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HEAVY METALS AND OTHER TRACE ELEMENTS IN THE BLOOD AND BREAST MILK FROM TWO DIFFERENT ROMANIAN AREAS

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Abstract

In order to highlight the impact of environmental pollution for Romanian nursing mothers we evaluated the concentration of heavy metals and trace elements in their blood and breast milk. The evaluation was done taking into account the pollution level of mother's residence: Bucharest (an industrial and heavy transited town, identified as the highly polluted area) and a small town (without industrial plants, identified as less polluted area). The determination of heavy metals and trace elements was performed by induced coupled plasma mass spectrometry (ICP-MS). Comparing the mean concentrations for the more toxic elements, in Bucharest and in the small town in colostrum, respectively, we found: Cd (0.6 μg/L vs 0.3 μg/L), Pb (<LoD vs 0.2 μg/), As (5.5 μg/L vs 3.8 μg/L), Cr (5.4μg/L 5.5μg/L). Our results, in good agreement with those reported in literature, evidence the influence of environmental pollution on breast milk and blood trace element levels, suggesting the need of a better environment management for a better public health.

*Key words:*blood, breast milk, heavy metals, ICP-MS, pollution

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1. Introduction

About 60 chemical elements are found in the human body, but what all of them are doing there is still unknown. Roughly 96% of the mass of the human body is made up of just four elements: oxygen, carbon, hydrogen and nitrogen, with a lot of that in the form of water. The remaining 4 percent is a sparse sampling of the periodic table of elements. Some of the more prominent representatives are called macrominerals, whereas those appearing only at the level of parts per million or less are referred to as trace elements (Forrer et al., 2001). Some of these elements are necessary for the human body to function and others have no function or are very toxic, even at very low levels.

The essential elements perform various functions, including the building of bones and cell structures, regulating the body's pH, carrying electric charge, and driving chemical reactions. Although required in very small amounts, trace elements such as iron, iodine, fluoride, copper, zinc, chromium, selenium, manganese and molybdenum are vital for maintaining health. These essential trace elements are part of enzymes, hormones and cells in the body. In addition to these, several nonessential elements, including the toxic "heavy metals", can be found in the human fluids and tissues (Deurenberg, 2013; Fraga, 2005; Parson and Barbosa, 2007; Preda et al., 2016).

To determine how the exposure to different pollution levels influences the content of metals in the

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human body, we analyzed the concentration of 11 elements, heavy metals and essential elements, in samples of breast milk and blood collected from two different geographical areas in Romania.

An important toxicity stress due to the exposure to heavy metals have been observed on plant growth (Pavel et al., 2013, Forer et al., 2001). The presence of heavy metals in blood and tissues has a great health impact especially for vulnerable groups as children (Burada et al., 2017; Howard, 2002; Demetrescu and Ionita, 2010; Demetrescu et al., 2009a) and lactating women. The maternal milk can transfer toxic elements from the mother to the baby (Almeida, 2008). In this context, in the last 20 years, many discussions regarding the relations between analytical data about milk composition, human exposure to pollution and social and environmental parameters took place (Chierci et al., 1999; Leotsinidis et al., 2005; Lonnerdal, 2000; Tholin et al., 1995).

The negative influence of heavy metals on health is determined by the dose which mainly depends on the pollution levels. For Romania, the Report of Environmental Agency (ECOPOLIS, 2011; Vatasescu et al., 2011) has established Bucharest as an area with a high pollution level. According to this analysis, Bucharest has frequently over polluted air (92 days in a year), presenting a high load of particulate matter (PM), over the PM10 daily mean value in EU, that may not exceed 50 µg/m3 more than 35 times in a year. In the present study were selected two areas in Romania with different pollution levels and one group of women from each area was investigated.

Using inductively coupled plasma mass spectrometry (ICP-MS), the concentrations of heavy metals and other elements in breast milk and blood samples were determined. The novelty of the research is focused on the comparison of heavy metals content in breast milk and blood from lactating women living in an area with high pollution levels (Bucharest) and “reference” lactating women, from a small town, an area with less pollution levels. The results obtained from both groups were statistically compared.

