Journal of Microbiology,
Biotechnology and
Food Sciences

Tóth et al. 2012 : 1 (February Special issue) 828-836

REGULAR ARTICLE

IMPACT OF BIOSLUDGE APPLICATION ON HEAVY METALS CONTENT IN SUNFLOWER

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ABSTRACT

The application of decomposed substrate after continual biogas production is one of the possible ways how to use alternative energy sources with following monitoring of its complex influence on the hygienic state of soil with the emphasis on heavy metal input. The substances from bilge and drain sediments from water panels, also biosludge gained by continual co-fermentation of animal excrements belong to these compounds. The biosludge application is connected with possible risk of cadmium and lead, also other risky elements input into the soil. The analyses of applicated sludge prove that the determined heavy metals contents are compared with limitary value. These facts - hygienic state of soil, pH influence this limitary value and biosludge is suitable for soil application. The total heavy metals content in soil is related to the increased cadmium, nickel, chromium and cobalt contents. The analyses of heavy metals contents in sunflower seeds show that the grown yield does not comply with the legislative norms from the stand point of heavy metals content due to high zinc and nickel contents. Copper, cadmium, lead, chromium contents fulfil limitary values, for cobalt content the value is not mentioned in Codex Alimentarius. The nickel value in the control variant seeds is 2.2 times higher than the highest acceptable amount, then in variant where the sludge was applicated the nickel content was increased by 1.6 times. In the case of zinc there was increasing content in individual variants 4.7, or 4.8 times. The direct connection with the higher accumulation of zinc and nickel in soil by the influence of biosludge application is not definitely surveyed, the increased heavy metals contents in sunflower were primarily caused by their increased contents in soils.

Keywords: biosludge, sunflower, heavy metals

INTRODUCTION

The biosludge application in the liquid form or in the form of dewatered sludge on the arable soil is possible, however, it is necessary to take into consideration the requirements of agricultural crops. The types of soils must fulfil certain criteria, i.e. suitable pH, sorptive capacity, content of noxious substances in soil and sludge, etc. One of the options in utilization of the alternative energy sources is the application of rotted out substrate after the continual biogas production and the following observation of its complex impact on the hygienic soil state with the consideration of the heavy metal input. There belong waste sludges from sewage water treatment plant and bilgewater sediments of water hydroelectric dams as well as biosludge obtained from the continual co-fermentation of animal excrements. The application of the biosludge is linked with the possible risk of cadmium and lead input along with the other harmful elements input into soil. Their quantity and form in the coaction with the soil qualities can mean the risk of their input into the plant commodities grown on these soils (**Chlpík, Pospíšil, 2004**). The mobility and immobility of heavy metals in soil are influenced by the following qualities: soil reaction, organic substance, mineral composition, oxides content (**Vollmannová et al., 2004**). The microelements present in sludges can influence positively their supply in soil. However, in practice they mean a risk as the microelement content is often high (Cu, Zn, Mo). The same refers to Pb, Cr, Ni, Hg, Cd and As. It is precisely the content of heavy metals and toxic elements which limits the utilization of biosludges as a fertilizing substrate. The activity of the soil microorganisms results in the bioaccumulation of metals in plants and subsequently, heavy metals in fodders get into animal organisms (**Petrová, 2005**). Biaccumulation often results in the physical changes, e.g. reduction or oxidation of elements (As, Mn, Se, Tc) and also their methylation (Hg, Cd, Pb, Te), which leads to the essential change of their physiological effects. In compounds the toxical effects between the particular metals can grow stronger (synergism Cd+Zn, Ni+Zn, Hg+Cu and others) but also weaken (antagonism Se+Cd, Se+Hg). The cadmium intake by the plant roots is determined by its solubility in soil, soil pH, content of alkalic metals and the metals of alkalic soils, as well as the presence of other heavy metals. Cadmiun is more available for plants at more acid soils than at soils with a higher pH (**Bajčan et al., 2006; Tóth et al., 2006, Stanovič et al.[a], 2011**). Many authors (**Hegedűsová et al., 2006; Tomáš et al., 2005, Stanovič et al.[a,b], 2011**) found out that the addition of the humic substances immobilized essentially soluble and replaceable forms of some heavy metals. The concentration scale of nickel in soils is wide and it varies most frequently from 1 to 300 mg.kg^{-1}. The average figures are between 30 – 80 $mg.kg^{-1}$. Nevertheless, there can also occur extremely high contents Ni (100 – 7 000 $mg.kg^{-1}$) (**Beneš, Piabanová, 1987; Makovníková, 2001**). For the evaluation of the relation of Ni and pH it is known that solubility of Ni is inversely related to the value pH, nickel is best soluble in the soils with pH 6.5 – 7.5. Nickel is more accessible in wet soils (5 – 15%) from the total content (**Zaujec, 1999**). **Beneš and Pabianová (1987)** claim that the organic substance has enormous ability to absorb nickel. In the topsoil nickel occurs in the organically bound forms out of which the part can be easily changed into chelates.

