HEAVY METALS AND PHYSICO-CHEMICAL COMPOSITION OF MATERNAL BREAST MILK AND COLOSTRUM

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Abstract

Mother's milk is a very valuable food for newborns, providing all the nutrients necessary for children's health. Colostrum is an important source of biologically active natural components and due to antimicrobial agents, can reduce gastrointestinal infections in newborns. Physical and chemical parameters were analyzed from maternal breast milk and colostrum for five days. The fat content of the colostrum shows the lowest values in the first postpartum days, after which it increases, reaching the highest values on day 5. Heavy metals in colostrum and mother's milk were evaluated considering their area of origin and all the samples were positive regarding Al, Pb, Rb, Sr, Cr. The variation in the metal concentration in maternal breast milk and colostrum could be due to their geographical origin and furtherly can affect the quality of milk.

Key words: Breast milk, colostrum, heavy metals, physico-chemical composition.

INTRODUCTION

Breast milk is an important nutritional source for the growth and development of newborns, an extremely complex and highly variable biofluid that has evolved over millennia to nourish infants and protect them from disease whilst, their own immune system matures (Fujita et al., 2012).

Colostrum contains a wide range of biologically active substances, including immunoglobulins, hormones, growth factors and at least 60 enzymes, ensuring immunological and anti-inflammatory properties against many diseases.In this century toxic heavy metals represent a major source of the ever-increasing problem of environmental pollution. Of these, lead (Pb), mercury (Hg), cadmium (Cd), zinc (Zn), copper (Cu) and iron (Fe) are the most common toxic heavy metals that can be present in the human milk, exhibiting harmful impact for the human body. When these elements arrive in the main feed sources, they can easily spread to all food chains, thus affecting the entire food chain.

Besides this aspect, another risk is bioaccumulation of heavy metals in living organisms which are considered primary elements of the food chain. It is observed that the human body, the last key point of the trophic chain retains the highest concentrations of heavy metals due to the bioaccumulation effect (Vinodhini & Narayanan 2008). Lead is a heavy metal naturally occurring in the earth crust and has nobeneficial impact on living organisms. Bioaccumulation of Pb arise in maternal bones, lately being released along with calcium intoher blood,and subsequently providingits pathway into breast milk.

Mercury is a toxic metal, ubiquitous in nature, is secreted in the breast milk from exposed mothers and may affect infant neurodevelopment (Johnson et al., 2009; Evers et al., 2005; Wu et al., 1991).

The development of cadmium (Cd) toxicity is known to be related to zinc (Zn) and copper (Cu) accumulation in the liver, kidney of animals (Sato & Nagai, 1989; Liu et al., 1992, 1994) and humans (Nordberg, 2004; Godt, 2006). During the lactation period, Cd is transported from maternal plasma to mammary gland and secreted into breast milk together with Cu and Zn (Sower et al., 1993; Kalkwarf et al., 1999).

Zinc is an essential trace element that has a complex biological role, playing the main

function in the activation of numerous enzymatic systems that can regulate and degrade pituitary hormones of appetite (Shay & Mangian, 2000).

Copper is a chemical element, soft, malleable, and ductile metal with high thermal and electrical conductivity. Deficiency of Cu, as well as toxicity, may present a concern for children, although precise copper concentrations have not been established yet for this age group (Lönnerdal, 1998).

Iron is the most common element on Earth, mainly forming Earth's outer and inner core. It is the fourth most common element estimated in the Earth's crust. Being a key factor, some of the bacteriostatic properties of human milk are associated with levels and/or bioavailability of this element in breast milk (Rowland et al., 1980).

Breast milk influences the intestinal microflora ensures the structural and functional maturity of mucous membranes, reduces the risk of allergies and autoimmune disorders, and contributes to the proper development of the gastrointestinal, central nervous, endocrine and immune systems (Leon-Cava et al., 2002). Due to the fact that heavy metals and trace elements are found in low concentrations in milk samples, ICP-MS is a suitable and precise analytical technique. In addition, the acid digestion assay has been considered to show the best results in sample preparation (Huynh et al., 2015; Dico et al., 2015). Therefore, the aims of this study were to evaluate the heavy metal profile and physicochemical composition of maternal breast milk and colostrum depending on the reference region. In Cluj, Sălaj, Bistrița-Năsăud, Turda, Baia-Mare, Satu-Mare, Târgu-Mures, Cehu Silvaniei and Carei no published study has evaluated the levels of heavy metals in breast milk and colostrum. Considering the toxic potential of these compounds and the importance of milk for children development, as a source of protein, fats and other essential nutrients we analyzed the physicochemical parameters to determine the influence of postpartum on protein: fat: lactose content of milk from the mothers used in that study. Moreover, the aim was to observe the differences between these parameters in both milk and colostrum samples and if high-fat content influences heavy metals assimilation.

