Journal of Elementology ISSN 1644-2296

Danilcenko H., Gajewski M., Jariene E., Paulauskas V., Mažeika R. 2016. *Effect of compost on the accumulation of heavy metals in fruit of oilseed pumpkin (Cucurbita pepo* L. var. *Styriaca).* J. Elem., 21(1): 21-31. DOI: 10.5601/jelem.2015.20.2.905

EFFECT OF COMPOST ON THE ACCUMULATION OF HEAVY METALS IN FRUIT OF OILSEED PUMPKIN (*CUCURBITA PEPO* L. VAR. *STYRIACA*)*

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Abstract

While it is true that heavy metals accumulate in soil easily, their removal is difficult. The organic fraction of municipal solid waste can be recycled into compost, although frequent application of biowaste may lead to the accumulation of heavy metals in soil. There is no deep-rooted tradition in Lithuania to make and use various biowaste composts, including the ones produced from municipal waste. The objective of this study has been to compare the accumulation of heavy metals in different parts of oilseed pumpkin fruit depending on the chemical composition of the compost the pumpkin plants had been supplied. The content of heavy metals (HM) was determined in the soil before the plant-growing experiment, and in biowaste composts of different origin. Green waste (GW) and municipal solid waste (MSW) composts were applied to soil as fertiliser. Heavy metal concentrations in the skin, flesh and seeds of the analysed pumpkin fruits did not exceed the maximum permissible concentrations established by the EU norms. Seeds of the investigated pumpkin cultivar Olivia are more sensitive to the effect of heavy metal accumulation than the other morphological fruit parts (skin and flesh). The highest amounts of the investigated heavy metals, except Cr, were determined in the seeds, while the lowest ones, except Cu, in the flesh of oilseed pumpkin fruits. The amount of heavy metals in pumpkin biomass was not directly related to their concentrations in the soil, which proves the fact that the transfer of heavy metals from soil to plant is determined primarily by metal bioavailability and by a plant species.

Keywords: oilseed pumpkins, green and municipal solid waste composts, heavy metals.

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* This publication is funded by European Social Fund and the Budget of the Republic of Lithuania (project „Eureka "E! 6855 – ECORAW „Higher functionality food products from organic vegetable raw materials").

INTRODUCTION

The industrial and technological growth of our civilisation enatils an increasing accumulation of inorganic contaminants in the air, soil and water. Taking into account the growing output of heavy metals, their high toxicity and the ability to accumulate in the human body, even if present in relatively low concentrations, the research on adverse effects of these elements should be given priority. Soil contamination with heavy metals is a global problem, to which there is no single solution.

Rational fertilisation with biowaste and compost increases amounts of organic substance in light soil as well as the content of macro- and microelements. However, heavy metals present in soil are environmental pollutants that cause toxic effects to plants. Heavy metals accumulate in soil easily, but their removal is difficult. The content of organic matter in soil is the key factor affecting the migration of metals in soil and their penetration into plants (Vulkan et al. 2002). The organic fraction of municipal solid waste can be recycled into compost, although it must be borne in mind that frequent application of biowaste may lead to the accumulation of heavy metals in soil. When compost enters soil, it decomposes and releases main plant nutrients, i.e. nitrogen, phosphorus, potassium, as well as trace elements. Yet, there is no deep-rooted tradition in Lithuania to make and use various biowaste composts, including the ones made from municipal waste. Today, safe recycling of waste combined with energy recovery, for example composting as well as anaerobic processing of organic waste, is given priority. Mixed municipal solid waste organic fraction is composted separately. More than 100 thousand tons of municipal waste compost is produced in Lithuania annually, showing an increasing tendency in organic waste recycling. Pumpkin fruits contain many biologically active substances. The nutritional value of seeds and oil is also very high and pumpkin seed oil is rich in unsaturated fatty acids (Danilčenko et al. 2011). These plants are also easy to grown, and therefore their global production is increasing markedly. There are reports showing that pumpkins tend to accumulate only low amount of nitrates or heavy metals (Nield, Lott 1989, Badr et al. 2010, Jariene et al. 2015).

The objective of the study has been to determine the accumulation of heavy metals in different parts of oil seed pumpkin fruits depending on the chemical composition of the applied compost.

