Heavy Metals in Several Soybean Varieties Cultivated in the Transylvanian Plain, Romania

Rajmund MICHALSKI1*, Marcel M. DUDA2, Paula N. ŞERDEAN2, Joanna KERNERT1, Katarzyna GRYGOYC1, Paulina PECYNA1, Edward MUNTEAN2,3

1 Institute of Environmental Engineering, Polish Academy of Sciences, Skłodowska-Curie 34 Street, 41-819 Zabrze, Poland, Phone: +48 608 584 875
2 University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania
3 Agricultural Research and Development Station Turda, Romania
*corresponding author: rajmund.michalski@ipis.zabrze.pl

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Abstract

Soybeans are species of legume that has become one of the most widely consumed food in the world, because they are easy to cultivate and very beneficial for human health. The quality of the cultivated plants, including the content of toxic heavy metals depends to a large extent on the contaminants present in the soils in which they are grown and the method of fertilization. Through this research we aim to highlight the degree of accumulation of cadmium, chromium, cobalt, copper, nickel, lead and zinc in relation with the cultivated soybean genotype and fertilization. Three soy varieties (Cristina, Felix and Onix) were cultivated in an experimental field located at Cojocna Experimental Didactic Center of the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca. The above-mentioned heavy metals were determined from the harvested mature soybean seeds using inductively coupled plasma-optical emission spectrometry, using an Avio 200 instrument. The obtained results demonstrated that the accumulation of heavy metals in soybean seeds is influenced by the cultivated genotype and fertilization; the Onix variety is more prone to the accumulation of heavy metals, while the highest values of the above listed metals were recorded in the case of fertilizing with „Fertitel”.

Keywords: fertilization, heavy metals, soybeans, ICP-OES spectrometry

Introduction

Soybean (Glycine max (L.) Merr.) is a species of legume (family Fabaceae) that has become one of the most widely consumed food in the world, because it is easy to cultivate and are very useful for human health (Graham and Vance, 2003). Soybean, originated from Manchuria (China), was cultivated in this part of the world (including Japan and Korea) for more than 7000 years, being considered in the past as a holy plant. In Europe it was cultivated only late, starting with the early 1700s, while in North America only in 1765, being taken by the British. It is also called the “golden plant” or “wonder plant” because it could solve most of the necessary protein supply in human nutrition (Muntean et al. 2014). Soybean is considered as being the “plant of the future”, not only for its important nutritional value and benefits for human health, but also for animal feed and industrial applications (Singh et al., 2010);
soybeans have a good mix of amino acids and fatty acids that make their proteins and lipids fit into most uses (Erickson, 1995).

A growing body of literature has reported on the many beneficial health effects of soybeans, including the improvement of lipid profiles (Anderson et al. 1995, Potter et al. 1998) and the reduction in hormone-dependent diseases or conditions such as menopausal symptoms and cycles (Adlercreutz et al. 1992), breast cancer and possibly prostate cancer and osteoporosis (Potter et al. 1998); interpopulation comparisons have indicated the lower incidences of many of these diseases or conditions in populations who consume soy, such as the Japanese and Chinese (Ho et al., 2000; Messina et al. 1994). Soy is one of the major sources of isoflavones in the human diet (Knight et al. 1995). Although soy is a component of traditional Asian food, many of the studies on soy consumption have been conducted in Caucasian populations, among whom soy intake is rather low or almost nil (Anderson et al. 1997).

The health benefits of soybeans come from the nutrients, vitamins, and organic compounds including a significant amount of dietary fiber and a very large amount of protein (Hossain et al., 2003; James – 2003; Jenkins et al., 2003; Muntean et al. 2016). Soybean is also one of the most important sources of vegetable oil (Sing, 2010); the soybean oil is beneficial for human health, its consumption being associated with lower acute myocardial infarction risk compared, for example, with palm oil consumption (Anand et al., 2015). As for minerals, soybean seeds contain significant amounts of iron, manganese, phosphorus, copper, potassium, magnesium, zinc, selenium, and calcium.

Some heavy metals (e.g. Cu, Zn, Cd, Pb, Hg) are hazardous for human. They may lead to several acute or chronic adverse effects. Among heavy metals with no functions in plant tissues cadmium, lead and chromium are the most studied: in human body, cadmium can cause stomach irritations, lung damage, kidney diseases, probably cancer, the toxicity threshold in plant tissues being of 5-10 mg / kg DW; the main toxic effects of lead include hypertension, reproductive dysfunction, gastrointestinal track damage, nephropathy, neurological damage, the toxicity threshold in plant tissues being of 10-20 mg / kg DW; chromium (Cr⁶⁺) is genotoxic and carcinogen, its toxicity threshold in plant tissues being of 1-2 mg / kg DW (Forstner, 1995). More than that, it was proved that soybean production is significantly influenced by abiotic stresses generated among other factors by heavy metals (Desmukh et al., 2014). The quality of the cultivated plants, including the content of toxic heavy metals depends to a large extent on the contaminants present in the soils in which they are grown, as well as on the inputs from the fertilizers used (Murakami and Ae, 2009; Salazar et al., 2012). Therefore, their content has to be monitored and taken into account for health risk assessment.