MATERIALS AND METHODS

Sampling

Twenty milliliters of breast milk and colostrum samples were collected aseptically from each lactating mother using sterile breast pumps, in sterile containers and stored at 4°C until analyzes were performed. The samples were collected from Romanian localities such as: Cluj, Sălaj, Bistrița-Năsăud, Turda, Baia-Mare, Satu-Mare, Târgu-Mureş, Cehu Silvaniei and Carei.

Characteristics of the Lactating Mothers

In the present study analyzed colostrum and milk samples were collected from a total of 45 women (N = 5) per locality. Colostrum samples were obtained from day 1 to day 5 after postpartum. After three months, milk samples were harvested from the same mothers. The average age of the mothers was ranged between 25-30 years. Healthy mothers were included in the study, mothers suffering from any illnesses or infections were excluded from the present study. They did not smoke or drink alcohol and coffee during pregnancy and breastfeeding period.

Physico-chemical analysis

Lactoscan (Milk analyzer Lactoscan) device was used for physico-chemical analysis. A method previously reported by (Marchis et al., 2018). The following % of parameters were determined: fat, protein and lactose. A number of 5 samples were used for each locality.

Chemicals

The chemicals from the present work were of analytical reagent grade. Hydrochloric acid fuming HCl 37% (Merk, Germany), nitric acid HNO₃ 65% (Merk, Germany), ultrapure water, Milli-Q (Millipore, Bedford, MA, USA), Hydrogen peroxide H_2O_2 30% (Merk. Germany), and ICP multi-element standard solution 1000 (Merk. Darmstadt. mg/l Germany).

Quality Assurance

For the quantitative determination of the desired elements, the external calibration method was performed. With the help of interpolation, the concentration of the analyte in the unknown sample can be easily deter-

mined. Therefore, calibrations were performed with multi-element standard solutions (Merk, Darmstadt, Germany) at different concentration levels and then the calibration curves were drawn.

Mineralization of samples

Heavy metals determination was based on an assay previously reported by Coroian et al., (2017). Milk and colostral samples were subjected to microwave digestion with 8 ml Nitric acid (65% HNO₃) and 2 ml Hydrogen peroxide (30% H₂O₂). The milk samples were digested according to the following program: (i) temperature 145°C, pressure 30 (bar), ramp time 5 min, hold time 15 min; (ii) temperature 180°C, pressure 30 (bar), ramp time 1 min, hold time 10 min; (iii) temperature 120°C, pressure 30 (bar), ramp time 1 min, hold time 15 min; (iv) temperature 100°C, pressure 0 (bar), ramp time 1 min, hold time 10 min. Digested samples were allowed to cool to ambient temperature, transferred to polypropylene tubes and diluted to 25 ml of ultrapure water. The samples were filtered through a 0.45 um cellulose membrane filter. Simultaneously, blank samples were prepared.

Determination of minerals and heavy metals

For determination of heavy metals from colostrum and milk samples a number of 5 samples were used for each locality. Determination of minerals and heavy metals from milk and colostrum samples was evaluated by inductively coupled plasma mass spectrometry or ICP-MS used for the identification and quantification of Cr, Fe, Cu, Na, Mg, Cu, Al, Pb, Rb, Sr, and Zn elements at a concentration level of mg/L or higher concentrations by the appropriate dilution of the sample. Determination of Pb, Zn, Cu, Fe and Cr traces was determined according to SR EN 14082: 2003; LOD - 0.05 mg/l; LOQ -0.1 mg/L. As equipment was used in that study Digestor Berghoff MWS-3+ Microwave (Eningen, Germany) was used followed by ICP-MS ELAN DRC II Perkin-Elmer.

