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F 4883

Spatial heterogeneity of soil properties

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ABSTRACT

This paper illustrates the spatial variability of plant-available soil nutrients P, K and Mg and soil pH on two fields of an area of 54 and 67.5 ha, located around Český Brod. Both of these are orthic luvisols fields. The use of the coefficient of variation (*CV*) as a criterion of comparison for different soil properties on the two examined fields showed the following: the highest variability was in the available P; the second highest variability was in the available K; and the lowest variability of main non-mobile nutrients was in the available Mg. In addition, the lowest of all measured soil properties was in soil pH. An additional comparison method was used. This relates to the parameter of the field area proportion of different soil test levels. The spatial continuity of examined properties is illustrated on combined contour and image maps.

Keywords: soil properties; spatial heterogeneity; maps of soil variability; levels of variability

As Oliver (1999) suggested, the areas of land on the earth's surface are covered by soil, the type of which varies from place to place. This variability is caused by the interaction of independent soil forming processes. These processes are affected by numerous natural phenomena including climate, geology, relief, hydrology, micro biota and so on, all of which operate on different characteristic spatial scales. Soil properties can therefore vary at different scales of soil resolution, from millimetres to several kilometres.

James and Wells (1990), citing Waynick (1918), claim that there has long been an awareness of soil heterogeneity. The only method to evaluate the soil heterogeneity was the uniformity trial. However, although effective, its use was time-consuming (LeClerg et al. 1962 – in James and Wells 1990). This method evaluated the dividing of a field into small segments. The characteristics of each segment were then measured.

James and Wells state the general conclusion from uniformity trials to be:

- Soil fertility variations are not distributed randomly, but they are, to some degree, systematic; that is, contiuous field segments are more likely to be alike than are segments separated by some distance.
- Soil fertility is seldom distributed so systematically that it can be described by a mathematical formula.

Peck and Soltanpour (1990) suggested: in the past, before fertilizers were commonly used, it was relatively uncommon to find big differences in nutrient levels in different parts of a given field, except where extreme heterogeneity of soil types existed; now, the large differences found in nutrient levels of samples are usually not sampling or testing errors but actual variations in fertility patterns.

James and Wells (1990) divided the sources of field variability into two types: natural variation (soil-forming processes), and human variation (different cropping systems and associated fertilizer management). The degree of soil variability falls into three categories based on the variation between points separated: micro- (0–0.05 m), meso- (0.05–2 m), and macro- (> 2 m).

There is a further significant source of soil heterogeneity, caused by human variation that brings higher levels of variability. That relates to an extension of field boundaries of several smaller fields with different treatment histories. This practise creates larger fields and was very common in the Czech Republic.

It is difficult to separate different sources of spatial variability and their levels on any examined field without a recorded detailed history of the field. Overall information about the spatial distribution of the soil properties generally derives from observations, for example, soil sampling. The use of grid sampling to map soil test levels has been shown to give the most accurate maps (Frazen and Peck 1995).

Sampled properties can have large differences in the magnitude of their spatial variability. Wilding (1985) in Wollenhaupt et al. (1995) classified selected soil properties into low, medium and high categories of spatial variability according to their coefficient of variation. Wollenhaupt et al. (1995) quantified magnitudes of variability for soil properties, thus: available P has an interval of *CV* 39–157%; available K 31–61%; and soil pH 8–14%.

MATERIAL AND METHODS

In this study of spatial heterogeneity of soil properties two field sites were examined. Klučov field with an area of 54 ha was sampled in 1999 and Třebovle field with area of 67.5 ha was sampled in 2000. Both are orthic luvisols fields located in the area of Český Brod.

Soil samples were collected from soil profile (0–30 cm) using the point sampling method, with regular grid square

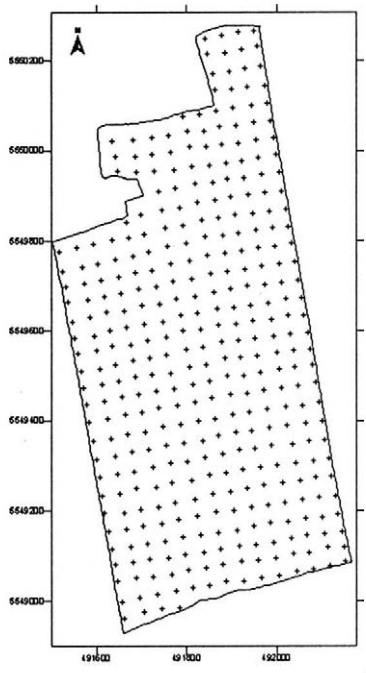

Figure 1. Soil sampling points (Klučov field)

pattern of 40 × 40 m across the whole fields. 14 individual core samples were taken from each point from a circle area with a radius of 3 m from the centre point. The total number of soil samples collected from Klučov field was 368 (Figure 1) and from Třebovle field 426 (Figure 2).

All point sample locations were recorded as x, y coordinates with GPS receiver Garmin GPS II+ in datum WGS-84. In 1999, GPS receiver was supported by the DGPS receiver RACAL (LandStar Europe) while the SA (Selective Available) error was included in GPS signals.

Both sets of samples were air-dried, the ground on 2 mm sieve and manually homogenized. To determine the available P, K and Mg Mehlich III solution method was used. Mehlich III is an official method of soil testing in the Czech Republic including soil test levels classification (intimation no. 477/2000). The Scalar (San System) segmented continuous flow analysis with photometric detector was used for the detection of P. The Spectr AA-300 (Varian) atomic absorption spectrometer was used for the detection of K and Mg. Soil pH was determined in 0.2 M KCl extract. An Acidimeter (Druopta) with a glass and calomel electrodes was used to determine pH.

All statistical analyses were processed in software SAS v. 8.00 (SAS Institute). Map plots were processed in software Surfer v. 7 (Golden Software) with the default girding method settings (interpolation – kriging with the default linear variogram).

Quality assurance of analytical data

Since there is a lack of reference material for the Mehlich III extraction method, other samples were used to evaluate the quality of the analytical data:

- repeated measures of one extraction of one sample weight from examined field soil sample (ISS – Internal Soil Sample from examined field)
- repeated measures of different weights of homogenized soil sample from the examined field (ISS – Internal Soil Sample from examined field)
- repeated measures of one extraction from internal reference material, soil sample from the International Soil-Analytical Exchange organized by WEPAL (1999), Wageningen, The Netherlands (IRM – Internal Reference Material); the median of results of the determined available K from published laboratories for this sample was 175 ppm with MAD 13.0 and for available Mg 231 ppm with MAD 21.0 in report 99.3

Quality assurance samples were posted at the beginning, in the middle and at the end of the main analysed sample sets to check analytical accuracy during the whole measuring process.

For statistical calculations of data from additional analysis median and MAD (median of absolute deviations) were used since in this case abnormal values have less influence on the estimated central value and the spread of this value. Another reason is that these small

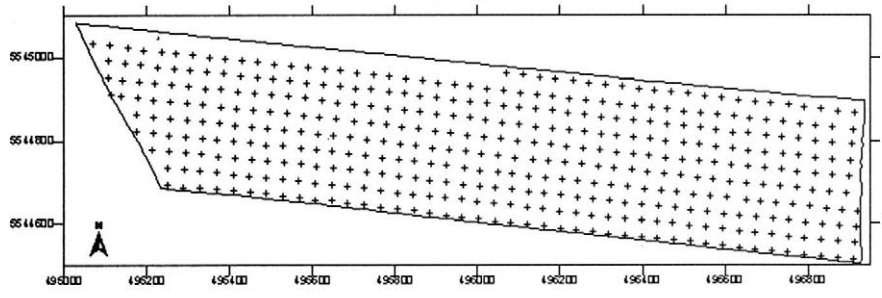

Figure 2. Soil sampling points (Třebovle field)

Table 1. The results of the quality assurance of the analysed data

Properties	Statistics	Klučov set				Třebovle set	
		n	ISS-repetition	*n*	ISS-weights	*n*	IRM-repetition
P	median ± MAD	9	35 ± 1	4	33 ± 2	–	a
ppm	*Z*-score		–		–		–
K	median ± MAD	29	212 ± 5	5	219 ± 10	9	171 ± 2
ppm	*Z*-score		–		–		1.95
Mg	median ± MAD	29	133 ± 2	5	136 ± 3	9	228 ± 8
ppm	*Z*-score		–		–		0.37
pH	median ± MAD	–	a	10	7.3 ± 0.3	–	a
	Z-score		–		–		–

n – number of samples, a – not measured

data sets do not usually have normal distribution. The *Z*-score was applied for the accuracy evaluation of the measured data with analysed internal reference material. For the Z-score the following formula was used:

$$Z = \frac{Y - Y(m)}{MAD}$$

where: Y is the reported value and $Y(m)$ is median of measured values; for evaluation of the accuracy was used *Z*-score classification with $|Z| \leq 2$ satisfactory, $2 < |Z| < 3$ questionable and $|Z| \geq 3$ unsatisfactory intervals

RESULTS AND DISCUSSION

It is problematic to attempt a separation of levels of spatial variability in different sources (natural variation and human variation) without detailed records of field history. However, it is possible to quantify the variability of the analytical system.

The statistical results of repeated measures of one extraction of soil sample from the examined field in Table 1 indicate very low deviation. A slightly higher deviation of repeated measures was indicated in different weights of one soil sample from this examined field. This deviation is influenced by analytical error as well as soil sample homogeneity. In addition to the Třebovle soil sample set analysis for K and Mg, IRM soil samples were used. This allowed to be *Z*-scores calculated. Both *Z*-score results 1.95 for K in Table 1 and 0.37 for Mg were under the level 2, which proves that the analysis of the main sets was processed with satisfactory accuracy. These validation analyses proved that the error in the analytical system is not the source of measured data variation.

For each field data set, a summary of statistics was calculated (Tables 2 and 3). The coefficient of variation (*CV*%) was used to compare the levels of variability of different soil property on both fields. The disadvantage of the use of this parameter is that parametric statistics require normal distribution of the measured data. As an additional comparison, the parameter of proportion of field area for different soil test levels was used. This parameter describes the spatial cover of the field belonging to different levels of the soil tests but does not include information on whether the total amount of area counted in some level is a compound of many smaller areas or if it is larger part of the field. This parameter also helps to better describe and understand the average statistic characteristics of some measured property for the whole field.

The spatial continuity of the soil properties is visually illustrated on the interpolated image maps combined with contour maps showing the boundaries of soil test properties for different levels. In the maps plotted in this paper, smaller or more minor areas of different test levels surrounded by a bigger area appear, which is an indication of the high resolution of the maps. That is to say the high sampling density.

Table 2. Summary statistics of Klučov field sets

Variable/parameter	P (ppm)	K (ppm)	Mg (ppm)	pH
Average	25.6	211	141	7.2
Standard deviation	14.3	85.9	21.2	0.35
Coefficient of variation (%)	56	41	15	5
Minimum	4	101	90	5.7
Median	22	192	139	7.3
Maximum	105	953	258	7.6
Skewness	1.69	3.49	1.48	–2.16

Table 3. Summary statistics of Třebovle field sets

Variable/parameter	P (ppm)	K (ppm)	Mg (ppm)	pH
Average	34.1	141	121	6.4
Standard deviation	13.5	35.8	16.9	0.55
Coeficient of variation (%)	39.5	25.4	14	8.7
Minimum	10.4	58.9	68.4	5
Median	31.3	136	122.4	6.4
Maximum	90.9	351	162.5	7.3
Skewness	1.04	1.86	–0.28	–0.35

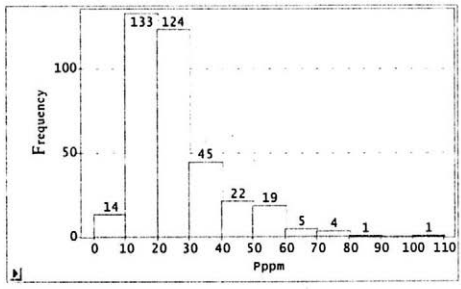

Figure 3. P data set histogram (Klučov field)

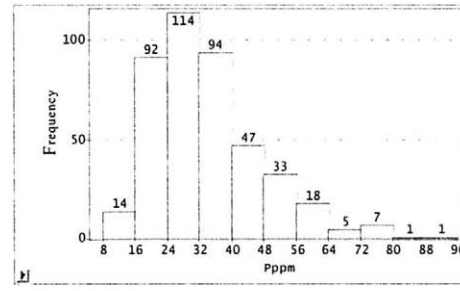

Figure 4. P data set histogram (Třebovle field)

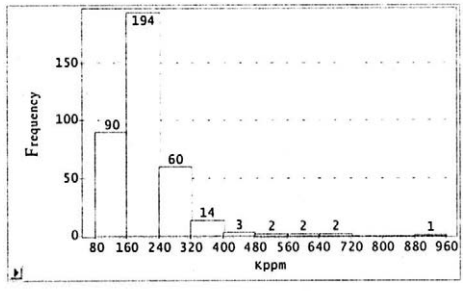

Figure 5. K data set histogram (Klučov field)

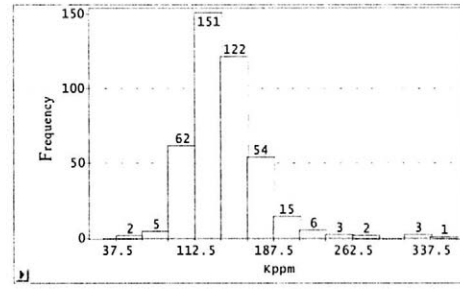

Figure 6. K data set histogram (Třebovle field)

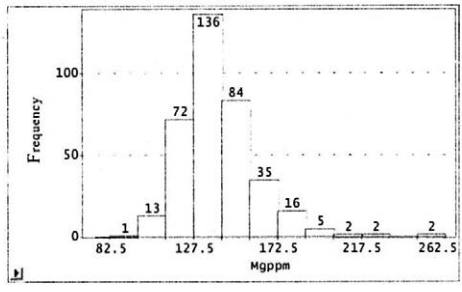

Figure 7. Mg data set histogram (Klučov field)

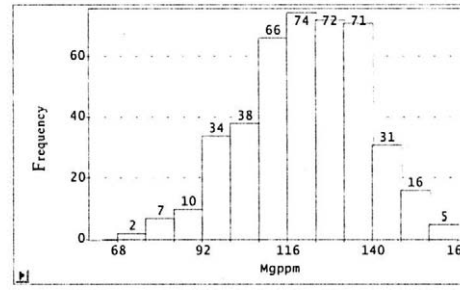

Figure 8. Mg data set histogram (Třebovle field)

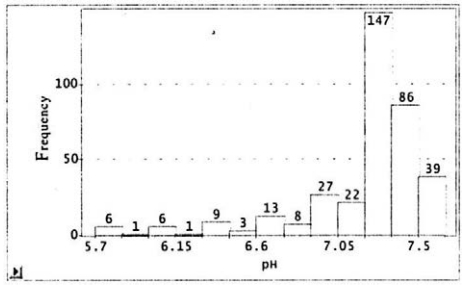

Figure 9. pH data set histogram (Klučov field)

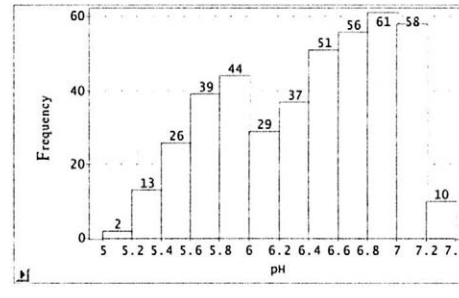

Figure 10. pH data set histogram (Třebovle field)

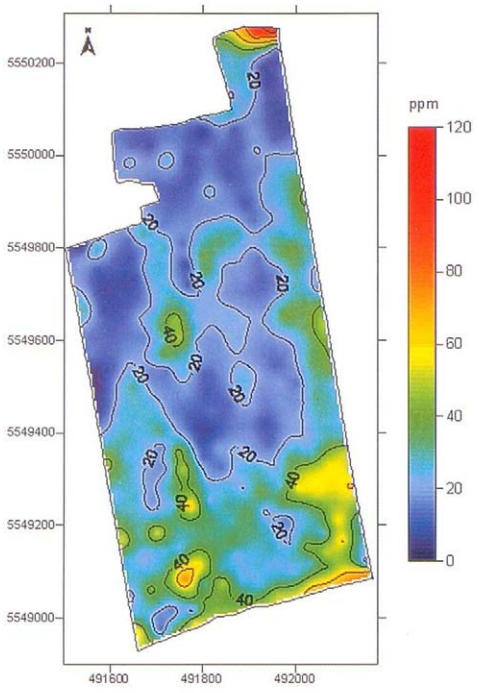

Figure 11. Soil available P map (Klučov field)

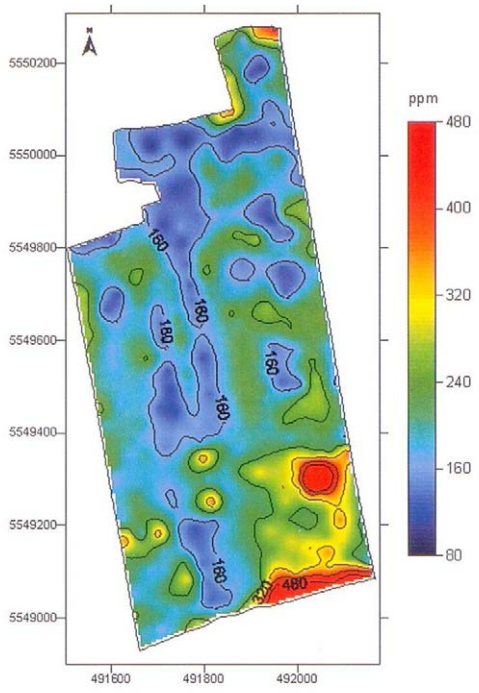

Figure 12. Soil available K map (Klučov field)

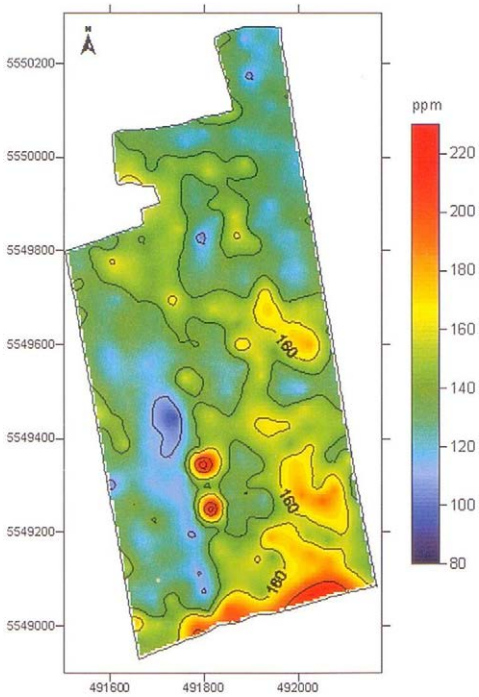

Figure 13. Soil available Mg map (Klučov field)

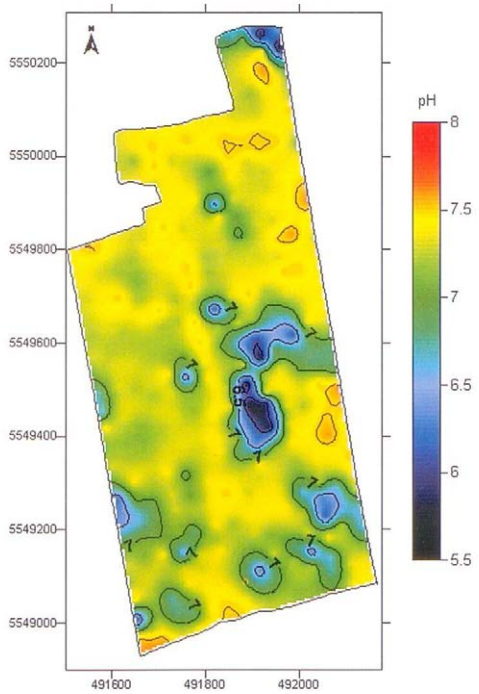

Figure 14. Soil pH map (Klučov field)

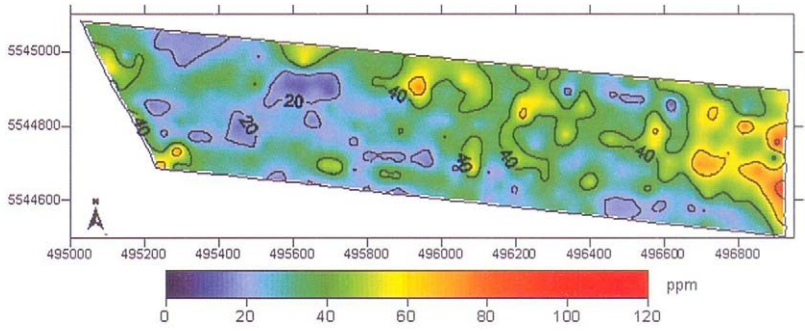

Figure 15. Soil available P map (Třebovle field)

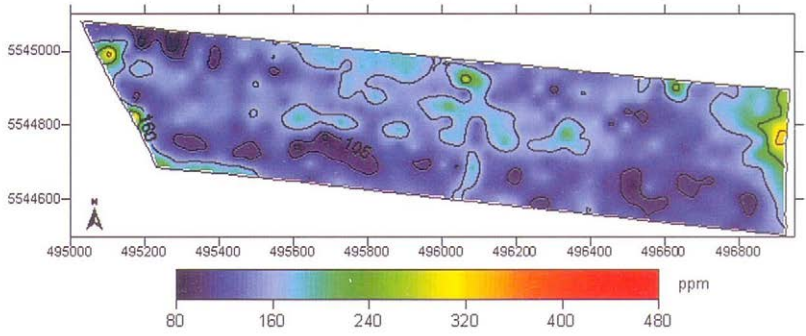

Figure 16. Soil available K map (Třebovle field)

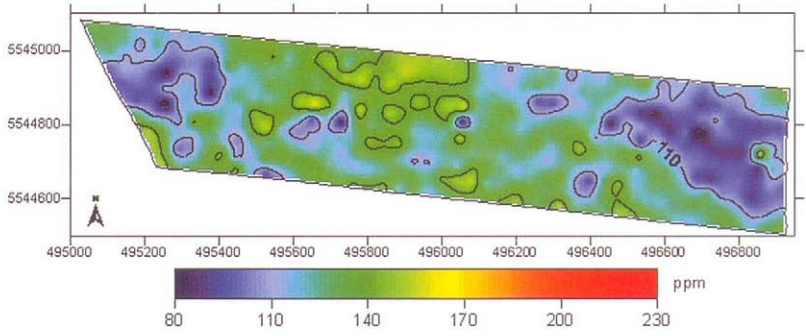

Figure 17. Soil available Mg map (Třebovle field)

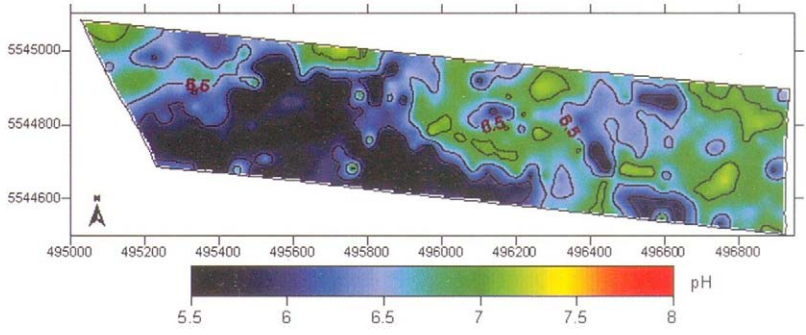

Figure 18. Soil pH map (Třebovle field)

Table 4. Proportions of field area for different soil test levels on Klučov field

ppm P	< 20	20–40	> 40
Area (ha)	22	26.2	5.9
%	41	48	11
ppm K	< 160	160–320	> 320
Area (ha)	10.9	40.6	2.6
%	20	75	5
ppm Mg	< 110	110–160	> 160
Area (ha)	0.7	46.9	6.5
%	1	87	12
pH	< 6.5	6.5–7.2	> 7.2
Area (ha)	2.3	17.2	34.6
%	4	32	64

Table 5. Proportions of field area for different soil test levels on Třebovle field

ppm P	< 20	20–40	> 40
Area (ha)	4.7	44.6	18.2
%	7	66	27
ppm K	< 160	160–320	> 320
Area (ha)	53.3	14	0.2
%	79	21	0
ppm Mg	< 110	110–160	> 160
Area (ha)	15.6	51.9	0
%	23	77	0
pH	< 6.5	6.5–7.2	> 7.2
Area (ha)	36.1	26.7	4.7
%	53	40	7

A comparison of soil properties variability (*CV*) according to published results (Wollenhaupt et al. 1995) shows that Klučov field set results (P 56%, K 41% and pH 5% in Table 2) are all inside given intervals. Třebovle field set results in Table 2 have a soil P coefficient of variability 39.5%, just at the lower border of given *CV* interval of P (39–157%) and the coefficient of variability for K data 25.4% is lower than the given interval (31–61%). The result of the soil pH coefficient of variability 8.7% was inside the given interval (8–14%).