MATERIAL AND METHODS

We determined the input of the heavy metals (Zn, Cu, Co, Ni, Pb and Co) into soil by the application of biosludge obtained from the continual co-fermentation of animal waste and energy crops under the conditions of the half-operational experiment at the Research Base of SUA in Kolíňany. The interest group is situated to the east of the village Kolíňany at the plot of ground called "Letisko – Airport". The north border is created by the common road Nitra – Zlaté Moravce, the eastern border is the airport VPP and south-west border is the riverbed of the local brook. The plot of ground has the east-west downslope with the altitude 160 – 180 m above sea level. The soil type: brown soil.

Experiment variants:

- Control: without biosludge application
- Variant 1: biosludge application – spring 50 $t.ha^{-1}$

During the half-operational experiments the following crops were cultivated: spring barley, sugar beet, silage corn, annual sunflower on the plots of 18 x 100 metres. We concentrated our attention on the biosludge impact on the husked achenes of the annual sunflower. We followed "Mandatory methodology of soil analyses, Monitoring System – Soil" in the process of sampling and adjustment of soil. For the evaluation of the soil hygiene the total contents of heavy metals in extract HNO_3 + $HClO_4$ +HF were set. The analyses of soil samples were carried out by the method of the flame absorption spectrophotometry. The total contents of heavy metals include all their forms occuring in soil and in accordance with the Decree of the Ministry of Agriculture of SR on the highest permissible values of the harmful substances in soil No. 531/1994 they are stipulating for the evaluation of soil hygiene. In order to evaluate the bioavailability of heavy metals we studied the distribution of those metals in particular soil types through the method of the selective sequential extraction (SSE) based on the methodology of Ziehen and Brümmer in depth 0 – 0.1m. In this depth there is the most active intake of heavy metals and therefore the evaluation of their bioavailability is the most important.

We set the following seven fractions:

1. **fraction** : *mobile forms of heavy metals,* Extr. : 1 M NH_4NO_3
2. **fraction** : *easily available forms of heavy metals*, Extr. : 1 M NH_4OAc
3. **fraction** : *heavy metals Mn – oxides-bound,* Extr. : 0.1 M NH_2OHHCl + 1 M NH_4OAc
4. **fraction** : *metals organic material-bound,* Extr. : 0.025 M NH_4-EDTA
5. **fraction** : *metals amorphous Fe – oxides-bound,* Extr. : 0.2 M NH_4OAc
6. **fraction** : *metals crystalline Fe – oxides-bound,* Extr. : 0.1 M ascorbic acid + 0.2M NH_4-oxalate
7. **fraction** : *residual fraction,* Extr. : 65 % HNO_3, 72 % $HClO_4$

The analysis of plant material was carried out according to the methodology set by the Codex Alimentarius of SR and the related regulations. Sunflowers were harvested in full ripeness. Sunflower achenes were adapted for analysis and the heavy metal content was tested only in husked seeds of achenes. The samples were homogenized and the heavy metals were determined by the method AAS. The measured contents were compared with the highest permissible amounts of foreign substances in sunflower and oil plants according to the Decree of Ministry of Agriculture SR and Ministry of Health Care No. 608/2004-100.

RESULTS AND DISCUSSION

We studied the impact of the biosludge application (rottted substrate) after the production of biogas at the research base in Kolíňany within the half-operational experiment. In accordance with the methology the biosludge was applied into soil in the dose 50 $t.ha^{-1}$ in 2009. The biosludge analysis related to the content of heavy metals is given in the table 1.