RESULTS AND DISCUSSIONS

Maternal breast milk samples were analyzed for seven heavy metals, Pb, Rb, Cr, Zn, Sr, Cu, Al and four trace elements, Na, Mg, Ca and Fe obtained from different regions of Romania. Heavy metals and essential mineral concentrations from different regions of Romania are described in Table1 and Table 2. Values represent the average of concentrations \pm standard deviation.

Table1. Essential mineral concentrations from breast milk

A roo/motol	Average ±s.d. (mg/l)						
/srea/metai	Na	Mg	Ca	Fe			
Cluj	178.79±2.39	36.57±0.74	640.98±2.47	1.76±0.05			
Sălaj	147.18±3.20	29.08±0.71	614.63±2.50	0.71±0.02			
Bistrița- Năsăud	71.22±2.83	30.33±1.07	590.88±2.55	0.85±0.04			
Turda	158.86±0.93	32.09±0.74	608.94±2.63	0.91±0.01			
Baia-Mare	178.07±2.27	25.53±0.51	578.00±4.71	1.20±0.02			
Satu-Mare	188.26±2.41	37.71±0.82	555.66±3.85	1.09±0.02			
Târgu- Mureș	181.03±3.05	40.36±0.57	380.98±5.98	0.91±0.02			
Cehu Silvaniei	157.54±3.75	34.25±0.90	479.90±2.12	1.07±0.03			
Carei	166.79±3.29	35.37±0.87	636.78±3.39	1.07 ± 0.01			

The highest Na counts from breast milk where observed in the samples from Satu-Mare (188.26±2.41) following in descending order by > Târgu-Mures (181.03 \pm 3.05) and Cluj (178.79±2.39) > Baia-Mare (178.07±2.27) > Carei (166.79±3.29) > Turda (158.86±0.93) > Cehu Silvaniei (157.54±3.75) > Sălai (147.18 ± 3.20) and Bistrita-Năsăud (71.22±2.83) mg/l, respectively. Magnesium levels from samples acquired from Târgu-Mureş presented elevated values compared with another regions. Altun et al. (2018) using ICP-MS noticed higher values of sodium compared with our results and the values ranged between (44.7-1703) mg/l in breast milk samples from Turkey. According to Björklund et al. 2012 Na presented increased concentration 217±77 mg/l compared to our results. Similar concentrations of Mg was found in the milk from Satu-Mare (37.71±0.82), Cluj (36.57±0.74), Carei (35.37±0.87) and Cehu Silvaniei (34.25±0.90). Our results are in line with those reported by Altun et al. (2018) and Björklund et al. (2012) with the Mg mean values of 32.6±15.5 and 28±4.8 mg/l. The most decreased values of magnesium parameter resulted in Baia-Mare (25.53±0.51) and Sălaj (29.08±0.71) mg/l samples.

One of the most important mineral for skeletal system development and namely calcium presented high values in the area of Cluj (640.98 ± 2.47) followed by Carei (636.78 ± 3.39) > Sălaj (614.63 ± 2.50) > Turda (608.94 ± 2.63) >

Bistrița-Năsăud (590.88 \pm 2.55) > Baia-Mare (578.00 \pm 4.71) > Satu-Mare (555.66 \pm 3.85) > Cehu Silvaniei (479.90 \pm 2.12) and Târgu-Mureş (380.98 \pm 5.98), mg/l, respectively. Ca levels from Swedish and Turkish mother milk demonstrated lesser concentrations compared to our results, between 305 \pm 45 and 193 \pm 53.2 mg/l, respectively (Altun et al., 2018; Björklund et al., 2012). Iron is a transition metal, playing an important key-role in human metabolism and is responsible for oxygen transport and oxygen storage in the muscular system. Fe showed increased concentrations in maternal breast milk from Cluj (1.76 \pm 0.05) and similar values among the samples were found in samples from Baia-Mare (1.20±0.02). Satu-Mare (1.09±0.02), Cehu Silvaniei (1.07±0.03) and Carei (1.07 ± 0.01) . In addition, iron content from Târgu-Mures (0.91±0.02) and Turda (0.91±0.01) specimens demonstrated a similarity between values. Moreover, minimal concentration of Fe was observed in samples from (0.71 ± 0.02) Bistrița-Năsăud Sălai and (0.85±0.04), mg/l. Futhermore, resulted Fe values in breast milk are higher than concentrations reported in studies from Australia (Mohd-Taufek al., 2016). et Sweeden (Björklund et al., 2012), and Turkey (Altun et al., 2018), 47±99, 339±134 µg/l and 1.65±1.43 mg/l. respectively.