2. Experimental

2.1. Study groups and sample collection

Identification of study groups took place after selecting Romanian areas with different heavy metals contamination due to air pollution. The first selected region was Bucharest, an area with high air pollution level. The second region was a small town, without important industrial plants, considered as “reference” area. The study was approved by the Ethical Committee of the Hospital of Obstetrics and Gynecology Prof. Dr. PanaitSarbu and all women participating in the study gave their written consent. They have answered to a detailed questionnaire, established by the Ethical Committee of the Institution, with 5 types of questions: a) General questions; b) Questions regarding their home comfort and living conditions; c) Questions regarding their health state; d) Educational factors and socio-economic situation of the family; e) Vitamins consumption.

In more detail, these questions were addressing: a) two general questions including place and date of birth; b) 29 questions about living conditions including the level of cleaning, garden and animals around the house, traffic, persons in family working in conditions with high exposure to Mn, As, Pb, Co, Ni, Cu, Zn and Se; c) two questions about their health regarding anemia and calcium deficiency; d) four questions about educational level and socio-economic situation of family; e) two questions about vitamins and minerals intake during pregnancy.

The samples of blood and breast milk were collected from women with ages between 17 and 43 years, with similar health conditions. The average age of women from Bucharest was 30 years, ranging from 23 to 43 years. For women of the “reference” group the average age was 26 years, ranging from 17 to 34 years.

2.2. Sampling

Blood samples were collected into 2 mL containers with EDTA as anticoagulant. Serum samples were collected into vacutainer tubes without anticoagulant. The samples were centrifuged at 3000 rpm, using a Hettich-EBA 8S centrifuge, then the serum was extracted with a micropipette. Breast milk samples were collected into 50 mL sterile containers as required by the Hospital’s protocol. Before analysis all the samples were stored in a freezer.

2.3. Determination of heavy metals concentrations

For all ICP-MS determinations was used a Perkin Elmer Sciex ELAN DRC-e instrument operated in the working conditions described in Table 1 (Demetrescu et al., 2009b). A multi-element reference standard, XXI CertiPUR (Merck Millipore) (containing Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, In, K, Li, Mg, Mn, Ni, Pb, Rb, Se, Na, Ag, Sr, Tl, V, U and Zn at a concentration 10 µg/mL) was used to prepare calibration standards for the calibration curve. For Mn, As, Pb, Co, Ni, Cu, Zn, Se, Ca, Cr, Cd the detection limits and the quantification limits, measured on ICP-MS equipment, were 0.002, 0.026, 0.001, 0.001, 0.004, 0.006, 0.010, 0.070, 1.104, 0.013, 0.002 µg/L and 0.004, 0.052, 0.002, 0.008, 0.012, 0.020, 0.140, 2.208, 0.026, 0.004, respectively.

In blood it was measured Mn, Ca, Pb, Cr, Cd, As and Ni and in serum Co, Cu, Zn and Se.

The samples were prepared as follows:

1) 1 mL serum + 9 mL solution containing 2% HNO_3 and 0.01% (v/v) TritonX-100

2) 1 mL colostrum + 9 mL solution containing 2% HNO_3 and 0.01% (v/v) TritonX-100

3) 1 mL blood + 9 mL solution containing 1% (v/v) ammonium hydroxide, 0.01% (m/v) disodium EDTA and 0.1% (v/v) Triton X-100.

Table 1. ICP-MS operating conditions

ICP-MS instrument	Perkin Elmer SCIEX ELAN DRC-e
Plasma conditions	
Rf power	1500 W
Plasma gas flow	15 $L.min^{-1}$
Aerosol gas flow	1.2 $L. min^{-1}$
Mass spectrometer settings	
Resolution	Normal
Dwel time	500 ms
Sweeps/reading and Points/spectral peak	5 and 1 respectively
Readings/replicate	400