Table 1 Content of heavy metals in applied biosludge and comparison with the boundary value (pursuant to the Law No. 188/2003 Code) in $mg.kg^{-1}$

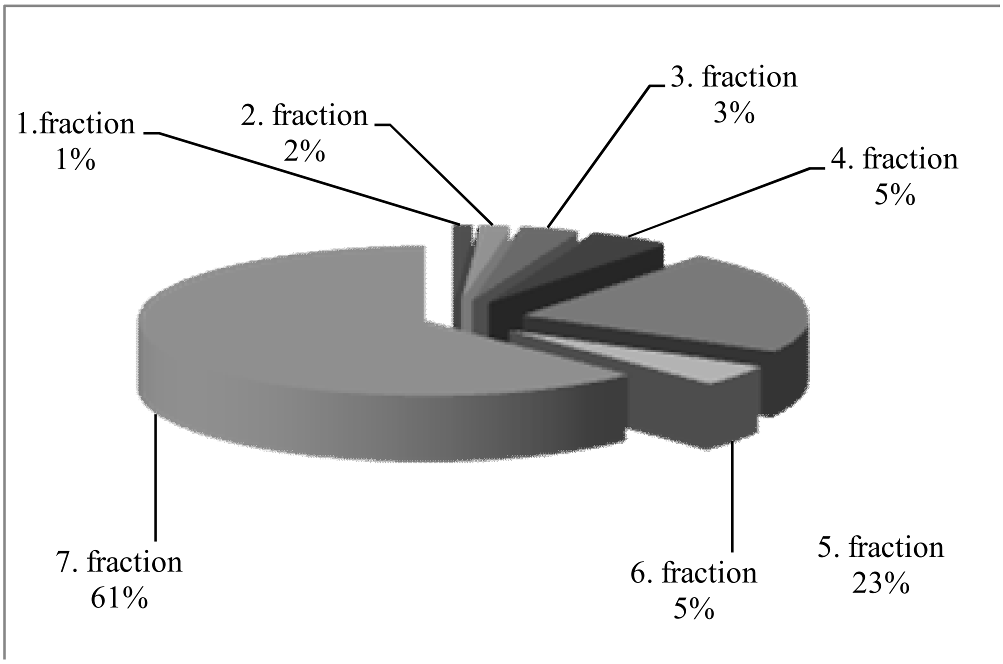
	Zn	Cu	Cd	Pb	Cr	Ni	Co
Sludge	1.178	0.243	0.010	0.088	0.068	0.127	0.076
Boundary value	2500	1000	10	750	1000	300	-

As it is evident from the analysis of the applied sludge, the content of the heavy metals did not exceed the boundary value for the content of studied the studied heavy metals in any trial. Based on the analyses we can state that the biosludge is suitable for the application into the agricutural soil. Within the half-operational experiment the soil analysis was carried out aimed at the heavy metals content in both control variant and variant where sunflower was cultivated within the sowing plan. The results of soils analyses are given in table 2.

Table 2 Total content of studied heavy metals in soil and comparison with the highest permissible amounts (NPM) in soil according to Decree of MA SR No. 531/1994-540 v $mg.kg^{-1}$

nom.	variant	Depth of samples (m)	pH	Zn	Cu	Cd	Pb	Cr	Ni	Co
1	**Control**	0-0.10	3.40	71.60	24.00	**0.80**	32.80	126.0	**39.60**	**20.00**
2		0.20-0.35	4.60	71.40	23.80	**0.81**	34.40	125.70	**40.20**	**21.40**
3		0.35-0.45	4.70	71.50	23.90	**0.94**	34.00	125.10	**40.90**	**21.30**
4	**50 tons biosludge**	0-0.10	5.80	67.20	27.60	**1.20**	34.00	**138.40**	**41.60**	18.80
5		0.20-0.35	5.94	70.40	26.80	**1.04**	34.00	**146.40**	**43.20**	**20.00**
6		0.35-0.45	5.40	70.80	24.00	**1.16**	32.80	**134.40**	**39.20**	19.60
The highest permissible quantities of heavy metals for:										
Total content				140	36	0.80	35	130	35	20

The results in table 2 show that the content of zinc, copper and lead is in both control variant and variant where biosludge was used under the limitary value. The biosludge application resulted in the increased content of chrome in soil, where the limitary value was exceeded by 7% due to its content in soil. The rise of chrome content in the variant, where biosludge was used, achieved 10.1% in comparison with the control variant without fertilizer. The cobalt content can be considered to be limitary where its content in soil in some sample depths is higher than the highest permissible quantity. The comparison of the control variant and the variant with the biosludge demonstrates that the chrome content was not increased after the application of sludge into soil.