MATERIAL AND METHODS

In 2012–2013, an experiment was established on limnoglacial loam overlaying boulder clays, carbonate deeper gleyic luvisol (*Calearie Luvisol*), situated in the Sakiai region (Lithuania). The experiment was located on a certified organic field, with known soil richness, which was adequate to satisfy the test plant's needs. The oilseed pumpkin cultivar Olivia was planted. Soil was not additionally fertilized with mineral fertilisers and pesticides were not used, either. Composite soil samples were taken with a sampling auger from 12 randomly selected points of each treatment replicate, from the surface soil layer 0-20 cm deep. They were collected before pumpkin planting. Soil samples were air-dried in open plastic boxes and, having removed small stones, remains of roots and other organic plant parts, they were crushed. Homogenised soil was sieved through a 1 mm mesh size sieve. Soil samples were analysed for pH_{KCl}, organic matter, while phosphorus and potassium were extracted with ammonium-lactate according to the Egner-Riehm-Domingo method. The soil agrochemical characteristics were as follows: slightly acidic to neutral reaction, medium humus content, phosphorus rich (265 mg kg^{-1}) and potassium rich (165 mg kg^{-1}) soil. In the experimental area, the soil was drained by drainage ditches and the relief was artificially levelled.

Heavy metal (HM) content in the soil was determined before the plant-growing experiment, as well as in biowaste compost of different origin (Table 1).

Table 1

Amounts of heavy metals in soil and compost before the experiment (mg kg^{-1} DM)

Heavy metal	Heavy metals concentration (mg kg^{-1} DM)		
	soil before compost application	GW compost	MSW compost
Cd	0.28	0.13	2.22
Cr	11.87	7.90	32.15
Pb	10.51	20.35	138.93
Cu	16.73	18.85	212.50
Zn	250.33	105.00	613.50

Two different types of waste-derived organic matter composts were applied to soil as fertiliser, namely green waste (GW) compost and municipal solid waste (MSW) compost. Composition and agrochemical properties of the selected composts were evaluated as well. MSW compost was found to contain from 5 to 20% of different inert materials, i.e., glass fragments, non-degraded residues of plastics as well as metals, stones and rubble. Before fertilizing, municipal recycled organic material was sieved and these residual materials were removed. Municipal solid waste compost was obtained from the Utena Regional Waste Management Centre and green waste compost originated from the Alytus Regional Waste Management Centre. The content of organic matter and the content of total nitrogen were determined to be higher in GW compost, while the contamination with heavy metals was shown to be lower than in MSW compost (Table 1). The phosphorus content was higher in MSW compost. Batches of GW or MSW compost, after sieving, were added and mixed with the soil at the end of the first decade of May, at a rate of 6 kg m^{-2} of compost to each of the four replications. The application dose was selected according to the methodology used by Ghaly and Alkoaik (2010).

Oilseed pumpkins of the cultivar Olivia were grown in the following manner: interlinearly – with a 2-meter distance between the lines and with a 2-meter distance between the plants. The replication variants in each replication block were distributed randomly. Pumpkin fruits were harvested during the last decade of September. To fully mature, fruits were kept in a storehouse for two weeks at 12-16°C, in relative humidity of 60%.

For determinations of the heavy metals content, 5.0 kg of oilseed pumpkin fruits were collected randomly from each replication. Fruit samples were washed with deionised water and a collective sample of 1.5 kg was made up. For chemical analysis, 100 g of seeds from each sample was used. The skin and flesh from pumpkin fruits were treated separately. All samples were weighed and oven-dried at 70-80°C for 24 h. The dry biomass was ground to fine particles using a stainless steel mill. The content of dry matter (DM) was determined by drying a sample at 105°C to constant weight (LST ISO 751:2000).

Ground samples of the skin, flesh and seeds of pumpkin fruits were acid-digested and analysed. Heavy metals (Cd, Pb, Cr, Cu and Zn) were determined using inductively coupled plasma mass spectrometer (ICP-MS, Thermo Finnigan MAT, Bremen, Germany) according to the standard method (LST EN 15621:2012). All analyses were carried out in the Laboratory of Agricultural and Food Science Institute of Aleksandras Stulginskis University and in the Agrochemical Laboratory of Lithuanian Research Centre for Agriculture and Forestry. All analyses were conducted in triplicates.

The data were statistically treated using Anova data analysis and management module of the version of Statistica. For the evaluation of the analyses, one factor analysis of variance was carried out. Averages of separate treatments were calculated, the standard deviation and the least significant difference at a 95% probability level were estimated using the Fisher's LSD test ($P < 0.05$). Simple linear and regression analyses were applied for calculating relationship between the amount of heavy metals in the soil and in different parts of oil pumpkin fruits.

