18** [1.12–1.24]	< LOD-31.0	0.30	0.41	1.20	3.42	4.78
		Men[a]	548	1	**1.25** [1.15–1.34]	< LOD-31.0	0.26	0.38	1.28	3.72	5.13
		Women[b]	535	1	**1.11** [1.04–1.19]	< LOD-10.2	0.31	0.43	1.11	3.09	4.06
Hg in urine	***µg/L***	*Total*	1055	12	**0.44** [0.41–0.48]	< LOD-12.3	0.05	0.11	0.45	2.22	3.47
		Men[a]	527	2	**0.56** [0.50–0.62]	< LOD-12.3	0.08	0.12	0.56	2.67	3.80
		Women[b]	528	10	**0.35** [0.31–0.39]	< LOD-7.05	0.04	0.08	0.36	1.74	2.93
	µg/g crt	*Total*	1020	12	**0.47** [0.43–0.50]	< LOD-8.67	0.06	0.11	0.51	1.78	2.48
		Men[a]	516	2	**0.42** [0.38–0.46]	< LOD-7.92	0.06	0.09	0.44	1.50	2.19
		Women[b]	504	10	**0.52** [0.47–0.58]	< LOD-8.67	0.07	0.11	0.59	1.92	2.64
	µg/L SG	*Total*	989	12	**0.49** [0.46–0.53]	< LOD-10.2	0.07	0.13	0.53	1.98	2.83
		Men[a]	495	2	**0.59** [0.54–0.65]	< LOD-10.2	0.10	0.15	0.61	2.30	3.30
		Women[b]	494	10	**0.41** [0.37–0.46]	< LOD-7.88	0.05	0.10	0.46	1.66	2.25
Hg in hair	***ng/g***	*Total*	947	0	**275** [258–292]	10.0–7068	48.0	79.5	303	867	1203
		Men[a]	444	0	**282** [256–311]	11.0–7068	46.0	77.0	306	984	1396
		Women[a]	503	0	**268** [248–290]	10.0–1947	52.3	82.0	300	731	993
Hg in milk	***µg/L***		470	18	**0.14** [0.13–0.16]	< LOD-3.39	0.02	0.04	0.16	0.51	0.64
As in blood	***µg/L***	Total	1084	1	**0.89** [0.85–0.94]	< LOD-28.9	0.35	0.40	0.77	2.62	3.74
		Men[a]	548	1	**0.83** [0.79–0.88]	< LOD-22.4	0.36	0.40	0.72	2.12	3.40
		Women[b]	536	0	**0.96** [0.89–1.03]	0.20–28.9	0.32	0.39	0.81	2.90	5.16
As in urine	***µg/L***	*Total*	812	0	**6.37** [5.89–6.90]	0.26–1026	1.28	1.68	5.63	30.7	54.2
		Men[a]	402	0	**7.79** [6.94–8.74]	0.48–1026	1.57	2.09	6.40	37.0	71.6
		Women[b]	410	0	**5.24** [4.72–5.82]	0.26–266	1.06	1.45	4.85	23.1	35.1
	µg/g crt	*Total*	812	0	**6.70** [6.24–7.19]	0.37–499	1.71	2.17	5.84	26.6	50.5
		Men[a]	402	0	**5.80** [5.22–6.43]	0.76–499	1.52	1.85	4.80	24.5	52.9
		Women[b]	410	0	**7.71** [7.02–8.48]	0.37–219	2.15	2.53	6.79	28.6	48.8
	µg/L SG	*Total*	804	0	**7.22** [6.72–7.75]	0.71–672	1.87	2.37	6.24	28.6	55.7
		Men[a]	397	0	**8.27** [7.44–9.21]	0.97–672	2.27	2.58	6.69	37.6	84.5
		Women[b]	407	0	**6.31** [5.75–6.93]	0.71–269	1.79	2.15	5.81	21.6	37.1
As in milk	***µg/L***		470	9	**0.18** [0.17–0.19]	< LOD-3.70	0.07	0.08	0.16	0.49	0.79

Notes: Different letters ([a,b]) indicate that the study groups differ statistically significantly. *The first sampling period's results are excluded. The sex-related difference may (also) be on account of the lactating status of women.

respectively, exceeded the HBM I Cd value of µg/L in urine (Schulz et al., 2011). The Cd levels in the study population were generally low and similar to those reported in the general populations studied in Europe (Baeyens et al., 2014; Becker et al., 2003, 2002; Castaño et al., 2012; Černá et al., 2012; Nisse et al., 2017) and North America (CDC, 2018a; Haines et al., 2017). The levels in the blood of the male study group were lower than those reported for the male military recruits examined in 2001 (median = 0.5 µg/L, n = 463, age = 18–27 years, non-smoking) who were living in nine health regions (Eržen and Zaletel Kragelj, 2004), which mostly overlap with the present study areas. In the absence of analytical issue (LODs between the studies were equivalent), higher levels in the non-smoking military recruits than in the present male study group could hypothetically be due to longer periods spent outdoors by the first group, increasing their contact time with dust.

3.3.2. Geographic variability within the country

Cadmium levels in blood and urine were more or less comparable

Table 3
Levels of essential elements in blood and urine (and breast milk of females) of the study population of men and lactating *primiparous* women from Slovenia. crt – creatinine, SG – specific gravity.

Biomarker	Unit	Population group	N	< LOD	GM [95% CI]	min-max	P5	P10	P50	P90	P95
Mn in blood	***µg/L***	Total	811	0	**13.8** [13.5–14.2]	5.69–40.5	8.18	9.02	13.3	22.1	26.2
		Men[a]	402	0	**11.1** [10.8–11.4]	5.69–29.8	7.64	8.26	11.0	15.0	16.4
		Women[b]	409	0	**17.2** [16.7–17.7]	5.83–40.5	10.4	11.5	17.3	25.9	28.0
Se in blood	***µg/L***	Total	1084	0	**105** [103–106]	53.9–226	74.2	80.5	104	138	152
		Men[a]	548	0	**115** [114–117]	60.3–226	87.3	91.7	113	151	166
		Women[b]	536	0	**94.6** [93.1–96.1]	53.9–176	70.7	75.1	94.5	119	127
Se in urine	***µg/L***	*Total*	812	0	**13.5** [12.7–14.2]	0.50–121	2.92	4.61	14.3	35.7	46.0
		Men[a]	402	0	**19.5** [18.3–20.8]	2.81–121	6.05	8.01	20.1	45.8	52.0
		Women[b]	410	0	**9.36** [8.68–10.1]	0.50–51.9	2.40	2.95	10.2	24.7	28.9
	µg/g crt	*Total*	812	0	**14.1** [13.8–14.5]	1.00–134	8.26	9.42	14.1	21.4	24.0
		Men[a]	402	0	**14.5** [14.0–15.0]	1.57–54.3	8.32	9.93	14.5	22.4	24.3
		Women[b]	410	0	**13.8** [13.3–14.3]	1.00–134	8.10	9.13	13.6	20.6	23.6
	µg/L SG	*Total*	804	0	**15.2** [14.7–15.7]	2.17–138	6.90	8.11	14.8	29.3	34.1
		Men[a]	397	0	**20.7** [19.9–21.6]	3.13–64.7	10.6	12.4	21.0	34.0	38.0
		Women[b]	407	0	**11.2** [10.8–11.7]	2.17–138	6.01	7.16	11.5	17.4	20.5
Se in milk	***µg/L***		470	0	**12.6** [12.3–13.0]	5.36–38.1	8.13	9.11	12.6	17.6	19.3
Cu in blood	***µg/L***	Total	1084	0	**951** [941–961]	532–2004	737	766	940	1196	1262
		Men[a]	548	0	**847** [839–856]	532–1404	708	738	844	976	1041
		Women[b]	536	0	**1070** [1057–1083]	657–2004	857	908	1062	1262	1364
Cu in urine	***µg/L***	*Total*	812	223	**5.39** [5.06–5.75]	< LOD-67.3	< LOD	< LOD	6.27	17.2	22.3
		Men[a]	402	81	**6.26** [5.74–6.83]	< LOD-64.5	< LOD	< LOD	7.07	17.7	21.6
		Women[b]	410	142	**4.66** [4.24–5.12]	< LOD-67.3	< LOD	< LOD	5.04	16.8	22.5
	µg/g crt	*Total*	812	223	**5.66** [5.37–5.97]	< LOD-86.2	< LOD	< LOD	5.84	14.2	19.6
		Men[a]	402	81	**4.66** [4.34–5.00]	< LOD-41.1	< LOD	< LOD	4.94	9.36	13.5
		Women[b]	410	142	**6.86** [6.37–7.40]	< LOD-86.2	< LOD	< LOD	7.09	17.3	24.9
	µg/L SG	*Total*	804	223	**6.07** [5.75–6.40]	< LOD-80.2	< LOD	< LOD	6.50	15.2	19.4
		Men[a]	397	81	**6.64** [6.16–7.15]	< LOD-43.9	< LOD	< LOD	7.40	15.8	19.3
		Women[b]	407	142	**5.56** [5.15–6.00]	< LOD-80.2	< LOD	< LOD	5.45	15.1	19.5
Cu in milk	***µg/L***		470	0	**355** [346–365]	99–954	221	250	360	525	578
Zn in blood	***µg/L***	Total	1084	0	**6606** [6549–6663]	3010–11733	5150	5518	6636	7806	8285
		Men[a]	548	0	**6494** [6419–6571]	3400–10301	5082	5460	6553	7646	8050
		Women[b]	536	0	**6721** [6636–6807]	3010–11733	5274	5647	6768	8011	8494
Zn in urine	***µg/L***	*Total*	812	0	**276** [261–291]	13.0–2213	71.9	98.4	287	708	923
		Men[a]	402	0	**297** [275–322]	17.3–2213	78.2	104	317	774	978
		Women[b]	410	0	**256** [237–277]	13.0–1921	66.1	93.9	267	661	824
	µg/g crt	*Total*	812	0	**290** [277–239]	19.7–3371	96.6	128	307	619	754
		Men[a]	402	0	**221** [208–235]	19.7–1283	75.0	112	232	454	528
		Women[b]	410	0	**377** [356–400]	23.1–3371	131	181	401	742	972
	µg/L SG	*Total*	804	0	**311** [297–325]	15.8–3458	109	148	323	663	816
		Men[a]	397	0	**315** [295–336]	15.8–1400	93.1	148	334	689	887
		Women[a]	407	0	**307** [289–325]	45.8–3457	110	147	313	639	771
Zn in milk	***µg/L***		470	0	**1935** [1842–2032]	207–7904	741	1005	2063	3708	4404