An average value of the P data set in Table 2 together with histogram (Figure 3) show that major parts of Klučov field have a very low P test level according to classification. A slight asymmetry of P data set distribution (Figure 3) quantified in the parameter of skewness (1.69) in Table 2 appears in the P map as smaller areas of higher concentration of available P. The total area with P level over 40 ppm is then only 11% of the whole field area (Table 4). The Klučov field P map (Figure 11) shows that areas of higher test levels (over 40 ppm of P, 11% area of field) are in the southern part of the field. The northern small erratic area of high values is caused by samples taken close to the field boundary where it is not representative for the whole northern part of the field. The average value of the P data set of the Třebovle field in Table 3 has a slightly higher value compare to the Klučov field P average but still according to the Mehlich III classification falls in very low soil P test level. Only 7% area of the field (Table 5) has P test values less than 20 ppm. Třebovle field P map (Figure 15) shows higher values (over 40 ppm P, 27% area of the field) mainly in the east part of the field. Figure 4 shows slight asymmetry of P test values in Třebovle field set illustrated with the coefficient of skewness 1.04 in Table 3. P values of Třebovle field set have a lower level of variation (*CV* 39.5%) compare to Klučov field, but higher average value of soil P test.

The results of available K analysis of the Klučov field set in Table 2 indicate high range of the data values, which cover low, medium, high and very high soil K test levels according to the Mehlich III classification. This data set has a positive asymmetry of the distribution (Figure 5) illustrated with a high value of the coefficient of skewness 3.49 in Table 2. There are several point samples with K test over 320 ppm which appear on the map of K (Figure 12) either as small erratic areas in the south-west and the north-east parts of the field or as a bigger area in the south-east part of the field. The average value of soil K test (Table 2) falls in the medium level according to Mehlich III classification and also 75% area of this field (Table 4) has medium K test level. The coefficient of variation of available K on the Klučov field reached only 41% despite the high range of the data. Třebovle field K test indicate low level of variability (*CV* 25.4%), low average value of K data set with 79% area of the field with a low level of available K according to the Mehlich III classification. Třebovle field K map (Figure 16) illustrates low variability with several erratic higher values at the boundary of the field. The histogram (Figure 6) of this field K data set shows a slight asymmetry with the coefficient of skewness 1.86 (Table 3).

The statistical results of Mg on both fields show a lower level of variability (Klučov set with *CV* 15% and Třebovle set with *CV* 14%). This indicates, that there is not effect of the different cropping history and Mg fertiliser was not used. The ranges of the data sets fall into the intervals of very low and low-test levels according to the Mehlich III classification. The average values of both sets fall into low Mg test level. Klučov field has 87% area of a low Mg test level and Třebovle field has 77% area of a low Mg test level (Table 5). Klučov field Mg map (Figure 13) shows higher values in the south-east part of the field. Třebovle field Mg map (Figure 17) shows areas of lower values in the eastern part of the field. The histogram of the both data sets in Figures 7 and 8 indicates fairly normal distribution.

Soil pH test on the Klučov field has the average value 7.2 with a very low level of variation (*CV* 5%) in Table 2. This data set has a higher negative asymmetry of the data distribution (Figure 9) with the coefficient of skewness –2.16 (Table 1). This negative asymmetry is caused by a minor number of soil samples, which tested values under pH 6.5 (4% area of the field). Klučov field pH map

(Figure 14) shows several smaller areas with values under the value 6.5 spread mainly in the southern part of the field. Třebovle field set tests the average soil pH 6.4 (Table 3), which is a slightly lower compared to Klučov data set. The coefficient of skewness of the Třebovle field set –0.35 (Table 3) indicates very low negative asymmetry of the data. The double peak histogram (Figure 10) with the Třebovle pH data set indicates that this data set is compound of two different distributions. The Třebovle field map of pH (Figure 10) shows that the eastern part of the field test mainly lower values of pH (53% area of the field tests pH under 6.5) and the western part of the field tests higher values from 6.5 to 7.3. This indication suggests that the field might be a compound of two different fields with extended boundaries.

CONCLUSIONS

The results of the two examined fields of 54 and 67.5 ha area indicate that all analysed soil properties have different degrees of spatial variability according to the soil property. Generally, the highest variability in the analysed soil properties is that of available P; the second highest level is that of variability has available K; a lower variability is indicated in that of available Mg; and the lowest variability has soil pH. The coefficient of variation (*CV*) as a criterion of comparison has been used.

Also, the levels of the spatial variability differ from field to field. Klučov field has higher variability (*CV*) of soil available P and K, while Třebovle field has a slightly higher level of variability of soil pH. The variability of soil available Mg is basically at the same level in both fields.

By the way of an additional comparison, the parameter of the proportion of the field area for different soil test levels helps to understand the average value calculated for the whole field. The spatial continuity of the soil properties is illustrated visually on the interpolated maps.

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ABSTRAKT

Prostorová heterogenita agrochemických vlastností půd

Na dvou pozemcích v oblasti Českého Brodu o výměrách 54 a 67,5 ha byla sledována prostorová variabilita rostlinám přístupných živin v půdě P, K, Mg a půdního pH. Oba pozemky patří do klasifikace hnědozem typická. Pro srovnání různých půdních faktorů na obou sledovaných pozemcích byl použit koeficient variability (*CV*). Ukázalo se, že nejvyšší variabilitu vykazoval fosfor, druhou nejvyšší úroveň variability draslík a nejnižší variabilitu z měřených přístupných živin hořčík. Nejnižší variabilitu ze všech měřených půdních faktorů mělo půdní pH. Pro další srovnání prostorové variability byl stanoven podíl plochy pozemku přísluřející k vymezené úrovni měřeného faktoru. Prostorové rozložení měřených půdních faktorů je znázorněno v kombinovaných vrstvách konturové a rastrové mapy.

Klíčová slova: půdní faktory; prostorová heterogenita; mapy půdní variability; úrovně variability

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The differences in the interpolation methods for mapping spatial variability of soil properties

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ABSTRACT

In this study kriging and inverse distance weighting methods were used to compare their performance. Ordinary kriging with the default linear variogram and with the modelled variogram was implemented. Inverse distance weighting method with powers p = 1, 2, and 4 was used. Two data sets of high sampling density on the field of an area of 54 ha with a higher and a lower variability were used in the comparison. The results show that the choice of the interpolation method for characterizing the spatial heterogeneity of the soil properties does affect the resulting map. Kriging with the modelled variogram produce smoothed maps, while kriging with the default linear variogram show all levels of the measured values. Inverse distance weighting method tends to generate maps with a pattern of round areas, which size is strongly dependent on the used value of the power p.

Keywords: soil properties; mapping spatial variability; interpolation methods; kriging; inverse distance weighting

The accuracy of spatial maps of soil properties depends on many factors such as sample density, sample configuration and interpolation method (Gotway et al. 1996). Since the point sampling method is used for the spatial description of soil heterogeneity, it is necessary to use some of interpolation methods to predict values in areas we have not sampled. Gridding, or interpolation fills holes (not sampled points) by interpolating or extrapolating Z values at the locations where no data exists using the measured data values (Golden Software 1999).

Kriging and inverse distance weighting methods are commonly used interpolation techniques to obtain the spatial maps. Isaaks and Srivastava (1989) suggested, that using point estimation some method perform very well according to some criteria, while they may not do as well according to other criteria.

While the interpolation technique influence the resulting spatial description, it is important to assess the differences of the gridding methods. Kriging and inverse distance weighting methods are linear interpolators where predictor at any location is assumed to be a linear combination of the available data surrounded by the estimated point:

$$z(x_0) = \sum_{i=1}^{n} \lambda_i z(x_i)$$

where: z signifies estimate at x_0 point, n is the number of points used to interpolate at each node, λ_i are weights and $z(x_i)$ are z values at the i^{th} point; the methods differ on the choice of the weights λ_i

With the inverse distance weighting method approaches, data are weighted during interpolation such as that the influence of one point relative to another declines with the distance from the grid node (Golden Software 1999). The weights for inverse distance weighting method are given by

$$\lambda_i = \frac{[d(x_i, x_0)]^{-p}}{\sum_{i=1}^{n} [d(x_i, x_0)]^{-p}}$$

where: $d(x_i, x_0)$ is the distance between x_i and x_0; weighting power, p, controls how fast the weights tend to zero as the distance from the grid node increases

In kriging, data carry different weights according to their positions both in relation to the unknown point and to one another (Oliver and Webster 1991). To obtain the kriging weights variogram must be modelled, which provides knowledge about the underlying spatial relationships in a data set as well. Kriging incorporate distance through the semivariance in the variogram:

$$\gamma[d(x_i, x_0)] = \mathrm{var}[z(x_i) - z(x_0)]$$

The weights for the estimates are chosen to minimize the estimation variances. Unbiasedness of the estimations is given by the equation:

$$\sum_{i=1}^{n} \lambda_i = 1$$

Theoretical details of the minimization procedure can be found in Isaaks and Srivastava (1989).

MATERIAL AND METHODS

Geostatistical methods for mapping soil properties, kriging and inverse distance weighting (IDW), were used to compare their performance. Two data sets from one examined field site (Klučov field) were used in the comparison.

First data set is the result of determined plant available P in soil with high level of variability (*CV* 56%) and the second is the result of determined plant available Mg in soil with low level of variability (*CV* 15%). These data sets are based on regular square point sampling, where the spacing between points is 40 m in both the north-south and the east-west directions. Detail description of soil sampling, methods of the data determination and analytical quality assurance is in Brodský et al. (2001).

Ordinary point kriging with default linear variogram (denoted K1), ordinary point kriging with modelled variogram (denoted K2) and inverse-distance weighting methods with distance powers $p = 1$, $p = 2$, and $p = 4$ (denoted IDW1, IDW2, and IDW4) were implemented with both data sets. The default linear variogram in Surfer 7 software is supposed to be gridding method, which gives good results for most XYZ data sets. An exponential variogram model was used to fit empirical variogram for both data sets (Figures 1 and 2) using GS+ v. 5 (Gamma Design Software). All estimations made with inverse distance weighting method were obtained using GS+ v. 5 (Gamma Design Software). Since the typical value of the power p used for IDW is $p = 2$, $p = 1$ as smaller value and $p = 4$ as a higher value were used to evaluate differences in the estimations influenced by chosen value of the power.

All maps were plotted from estimated grid data at points located on a regular square grid of 5 m. For evaluation differences of the performance of the interpolation methods residuals were computed for each grid data file of 40 m grid. The formula used to compute a residual value is:

$$z_{res} = z_{dat} - z_{grd}$$

where: z_{res} is the residual value, z_{dat} is the z value in the input data file (measured value), and z_{grd} is the z value of the surface at the XY coordinate from the grid file (after interpolation)

The summary statistics include ranges of the estimate values for the different interpolations were calculated. An additional parameter, field area proportion of different soil test levels, was used to evaluate changes in plotted maps of the two data sets with the different interpolation technique.

RESULTS AND DISCUSSION

For each data set six different interpolation methods were used. The univariate description of the measured data (input file) and the estimates (calculated from the residuals) are summarized in Table 1 for P data set and in Table 2 for Mg data set.

These results indicate that the choice of the interpolation method has no influence on the average value of the calculated estimates compared to the measured data. There was only slightly lower average of P data for inverse distance weighting interpolation method with power $p = 1$ indicated in Table 1. Using non-parametric statistics, the median calculated for P data after kriging with modelled variogram indicated slightly higher value (24 ppm) compared to the measured data in Table 1. The results for Mg data did not show any slight differences. Since the all used interpolation techniques are linear interpolators, the correlation coefficients for these methods indicate very high value without any significant differences.

The differences were indicated in the parameters describing variability and the range of the data. Generally, these parameters show that implementation of the interpolation methods lower coefficient of variation and the range of the data, or reach the same level. The coefficient of variation 37% for P data set and kriging with the modelled variogram together with the range 45 ppm indicate the highest reduction in calculated estimates (Table 1). This is caused by the nugget effect in the modelled variogram. The same situation became for Mg data set in Table 2. Kriging with the modelled variogram showed the highest reduction in calculated estimates. The coefficient of variation changed from 15 to 11% and the range from

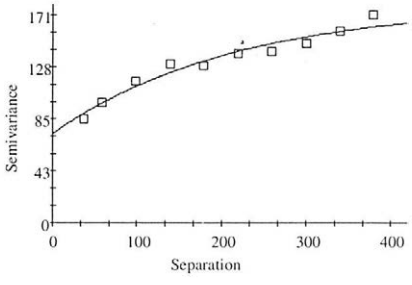

Figure 1. P data set empirical variogram and fitted model; the fitted exponential model has parameters: a nugget effect of 73.2 ppm², a sill of 179.8 ppm², and a range of 278 m

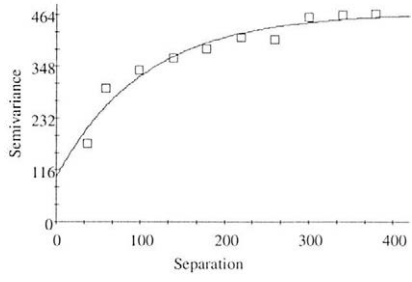

Figure 2. Mg data set empirical variogram and fitted model; the fitted exponential model has parameters: a nugget effect of 100 ppm², a sill of 465.7 ppm², and a range of 104.4 m

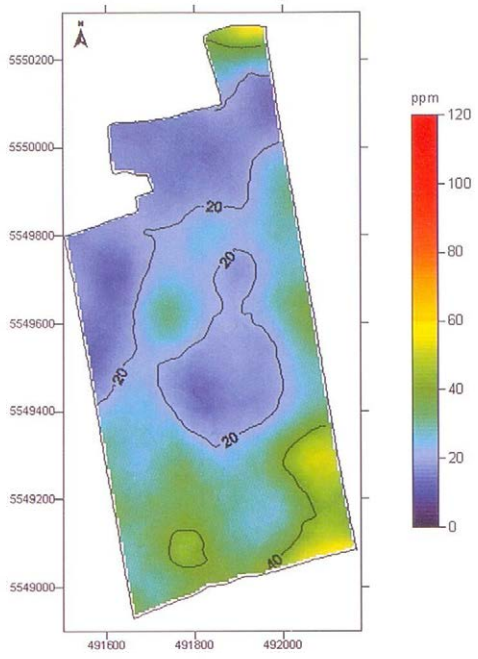

Figure 3. P map, kriging with modelled variogram

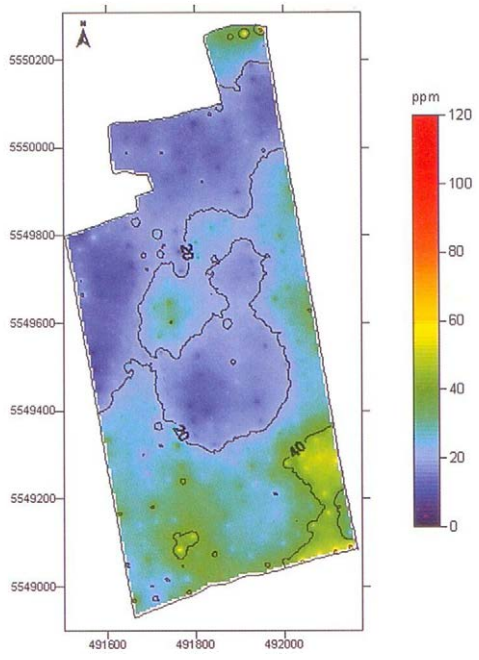

Figure 4. P map, inverse distance weighting (p = 1)

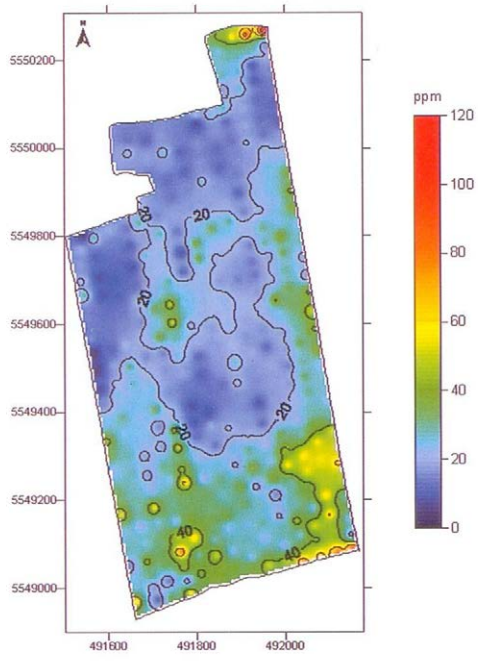

Figure 5. P map, inverse distance weighting (p = 2)

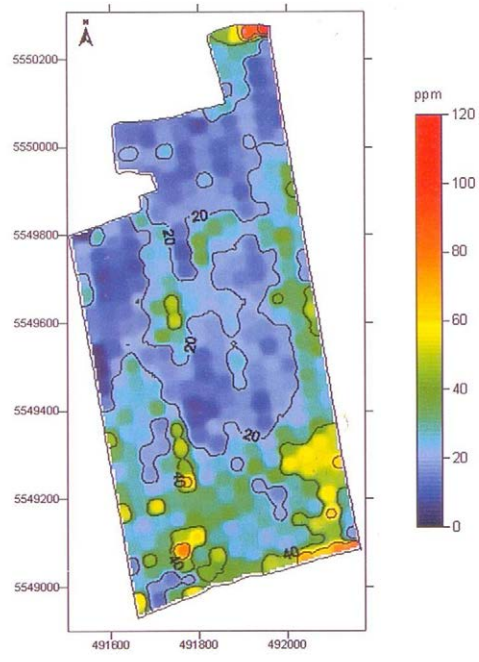

Figure 6. P map, inverse distance weighting (p = 4)

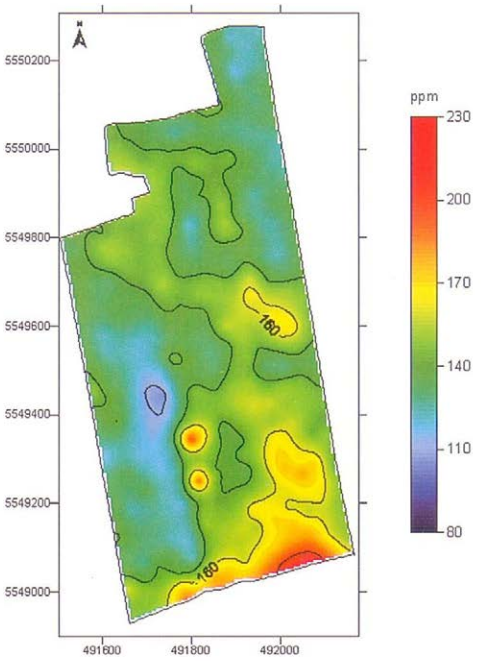

Figure 7. Mg map, kriging with modelled variogram

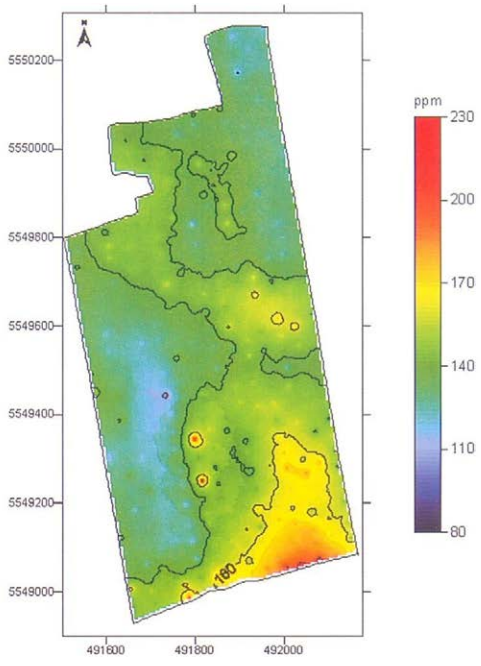

Figure 8. Mg map, inverse distance weighting (p = 1)