Table 2. Heavy metals concentrations from maternal milk samples

Area	Average ±s.d. (mg/l)								
	Pb	Rb	Cr	Sr	Cu	Zn	Al		
Cluj	0.04±0.01	1.03±0.04	0.30±0.01	0.22±0.03	0.97±0.08	1.56±0.03	1.87±0.12		
Sălaj	0.08±0.00	0.90±0.02	0.41±0.02	0.27±0.02	1.87±0.06	1.33±0.03	2.20±0.15		
Bistrița-Năsăud	0.01±0.00	1.09 ± 0.01	0.20±0.01	0.28±0.03	0.05±0.01	0.18±0.03	1.61±0.18		
Turda	0.03±0.01	1.12±0.01	0.57±0.02	0.45±0.03	0.64±0.16	0.86±0.03	2.93±0.28		
Baia-Mare	0.05±0.01	1.13±0.02	0.76±0.03	0.56±0.03	2.87±0.08	1.13±0.03	4.97±0.05		
Satu-Mare	0.06±0.01	1.16±0.02	0.80±0.01	0.50±0.02	1.24±0.09	1.33±0.06	5.79±0.20		
Târgu-Mureș	0.03±0.14	1.15±0.03	0.88±0.03	0.38±0.01	2.25±0.11	1.53±0.03	4.03±0.06		
CehuSilvaniei	0.02±0.01	$1.00{\pm}0.08$	0.36±0.01	0.39±0.01	0.59±0.08	0.95±0.03	3.97±0.23		
Carei	0.09±0.02	1.20±0.04	0.60±0.01	0.40±0.01	1.08±0.10	1.05±0.03	4.02±0.06		

From the detected concentrations of toxic heavy metals, lead was found in relative reduced amounts in breast maternal milk samples compared among other metals. The highest concentration of Pb mg/l was identified in maternal milk from Carei (0.09 ± 0.02) followed by reduced amounts in the areas of >Sălaj (0.08 ± 0.00) and > Satu-Mare (0.06 ± 0.01) > Baia-Mare (0.05±0.01) > Cluj (0.04±0.01) > Târgu-Mureş $(0.03\pm0.14) >$ Turda (0.03 ± 0.01) > Cehu Silvaniei (0.02 \pm 0.01) > Bistrita Năsăud (0.01 ± 0.00) , respectively. According to World Health Organisation, the acceptable Pb concentrations in breast milk are reported to reach values between 2 and 5 ng/g (Choi et al., 2008). Swedish maternal breast milk samples demonstrated reduced levels of Pb (1.5±90 μ g/l) compared to ours. Chao et al. (2014) findings showed that Pb levels varied between different lactation stages indicating values ranged between (0.45 and 22.36) ng/ml. Rb did not present significant variations among the samples and comprise the values in ranged between 0.90-1.20 mg/l. Values of Rb were reported in a study of Björklund et al. (2012) showed decreased concentrations (714±108 µg/l) of Rb compared to those obtained by us. Cr levels of breast milk varied between 0.88 and 0.20 mg/l. Recommended Cr intakes in maternal breast milk for infants aged from < 6months should comprise doses ranged from 10-40 ug (Anderson, 1998). Elevated amounts of chromium were find areas such as Târgu-Mures (0.88±0.03) and Satu-Mare (0.80±0.01) and the most reduced in samples from Bistrita-Năsăud (0.20±0.01) and Cluj (0.30±0.01) (mg/l). In contrast to our result, Cr was find in lower concentrations in the samples analyzed by Björklund et al. (2012) with a mean of 0.30±0.27 µg/l. Maternal breast milk strontium concentrations values resulted between 0.22 and 0.56 mg/l. Lowest Sr content was detected in Cluj (0.22 \pm 0.03) followed by Sălaj (0.27 \pm 0.02) and Bistrița-Năsăud (0.28 \pm 0.03). Lower levels of Sr from human milk samples are reported in Swedish mother milk specimens comprising means of 33 \pm 12 µg/l.

