Assessing the level of heavy metals in different geographical areas in Romania

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Abstract. The concentration of heavy metals in the meat was analysed in seven geographical areas in Romania. The concentrations of heavy metals in the meat was determined with spectrophotometric method. In the present study, the higher heavy metals concentration was found in Mureș, Satu–Mare and Salaj areas, while the lower concentration was observed in Brasov and Bistrița–Nasaud areas. The geographical areas considered in this study are the most polluted areas of Romania and have the highest heavy metals concentration in meat.

Keyword: Carob pods syrup, fermentation, Lactobacillus bulgaricus.

Introduction
The heavy metals presence in food can cause adverse effects on human health. There are reports of various diseases, malformations and disorders of the human body due to the toxicity of heavy metals [ARUP, 2003]. After absorption, the aluminum (Al) is distributed unequally to all tissues in the human body.

Normal levels of Al in the serum are about 1–3 μg/L.

Normal levels reported in human bone tissue are between 5 and 10 mg/kg. Al has also been found in human skin, gastrointestinal tract, lymph nodes, supranasal glands in most organs and tissues. In humans, after ingestion, it reaches the blood where it is discharged into the kidneys, after which it is excreted in the urine [ATSDR, 2008; EFSA, 2008], while unabsorbed Al is excreted via faeces [EFSA, 2008]. Laboratory studies on the cadmium (Cd) level during pregnancy have had deleterious effects on young animals.

The nervous system was the main target in laboratory animals.

Exposure to Cd before birth can causes headaches, dizziness, nausea and diarrhea. Long–term exposure to Cu dust can irritate the nose, mouth, eyes and causes headaches, dizziness, nausea and diarrhea.

If we drink water with higher levels of Cu than normal levels, we could suffer side effects such as: nausea, vomiting, abdominal cramps, diarrhea.

Besides, a high consumption of Cu can cause liver and kidney problems. Cu is not considered to be carcinogenic for humans [ATSDR, 2004]. Food contamination by heavy metals is considered to be a threat and its exposure for a long time has had a great impact on the health status.

Heavy metals are those elements, which have specific gravity greater than five. Elements which fit this category are Cd, arsenic (As), vanadium (V) and many more [HENRY and MILES, 2001 cited by KHAN et al., 2016].

These compounds resulting from human activities are sources of pollution and are continuously released into ecosystems. They pose an elevated risk also because of their accumulation in food chains [KHAN et al., 2016].

Metals can also be essential for the human body, like manganese (Mg), iron (Fe), Cu and many others. If found in excess or deficiency, they can cause serious metabolic disturbances.
Although these heavy metals are natural components of the environment, industrialization had a substantial influence in their wider diffusion and dispersal [RAJAGANAPATHY et al., 2011 cited by LUKACOVA, 2014].

Environmental contamination is also caused by metal corrosion, soil erosion of metal ions, atmospheric deposition and leaching of heavy metals. Industrial sources such as coal burning, petroleum combustion and nuclear power also lead to heavy metal pollution [TCHOUNWOU, et al., 2014].

The most popular sources are pesticides, fossil fuels, waste disposals, mining industries, farms [LUKACOVA, 2014].

Cd exposure leads to kidney and bone problems. Moreover, it has been found as a potential carcinogen responsible for lung cancer [KHAN et al., 2016].

Meat is a very convenient and rich source of proteins and other nutrients [RAJAGANAPATHY et al., 2011 cited by LUKACOVA, 2014].

Several studies performed on diverse types of meat and certified by WHO have concluded that even a small amount of heavy metals like Cd can lead to improper consequences [LUKACOVA, 2014].

It is concerning that heavy metals do not enter our body just by eating contaminated food but also by smiling or by skin. Cd is mainly found in food which is considered the principal environmental source for it. It is responsible for kidney dysfunction, hypertension, lung damage and hepatic injury [JOHN AND JEANNE, 1994 cited by AIZUHAIRI et al. 2015, STOLERU, et al., 2019].

Moreover, its concentration increases 50 to 60 times when present in carnivores. Processing technologies in food production affect the concentration of heavy metals. When animals eat polluted food, they clear the path to creating deposits in meat [SABIR et al., 2003 cited-by AI-ZUHAIRI et al., 2015]. Meat in general is a valuable source of essential protein, fat and other critical elements. Furthermore, it is mandatory for growth and maintenance of optimal health parameters [AI-ZUHAIRI et al., 2015]. Apart from proteins, meat has fat which is a reliable source of vitamins B6, B12 phosphorus, Zn and Fe [AKAN et al. 2010, WILLIAMS 2007 cited-by NKANSAH and ANSAH, 2014]. The toxic substances found in meat tissues can be caused by a variety of sources such as the presence of pesticides, animal drugs, polluted meat and other industrial products [FATHY et al, 2011 cited-by NKANSAH and ANSAH, 2014].

