Correlations between fertilizer use, potentially toxic element content and enzyme activity in Hungarian soils

Thesis of Ph.D. dissertation

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Gödöllő

2016
Ph.D. School: Ph.D. School of Environmental Sciences

Discipline: Soil sciences, agrochemistry, environmental chemistry

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Introduction and objectives

Mineral fertilisers applied on agricultural soils serve as a source of trace elements for cropped plants. These chemicals might also contain different pollutants, among others, heavy metals. By unreasonable doses and repeated fertiliser application total heavy metal concentration of soils might increase (Gimeno-Garcia et al., 1996).

Changes in soil environment can influence directly the activity and diversity of soil microbiota. Agricultural practice is one of the most important factors causing serious changes in both features and processes of soil. Microorganisms react quickly to these changes and they can also adapt fast to altering environmental conditions. Shifts in microbial populations and activity often precede detectable changes of physical and chemical properties of soil. Therefore they can be used as early warning signs of actual soil state (Dick, 1994; Pankhurst & Lynch, 1995; Jangid et al.; 2008).

In small quantities some heavy metals stimulate microbial reproduction being structural and functional components of several enzymes and other biologically active compounds. In spite of this, in larger quantities these metals might have a strongly harmful effect on natural regulating systems of soil. Namely, they influence redistribution and spread of microbe populations, prefer tolerant microbe species and they also decrease the quantity of microbial biomass (Brookes et al., 1986; Stephen et al., 1999; Kabata-Pendias & Pendias, 2001). Moreover, the same element might be useful or essential but still toxic when being present in larger concentration (Simon, 1999; Kabata-Pendias, 2004). Most of the studies regarding the relationships between heavy metals and soil microbiota had been carried out within laboratory conditions. Another huge part of these studies had been conducted on industrial pollution sites (Ellis et al., 2002; Szili-Kovács et al., 2006; Li et al., 2009). Large scale heterogeneity of microorganisms makes it more difficult both to assay them within field conditions and to carry out representative sampling (Vályi et al., 2013).

This work has been funded by TDR project (KEOP-6.3.0/2F/09-2009-0006). My own work within this project was collection, analysis and evaluation of data as well as performance of enzyme activity measurements.

The main objective of this research was to ascertain whether there is detectable toxic element load originating from fertilizer use in Hungary in the test areas. Namely, to find out whether there is a detectable correlation between different levels of fertilizer uses and the concentration of potentially toxic elements in soil. Possible correlations between applied fertilizer quantities (known from the farming logs) and potentially toxic element concentrations of the soil samples were analysed. Some basic soil parameters determining behaviour of metals in soils as well as microbial processes were also examined. Microbial activity of soil samples were measured applying fluorescein-diacetate (FDA) and sucrose (invertase) enzyme activity tests as well as substrate induced respiration (SIR) measurement in order to feature actual soil state and to assay
effects of potentially toxic elements. The analysed soil samples resulted from a nation-wide sampling survey being representative of both soil type and intensity of cultivation.

Specific questions were as follows:

1. How large is the potentially toxic element concentration of fertilised, cultivated lands in Hungary? Was there an elevated concentration of the analysed potentially toxic elements in the experimental soils as high as to be statistically provable presumably as a consequence of fertiliser use?
2. Which of the measured basic soil parameters have the biggest effect on the potentially toxic element content of the analysed soils?
3. Based on the results of the analysed three microbial parameters, does the potentially toxic element content of cultivated soils in Hungary pose a real risk on microbial activity? Which of the measured basic soil parameters show the closest relationship to these microbial variables?

Materials and methods

Selection and characterisation of the sampling sites

Selection method of the sampling sites followed two different aspects. One aspect was to examine farms applying presumably the highest doses of fertilizers, pesticides and organic manures country-wide. The other covered farms located on good quality production sites (production site category I and II) of Hungary having a loam or loamy soil texture. Preliminary knowledge of the applied N, P₂O₅ and K₂O active ingredient doses was also a must. All of the sampling sites were designated on cultivated lands. A total of 129 sampling sites were selected throughout the country. Location of these sites is demonstrated on Figure 1.

Soil sampling

Soil sampling was carried out between August 1 and November 30, 2011. Samples were taken from the 5 ha so-called RPRs (Representative Parcel Part) designated on a homogenous part of the sampling sites. Bulk samples were taken from 20 points of the diagonals of the RPRs, in 0-30 cm depth.
Measurement methods

Aqua regia soluble „total” potentially toxic element content of soil samples was measured according to the Hungarian Standard MSZ 21470-50:2006 by ICP-OES in MTA ATK TAKI as well as in Soil Laboratory of Velence. Distilled water soluble element concentrations were also measured from nearly half of all samples. However, these results did not reach detection limits for most of the analysed elements, therefore this measurement has been left out of the dissertation.