Notes: Different letters ([a,b]) indicate that the study groups differ statistically significantly. The sex-related difference may (also) be on account of the lactating status of women.

among the study areas, with some minor but significant differences (Table S1, S9—S11). Although the highest Cd levels in soil existed in the industrially contaminated area of KR and were two to six times higher than in other areas (Table S39), this was not reflected in the HBM levels. As the Cd levels in breast milk were mostly below the LOD and the latter differed between the study periods, comparison among the study areas was not performed (Table S30). According to the type of study area, there were no significant differences in Cd levels in blood, while higher urinary Cd levels were observed in rural than in urban and/or contaminated areas (Table 4), which could hypothetically be associated with increased levels in crops due to the application of artificial phosphate fertilisers (Roberts, 2014).

3.3.3. Predictors of exposure

In line with tobacco smoking and the diet, generally recognised as the main sources of Cd exposure (ATSDR, 2012), our study showed the association mainly with smoking and to a minor extent, with the consumption of certain food items (i.e., game) (Table 4). This finding was particularly evident in blood, the matrix which was confirmed as a superior exposure biomarker versus urinary Cd at low exposure levels in cases when 24-h urine is unattainable (Stajnko et al., 2017). Male smokers had on average 5 times higher Cd levels in their blood than non-smokers, while female smokers had 1.2 times higher levels than non-smoking women. Urinary levels (SG normalised) did not show association with smoking (Table 4). The highest two values observed in the blood (> 4 µg/L) were represented by male heavy smokers (both 20 cigarettes/day) (Table S1), while the highest urine value, regardless of the normalisation method (3.65 µg/L, 2.79 µg/g crt or 3.65 µg/L SG) was found in the contaminated area of ZA and was not associated with smoking (Tables S9–10). Association between blood Cd and smoking is evident from various national or regional HBM studies on a general adult populations (Becker et al., 2002; Černá et al., 2012; Nisse et al., 2017; Stajnko et al., 2017), and in contrast to our study also for crt-normalised urine levels (Becker et al., 2003; Castaño et al., 2012; CDC, 2018a; Den Hond et al., 2015; Nisse et al., 2017) or non-normalised levels (Becker et al., 2003; Hoet et al., 2013).

The dietary source associated with Cd levels in blood or urine was vegetable but was found significant only in the rural area of SP ($p = 0.041$, model $R^2 = 0.36$, $p < 0.001$, $n = 78$) and the industrially contaminated area of ZA ($p = 0.008$, model $R^2 = 0.26$, $p < 0.001$, $n = 94$). On the contrary, we found no increased Cd exposure due to vegetable consumption in the industrially contaminated area of CE,

Table 4
Multiple linear regression: predictors of elements exposure in the Slovenian study population.

		Estimate of change (95% CI), multiplicative factor		
		Total population	Women, lactating	Men
Cd in blood (μg/L)	*Model R*[b]	*0.17 (p < 0.001)*	*0.04 (p = 0.003)*	*0.30 (p < 0.001)*
		N = 973	*n = 471*	*n = 487*
Type of study area		ns	ns	ns
Sex[a]	Men	1.00	–	–
	Women	**1.58 (1.39–1.79)****	–	–
Age (years)	≤25	1.00	1.00	
	25–35	**1.14 (1.02–1.28)***	**1.19 (1.02–1.38)***	ns
	> 35	**1.28 (1.08–1.51)***	**1.34 (1.01–1.77)***	1.22 (0.98–1.50)#
Smoking	No	1.00	1.00	1.00
	Yes	**2.16 (1.88–2.48)****	**1.21 (1.03–1.43)***	**5.05 (4.03–6.32)****
Consumption of game	No		1.00	
	Yes	ns	**1.22 (1.02–1.45)***	ns
Cd in urine (μg/L SG)	*Model R*[b]	*0.02 (p = 0.002)*	*0.008 (p = 0.214)*	*0.02 (p = 0.017)*
		N = 892	*n = 424*	*n = 455*
Type of study area	Rural	1.00		1.00
	Urban	0.90 (0.80–1.01)#	ns	0.88 (0.76–1.02)#
	Contaminated	**0.88 (0.79–0.97)***	ns	0.89 (0.78–1.01)#
Sex[a]		ns	–	–
Age (years)	≤25	1.00		1.00
	25–35	**1.14 (1.03–1.27)***	ns	ns
	> 35	**1.28 (1.11–1.49)***	ns	**1.27 (1.07–1.51)***
Smoking		ns	ns	ns
Pb in blood (μg/L)	*Model R*[b]	*0.18 (p < 0.001)*	*0.11 (p < 0.001)*	*0.19 (p < 0.001)*
		n=958	*n=463*	*n=480*
Sex[a]	Men	1.00	–	–
	Women	**1.10 (1.01–1.19)***	–	–
Age (years)	≤25	1.00		1.00
	25–35	**1.11 (1.03–1.19)***	1.09 (0.99–1.20)#	1.12 (1.00–1.25)#
	> 35	**1.19 (1.07–1.32)***	1.18 (0.99–1.41)#	**1.20 (1.05–1.38)***
Game consumption	No	1.00		1.00
	Yes	**1.11 (1.03–1.20)***	ns	**1.11 (1.00–1.23)***
Smoking	No	1.00	1.00	1.00
	Yes	**1.15 (1.05–1.25)***	**1.12 (1.00–1.24)***	**1.17 (1.01–1.36)***
Alcohol consumption	No	1.00	1.00	1.00
	Yes	**1.18 (1.11–1.25)****	**1.13 (1.04–1.22)***	**1.23 (1.12–1.36)****
Type of water supply	Public	1.00		1.00
	Bottled	ns	ns	ns
	Private	**1.24 (1.08–1.43)***	ns	**1.35 (1.08–1.69)***
Pb concentration in moss (twofold change)		**1.12 (1.09–1.14)****	**1.15 (1.09–1.21)****	**1.12 (1.09–1.16)****
Pb in urine (μg/L SG)	*Model R*[b]	*0.22 (p < 0.001)*	*0.11 (p < 0.000)*	*0.07 (p < 0.001)*
		n=733	*n=351*	*n=369*
Sex[a]	Men	1.00	–	–
	Women	**0.61 (0.70–0.54)****	–	–
Age (years)	≤25	1.00	1.00	1.00
	25–35	ns	ns	ns
	> 35	**1.32 (1.11–1.57)***	**1.69 (1.23–2.31)***	1.22 (0.97–1.53)#
Smoking	No	1.00	1.00	1.00
	Yes	**1.36 (1.15–1.61)****	**1.30 (1.06–1.59)***	**1.51 (1.11–2.04)***
Alcohol consumption	< once/month	1.00	1.00	1.00
	≥once/month	**1.24 (1.11–1.39)****	**1.23 (1.05–1.44)***	**1.26 (1.06–1.49)***
Pb concentration in moss (twofold change)		**1.11 (1.07–1.16)****	**1.18 (1.10–1.26)****	**1.12 (1.04–1.21)***
Hg in blood (μg/L)	*Model R*[b]	*0.18 (p < 0.001)*	*0.14 (p < 0.001)*	*0.20 (p < 0.001)*
		N = 734	*n = 352*	*n = 370*
Age (years)	≤25	1.00	1.00	1.00
	25–35	ns	ns	ns
	> 35	**0.72 (0.58–0.91)***	0.69 (0.47–1.03)#	**0.71 (0.53–0.97)***
Consumption of fresh seafood	Less than once/month	1.00	1.00	1.00
	1-3 times/month	**1.34 (1.17–1.54)****	**1.28 (1.06–1.55)***	**1.42 (1.16–1.74)****
	At least once/week	**1.89 (1.56–2.28)****	**1.82 (1.42–2.33)****	**2.06 (1.50–2.83)****
Consumption of canned seafood	Less than once/month	1.00	1.00	1.00
	1-3 times/month	**1.40 (1.22–1.61)****	**1.41 (1.16–1.71)****	**1.41 (1.14–1.75)***
	At least once/week	**1.65 (1.43–1.91)****	**1.39 (1.13–1.70)***	**1.91 (1.53–2.38)****
No. of amalgam fillings	0–3	1.00	1.00	1.00
	4–9	ns	1.17 (0.98–1.39)#	ns
	> 9	**1.35 (1.10–1.65)***	1.29 (0.98–1.70)#	**1.48 (1.09–2.01)***
Hg concentration in soil (twofold change)		**1.10 (1.05–1.14)****	**1.09 (1.01–1.18)***	**1.10 (1.05–1.17)****
Hg concentration in water (twofold change)		**1.10 (1.01–1.21)***	**1.13 (1.00–1.28)***	ns

(*continued on next page*)

Table 4 (*continued*)