Table 2. Metals concentrations in COLOSTRUM samples

Bucharest area (12 samples)	**Mn (µg/L)**	**As (µg/L)**	**Pb (µg/L)**	**Co (µg/L)**	**Ni (µg/L)**	**Cu (µg/L)**	**Zn (µg/L)**	**Se (µg/L)**	**Ca (mg/L)**	**Cr (µg/L)**	**Cd (µg/L)**
Mean	19.2	5.5	<LoD	0.85	44	685	6960	118	215	5.39	0.6
St. Dev.	11.8	2	<LoD	1	24.4	265	2694	65	61	2.58	0.8
Median	17.4	5.3	<LoD	0.52	35.3	732	7850	101	204	6.12	0.3
Minimum	2.3	2.9	<LoD	<LoD	14.8	290	1145	47	107	0.53	<LoD
Maximum	45	8.9	<LoD	2.8	88	1141	10206	230	334	8.87	2.1
Small town area (7 samples)	**Mn (µg/L)**	**As (µg/L)**	**Pb (µg/L)**	**Co (µg/L)**	**Ni (µg/L)**	**Cu (µg/L)**	**Zn (µg/L)**	**Se (µg/L)**	**Ca (mg/L)**	**Cr (µg/L)**	**Cd (µg/L)**
Mean	14.2	3.8	0.2	0.24	35	537	8250	104	234	5.46	0.3
St. Dev.	7.1	0.8	0.53	0.49	21.2	141	2818	55	74	2.16	0.4
Median	11.9	3.9	<LoD	<LoD	32.7	472	8156	93	209	5.82	<LoD
Minimum	6.8	2.5	<LoD	<LoD	8	371	4908	53	136	0.56	<LoD
Maximum	29	4.9	1.42	1.34	74	738	13595	220	330	8.87	1

2.4. Statistical analysis

The study was conducted in one group divided by geographical area of residence. Due to the large individual variability of results, for a better description of each element, the follows indicators were calculated: mean, standard deviation, median, minimum and maximum (the values <LoD were considered 0 in the calculations). For assessment of data distribution Kolmogorov-Smirnov test was applied. For comparison of means, two sample paired Student's t-test was used; data relationship was tested using Pearson correlation coefficients (r). Significance was considered for $p<0.05$. Box-and-whiskers plots were used for suggestive presentation of comparative data set. Statistical analysis was performed with Epi Info (TM) 3.5.3 software package.

3. Results and discussion

Tables 2-5 summarize results of the ICP-MS analysis of breast milk (colostrums) and blood samples of women from both areas (highly polluted and less polluted-small town).

The results obtained from colostrum analysis are similar with those reported in other studies performed in different countries (Almeida et al., 2008; Hamzaoglu et al., 2014; Turan et al., 2001).

From Table 2 it can be observed that, in the colostrum samples from the less polluted area, the concentrations of elements except Pb, Zn, Cr and Ca are lower than the ones from Bucharest samples.

The concentrations of Zn and Cu showed wide variations, more accentuated for Zn. These individual variations can be related to age, nutrition, and socio-economic level. Different research studies (Fransson et al., 1984; Melnikov et al., 2007; Silvestre et al., 2001; Trugo et al., 1988) evaluated the levels of Cu and Zn in human colostrum and found similar values in different countries, but also quite variable.

Table 3 shows that for Bucharest (high polluted area), the upper limit of the reference range was exceeded for Mn, Co, Ni, Se and Cr, and low values, under the lower limit of the biological literature reference range, as established by Sariego-Muñizet al. (2001) were obtained for: Cu, Zn and Ca. Except for Ca, Zn, Cu, Co and Cr which have mean values of concentrations under the lower limit of reference range, or Se, whose mean concentration is slightly above the upper limit, the other elements have mean values well within the reference interval or lower than the literature reference values. From Table 3 for small-town (less polluted area) wecan observe mean values higher than the limit for Ni and Se and values under the lower limit of the biological reference interval for Co and Cu. There are some elements that present maximum concentrations above the limit, like: Zn (1831 µg/L) and Ca (134 mg/L). From Table 4 it can be observed that for Bucharest (high polluted area), none of the mean concentrations is exceeding the upper limit of the biological reference interval. But the maximum values for Mn, Ni, Se, Ca and Cr are above the upper limit and for Co and Cu are below the lower limit.