Further physical and chemical analyses were carried out in Velence according to the further standards:
- Plasticity index according to Arany (Kₐ): MSZ 08-0205:1978;
- Humus content (H%, m/m%): MSZ 21470-52:1983;
- Water soluble total salt content (salt content, m/m %): MSZ 21470-2:1981;
- Carbonate content (CaCO₃ %, m/m %): MSZ-08-0206-2:1978;

Consistency number derived from the plasticity index values according to Arany has also been counted (Stefanovits, 1992).

Microbial assays conducted in MTA ATK TAKI were carried out on dried and re-wetted (to pH 2.5 water holding capacity) soil samples after 10 days of incubation and involved the following widely applied methods:

*FDA hydrolysis* is based on the process of soil enzymes hydrolysing colourless fluorescein-diacetate added to the soil. Released coloured fluorescein can be measured by spectrophotometry. Analyses were carried out as described by Schnürer and Rosswall (1982) and optimised for soil samples by Adam and Duncan (2001). Measurements were done in three replicates and a control on a
Determination of sucrose (invertase) enzyme activity is founded on quantitative measurement of reductor monosaccharides emerging from the hydrolysis of sucrose (Szili-Kovács, 2004). These assays were carried out on the basis of the Hungarian Standard MSZ-08-1721-2:1986 in three replicates and one control for each sample. Colour intensity was measured by a Helios Beta Thermo Spectronic type spectrophotometer in a wave length of 540 nm. For the determination of the results given in mg glucose/g soil/24 hour calibration curves were used.

Substrate induced respiration (SIR) method is grounded on a so-called respiration answer given by the microbial biomass in the presence of an easily utilisable substrate (glucose) being in saturated concentration (Szili-Kovács, 2004; Mirsal, 2008). Released CO$_2$ was measured in each glass tubes by a FISONS GC8000 gas chromatograph. Results were presented in µg CO$_2$-C/g soil/hour units.

Fertiliser output data were known from a preliminary survey made among the farmers. Prior to sampling, farmers gave full details of the amount of applied active ingredients (N, P$_2$O$_5$, K$_2$O kg/ha, each) on their fields in the years 2008/2009, 2009/2010 and 2010/2011. The average data of these three years were calculated for each ingredient.

Statistical analysis of the data was performed with the programme StatSoft Statistica (Version 12 and 13). For the statistical analyses outlier data were deleted because of the bias they have caused in the results. Linear correlations were evaluated by correlation analysis at a significance level of p<0.05. Samples were independent. The results of the normality test Shapiro-Wilk showed not all of the parameters to be normally distributed. Therefore further analyses were accomplished with Mann-Whitney U-test, Kruskal-Wallis test and Bonferroni post-hoc test.

Results and discussion

Evaluation of correlations between fertiliser use and potentially toxic element content of the investigated soils

The results of correlation analysis between potentially toxic element content of the soil samples and the applied fertiliser quantities showed a weak and negative correlation between potassium output and the concentration of Co, Cr, Ni, Pb, Sn and Zn. Nitrogen and phosphorous output did not prove linear correlation with either of the potentially toxic elements. Thus, increased element content could not be detected on the sites with higher N, P and K application.

In order to investigate joint effects of NPK fertiliser output 5 groups were created of the samples according to the applied fertiliser doses (N+P+K, kg/ha) (Table 1).
Table 1. Five categories created from the applied fertiliser doses and dose ranges. n stands for number of samples.

<table>
<thead>
<tr>
<th>Fertiliser category</th>
<th>Dose ranges</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>N+P+K = 0 kg/ha</td>
<td>17</td>
</tr>
<tr>
<td>1</td>
<td>0 kg/ha &lt; N+P+K ≤ 100 kg/ha</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>100 kg/ha &lt; N+P+K ≤ 200 kg/ha</td>
<td>47</td>
</tr>
<tr>
<td>3</td>
<td>200 kg/ha &lt; N+P+K ≤ 300 kg/ha</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>300 kg/ha &lt; N+P+K</td>
<td>4</td>
</tr>
</tbody>
</table>

Results of the Kruskal-Wallis test performed on the five categories showed all of the elements to be significantly correlated (p<0.05) to fertiliser output doses except for As and Ba. To find out the specific categories being different from each other post-hoc tests were applied. Sampling sites with no fertiliser output proved to have a lower concentration of potentially toxic elements (except for As, Ba and Se) than those with either N, P or K output. This was only true until 100 kg/ha N+P+K active ingredient doses. According to these results, no trend could be stated supporting a tendency of increasing fertiliser doses causing higher potentially toxic element concentration. Besides, huge differences could be observed among the elements.