		Estimate of change (95% CI), multiplicative factor		
		Total population	Women, lactating	Men
Hg in urine (μg/g SG)	*Model R*[b]	*0.17 (p < 0.001)*	*0.13 (p < 0.001)*	*0.18 (p < 0.001)*
		N = 699	*n = 331*	*n = 358*
Sex[a]	Men	1.00	–	–
	Women	**0.68 (0.58–0.80)****	–	–
Age (years)		ns	ns	ns
Consumption of fresh seafood	Less than once/month	1.00		1.00
	1-3 times/month	ns	ns	**1.35 (1.06–1.71)***
	At least once/week	**1.29 (1.00–1.65)***	ns	ns
Consumption of canned seafood	Less than once/month	1.00		1.00
	1-3 times/month	**1.25 (1.04–1.51)***	ns	**1.30 (1.02–1.65)***
	At least once/week	**1.36 (1.12–1.66)***	ns	**1.45 (1.12–1.86)***
No. of amalgam fillings	0–3	1.00	1.00	1.00
	4–9	**1.89 (1.60–2.24)****	**2.13 (1.64–2.76)****	**1.81 (1.47–2.24)****
	> 9	**3.08 (2.33–4.06)****	**2.99 (1.94–4.16)****	**3.18 (2.25–4.49)****
Hg concentration in soil (twofold change)		**1.09 (1.03–1.15)***	ns	**1.10 (1.04–1.18)***
Hg in hair (ng/g)	*Model R*[b]	*0.22 (p < 0.001)*	*0.20 (p < 0.001)*	*0.25 (p < 0.001)*
		N = 845	*n = 448*	*n = 397*
Sex		ns	ns	ns
Consumption of fresh seafood	Less than once/month	1.00	1.00	1.00
	1-3 times/month	**1.44 (1.26–1.65)****	**1.23 (1.03–1.46)***	**1.71 (1.38–2.12)****
	At least once/week	**2.32 (1.93–2.80)****	**1.91 (1.53–2.39)****	**3.00 (2.17–4.14)****
Consumption of canned seafood	Less than once/month	1.00	1.00	1.00
	1-3 times/month	**1.42 (1.24–1.63)****	**1.44 (1.22–1.71)****	**1.41 (1.13–1.75)***
	At least once/week	**1.83 (1.58–2.11)****	**1.69 (1.39–2.05)****	**1.95 (1.56–2.44)****
Hg concentration in soil (twofold change)		**1.19 (1.12–1.27)****	**1.24 (1.13–1.36)****	**1.18 (1.08–1.29)****
As in blood (μg/L)	*Model R*[b]	*0.13 (p < 0.001)*	*0.12 (p < 0.001)*	*0.14 (p < 0.001)*
		N = 948	*n = 460*	*n = 473*
Sex[a]	Men	1.00	–	–
	Women	1.13 (0.99–1.30)#	–	–
BMI (10% change)		**0.998 (0.997–1.000)***	**0.998 (0.996–1.000)***	ns
Consumption of fresh seafood	Less than once/month	1.00	1.00	1.00
	1-3 times/month	**1.27 (1.13–1.41)****	**1.24 (1.04–1.47)***	**1.29 (1.12–1.49)***
	At least once/week	**1.83 (1.57–2.14)****	**1.92 (1.54–2.41)****	**1.73 (1.39–2.15)****
Consumption of canned seafood	Less than once/month	1.00	1.00	1.00
	1-3 times/month	ns	1.16 (0.98–1.38)#	ns
	At least once/week	**1.15 (1.02–1.30)***	ns	**1.20 (1.02–1.40)***
Consumption of frozen seafood	Less than once/month	1.00		1.00
	1-3 times/month	1.11 (0.99–1.22)#	ns	1.14 (0.99–1.32)#
	At least once/week	**1.17 (1.00–1.37)***	ns	**1.42 (1.15–1.74)***
As concentration in water (twofold change)		**1.06 (1.02–1.12)***	ns	**1.07 (1.00–1.14)***
As in urine (μg/L SG)	*Model R*[b]	*0.06 (p < 0.001)*	*0.06 (p = 0.001)*	*0.05 (p = 0.001)*
		N = 728	*n = 350*	*n = 365*
Sex[a]	Men	1.00	–	–
	Women	**0.74 (0.63–0.86)****	–	–
BMI (10% change)		**0.997 (0.996–0.999)***	0.998 (0.995–1.000)#	0.997 (0.994–1.000)#
Consumption of fresh seafood	Less than once/month	1.00	1.00	1.00
	1-3 times/month	**1.22 (1.03–1.44)***	ns	1.26 (0.98–1.63)#
	At least once/week	**1.52 (1.20–1.93)***	**1.63 (1.21–2.20)***	ns
Consumption of canned seafood	Less than once/month	1.00		
	1-3 times/month	ns	ns	ns
	At least once/week	1.17 (0.97–1.42)#	ns	1.26 (0.96–1.66)#
Consumption of frozen seafood	Less than once/month	1.00	1.00	1.00
	1-3 times/month	ns	ns	ns
	At least once/week	**1.47 (1.15–1.87)***	1.34 (0.95–1.89)#	**1.54 (1.07–2.21)***
As concentration in water (twofold change)		ns	ns	ns
Mn in blood (μg/L)	*Model R*[b]	*0.42 (p < 0.001)*	*0.17 (p < 0.001)*	*0.01 (p = 0.180)*
		N = 711	*n = 337*	*n = 361*
Type of study area	Rural	**1.06 (1.00–1.11)***	**1.09 (1.00–1.18)***	
	Urban	ns	**1.09 (1.01–1.18)***	ns
	Contaminated	1.00	1.00	ns
Sex[a]	Men	1.00	–	–
	Women	**1.64 (1.54–1.75)****	–	–
BMI (10% change)		**0.985 (0.972–0.999)***	0.982 (0.963–1.001)#	ns
Type of water supply	Private	1.00	1.00	
	Public	**1.12 (1.01–1.23)***	**1.17 (1.01–1.34)***	ns
	Botted	**1.19 (1.05–1.35)***	**1.27 (1.05–1.53)***	ns
Consumption of game	Less than once/month	1.00	1.00	
	Once/month or more	**1.06 (1.01–1.11)***	**1.10 (1.00–1.21)***	ns

(*continued on next page*)

Table 4 (*continued*)

		Estimate of change (95% CI), multiplicative factor		
		Total population	Women, lactating	Men
Consumption of poultry	Once/week or less	1.00	1.00	
	More than once/week	**0.94 (0.90–0.98)***	**0.90 (0.85–0.96)***	ns
Consumption of tea or coffee	Once/week or less	1.00		
	2-6 times/week	0.94 (0.89–1.00)#	ns	ns
	At least once/day	ns	ns	ns
Intake of dietary supplements[b]	No	1.00	1.00	1.00
	Yes	**0.95 (0.90–1.00)***	**0.87 (0.81–0.93)****	**1.08 (1.00–1.16)***
Se in blood (μg/L)	*Model R*[b]	*0.30 (p < 0.001)*	*0.15 (p < 0.001)*	*0.11 (p < 0.001)*
		N = 943	*n = 455*	*n = 473*
Type of study area	Rural	1.00	1.00	1.00
	Urban	**1.08 (1.05–1.12)****	**1.12 (1.07–1.17)****	**1.05 (1.00–1.10)***
	Contaminated	**1.08 (1.05–1.12)****	**1.15 (1.10–1.20)****	ns
Sex[a]	Men	1.00	–	–
	Women	**0.82 (0.79–0.85)****	–	–
Consumption of fresh seafood	Less than once/month	1.00	1.00	1.00
	1-3 times/month	**1.04 (1.01–1.07)***	**1.04 (1.00–1.08)***	**1.04 (1.00–1.09)***
	At least once/week	**1.08 (1.04–1.12)****	**1.07 (1.01–1.12)***	**1.10 (1.03–1.16)***
Consumption of canned seafood	Less than once/month	1.00	1.00	1.00
	1-3 times/month	ns	ns	ns
	At least once/week	**1.07 (1.04–1.10)****	**1.04 (1.00–1.09)***	**1.08 (1.04–1.13)****
Consumption of nuts	Less than once/week	1.00	1.00	1.00
	Weekly	**1.04 (1.01–1.06)***	1.03 (0.99–1.06)#	**1.04 (1.00–1.08)***
Intake of dietary supplements[b]	No	1.00		1.00
	Yes	**1.03 (1.00–1.05)***	ns	**1.04 (1.00–1.08)***
Cu in blood (μg/L)	*Model R*[b]	*0.50 (p < 0.001)*	*0.16 (p < 0.001)*	*0.13 (p < 0.001)*
		N = 968	*n = 469*	*n = 484*
Type of area	Urban	1.00	1.00	1.00
	Rural	**1.02 (1.00–1.04)***	**1.07 (1.04–1.11)****	1.03 (1.00–1.06)#
	Contaminated	**1.03 (1.01–1.05)***	**1.03 (1.01–1.06)***	**1.03 (1.00–1.05)***
Sex[a]	Men	1.00	–	–
	Women	**1.24 (1.21–1.27)****	–	–
Alcohol consumption	< once/month	1.00	1.00	
	≥once/month	0.98 (0.96–1.00)#	0.97 (0.95–1.00)#	ns
BMI, 10% change		**1.015 (1.010–1.021)****	**1.014 (1.007–1.022)***	**1.021 (1.013–1.029)****
Zn in blood (μg/L)	*Model R*[b]	*0.07 (p < 0.001)*	*0.06 (p < 0.001)*	*0.07 (p < 0.001)*
		N = 963	*n = 467*	*n = 481*
Type of area	Rural	1.00		1.00
	Urban	**1.03 (0.99–1.05)***	ns	**1.06 (1.02–1.10)***
	Contaminated	**1.02 (1.02–1.05)***	ns	**1.05 (1.02–1.09)***
Sex[a]	Men	1.00	–	–
	Women	**1.11 (1.08–1.14)****	–	–
Fruit consumption	Once/week or less			1.00
	Couple of times/week	ns	ns	ns
	Daily	ns	ns	**0.96 (0.93–1.00)***
Consumption of fresh seafood	Less than once/month	1.00	1.00	
	1-3 times/month	**1.02 (1.00–1.05)***	1.03 (1.00–1.06)#	ns
	At least once/week	**1.04 (1.01–1.07)***	**1.05 (1.01–1.09)***	ns
Intake of dietary supplements[b]	No	1.00	1.00	
	Yes	**0.97 (0.95–1.00)***	**0.96 (0.94–0.99)***	ns

Notes: Margin of statistical significance: #p < 0.1, *p < 0.05, **p ≤ 0.001, ns – not significant. Variables with significance of p < 0.05 in at least one group are bolded.