Table 3. Metals concentrations in blood/serum of mothers

Bucharest area (19 samples)	**Mn (μg/L)**	**As (μg/L)**	**Pb (μg/L)**	**Co (μg/L)**	**Ni (μg/L)**	**Cu (μg/L)**	**Zn (μg/L)**	**Se (μg/L)**	**Ca (mg/L)**	**Cr (μg/L)**	**Cd (μg/L)**
Mean	5	2	8	1.1	4.1	259	728	201	79	0.78	0.4
St. Dev.	2	1	3	2	1.6	158	242	83	23	0.27	0.1
Median	5	2	7	0.7	4.7	214	681	199	77	0.82	0.4
Minimum	1	1	3.6	0.3	0.7	42	460	36	32	0.24	0.1
Maximum	11	4	16	9.6	6	521	1303	329	118	1.29	0.6
Literature reference values	10	10	4-47	1.2-3.6	1.2-4.6	800-1300	800-1400	60-200	85-105	1.00-1.20	0.6
Small town area (14 samples)	**Mn (μg/L)**	**As (μg/L)**	**Pb (μg/L)**	**Co (μg/L)**	**Ni (μg/L)**	**Cu (μg/L)**	**Zn (μg/L)**	**Se (μg/L)**	**Ca (mg/L)**	**Cr (μg/L)**	**Cd (μg/L)**
Mean	5	3	7	0.7	7.6	356	929	268	97	1.04	0.4
St. Dev.	1	1	2	0.1	8.9	149	353	65	21	0.21	0.1
Median	5	3	7	0.7	5.4	373	806	259	99	1.05	0.5
Minimum	3	1	4	0.4	3	70	473	167	53	0.72	0.3
Maximum	8	5	13	0.9	38	567	1831	390	134	1.37	0.6

Table 4. Metals concentrations in blood and serum of umbilical cord and serum

Bucharest area (14 samples)	**Mn (μg/L)**	**As (μg/L)**	**Pb (μg/L)**	**Co (μg/L)**	**Ni (μg/L)**	**Cu (μg/L)**	**Zn (μg/L)**	**Se (μg/L)**	**Ca (mg/L)**	**Cr (μg/L)**	**Cd (μg/L)**
Mean	6	2	8	0.6	4.2	264	718	200	81	0.83	0.4
St. Dev.	3	1	4	0.2	1.7	178	212	84	28	0.34	0.2
Median	5	2	7	0.6	4.7	214	744	221	86	0.82	0.4
Minimum	1	1	<LoD	<LoD	1.7	31	333	69	37	0.32	<LoD
Maximum	15	3	18	0.9	6.9	523	1218	361	125	1.36	0.6
Literature reference values	10	10	4-47	1.2-3.6	1.2-4.6	800-1300	800-1400	60-200	85-105	1.00-1.20	0.6
Small town area (11 samples)	**Mn (μg/L)**	**As (μg/L)**	**Pb (μg/L)**	**Co (μg/L)**	**Ni (μg/L)**	**Cu (μg/L)**	**Zn (μg/L)**	**Se (μg/L)**	**Ca (mg/L)**	**Cr (μg/L)**	**Cd (μg/L)**
Mean	5	2	7	0.6	4.4	295	686	183	77	0.83	0.4
St. Dev.	2	1	3	0.2	1.9	206	268	120	30	0.40	0.2
Median	5	2	6	0.6	4.4	347	607	164	77	0.87	0.4
Minimum	3	1	4	0.3	2	31	333	17	37	0.31	0.1
Maximum	8	3	13	0.9	8.2	594	1167	381	114	1.39	0.6

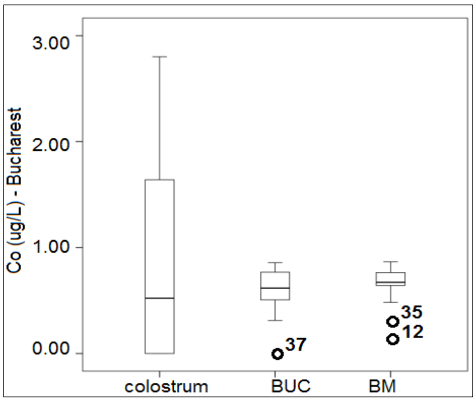
Table 5. Metals concentrations in mother's blood - with and without vitamins and minerals intake