Through this research we aim to highlight the degree of accumulation of cadmium, chromium, cobalt, copper, nickel, lead and zinc in relation with the cultivated soybean genotype and fertilization, this being new approach dealing with the influence of the fertilizers on the quality of the soybean seeds.

**Materials and methods**

**Plant materials:**

Three soy varieties (Cristina, Felix and Onix - creations of the Turda Agricultural Research Station), cultivated in an experimental field located at Cojocna Experimental Didactic Center of the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca. The experimental factors were: soybean variety (A1=Cristina, A2=Felix, A3=Onix), fertilization (B0=Basic fertilization, B1=basic fertilization + "Agrofeed" foliar fertilizer, B2 = basic fertilization + "Fertitel" foliar fertilizer).

The basic fertilization (B0) was achieved with N 68 kg and P total 46 kg in two stages (the preparation of the seedbed, 150 kg of ammonium nitrate [33.5% N] + sowing 18-46-0 DAP 100 kg starter); foliar fertilization, one application immediately after flowering, with "Agrofeed" (B1) (19-19-19 + Mg + ME), 8 kg/ha in 400 L water and "Fertitel" (B2) (N6,7 P6 K4,2 + microelements) 2 l / ha in 400 L water. The basic fertilization was made in the preparation of the germinating bed, and the foliar fertilizers were applied at the time of the appearance of the floral buds. The soil is argilioiluvial chernozem type with a succession of Am - Bty - C horizons, having a humus content of approx. 3.5%, a mobile phosphorus content above 4.5 mg P₂O₅ / 100 g soil (AL), a mobile potassium over 30 mg K₂O / 100 g soil (AL) and neutral pH.
Plant analysis

Around 1 g of homogenized samples of the harvested seeds were dried and mineralized by using HNO$_3$ and HCl. Inductively coupled plasma-optical emission spectrometric analysis was accomplished with an Avio 200 instrument (Perkin Elmer), using the following wavelengths for the selected metals: Cd – 226,502 nm; Co – 228,616 nm; Cr – 267,716 nm; Ni – 231,393 nm; Pb – 220, 353 nm; Zn – 206,200 nm. The main instrument parameters were: plasma gas flow - 12 L/min, nebulizer gas flow 0.7 L/min, auxiliary gas flow 0.2 L/min, sample flow 1.0 L/min, scanning mode - peak area.

Data Analysis

The data matrix was prepared and processed in Excel (Microsoft, USA), then principal component analysis (PCA) and cluster analysis were performed using MatLab (The Mathworks, USA) after mean center preprocessing.

Results and discussions

The obtained results are summarized in Table 1, demonstrating that the Onix variety soybean seeds, especially when additionally fertilized with „Fertitel“, have accumulated the highest amounts of copper, nickel and zinc (up to 72.9 mg/ kg overall heavy metals’).

The results show that the seeds of the three soybean varieties, in the experimental fertilization variants, do not accumulate significantly cadmium. An exception is the first variant, where the content is much larger than the other and cannot be explained. Higher cobalt content occurred to Onix, especially additional fertilized with „Fertitel" (0.35 mg/kg). In terms of chromium, no significant amounts were determined. Higher quantities of copper were recorded in additional fertilized with „Fertitel" on the Onix (13.19 mg/kg) and Cristina additional fertilized with „Agrofeed" (12.97 mg/kg). Higher nickel accumulations were found in additional fertilized with „Fertitel"on the Onix (9.94 mg/kg) and Felix additional fertilized with „Agrofeed" (8.33 mg/kg). The only seed sample to which lead was found (at a fairly high dose, 0.25 mg/kg) was the fertilized with „Fertitel" on the Onix variety. The seventh element, zinc, is found in larger concentrations, also in the Onix variety fertilized with „Fertitel" (50.16 mg/kg) and fertilized with only N 68 kg and P 46 kg (49.07 mg/kg).

The obtained results demonstrated that the Onix variety soybean seeds, especially those obtained after the additional fertilization with „Fertitel", have accumulated overall the highest amounts of cobalt (0.35 mg/kg), copper (13.19 mg/kg), nickel (9.94 mg/kg), lead (0.25 mg/kg) and zinc (50.16 mg/kg). The same variety accumulated significant amounts of cobalt (0.30 mg/kg) and zinc (49.07 mg/kg) in the fertilized variant with N 68 kg and P 46 kg.