[a] The sex-related difference may (also) be on account of the lactating status of women.

[b] Supplements containing minerals were taken into account.

where elevated levels of Cd in locally grown vegetables were found (Bešter et al., 2013), which might be a consequence of the local recommendation about consuming purchased rather than home-grown vegetables (Bešter et al., 2013).

Game consumption was shown as an additional potential source of Cd in the blood of the female study group (Table 4). Although Cd concentration is known to be much higher in game kidney and liver than in game meat, several studies across Europe reported a considerable percentage of game meat exceeded the maximum level 0.050 mg/kg wet weight set by Commission Regulation (EC) 1881/2006 or other (Doganoc and Gačnik, 1995; Mitranescu et al., 2011). Furthermore, as observed by Danieli et al. (2012) in their study involving a hunter population and its household members from central Italy, more frequent game consumption could contribute to higher Cd exposure levels.

Both, blood and urinary Cd concentration increased with age, as observed from the statistical model for the whole population (Table 4), which is in agreement with the literature data (ATSDR, 2012) and has been confirmed in various national or regional HBM studies (Castaño et al., 2012; Den Hond et al., 2015; Hoet et al., 2013; Mortensen et al., 2011; Nisse et al., 2017).

3.4. Lead

3.4.1. Basic exposure levels

In line with decreasing Pb exposure levels in industrialised countries starting from the 1990s due to the initiation of unleaded gasoline usage, our study population showed low mean Pb levels in all matrices (Table 2). The observed exposure in males (GM = 19.3 μg/L of blood, Table 2) was lower than that observed in 2001 in a population of male military recruits residing in nine health regions across the country (overall mean = 37.4 μg/L of blood, n = 463, age = 18–27 years) (Eržen and Zaletel Kragelj, 2004). Moreover, the majority of our study population was within the concentration range generally believed not to present elevated health risk (Tables S2 and S24). Only two men from the Pb-smelter area exceeded the population-based reference value of 90 μg/L in the blood (Schulz et al., 2011). In the lack of international consensus for population groups of increased susceptibility (pregnancy, *postpartum*), we referred to the guidelines regarding the screening and management of pregnant and lactating women in the US (Committe on Obstetric Practice, 2012): six women in our study group exceeded the blood Pb level of 50 μg/L (three from the contaminated areas of RA, KR, and ZA, and three from the urban area of LJ), which presents 1% of the study population, similarly to the US population.

3.4.2. Geographic variability within the country

Despite the generally low levels, our study population showed a distinctive geographical pattern of Pb exposure, with peak levels in the Pb-smelter area of RA (GM = 27.2 μg/L, Table S2). As the participants in our study lived in the wider area of RA, we divided them into two subgroups: one living in the vicinity of the Pb-smelter area with higher environmental contamination (Upper RA, n = 22, GM = 39.9 μg/L) and the other living in Lower RA with lower environmental contamination (n = 57, GM = 23.5 μg/L) (Table S2, $p < 0.001$). These results confirm those from the local monitoring of the Pb-smelter community, specifically mothers and preschool and school children, which has been ongoing since 1976. Following the installation of an emission control system in 1978, the Pb levels in blood have decreased slowly from the median levels of 400–500 μg/L in the three study groups (n = 93) to 41–284 μg/L (95% of all values) in 1990 (n = 131), as opposed to the range of 25–123 μg/L in the control area in 1984 (n = 84) (Prpić-Majić et al., 1992, 1984). These findings indicated that Pb-contaminated soil from past exposure was the major source of increased Pb exposure in a population (Prpić-Majić et al., 1992). In 2001–2002, elevated Pb levels in blood (> 100 μg/L) could still be found in over one-third of the studied children from RA area (age = 3 years, n = 47) (Eržen and Janet, 2005), and in year 2017 in one-fifth of the children (age = 3 years, n = 90) (unpublished data). Male military recruits living in nine health regions (Eržen and Zaletel Kragelj, 2004) also showed the highest Pb levels in blood in the RA area (mean = 60.7 μg/L).

3.4.3. Predictors of exposure

Adjusting for potential covariates and confounders revealed that living in Upper RA accounted for about two times higher Pb levels in blood and urine (crt- or SG-normalised) (models not presented). However, as a more predictive variable of Pb contamination, instead of the study area, Pb concentration in moss (Table S40) was used in the statistical models (Table 4). The Pb concentration in the moss species *Hypnum cupressiforme* indicates Pb atmospheric deposition (ICP Vegetation Coordination Centre, 2010). Owing to the extreme persistence of Pb in soil, this and other studies (Prpić-Majić et al., 1992; Zahran et al., 2013) showed that current airborne Pb is most probably from legacy sources in industrialised areas. The following are additional significant determinants of Pb levels in blood that were identified from the statistical models: age (19% higher in participants > 35 years than in those ≤ 25 years), type of water supply (24% higher in private than in public), alcohol consumption (18% higher in those consuming it ≥ once/month than in those < once/month), smoking (15% higher in smokers than in non-smokers) and game consumption (11% higher in consumers than in non-consumers) (Table 4). Estimation of human exposure to Pb mostly relies on levels in blood (Bergdahl and Skerfving, 2008), representing a combination of recent (2–3 months) and past Pb exposure, whereas urinary Pb is considered as an indicator of recent exposure, which is more variable within individuals and does not have a linear correlation with blood Pb (Skerfving and Bergdahl, 2015). Despite that, we identified a similar pattern of exposure using urinary Pb levels, except for the game consumption (Table 4).

The identified predictors were in line with generally known sources of exposure, revealed after the initiation of unleaded gasoline use (Jakubowski, 2012), except in RA, where lifestyle was not found as a significant determinant of Pb exposure. In this area, Pb concentration in moss or soil was the only significant covariate associated with the overall Pb levels in blood and urine (Table S38). Among the male residents of RA, private water supply was additionally found to contribute to 4.5 times higher urinary Pb levels as opposed to bottled water use (Table S38). Increasing Pb levels in blood with increasing levels in tap water have been reported elsewhere due to Pb leaching from the water pipe system (Moore et al., 1977; Pocock et al., 1983). Various studies involving the general adult population whose mean Pb levels ranged between 30 and 90 μg/L of blood confirmed smoking and wine consumption as relevant determinants of Pb exposure (Alimonti et al., 2005; Forte et al., 2011; Hense et al., 1992; Weyermann and Brenner, 1997). While smoking contributes to Pb exposure through the Pb content in tobacco plants, it is commonly argued that alcohol consumption contributes through crystal glassware containing Pb. However, other factors, such as different metabolism rates of drinkers, are possible (Grandjean et al., 1981; Graziano and Blum, 1991). To our best knowledge, the present study is the first to demonstrate smoking and alcohol as significant determinants of Pb exposure at lower levels than reported previously, while increasing Pb levels with age are known (Lee and Kim, 2014).

3.5. Mercury

3.5.1. Geographic variability within the country

The highest Hg levels in the study population's blood and hair were found in the coastal part of Slovenia (KP) (Tables S3 and S37), corresponding to the coastal area residents' lifestyle, which includes the Mediterranean diet. Inhabitants of Mediterranean countries are known to have higher Hg levels in their blood and/or hair than residents of other European countries (Castaño et al., 2015; Višnjevec et al., 2014) because of higher consumption of fish and other seafood, which are the dominant sources of organic Hg exposure (NRC, 2000; UNEP/WHO, 2008). The questionnaire data confirmed that the highest percentage (37%) of participants consuming seafood at least once per week lived in KP, while the percentages in the other study areas ranged between 4% and 19%. The urinary Hg levels (volume expressed, crt- or SG-normalised) were also among the highest in KP (Tables S15–S17) but did not differ statistically from most of the other areas. The Hg levels in breast milk differed minimally among the study areas (Table S32).

The second highest Hg levels in blood and hair were observed in GO and did not differ significantly from KP. Regardless of the normalisation method, the urinary Hg levels of the GO residents were among the highest as well (Tables S15–S17). In the GO area, 11 male participants lived in the former Hg mine area (town of Idrija), and their median creatinine (0.96 μg/g crt, range = 0.3–2.4 μg/g crt) or SG normalised levels in urine (0.90 μg/L SG, range = 0.33–2.35 μg/L SG), but not in blood (2.1 μg/L, range = 1.1–15.0 μg/L) or in hair (466 ng/g, range = 310–5740 ng/g), were somewhat higher than in the overall GO area (0.56 μg/g crt, 0.60 μg/L SG, 2.02 μg/L and 429 ng/g, respectively) (Tables S3, S16, S17, S37), indicating elevated inorganic Hg exposure in the town of Idrija. In this area, increased exposure due to inhalation of Hg vapour in ambient air is still evident despite the mine's closure in the

1990s, with other potential sources of Hg identified (in this site) as ingestion of drinking water and locally grown food or fish from Idrijca River (Horvat et al., 2004; Kobal et al., 2017; Miklavčič et al., 2013b; Žižek et al., 2007). The blood and urine Hg levels of the inhabitants of the former Hg mine area were previously shown to be higher than in a control rural area but did not differ from the ones found in the urban area of LJ (Kobal et al., 2017), similar to our study's findings.