Lowering tendency after category 1 could have been caused by several factors. Plant accumulation might occur only at higher concentrations, toxic elements might get into soil solution which form is easier to be leached out. Provided that the marked differences were an effect of fertiliser use, it might be supposed that within acidifying conditions caused by increasing fertiliser application (Stefanovits et al., 1999; Iturri & Buschiazzo, 2016) large proportion of potentially toxic elements might be mobilised. Plant uptake might also be enhanced (Li et al., 2014) resulting in lowering element concentrations in the upper soil layers.

Increase in the element content between category 0 and 1 might also result from other components of a more intensive agricultural practice. Large quantities of potentially toxic elements are loaded into soil via pesticide application (Jepson, 2001). Huge interquartile ranges might imply environmental risks as extreme differences might occur depending on the properties of a given soil. According to the results of the sampling sites it can be assumed that toxic element content of Hungarian soils is mainly influenced by other factors rather than agricultural load.

Evaluation of correlations between potentially toxic element content and basic soil properties of sampled cultivated soils in Hungary

Based on the results of the correlation analysis potentially toxic element content of sampled soils is mainly influenced by humus content, consistency and salt content, regarding basic soil parameters. These positive correlations refer to
the fact that the mentioned parameters increase potentially toxic element content of the soil. Humus content proved to correlate with ten of the measured elements out of which relationships they were moderate strength with As, Ba, Cd, Cr, Cu, Ni and Zn. No correlation was found between humus content and Mo and Se, being usually in anionic form in the soil. As for consistency (Plasticity index according to Arany, K_A) except for Cd, Mo and Pb all of other measured elements showed significant correlations. Moderate strength correlation was found with the same elements as in the case of humus content except for Cd. pH(H_2O) turned out to have only weak correlation with Cd and Cu. All of the significant relationships found between carbonate content (%) and potentially toxic elements (in this case Co, Cr, Ni, Pb, Sn and Zn) proved to be weak and negative. Stronger correlation than moderate was not found between any of the parameter pairs. Distribution of Cd, Mo, Pb and Se was far from normal in the analysed samples. Besides, in the cases of Cd, Mo and Se uncertainty of measurements caused by extremely low concentrations might also bias the results. These facts might contribute the lack of correlation with consistency and humus content. According to literature data, Pb is the most strongly bound element in the soil out of the analysed elements. Its binding to organic compounds might be as strong even at low organic matter content as not to show changes at higher rates as well.

Statistical analyses of correlations with texture were performed using the same method as for fertiliser dose effects. Samples can be ranged into five texture classes: sand, sandy loam, loam, clay loam and clay. Results of the Kruskal-Wallis test showed all of the measured elements to correlate with the texture classes of the samples with the exceptions of Cd and Mo. The following post-hoc tests produced slight differences among the elements but the tendency of increasing concentrations with raising clay content seemed to be usual. In the cases of As, Ba, Co, Cr, Cu, Ni, Pb and Zn element concentration in sandy soils were found to be different (lower) either from the concentrations in clay loam or in clay soils, or from both of them. This correlation is important from the aspect of microbes: it could influence the effects of analysed elements on soil microbial activity.

**Evaluation of correlations of microbial activity with basic soil properties and with potentially toxic element content of sampled cultivated soils in Hungary**

According to linear correlation analyses, upward relationships were found between the three measured microbial parameters. Moderate strength correlation between FDA and sucrose enzyme activities (0,46***), as well as between sucrose activity and SIR (0,62***), whereas correlation was weak between FDA activity and SIR (0,34***). Haynes (1999) and Stark et al. (2008) has found strong correlations between the size of microbial biomass and different enzyme activities (FDA among others) as well.

Possible connections between basic soil properties and microbial parameters were also examined in order to explore the bias they might cause in the correlations between potentially toxic elements and microbial activity.
Correlation analysis resulted all of examined basic soil properties to have statistically provable connection to FDA activity. These relationships were weak strength in every cases and negative with pH and carbonate content, whereas positive with the others. Weak upward correlation was found between sucrose enzyme activity and humus content, consistency and salt content, whereas the correlation was weak and downward with carbonate content. SIR was found in weak relationship with pH, consistency and salt content whereas in moderate with humus content. These connections were all positive.