Mothers with vitamins intake (16samples)	**Mn (μg/L)**	**As (μg/L)**	**Pb (μg/L)**	**Co (μg/L)**	**Ni (μg/L)**	**Cu (μg/L)**	**Zn (μg/L)**	**Se (μg/L)**	**Ca (mg/L)**	**Cr (μg/L)**	**Cd (μg/L)**
Mean	5	2	8	1.2	4.3	255	831	216	86	0.83	0.4
St. Dev.	2	1	3	2.2	1.5	145	338	64	23	0.23	0.1
Median	5	2	7	0.7	4.9	228	725	202	91	0.84	0.4
Minimum	1	1	4	0.4	0.7	63	508	68	53	0.34	0.2
Maximum	11	4	16	9.6	5.7	505	1831	325	127	1.29	0.5
Literature reference values	10	10	4-47	1.2-3.6	1.2-4.6	800-1300	800-1400	60-200	85-105	1.00-1.20	0.6
Mothers without vitamins intake (17 samples)	**Mn (μg/L)**	**As (μg/L)**	**Pb (μg/L)**	**Co (μg/L)**	**Ni (μg/L)**	**Cu (μg/L)**	**Zn (μg/L)**	**Se (μg/L)**	**Ca (mg/L)**	**Cr (μg/L)**	**Cd (μg/L)**
Mean	5	2	8	0.7	6.8	343	796	242	88	0.94	0.4
St. Dev.	2	1	3	0.1	8.2	165	282	97	25	0.31	0.1
Medium	5	3	7	0.7	4.8	397	702	249	89	0.96	0.4
Minimum	2	1	4	0.3	1.9	42	460	36	32	0.24	0.1
Maximum	8	5	13	0.9	38.3	567	1362	390	134	1.37	0.6

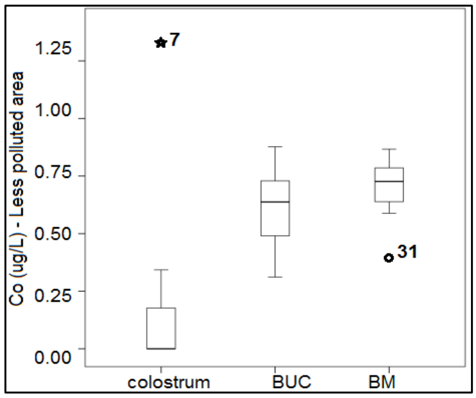
Also the mean concentrations of Co, Cu, Zn, Ca and Cr are under the lower limit of the reference interval. The metals concentrations of blood from umbilical cord of children born in less polluted area, as it can be seen from Table 4, are similar to those from Bucharest.

Several reports (e.g. Bloxam, 1989) indicate that maternal Fe supplementation or therapy may have an adverse effect on Zn status. In cases where Zn intake is already low or inadequate, supplementation of Fe might possibly reduce its availability to the fetus to an extent that it could compromise fetal growth. A large number of congenital malformations in fetus could be related to low maternal levels of Zn as has been shown in various experimental studies (O'Brian et al., 2000).

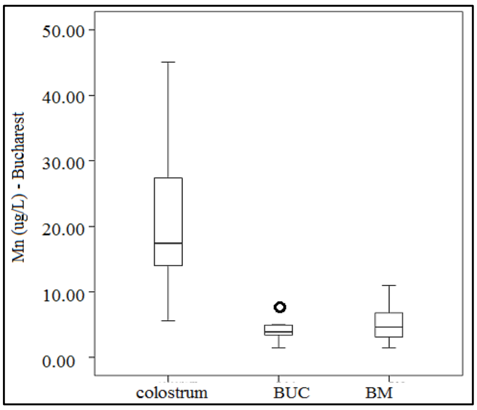
As can be seen from the Table 4, trace elements as Zn, Mn and Cr have values in the same domain with the one reported in literature (Ali et al., 2012; Örun et al., 2012). For mother's blood – with vitamins and minerals intake living in Bucharest (highly polluted area), (Table 5) we obtained maximum concentrations values that exceeded the limit for the following elements: Mn, Ni, Zn, Se, Ca, Cr and Co; and minimum values situated under the lower limit of the reference interval for the following elements: Cu, Zn, Ca and Co. For mother's blood – without vitamins and minerals intake, living in small-town (low polluted area), (Table 5) we found maximum concentrations that exceeded the limitfor the following elements: Ni, Se, Ca and Cr and minimum values under the lower limit of the reference interval for the following elements: Cu, Zn, Ca and Co. The mean concentrations of Mn, As, Co, Cu, Zn, Cr and Cd were below the limit and only the mean concentrations of Ni and Se exceed the limit. In Fig. 1a-j, the box-and-whiskers plots put in evidence the observed values for, Co, Mn, Se, Zn and Pb, for women from both studied areas and for all kind of samples analyzed.