3.5.2. Predictors of exposure

The geographical trend of the highest levels in the surroundings of the Idrija Hg mine (Gosar et al., 2016; Kocman et al., 2011) was confirmed in the statistical models by the positive correlations between Hg concentrations in the soil and in blood, urine and hair. Moreover, Hg concentrations in tap water showed correlations with Hg levels in blood (Table 4). In general, the Hg levels in hair and blood clearly showed exposure that was predominantly associated to seafood consumption with peak exposure in the coastal area. The participants who consumed seafood at least once per week had on average 89% higher Hg levels in their blood and 2.3 times higher Hg levels in their hair than those eating seafood less than once per month, whereas having more than 9 amalgam fillings versus a maximum of 3 contributed to 35% higher Hg levels in blood (Table 4). In contrast, urinary Hg levels depended mainly on the presence of dental amalgams (3 times higher levels in participants with > 9 amalgams than in those with ≤3), with no distinct geographical pattern identified. Seafood consumption contributed to approx. 30% increase in urinary Hg levels (Table 4), confirming that a certain proportion of urinary Hg originates from fish consumption, as first shown by Sherman et al. (2013), who used a stable isotope technique to prove it as a result of demethylation processes in the body (Sherman et al., 2013). Both fresh and canned, but not frozen seafood consumption was significantly associated with Hg levels in blood, hair and urine (Table 4).

3.5.3. Basic exposure levels

Overall, as observed recently in the European-scale pilot HBM study (Den Hond et al., 2015), the Hg levels in hair found in Slovenia were within the European average. In the present study, the percentages of the female study population exceeding the US Environmental Protection Agency (EPA)-based threshold value of 1000 ng/g of hair for developmental neurotoxicity risk (US EPA, 2001) were 22% in the coastal area of KP and 6% in the other study areas. Referring to the updated biological limit of 0.58 μg/g hair, which is based on the outcomes of the highly exposed Faroese population, but is supported by the recent studies of developmental neurotoxicity at low exposure levels (Bellanger et al., 2013), 18% of women in our study were above this cut-off level, 59% in the coastal area (KP). In the absence of a consensus value protecting child development, the data for the susceptible population group (pregnancy and *postpartum*) at low levels of exposure, similarly as in case of Pb, are critically lacking. Among men, four exceeded the HBM II value of 15 μg/L of blood (Schulz et al., 2011); two of them were frequent seafood consumers (residents of the coastal area), while for the other two, no known source of exposure was identified (neither seafood, amalgam nor study area). Ten participants exceeded the HBM I value of 5 μg/g crt in urine (Schulz et al., 2011); among them, no one was from the Hg mine area (Table S13).

Regarding the evaluation of Hg levels in breast milk, scarce data in the literature is available concerning the general population of women. In comparison to other studies, the Hg levels in our study population were very low and geographically independent (Table S32). The previously reported level for mature milk of the women from LJ, using radiochemical neutron activation analysis, was 1 ± 0.2 μg/L (Kosta et al., 1983), while the findings of a recent cohort study are similar to those for our study population (median = 0.2 ng/g, range = LOD–2.9 ng/g, n = 284) (Miklavčič et al., 2013a). In contaminated areas of South Africa and Asia, 10 or 20 times higher concentrations than those in our study were reported in breast milk as a consequence of Hg vapour inhalation or consumption of contaminated fish (Böse-O'Reilly et al., 2008).

3.6. Arsenic

3.6.1. Basic exposure levels

In our study, 161 participants (20%) exceeded the population-based reference value of 15 μg/L of urine, established for people who did not consume seafood 48 h prior to the sampling (Schulz et al., 2011). Within this subgroup, speciation analysis was performed to check for the presence of inorganic As species (n = 138, total As GM = 39.8 μg/L, range = 16–1030 μg/L). The concentrations ranged as follows: AsIII = < LOD–6.1 μg/L (GM = 0.6 μg/L), AsV = < LOD–2.1 (GM = 0.1 μg/L), DMA = < LOD–54.7 μg/L (GM = 3.9 μg/L) and MMA = < LOD–6.6 μg/L (GM = 0.2 μg/L). Their respective percentages were 0.1–80% (GM = 9.6%), 0.1–37% (GM = 1%), 21–100% (GM = 71%) and 0.2–55% (GM = 4%). The respective numbers of samples below LOD were 33, 124, 5, and 84. It is known that the major form of dietary As that predominates in seafood is organic compound AsB (Horvat et al., 2012; Hughes, 2006), whose elevated concentrations can be found in urine at least three days after the consumption of such food (Navas-Acien et al., 2011). Therefore, AsB was determined in samples exceeding 100 μg total As/L of urine (n = 12). The percentages ranged from 65% to 97%, while the levels of inorganic As and MMA were negligible, and the proportion of DMA was low (3–35%). These results excluded any significant environmental exposure to inorganic As and confirmed the exposure mainly to dietary organic As. Some types of seafood can also be rich in DMA (especially mussels, shrimps and some fish) or arsenosugars and also inorganic As (e.g., algae and animals feeding on them) (Cullen and Reimer, 1989; Taylor et al., 2017). Therefore, in some cases, urinary DMA that was found in our samples (together with MMA) may also come from seafood consumption, although both are among the commonly used biomarkers of inorganic As exposure (Aylward et al., 2014; Šlejkovec et al., 2008).

3.6.2. Geographic variability within the country

Similar to the case of Hg, As levels in blood and urine (regardless of the normalisation method) were the highest in the coastal area of KP (Table S4, S18−S20). This finding already indicates that the primary source of total As in our study population was seafood, as previously reported for the Slovene population of pregnant women (Miklavčič et al., 2013a). Among other study areas, excluding the coastal area of KP, total As levels in blood and urine were more or less similar. However, including the potentially As-contaminated areas of Slovenia (ZA, GO, RA, CE and KR) as a confounder in the statistical models, 18% higher As levels in blood were observed in female residents ($p = 0.061$), and 19% higher SG-normalised urinary levels ($p = 0.018$) were found in male and female residents of these areas (models not presented). These results confirm slightly elevated exposure in the listed As-contaminated areas but still below the level regarded as posing a health risk.

3.6.3. Predictors of exposure

Consistent with the speciation analysis, the regression analysis showed that the main predictor of total As exposure was seafood, confirming the predominant exposure to dietary organic As. Consumption of fresh seafood at least once per week increased As levels in blood, on average, by 83% and in urine by about 50% in comparison to intake of less than once per month (Table 4). Consumption of canned and frozen seafood contributed to As levels in blood to a lower extent than fresh seafood, while in urine, the contribution from frozen seafood was also considerable, particularly in men, increasing the urinary As levels by 54% (Table 4). Due to its fast clearance from blood, As levels in blood are normally not used as indicators of chronic exposure to low levels of As (Horvat et al., 2012). However, our study showed similar patterns in blood and urine, probably indicating constant dietary habits

of our study participants.

The As levels in blood also showed a significant association with As concentrations in tap water (Table 4). Drinking water, depending on the geographic region, is the most important medium for inorganic As exposure (Hughes, 2006; Nordstrom, 2002). In Slovenia, As levels in drinking water are generally low (below 10 μg/L); however, certain mineral water springs may contain elevated levels, up to 60 μg inorganic As/L (Van Elteren et al., 2002). Total As concentration in tap water amounting to 0.50 μg/L was observed in ZA, while in the other study areas, the mean concentration was 0.14 μg/L ($p < 0.001$) (Table S42), consistent with the concentration in the vicinity of Znojile mine in Sava Valley, contaminated due to past mining activities (Perharič et al., 2017). Of the 17 samples from ZA that were included in the speciation analysis, none showed elevated inorganic As species, with the levels of AsV, AsIII and MMA below the LOD, and DMA ranging from < LOD to 9.3 μg/L (median = 3.8 μg/L). Likewise, in their study, Perharič et al. (2017) reported no excessive urinary As concentrations, indicating that the levels observed in this particular region did not pose elevated health risks.

3.7. Manganese

3.7.1. Basic levels

As an essential element, Mn has a normal range of 4–15 μg/L of blood (Barceloux, 1999; Burtis et al., 2012). However, Mn levels are known to increase during pregnancy, which is due to changes in absorption, metabolism or tissue mobilisation related to increased oestrogen and progesterone concentrations to satisfy its higher demand during pregnancy (Gunier et al., 2014). The increase in mean blood Mn was demonstrated in the Quebec population of pregnant women (1st trimester: 8.5 μg/L; 3rd trimester 15.6 μg/L) (Takser et al., 2004) and agricultural community of California (26th week of gestation: 14.6 μg/L; delivery: 20.7 μg/L) (Gunier et al., 2014). Consistent with this, 70% of women in our study exceeded the upper reference value of 15 μg/L, while among the men, 10% exceeded the upper level. Among all the participants, no one's level was below the lower reference level of 5 μg/L (Burtis et al., 2012) (Table S5). Similar levels to those of our study (GM_{women} = 17.2 μg/L, Table 3) were reported for maternal blood at 6 weeks *postpartum* in Northern Norway (GM = 17.3 μg/L, n = 211) (Hansen et al., 2011). On the other hand, the US population of non-pregnant women showed markedly lower blood levels than ours (GM ~ 10 μg/L), while men only slightly lower (GM ~ 8.8 μg/L) (CDC, 2018b).