Soil consistency influenced all three microbial parameters significantly according to Kruskal-Wallis test. Microbial activity raised in accordance with increasing clay content. FDA activity was lower in sandy soils than in clay loam and clay soils (Figure 2). Sucrose activity and SIR were both detected to be lower in either loam, clay loam or clay samples (Figure 3 and 4).

Figure 2. FDA activity of samples grouped by texture. Letters a and b refer to significantly different groups according to post-hoc tests.
Figure 3. Sucrose activity of samples grouped by texture. Letters a and b refer to significantly different groups according to post-hoc tests.

Figure 4. SIR values of samples grouped by texture. Letters a and b refer to significantly different groups according to post-hoc tests.

Based on literature data FDA and sucrose activity as well as SIR all depend on the content of clay minerals and humus of soil, as these can produce favourable conditions for the accumulation of enzymes (Schnürer & Rosswall, 1982; Brezovicskiné & Anton, 1985; Szili-Kovács et al., 2009). Furthermore, some publishers could detect stronger relationships between physicochemical properties of soil and microbial biomass than between the latter one and heavy metal concentration (Zhang et al., 2016).
Bacteria and fungi react differently to the changes in pH and carbonate content of soil. As FDA and SIR measures both microbial groups no obvious explanation can be given to the detected correlations. Although high salt content might reduce microbial activity of the soil solution (Füzy, 2007), the salt content of the analysed soil samples did probably not reach a limit detectably lowering the activity. Besides, the possibility of the spread of salt tolerant species makes it more difficult to follow the changes.

All of the statistical relationships found between measured potentially toxic elements and microbial parameters proved to be positive. Except for Se, FDA activity correlated with all of the potentially toxic elements among which the relationship was moderate with Co, Cr, Ni, Pb, Sn and Zn whereas it was weak with As, Ba, Cd, Cu and Mo. Sucrose enzyme activity was found to be in moderate correlation with Ba and in weak with Cr, Ni, Sn and Zn. Between SIR and Ba the relationship turned out to be moderate as in the case of sucrose and it was weak with Cr, Cu, Ni, Se, Sn and Zn elements.

In the experiment of Uzinger (2010) conducted on acidic soil with low organic matter content the correlations between aqua regia soluble Cr content and FDA as well as sucrose activity proved to be moderate and downward as a consequence of high dose metal salt application and after 8 weeks of incubation. Besides, no significant effect was found as the result of Pb and Zn application. Zn, as an essential element, might have contributed to microbial processes whereas Cr application did lower pH (to pH<4,5) of the experimental soil remarkably (Szécsy et al., 2011).

Toxicity of heavy metals usually increases in inverse ratio to clay content, and also in inverse ratio to organic matter content of soil (Doelman & Haanstra, 1986; Moreno et al., 2003; Xian et al., 2015). Therefore the effects of different potentially toxic elements on microbial activity were evaluated from the aspect of soil texture, namely if there were any difference between the effects of the elements on different soil textures. Figure 5 illustrates Zn – FDA activity diagrams of the samples grouped by texture.
Figure 5. Concentration of Zn and FDA activity values of analysed soil samples grouped by texture.

These sample diagrams and the others presented only in the dissertation show increasing element concentration and microbial activity in line with increasing consistency. Moreover, decreasing effect of the elements on microbial activity can not be seen even on sandy soils, meaning no more toxic effect on sandy soils than on clay soils. The reason for this might be the originally low potentially toxic element concentration in the samples.

There is lack of comparative soil biology studies conducted on different soil types in Hungary. Earlier experiments had mostly been set up using only one soil type as well. Moreover too general consequences are being drawn from short-time laboratory experiments based on one single soil and among controlled conditions resulting in confusing achievements regarding heavy metal effects on soil biological activity (Szili-Kovács et al., 2009; Vig et al., 2003).

Publications investigating impacts of potentially toxic elements on soil microbiota usually deal with initially extremely contaminated or with artificially polluted soil samples. Regarding the latter case, the lowest element load concentrations are even higher than the concentrations of natural, unpolluted soils (Yang, 2006; Khan et al., 2007; Wang et al, 2007; Gamalero et al., 2012; Jiang et al., 2015).
It is assumed that clay content may obscure occurrent hindering effect of potentially toxic elements in statistics. According to Chodak et al. (2013) the assay of the effects of heavy metals on microbiota is difficult because of other disturbing environmental factors. Analysis of local properties of soil is crucial in the case of studying extent areas as these features might hinder possible influences of heavy metals.