Principal component analysis was based on three variables (concentrations of Cu, Zn and Ni), leading to a model which explains 90.90% of variance; it revealed a close correlation between the content of Zn and Ni in the analyzed samples (Fig.1). Cluster analysis revealed a similar contamination for both basic fertilization and that with additional „Fertitel" - samples A3B2

Table 1. Heavy metals from soybean seeds [mg/kg]

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Cd</th>
<th>Co</th>
<th>Cr</th>
<th>Cu</th>
<th>Ni</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1B0</td>
<td>0.05</td>
<td>0.10</td>
<td>0.05</td>
<td>12.33</td>
<td>7.69</td>
<td>N.D.</td>
<td>43.38</td>
</tr>
<tr>
<td>A1B1</td>
<td>0.05</td>
<td>0.15</td>
<td>0.10</td>
<td>12.97</td>
<td>7.58</td>
<td>N.D.</td>
<td>41.45</td>
</tr>
<tr>
<td>A1B2</td>
<td>0.05</td>
<td>0.10</td>
<td>0.10</td>
<td>9.88</td>
<td>6.93</td>
<td>N.D.</td>
<td>40.96</td>
</tr>
<tr>
<td>A2B0</td>
<td>N.D.</td>
<td>0.10</td>
<td>N.D.</td>
<td>9.18</td>
<td>2.29</td>
<td>N.D.</td>
<td>44.26</td>
</tr>
<tr>
<td>A2B1</td>
<td>N.D.</td>
<td>0.15</td>
<td>N.D.</td>
<td>8.28</td>
<td>8.33</td>
<td>N.D.</td>
<td>41.74</td>
</tr>
<tr>
<td>A3B0</td>
<td>N.D.</td>
<td>0.30</td>
<td>N.D.</td>
<td>11.99</td>
<td>8.00</td>
<td>N.D.</td>
<td>49.07</td>
</tr>
<tr>
<td>A3B1</td>
<td>N.D.</td>
<td>0.25</td>
<td>N.D.</td>
<td>9.85</td>
<td>8.15</td>
<td>N.D.</td>
<td>44.59</td>
</tr>
<tr>
<td>A3B2</td>
<td>0.05</td>
<td>0.35</td>
<td>0.05</td>
<td>13.19</td>
<td>9.94</td>
<td>0.25</td>
<td>50.16</td>
</tr>
</tbody>
</table>

*N.D.: not detected*
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Our data reveals generally lower heavy metals' contents in soybeans' grains in comparison with previously reported data:

- for cadmium, 0.09-0.2 mg/ kg (Salazar et al., 2012), <0.05 mg/ kg (Lavado et al., 2001), 1-8 mg/ kg (Shute and Macfie, 2006), 0.15-3.61 mg/ kg (Arao et al., 2003), 0.81 - 5.48 mg/kg (Zhou et al., 2013)
- for chromium 0.93 - 1.2 mg/ kg (Lavado et al., 2001),
- for copper 17.10 - 20.83 mg/ kg (Lavado et al., 2001)
- for lead, 1.52 - 2.55 mg/ kg (Salazar et al, 2012), 0.80-0.85 mg/ kg (Lavado et al, 2001), 4.69 -20.5 mg/kg (Zhou et al, 2013)

and A3B0 (Fig.3); these samples have the highest loadings on PC1 (Fig.2), accumulating overall 72.9 and 69 mg heavy metals/ kg.

Figure 1. Biplot for experimental data

Figure 2. Dendrogram obtained by cluster analysis using Ward's method & Mahalanobis distance
• for nickel 4.26 - 4.39 mg/ kg (Lavado et al., 2001)
• for zinc, 29.52 - 36.9 mg/ kg (Salazar et al., 2012), 43.50-44.85 mg/kg (Lavado et al., 2001), 60-120 mg/ kg (Shute and Macfie, 2006), 49.6 to 135 mg/kg (Zhou et al., 2013)

However, these values originate from studies with other objectives, dealing with different soybeans species which were subjected to certain environmental conditions; hence a direct comparison is not appropriate. It is important to emphasize that the values found in all samples did not exceed 0.2 mg/ kg Cd, the level given by the EU directive relating to maximum levels of certain contaminants in foodstuffs (EC 1881/2006), while there are no imposed limits for the other studied heavy metals.

Conclusions

The accumulation of heavy metals in soybean seeds seems to be influenced by the cultivated genotype and fertilization. The results of this experience show that the Onix variety is more prone to the accumulation of heavy metals, especially cobalt, copper, nickel, lead and zinc. In case of fertilizing with „Fertitel”, the highest values of the above listed metals were recorded.

The used fertilizers proved to pose no health risk for consumers from the point of view of the cadmium and lead content in seeds, their concentrations being under 0.2 mg/ kg in all cases.

References

near different emission sources on quality, accumulation and food safety in soybean [Glycine max (L.) Merrill].
CABI, p. 18.