When heavy metals accumulate in high doses, they give birth to metabolic anomalies. Cd, the heavy metal of high concern is known to specifically affect the proximal tubular cells. Also, it can cause bone demineralization which further leads to serious health issues [SOLIDUM et al., 2013 cited-by NKANSAH and ANSAH, 2014].

Sadly, studies have shown that children are more prone to some heavy metal toxicity [NKANSAH and ANSAH, 2014].

These metallic elements are called systematic toxins because of their ability to induce multiple organ damage even at lower levels of exposure.

Their bioavailability is significantly influenced by temperature, adsorption, sequestration and phase association. In biological systems, these compounds alter cellular organelles and components like mitochondria, cellular membrane, lysosomes, nuclei and several enzymes.

They interact with the DNA causing damage and conformational changes that result in cell cycle modulation, apoptosis or carcinogenesis [TCHOUNWOU, et al., 2014].

By nature, animal liver is a very good provider of Fe and other essential elements. Nonetheless, it might contain higher amounts of heavy metals which tend to accumulate in liver tissues [LEONTPOULOS, et al., 2015, SAMFIRA, et al., 2015].

The purpose of this study was to assess the level of heavy metals in meat according to the area.

Material and methods

Five samples of pork meat were used for each area. The pork meat in this study comes from animals raised in traditional system.

The feeding included local forages with no nutritional supplements. After harvesting, the samples were kept in the freezer until heavy metal analysis.

The analysis was carried through ICPMS ELAN DRC II PerkinElmer spectrophotometry.
The reagents for heavy metal detection were: HNO₃ 65 % (Merk, Germany); HCl 7 % (Merk, Germany); analytical purity H₂O₂ 30 % (Merk, Germany); multi element stock solution 1000 mg/L (Merk, Darmstadt, Germany); ultrapure water, MilliQ (Millipore, Bedford, MA, USA).

The equipment used consisted of a Berghoff MWS3+ (Eningen, Germany) microwave digester and ICPMS ELAN DRC II PerkinElmer.

The pork meat samples were in the first instance mineralized for compatibility with the mass spectrophotometer.

The sample mineralization process implies microwave digestion of 1 g meat with 8 mL HNO₃ 65 % and 2 mL H₂O₂ 30 %. After cooling at room temperature, the sample was diluted with 25 mL ultrapure water and then filtered with a membrane filter (0.45 μm cellulose).

After completing this procedure, every element’s concentration can be measured with ICPMS.

Values obtained for heavy metals were interpreted with the ANOVA program (JMP version 12, SAS Institute, USA).

Results and discussion

The results obtained using AAS analysis of heavy metals in meat samples has been shown in Table 1 and Table 2.

### Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Li (µg/L)</th>
<th>Sr (µg/L)</th>
<th>Ba (µg/L)</th>
<th>Ti (µg/L)</th>
<th>Zn (µg/L)</th>
<th>Ca (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salaj</td>
<td>12.96±0.89</td>
<td>130.73±2.08</td>
<td>262.23±7.26</td>
<td>80.31±2.74</td>
<td>18.92±0.47</td>
<td>2831.14±76.12</td>
</tr>
<tr>
<td>V%</td>
<td>19.32</td>
<td>3.96</td>
<td>6.19</td>
<td>7.63</td>
<td>5.51</td>
<td>6.01</td>
</tr>
<tr>
<td>Cluj</td>
<td>8.12±0.36</td>
<td>21.75±0.44</td>
<td>159.62±1.87</td>
<td>59.45±1.48</td>
<td>9.66±0.24</td>
<td>3060.85±121.22</td>
</tr>
<tr>
<td>V%</td>
<td>10.03</td>
<td>4.49</td>
<td>2.61</td>
<td>5.58</td>
<td>4.45</td>
<td>8.83</td>
</tr>
<tr>
<td>Dej</td>
<td>11.98±0.57</td>
<td>55.64±2.66</td>
<td>178.3±2.02</td>
<td>26.40±1.13</td>
<td>18.23±0.59</td>
<td>2945.74±27.91</td>
</tr>
<tr>
<td>V%</td>
<td>10.58</td>
<td>10.64</td>
<td>2.53</td>
<td>9.56</td>
<td>7.26</td>
<td>2.12</td>
</tr>
<tr>
<td>Bistrița–Nasaud</td>
<td>3.08±0.31</td>
<td>11.14±0.71</td>
<td>185.86±3.48</td>
<td>13.41±0.25</td>
<td>13.80±0.48</td>
<td>3346.42±83.25</td>
</tr>
<tr>
<td>V%</td>
<td>22.70</td>
<td>14.28</td>
<td>2.99</td>
<td>4.12</td>
<td>7.85</td>
<td>5.56</td>
</tr>
<tr>
<td>Mures</td>
<td>24.16±0.68</td>
<td>73.02±1.67</td>
<td>128.54±2.47</td>
<td>109.85±2.22</td>
<td>23.41±0.62</td>
<td>2862.90±44.92</td>
</tr>
<tr>
<td>V%</td>
<td>6.32</td>
<td>5.12</td>
<td>4.30</td>
<td>4.51</td>
<td>5.95</td>
<td>3.86</td>
</tr>
<tr>
<td>Satu Mare</td>
<td>14.32±0.54</td>
<td>89.89±1.31</td>
<td>114.84±2.46</td>
<td>50.17±2.46</td>
<td>21.22±0.42</td>
<td>2721.66±55.47</td>
</tr>
<tr>
<td>V%</td>
<td>8.50</td>
<td>3.25</td>
<td>4.80</td>
<td>10.96</td>
<td>4.45</td>
<td>4.56</td>
</tr>
<tr>
<td>Brasov</td>
<td>6.09±0.63</td>
<td>10.72±0.52</td>
<td>102.45±2.93</td>
<td>11.54±0.46</td>
<td>9.82±0.38</td>
<td>5745.16±67.71</td>
</tr>
<tr>
<td>V%</td>
<td>23.00</td>
<td>10.83</td>
<td>6.40</td>
<td>8.95</td>
<td>8.55</td>
<td>2.64</td>
</tr>
</tbody>
</table>

V – coefficient of variability; X – average value; sx – Standard error of average.

A relatively high Cd level in food is the result of alloys used in agriculture (foodstuff and residual waters). Cd can cause acute intoxications, especially to children, pregnant women and people suffering from illnesses [SUHAROSCHI, 2013; COROIAN, 2017; GEORGIEVA, et al., 2018]. Li was detected in all samples from every geographic area. The values varied from 3.08±0.31 µg/L (Bistrița–Nasaud) to 24.16±0.68 µg/L (Mures).

Recently, a number of studies have suggested the neuroprotective effects of Li in models of several neurodegenerative diseases including PD (Parkinson disease).

However, the exact mechanisms underlying this neuroprotection remain unclear [LINGLING HOU, et al., 2015].

Also, Zn was found in every samples and results varied between 9.66±0.24 µg/L (Cluj) and 23.41±0.62 µg/L (Mures). Zn is an essential element in animal and human diet. Too little Zn can cause problems, however, too much Zn is harmful to human health (nausea and vomiting, epigastric pain, abdominal cramps and diarrhea) [PLUM et al., 2010].

The amount of Al found in samples varied in the range of 1.68±0.34 µg/L (Cluj) to 9.42±0.21 µg/L (Mures).

Many researchers have reported that Al can be toxic to the central nervous, skeletal, and hematopoietic systems, and Al has been controversially implicated in Alzheimer’s disease, osteomalacia, and dialysis encephalopathy [NORDBERG et al. 2007; AGUILAR et al. 2008; SHE et al. 2012], although this remains to be clearly demonstrated [SATO et al., 2014; BUTNARIU, et al., 2015].

Cd is a nonessential heavy metal and ubiquitous potential environmental
pollutant. Although the kidney proximal tubule is an important target for Cd, the underlying cellular mechanisms of Cd induced renal toxicity remain elusive.

Numerous studies have demonstrated that Cd induces apoptotic cell death in various cell types via several apoptotic pathways, including mitochondria mediated apoptotic cell death.

In the epithelial cells of renal proximal tubules, Cd can also induce apoptotic cell death in vivo and in vitro, which suggests that cell death of the epithelial cells through the apoptotic pathways is one of the key events in Cd induced renal toxicity [FUJWARA et al., 2012; BONCIU et al., 2018].

By comparison, other studies conducted on pork meat from Nigeria analysed the differences between different methods of growing.

They found Zn in pork meat (free ranger pig muscle) with a value ranging from 34.64 to 231.08 µg/g (15 meat samples). The mean value of Cu was 2.25 µg/g and the range were 0.89–4.23 µg/g. Ni had values between 0.20–1.37 µg/g and Cd was below the detection limit of 0.002 µg/g. On the other hand, concentrations of heavy metals in confined pigs were slightly different.