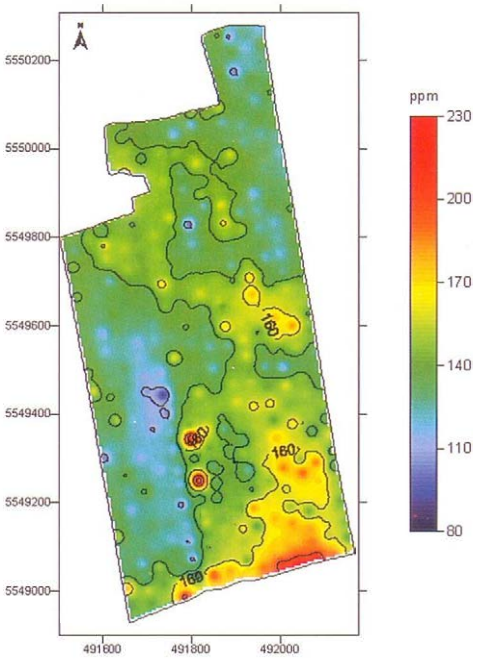

Figure 9. Mg map, inverse distance weighting (p = 2)

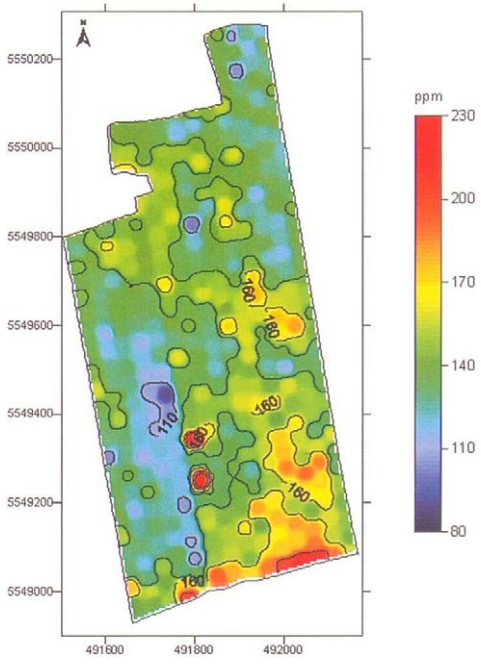

Figure 10. Mg map, inverse distance weighting (p = 4)

Table 1. Summary statistics of measured available P data and residuals for different interpolation methods

Parameter	Origin data	Interpolation method				
		K1	K2	IDW1	IDW2	IDW4
Average	25.6	25.7	25.5	24.5	25.5	25.6
Standard deviation	14.3	13.8	9.5	10.8	13.9	14.3
CV	56	54	37	44	55	56
Min	4	4	11	5	4	4
Median	22	22	24	22	22	22
Max	105	102	57	66	101	105
Range	101	98	45	61	97	101
Correlation coefficient		0.999	0.96	0.968	0.999	0.999

K1 – kriging with the default linear variogram
K2 – kriging with the modelled variogram
IDW1 – inverse distance weighting method with power $p = 1$
IDW2 – inverse distance weighting method with power $p = 2$
IDW4 – inverse distance weighting method with power $p = 4$

168 to 111 ppm. The observed reduction for kriging interpolation method with the default linear variogram was very low.

The differences in inverse distance weighting interpolation method are dependent on the value used for power p. The results show that the highest change is in situation with power $p = 1$. Coefficient of variation in P data set changed from 56 to 44% (Table 1) and in Mg data set changed from 15 to 12% (Table 2). The range of P data set changed from 101 to 61 ppm and range of Mg data set changed from 168 to 119 ppm. These reductions in both data sets decrease with the increase of the used value of the power p. For inverse distance weighting interpolation method with power $p = 2$ and P data set was a slight reduction in the coefficient of variation 55% and the range 97 ppm indicated. For Mg data set was not any change in the coefficient of variation indicated, while the range of the data changed from 168 to 160 ppm. When the value of power $p = 4$ was used, not any changes in the coefficient of variation, nor in the range were indicated.

The field area proportion of different soil test levels, as an additional parameter, was used to evaluate changes in plotted maps with the different interpolation technique. The results for P data set are in Table 3 and for Mg data set are in Table 4.

Because of the lack of true map for different interpolation techniques comparison, one of the interpolated maps has to be chosen. According to the literature, kriging with the default linear variogram is one of the more flexible methods and is useful for gridding almost any type of data set. Also changes in the data set (residuals) after gridding are very slight. The maps from this interpolation method can be chosen to compare other techniques.

Generally, there were not enormous changes indicated despite the visual changes in the distribution maps. In the P data set was for kriging with the modelled variogram and the area calculated for values under 20 ppm indicated reduction from 41% (calculated from map with kriging with default linear variogram) to 36% of total field area. The area calculated for values over 40 ppm in P data set and K2 method was reduced from 11 to 8%. Consequently, area for interval between 20 to 40 ppm indicates the highest value 56% (part of field area) of all used interpolation methods. The highest reduction of the area cal-

Table 2. Summary statistics of measured available Mg data and residuals for different interpolation methods

Parameter	Origin data	Interpolation method				
		K1	K2	IDW1	IDW2	IDW4
Average	141.4	141.5	141.5	141.5	141.4	141.4
Standard deviation	21.2	20.3	16.1	16.6	20.7	21.2
CV	15	14	11	12	15	15
Min	90	91	105	101	91	90
Median	139	139	139	139	139	139
Max	258	245	216	220	251	258
Range	168	154	111	119	160	168
Correlation coefficient		0.999	0.939	0.968	0.999	0.999

Table 3. Field area proportion for different P test levels and interpolation methods

ppm P	< 20	20–40	> 40
K1			
Area (ha)	22	26.2	5.9
%	41	48	11
K2			
Area (ha)	19.7	30.1	4.2
%	36	56	8
IDW1			
Area (ha)	23.2	28.2	2.6
%	43	52	5
IDW2			
Area (ha)	22.8	27	4
%	42	50	7
IDW4			
Area (ha)	23.1	25.3	5.5
%	43	47	10

Table 4. Field area proportion for different Mg test levels and interpolation methods

ppm Mg	< 110	110–160	> 160
K1			
Area (ha)	0.7	46.9	6.5
%	1	87	12
K2			
Area (ha)	0.2	47	5.8
%	0	87	11
IDW1			
Area (ha)	0	49	5
%	0	91	9
IDW2			
Area (ha)	0.3	48	5.7
%	1	89	11
IDW4			
Area (ha)	0.9	46.7	6.4
%	2	86	12

culated for values over 40 ppm was indicated for inverse distance weighting interpolation method, from 11 to 5%. For this interpolation method and P values situation over 40 ppm shows that with the increase of the power p the reduction of the field area proportion of different soil test levels decrease.

In the Mg data set (Table 4) was the area calculated for values under 110 ppm very small. For kriging with the default linear variogram was calculated area of 0.7 ha, 1% of the whole field area, for kriging with the modelled variogram then only 0.2 ha, and for inverse distance weighting method with power $p = 1$ was even not indicated at all. For values of Mg between 110 and 160 ppm was the highest change indicated with inverse distance weighting method with power $p = 1$, from 87% for K1 situation to 91% of whole field area. For values of Mg over 160 ppm was the highest reduction of the field area calculated for inverse distance weighting method with power $p = 1$, from 12 to 9%. For the situation of IDW interpolation method, the reduction of field area proportion of Mg values (over 160 ppm) decreases with the increase of the power p. Consequently, the field area calculated for IDW method with power $p = 4$ has the same value (12%) as the use of kriging with the default linear variogram. Both kriging interpolation methods indicated only very slight differences with Mg data set.

The spatial distribution of the data is illustrated in the maps. For kriging with the default linear variogram and P data set is map in Brodský et al. (2001, Figure 11). Map for kriging with the modelled variogram with P data set is in Figure 3. Maps for inverse distance weighting method and P data set are in Figure 4 for power $p = 1$, Figure 5 for power $p = 2$, and Figure 6 for power $p = 4$. Spatial map for kriging with the default linear variogram and Mg data set is map in Brodský et al. (2001, Figure 13). Map for kriging with the modelled variogram with Mg data set is in Figure 7. Maps for inverse distance weighting method and Mg data set are in Figure 8 for power $p = 1$, Figure 9 for power $p = 2$, and Figure 10 for power $p = 4$.

There are obvious visual differences between the maps plotted with different interpolation methods. These differences are emphasized with the used contour map layer. In the maps of kriging with the default linear variogram is shown that small erratic areas (local extremes) surrounded by contour lines appear as a part of bigger area. The kriging with the modelled variogram creates smoothed maps. This smoothing effect is caused by the nugget effect in the modelled variogram. Maps are divided in larger areas surrounded by contour lines of some level. Small erratic areas in the previous maps, usually created from one point sample of extreme value without support from close surrounded samples, disappeared in the maps where the modelled variogram with the nugget effect was used.

The maps plotted with the inverse distance weighting interpolation method implemented are similar to some degree. The similarity is in round areas appearance in the maps, while the main difference is in the size or area of the circles. The size of these areas increases with the increases of the power p. Another result of this interpolation method's implementation is that with power $p = 1$, local extremes (small erratic areas) appear in the maps as small dots. Larger areas belonging to some soil test level are very similar to those in the maps of kriging with the modelled variogram. The contour lines plotted with inverse distance weighting method and power $p = 1$ are not as smooth as with the kriging method.

CONCLUSIONS

In this study, two data sets of high sampling density on the field of an area of 54 ha with higher and lower variability were used to compare performance of the five

different interpolation methods. The results show that the choice of the interpolation method for characterizing the spatial heterogeneity of the soil properties does affect the resulting map.

The use of the different interpolation technique has not any influence on the parameters of the average and the correlation coefficient. In the data set with the higher coefficient of variation, the higher changes in parameter of variability occur as well as in the parameter of the field area proportion of different soil test levels. The visual differences in the plotted maps are obvious in the changes of the small erratic areas, local extremes.

All maps constructed from some interpolation methods are to some degree subjects of uncertainty. The reason of the mapping of measured properties should be considered before some of the interpolation methods is chosen.

Kriging with the modelled variogram produces smoothed maps, where the field is divided into larger areas of some level of the measured property. Kriging with the default linear variogram shows all levels of the measured values. Smaller areas in larger areas do not indicate any repeated patterns in the maps caused by the used interpolation method. The size and the shape of the areas is simply dependent on the input data. Changes in the estimated values (calculated from the residuals) were very small. Inverse distance weighting method tends to generate maps with a pattern of round areas, which size is strongly dependent on the used value of the power p.

There are also differences in the ease of the implementation of the interpolation methods. The lack of automation and the subjectivity in variogram model selection disadvantage kriging with the modelled variogram. The important advantage of the kriging with the modelled variogram method is that it allows a measure of the error in each estimated point. The use of the default linear variogram in kriging makes this method fully automated, but the above-mentioned advantage of this method cannot be used. The advantage of the inverse distance weighting method is its ease of automation. The parameter, which has to be chosen, is the power p. While the changes with the different value of the power p are obvious, the effects have to be considered.

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ABSTRAKT

Rozdíly v interpolačních metodách pro mapování prostorové variability vlastností půd

Pro srovnání rozdílů interpolačních technik byly použity metody kriging a inverse distance weighting. Běžný kriging byl použit s předem nastaveným lineárním variogramem a s modelovaným variogramem. Metoda inverse distance weighting byla použita s nastavenými parametry power p = 1, 2 a 4. Pro srovnání těchto metod byly využity dva soubory dat s vysokou a nízkou úrovní variability. Data byla získána z pozemku o výměře 54 ha s vysokou hustotou vzorkování. Výsledky ukázaly, že výběr interpolační metody ovlivňuje výslednou mapu popisující prostorové rozložení měřeného faktoru. Kriging s modelovaným variogramem vytváří vyhlazenou mapu, přičemž kriging s nastaveným lineárním variogramem zobrazuje všechny úrovně měřených dat. Interpolační metoda inverse distance weighting vytváří mapy s opakujícím se vzorem ploch kruhového tvaru, jejichž velikost závisí na zvoleném parametru power p.

Klíčová slova: půdní faktory; mapování prostorové variability; interpolační metody; kriging; inverse distance weighting

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Determination of cytotoxicity of selected liquid fertilizers by pollen bioassay

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ABSTRACT

Pollen from *Brassica campestris* L. subsp. *oleiferae* DC. cultivar Toria cultivated *in vitro* conditions is useful for bioassay of xenobiotics. Test of toxic effect by using germination of pollen grains (male gametophyte) is very simple method. Maximum tested dose for pollen biotest was calculated from field dose. For liquid nitrogen fertilizer DAM 390 (Urea Ammonium Nitrate Solution, UAN) toxic values were calculated LD_{50} 4.25 $mL.kg^{-1}$ pollen, LC_{90} 22.9 $\mu L.mL^{-1}$ cultivated medium and NOEC 9.54 $pL.mL^{-1}$ cultivated medium. For liquid fertilizer Vegaflor contained macro- and microelements toxic values were calculated LD_{50} 943 $nL.kg^{-1}$ pollen, LC_{90} 6.43 $\mu L.mL^{-1}$ cultivated medium and NOEC 6.68 $fL.mL^{-1}$ cultivated medium. Toxic values was also calculated for the whole sum of active ingredients of Vegaflor (LD_{50}, NOEL, NOEC, LC_{90} etc.). Our results confirmed necessary to restrict application of fertilizers during the both pollen evolution and germination time of mature pollen grains on stigma for crops cultivated for reproduction and plant breeding. The yield of seeds will not decrease after application of tested fertilizers.

Keywords: fertilizer; pollen; cytotoxicity; ecotoxicity; plant breeding; *Brassica campestris*

The tests of effect of xenobiotics on living organisms are evaluated in many papers (Warne et al. 1998, Cotelle and Ferard 1999, Warne and Westbury 1999). Steinberg et al. (1995) reported biological and ecological systems responding to effects on the various levels of aggregation, from molecule to ecosystems. In many, but not all instances, in this tier the sensitivity of responses decrease, whereas the ecological relevance increases (Table 1).

In principle, ecotoxicity tests can be carried out at any level in the biological hierarchy of systems ranging from molecules to ecosystems (Calow 1993). Shaw and Chadwick (1995) also reviewed effects on biotic systems according to the procedure of OECD. This resulted in OECD testing procedures used to assess toxicity of the substances to biotic environment devided into three categories of plant based on the major human food plants groups (category 1 – *Monocotyledonidae*: rye, grass, rice, oats, wheat and sorghum; category 2 – *Brassiceae*: e.g. mustard, rape, radish, turnip and Chinese cabbage; category 3 – *Fabaceae*: vetch, mung beans, red clover, fenugreek, lettuce and cress). The test aims to shed light upon the consequence of soil contamination by the test chemical or agrochemical. The plant growth test involves measuring the effects of the test chemical on seed germination (LC_{50} lethal concentration in 50% of a test population or IC_{50} concentration at which 50% inhibition root growth) and root and shoot growth (EC_{50} a concentration necessary to give a toxic effect in 50% of a test population). The latter are measurements including plant root and shoot growth and evaluation of the biomass production.

Papers (Jandurová and Pavlík 1995, Pavlík and Jandurová 1998) and Table 1 show specific suitability of pollen germination for bioassay of toxicity. Properties of pollen – male gametophyte (sensitivity and ecological relevance) are useful for cytotoxic observation (Strube et al. 1991, Kristen 1997, Pavlík and Jandurová 1999) and also for ecotoxic evaluation of xenobiotics (Pfahler 1992, Jung et al. 1999, Abo-Elkhier and Abd El-Shafy 2000, Pavlík and Jandurová 2000). The percentage of pollen germination responds to changes of environmental properties and is also influenced by tested substances in conditions *in vitro* (Holub and Ostrolucká 1983, Wolters

Table 1. Spectrum of biological responses of test systems to stressors

Targets	Effects	Sensitivity	Ecological relevance
Molecule	genotoxicity, immoresponse, enzyme inhibition, etc.	highest	low
Cell	mutagenicity, death, change in reproduction, metabolic changes, etc.	high	middle
Organisms	death, metabolic changes, changes in reproduction and growth, etc.	middle	high
Ecosystems	changes in genetic information, loss in ecological functions, etc.	low	highest

Table 2. Doses of fertilizers added into nutrient medium

Fertilizers	Unit	Treatments								
		K	H	G	F	E	D	C	B	A
DAM 390	nL	0	0.04	0.4	4	40	400	4 000	10 000	40 000
Vegaflor	pL	0	1.6	16	160	1 600	16 000	160 000	400 000	1 600 000

K = control treatment

and Martens 1987, He et al. 1995). These observations of cytotoxicity and/or ecotoxicity on the cellular level are difficult to detect on plant sporophyte *in vivo* tests.

The main objective of our study was focused on determination of cytotoxicity of two liquid fertilizers DAM 390 and Vegaflor. Both fertilizers are applied in period of the vegetative plant growth and also in the period before flowering and therefore viability of pollen can be affected.

MATERIAL AND METHODS

Characterization of fertilizers

DAM 390 contains 30% N in 1 kg of solution (25% in an ammonium form, 25% in a nitrate form and 50% in an amidic form) (Vaněk et al. 1998). For any crops it is recommended to apply this fertilizer in the period before flowering, for rape in the period from early spring to yellow flower buds. According to crop the applied dose fluctuates from 10 to 40 kg N.ha^{-1} (Vašák and Mikšík 2001). Vegaflor classified as liquid fertilizer used for wide range of crops. According to crop the concentrations 0.2–0.25% are applied during the plant growth. Vegaflor contains 75–85 g N, 34–37 g P, 62–70 g K, 90–132 mg Fe, 80–115 mg Mn, more than 75 mg B, 40–115 mg Cu, 64–86 mg Zn, 10–20 mg Ni, 5–15 mg Mo, 3–7 mg Co and plant growth stimulators (4–7 mg a-naphthylacetic acid, nicotinic acid amide and 6-benzylaminopurine) in 1 L solution.

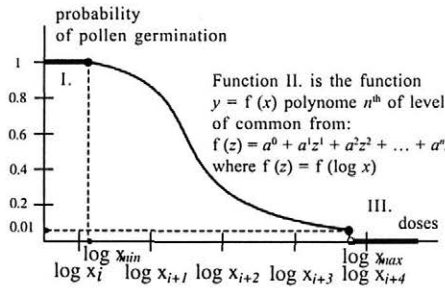

Figure 1. Function of dependence of pollen germination on concentration of agrochemicals (fertilizers)

Bioassay

Doses of the tested fertilizers used for bioassay are summarized in Table 2. Pollen from cultivar Toria of *Brassica campestris* L. subsp. *oleiferae* DC. (Williams and Hill 1986) was used for test. Cultivation of plants, collection of pollen, pollen cultivation conditions and evaluation of pollen germination has been formerly reported by Pavlík and Jandurová (2000). The pollen was germinated in 1 mL of Roberts' medium (Roberts et al. 1983). There were 8 μL of Roberts' medium without fertilizers as the control treatment (K) and other treatments (A, B, C, D, E, F, G and H) were prepared as mixtures of 8 μL of Roberts' medium with the appropriate volumes of fertilizer. For treatments A, B, C and D fertilizer DAM 390 was added into solution without dilution (Table 2). The calculation of fertilizer concentration used in the test treatment is derived from doses used in field application. This calculation of doses for *in vitro* test converted from field doses was reported by Pavlík and Jandurová (2000). Field dose was calculated for DAM 390 10 mL.m^{-2} and for Vegaflor 0.4 mL.m^{-2}. Treatment A (maximum dose) for DAM 390 contains 39 kg N.ha^{-1} and for Vegaflor 1% concentration of solution.

Histochemical staining methods were used to detect activities of enzymes alkaline phosphatase, nonspecific esterases, succinate dehydrogenase and peroxisomal catalases, in pollen grains after treatment and the evaluation of enzymes activities was described by Pavlík and Jandurová (2000).

Evaluation of toxicity, effect on living organisms, principles methods using in environmental monitoring are mentioned by Krejčík (2000), Warne et al. (1998), Warne and Westbury (1999) and Shaw and Chadwick (1995).

Statistics

Programs Quattro Pro 3.01 for DOS, Statgraphics 4.0 for DOS and Delta Graph Professional 2.0 for Windows were used for calculation. The curve describing the effect of fertilizers on pollen germination is showed in Figure 1 (Pavlík and Jandurová 2000). It is not possible to include extreme measured values (dependent variable $y_i = 0$ or $y_i = 1$) for calculating the regression equation that follows from single doses (independent variable x_i) (Pavlík and Jandurová 2000).

Table 3. Some factors involved in the statistical characteristics of the methods

Factors[a]	Average	Variance	*SD*	*SE*	*CV*
A	6.5 mg	2.8 mg	1.7 mg	0.3 mg	26.2 %
B2	53.1 %	6.4 %	2.5 %	0.8 %	4.7 %
D2	7.4 %	3.8 %	2.0 %	0.2 %	26.6 %

[a] A mass of six anthers in mg
B2 % pollen germination of control from one collection measured after 2 h
D2 % nonhydrated and nongerminated pollen grains in all variants from one collection measured after 2 h
SD = standard deviation, *SE* = standard error, *CV* = coefficient of variation

Table 4. Regression describing the effect of fertilizers on pollen germination after 2 hours

Fertilizers	f (*x*)					
DAM 390	$-0.0002173x^4 + 0.002919x^3 - 0.01021x^2 - 0.1379x + 1.142$					
Vegaflor	$-0.003768x^3 + 0.06575x^2 - 0.4037x + 1.290$					
Fertilizers	R_4^2	R_3^2	R_2^2	R_1^2	R_0^2	R^2
DAM 390	0.77	0.85	0.93	0.98	0.98	0.90
Vegaflor	–	0.59	0.69	0.83	0.95	0.77

RESULTS AND DISCUSSION

Statistical characteristics of the well reproducible used method are shown in Table 3. Figure 2 showed that the doses of both used fertilizers decreased pollen germination. Calculated regression curves (showed on Figure 2) described the effect of fertilizers on pollen germination are showed in Table 4, which we used to calculate by Statgraphics from the value of probability of germination (y) to value of fertilizer doses (x) (Table 5).