3.7.2. Predictors of Mn status

In accordance with the primary source of Mn intake in the general population through food, adjusting for potential covariates and confounders, our study showed higher Mn levels in the blood of women consuming bottled water (27%) or water from the public supply (17%) than in those using water from private supplies, and somewhat higher in participants consuming game meat (Table 4). An inverse association was observed with poultry consumption in women, likewise for the intake of dietary supplements containing minerals (Table 4). The observed inverse associations may result from co-linearity with some other dietary or lifestyle-related variables that were not covered by our study protocol or in case of dietary supplements from decreased Mn absorbance in the presence of other elements (e.g. iron - Fe) (Kim, 2018).

Along with the dietary determinants, the statistical models (Table 4) indicated slightly higher levels in rural areas, as well as in women in urban areas, compared with contaminated areas. Although the levels in blood did not reflect the levels in soil (Table S43), similar results were observed in pregnant women from Quebec – those who lived in urban and/or agricultural areas had significantly higher Mn levels in their blood compared with the residents of small villages; and higher levels were associated with pesticide spraying less than 1 km from their houses (Takser et al., 2004). The association between Mn exposure and agricultural activities (i.e., the use of Mn-containing fungicides) is further supported by the Mn levels in the blood or hair of pregnant women from the Californian agricultural community (Gunier et al., 2014) and the Costa Rican community of pregnant women (Mora et al., 2014).

Due to its essentiality for maternal health and foetal development, particularly during late pregnancy and neurotoxic potential at excessive levels (Horning et al., 2015), Mn uptake and efflux is highly regulated. Dietary deficiency of Fe can lead to excess absorption of Mn, and it has been shown that interaction between Fe with Mn is the major factor determining sex-specific differences in blood Mn levels (Lee and Kim, 2014). Our study did not monitor Fe levels in blood, however this should be done in future to assure comparability among different study groups and proper interpretation of dose-response relationships.

3.8. Selenium

3.8.1. Basic levels

The majority of our study population had Se levels in their blood within the reference range relevant for European population (Wilhelm et al., 2004): 24% of men and 9% of women exceeded their respective upper reference levels of 130 and 120 μg/L, and only a few participants had levels below the lower reference values of 79 and 60 μg/L, respectively (Table S6). Compared with previous observations in smaller groups of healthy Slovene adults, our study showed somewhat higher Se levels in blood (GM = 105 μg/L). A group of Slovene residents (n = 43) had a mean level of 87 ng/g (SD = 13 ng/g) (Mazej et al., 2003) and 30–62-year-old men living in central and southwestern Slovenia had a mean level of 89.3 μg/L of blood (range = 62–138 μg/L) (Kobal et al., 2004). These findings suggest an increasing time trend for the Slovenian population, which was reported for the Czech adult population monitored between 1996 and 2011 (n = 2414), with a median level of 73.2 μg/L of whole blood in 1996 and 91.5 μg/L in 2001 (Batáriová et al., 2005). The observed Se level in a female study group (95.0 μg/L, Table 3) was lower than those recently determined in the blood of adult non-pregnant women (aged 30–51 years) from central Slovenia (GM = 105 μg/L, range = 68.7–142 μg/L, n = 170) (not published), which is believed to be primarily due to the lactation status of participating women. Selenium levels in serum or plasma are known to decrease with increasing gestational period, due to increased Se requirement to maintain anti-oxidative activity (Izquierdo Álvarez et al., 2007; Kobal et al., 2017). In line with the known higher Se status in the US population (Combs et al., 2011), the most recent Canadian (2009–2011) and US surveys (2011–2016) reported considerably higher levels in the whole blood of their healthy adult population compared with the levels in our study population, GMs of both populations being around 190 μg/L, and in urine of the Canadian population 44 μg/g crt (CDC, 2018b; Health Canada, 2013).

Based on the observed Se levels in breast milk (Tables 3 and S34), with 19% of women below the lower reference value of 10 μg/L, and eight women above the upper value of 25 μg/L (Iyengar, 1998), Slovenia is ranked among the countries with relatively low Se levels (GM = 12.6 μg/L). Similar levels were observed in a group of mothers from a neighbouring region (Northeast Italy, n = 62, 12 ± 3 μg/L) (Mazej et al., 2004). In the past, mature milk of Slovene mothers (Kosta et al., 1983) showed even a lower range (8 ± 2 μg/L, 7–12 μg/L, n = 6).

3.8.2. Predictors of Se status

Based on the statistical models, sex/physiological status was the most distinctive predictor of Se blood levels, men having about 20% higher levels than lactating women. Among the dietary predictors, seafood consumption contributed, on average, up to 10% higher Se levels in blood, while smaller contributions from nuts and intake of dietary supplements were revealed, with the latter only found in men

(Table 4). Fish, a protein-rich food, was previously reported as containing among the highest Se levels on the Slovenian market (Smrkolj et al., 2005). In addition to sex and dietary items, rural areas showed lower Se levels in blood than urban or contaminated areas (Table 4). Unfortunately, Se concentration in soil was not available systematically across the country's regions; therefore, we were unable to check for the correlation between Se levels in blood and soil, which could be present if Se supplementation was used in agriculture (Carvalho et al., 2003).

3.8.3. Geographic variability within the country

Among the specific study areas, LJ was among those with the highest Se levels in blood (GM = 116 μg/L) and in milk (GM = 15.2 μg/L) (Tables S6 and S34). Similar Se levels in breast milk were observed in a recent birth cohort study, which collected breast milk samples 6–8 weeks after delivery (the same as in the present study) and recruited women from LJ and its surroundings (median = 17 ng/g, range = 1.7–69 ng/g, n = 287) (Miklavčič et al., 2013a).

3.9. Copper

3.9.1. Basic levels

Inadequate Cu levels in blood of our study population were more frequent than excessive levels; 31% of men and women had levels below their respective lower reference levels of 800 and 1000 μg/L (Iyengar, 1998). The respective percentages of men and women exceeding the upper reference levels of 1100 and 1400 μg/L were 3% and 4% (Table S7). In contrast to blood, excessive Cu levels in breast milk were more likely than inadequate; 55% of the women had Cu levels above the upper reference value of 400 μg/L and only a few (n = 11) below the lower value of 200 μg/L (Iyengar, 1998) (Table S35). The previously reported levels in the mature milk of women living in LJ were comparable to the mean level in our study (range = 20–500 μg/L, mean = 300 μg/L, n = 7) (Kosta et al., 1983). The sex difference observed in our study population (Table 3) was consistent with the Canadian survey (2009–2011) showing higher blood levels in women (990 μg/L) than men (810 μg/L), similarly also in urine (10 μg/g crt in women; 7.9 μg/g crt in men) (Health Canada, 2013), and also with the US survey with higher serum Cu in women than in men (CDC, 2018b). The Cu levels in serum were demonstrated to increase during the course of pregnancy due to increased physiological need (Izquierdo Álvarez et al., 2007), which might explain the somewhat higher level in the whole blood of Slovenian lactating women compared with Canadian adult non-lactating women (Health Canada, 2013). When comparing specific study areas, no distinct geographical pattern was identified in any of the matrices studied (Table S7, S24–26, S35).

3.9.2. Predictors of Cu status

The most distinctive predictor of Cu in our study population was sex, followed by the type of study area, age and alcohol consumption (Table 4). The levels in rural and contaminated areas prevailed over urban areas. Rural areas may be significant sources of Cu intake due to the potential Cu-containing soil treatments applied in order to prevent Cu deficiency in soils and in livestock or to stimulate the growth of pigs and broiler chickens or due to the use of Cu sulphate as fungicide in agriculture (www.copper.org). A significant influence from the contaminated sites due to industrial activities was somewhat lower than the effect observed in rural areas.

An inverse association between Cu levels in blood and alcohol consumption probably indicates altered Cu metabolism due to alcohol intake (Keen et al., 1998). However, the observed association may also result from co-linearity with some other dietary or lifestyle-related variables that were not covered by our study protocol (Table 4).

3.10. Zinc

3.10.1. Basic levels

According to the reference levels established for blood and breast milk (Iyengar, 1998), the Zn levels in the blood of 37% of the women and 29% of the men were above the upper reference value of 7000 μg/L, while 19% of the women and 26% of the men had levels below the lower value of 6000 μg/L (Table S8). Among women, 71% had Zn levels in their breast milk above 2000 μg/L and 10% had levels below 1000 μg/L (Table S36). The previously reported levels in the mature milk of women living in LJ were comparable to those found in our study (range = 300–3100 μg/L, mean = 1400 μg/L, n = 7) (Kosta et al., 1983). The Canadian survey (2009–2011) showed similar levels in adult men (GM = 6400 μg/L of whole blood and 270 μg/g crt in urine), but the levels in women were much lower than our study's findings (5800 μg/L and 230 μg/g crt, respectively) (Health Canada, 2013), which could be due to the lactating status of our female study group. Supporting this assumption, a case study on 45 pregnant women showed a decreasing trend in Zn levels in plasma during pregnancy, but the levels in whole blood and red cells showed a significant rise (Qvist et al., 1986).