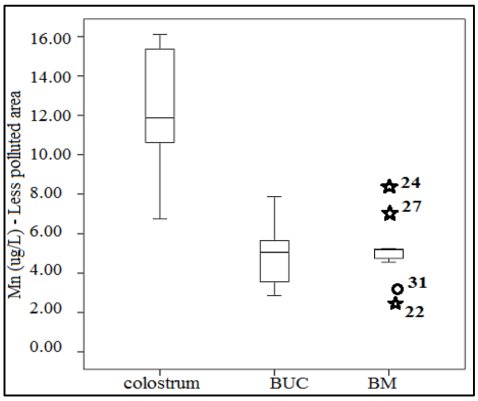
(a)



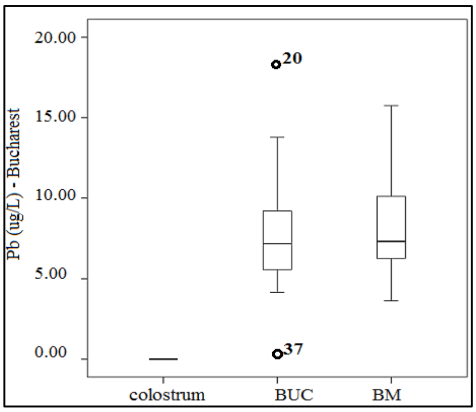
(b)



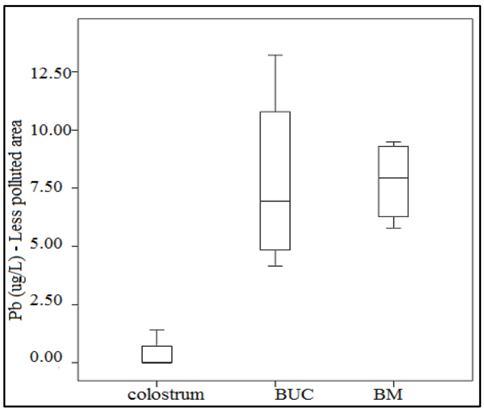
(c)



(d)



(e)



(f)