Figue 1 Percentage of nickel presence in soils in particular fractions

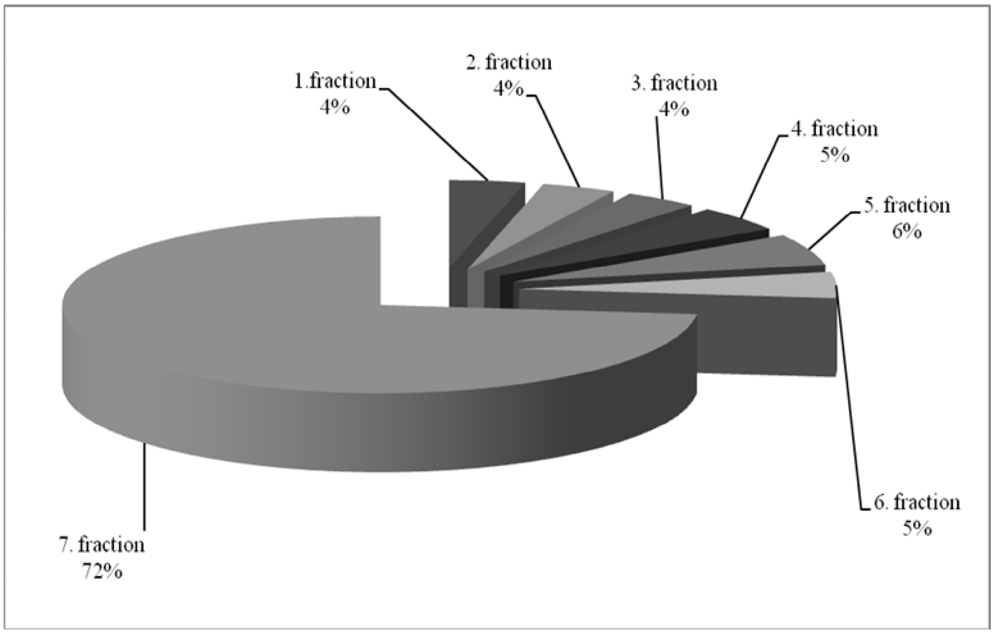


Figure 2 Percentage of cadmium presence in soils in particular fractions

The cadmium and nickel contents are higher in all sample depths and variants than the legal norm permits. The comparisons of particular variants show that the cadmium content was increased on average by 29% and nickel content by 3.5% after the application of the biosludge into soil. We determined 7 fractions of the studied heavy metals by the method SSE. Based on the results of soil analyses we state the soil analyses by this method only for cadmium and nickel, because there is the highest precondition of their input into the plant production according to their contents in soil (fig.1, 2). From the viewpoint of availability and acceptability of heavy metals the important fractions are 1 – 4, which represent those forms of heavy metals that are acceptable by plants. The acceptable forms of nickel in soil create 11% out of the whole amount. But there is also significant presence of the fraction 5 (23%). Taking into account the given value pH of soil this fraction is a potential risk as the release of the acceptable nickel ions into soil can occur with the detected soil qualities. The share of the mobile forms of cadmium in soil is 14% (fractions 1 – 4). The potential mobilising forms (fractions 5 and 6) represent 11%. The residual fraction (72%) creates a high share of unacceptable cadmium forms for plants. The analysis of sunflower achenes is given in table 3. It shows that from the viewpoint of heavy metal content the grown yield does not comply with the valid legislative norms due to the high zink and nickel content. The contents of copper, cadmium, lead and chrome comply with the limitary values. The Codex Alimentarius does not set the limitary value for the cobalt content. The nickel content in achenes is higher 2.2 times in the control variant than it is the highest permissible quantity. In the variant with the sludge application the nickel content was exceeded 1.6 times. The zink content was exceeded 4.7 times or 4.8 times in the particular variants.

Table 3 Content of heavy metals in husked achenes of sunflower and comparison with the highest permissible quantities according to Codex Alimentarius of SR (Decree of MA SR and MHC SR No. 608/2004-100) v $mg.kg^{-1}$

	Zn	Cu	Cd	Pb	Cr	Ni	Co
Control	**47.30**	15.90	0.43	0.49	0.50	**6.60**	0.39
Biosludge	**48.60**	16.30	0.45	0.70	0.45	**5.00**	0.15
The highest permissible quantities of heavy metals according to Codex Alientarius of SR							
	10.0	25.0	0.50	1.0	4.0	3.0	x

CONCLUSION

Based on the soil analyses we can conclude that the state of soil hygiene proves higher contents of some heavy metals in soils. Their measured contents are higher than the legislative limitary value allows for their contenst in soil. The total content of heavy metals in soil shows exceeding of cadmium, nickel, chrome and cobalt content in comparison with the limitary value. The detected facts - hygienic state of soil. pH of soil resulted in the increased intake of zink and nickel by sunflower. The analysis of the heavy metal content in sunflower achenes proves that the grown yield does not comply with the valid legislative norms due to the high zink and nickel content. The copper, cadmium, lead and chrome contents comply with the limitary values. The Codex Alimentarius does not set the limitary value for the cobalt content. The nickel content in achenes is 2.2 times higher in the control variant than it is the highest permissible quantity. The nickel content is 1.6 times higher in the variant with the sludge application. The zinc content exceeded in the particular variants 4.7 or 4.8 times. The direct relationship between the increased accumulation of zinc and nickel in soil and the biosludge application cannot be deduced unambiguously. The increased contents of heavy metals in sunflower were caused primarily by their increased contents in soil.