The evaluation of ecotoxicity of a given dose is possible when optimum conditions for pollen germination of donor plants are determined (Jandurová and Pavlík 1995). If the presence of xenobiotic compounds is the only one significant difference between control (optimal conditions) and treatment the difference should be explain as a direct reaction to the stress factor. Pollen germination of *Brassica campestris* in this selected *in vitro* conditions was very sensitive for broad interval of tested xenobiotics as well as for potential toxic substances (Pavlík and Jandurová 1998, 1999 and 2000). This test was used for determination of cytotoxic effect of liquid fertilizers (Figure 2, Table 5). The results showed pollen germination of *Brassica campestris* cultivar Toria cultivated in selected conditions is in useful to biotest of broad spectrum of substances. In Figure 2 maximum doses from first to third logarithmic doses correspond to field doses of tested fertilizers. Any different biotests (Pavlík et al. 2000) do not make many possibilities of results application as in this bioassay (Table 5 – LD_{50} lethal dose, LC_{50} lethal concentration, NOEC – no-effect concentration, NOEL – no-effect level, LD_{50}, LC_{90} etc.).

The results of different pollen bioassays confirmed (Stanley and Linkens 1974, Wolters and Martens 1987, Strube et al. 1991) that potentially toxic substances decreased pollen germination. Decrease of pollen germination can be explained by interaction between toxin and/or receptor, and/or enzymes and/or NA (nucleic acid). We

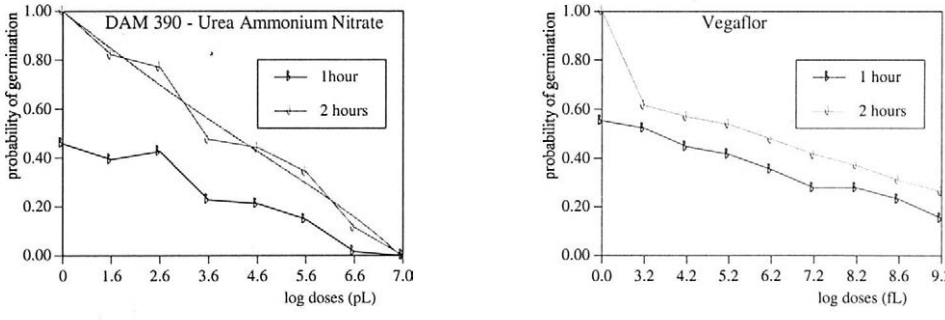

Figure 2. Influence of fertilizers on probability of pollen germination

Table 5. Calculate doses of fertilizer X_n (fL), of a.i. X_{na} (pg), and toxic doses of fertilizer X_{nb} (nL.kg^{-1}) and of active ingredients X_{nc} (μg.kg^{-1}) from Y_n[a], according to the function f (x) or their toxicity concentration (1 mL testing medium), respectively

Fertilizers or active ingredients		Probability of pollen germination Y_n							
		0	0.001	0.1	0.25	0.5	0.75	0.9	1
X_n	DAM 390	$8.79 \cdot 10^{10}$	$8.68 \cdot 10^{10}$	$2.29 \cdot 10^{10}$[c]	$2.20 \cdot 10^{9}$	$2.74 \cdot 10^{7}$[e]	$4.38 \cdot 10^{5}$	$4.37 \cdot 10^{4}$[g]	$9.54 \cdot 10^{3}$[i]
	Vegaflor	$1.81 \cdot 10^{10}$	$1.79 \cdot 10^{10}$	$6.43 \cdot 10^{9}$[c]	$7.76 \cdot 10^{8}$	$6.08 \cdot 10^{3}$[e]	$6.66 \cdot 10^{1}$	$1.51 \cdot 10^{1}$[g]	6.68[i]
X_{na}	NH_4NO_3	$4.82 \cdot 10^{10}$	$4.76 \cdot 10^{10}$	$1.29 \cdot 10^{10}$[c]	$1.20 \cdot 10^{9}$	$1.51 \cdot 10^{7}$[e]	$2.40 \cdot 10^{5}$	$2.39 \cdot 10^{4}$[g]	$5.23 \cdot 10^{3}$[i]
	Vegaflor[b]	$3.67 \cdot 10^{9}$	$3.63 \cdot 10^{9}$	$1.30 \cdot 10^{9}$[c]	$1.57 \cdot 10^{8}$	$1.23 \cdot 10^{3}$[e]	$1.35 \cdot 10^{1}$	3.05[g]	1.35[i]
X_{nb}	DAM 390	$1.36 \cdot 10^{10}$	$1.35 \cdot 10^{10}$	$3.56 \cdot 10^{9}$[d]	$3.41 \cdot 10^{8}$	$4.25 \cdot 10^{6}$[f]	$6.80 \cdot 10^{4}$	$6.77 \cdot 10^{3}$[h]	$1.48 \cdot 10^{3}$[j]
	Vegaflor	$2.81 \cdot 10^{9}$	$2.78 \cdot 10^{9}$	$9.98 \cdot 10^{8}$[d]	$1.20 \cdot 10^{8}$	$9.43 \cdot 10^{2}$[f]	$1.03 \cdot 10^{1}$	2.34[h]	1.04[j]
X_{nc}	NH_4NO_3	$7.48 \cdot 10^{9}$	$7.39 \cdot 10^{9}$	$1.95 \cdot 10^{9}$[d]	$1.87 \cdot 10^{8}$	$2.33 \cdot 10^{6}$[f]	$3.73 \cdot 10^{4}$	$3.71 \cdot 10^{3}$[h]	$8.11 \cdot 10^{2}$[j]
	Vegaflor[b]	$5.69 \cdot 10^{8}$	$5.63 \cdot 10^{8}$	$2.02 \cdot 10^{8}$[d]	$2.44 \cdot 10^{7}$	$1.91 \cdot 10^{2}$[f]	2.09	0.47[h]	0.21[j]

$f(x) = a_n x^n + a_{n-1} x^{n-1} + \ldots + a_1 x^1 + C = 0$; $X_{na} = X_n f$; where f is converting factor from dose fertilizer on dose active ingredients (a.i.) these fertilizers. $X_{nb} = X_n m_{6p}^{-1}$, where m_{6p} is average mass of six anthers, which were used in bioassay, $X_{nc} = (X_n f) m_{6p}^{-1}$ [a] calculated on whole summary tested pollen, [b] calculated on whole summary a.i. of Vegaflor, [c] LC_{90}, [d] LD_{90}, [e] LC_{50}, [f] LD_{50}, [g] LC_{10}, [h] LD_{10}, [i] NOEC, [j] NOEL

can see possible inhibition of activities of enzymes important for germination on Figure 3. Decreased germination of heterozygous pollen grains of *Brassica campestris* (segregating population) is the basic presumption for investigation of cytotoxicity as well as ecotoxicity. Pollen is suitable biological material for investigation of toxic effect to organisms (male gametophyte), cell, as resulted from the papers (Strube et al. 1991, Sawidis and Reiss 1995, Kristen 1997).The presence of toxin can evoke non specific selection changes in the genetic structure of population. These changes could be expressed as different germination or viability of pollen grains in tested population.

These results showed significant difference between DAM and Vegaflor in influence on pollen germination. Low doses of Vegaflor decreased strongly pollen germination in contrast to DAM. Stronger effect of Vegaflor doses can be explained by microelements concentration of tested fertilizer. These results confirm observation of Chaney and Strickland (1984) and Holub and Ostrolucká (1983). They mentioned phytotoxic effect of heavy metals (Cd, Pb) as well as microelements (Cu, Zn, Fe). This difference of fertilizers effect was reduced in higher doses, which confirm cytotoxicity of high concentration of ammonium ions for plants.

Toxic effect on pollen (LD_{10}, LC_{10}) was found for very low concentrations of fertilizers (Table 5). These concentrations probably do not affect the crop yield. On the basic of our results, we recommended the restriction application in the period from yellow buds to flowering time for liquid fertilizers to present risk of non specific selection pressure and non specific changing in gene pool of breeding population. The yield of seeds will not decrease after application of tested fertilizers.

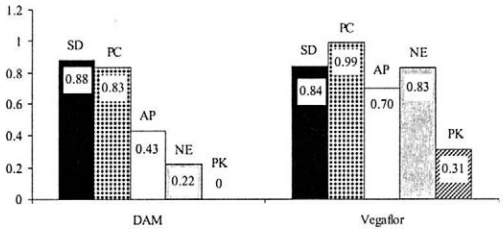

Figure 3. Influence of fertilizers (B variant) on activity of individual enzymes in relationship to germination of pollen grains (2 hours control = 100%); SD = succinate dehydrogenase, PC = peroxisomal catalases, AP = alkaline phosphatase, NE = non-specific esterases, PK = pollen germination

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ABSTRAKT

Stanovení cytotoxicity vybraných kapalných hnojiv pylovou biozkouškou

Pyl z *Brassica campestris* L. subsp. *oleiferae* DC., odrůda Toria, kultivovaný v podmínkách *in vitro*, se ukázal jako vhodný k biozkoušce na široké spektrum xenobiotik. Test toxického vlivu za pomoci klíčení pylových zrn (samčího gametofytu) představuje velmi jednoduchou metodu. Maximální testovací dávka pro pylové biotesty byla vypočtena z polních dávek. U kapalného dusíkatého hnojiva DAM 390 byly stanoveny toxické hodnoty LD_{50} (letální dávka) 4,25 $ml.kg^{-1}$ pylu, LC_{90} (letální koncentrace) 22,9 $\mu l.ml^{-1}$ kultivačního média a NOEC (neefektivní koncentrace) 9,54 $pl.ml^{-1}$ kultivačního média. Pro kapalné hnojivo Vegaflor, které obsahuje makro- i mikroelementy, byly vypočteny hodnoty LD_{50} 943 $nl.kg^{-1}$ pylu, LC_{90} 6,43 $\mu l.ml^{-1}$ kultivačního média a NOEC 6,68 $fl.ml^{-1}$ kultivačního média. Toxické hodnoty byly stanoveny také pro celkovou sumu aktivních složek Vegafloru (LD_{50}, NOEL – neefektivní dávka, NOEC, LC_{90} apod.). Z výsledků vyplývá, že je vhodné omezit aplikaci hnojiv během vývoje pylu a v době klíčení zralých pylových zrn na blizně u semenářských porostů a při šlechtění rostlin. Výnos semen po aplikaci hnojiva nebude snížen.

Klíčová slova: hnojivo; pyl; cytotoxicita; ekotoxicita; šlechtění rostlin; *Brassica campestris*

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Differential lead tolerance of rice and black gram genotypes in hydroponic culture

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ABSTRACT

High yielding cultivars of black gram (*Vigna mungo* L.) and rice (*Oryza sativa* L) were tested for their tolerance to different levels of lead (Pb^{2+}) (0, $4.83.10^{-4}$, $9.65.10^{-4}$, $1.93.10^{-3}$ and $3.86.10^{-3}$ mol.l^{-1}) in nutrient solution at pH 6.8 under controlled environmental conditions. Root length, shoot length, root/shoot dry biomass and root/shoot tolerance index (RTI and STI) were used as markers of lead toxicity. Root measurements on 9th day after root emergence yielded significant differences among twelve cultivars of black gram and twenty cultivars of rice. Black gram cultivars G-31, UPU-1 and UPU-2 showed better root growth in the presence of $1.93.10^{-3}$ mol.l^{-1} lead. In case of rice, Bharati, Deepa and Annapurna showed better root growth as compared with other cultivars. Root tolerance index (RTI) and shoot tolerance index (STI) of G-31, UPU-1 and UPU-2 of black gram and Bharati, Deepa and Annapurna of rice were relatively higher indicating their relative tolerance to lead. Enzyme activities (peroxidase, catalase and G6PDH) were higher in tolerant cultivars than in non-tolerant ones. Based on the growth parameters, twelve cultivars of black gram and twenty cultivars of rice were ranked in respect of their tolerance to lead. This method can be employed for quick screening of mung bean and rice cultivars for lead tolerance in breeding programmes.

Keywords: black gram; rice; peroxidase; catalase; G6PDH; lead toxicity; nutrient culture; screening

Lead was identified as one of the most commonly occurring contaminants, it is considered as an extremely toxic pollutant of the biosphere resulting from various agricultural, lead mining, primary and secondary lead smelters and industrial activities (Hafen and Brinkmann 1996). Lead is an abundant metal and inhibiting photosynthesis, calcium metabolism and ATP (adenosine triphosphate) synthesis. The toxic effects of lead in aquatic ecosystems were reported by various authors (Swaine 1978, Ye et al. 1997a, b). Lead in soils and solutions causes stunted growth and chlorosis. Phyto-toxicity, however, varied with the concentration of Pb in the soil solution as well as with the plant species (Jones et al. 1973, Burzynski 1987, Wierzbicka and Obidzinska 1998). Hydroponics have been employed to assess tolerance or the efficient utilization of nutrients in several crops (Hewitt 1966). Studies on hydroponics by non-destructive methods allow easy observations for screening of selected plants on the basis of relative growth rate. Therefore, identifying lead tolerant genotypes for better growth and productivity in Pb-toxic and infertile soils could be the best strategy to circumvent Pb toxicity. The present investigation was intended to identify the Pb-tolerant cultivars on the basis of root elongation, biomass production in hydroponic cultures. This study could also be a prerequisite for establishing a breeding programme for lead tolerance in rice (*Oryza sativa* L.) and black gram (*Vigna mungo* L.).

MATERIAL AND METHODS

Plant material and environmental condition. High yielding varieties of rice (Bharati, Deepa, Annapurna, Ratna, Subhadra, Vaghari, Hamsa, Hema, Pragati, Kusuma, Nilgiri, Khandagiri, Rudra, Vikram, Panidhan-1, Sakti, Sankar, Panidhan-2, Aswathi and Pusa-2-21) and black gram (G-31, UPU-2, UPU-1, T-27, H-10, T-77, T-9, Mash-48, Pusa-1, Khargone-3, BGVB-4 and LBG-263) were collected from the Department of Plant Breeding and Genetics, Orissa University of Agriculture and Technology, Orissa, India. Seeds were treated with detergent solution Teepol (Glaxo, India) for 10 min and washed with running tap water for 15 min. Furthermore, the seeds were sterilized with 0.1% aqueous mercuric chloride solution for 20 min and were sown over plastic nets on glass trays (12 × 15 × 7 cm) (Borosil, India) containing the Hoagland nutrient solution (Hoagland and Arnon 1950). Ten seeds were cultured in 300 ml of nutrient solution. The trays were kept in a growth room at 25 ± 2°C under cool, white flourescent lamp (55 µmol.m^{-2}.s^{-1}) with 16-h photoperiod. The pH of the nutrient solution was adjusted to 6.8 using 0.1 mol.l^{-1} HCl or 0.1 mol.l^{-1} KOH; the solution was changed at 3-d-intervals to maintain the desired level of nutrients and the pH. The concentrations were selected on the basis of preliminary experiment within a range that produced growth inhibition. Lead was used in the form of lead sulfate at 0, $4.83.10^{-4}$, $9.65.10^{-4}$, $1.93.10^{-3}$ and $3.86.10^{-3}$ mol.l^{-1}.The experiment was laid in a completely randomized block design with three replications. The experiments were repeated three times. The length of the primary root and the shoot and number of lateral roots/shoot were measured at 3-d-intervals from the date of root emergence up to the 9th day. The rate of root length in each experiment was determined by subtracting the length of the root recorded on the day of germination from that noted on the 9th day. Tolerance index (TI) for the

tested plants was calculated using the formula: TI (%) = mean root or shoot length in solution with Pb/mean root or shoot length in solution without Pb × 100.

Biomass analysis. For biomass analysis, plants were harvested after 9th day of root emergence in the nutrient solution with and without Pb treatment. Shoot and root were separated, the fresh weight measured and then kept at 70°C in an oven till getting the constant weight.

Determination of lead. For lead analysis, plants grown in nutrient solution in presence ($1.93.10^{-3}$ mol.l^{-1}) and absence of Pb were harvested after 9th day and washed with distilled water, oven dried at 70°C and 500 mg of grounded biomass were pre-digested in 10 ml concentrated HNO_3 for 12 h followed by digestion with 5 ml diacid mixture, i.e. nitric acid (HNO_3): perchloric acid ($HClO_4$) in the ratio of 3:2. Distilled water was then added to the digested samples which were then filtered by Whatman-42 filterpaper and, after suitable dilution, Pb was determined by inductively coupled plasma spectrometry (ICP 8410 Plasmascan) (Australia) using wavelength of 220.353 nm for Pb.

Peroxidase activity. Fresh shoots (100 mg) were collected from plants grown in solution with $1.93.10^{-3}$ mol.l^{-1} Pb and without Pb and homogenized with mortar and pestle in 4 ml of cold 0.1 mol.l^{-1} phosphate buffer (pH 6.1) containing 30 mg of insoluble PVP (polyvinylpyrrolidone)

Table 1. Shoot length, root length and number of lateral roots/plant of twelve blackgram and twenty rice cultivars in nutrient solution with 0.0 and $1.93.10^{-3}$ mol.l^{-1} lead after 9th day of root emergence; values are means of twenty replicates; repeated three times

Cultivars	Root length (cm)		Shoot length (cm)		Number of lateral roots/plant	
Lead conc. (mol.l^{-1})	0	$1.93.10^{-3}$	0	$1.93.10^{-3}$	0	$1.93.10^{-3}$
			Oryza sativa			
Ratna	5.76 ± 0.6j	4.45 ± 0.8l	7.67 ± 0.5n	6.81 ± 0.7m	3.27 ± 0.4	2.32 ± 0.5
Pusa-2-21	4.26 ± 0.7a	2.54 ± 0.6a	4.81 ± 0.8a	2.96 ± 0.6a	1.70 ± 0.3	0.96 ± 0.3
Annapurna	6.08 ± 0.8n	4.92 ± 0.7m	8.11 ± 0.9q	7.94 ± 0.7n	3.21 ± 0.6	2.87 ± 0.5
Pragati	5.80 ± 0.9k	4.06 ± 0.6h	6.91 ± 0.8k	5.11 ± 0.8i	2.22 ± 0.5	1.83 ± 0.8
Hema	5.86 ± 0.5l	4.13 ± 0.9i	7.36 ± 0.7o	5.57 ± 0.4j	2.82 ± 0.6	2.21 ± 0.5
Panidhan-1	5.37 ± 0.8f	3.41 ± 0.7e	5.23 ± 0.5d	3.55 ± 0.8d	2.26 ± 0.7	1.81 ± 0.4
Panidhan-2	5.26 ± 0.4d	3.23 ± 0.6d	5.12 ± 0.8c	3.32 ± 0.6b	2.11 ± 0.9	1.67 ± 0.5
Kusuma	5.74 ± 0.6j	4.00 ± 0.7h	6.36 ± 0.8i	4.60 ± 0.7h	2.76 ± 0.5	2.01 ± 0.3
Bharati	6.02 ± 0.8n	6.10 ± 0.8n	7.09 ± 0.9l	8.37 ± 0.9o	3.87 ± 0.4	3.82 ± 0.6
Deepa	6.32 ± 0.7o	6.12 ± 0.9n	7.87 ± 0.6p	9.06 ± 0.8p	4.12 ± 0.5	3.79 ± 0.4
Sakti	4.48 ± 0.8b	2.76 ± 0.8b	5.01 ± 0.8b	3.31 ± 0.7b	1.83 ± 0.5	1.10 ± 0.3
Hamsa	5.63 ± 0.7h	4.01 ± 0.5h	6.69 ± 0.9j	5.20 ± 0.8i	2.82 ± 0.6	1.98 ± 0.8
Vikram	4.67 ± 0.6c	2.98 ± 0.7c	5.10 ± 0.4c	3.50 ± 0.6c	1.88 ± 0.7	1.17 ± 0.5
Nilgiri	5.68 ± 0.5h	3.92 ± 0.8g	5.76 ± 0.7f	4.10 ± 0.8f	2.36 ± 0.6	1.77 ± 0.6
Subhadra	5.81 ± 0.8k	4.32 ± 0.6k	7.56 ± 0.9n	6.22 ± 0.9l	2.98 ± 0.5	2.02 ± 0.6
Khandagiri	5.70 ± 0.6i	3.85 ± 0.7g	6.10 ± 0.8h	4.30 ± 0.7g	2.88 ± 0.4	2.16 ± 0.7
Rudra	5.53 ± 0.7g	3.60 ± 0.9f	5.92 ± 0.6g	4.20 ± 0.8g	2.92 ± 0.5	2.00 ± 0.5
Sankar	5.31 ± 0.8e	3.27 ± 0.8d	5.66 ± 0.7e	3.71 ± 0.6e	2.51 ± 0.7	1.87 ± 0.5
Vaghari	5.92 ± 0.7m	4.24 ± 0.5j	7.22 ± 0.5m	5.90 ± 0.7k	3.32 ± 0.6	2.11 ± 0.6
Aswathi	5.32 ± 0.8e	3.18 ± 0.7d	5.62 ± 0.9e	3.60 ± 0.8d	2.76 ± 0.8	1.96 ± 0.5
			Vigna mungo			
LBG-263	4.91 ± 0.8a	3.52 ± 0.8a	5.07 ± 0.8a	3.61 ± 0.7a	2.15 ± 0.7	0.76 ± 0.5
BGVB-4	5.10 ± 0.9b	3.81 ± 0.7b	5.31 ± 0.6b	3.80 ± 0.6b	2.23 ± 0.5	1.10 ± 0.4
T-9	6.12 ± 0.7f	4.80 ± 0.6f	5.81 ± 0.7f	4.44 ± 0.8e	3.01 ± 0.7	2.00 ± 0.6
T-27	6.27 ± 0.8h	5.07 ± 0.7h	5.76 ± 0.9e	4.86 ± 0.7g	2.83 ± 0.6	1.61 ± 0.5
T-77	6.36 ± 0.6i	5.12 ± 0.4i	5.88 ± 0.6g	4.53 ± 0.6f	2.76 ± 0.8	1.87 ± 0.7
H-10	6.22 ± 0.9g	5.01 ± 0.8g	6.82 ± 0.8j	5.42 ± 0.9h	2.83 ± 0.5	2.14 ± 0.8
Pusa-1	5.71 ± 0.7e	4.30 ± 0.7e	5.53 ± 0.9d	4.01 ± 0.5d	2.72 ± 0.4	1.90 ± 0.6
UPU-1	6.86 ± 0.9k	5.82 ± 0.6k	6.74 ± 0.7i	5.81 ± 0.7j	3.02 ± 0.6	2.83 ± 0.7
UPU-2	6.67 ± 0.6j	5.66 ± 0.8j	6.57 ± 0.7h	5.73 ± 0.6i	2.98 ± 0.8	2.11 ± 0.6
Khargone-3	5.31 ± 0.7c	4.02 ± 0.6c	5.41 ± 0.8c	3.93 ± 0.5c	2.61 ± 0.6	1.11 ± 0.5
G-31	6.72 ± 0.5j	5.93 ± 0.7l	7.71 ± 0.6k	6.82 ± 0.7k	3.42 ± 0.6	3.06 ± 0.4
Mash-48	5.40 ± 0.5d	4.12 ± 0.4d	5.50 ± 0.5d	4.03 ± 0.4d	2.26 ± 0.7	1.80 ± 0.6

Within a column means having a letter in common of black gram and rice cultivar are not significantly different at $P \leq 0.05$ level by Duncan's multiple range test

and 15 mg sodium ascorbate. The homogenate was filtered through four layers of miracloth and centrifuged at 12 000 G for 10 min at 4°C. The supernatant was used for the peroxidase assay. The assay mixture contained 0.1 mol.l^{-1} phosphate buffer (pH 6.1), 0.004 mol.l^{-1} guaiacol, 0.003 mol.l^{-1} H_2O_2 and 0.4 ml of crude enzyme extract. The total reaction volume was 1.2 ml. The rate of change in absorbance (OD) at 420 nm was measured using a double beam UV-Spectrophotometer (Jasco, UVIDEC-650, Japan). The levels of enzyme activity were expressed as μmoles H_2O_2 destroyed/min/mg protein (Bergmeyer et al. 1974). Soluble proteins in the supernatant were determined according to Bradford (1976) using bovine serum albumin as standard.