However, increased Sr numbers were detected in Baia-Mare (0.56 ± 0.03) followed by a slight decrease in Satu-Mare (0.50 ± 0.02) >Turda (0.45 ± 0.03) > Cehu Silvaniei (0.39 ± 0.01) > Târgu-Mureş (0.38 ± 0.01) > Bistrița-Năsăud (0.28 ± 0.03) > Sălaj (0.27 ± 0.02) > and Cluj (0.22 ± 0.03) (mg/l), respectively. Copper plays an important role in the processes of heme and hemoglobin biosynthesis. Therefore, its deficiency as well as iron can cause anemia. Zinc stimulates the hormonal activity of the pituitary gland contributing to the normal development of the body increasing its weight.

However, deficiency of this trace element leads to growth retardation and weight loss. Greatly increased copper values were noticed in the samples from Baia-Mare (2.87±0.08) and Târgu-Mureş (2.25±0.11). Intermediate values were indicated for areas such as Sălaj (1.87 ± 0.06) and Satu-Mare (1.24 ± 0.09) followed by decreased concentrations in Carei (1.08 ± 0.10) > Cluj (0.97 ± 0.08) > Turda (0.64 ± 0.16) > Cehu Silvaniei (0.59 ± 0.08) and Bistrița-Năsăud (0.05±0.01) (mg/l), with the minimal amount of Cu. Copper concentrations from the present study are higher compared to results reported by others (Mohd-Taufek et al., 2016; Björklund et al., 2012; Altun et al., 2018). Specimens from the region Cehu Silvaniei exhibit similar concentrations of Cu (0.59 ± 0.08) compared to the region of Sanlıurfa from Turkey (0.54±0.46), mg/l. According to European Union Scientific Food Committee, the recommended doses of Cu and Zn for 6-11 month children are 0.3 mg/d and 4.0 mg/d, respectively (Mandic et al., 1996).

Another important mineral indicator, zinc, was detected in predominant concentrations in samples from Cluj (1.56 ± 0.03) and Târgu-Mureş (1.53 ± 0.03) (mg/l). Sălaj and Satu-Mare Zn values revealed a similarity among concentrations (1.33 ± 0.03) , (1.33 ± 0.06) , respectively. Decreased Zn values were found in Bistrița-Năsăud (0.18 ± 0.03) milk samples. Cu and Zn concentrations from human milk

samples are reported by various authors (Mohd-Taufek et al., 2016; Björklund et al., 2012; Altun et al., 2018).

The results regarding Zn concentrations are in concordance with Taufek et al. (2016) that obtained similar concentrations of this element in milks samples (1390±211 µg/l). Slightly elevations of Zn are presented in the study of Björklund et al. (2012) and Altun et al. (2018) with the means of 3471 ± 979 µg/l and 2.89 ± 3.23 mg/l. According to the study of Levi et al. (2018) milk obtained from Argentina mothers was subjected to acid digestion through ICP-MS showing median means for elements such as Na, Mg, Ca, Zn as follows 139/101 (28-1360), 31/30 (15-52), 247/246 (151-370) and 1.7/1.4 (0.17-7.9) (ng/g), respectively.

Predominantly high concentrations of Al were samples from Satu-Mare detected in (5.79±0.20), Baia-Mare (4.97±0.05), Târgu-Mures (4.03±0.06) and Carei (4.02±0.06). Aluminum is exerting nephrotoxic and hepatotoxic potential at low concentrations leading to poisoning in children and adults Chao et al. (2014). Average concentrations of aluminum were noticed in breast milk from Cehu Silvaniei (3.97±0.23), Turda (2.93±0.28) and Sălaj (2.20±0.15) (mg/l), respectively. The lowest values for that parameter was registred in Bistrița-Năsăud (1.61±0.18) and Cluj samples (1.87±0.12) (mg/l), respectively. Aluminum and lead are heavy metals with neurotoxic potential, and are responsible to induce nervous system disorders in children Rebelo and Caldas (2016). Further, lesser concentrations are reported in maternal breast milk specimens from Swedish healthy mothers with a mean of $185\pm584 \mu g/l$.

Cadmium, lead, aluminum, and copper are heavy metals presenting mainly negative impact on the development of biological organisms, and according to governmental regulations, the limits of these elements in food should be restricted. Contamination of breast milk with heavy metals from Lebanon showed different loads of arsenic (2.36 ± 1.95) , cadmium (0.87 ± 1.18) and lead (18.17 ± 13.31) (µg/l) (Bassil et al., 2018).