Results of the dissertation suggest that regarding present cultivation practice in Hungary the concentration of potentially toxic elements in the sampled cultivated soils does not reach a concentration that could impede microbial activity. This is only true for the average, therefore they still mean risk on microbes. The possibility could not be excluded that potentially toxic element load into the soil via cultivation could be as high as to lower microbial activity in the soils of Hungary.

**New scientific results**

1. Concentration of potentially toxic elements in the sampled cultivated Hungarian soils did not show statistically provable increase as the result of present fertilizer application.
2. Correlation between the concentration of potentially toxic elements and clay content, as well as humus content – already known from soil sciences – has been detected on the sampled areas within cultivated conditions. The rates of these correlations within the elements has been different. As, Ba, Co, Cr, Cu, Ni, Sn and Zn were found to correlate with both consistency and humus content. Cd and Pb correlated only with humus content, whereas Se only with consistency of the samples.
3. According to the results of FDA and sucrose enzyme activity as well as SIR measurements of the explored cultivated areas it can be confirmed that potentially toxic element levels of the Hungarian soils besides present fertilizer use does not pose a threat to microbial activity of soils yet. It has been verified within cultivated conditions that microbial activity is higher in soils with higher humus content and higher consistency.

**Conclusions and suggestions**

On the basis of our results potentially toxic element concentration of cultivated soils in Hungary did not represent statistically provable increase as a consequence of present fertiliser use regarding several soil types. Besides chemical properties of the elements are different therefore local accumulation might still occur. The possibility could not be excluded that potentially toxic element load into the soil via cultivation could be as high as to lower microbial activity in the soils of Hungary.

Significant correlation was detected between some of the toxic elements concentrations and certain soil parameters. These correlations were probably
caused by other variables but fertiliser application as the samples were taken from several soil types. Finding definite answers has been more difficult because the element content of sampled soils before fertiliser application were not known. Although strong bound of heavy metals to clay minerals is well documented this correlation is important in this study because this might have an impact on the effects of analysed elements on soil microbiota. On the score of the examined areas it can be assumed that toxic element concentration of the Hungarian soils is mainly influenced by geochemical background values and local specifications of soil forming processes as well as by further soil features rather than toxic element load with agricultural origin.

Correlations found between potentially toxic elements and microbial activity values were all positive meaning that the analysed elements did not decrease microbial activity. Based on the studied cultivated soils the level of potentially toxic elements besides present cultivation methods in Hungary is not as high as to impede microbial activity. Results showed that microbial parameters accelerated with increasing consistency. Grouping of the samples according to their texture indicated that analysed elements did not impede microbial activity even on sandy soil samples. This means that as opposed to literature potentially toxic elements did not turn out to be more toxic on microbes on the sandy soil samples than on clay soils. Explanation of this fact and of the positive relationships between toxic elements and microbial variables should be the originally low element concentration.

The analysed soil samples were taken from cultivated conditions. In this case evaluation of the results is more difficult because of the large number of uncontrolled variables opposite to a planned and set-up experiment. Besides our version is closer to real situations. Large number of different soils is beneficial by representativity. However, this also makes the assessment of detected effects more complicated as the correlations do not represent real effects in every cases. Some soil properties, first of all humus content and clay content may hide possible effects.

High concentration of potentially toxic elements in the cultivated soils of Hungary usually originates in geology or comes from industrial, or mining sources. Monitoring of pollutions brought to the soil via fertiliser application is missing in Hungary. Maintenance of soil monitoring programmes and the monitoring of soil biological parameters has a great importance also in view of potential pollutions originating from fertilizer use. The monitoring of the latter should be long-term and in correspondence with the analysis of soil parameters. Increased attention to soils with weak production characteristics (e.g. sandy soils, acidic soils) should be paid in the course of fertilizer application. Because of evaluation problems arising during this work it would be advisable to conduct similar surveys on a long-term with control soils of the same type. Since the effects of potentially toxic elements has mostly been analysed in experiments with high doses, there is a need for assaying the effects of lower element concentrations of natural, uncontaminated conditions as well.
Related publications

ARTICLES

1. Peer-reviewed research articles
1.1. With impact factor (according to WEB OF SCIENCE), in English
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TÓTH E., GELYBÓ GY., HOREL Á., KÁSA I., SZÉCSY O., BIRKÁS M., FARKAS CS. (2014): A talajbolygatás rövid távú hatása a talaj szén-dioxid

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