Table.2. Tolerance index of shoot (STI) and root (RTI) of twenty rice (*Oryza sativa* L.) and twelve black gram (*Vigna mungo* L.) cultivars in 1.93.10^{-3} mol.l^{-1} lead; values are mean of 20 samples

Cultivars	Tolerance index (%)	
	STI	RTI
	Oryza sativa	
Ratna	88.78	77.25
Pusa-2-21	61.66	59.62
Annapurna	97.90	80.92
Pragati	73.95	70.00
Hema	75.67	70.47
Panidhan-1	67.87	63.50
Panidhan-2	64.84	61.40
Kusuma	72.32	69.68
Bharati	118.05	103.98
Deepa	115.12	96.83
Sakti	66.00	61.60
Hamsa	77.72	71.22
Vikram	68.62	63.81
Nilgiri	71.18	69.01
Subhadra	82.27	74.35
Khandagiri	70.49	67.54
Rudra	69.25	65.09
Sankar	65.37	61.58
Vaghari	81.94	71.62
Aswathi	64.05	59.77
	Vigna mungo	
LBG-263	71.20	71.42
BGVB-4	71.56	74.50
T-9	76.41	78.43
T-27	84.37	80.86
T-77	77.04	80.50
H-10	79.47	80.54
Pusa-1	72.33	75.43
UPU-1	86.20	84.83
UPU-2	87.21	84.85
Khargone-3	72.64	75.47
G-31	88.45	88.24
Mash-48	73.27	75.92

Catalase activity. Fresh shoots (100 mg) were collected from plants grown in solution containing 1.93.10^{-3} mol.l^{-1} Pb and without Pb and homogenised in 0.1 mol.l^{-1} sodium phosphate buffer (pH 7.0) and centrifuged at 1000 G for 10 min at 4°C. The supernatant was used as the enzyme extract for catalase. For the catalase assay, 1 ml of the enzyme extract was added to the reaction mixture containing 1 ml of 0.1 mol.l^{-1} H_2O_2 and 3 ml of 0.1 mol.l^{-1} sodium phosphate buffer (pH 7.0). The reaction was stopped by adding 10 ml of 2% H_2SO_4 after 1 min of incubation at 20°C. The acidified reaction mixture with or without enzyme extract was titrated against 0.1 mol.l^{-1} $KMnO_4$ to determine the quantity of H_2O_2 utilized by the enzyme. The catalase activity was expressed as μmoles H_2O_2 destroyed/min/mg protein (Bergmeyer et al. 1974). Soluble proteins in the supernatant were determined according to Bradford (1976) using bovine serum albumin as standard.

Glucose-6-phosphate dehydrogenase. Fresh shoots (100 mg) were collected from plants grown in solution containing 1.93.10^{-3} mol.l^{-1} Pb and without Pb and homogenised in 0.1 mol.l^{-1} Tris-HCl (pH 7.8) containing 0.001 mol.l^{-1} EDTA and 0.001 mol.l^{-1} dithiothreitol. The homogenates were centrifuged at 1000 G for 10 min and the supernatant was used for enzyme assays. Activity of the enzyme was measured according to Bergmeyer et al. (1974). The enzyme activity was referred to the protein content of each sample and expressed as percentage of control. Soluble proteins were determined in the supernatant according to Bradford (1976) with bovine serum albumin as standard.

Statistical analysis. In order to ascertain the significant differences of growth among various cultivars of black gram and rice, an ANOVA test was performed (Sokal and Rohlf 1973). Regression analyses were performed to assess the response of root length of different cultivars of rice and black gram to lead over the time of exposure. Effects of lead on growth variables at each level were noted with the separation of mean using the Waller-Duncan multiple range test (Harter 1960).

RESULTS AND DISCUSSION

Twelve black gram and twenty rice cultivars were exposed to four levels of lead (0, 4.83.10^{-4}, 9.65.10^{-4}, 1.93.10^{-3} and 3.86.10^{-3} mol.l^{-1}). The cultivars responded differently in terms of seed germination, elongation of shoot and root and root/shoot biomass. Our results show that the seed germination was not affected at 4.83.10^{-4} to 1.93.10^{-3} mol.l^{-1} of lead (data not shown). At higher concentration of Pb (3.86.10^{-3} mol.l^{-1}), germination rate declined. The percentage of germination reached maximum in the nutrient solution without lead 3-day after sowing. A high degree of variation in the response was observed at 1.93.10^{-3} mol.l^{-1} lead rather than other concentrations, therefore, this concentration was chosen to compare the performance of different cultivars. The mean root and shoot length and number of laterals per shoot for all the

Table 3. Correlation coefficients (r^2) between different levels of lead concentration and root length at four time intervals for twelve cultivars of blackgram and twenty cultivars of rice in nutrient solution

Cultivars	Days	Pb conc. vs. root length
	Vigna mungo	
LBG-263	3	0.974
	6	0.937
	9	0.975
BGVB-4	3	0.976
	6	0.979
	9	0.965
T-9	3	0.979
	6	0.991
	9	0.972
T-27	3	0.982
	6	0.966
	9	0.957
T-77	3	0.995
	6	0.954
	9	0.966
H-10	3	0.984
	6	0.980
	9	0.965
Pusa-1	3	0.989
	6	0.970
	9	0.986
UPU-1	3	0.958
	6	0.972
	9	0.987
UPU-2	3	0.949
	6	0.958
	9	0.957
Khargone-3	3	0.988
	6	0.982
	9	0.976
G-31	3	0.959
	6	0.953
	9	0.972
Mash-48	3	0.980
	6	0.989
	9	0.993
	Oryza sativa	
Ratna	3	0.943
	6	0.960
	9	0.634
Pusa-2-21	3	0.968
	6	0.956
	9	0.985
Annapurna	3	0.941
	6	0.946
	9	0.959
Pragati	3	0.953
	6	0.977
	9	0.992
Hema	3	0.981
	6	0.968
	9	0.984
Panidhan-1	3	0.989
	6	0.995
	9	0.999
Panidhan-2	3	0.945
	6	0.903
	9	0.919
Kusuma	3	0.952
	6	0.916
	9	0.920
Bharati	3	0.357
	6	0.896
	9	0.365
Deepa	3	0.926
	6	0.993
	9	0.948
Sakti	3	0.986
	6	0.975
	9	0.991
Hamsa	3	0.921
	6	0.880
	9	0.892
Vikram	3	0.943
	6	0.937
	9	0.973
Nilgiri	3	0.957
	6	0.956
	9	0.978
Subhadra	3	0.983
	6	0.978
	9	0.985
Khandagiri	3	0.940
	6	0.909
	9	0.950
Rudra	3	0.961
	6	0.960
	9	0.977
Sankar	3	0.752
	6	0.755
	9	0.807
Vaghari	3	0.937
	6	0.946
	9	0.950
Aswathi	3	0.858
	6	0.871
	9	0.869

cultivars of black gram and rice are presented in Table 1. Root length in black gram (cvs. G-31, UPU-1 and UPU-2) and rice (cvs. Deepa and Annapurna), however, decreased by 11.7, 15.1 and 15.2 and 3.2 and 19.0 percent, respectively, in the presence of lead as compared to their respective controls, while in BGVB-4 and LBG-263 of black gram and Aswathi and Pusa-2-21 of rice, the root length was reduced by 25.2 and 28.3 and 40.2 and 40.4 percent, respectively. The cultivars BGVB-4, LBG-263 and Khargone-3 of black gram and Aswathi, Pusa-2-21, Sankar and Panidhan-2 of rice showed stunted growth with chlorotic leaves at $1.93.10^{-3}$ mol.l^{-1} of Pb. Several reports show in-

hibition of growth of plant and physiological disorder in presence of lead in soil (Bazzaz et al. 1975, Wierzbicka and Obidzinska 1998). Trollope and Evans (1976) suggested that Pb-induced chlorosis in crop plants could be due to changes in Fe:Pb ratios. In others, lead toxicity appeared to induce calcium deficiency or reduce water transport problems (Zimdahl and Arvik 1973). Taylor and Foy (1985) suggested that the root tolerance index (RTI) is one of the most important markers to screen genotypes and varieties for metal tolerance. Tolerance index (TI) derived from ratios between the data of the treatment and the control solutions have been useful to characterize individual populations for metal tolerance. Our observations therefore provide further evidences that G-31, UPU-1 and UPU-2 of black gram and Bharati, Deepa and Annapurna of rice were tolerant to lead having RTI values as 88.24, 84.85, 84.83 and 103.98, 96.83 and 80.92, respectively (Table 2). The regression equations and correlation coefficients between lead concentration and root length at three different intervals in different cultivars of black gram and rice are presented in Table 3. The slopes of the regressions of root length and lead concentration clearly show the differences in response of various cultivars of black gram and rice.

The accumulation of lead in root and shoot of G-31, UPU-1 and UPU-2 of black gram and Bharati, Deepa and Annapurna of rice were found to be higher than other cultivars tested (Table 4). Lead accumulation was more in root than shoot (stem + leaves) in all cultivars of black gram and rice. Similar results were reported earlier in other crops (Lane and Martin 1977, Burzynski 1987, Wierzbicka 1999). This fact is that the primary importance in the uptake and transport of water by plants associated with lead. The basic strategies of metal tolerance is metal accumulation where there is no such restriction and metals are accumulated in a detoxified form; detoxification may result from cell wall binding, active pumping of ions into vacuoles, complexing by organic acids and possibly by specific metal-binding proteins, and alteration of membrane structures (Verkleij and Schat 1990, Jiang and Liu 1999).

Root and shoot biomass production were in accordance with shoot and root length, G-31 of black gram had 51.09 percent increase in root biomass as compared to the control. The cultivars UPU-2, UPU-1, H-10, T-27 and T-77 showed 36.87, 30.23, 23.02, 12.70 and 4.33 percent increase in root biomass (Table 5). The results presented in Table 5 indicated that Khargone-3, Pusa-1, Mash-48, T-9, BGVB-4 and LBG-263 were sensitive to lead toxicity showing 3.29–9.79 percent reductions in the root biomass as compared to the respective controls. Rice cultivars such as Bharati, Deepa, Annapurna, Vaghari, Subhadra and Ratna showed increased root biomass in the presence of lead compared to their control. The shoot/root biomass ratio varied in the presence of lead compared to control (Table 5). The underground biomass was higher than the above ground biomass in most of the cultivars tested. It may be due to the greater accumulation of lead to the root (Wierzbicka 1999). Acceleration in the enzyme activities is believed to play an important role under conditions of metal stress (Van Assche and Clijsters 1990) and therefore may have a subtle role in metal tolerance. Catalase, peroxidase and G6PDH activity increased significantly in tolerant cultivars of black gram and rice (Table 6). The activity increased 8.0–38.4% of rice and 9.3–32.8% of black gram in case of peroxidase, 3.9–29.9% of rice and 8.73–19.3% of black gram in case of catalase and 15.07–121.4% of rice and 49.2–127.1% of black gram in case of G6PDH, respectively, as compared to control. Greater enzyme activity of catalase and peroxidase indicates that the tolerant plants were under stress, these features are

Table 4. Lead content ($\mu g.g^{-1}$) in twelve black gram and rice cultivars in presence of $1.93.10^{-3}$ $mol.l^{-1}$ lead after 9 days of culture; values are means of 10 replicates; repeated three times

Cultivars	Pb content ($\mu g.g^{-1}$) ± s.e.	
	shoot	root
	Oryza sativa	
Ratna	10.6 ± 1.3l	13.6 ± 1.8k
Pusa-2-21	3.40 ± 1.0a	4.63 ± 0.9a
Annapurna	12.2 ± 0.8n	16.4 ± 1.0l
Pragati	6.91 ± 1.1f	9.60 ± 1.3g
Hema	7.82 ± 1.2h	10.2 ± 0.7h
Panidhan-1	7.61 ± 0.8g	9.61 ± 1.1g
Panidhan-2	5.23 ± 0.9c	6.72 ± 1.0c
Kusuma	9.71 ± 0.7k	11.4 ± 0.9i
Bharati	12.4 ± 1.3n	16.7 ± 2.2l
Deepa	11.6 ± 1.2m	13.6 ± 1.7k
Sakti	6.43 ± 1.0d	8.21 ± 1.3e
Hamsa	8.32 ± 0.9i	10.3 ± 1.4h
Vikram	6.72 ± 1.1e	8.62 ± 1.0f
Nilgiri	9.12 ± 0.7j	11.3 ± 0.6i
Subhadra	10.3 ± 1.4l	12.8 ± 1.6j
Khandagiri	8.42 ± 0.9i	11.7 ± 0.8i
Rudra	7.81 ± 0.8h	10.4 ± 1.0h
Sankar	6.41 ± 0.6d	7.82 ± 0.8d
Vaghari	9.80 ± 1.0k	12.3 ± 0.7j
Aswathi	4.32 ± 0.8b	5.62 ± 1.1b
	Vigna mungo	
LBG-263	5.62 ± 1.3a	7.22 ± 1.1a
BGVB-4	7.12 ± 0.9d	9.41 ± 1.2c
T-9	6.91 ± 1.5c	10.3 ± 1.3e
T-27	9.72 ± 0.8g	11.6 ± 0.9f
T-77	10.4 ± 1.2h	12.3 ± 1.0f
H-10	9.21 ± 1.1f	10.1 ± 1.3e
Pusa-1	6.32 ± 0.9b	9.41 ± 1.0c
UPU-1	9.71 ± 1.7g	13.6 ± 1.1g
UPU-2	10.4 ± 1.3h	15.4 ± 1.8h
Khargone-3	7.42 ± 0.9e	8.42 ± 1.3b
G-31	10.9 ± 1.7h	17.6 ± 2.4i
Mash-48	6.82 ± 1.2c	9.80 ± 1.5d

Within a column means having a letter in common of black gram and rice cultivar are not significantly different at $P \leq 0.05$ level by Duncan's multiple range test

Table 5. Biomass yield of twelve black gram and twenty rice cultivars in absence and presence ($1.93.10^{-3}$ mol.l^{-1}) of lead after 9th day of root emergence; values are means of 20 replicates; repeated thrice

Cultivars	Biomass yield (mg/plant)					
	shoot		root		shoot/root	
	0	$1.93.10^{-3}$	0	$1.93.10^{-3}$	0	$1.93.10^{-3}$
			Oryza sativa			
Ratna	26.7 ± 0.9d	28.6 ± 0.8g	28.7 ± 0.8f	30.2 ± 0.8i	0.93	0.99
Pusa-2-21	30.2 ± 1.0g	20.5 ± 1.1a	29.7 ± 0.8g	27.4 ± 0.9f	1.01	0.74
Annapurna	23.7 ± 1.2b	24.3 ± 0.8d	22.6 ± 0.9b	25.4 ± 0.7d	1.04	0.95
Pragati	29.8 ± 1.1f	24.2 ± 1.0d	30.8 ± 1.0h	28.6 ± 0.9g	0.96	0.84
Hema	28.4 ± 0.9e	23.3 ± 0.9c	27.5 ± 0.7e	25.7 ± 0.8d	1.03	0.90
Panidhan-1	31.2 ± 0.6h	24.7 ± 0.8d	30.2 ± 1.3h	26.7 ± 1.0e	1.03	0.92
Panidhan-2	35.8 ± 0.9k	25.8 ± 0.9e	32.7 ± 0.8i	22.3 ± 0.8a	1.09	1.15
Kusuma	32.7 ± 0.8i	28.2 ± 0.8g	30.8 ± 0.9h	30.0 ± 1.1i	1.06	0.94
Bharati	25.2 ± 1.1c	28.3 ± 1.0g	24.6 ± 1.0c	30.3 ± 0.6i	1.02	0.93
Deepa	26.5 ± 0.8d	29.1 ± 0.8h	27.7 ± 0.9e	33.5 ± 0.7k	0.95	0.86
Sakti	32.7 ± 0.9i	25.6 ± 0.6e	27.8 ± 0.7e	27.1 ± 0.4f	1.17	0.94
Hamsa	32.1 ± 0.8i	29.3 ± 0.9h	30.2 ± 0.8h	32.4 ± 0.8j	1.06	0.90
Vikram	34.7 ± 0.9j	26.6 ± 1.0f	33.7 ± 1.2j	30.2 ± 1.1i	1.02	0.88
Nilgiri	35.6 ± 1.3k	30.8 ± 1.1i	32.2 ± 1.0i	32.6 ± 0.9j	1.10	0.93
Subhadra	23.5 ± 1.2b	22.1 ± 1.0b	21.2 ± 0.8a	24.8 ± 0.6c	1.10	0.89
Khandagiri	30.7 ± 1.0g	23.6 ± 0.8c	28.7 ± 0.7f	29.4 ± 0.8h	1.06	0.80
Rudra	29.7 ± 0.8f	21.4 ± 0.7b	26.2 ± 0.9d	24.6 ± 0.9c	1.13	0.86
Sankar	30.2 ± 0.7g	24.5 ± 0.9d	28.2 ± 0.6f	30.4 ± 0.5i	1.07	0.80
Vaghari	22.3 ± 0.9a	23.3 ± 0.5c	20.7 ± 1.0a	24.3 ± 0.9c	1.07	0.95
Aswathi	29.7 ± 0.8f	20.2 ± 0.9a	27.4 ± 0.7e	23.2 ± 0.8b	1.08	0.87
			Vigna mungo			
LBG-263	36.2 ± 0.6f	24.3 ± 0.8a	30.2 ± 1.0b	28.7 ± 1.3a	1.19	0.84
BGVB-4	35.7 ± 0.9e	28.8 ± 0.6c	32.2 ± 0.8c	30.2 ± 1.0b	1.10	0.95
T-9	32.8 ± 0.8c	27.6 ± 0.7b	30.9 ± 0.8b	29.3 ± 0.6a	1.06	0.94
T-27	34.6 ± 0.9d	32.3 ± 0.8e	30.7 ± 0.9b	34.6 ± 0.9c	1.12	0.93
T-77	37.5 ± 0.8g	33.0 ± 0.9e	34.6 ± 0.8e	36.1 ± 0.7e	1.08	0.91
H-10	30.8 ± 1.0a	28.1 ± 0.9c	27.8 ± 1.1a	34.2 ± 0.8c	1.10	0.82
Pusa-1	36.7 ± 0.7f	31.3 ± 0.8d	32.1 ± 0.9c	34.6 ± 0.7c	1.14	0.90
UPU-1	32.4 ± 0.6c	36.7 ± 1.1g	30.1 ± 0.8b	39.2 ± 1.0g	1.07	0.93
UPU-2	31.5 ± 0.9b	35.7 ± 0.8f	28.2 ± 1.0a	38.6 ± 0.8f	1.11	0.92
Khargone-3	37.4 ± 1.2g	32.3 ± 0.9e	33.7 ± 0.6d	30.4 ± 1.1b	1.10	1.06
G-31	30.6 ± 0.9a	36.7 ± 0.8g	27.4 ± 0.8a	41.4 ± 0.7h	1.11	0.88
Mash-48	38.4 ± 0.8h	32.6 ± 0.7e	36.4 ± 0.9f	35.2 ± 0.8d	1.05	0.92

Within a column means having a letter in common of black gram and rice cultivar are not significantly different at $P \leq 0.05$ level by Duncan's multiple range test

often associated with metal tolerance (DeVos and Schat 1991). Nashikkar and Chakrabarti (1994) reported that catalase and peroxidase activities were indicators of heavy metal toxicity and subsequent stress situation in plants. Several mechanisms of heavy metal tolerance in plants were proposed which include production of intracellular metal binding compounds, alteration of metal compartmentation patterns, alteration of cellular metabolism and alteration of membrane structure (Verkleij and Schat 1990); once absorbed, toxic metals were not completely inert, but stimulate the activity of certain enzymes (Van Assche and Clijsters 1990). The regressions of root length versus different concentrations of lead at 3rd, 6th and 9th day of exposure also confirmed varied response in the cultivars of black gram and rice. Therefore, it was possible to categories black gram cultivars based on their relative tolerance to lead as: G-31 > UPU-2 > UPU-1 > T-27 > H-10 > T-77 > T-9 > Mash-48 > Pusa-1 > Khargone-3 > BGVB-4 > LBG-263 and rice cultivars as: Bharati > Deepa > Annapurna > Ratna > Subhadra > Vaghari > Hamsa > Hema > Pragati > Kusuma > Nilgiri > Khandagiri > Rudra > Vikram > Panidhan-1 > Sakti > Sankar > Panidhan-2 > Aswathi > Pusa-2-21.