Concentrations of Na, Mg and Ca from colostrum are represented in Figure 1. Sodium concentration from maternal colostrum varied between 142.7 and 189.2 mg/l. Bistrița-Năsăud concentrations demonstrated the lowest Na content (88.56 mg/l) compare to samples from other regions. Additionally, a similar fact was observed in our breast milk samples from the

same region (71.22 mg/l). Calcium values from maternal colostrum were in the range of 521.3-632.1 mg/l. Baia-Mare and Târgu-Mureş samples revealed the highest content of Ca.



Figure 1. Values for essetial minerals obtained from colostrum depending on the reference region

Interestingly, by using ICP-MS, our study revealed elevated calcium contents from maternal milk samples and colostrum and that may be attributed due to Romanian cultural gastronomy consisted of different traditional types of cheeses and other dairy products. Magnesium from colostral specimens presented values in the range between 25.03 and 42.1 mg/l. Moreover, increased numbers were registered in the colostrum samples obtained from Târgu-Mures and the lowest in the region of Baia-Mare. These results can be correlated with Mg concentrations that were acquired from maternal breast milk samples with the similar values in a range 25.53 and 40.36 mg/l, respectively.

Figure 2 indicates the content of heavy metals, Pb, Rb, Cr, Sr, Cu, Zn Al, and Fe from maternal colostrum depending on the reference region. From the analyzed heavy metals from colostrum, lead being the most toxic was in the range of 0.01-0.09 mg/l. Pb values in colostrum from Cluj indicated lowest concentration. However, samples from other areas exhibit elevated concentrations of Pb. Hence, Pb concentration from the present study was higher in colostrum compared to breast milk samples, a similar characteristic remarked in a study of Chao et al. (2014). Lead concentrations in Croatian (non-smoker) transitional milk was 3.4 μ g/kg dry matter; mature milk showed lower values of 2.6 and colostrum Pb concentrations were predominant, 5.0 μ g/kg dry matter, respectively (Letinić et al., 2016).

Suciu et al. (2008) results denoted that Câmpia Turzii (Turda) region is considered least contaminated area showing Cu, Cr, Pb, values varying from 15.70 to 63.20 ppm, 20.70 to 62.40 ppm and 27.00 to 868.60 ppm, respectively. Rb and Cr in mother colostrum samples collected from different regions of Romania presented levels ranged between 0.83-1.36 and 0.26-1.02 mg/l, respectively. Highest rubidium concentrations detected in colostrum were noticed in Turda samples, and chromium increased concentrations are attributed to Sălaj samples. The excessive level of Sr among all samples was 0.66 mg/l, identified in colostrum from Baia-Mare. Instead, the lowest and the highest concentrations of Cu from the studied localities were observed in Bistrita-Năsăud 0.03 mg/kg and Baia-Mare 2.83 mg/kg



Figure 2. Heavy metal concentrations obtained from colostrum depending on the reference region

Rodna (Bistrita-Năsăud) samples from mining area soil presented Cu variations among the means from 8.5 to 108 mg/kg and Pb 3.5-4712 mg/kg (Nimirceag, 2012). Interestingly, >90% of samples had increased lead concentrations above the normal values. Concentrations of Zn in the samples with the highest concentration was 1.58 (Satu-Mare), followed in order by reduced concentrations in Baia-Mare (1.55) >Târgu-Mures (1.54) > Turda (1.47) > Sălaj (1.42) > Bistrita-Năsăud (1.39) > Cluj (1.32)>Carei (1.08) and Cehu Silvaniei (1.03) mg/l, respectively. In a Ph.D. thesis reported soil samples from Zlatna (central Transilvania) locality showed numerical variations for Pb (160.5-563), Cd (0.94-3.28), Cu (111-446.5) and Zn (84-576.5) (mg/kg) (Buzgău, 2013). According to Geana et al. (2011) study, Cr concentrations from soil samples of Sălaj showed means of 16.36 and for Cluj-Tarnita area 83.41 mg/kg. Zinc amounts for Sălaj samples presented values of 22.2 and 150.44 mg/kg for Cluj-Tarnita locality. Moreover, for either locality Pb varied between 1.44 and 9.88 mg/kg. We found that aluminum concentrations were the highest in colostrum from Târgu-Mureş (6.12) followed by Baia-Mare (5.66). Senila et al. (2011) remarked that Baia-Mare