RESULTS AND DISCUSSION

The content of dry matter in plants is one of the most important indicators of the quality of chemical composition, which determines the quality of processing products and their yield (Souci et al. 1994). It is claimed that genetic characteristics of the cultivars determine accumulation of the dry matter by 75%, meteorological conditions by 14% and other factors by 11%. There is about 6-20% of dry matter in a pumpkin fruit. Our experiment showed that the significantly highest content of dry matter was determined in the skin and flesh of the cv. Olivia pumpkins from the control variant, accor-

Table 2

Dry matter content in the morphological parts of cv. Olivia oilseed pumpkin fruits (%)

Morphological part of pumpkin fruit	Dry matter content (%)		
	control	fertilised with GW compost	fertilised with MSW compost
Skin	11.50*a**	8.26*b*	7.10*c*
Flesh	5.67*a*	4.69*ab*	3.89*b*
Seeds	93.60*a*	93.89*a*	93.67*a*

* The average values marked by the same letter in a row have no significant differences at $P \leq 0.05$.

dingly 11.50%, 5.67%. The dry matter content in oil seeds from all the variants was very similar and ranged from 93.60% up to 93.89% (Table 2).

pH is one of the main factors affecting the concentration of heavy metals in the soil and determining amount of soluble and plant-absorbed heavy metals. The solubility as well as bioavailability of metals has a tendency to increase when the pH decreases and vice versa (Khan et al. 2009). In our experiment, the growing medium was neutral or slightly acidic, so this factor should not have adversely influenced heavy metals solubility and soil-to--plant mobility.

Cd. The proper growth and development of plants depends primarily on the availability of adequate amounts of nutrients. Apart from macroelements, there are also microelements such as copper and zinc, which are necessary for maintaining proper functions of an organism (Mccall et al. 2000). There are also such elements in the natural environment which do not have any beneficial physiological role, e.g. cadmium or lead (Waalkes 2003, Tran, Popova 2013). Such elements are known as non-biogenic. Our experiment showed that compost application did not have significant influence on the cadmium accumulation in the different parts of oilseed pumpkin fruits. The concentration of cadmium varied from 0.011 to 0.014 mg kg^{-1} in the skin and flesh regardless of what compost was applied (Table 3). Other researchers

Table 3

Cd concentration in different morphological parts of cv. Olivia oilseed pumpkin fruits (mg kg^{-1} DM)

Morphological part of pumpkin fruit	Cd concentration (mg kg^{-1} DM)		
	control	fertilised with GW compost	fertilised with MSW compost
Skin	0.011*a**	0.014*a*	0.014*a*
Flesh	0.011*a*	0.013*a*	0.013*a*
Seeds	0.022*a*	0.016*a*	0.018*a*

* The average values marked by the same letter in a row have no significant differences at $P \leq 0.05$.

have determined similar Cd content in the flesh of great pumpkins. Cadmium is known to be one of the most toxic heavy metals, since it inhibits many vital processes of living organisms (*Directive* ... 2006, WYSZKOWSKA et al. 2013). The highest Cd content was found in the pumpkin seeds of the control variant – 0.022 mg kg^{-1}. However, no significant difference between the content of cadmium in different variants was determined (Table 3). Nevertheless, these values did not exceed the maximum allowable concentrations recommended for vegetables by the EU normative document (Commission Regulation 2006) – Table 3.

An antagonistic interaction between heavy metals Cu and Cd was determined in cucumbers – *Cucumis sativus* L. (AN et al. 2004). A weak positive correlation was identified between the Cu content in the soil and the Cd content in the skin of our examined pumpkin fruit (r = 0.32). Copper, which was in the soil, increased the content of cadmium in the fruit. The above mentioned correlations were not found in the oilseed pumpkin flesh and seeds. According to ROUT and DAS (2003), Zn is a potential antagonist of Cd. Our experiment showed a positive moderate correlation between the content of Zn in the soil and the content of Cd in the peel (r = 0.59). We assume that other factors determine the content of Cd in the skin by 65% and the composition of the compost used determines it by 35%. Although cadmium is not essential for the growth and development of plants, it is readily taken up by the root system and therefore disturbs the uptake of other elements (DAVIES, WHITE 1981, TRAN, POPOVA 2013), especially those elements that have similar properties (valence, atom radius).