Hydroponic culture is efficient in the determination of lead tolerance in black gram (*Vigna mungo* L.) and rice (*Oryza sativa*) cultivars. Among the tested cultivars, it

Table 6. Effect of lead ($1.93.10^{-3}$ mol.l^{-1}) on the enzyme activity of different cultivars of black gram (*Vigna mungo* L.) and rice (*Oryza sativa* L.); peroxidase and catalase activities are expressed as μmoles H_2O_2 destroyed per min per mg protein and glucose-6-phosphate dehydrogenase activity is expressed as *n*mole NAD (P) H oxidised per min per mg protein; parentheses indicate percent of enzyme activity increase (+) or decrease (–) as compared to the control; values given are the averages of three assays

Cultivars	Enzyme activity (mean ± s.e.)		
	peroxidase	catalase	G6PDH
	Oryza sativa		
Ratna	37.91 (+28.2)	27.58 (+11.8)	185.6 (+105.7)
Pusa-2-21	28.11 (+9.12)	27.14 (–3.94)	95.8 (+15.07)
Annapurna	37.11 (+30.0)	32.54 (+15.7)	172.8 (+82.6)
Pragati	34.82 (+15.6)	29.43 (+19.0)	132.3 (+54.3)
Hema	36.33 (+10.9)	28.94 (+21.7)	143.2 (+62.9)
Panidhan-1	28.91 (+8.60)	24.94 (+12.3)	122.6 (+32.2)
Panidhan-2	36.72 (+13.6)	29.58 (+7.36)	117.8 (+17.3)
Kusuma	34.52 (+16.2)	25.34 (+15.9)	124.2 (+27.2)
Bharati	43.27 (+38.4)	36.24 (+25.3)	212.6 (107.9)
Deepa	40.57 (+17.2)	31.74 (+29.9)	183.5 (+86.4)
Sakti	30.12 (+8.53)	22.56 (+23.7)	134.2 (+30.6)
Hamsa	38.54 (+23.6)	25.23 (+17.7)	162.8 (+75.4)
Vikram	27.24 (+9.48)	27.31 (+11.3)	178.6 (+121.4)
Nilgiri	35.21 (+17.1)	34.32 (+13.9)	170.5 (+102.2)
Subhadra	37.32 (+32.4)	31.82 (+21.3)	175.6 (+79.5)
Khandagiri	30.16 (+9.51)	34.13 (+14.8)	182.6 (+61.9)
Rudra	33.46 (+13.4)	33.21 (+10.2)	153.8 (+54.9)
Sankar	31.19 (+8.0)	27.02 (+8.55)	140.6 (+17.1)
Vaghari	32.51 (+21.1)	31.08 (+21.7)	173.6 (+83.7)
Aswathi	30.92 (+9.52)	26.10 (+3.90)	102.7 (+22.6)
	Vigna mungo		
LBG-263	23.23 (+9.0)	22.21 (+10.3)	134.6 (+49.2)
BGVB-4	31.11 (+9.1)	21.04 (+12.3)	138.2 (+55.8)
T-9	30.01 (+23.4)	23.64 (+15.8)	156.8 (+60.8)
T-27	33.21 (+24.3)	20.11 (+13.9)	162.7 (+75.3)
T-77	35.21 (+15.7)	25.42 (+19.2)	174.8 (+77.1)
H-10	29.76 (+31.4)	26.57 (+18.4)	176.2 (+78.7)
Pusa-1	31.27 (+19.3)	32.42 (+19.1)	160.9 (+72.6)
UPU-1	36.23 (+28.5)	29.32 (+18.1)	192.8 (+89.4)
UPU-2	34.11 (+30.0)	27.54 (+19.1)	212.6 (+89.1)
Khargone-3	28.52 (+17.3)	20.16 (+8.73)	126.6 (+65.7)
G-31	36.14 (+32.8)	26.32 (+18.7)	278.4 (+127.1)
Mash-48	32.14 (+25.5)	30.53 (+18.9)	152.7 (+68.9)

appeared repeatedly that the G-31, UPU-1 and UPU-2 of black gram and Bharati, Deepa and Annapurna of rice were tolerant to lead. It is possible to select black gram and rice for lead tolerance in progeny tests in hydroponics for breeding programmes. Further studies are warranted to unravel the hidden facts on the mechanism of lead toxicity in black gram and rice.

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ABSTRAKT

Tolerance genotypů rýže a fazolu mungo ke zvýšeným koncentracím olova v hydroponii

Tolerance vysoce produktivních odrůd fazolu mungo (*Vigna mungo*) a rýže (*Oryza sativa* L.) k rozdílným koncentracím olova (Pb^{2+}) (0, 4,83.10^{-4}, 9,65.10^{-4}, 1,93.10^{-3} a 3,86.10^{-3} mol.l^{-1}) byla sledována v živném roztoku o hodnotě pH 6,8. Jako markery toxicity olova byly sledovány délka kořenů, délka nadzemních částí, sušina biomasy kořenů a nadzemních částí a indexy tolerance kořenů a nadzemních částí (RTI a STI). Hodnocení kořenů devátý den po vzejití ukázalo významné rozdíly mezi 12 odrůdami fazolu mungo a 20 odrůdami rýže. Odrůdy fazolu mungo G-31, UPU-1 a UPU-2 vykazovaly v přítomnosti 1,93.10^{-3} mol.l^{-1} olova v živném roztoku vyšší kořenový růst. V případě rýže byl zjištěn nejvyšší kořenový růst u odrůd Bharati, Deepa a Annapurna. Index tolerance kořenů (RTI) a index tolerance nadzemních částí (STI) u odrůd fazolu mungo G-31, UPU-1 a UPU-2 a u odrůd rýže Bharati, Deepa a Annapurna byl relativně vyšší, což naznačovalo jejich výraznější toleranci k olovu. Enzymová aktivita (peroxidázy, katalázy a G6PDH) byla vyšší u tolerantních odrůd než u odrůd netolerantních. Na základě sledovaných růstových parametrů bylo určeno pořadí 12 odrůd fazolu mungo a 20 odrůd rýže, pokud jde o jejich toleranci k olovu. Tuto metodu lze použít ve šlechtitelských programech k rychlému screeningu tolerance odrůd fazolu mungo a rýže k olovu.

Klíčová slova: fazol mungo; rýže; peroxidáza; kataláza; G6PDH; toxicita olova; živná kultura; screening

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Isolation and basic characterization of Zn-binding compounds in spinach biomass

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ABSTRACT

Zinc accumulation in spinach biomass and Zn-binding forms in above ground biomass were investigated on Fluvisols treated by sewage sludge in model pot experiment. For sequential analysis of dry biomass five solvents were used – petroleum ether, ethyl acetate, methanol, methanol + H_2O & H_2O, methanol + H_2O + HCl. Compounds contained in individual fractions were indicated using IR spectrum. Petroleum ether and ethyl acetate fractions formed the lowest part of isolated fractions (3.2% treatment without sludge, 1.3% sludge treatment) and the lowest Zn content was determined in both fractions (3.36% treatment without sludge, 0.43% sludge treatment). Portion of methanol fraction (carboxylic acid, aromatic and pyrrole substances) was the highest from isolated fractions at the treatment without sludge (36.2%). Fraction methanol + H_2O & H_2O contained oligopeptides, extractable polypeptides, proteins. Phytochelatins, methallothioneins and chelating agents (mugineic acid, deoximugineic acid, avenic acid, nicotinamine) can be extracted in this fraction. Fraction methanol + H_2O + HCl contained compounds isolated from cell walls and cytoskeleton after hydrolysis (phytin). The majority of Zn (69%) was found at sludge treatment in this fraction. Zn-binding compounds indicated in this fraction were important for intoxication of plants. Non-extractable residues formed portion of 37.6% at the treatment without sludge and 46.6% at sludge treatment. Zn content in both treatments was very low.

Keywords: zinc; Zn-binding compounds; sequential extraction; spinach biomass

Zinc, the essential micronutrient, occurs in plant either as a free ion, or as a complex with a variety of low molecular weight compounds, storage metalloproteins and insoluble forms associated with the cell wall. Zinc can become inactivated within the cell either by ligand formation or by complexation with phosphorus. Depending on plant species and plant part, Zn portion in the range 58%–91% of total Zn content may be soluble (Walker and Welch 1987, Brown et al. 1993). This soluble Zn part is considered to be the physiologically active fraction. The Zn is introducing a medium mobility in plants and is mostly accumulated in root tissues and seeds especially when Zn supply is high (Tlustoš 1999). In older leaves Zn can become very immobile (Rinne and Langston 1960). The rate of Zn transport to younger tissues is particularly depressed in Zn deficient plants (Loneragan 1975).

The metabolic functions of zinc are based on its tendency to form tetrahedral complexes with N, O and S-ligands and thereby plays a catalytic and structural role in enzyme reactions (Vallee and Auld 1990). There is a large number of enzymes in which zinc is a component of enzyme structure (Marschner 1995). Within enzymes with catalytic zinc function plays an important role the carbonic anhydrase – key enzyme for photosynthesis, while alcohol dehydrogenase (this enzyme contains two zinc atoms per molecule, one with catalytic and the other with structural function) and the proteins involved in DNA replication and gene expression belong to the compounds with structural zinc function. This role of Zn in protein molecules (zinc metalloproteins) has been investigated in last decades (Coleman 1992). Zinc is the metal component in a number of other enzymes – alkaline phosphatase, phospholipase, carboxypeptidase, RNA polymerase etc. (Coleman 1992). Zn deficiency also reduces auxin level, increase in plasma membrane permeability, for example in bean roots (Pinton et al. 1993).

Zinc toxicity is a problem in areas of natural Zn deposits, spoil heaps from mining and around zinc smelters. Toxicity causes restricted growth and leaf chlorosis resembling Fe deficiency (Brown et al. 1993). The increase of Zn accumulation in plant biomass can also be due to the application of sewage sludge to the soil (Balík et al. 1998, 2000, Tlustoš et al. 2001).

The knowledge of binding forms of zinc in vegetative plant organs is poor. Separation analyses are mostly focused on isolation of one group of compounds and relatively few studies have been concerned with sequential fractionation of plant biomass. Therefore the main objective of our study was focused on sequential fractionation of spinach biomass, partial characterisation of Zn-binding compounds and determination of zinc concentration in these fractions.

MATERIAL AND METHODS

The Zn accumulation in spinach biomass was investigated on Fluvisols (pH_{KCl} = 4.8, C_{ox} = 0.82%, CEC = 77 mval.kg^{-1}, total content of Zn = 70.9 mg.kg^{-1}) treated by sewage sludge in model pot experiment. Fresh homogenous sewage sludge with 26–28% of dry matter and total content of Zn 1438 ± 10 mg.kg^{-1} was used in this experiment. 5 kg of soil was thoroughly mixed with 0.5 g N, 0.16 g P, and 0.4 g K applied in ammonium nitrate and potassium hydrogen phosphate at control treatment and with same amount of nutrients plus fresh sewage sludge in equivalent to 20 Mg.ha^{-1} at observed treatment. Soil mixture was filled into plastic pots and sown by spinach seeds. Soil moisture was regularly controlled and kept on 60% of MWHC. Both treatments were prepared with three replications. Spinach (*Spinacia oleracea* L.) var. Monores was planted up to full leaves development. After harvest an above ground biomass was gently washed with deionised water, dried, ground and analyzed for total zinc content. Plant material was decomposed by dry ashing procedure (Mader et al. 1989). The determination of Zn concentration was performed by atomic absorption spectrometry (VARIAN SpectrAA-300) with flame atomization. Quality of plant analyses was controlled by reference material RM 12-02-03 Lucerne with certified contents of Zn = 33.2 ± 0.5 mg.kg^{-1} and obtained Zn = 33.2 ± 1.4 mg.kg^{-1}.

Sequential analysis of spinach dry matter was conducted according to design extraction scheme (Table 1) which allow us to determine Zn in five fractions. That is, typically non-polar, highly lipophilic solvents immiscible with water are given at the beginning of this series (fractions A, B), and polar hydrophilic solvents completely miscible with water are found as the third step (fraction C). At the end of the series the mixture solvents are methanol + H_2O & H_2O (fraction D) and methanol + H_2O + HCl (fraction E). This extraction series is used for characterisation of natural plant substances in isolated fractions. Fractions were extracted to constant weight and evaporated to dryness. Variability of isolated fraction weights was to 1%. IR spectrum of isolated fractions was measured by IR spectrometer (Brucker IFS 88). Isolated fractions were analysed in micro tablets with KBr. Owing to difficult extractions analyses were not replicated.

Evaporated isolated fractions A, B, C, D were dissolved in mixture 1 ml HNO_3 conc. + 1 ml H_2O using ultrasonic bath. Fraction E (methanol + H_2O + HCl) was decomposed in a mixture of HF conc. + HNO_3 conc. (1:2) at the temperature 150°C. Mixture was evaporated to dryness and the residue was dissolved in 1 ml 1.5% HNO_3 using ultrasonic bath. Non-extractable residues (fraction F) were decomposed by dry ashing procedure and ash was dissolved in 1 ml of 1.5% HNO_3. Before measurement fraction solutions were diluted by 1.5% HNO_3 up to 10 ml. The determination of element concentration was performed by atomic absorption spectrometry (VARIAN SpectrAA-300) with flame atomization.

Table 1. Sequential extraction scheme

Fractions	Extraction solvents	Process of fractionation
A	petroleum ether	5 fold 20 ml
	↓	
B	ethyl acetate	5 fold 20 ml
	↓	
C	methanol	7 fold 20 ml
	↓	
D	methanol + H_2O (1+1; v/v)	3 fold 20 ml
	H_2O	1 fold 30 ml
	↓	
E	methanol + H_2O + HCl (36%) (49.3 + 49.3 + 1.4; v/v/v)	1 fold 15 ml
	↓	1 fold 5 ml
F	non-extractable residues	

RESULTS AND DISSCUSION

After application of sludge to sandy soil with low CEC Zn accumulation in spinach biomass was increased significantly. Total Zn content in spinach biomass at sludge treatment was more than two fold higher in contrast to treatment without sludge (Figure 1).

The results of sequential analysis of spinach biomass showed different portions of individual isolated fractions at the treatments (Figure 2) and different portions of Zn in these fractions (Figure 3). The soluble Zn part (fractions A, B, C, D) was 59% from total Zn content in spinach grown at treatment without sludge. Portion of soluble Zn in plant biomass from 58 to 91% mentioned also Walker and Welch (1987) and Brown et al. (1993). Portion of soluble Zn part was reduced at sludge treatment in contrast to treatment without sludge and it was found 29% from total Zn. Petroleum ether and ethyl acetate fractions (A and B) formed the lowest part of isolated fractions (3.2% treatment without sludge, 1.3% treatment with sludge) and the lowest Zn content was determined in both fractions (3.36% treatment without

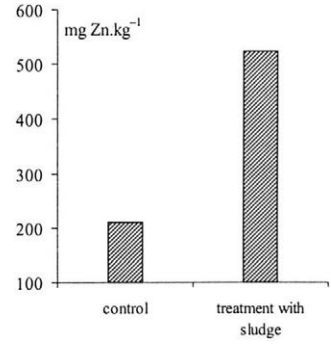

Figure 1. Total Zn content in spinach biomass (mg.kg^{-1})

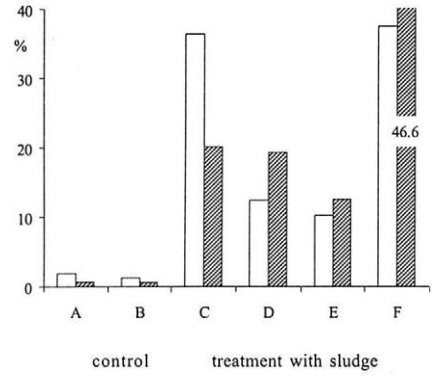

Figure 2. Portion of fractions isolated from spinach biomass (%)

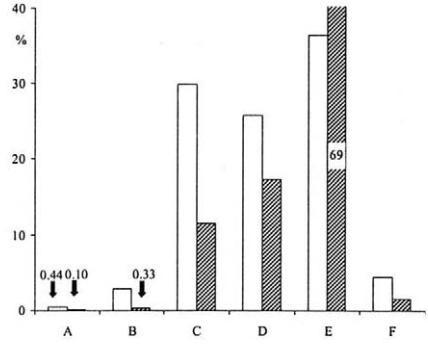

Figure 3. Zn content in isolated fractions (%)

sludge, 0.43% treatment with sludge). These fractions consisted of the least polar substances (Table 2). Zn ions in petroleum ether fraction were located either in intracellular space or Zn was bound to long chain hydrocarbons in membranes by chelate bindings. Brown et al. (1993) also reported only a small percent of Zn present as free ions in plant tissues. Zinc in ethyl acetate fraction was probably bound to saturated and unsaturated fatty acids. These organic compounds in both fractions can be significant for membrane transport.

Methanol fraction (C) mainly contained carboxylic acids, aromatic and pyrrole substances. Brooks (1998) has been suggested formation of zinc-citrate or zinc-malate complexes in plants. Portion of methanol fraction at the treatment without sludge was the highest (36.2%) from isolated fractions and twelve fold higher in contrast to the sum of fractions A and B. Amount of C fraction was lower (20.2%) at sludge treatment. The lower weight of C fraction at sludge treatment may be showed that metabolism of plant natural products was inhibited by toxins at this treatment. Zn contents at both treatments were not significantly different (63.0 mg $Zn.kg^{-1}$ at treatment without sludge, 60.9 mg $Zn.kg^{-1}$ at sludge one). These results showed strong influence of toxins in the metabolism of different substances (cytochrome P450, glutamate kinase – biosynthesis of free proline) in this fraction at sludge treatment (Talanova et al. 1999). Metabolism of Zn-binding compounds was not influenced.

Portion of fraction D (methanol + H_2O & H_2O) was only 12.2% at treatment without sludge, at sludge treatment was similar as fraction C (19.3%) (Figure 2). Zn content determined in this fraction (25.8%) and fraction C was not significantly different at the treatment without sludge (Figure 3). Zn content at sludge treatment was higher (17.3%) in contrast to fraction C. Results showed that biosynthesis of organic substances at sludge treatment were not limited by toxins so strongly in contrast to fraction C. Major compounds indicated, using IR spectrum, were compounds with amidic bound – oligopeptides, extractable polypeptides, proteins, phytochelatins and metallothioneins (Kotrba et al. 1999, Leopold et al. 1999, Rauser 1999, Sanita di Toppi and Gabbrielli 1999). Finally, chelating agents – mugineic acid (Takemoto et al. 1978), deoxymugineic acid, avenic acid (Fushiya et al. 1981), amino acid nicotianamine (Buděšínský et al. 1980, Rudolph et al. 1985) can be presented in this fraction. Nicotianamine has an optimal molecular structure for chelating iron ions and is considered a possible phytosiderophore with an essential function in cellular iron transport and/or metabolism. Walker and Welch (1987) reported these compounds form stable anionic complexes with several trace elements. All these cited compounds can be extracted to this fraction D. In this reason fraction D is one of most important fractions for metals metabolisms. Sanita di Toppi and Gabbrialli (1999) reported response mechanisms to metals at cellular level. In response to stress the

Table 2. Substances in isolated fractions indicated using IR spectrum

Fraction	Substances
A	saturated and unsaturated long chain hydrocarbons with or without keto group
B	saturated and unsaturated fatty acids
C	different carboxylic acids, probably not fatty acids, but acids for example from citric cycle or free amino acids, aromatic substances and pyrrole substances
D	substances with amidic bond, for example oligopeptide or extractable polypeptide and proteins, extractable soluble oligosacharides
E	substances with amidic bond, extractable hydrolysate substances from cell walls or cytoskeleton (phytin, oligosacharides, polysacharides, proteins, glycoproteins)

plant cell can form defense system such as synthesis of phytochelatins and metallothioneins. Geiken et al. (1998) reported the effect of metal stress on photochemical activity and protein behaviour of photosystem II, mainly for cytochrome B_{559}. Aromatic substances were indicated using IR spectrum, too.

Fraction E (methanol + H_2O + HCl) contained compounds isolated from cell walls and cytoskeleton after hydrolysis. One of major compounds was phytin or different salts of phytic acid (Prošková 1988). Zinc was very tightly bound to phytic acid and formation of zinc-phytic acid complexes increased the resistance to hydrolysis (Marschner 1995). Lignins were the other compound extracted in this fraction. Morrison et al. (1981) performed sequential analysis of plants and have been suggested importance of lignins to bound metals.

Portion of fraction E was lower in contrast to fractions C and D, mainly at sludge treatment. The majority of Zn (361 $mg.kg^{-1}$) was found in spinach biomass at sludge treatment (69% of total Zn) (Figure 3). This fraction of treatment without sludge contained 77 $mg.kg^{-1}$ (36.5% of Zn). Zn of this fraction was insoluble. Zn-binding compounds in this fraction were very important for intoxication of plants.

In fraction F non-extractable residues were determined. Compounds contained in residues were very difficult to be extractable. Portion of 37.6% at treatment without sludge and 46.6% at sludge treatment was found in spinach biomass (Figure 2). Zn content in both treatments was very low (9.3 mg $Zn.kg^{-1}$ at treatment without sludge, 8.4 mg $Zn.kg^{-1}$ at sludge treatment) (Figure 3).

The application of sequential analysis for spinach biomass extraction makes to investigate complex effect of Zn and other toxins on plant metabolism. The analysis provides possibility to isolate Zn-binding compounds and metabolites which biosynthesis was affected by zinc. It would be possible to investigate relationship of Zn or other metals and biosynthesis of stress metabolites and their effect on decrease of biosynthesis of some compounds in plant biomass.