area is highly polluted by heavy metals. Baia-Mare is considered polluted due to processing wastes from non-metallic ores and anthropogenic activities such as Pb, Zn and Cu refineries Damian et al. (2008). Lowest values of Al mg/l are represented in Clui (1.02) and Bistrita-Năsăud (1.06) colostral specimens. Iron concentration in colostrum was not much different among the samples and was between the level of 0.87 and 1.57 mg/l. In both types of samples, predominant Fe values were detected in Cluj. On another hand, decreased amounts in either sample were observed in Sălaj region. Heavy metal content measurements obtained from Rovinari (South-West of Romania) soil samples between 2009 and 2010 presented values for copper depending on sampling depths varied from 11.4 to 157.4 mg/kg. Most elevated Cu concentration was 157.4 mg/kg. being over the alert threshold. Soil zinc concentrations presented differences in 2009 between 38.6 and 118.4 and for 2010 values from 25.8 to 91.8 mg/kg. Regarding Pb, in 2009 the values ranged from 3.2 to 20.8 and for 2010 the means were 4.4 to 11.0 mg/kg. Figure 3 indicates means \pm standard deviation,

for fat content analyzed from mother colostrum during the days 1-5.



Figure 3. Fat content from maternal colostrum depending on the region

The fat content varied between 3.046 ± 0.06 for the 1st day (Sălaj) and 4.49 ± 0.22 g/100 ml for 5th day, postpartum period.

It can be noticed that regardless of the sampling area, the fat content shows the lowest mean values on day 1 (2.80-3.26), after which this parameter gradually increases reaching the highest values on day 4 (3.68-4.02) and day 5 postpartum (3.98-4.61) g/100 ml.

The fat content for the colostrum period is within the characteristic values for the postpartum period. Lactose content evaluated from mother colostrum during the days 1-5 is shown in Figure 4. The lactose content is influenced by the colostral period, a physiological phenomenon reported by Shi et al. (2011) that observes the highest lactose levels during the colostral period and decreases in transitional milk and reaching the lowest values in mature milk. Proteins and lipids are behaving in a similar way.

Protein content obtained from maternal colostrum during the days 1-5 is depicted in Figure 5.



Figure 4. Lactose content from maternal colostrum



Figure 4. Protein content from maternal colostrum

Protein composition resulted in maternal colostrum. behaves as lactose and fat. previously described parameters. During the 1st-day the protein profile showed lesser values of means (1.73 and 2.06%), compared with the other days. Notably, regardless of regions between the 2^{nd} (2.06-2.53), 3^{rd} (1.96-2.73) and 4th-day (2.07-2.70), the protein profile moderately elevates reaching the highest concentrations on day 5 (2.31-2.79%), respectively. Moreover, samples from Cehu Silvaniei (2.77±0.07) and Bistrita-Năsăud (2.79 ± 0.05) exhibited the highest protein

contents compared to other regions during postpartum, while Turda (2.41 ± 0.08) and Baia-Mare (2.31 ± 0.04) %, presented decreased mean values.

Figure 6 indicates the total average of values \pm standard deviation of physico-chemical parameters from all the localities described in the present study.



Figure 6. Basic physico-chemical composition of maternal milk

From the present study, it can be observed that maternal breast milk exhibits lower concentrations of protein (1.27 ± 0.07) and fat (3.52 ± 0.15) , compared to colostrum nutritional parameters.

Moreover, lactose from milk samples showed an increase compared to maternal colostrum samples.

Maternal breast milk samples from collected from Indian women presented a median fat composition of 3.02% slightly decreased compared to our average value of 3.52 ± 0.15 , Bedi et al. (2013).

CONCLUSIONS

The physico-chemical parameters of colostrum are influenced by postpartum, as can be observed on day 5 of postpartum when the values are reaching the highest increases. Maternal milk and colostrum have a balanced compositional and nutritive matrix for the development of young children. Lactose was the component with the highest level in the mother's milk. Hence, it may be concluded that the variation in the metal concentration in maternal breast milk and colostrum could be due to their geographical origin. The high level of heavy metals could potentially affect the breast milk and therefore the infant health.

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