Pb. The analysis of our experimental data showed that the content of lead in all morphological parts of oilseed pumpkins did not exceed the allowed maximum acceptable concentration (MAC) according to the legislative normative document (Commission Regulation 2006). Regardless of the variant in which the oilseed pumpkins were grown, the content of lead in the skin of the fruits was similar, i.e., from 0.03 to 0.04 mg kg^{-1}. Significantly more Pb was found in the flesh and seeds of the control variant (Table 4). These results indicate that pumpkins do not tend to accumulate Pb in aboveground biomass. It is known that plants poorly accumulate the majority of

Table 4

Pb concentration in different morphological parts of cv. Olivia oilseed pumpkin fruits (mg kg^{-1} DM)

Morphological part of pumpkin fruit	Pb concentration (mg kg^{-1} DM)		
	control	fertilised with GW compost	fertilised with MSW compost
Skin	0.04*a**	0.04*a*	0.03*a*
Flesh	0.04*b*	0.02*a*	0.03*a*
Seeds	0.09*c*	0.04*a*	0.06*b*

* The average values marked by the same letter in a row have no significant differences at $P \leq 0.05$.

heavy metals, including lead, even when their concentration in the soil is relatively high, because metals appear as practically insoluble, immobile and thus hardly bioavailable compounds. Hence, the concentration of lead in plants is usually lower than 50 mg kg^{-1}. As mentioned previously, a synergetic interaction between cadmium and lead was determined in cucumbers – *Cucumis sativus* L. (An et al. 2004). The analysis of our research results showed that no such interaction was determined between Cd and Pb in the analysed morphological parts of oil pumpkin fruits (Table 4).

Cu. Copper is an important biogenic element and an elevated concentration of its compounds may be found almost in all food raw materials. More than 20 mg kg^{-1} of copper may be found in waste or by-products of animal origin, animal offal, various kinds of shellfish and nuts. If agricultural raw materials have been treated with pesticides containing copper compounds, it is very likely that copper will accumulate in the biomass, and a higher content of this heavy metal will also be found in food products produced from such material (Danilcenko et al. 2011). Our research results showed that significantly the highest amounts of Cu had accumulated in the skin and seeds when oilseed pumpkin plants where grown on soil treated with green waste compost, accordingly 6.31 and 13.70 mg kg^{-1}, and in flesh, when cv. Olivia pumpkins were grown using municipal waste compost (7.88 mg kg^{-1}) – Table 5.

Table 5

Cu concentration in different morphological parts of cv. Olivia oilseed pumpkin fruits (mg kg^{-1} DM)

Morphological part of pumpkin fruit	Cu concentration (mg kg^{-1} DM)		
	control	fertilised with GW compost	fertilised with MSW compost
Skin	4.73*a**	6.31*b*	5.54*ab*
Flesh	4.35*a*	3.92*a*	7.88*b*
Seeds	11.65*a*	13.70*b*	12.63*ab*

* The average values marked by the same letter in a row have no significant differences at $P \leq 0.05$.

Other researchers state that the concentration of Cu in plants varies within the range of 4-12 mg kg^{-1} and the phytotoxic concentration of this metal starts from 30 mg kg^{-1} (Athar, Ahmad 2002). According to other researchers, the content of copper found in the pumpkin seeds varies from very low (under detection limit) to 24.49 mg kg^{-1} (Badr et al. 2010) – Table 5.

Our results concerning the Cu content in pumpkin flesh were similar to other published data. It was established that Cu concentrations in all the tested great pumpkin fruit samples were at very similar levels and varied between 0.563 and 0.792 mg kg^{-1} in cv. Rouge vif d'Etampes pumpkin peel having the lowest amount and in cv. Muscade de Provence pumpkin flesh having the highest values (Jariene et al. 2015).

Cr. When growing oilseed pumpkins on soil fertilised with green waste compost, the significantly highest values of chromium were determined in the peel of the pumpkin fruit, i.e., 1.74 mg kg^{-1} (Table 6). Significantly higher values of chromium were identified in the flesh when GW compost had been used (1.04 mg kg^{-1}) as well as in the control experiment (0.9 mg kg^{-1}) than after MSW compost was used (0.35 mg kg^{-1}). It was established that significantly the lowest amount (0.82 mg kg^{-1}) of Cr was accumulated in seeds, when cv. Olivia was grown in the control soil. There are some reports implicating high accumulation of Cr in pumpkin skin and flesh, while in pumpkin seeds the Cr concentrations vary from 0.01 mg kg^{-1} up to 0.28 mg kg^{-1} depending on the cultivars (Jariene et al. 2015) – Table 6.