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ABSTRAKT

Izolace a základní charakteristika sloučenin vázajících Zn v biomase špenátu

V nádobovém vegetačním pokusu na fluvizemi byl pěstován špenát po aplikaci čistírenského kalu. Z nadzemní biomasy špenátu bylo extrahováno pět frakcí s použitím petroléteru, etylacetátu, metanolu, metanolu + H_2O & H_2O a metanolu + H_2O + HCl. Ve frakcích byl stanoven obsah Zn a sloučeniny obsažené ve frakcích byly charakterizovány pomocí IČ spekter. Petroléterová a etylacetátová frakce tvořily nejmenší podíl z extrahovaných vzorků (3,2 % varianta bez kalu, 1,3 % varianta s kalem). V těchto frakcích byl stanoven nejnižší podíl z celkového obsahu Zn (3,36 % varianta bez kalu, 0,43 % varianta s kalem). Frakce extrahovaná metanolem (karboxylové kyseliny, aromatické a pyrrolové látky) tvořila ve variantě bez kalu nejvyšší podíl 36,2 %, ve variantě s kalem pouze 20,2 %. Frakce metanol + H_2O & H_2O obsahovala oligopeptidy, extrahovatelné polypeptidy, proteiny. Proto mohly být extrahovány fytochelatiny, methallothioneiny a chelatové produkty (mugineová kyselina, deoximugineová kyselina, avenová kyselina, nicotinamin). Frakce metanol + H_2O + HCl obsahovala sloučeniny izolované po hydrolýze z buněčných stěn a cytoskeletu (fytin). V biomase špenátu pěstovaného na variantě s kalem bylo 69 % Zn obsaženo v této frakci. Sloučeniny vázající Zn v této frakci ovlivnily intoxikaci rostlin. Podíl neextrahovatelného zbytku tvořil ve variantě bez kalu 37,6 % a ve variantě s kalem 46,6 %. V této frakci byl stanoven nízký obsah Zn.

Klíčová slova: zinek; sloučeniny vázající Zn; sekvenční extrakce; špenát

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The availability of Cd in aerobically and anaerobically incubated sewage sludge amended by bentonite

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ABSTRACT

During the evaluation of sewage sludge application on the agricultural land, the total content of potentially toxic elements usually plays a major role, but only available portion of element can be taken by plants. Main objective of the investigation was focused on determination of Cd availability in sewage sludge incubated under aerobic and anaerobic environment and amended by bentonite. Stabilised sludges from five different locations with different properties were incubated for eight months with presence of air (aerated every other week) and without air (pressed into pot, covered and sealed) under controlled conditions (20°C, 80% humidity). Each sludge was incubated as control treatment and with addition of two rates of bentonite in three replications. The available content of Cd was extracted by 1 mol.l^{-1} NH_4NO_3 every other month and sequential extraction was made twice during incubation. Results showed great differences in Cd availability among tested sludges. Aerobically incubated sludges increased mean amount of mobile Cd in control as well as in bentonite treatments during incubation. Sludge incubated under anaerobic conditions released majority of Cd at the end of incubation. The application of bentonite decreased mean Cd availability under both conditions. Sequential analyses showed transformation of Cd bounds and Cd movement into labile exchangeable and oxide fractions in sludges incubated with presence of air and stable Cd distribution in sludge incubated under lack of air.

Keywords: sewage sludge; cadmium; availability; sequential analyses; bentonite; aerobic incubation; anaerobic incubation

Growing concern on the improvement of environmental purity affected the quality of waste materials through the world introducing decreasing content of heavy metals in sewage sludge over last decade (Smith 1996). We can assume that the total content of element plays usually a major role in the long term assessment of the rate and interval of application, but only available portion of element can immediately affect element accumulation in plant biomass (Tlustoš et al. 1998). Cadmium belongs to the most mobile elements and its plant availability is affected by a wide range of factors in the soil as well as in applied ameliorative material (Adriano 1986). Among them access of air (McBride 1989) as well as addition of materials with high sorption capacity (Chlopecka and Adriano 1997) can substantially affect the availability of metals for plants. There were different adsorptive materials as natural zeolite (Chlopecka and Adriano 1996), bentonite (Sims and Boswell 1978, Hlušek and Richter 1992), phosphatic clay (Gonzales et al. 1992) tested on contaminated soils or soils amended with sewage sludge to decrease uptake of metals by plants. Tlustoš et al. (1996) showed reduction of Cd content in oat biomass by 40% planted at soil amended with bentonite. Similarly, Eriksson (1988) found reduced Cd content in ryegrass and oil seed rape biomass grown on clay substrate compared to sand one. Arnfalk et al. (1996) tested 14 different types of minerals and soil materials for their adsorption of metals and found montmorillonite contained in bentonite and in smectite as a mineral with highest element binding capacity. However, while the effect of addition of sorption materials into soil and their effect on plant uptake are described well, there is no available information on the fluctuation of metal availability in sewage sludge treated by such materials.

The main objective of our investigation was focused on determination of Cd availability in sewage sludge incubated under aerobic and anaerobic conditions and amended by two rates of bentonite.

MATERIAL AND METHODS

Anaerobically stabilised sewage sludge was obtained from five waste water factories in the Czech Republic situated in towns of different population Tábor, Protivín, České Budějovice, Kladno and Praha. Each sewage sludge showed slightly different properties (Table 1). Three treatments were set up for each sludge: control treatment, sludge with addition of bentonite (10% w/w of dry solid) – treatment sewage sludge + bentonite I, sludge with addition of bentonite (30% w/w of dry solid) – treatment sewage sludge + bentonite II. Each treatment was carried out in triplication.

Before the experiment each sewage sludge was intensively homogenized to minimize heterogeneity of material used in the experiment. The components of each replication were weighted separately, thoroughly mixed in a vessel and homogeneous matter was inserted into the plastic pot diameter 23 cm. The amount of mixture differed from water content of sewage sludge and from added material. All treatments were incubated as follows:

Table 1. Physical and chemical properties of five sewage sludges and bentonite

Parameter	Sewage sludge					Bentonite
	Tábor	Kladno	Protivín	Č. Budějovice	Praha	
Dry matter (%)	19	18	16	21	29	
Zn (mg.kg^{-1})*	1829	1945	471	1266	1524	143
Cd (mg.kg^{-1})*	1.1	2.5	0.95	6.1	4.7	0.12
Total N (%)*	6.1	7.4	8.3	5.4	4.1	
C_{ox} (%)*	28.3	34.0	33.3	25.1	20.8	
pH/$CaCl_2$	7.2	6.9	7.3	7.7	7.4	

* in dry matter

1. under aerobic conditions, each 14 days mixed thoroughly, air conditioned, and watered (60% of matter lost was added as water to control moisture content)
2. under anaerobic conditions, homogeneic mixture was pressed into a pot, covered by plastic bag and sealed by elastic band. Pots were incubated in controlled incubation room (20°C and 80% relative humidity). The samples were taken at 2nd, 4th, 6th and 8th month from all five sewage sludges and stored in the freezer (–26°C) until beginning of analyses.

The availability of Cd in treated and untreated sewage sludge samples was determined by 1 mol.l^{-1} NH_4NO_3 solution (Pruess et al. 1991). The available content of Cd was determined in samples of 2nd, 4th, 6th and 8th month of incubation and sequential analysis of Cd (Száková et al. 1999) at 2nd and 8th month in the fresh material, included untreated sludge (beginning of experiment). For total content of Cd, the samples were decomposed by dry ashing procedure and ash was dissolved in diluted Aqua Regia (Mader et al. 1998). For the determination of Cd

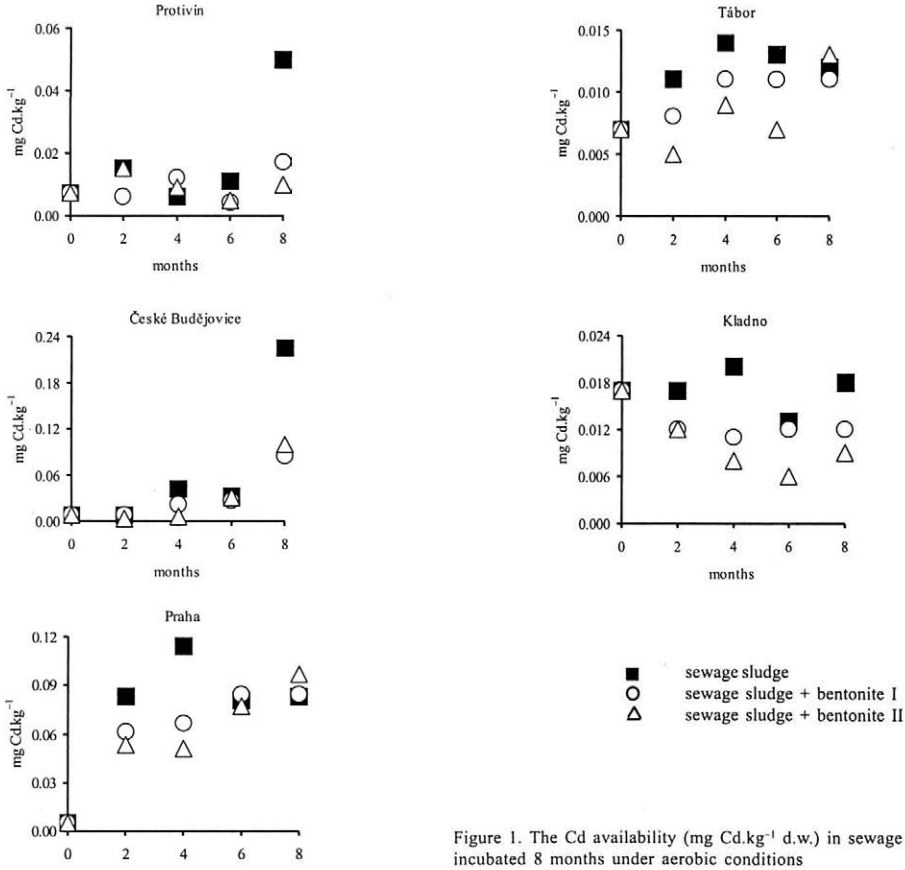

Figure 1. The Cd availability (mg Cd.kg^{-1} d.w.) in sewage sludge incubated 8 months under aerobic conditions

atomic absorption spectrometer (AAS) Varian SpectrAA-300 was used equipped by flame and flameless atomizers in Trace laboratories of Chemistry and Agrochemistry Departments. Quality of analyses of total Cd sludge content was controlled by reference material RM 12-03-12 Sludge. Certified value of Cd in reference material was 1.97 ± 0.21 mg.kg^{-1} and obtained value was 2.04 ± 0.35 mg.kg^{-1}.

RESULTS AND DISCUSSION

Tested stabilised sewage sludge showed different properties. Sludge from Protivín contained the lowest content of dry matter (16%) with high amount of organic matter (33%) and extremely low content of investigated heavy metals. On the other hand, Praha sludge contained the highest amount of dry matter (29%), the lowest amount of organic compounds (21%), and three to five times higher content of Cd and Zn. The amount of available Cd in sludge before incubation was not affected by total content of Cd in sludge and fluctuated in the range from 0.005 (Praha) to 0.017 (Kladno) mg Cd.kg^{-1}. Different properties of sewage sludge affected the availability of Cd during incubation. Aerobically incubated sludges mostly increased amount of mobile Cd in control as well as in bentonite treatments (Figure 1). The highest increase of availability was found in Praha sludge with the lowest content of organic matter at the first half of incubation (0.11 mg Cd.kg^{-1}). The ability of organic matter to bound heavy metals confirmed Karapanagiotis et al. (1991). Similar trend of increasing availability with lower slope was determined in Tábor sludge, too. At both sludges were determined drops of pH confirming higher activity of microbial oxidation during incubation. The rest of sludges did not significantly change Cd availability during four months of incubation. Second half of aerobic incubation showed different pattern. Low Cd mobility in Protivín and České Budějovice sludges was significantly increased at the end of incubation and confirmed by drop of pH, as well.

Individual changes of sludge stability under aerobic environment were partly confirmed under the lack of air (Figure 2). Sludges from Protivín and České Budějovice showed similar pattern under lack of air and also sludge from Kladno released majority of Cd at the end of incubation. Content of available Cd in sludge Tábor fluctuated among ranges determined in fresh material during period of incubation. Only in Praha sludge was released significantly less Cd under anaerobic conditions than in presence of air.

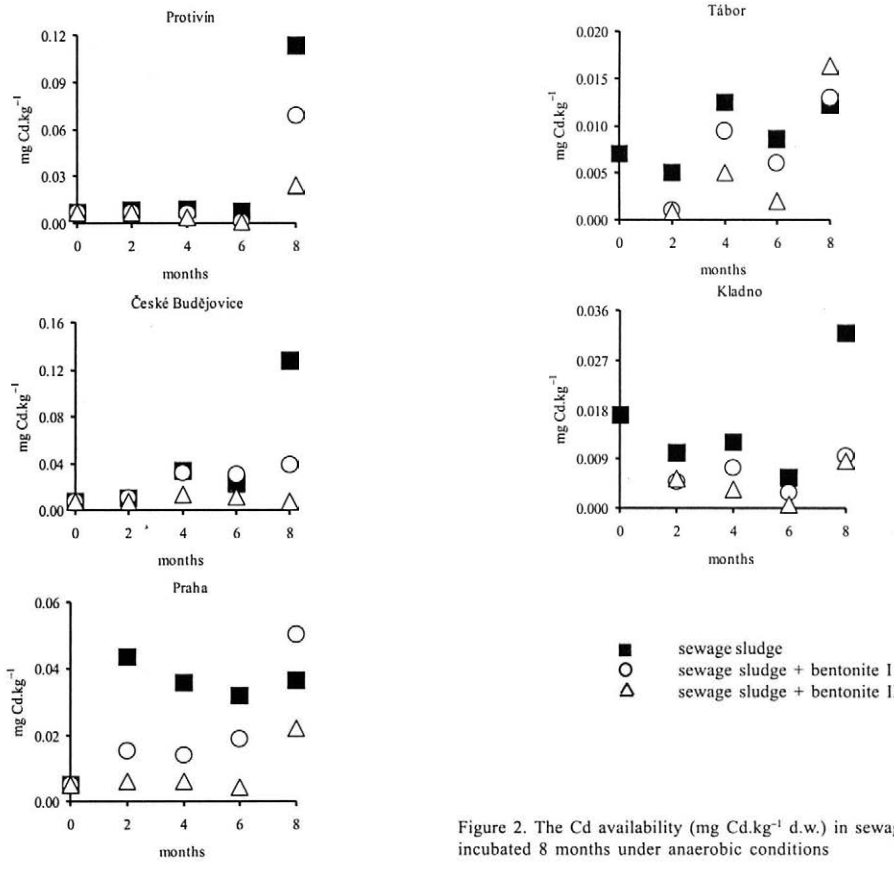

Figure 2. The Cd availability (mg Cd.kg^{-1} d.w.) in sewage sludge incubated 8 months under anaerobic conditions

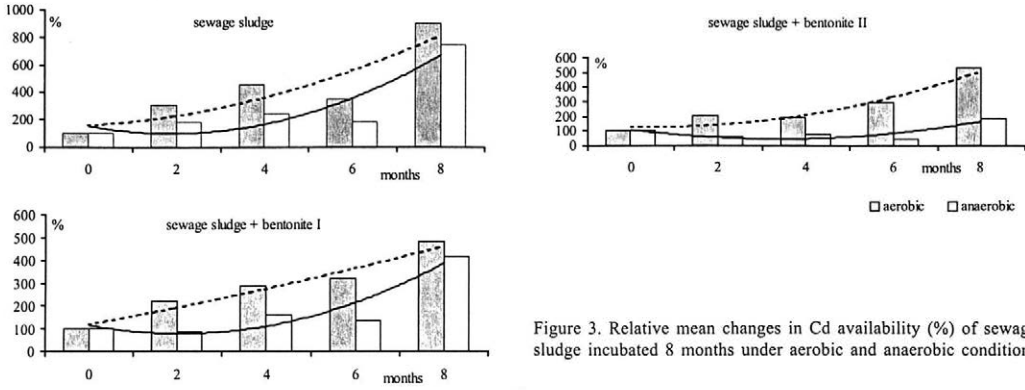

Figure 3. Relative mean changes in Cd availability (%) of sewage sludge incubated 8 months under aerobic and anaerobic conditions

Selection of sludges representing different quality of waste water and treatments at individual waste water plants showed a great importance of both factors for specific mobilization of metals during storage of sludge before application. Mean results presented in Figure 3 showed a slow growth of mobile Cd over the whole period of incubation with higher element values in presence of air than under anaerobic conditions. The presence of air led to increased Cd availability in the first half of experiment another growth at the end of experiment was followed by anaerobic treatments as well. The application of bentonite did not change the pattern of Cd availability but slightly increased differences between aerobically and anaerobically treated sludges (Figure 3).

The differences between Cd availability at aerobically and anaerobically incubated sludges are presented in Figure 4. The application of bentonite led to limitation of Cd mobility at the first half as well as at the second part of experiment. Our results with sludge treated by bentonite confirmed high binding capacity a such of material and also results published by Sims and Boswell (1978) and Hlušek and Richter (1992). Mean trend of decreasing Cd mobility was found at both aerobic and anaerobic treatments with similar slope. The anaerobically incubated sludges introduced always lower mean content of available Cd than sludges incubated in presence of air with the highest differences at the second rate of bentonite, where the portion of mobile Cd at anaerobic treatment was only 37% compared to aerobic one. The effect of rate of added bentonite played more important role in the first half of experiment than at the end. After eight months of incubation the differences in Cd mobility were found between treated and untreated sludges but the rate of bentonite played significant role under lack of air only. Regular conditioning of sludge probably damaged binding sites at treatment with higher amount of bentonite resulted in increased Cd mobility.

Fluctuation of Cd among main fractions during an incubation was determined by sequential extraction procedure. Majority of Cd in sludge was bound on organic matter (61%) and 32% of Cd contained residual portion of sludge. Only less than 2% of Cd was mobile in the material before incubation. The incubation of sludge significantly changed Cd distribution among main fractions. The presence of air supported oxidation of organic matter during incubation (Figure 5) and release of Cd into labile fractions. After two months of incubation organic and residual fractions contained 63% of Cd and after eight months 53%. Majority of element was bound at oxide fraction 28% and at exchangeable fraction 16% representing potentially available portion of element. Mo-

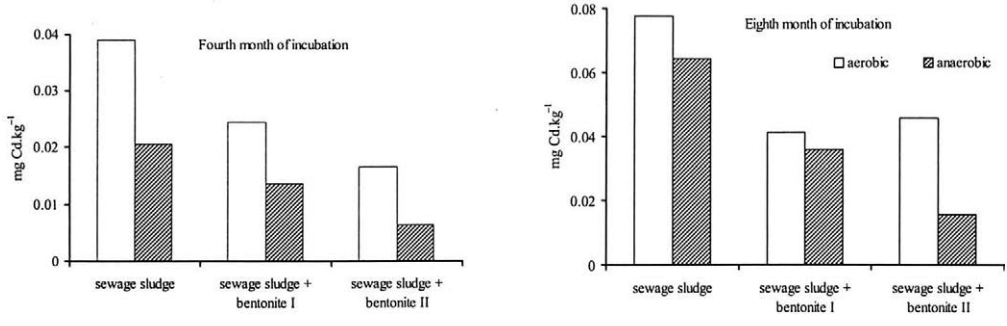

Figure 4. The comparison of mean Cd available content (mg.kg^{-1} d.w.) in sewage sludge incubated under aerobic and anaerobic conditions

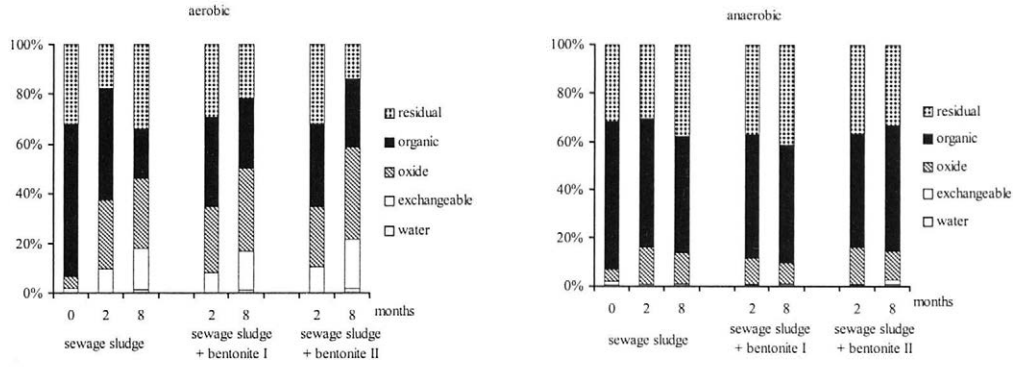

Figure 5. The distribution of Cd among main fractions (%) during eight months of incubation

bile portion of Cd determined by water extraction grew up consistently during incubation. Untreated sludges showed higher variation than sludges with bentonite addition. The application of bentonite slightly increased a portion of Cd in labile fractions mostly in the second half of incubation. Portion of Cd bound at oxide and exchangeable fractions was around 50% of total content and grew up at the treatment with high amount of bentonite. Differences between lower content of Cd released by ammonium nitrate at bentonite treatments than at the control sludge and higher Cd content in exchangeable fraction by acetic acid at the same treatments were caused by different extraction capacity of both solutions. Cd movement into mobile fractions determined by acetic acid has not been confirmed by ammonium nitrate extraction showing potential but not immediate risk of Cd availability for plants. The sludge incubation under lack of air has not substantially changed Cd distribution among main fractions. During incubation was found a slight reduction of Cd amount in organic fraction and its increase in oxide fraction at untreated sludges. The application of bentonite did not significantly change Cd distribution among fractions, only portion of exchangeable Cd went up slightly at bentonite treatments due to high bentonite sorption capacity.

The presence of air played an important role during sludge storage. Sludge stored under the presence of air lost a part of binding capacity due to oxidation of organic matter and mobilization of element from residual portion. Cd was mostly bound in labile fractions. The anaerobic environment did not substantially change Cd distribution among fractions. The bentonite addition supported Cd movement into labile fractions in aerobic conditions, but decreased element content in mobile fractions. Under the lack of air, bentonite has not affected Cd distribution, but showed higher capacity to bind mobile Cd.