3.10.2. Predictors of Zn status

The statistical model for the total population confirmed 11% higher levels in women than in men, with additional predictors being seafood consumption (positive association), dietary supplement intake (inverse association), and fruit intake (inverse association) (Table 4). Seafood is among the dietary sources of Zn, which was confirmed in the female study group, contributing to an average 5% higher level of Zn in the blood of those consuming seafood at least once per week compared with those who did so less than once per month. Although animal proteins are known major sources of dietary Zn (Lönnerdal, 2000), meat and eggs were not found to be significant sources of Zn in our study population; neither was freshwater fish (not presented). Vegetables and fruits are poor sources of Zn and contain high levels of phytate, which might inhibit Zn absorption from the intestines (Kelsay et al., 1979). This could explain a 4% lower level of Zn in the blood of men consuming fruit daily than those doing so weekly or less (Table 4). Although we would expect increased Zn levels in blood due to dietary supplement intake, the inverse association indicates the possibility of decreased Zn absorption in the presence of other minerals in the supplements (Guyette et al., 1983).

Besides the dietary predictors, higher Zn levels in blood were observed in contaminated and urban areas than in rural areas (Table 4). These results show that Zn status is obviously not compromised due to the elevated presence of metals in industrially active sites. Moreover, none of the elements (Cu, Cd or Hg) that could inhibit Zn absorption was found to have an inverse relationship with Zn (not presented). Adjusted SG-normalised levels showed significantly lower urinary Zn levels of the participants in contaminated areas than those in rural or urban areas (model not presented). This, together with elevated Zn levels in blood and moderate levels of toxic metals, could indicate increased uptake of Zn in a cellular anti-oxidative defence (as part of Cu-Zn superoxide dismutase and other antioxidative enzymes) in contaminated areas.

3.10.3. Geographic variability within the country

Despite relatively uniform levels among the study participants (Tables S8, S27-29, S36), the tendency towards higher levels in the coastal part (KP) of the country supported seafood as an important source of dietary Zn and did not reflect Zn concentrations in soil, which were two to five times higher in KR and Upper RA than in other study areas (Table S46).

Table 5
Proposed reference values (RV_{95}) for selected elements in the Slovenian population of men and lactating *primiparous* women of childbearing age (18–49 years).

Biomarker	Population group	N	P95 [95% CI]	RV_{95}
Cd in blood	Lactating women, non-smoking	452	0.86 [0.78–0.95]	0.9 µg/L
	Non-smoking men	492	0.68 [0.57–0.80]	0.7 µg/L
Cd in urine	Lactating women, non-smoking	410	0.75 [0.65–0.87]	0.8 µg/g crt
		416	0.67 [0.57–0.79]	0.7 µg/L
		399	0.56 [0.52–0.61]	0.6 µg/L SG
	Men, non-smoking	453	0.41 [0.36–0.47]	0.4 µg/g crt
		459	0.61 [0.54–0.70]	0.6 µg/L
		439	0.56 [0.50–0.63]	0.6 µg/L SG
Cd in milk	Lactating women, non-smoking	405	0.19 [0.16–0.22]	0.2 µg/L
Pb in blood	Lactating women, Pb-smelter area excluded	506	33.1 [30.5–36.0]	35 µg/L
	Men, Pb-smelter area excluded	499	42.4 [37.9–47.4]	45 µg/L
Pb in urine	Lactating women, Pb-smelter area excluded	380	1.52 [1.30–1.80]	1.5 µg/g crt
		380	1.12 [0.97–1.31]	1.1 µg/L
		377	1.10 [0.97–1.24]	1.1 µg/L SG
	Men, Pb-smelter area excluded	353	1.30 [1.15–1.47]	1.3 µg/g crt
		353	2.13 [1.86–2.44]	2.1 µg/L
		349	1.77 [1.58–1.97]	1.8 µg/L SG
Pb in milk	Lactating women, Pb-smelter area excluded	324	0.98 [0.77–1.25]	1.0 µg/L
Hg in blood	Lactating women consuming fish ≤ 3 times/month	431	3.65 [3.17–4.20]	4.0 µg/L
	Men consuming fish ≤ 3 times/month	479	4.78 [4.10–5.58]	5.0 µg/L
Hg in urine	Lactating women without amalgam dental fillings	84	2.54 [1.51–4.26]	3.0 µg/g crt
		87	3.73 [1.35–10.3]	4.0 µg/L
		80	2.06 [1.19–3.57]	2.0 µg/L SG
	Men without amalgam dental fillings	73	1.22 [0.81–1.84]	1.2 µg/g crt
		73	2.42 [1.24–4.73]	2.4 µg/L
		65	2.09 [1.15–3.81]	2.1 µg/L SG
Hg in hair	Lactating women consuming fish ≤ 3 times/month	404	841 [742–953]	900 ng/g
	Men consuming fish ≤ 3 times/month	388	1253 [1041–1510]	1200 ng/g
Hg in milk	Lactating women without amalgam dental fillings	84	0.53 [0.38–0.75]	0.5 µg/L
As in blood	Lactating women consuming fish ≤ 3 times/month	432	4.26 [3.25–5.56]	4.0 µg/L
	Men consuming fish ≤ 3 times/month	479	3.09 [2.41–3.95]	3.0 µg/L
As in urine	Lactating women consuming fish ≤ 3 times/month	324	41.6 [31.1–55.7]	40 µg/g crt
		324	33.2 [24.7–44.6]	30 µg/L
		321	33.3 [24.6–45.1]	30 µg/L SG
	Men consuming fish ≤ 3 times/month	353	47.5 [29.6–76.5]	50 µg/g crt
		353	65.7 [45.5–95.0]	70 µg/L
		348	84.2 [43.4–163]	80 µg/L SG
As in milk	Lactating women consuming fish ≤ 3 times/month	380	0.74 [0.58–0.94]	0.7 µg/L

3.11. Sex-related differences?

Among the potentially toxic elements, blood samples showed higher Cd levels in women than in men ($p < 0.001$) (Table 2), the difference that is typically observed in adult healthy populations (CDC, 2013; Černá et al., 2012; Health Canada, 2013), mainly due to low ferritin concentrations in women. Cadmium absorption is additionally enhanced during late pregnancy on account of upregulated Fe absorption (Lee and Kim, 2014). On the other hand, men had higher Pb and Hg levels in their blood than women ($p < 0.001$) (Table 2), consistent with the findings reported in the literature (Becker et al., 2002; Bjermo et al., 2013; CDC, 2018b; Černá et al., 2012; Health Canada, 2013) and with higher haematocrit levels in men and in case of Pb decreasing effect of oestrogen on blood Pb levels in women (Lee and Kim, 2014; Vahter et al., 2007). However, after adjustments for possible predictors and confounders (including haematocrit level), the Pb levels in blood showed 10% higher levels in women than in men (Tabe 4), speculatively due to increased release of Pb from bone compartment particularly during *postpartum* period (Manton et al., 2003). In case of the Hg, the difference was lost, which could be explained by the haematocrit level included as a confounder into the model (Table 4).

Sex-related difference in the levels of toxic metals in urine, expressed per volume or creatinine, was consistent with other HBM studies (higher in women than men) (Castaño et al., 2012; CDC, 2013; Puklová et al., 2010), but not in cases expressed per SG. Urinary Cd did not differ between men and women (Table 2), while Pb, Hg and As showed higher urinary levels in men than women (Table 2). The differences in SG-normalised levels were confirmed in the models (Table 4). It should be noted that crt-normalised levels present the risk of underestimating the exposure due to creatinine over-compensation, particularly for Cd and As (Balakrishnan et al., 2018; Drobná et al., 2016; Hoet et al., 2016; Skröder et al., 2018), therefore SG normalisation is suggested as a more reliable alternative.

Speciation analysis of the samples > 15 µg/L of urine (n = 138) showed no sex difference in the sum of As species (AsV, AsIII, MMA and DMA) ($p = 0.548$). Although on the margin of statistical significance, the percentage of AsIII was lower in women than in men ($p = 0.061$), while the percentage of DMA was higher in women ($p = 0.165$). The difference was observed also in a study including central European population (median total As = 8.0 µg/L of urine, n = 415) (Lindberg et al., 2007), supported by more efficient methylation in females due to the sex hormone influence on women of childbearing age. The oestrogen influence is even more pronounced in the pregnancy or lactation phase as was demonstrated in the recent work by Stajnko et al. (2019). The US survey (NHANES, 2003–2004, n = 2568) with GM for total As of 8.24 µg/g crt also showed slightly higher levels of DMA in women compared to men, but the reverse was observed when non-normalised levels were used (Caldwell et al., 2009).

Among the essential elements, women in our study population had significantly higher Mn, Cu and Zn levels in their blood than the men, which was confirmed by the models (Tables 3 and 4). This is consistent with the abovementioned normal increase in blood levels during the course of pregnancy (Gunier et al., 2014; Izquierdo Álvarez et al., 2007; Qvist et al., 1986). The sex-difference in Cu blood levels is typical, regardless of the pregnancy, because the levels of ceruloplasmin, a Cu-containing plasma protein, are elevated by oestrogen (Fitzgerald et al.,

Table 6
Proposed reference intervals ($RI_{2.5–97.5}$) for selected essential elements in the Slovenian population of men and lactating *primiparous* women of childbearing age (18–49 years).