Table 6

Cr concentration in different morphological parts of cv. Olivia oilseed pumpkin fruits (mg kg^{-1} DM)

Morphological part of pumpkin fruit	Cr concentration (mg kg^{-1} DM)		
	control	fertilised with GW compost	fertilised with MSW compost
Skin	1.00*b**	1.74*c*	0.51*a*
Flesh	0.91*b*	1.04*b*	0.35*a*
Seeds	0.82*a*	1.08*b*	1.05*b*

* The average values marked by the same letter in a row have no significant differences at $P \leq 0.05$.

Zn. Like copper, zinc is among the most plant required chemical microelements, without which plants could not embrace full life cycle. It plays an important role in catalysing biochemical reactions by participating in the formation of an enzyme-substrate system, protein translation, gene copying and multiplication of a genetic chain (Sekler et al. 2007, Sądej, Namiotko 2010). According to Ovca et al. (2011), the content of zinc in plant seeds depends not only on fertilization or chemical composition of the soil, but also on the level of their maturity level and vegetation period, and varies from 15 to 23.4 mg kg^{-1}. Our research showed that the zinc concentration in the skin of control oilseed pumpkin fruit (Table 7) was significantly the lowest (19.62 mg

Table 7

Zn concentration in different morphological parts of cv. Olivia oilseed pumpkin fruits (mg kg^{-1} DM)

Morphological part of pumpkin fruit	Zn concentration (mg kg^{-1} DM)		
	control	fertilised with GW compost	fertilised with MSW compost
Skin	19.62*a**	23.42*b*	22.65*b*
Flesh	10.27*a*	11.87*b*	19.37*c*
Seeds	93.43*a*	93.97*a*	101.33*b*

* The average values marked by the same letter in a row have no significant differences at $P \leq 0.05$.

kg^{-1}) compared with the other two variants. A tendency was observed to accumulate a significantly higher amount of Zn in the flesh and seeds (19.37 and 101.33 mg kg^{-1} accordingly) when pumpkins were cultivated on soil treated with MSW compost (Table 7).

Other researchers stated that higher bioavailability of heavy metals was observed in soils with a low content of humic acids. As the soil pH increases (within 6.5-7.5), metals – especially zinc and, to a lesser degree, copper – become less toxic to plants (Wyszkowska et al. 2013). Plant species or even cultivars might differ from one to another in the tolerance to excessive quantities of cadmium, copper and zinc and in their ability to absorb these elements (Vig et al. 2003, Sekler et al. 2007, Beyersmann, Hartwig 2008, Nadgórska-Socha et al. 2013). Our research showed that their positive average correlation (r = 0.57) was determined in the flesh of oilseed pumpkins; the content of zinc in the flesh could have increased by 32% due to the elevated content of cadmium in the soil. Some researchers have proven a synergetic interaction between cadmium and zinc (Georgieva et al. 1997).

Mostly, the concentrations of heavy metals are significantly higher in soil than in vegetables grown in the same soil. This indicates that only a small amount of soil heavy metals is transferred to the vegetables and the root system acts as a barrier to the translocation of heavy metals into the plant (Khan et al. 2009).

CONCLUSIONS

1. Concentrations of heavy metals in the skin, flesh and seeds of the analysed oilseed pumpkin fruits, cultivated on soil treated with 2 different types of compost from green waste and municipal waste, did not exceed the maximum permissible concentrations under the EU normative document. Seeds of the investigated oil pumpkin cultivar are more sensitive to the effect of heavy metal accumulation than the other morphological fruit parts (peel and flesh).

2. The highest amounts of the investigated heavy metals, except Cr, were determined in the seeds, while the lowest ones, except Cu, in the flesh of the fruit of oilseed pumpkins cv. Olivia.

3. The amount of heavy metals in the soil was not directly related to their concentration in oilseed pumpkin biomass, and this proves that concentrations of heavy metals in plant material are determined primarily by metal bioavailability, uptake processes as well as plant species.

ACKNOWLEDGMENTS

The authors appreciate the help of Lina Neverauskaite, M.S., in conducting this research.

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