Zn ranged between 19.15 to 46.45 µg/g. The range for Cu was 0.60–4.65 µg/g and for Ni, 0.05–1.62 µg/g.

The conclusion is that free ranger pigs tend to accumulate higher concentrations of the discussed heavy metals probably due to their movement freedom and feeding habits [CHISOM, 2009].

### Table 2.

Average value and variability for heavy metals by area by area

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(Al) (mg/kg)</th>
<th>(V) (mg/kg)</th>
<th>(Mn) (mg/kg)</th>
<th>(Ni) (mg/kg)</th>
<th>(Cu) (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salaj X±sX</td>
<td>5.70±0.26</td>
<td>0.86±0.06</td>
<td>0.63±0.06</td>
<td>0.51±0.09</td>
<td>0.37±0.03</td>
</tr>
<tr>
<td>V%</td>
<td>10.28</td>
<td>15.71</td>
<td>22.80</td>
<td>40.87</td>
<td>19.77</td>
</tr>
<tr>
<td>Cluj X±sX</td>
<td>1.68±0.34</td>
<td>0.71±0.07</td>
<td>0.17±0.05</td>
<td>0.14±0.07</td>
<td>0.57±0.11</td>
</tr>
<tr>
<td>V%</td>
<td>45.02</td>
<td>23.54</td>
<td>61.67</td>
<td>105.68</td>
<td>41.46</td>
</tr>
<tr>
<td>Dej X±sX</td>
<td>6.24±0.38</td>
<td>0.56±0.05</td>
<td>0.61±0.11</td>
<td>0.63±0.07</td>
<td>0.69±0.06</td>
</tr>
<tr>
<td>V%</td>
<td>13.52</td>
<td>21.41</td>
<td>41.21</td>
<td>25.99</td>
<td>19.08</td>
</tr>
<tr>
<td>Bistrița–Nasaud X±sX</td>
<td>2.68±0.24</td>
<td>0.29±0.08</td>
<td>0.15±0.03</td>
<td>0.11±0.04</td>
<td>0.43±0.07</td>
</tr>
<tr>
<td>V%</td>
<td>20.44</td>
<td>63.63</td>
<td>49.10</td>
<td>73.29</td>
<td>35.12</td>
</tr>
<tr>
<td>Mures X±sX</td>
<td>9.42±0.33</td>
<td>1.11±0.11</td>
<td>0.76±0.07</td>
<td>0.68±0.04</td>
<td>0.63±0.06</td>
</tr>
<tr>
<td>V%</td>
<td>7.89</td>
<td>21.61</td>
<td>20.42</td>
<td>14.34</td>
<td>21.63</td>
</tr>
<tr>
<td>Satu Mare X±sX</td>
<td>9.28±0.57</td>
<td>1.09±0.13</td>
<td>0.85±0.05</td>
<td>0.40±0.08</td>
<td>0.58±0.09</td>
</tr>
<tr>
<td>V%</td>
<td>13.66</td>
<td>27.31</td>
<td>13.80</td>
<td>43.77</td>
<td>35.23</td>
</tr>
<tr>
<td>Brasov X±sX</td>
<td>2.44±0.20</td>
<td>0.23±0.09</td>
<td>0.24±0.05</td>
<td>0.18±0.05</td>
<td>0.17±0.05</td>
</tr>
<tr>
<td>V%</td>
<td>18.68</td>
<td>87.98</td>
<td>45.69</td>
<td>60.60</td>
<td>59.06</td>
</tr>
</tbody>
</table>

V–coefficient of variability; X–average value; sx–Standard error of average;

In meat, the presence of heavy metals is a major issue and need a special focus, because these can have toxic effects on human and animal health [UMER et al., 2017; CAUNII et al., 2015].

The direct exposure of these toxic metals is due to use of waste and drain water of industrial area for irrigation of crops and by grazing of animals on these crops and soil, these contaminants become part of animal body and by consumption of these contaminated animals, these toxic metals enter in human body.

### Conclusions

The geographic area had an important impact on heavy metals accumulation. The highest values of strontium (Sr) and barium (Ba) were measured in Mureş for Li, Zn, Al and Salaj.

Probably, in the studied geographic areas, the concentration of heavy metals in the soil is low, but the animals absorbed the heavy metals in their organisms by oral intake and then it was accumulated in the tissues of animals.

The highest level of V was found in Mureş, while the lowest was observed in
Bistrița–Nasaud. Mn, Ni and Cu showed the lowest content in all studied areas, compared to other heavy metals.

In the present study, the level of heavy metals was influenced by the degree of pollution in these areas.

References


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