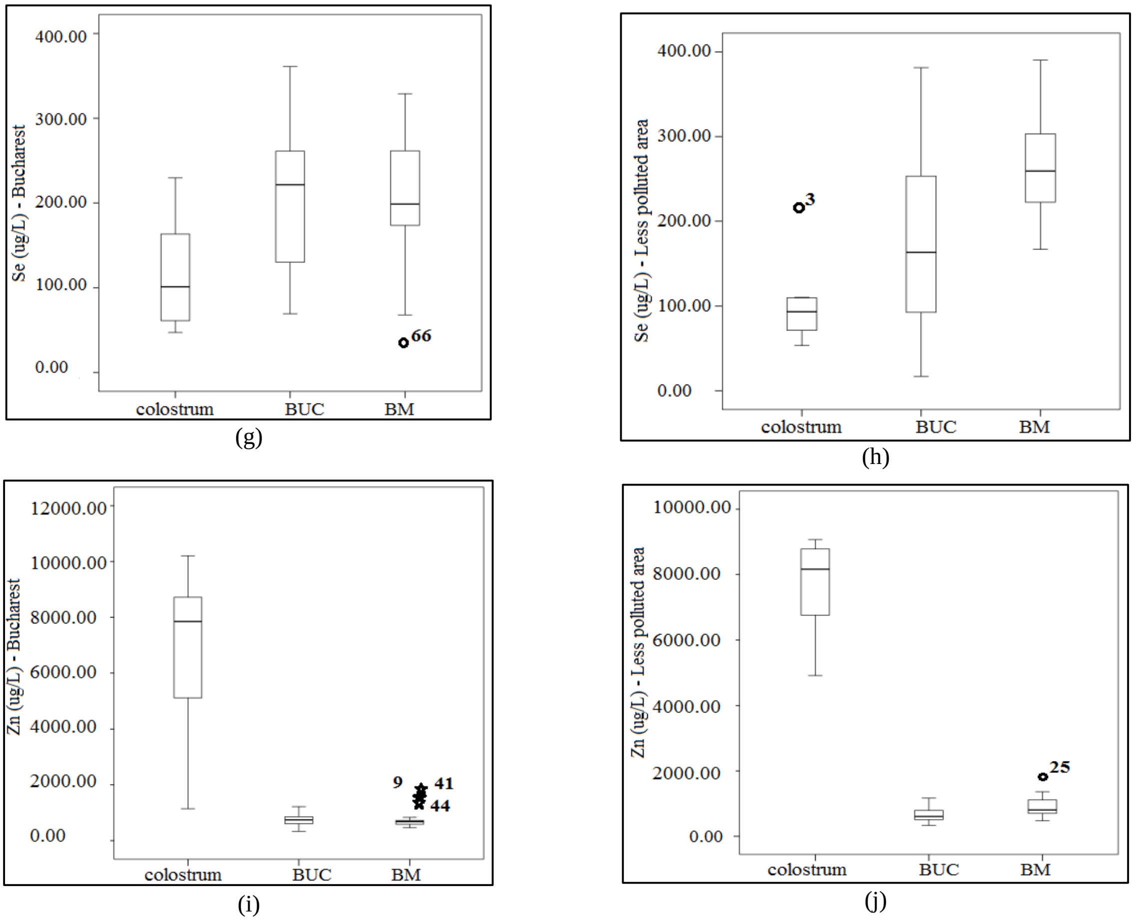


Fig. 1. Box-and-whiskers plots depicting the concentration of several trace elements in colostrum, mother's blood (BM) and umbilical cord blood (BUC)

From the univariate analysis, carried out for the "mother's residence" variable, it could be concluded that:

1. For the elements measured in colostrums, significant statistical differences were observed only for As: the concentration in the samples collected from Bucharest woman was 1.44 times higher than for those collected at the less polluted area.
2. For the elements measured in blood, significant statistical differences were found for: As, Se, Ca and Cr.

From the univariate analysis carried out for the "consumption of vitamins and minerals" variable, for the elements identified in the umbilical cord, no significant statistical differences were identified for the elements measured in the umbilical cord.

4. Conclusions

This work presents the ICP-MS determination of heavy metals and other trace elements in samples of colostrum, umbilical cord blood and mother's blood (whole blood and serum), collected from women living in Bucharest (considered as a highly polluted area and in a small-town (a less polluted area considered as "reference", and the obtained results were statistical analyzed. The results show that the concentrations in blood are much lower compared with the ones in colostrum. The values for Cr and Zn in colostrum are more than five times higher compared to the ones in blood, were for the majority of samples the values are under the lower limit of the reference interval. Also, the value for Ca is almost three times smaller in blood than in colostrum.

For all blood samples, Cu was significantly below the lower limit of reference interval. We also observed that more than 75% of the mothers from Bucharest have serum Zn concentrations below the lower limit of the reference interval in comparison with only 50% of the mothers from the less polluted area, probably as a result of their diet.

No significant differences were observed between the concentrations obtained for the blood samples of mothers with or without intake of vitamins and minerals. For all studied elements, the concentrations in blood are much lower comparative with the ones in colostrum, for example, the value for Ca is almost three times lower in blood than in colostrum. This study points out the risks of pregnant women exposure to air pollutants. They concentrate into the mother's organism and may be eliminated through colostrum in high concentrations, fact that may represent a hazard for babies' health and their further development.

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