Acknowledgments: This contribution is the result of the project implementation: Centre of excellence forwhite-green biotechnology, ITMS 26220120054, supported by the Research & Development Operational Programme funded by the ERDF.

REFERENCES

BAJČAN D. - LAHUČKÝ L. - STANOVIČ R. - TREBICHALSKÝ P. - TIMORACKÁ M. 2006. Rizikové látky v poľnohospodárskych plodinách Ipeľského regiónu. In *Výživa a potraviny pre tretie tisícročie „ Výživa a nádorové ochorenia“*. Nitra: SPU Nitra, 2006, p. 18-21. ISBN 80-8069-775-2.

BENEŠ S., PABIANOVÁ J. 1987. Přirozené obsahy, distribuce a klasifikace prvků v půdach. *Praha: VSŽ*, 1987. 205 p.

Codex Alimentarius, 2011, http://www.svps.sk/legislativa/legislativa_kodex.asp.

Decree of Ministry of Agriculture SR and Ministry of Health Care No. 608/2004-100.

HEGEDŰSOVÁ A., HEGEDŰS O., MUSILOVÁ J. 2006. Riziká kontaminácie pôd kadmiom. *First edition, Nitra :. Vyd. Fakulta prírodných vied UKF v Nitre*, 89 p. ISBN 80-8094-047-9.

CHLPÍK, J. - POSPÍŠIL, R. 2004. Plošná charakteristika mechanických a chemických vlastností pôdy na Výskumnej báze Slovenskej poľnohospodárskej univerzity v Nitre, lokalita Kolíňany. In *Acta fytotechnica et zootechnica*, vol. 7, 2004, no. 1., p. 6-10.

MAKOVNÍKOVÁ J. 2001. Distribúcia kadmia a olova v hlavných pôdnych predstaviteľoch Slovenska. In *Poľnohospodárstvo*, vol. 47, 2001, no. 12, p. 903-911.

PETROVÁ J. 2005. Vplyv kalov z ČOV a vyfermentovaného produktu pri výrobe bioplynu na úrodu kukurice siatej. *PhD thesis*. Nitra : SPU, 2005, 247 p.

STANOVIČ, R.- ÁRVAY, J. - JOBBÁGY, J. 2011[b]. The statistical parameters of heavy metals transfer in soil - plant system. In : *Potravinárstvo : vedecký časopis pre potravinárstvo.* 2011. vol. 5, special issue no., p. 205-211. ISSN 1338-0230.

STANOVIČ, R.- ÁRVAY, J. - TÓTH, J. 2011[a]. The influence of soil chemical properties on heavy metals mobility. In: *Potravinárstvo : vedecký časopis pre potravinárstvo*. 2011. vol. 5, special issue, p. 201-204, ISSN 1338-0230.

TOMÁŠ J. - BAJČAN D. - VOLLMANNOVÁ A. - ZAUJEC A. - JOMOVÁ K. 2005. Rizikové prvky okolia Rudnian a Krompách. In *Zborník z 57. zjazdu chemických spoločností. Tatranské Matliare, sept. 2005. ChemZi, Vol. 1/1, p. 103*, ISSN 1336-7242.

TÓTH T. - VOLLMANNOVÁ A. - MUSILOVÁ J. - BYSTRICKÁ J. - HEGEDUSOVÁ A. - JOMOVÁ K. 2006. Rizikové prvky antropogénneho pôvodu v pôdach stredného Spiša. In *Chemické list,* Vol. 100, 2006, no. 8, p. 701-702. *ISSN 0009-2770.*

VOLLMANNOVÁ A. - LAHUČKÝ L. - LAZOR P. - HEGEDUSOVÁ A. 2004. Kumulácia ťažkých kovov bôbom vo vzťahu k obsahu ich potenciálne biopritupných foriem v pôde. In *Chemické List, Vol. 98 no. 8*, 2004, p.532

ZAUJEC A. 1999. Cudzorodé látky a hygiena pôd. *Nitra: SPU, 1999. 103 p*. ISBN 80-7137-567-5