Biomarker	Population group	N	P2.5 [95% CI]	P97.5 [95% CI]	$RI_{2.5-97.5}$
Mn in blood	Lactating women	409	9.39 [8.63–10.2]	30.3 [28.3–32.5]	9.0–30 µg/L
	Men	402	7.13 [6.78–7.49]	17.6 [16.6–18.7]	7.0–18 µg/L
Se in blood	Lactating women	536	66.6 [64.1–69.1]	138 [131–144]	70–140 µg/L
	Men	548	81.5 [79.0–84.1]	179 [165–195]	80–180 µg/L
Se in urine	Lactating women	410	7.03 [6.58–7.52]	26.2 [24.7–27.8]	7.0–26 µg/g crt
		410	1.83 [1.41–2.38]	37.3 [32.4–42.9]	2.0–40 µg/L
		407	5.03 [4.46–5.68]	22.4 [20.9–24.1]	5.0–22 µg/L SG
	Men	402	7.69 [7.22–8.20]	28.1 [26.1–30.1]	8.0–28 µg/g crt
		402	4.87 [3.93–6.04]	68.4 [59.1–79.2]	5.0–70 µg/L
		397	8.72 [7.58–10.1]	44.3 [40.6–48.4]	9.0–45 µg/L SG
Se in milk	Lactating women	470	7.58 [7.16–8.02]	23.3 [20.6–26.5]	8.0–23 µg/L
Cu in blood	Lactating women	536	809 [784–835]	1432 [1380–1496]	800–1400 µg/L
	Men	548	668 [651–687]	1108 [1057–1161]	700–1100 µg/L
Cu in urine	Lactating women	410	1.41 [1.12–1.79]	36.7 [26.9–50.0]	1.5–40 µg/g crt
		410	1.50 [1.39–1.61]	30.0 [23.8–37.9]	1.5–30 µg/L
		407	1.16 [0.93–1.45]	30.2 [22.1–41.3]	1.0–30 µg/L SG
	Men	402	0.71 [0.30–1.67]	19.5 [15.9–23.8]	1.0–20 µg/g crt
		402	1.50 [1.32–1.70]	28.3 [24.5–32.7]	1.5–30 µg/L
		397	1.14 [0.74–1.77]	22.7 [20.4–25.3]	1.0–23 µg/L SG
Cu in milk	Lactating women	470	196 [183–211]	629 [591–668]	200–600 µg/L
Zn in blood	Lactating women	536	4869 [4628–5123]	9025 [8717–9344]	5000–9000 µg/L
	Men	548	4875 [4694–5062]	8383 [8181–8589]	5000–8400 µg/L
Zn in urine	Lactating women	410	98.9 [75.7–129]	1179 [1029–1350]	100–1200 µg/g crt
		410	52.0 [41.5–65.2]	1034 [898–1190]	50–1100 µg/L
		407	85.1 [67.8–107]	909 [807–1024]	85–900 µg/L SG
	Men	402	38.8 [13.0–116]	618 [564–677]	40–600 µg/g crt
		402	50.3 [35.0–72.3]	1335 [1116–1596]	50–1300 µg/L
		397	54.3 [25.1–118]	983 [883–1096]	50–1000 µg/L SG
Zn in milk	Lactating women	470	579 [461–728]	4799 [4438–5189]	600–5000 µg/L

2008). In contrast, physiological status of women resulted in their lower Se levels in blood (Tables 3 and 4), which normally does not differ according to the sex (Batáriová et al., 2005; Health Canada, 2013).

3.12. Reference values

To derive the RV_{95}, the population groups were selected by considering the following exclusion criteria: smoking (Cd), living in a Pb-smelter area (Pb), consuming fish more than thrice a month (Hg and As in blood and hair) and presence of dental amalgam fillings (Hg in urine). The selected population groups for each biomarker are defined in Table 5. For essential elements (Table 6), no exclusion criteria were considered. For all elements, the partition criteria used to characterise subsets of the reference population was sex.

The proposed levels for toxic elements are mainly comparable to the reference values reported for various populations internationally, as recently reviewed by Saravanabhavan et al. (2017). Among the elements, Hg in blood showed markedly higher reference values in Slovenian than in German, Czech and Canadian populations, and urinary Hg levels were higher in Slovenian than in German, Canadian and Belgian populations. However, it should be noted, that the percentage of our study population without amalgam fillings was very small and was therefore not representative for the Slovenian general population. Similar results were observed for urinary As, but it should be noted that our study lacked information on seafood consumption prior to the sampling; therefore, recent seafood consumption could not be excluded.

The reference intervals proposed for essential elements should be taken as informative because concentrations in whole blood and urine are not used as the most appropriate indicators of essential element status. When comparing the blood and urine RIs with the Canadian reference values (Saravanabhavan et al., 2017), similarities were observed for Zn and Cu, while Se levels were expectedly considerably lower in the Slovenian than the Canadian population. The reference levels for the selected elements are compared internationally in Table 7.

4. Conclusions

The presented study assessed, for the first time, exposure to selected elements in a Slovenian population of childbearing age (18–49 years), selected from lactating *primiparous* women and men. The complete dataset of potentially toxic and essential elements, accompanied by environmental and life-style data, and even distribution of male and female participants across diverse geographical areas of Slovenia enabled us to estimate the exposure levels, compare them geographically and identify the main predictors (lifestyle, particularly diet, and environmental) of exposure. Additionally, the first reference values for the selected Slovenian population have been established, providing important data for the sensitive period of pregnancy/lactation, which is critically lacking in the literature.

Limitations emerging from our study include a lack of parameters which interact crucially with trace elements, particularly in childbearing age, as discussed above (e.g. Fe status), small sample size per study area, and differences in spatial resolutions and the coverage of various datasets for HBM and environmental data, respectively. Despite that, the findings of the study provide the basis to establish recommendations regarding food intake for pregnant or lactating women (e.g. seafood and vegetable intake) and could help focus future biomonitoring efforts for specific metals, especially Pb and Hg, in the selected geographic areas.

Declaration of competing interests

The authors report no competing interests.

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Table 7
International comparison of reference values (RV_{95}) for the selected trace elements in general adult population.

Element/Survey	Study period	Population group (age in years)	N	Exclusion criteria	RV_{95}	Reference
Cd in blood						
Czech Republic	2005–2009	18–58	896	smoking	1.0 µg/L	Černá et al. (2012)
Germany, GerES III	1997–1999	18–69	3061	smoking	1.0 µg/L	Schulz et al. (2011)
Italy, PROBE	2008–2010	18–65	831	smoking	1.08 µg/L	Alimonti et al. (2011)
Canada	2012–2013	20–79	2507		0.83 µg/L	Saravanabhavan et al. (2017)
Cd in urine						
Czech Republic	2005–2009	18–58	896	smoking	1.3 µg/L	Černá et al. (2012)
Germany, GerES III	1997–1999	18–69	3128	smoking	0.8 µg/L	Schulz et al. (2011)
Belgium	2010–2011	> 18	620	smoking	0.7 µg/L[a]	Hoet et al. (2013)
Spain, BIOAMBIENT.ES	2009	18–65	691	smoking	0.74 µg/L	
Canada	2009–2011	20–79	1196		1.3 µg/L	Saravanabhavan et al. (2017)
Pb in blood						
Czech Republic	2001–2003	18–58	1188		75 µg/L	Černá et al. (2012)
Germany, GerES III	1997–1999	18–69	2303		70 µg/L (F)	Schulz et al. (2011)
			2342		90 µg/L (M)	
Italy, PROBE	2008–2010	18–65	1423		51.7 µg/L	Alimonti et al. (2011)
Spain, BIOAMBIENT.ES	2009	18–65	1880		56.8 µg/L	
Canada	2012–2013	20–79	3142		33 µg/L	Saravanabhavan et al. (2017)
Pb in urine						
Belgium	2010–2011	> 18	1001		2.81 µg/L	Hoet et al. (2013)
Canada	2009–2011	20–79	3210		1.9 µg/L	Saravanabhavan et al. (2017)
Total Hg in blood						
Czech Republic	2005–2009	18–58	1221	Seafood[b]	2.6 µg/L	Černá et al. (2012)
Germany, GerES III	1997–1999	18–69	2310	Seafood[c]	2.0 µg/L	Schulz et al. (2011)
Italy, PROBE	2008–2010	18–65	1423		5.16 µg/L	
Canada	2012–2013	20–79	1229		2.3 µg/L **	Saravanabhavan et al. (2017)
Total Hg in urine						
Czech Republic	2005–2009	18–58	1227		3 µg/L	Černá et al. (2012)
Germany, GerES III	1997–1999	18–69	1560	amalgams	1.0 µg/L	Schulz et al. (2011)
Canada	2012–2013	20–79	241		NA (F)	Saravanabhavan et al. (2017)
			217		0.73 µg/L (M)	
Total As in blood						
Italy, PROBE	2008–2010	18–65	1423		5.32 µg/L	
Canada	2007–2009	20–79	996		2.0 µg/L	Saravanabhavan et al. (2017)
Total As in urine						
Germany, GerES III	1997–1999	18–69	3924	Seafood[d]	15 µg/L	Schulz et al. (2011)
Belgium	2010–2011	> 18	1001	Seafood[e]	48.8 µg/L	Hoet et al. (2013)
Canada	2009–2011	3–79	2480		27 µg/L	Saravanabhavan et al. (2017)
Mn in blood						
Italy, PROBE	2008–2010	18–65	470		14.8 µg/L (F)	
			953		14.1 µg/L (M)	
Canada	2009–2011	20–79	1937		16 µg/L (F)	Saravanabhavan et al. (2017)
			1676		14 µg/L (M)	
Cu in blood, Canada	2009–2011	3–79	3124		1300 µg/L (F)	Saravanabhavan et al. (2017)
			2940		1000 µg/L (M)	
Se in blood, Canada	2009–2011	20–79	3598		240 µg/L	Saravanabhavan et al. (2017)
Zn in blood, Canada	2009–2011	20–79	947		6700 µg/L (F)	Saravanabhavan et al. (2017)
			821		7900 µg/L (M)	

[a] P95; **provisional due to high coefficient of variation.
[b] Average fish consumption ≥ 1 time per week.
[c] Average fish consumption > 3 times per month.
[d] Fish consumption 48 h prior to sample collection.
[e] Fish consumption within 96 h of sample collection.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2019.02.008.

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