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ABSTRAKT

Přístupnost Cd čistírenských kalů inkubovaných za aerobních a anaerobních podmínek a ošetřených bentonitem

Při hodnocení kvality čistírenských kalů se zpravidla sleduje celkový obsah rizikových kovů, přičemž rostlina reaguje pouze na přístupný podíl prvku. Hlavním cílem příspěvku bylo zjistit změny v přístupnosti Cd kalů inkubovaných v aerobním a anaerobním prostředí s přídavkem bentonitu. Stabilizované kaly z pěti čistíren byly inkubovány po dobu osmi měsíců za stabilních podmínek (20°C, 80% vlhkost) za přítomnosti vzduchu (promíchání vzorků každý druhý týden) a bez vzduchu (utlačení kalu do plastové nádoby a její utěsnění). Jednotlivé kaly byly inkubovány samostatně a s přídavkem dvou dávek bentonitu. Každá varianta byla třikrát opakována. Přístupný obsah Cd byl stanoven ve výluhu 1 mol.l^{-1} NH_4NO_3 každý druhý pokusný měsíc a sekvenční analýza Cd byla realizována dvakrát během inkubace. Získané výsledky potvrdily velmi vysoké rozdíly ve změnách přístupnosti Cd u jednotlivých kalů během inkubace. Během aerobní inkubace pravidelně rostl průměrný obsah přístupného Cd na neošetřených kalech stejně jako na kalech s přídavkem bentonitu. Při anaerobní inkubaci došlo k nárůstu přístupného Cd až na konci experimentu. Přídavek bentonitu vedl k poklesu přístupnosti Cd. Sekvenční analýza prokázala, že při aerobní inkubaci se významná část Cd vázaná v pevnějších vazbách přesunula do labilních forem, reprezentovaných významně vyšším podílem Cd v oxidové a výměnné frakci. Při anaerobní inkubaci nedošlo k výrazným změnám v zastoupení Cd v jednotlivých frakcích.

Klíčová slova: čistírenký kal; kadmium; přístupné formy; sekvenční analýza; bentonit; aerobní inkubace; anaerobní inkubace

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Dynamics of soil microbial activity and heavy metal availability after amendment of contaminated soils by lucerne substrate

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ABSTRACT

The dynamics of the soil microbial biomass and soil respiratory activity were studied during laboratory incubation with lucerne substrate in long-term contaminated soils from vicinity of a lead smelter. The microbial biomass of non-contaminated and contaminated arable soils did not differ significantly after 2 days of incubation, afterward till the end of incubation, the greater contents of microbial biomass were observed in more contaminated soils, probably due to a possible development of more resistant microbial strains. The greatest microbial biomass was determined in the less-contaminated grassland soil where the key role could play, in contrary to other contaminated grassland soils, the greater total organic carbon content (TOC) (3.5%). This could indicate that in this case the effect of organic matter was more important than heavy metal contamination. The respiration was after 2 days of incubation greater in arable contaminated soils than in the control. The important role of organic matter was manifested in grassland soils where the soil with 3.5% TOC showed lower respiratory activity indicating lower microbial biomass maintenance requirements than in contaminated soils with lower organic matter content. The significant relationships were obtained for DTPA-extractable lead and microbial biomass in all studied soils, whereas only few correlations were found for Cd and Zn.

Keywords: heavy metals; soil microbial biomass; respiratory activity; metabolic quotient (qCO_2); availability

Heavy metals belong to the most important pollutants of the environment. The organic matter and in particular the microbial pool and its activity can be significantly affected by pollution of soils by heavy metals (i.e. Brookes 1995, Giller et al. 1998). It is difficult to estimate the relationship between microbial pool and its activity in the soil and the actual metal toxicity, because there may be differences between laboratory experiments with laboratory addition of metal salts and the *in situ* situation where metals may be strongly bound on soil particles and their availability for the environment can be lower. The summary of results from such studies (Baath 1989) shows the enormous disparity between studies as to which metal concentrations are toxic. One of possibilities to estimate the soil heavy metal contamination is the determination of soil microbial communities which are in intimate contact with the soil environment and may give a more exact view about the real level of toxicity of heavy metals. Microbial properties as indicators of soil pollution by heavy metals are mainly: 1) the activities of whole microbial population, i.e. microbial respiration, soil N mineralization, 2) the size of the microbial population, eventually the combination of both activity and biomass data, giving specific activities of the microbial population (Brookes 1995). Both microbial biomass and respiratory activity measurements could serve as indicators of environmental stress due to metals also in non-experimental conditions (Barajas et al. 1999). The rate of respiratory activity in contaminated soils may depend upon the nature of soil organic matter and substrates which the microbial communities use. The metabolic quotient (qCO_2) described also as the specific respiration may indicate changes in the community composition and the change of physiological status of the community due to altered maintenance requirements (Insam et al. 1996). The contents of microbial biomass in contaminated soils often decrease compared to that in non-polluted soils, while CO_2 production and specific respiration increase, showing a greater energetic demand for survival in polluted soils (Brookes 1995, Leita et al. 1995, 1999, Barajas et al. 1999). However, studies focused on the evaluation of microbial activity in long-term contaminated soils indicate in accord with Insam et al. (1996) that the results could be more discussible.

The bioavailability of metals in soil is strongly influenced by the amount and quality of the organic matter which can interact with metals, forming complexes and chelates of varying stability (Leita et al. 1999). One potentially important organic surface are soil microorganisms which are small in size, but they exhibit a large area relative to the volume, a surface which may interact with dissolved trace constituents in the environment. Many microorganisms possess considerable-metal accumulating ability and may also exhibit substantial selectivity (Ledin et al. 1999). Soil microorganisms which are typically associated with the organic fractions of the soil are expected to influence the mobilization-immobilization equilibria of metals by changing the chemical composition of their immediate microenvironment (Leita et al. 1999).

The aim of the research was to assess the heavy metal toxicity to soil microbial pool and its activity in non-contaminated and long-term contaminated arable and grassland soils. The particular interest was pointed on the

dynamics of microbial biomass, its respiratory activity and specific respiratory rate during the incubation experiment after amendment of lucerne substrate. Possible relationships between DTPA-extractable heavy metal contents and soil microbial biomass were also studied.

MATERIAL AND METHODS

The sampling site is situated in vicinity of a lead smelter in Příbram, a historic mining and smelting town 60 km SW of Prague in the Czech Republic. The smelter has been operated for over two centuries, originally as a primary lead smelter utilising lead ores mined in the area. Metal mining has now been ceased in 1972, but a secondary lead smelter is in operation. (Kalac et al. 1991, Riuwerts and Farago 1996).

Arable (A, B, C) and grassland (D, E, F) soils were sampled in different distances from the smelter in order to receive lower and higher heavy metal contents. The soil D is a long-term grassland soil. The soils E and F were converted to grassland in 1995 because of their great heavy metal contamination. The soil samples were sampled from the depth 0–20 cm. The soils were after the manual remove of animals and plant debris sieved at < 2 mm and stored at 4°C till the beginning of experiment. The soil characteristics and the total contents of heavy metals are shortly described in Table 1, more precise data were reported by Mühlbachová and Růžek (2000).

Approximately 1 kg of each soil adjusted at 50% of its maximum water holding capacity (WHC) in plastic jars were placed in three replicates in greater plastic containers with tightly fitting lids at 27°C and conditioned 1 week before treating. The distilled water was added at the bottom of the containers to avoid drying of soils. A jar with 25 ml of 1M NaOH was placed to take up the CO_2-evolved. The containers were aerated daily to ensure a sufficient oxygen supply. After one week of preincubation the soils were treated with lucerne powder (N 2.5%, C 43%) in the dose 1000 μg $C.g^{-1}$ soil. Thereafter 50 g of soil of each treatment were weighed in three replicates and separately incubated in tightly closed jars containing 5 ml 1M NaOH to determine the CO_2-evolved. Soils were analysed at day 0, 2, 7, 14, 21 and 28 of the incubation for the content of microbial biomass, respiratory activity and DTPA heavy metal contents.

The measurements of the soil microbial biomass (B_c) were performed using the fumigation-extraction method (F.E.) according to Vance et al. (1987). Organic C in soil extracts was determined by digestion with $K_2Cr_2O_7$ and titration of excess dichromate with $(NH_4)_2Fe(SO_4)_2.6\,H_2O$. The microbial biomass C was calculated from the relationship: $B_c = 2.64\,E_c$, where E_c is the difference between organic C extracted from the fumigated and non-fumigated treatments, both expressed as μg $C.g^{-1}$ oven dry soil.

The CO_2-C evolved was determined as amount of organic carbon released as CO_2 after absorption in NaOH and precipitation with $BaCl_2$ was analysed by titration and with standard HCl. The metabolic quotient (qCO_2) was calculated according to Anderson and Domsch (1990) equation: $qCO_2 = \mu g\ CO_2\text{-}C.\mu g\ C_{Bc}^{-1}.h^{-1}$.

Table 1. Total organic carbon (TOC), pH, and total heavy metal content in arable (A, B, C) and grassland (D, E, F) soils from vicinity of a lead smelter of Příbram

Soil	TOC (%)	pH/H_2O	Pb	Cd	Zn
			mg.kg^{-1} soil		
A	1.41	5.28	58.7	0.30	50.35
B	1.63	7.21	595.7	3.31	295.9
C	1.51	7.00	1363.6	5.45	287.1
D	3.50	5.75	359.4	2.24	93.9
E	2.10	6.31	1162.6	4.99	240.6
F	2.23	6.98	2204.5	8.34	315.2

DTPA-extractable fractions of heavy metals were extracted from 10 g weighing samples of soil with 20 ml of extracting solution (0.005M DTPA, 0.01M $CaCl_2$ and 0.1M TEA adjusted to pH 7.3) according to Lindsay and Norvell (1978) procedure. DTPA extracts were filtered on Schleicher and Schuell filters No. 310645 and clear solutions were then analysed for metal content using the ICP spectrometer Trace Scan f. Thermo Jarrell Ash.

RESULTS AND DISCUSSION

The microbial biomass dynamics in arable and grassland soils contaminated with heavy metals during incubation of soils with lucerne powder is shown in Figures 1a, b. The

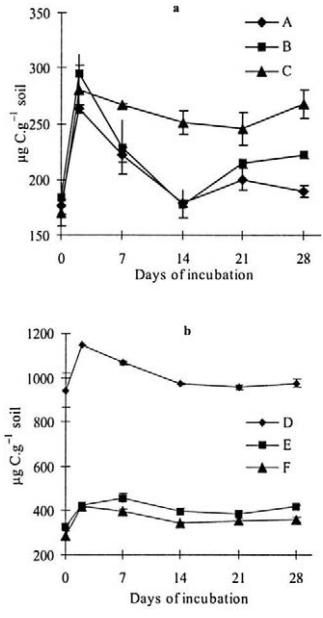

Figure 1. Dynamics of microbial biomass (y-axis) in arable (a) and grassland (b) soils during laboratory incubation with lucerne substrate; the vertical bars indicate a standard deviation

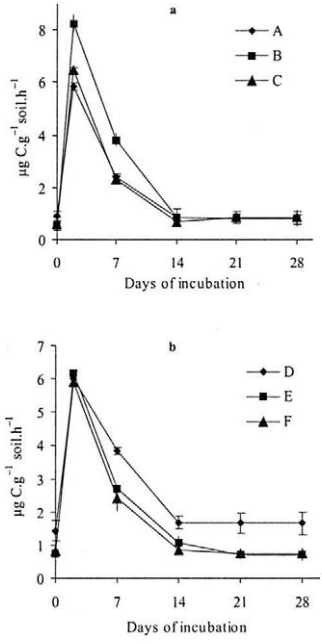

Figure 2. Respiratory activity (y-axis) in arable (a) and grassland (b) soils during laboratory incubation with lucerne substrate; the vertical bars indicate a standard deviation

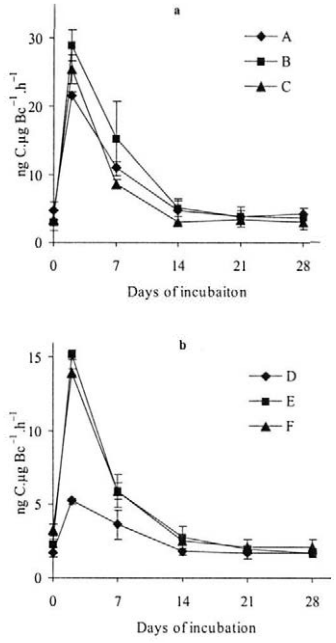

Figure 3. Metabolic quotient qCO_2 (y-axis) in arable (a) and grassland (b) soils during laboratory incubation with lucerne substrate; the vertical bars indicate a standard deviation

microbial biomass increase in arable soils did not show any significant difference between less and more contaminated soils after two days of incubation, afterward it decreased the most in the non-contaminated arable soil. Although the microbial biomass in soil B also decreased, it remained significantly greater than in the soil A throughout the incubation. Exception was the measure after two weeks of incubation when the microbial biomass contents in soils A and B were the lowest. The greatest contents of microbial biomass during the overall time of the incubation were observed in the most contaminated arable soil C (Figure 1a). In comparison to arable soils, the grassland soils having greater organic matter content demonstrated the greater microbial biomass compared to that in arable soils throughout incubation. The microbial biomass in soil D was about three times greater than in soils E and F (Figure 1b). A possible explanation may be rather than lower heavy metal pollution, the significantly greater content of organic matter in the soil D compared to the soils E and F. In fact, the native microbial biomass of soils from Příbram area, irrespective to their heavy metal content, well correlated with the total organic carbon content (Mühlbachová and Růžek 2000). The dynamics of the microbial biomass during incubation was in the more contaminated soil F lower compared to the soil E, indicating that the microbial pool might be affected by metal stress. Brookes (1995), Chander and Brookes (1991), Giller et al. (1998), Kelly et al. (1999), Leita et al. (1995), Šimon (2000) found significantly lower microbial biomass contents in polluted soils. In fact, these authors often performed their experiments on experimentally contaminated soils, eventually on soils from field experiments in which the exposition time of pollution by heavy metals was shorter. On the contrary, in this study of soils polluted for more than 200 years, the heavy metal complexes in soils could achieve such an equilibrium, metals could become less available for soil environment and therefore less toxic for microbial communities. The resistance of soil microbial populations to heavy metal effects has been studied in several studies. Microbial population sensitive and resistant to Pb contamination have been found in metal-stressed soils, but also in soils with no previous exposure to lead (Roane and Kellog 1996). The increase of micromycetes resistant populations compared to more sensitive bacteria reported by Šimon (2000) may also explain the results obtained in this experiment.

The respiratory activity during soil incubation is reported in Figures 2a, b. The basal respiration in arable soils ranged between 0.537 and 0.745 µg $C.g^{-1}$ $soil.h^{-1}$ at the beginning of experiment. During the first two days of incubation the respiration increased in contaminated arable soils B and C more than in the soil A. Thereafter the respiration decreased and after 14 days of incubation it was found to be near initial values in all arable soils. The greatest respiratory activity in grassland soils was obtained in the soil D (1.432 µg $C.g^{-1}$ $soil.h^{-1}$) and represented twice higher levels than found in soils E and F. Similar values of respiration activity (5.895–6.174 µg $C.g^{-1}$ $soil.h^{-1}$)

Table 2. Simple linear correlation coefficients ($P < 0.05$) between DTPA-extractable Pb, Cd and Zn and content of soil microbial biomass in arable (A, B, C) and grassland (D, E, F) soils

Soil	Pb	Cd	Zn
A	0.88	ns	0.60
B	0.83	ns	ns
C	0.81	ns	ns
D	0.92	ns	ns
E	0.85	0.75	ns
F	0.88	0.54	0.66

ns – non-significant

were measured in soils D, E, and F after the first two days of incubation. Thereafter the respiratory activity in contaminated soils E and F decreased more rapidly and from the 14th day of incubation similarly as in arable soils the respiration was found to be close to initial values and thereafter decreased only slowly.

The metabolic quotient (qCO_2) determining the respiratory activity per unit of microbial biomass can be a more representative measure of heavy metal stress of soils than the simple determination of soil respiration (Anderson and Domsch 1990, Bajaras et al. 1999). The qCO_2 was after two days of incubation significantly higher in more contaminated arable soils B and C compared to the non-contaminated soil A and about three times greater in grassland soils E and F compared to the soil D (Figures 3a, b). Afterwards, the values of the metabolic quotient decreased, the relatively lowest decrease was observed in the less contaminated soil D. The values of the qCO_2 in grassland soils E and F decreased after they reached the maximum of 58.5% and 56.8% compared to 40.8% obtained for the soil D on the 7th day of incubation. Thereafter the values of qCO_2 in soils decreased during the next three weeks near to their original levels.

Insam et al. (1996) dispute the assumption that heavy metal stress on the soil microflora may be easily detected by an increased qCO_2 irrespective to other soil properties. In contrast to the data of Fließbach et al. (1994), Insam et al. (1996) did not report any increase of the qCO_2 as a result of heavy metal contamination. In fact, the elevated measures of qCO_2 were found in studies where the heavy metal contaminated substrates (sewage sludges or municipal solid waste composts) were amended (Leita et al. 1999). The results obtained in the performed incubation experiment with lucerne substrate show that the simple measure of respiratory activity or value of qCO_2 could give more valuable data only in contaminated soils with similar organic matter content (Figures 2, 3). However, the same evaluation of results in the studied grassland soil is more difficult to assess because of the effect of organic matter on qCO_2 measured in grassland soils.

DTPA-extractable Pb well correlated with microbial biomass content in all studied soils, whereas the significant correlations for Cd and Zn were found only in two of them (Table 2). Only few relationships obtained for the microbial biomass and Cd and Zn DTPA-extractable contents cannot give more precise estimation of the role of soil microorganisms on their mobility in this incubation experiment. The DTPA-extractable Pb increased significantly in function of microbial biomass (Figures 4a–f). Leita et al. (1995, 1999) suggest that microorganisms can be significantly involved in mobilization-immobilization processes of toxic elements in soils. In their experiments, the available fractions of some toxic elements decreased in correspondence to the microbial biomass content after soil

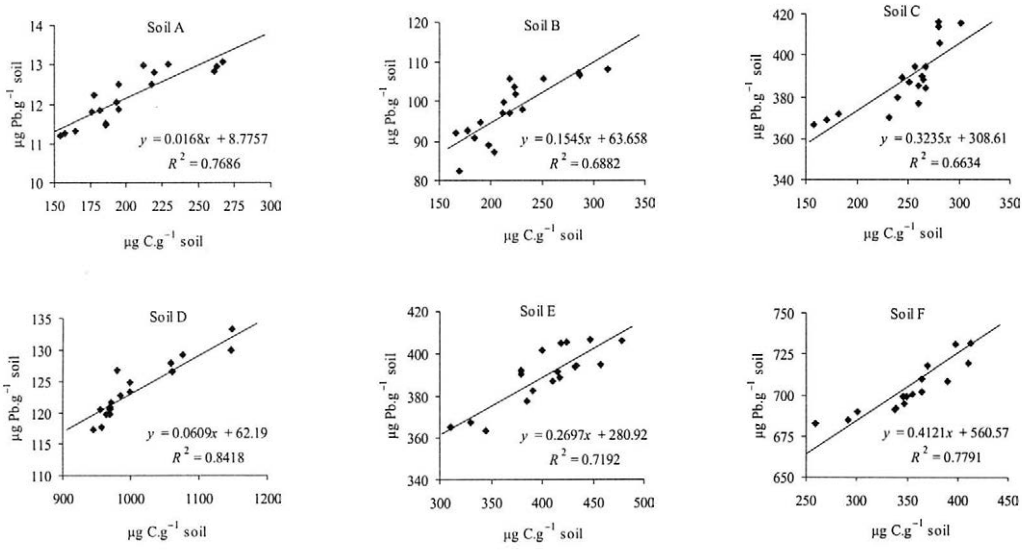

Figure 4. Relationships between soil microbial biomass (x-axis) and DTPA-extractable Pb (y-axis) in arable (A, B, C) and grassland (D, E, F) soils during laboratory incubation with lucerne substrate

treatment by metal salts (Leita et al. 1995), or increased in soils amended by contaminated municipal solid waste composts (Leita et al. 1999). Metals in soils are not all available because of chelation by organic molecules and the occurrence of chemical forms which cannot be taken up directly. Microorganisms may alter metal availability in their vicinity due to localised acidification of the environment, or production of compounds which complex metals. For example, iron-oxidising bacteria, which reduce iron pyrits to $FeSO_4$ and H_2SO_4, can cause an extreme acidification causing increases in metal availability (Giller et al. 1998). One of possible explanations of increased Pb DTPA-extractability may be also the greater microbial activity during the incubation experiment.

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ABSTRAKT

Dynamika půdní mikrobiální aktivity a přístupnost těžkých kovů po přídavku vojtěškového substrátu do kontaminovaných půd

V laboratorním inkubačním pokusu byl sledován vliv přídavku organického substrátu (vojtěškové moučky) na syntézu mikrobiální biomasy a půdní respirační aktivitu u půd dlouhodobě kontaminovaných těžkými kovy pocházejícími z hutní činnosti. Obsah mikrobiální biomasy se v nekontaminované a v kontaminovaných orných půdách po dvou dnech inkubace statisticky významně nelišil, poté až do konce pokusu zůstával vyšší u více kontaminovaných půd, což mohlo být způsobeno syntézou rezistentnějších mikrobiálních kmenů. Nejvyšší obsah mikrobiální biomasy byl stanoven u méně kontaminované zatravněné půdy, u které však klíčovou úlohu ve srovnání s ostatními kontaminovanými zatravněnými půdami měl vyšší obsah celkového organického uhlíku (TOC) (3,5 %), což naznačuje, že vliv organické hmoty na syntézu mikrobiální biomasy byl větší než vliv těžkých kovů. Respirační aktivita byla po dvou dnech inkubace větší v orných kontaminova-

ných půdách než v kontrolní půdě nekontaminované. Výsledky pokusů nasvědčují, že v zatravněných půdách hrál důležitou roli obsah organické hmoty, kde půda s 3,5% TOC pravděpodobně proto, že mikroorganismy měly v tomto případě nižší energetické nároky na svůj rozvoj, vykazovala nižší respirační aktivitu než v půdy více kontaminované s nižším obsahem organické hmoty. Mezi DTPA-extrahovatelnými obsahy Pb a mikrobiální biomasou byly zjištěny statisticky významné závislosti ve všech sledovaných půdách, zatímco pro Cd a Zn byly závislosti nalezeny jen u dvou půd.

Klíčová slova: těžké kovy; půdní mikrobiální biomasa; respirační aktivita; metabolický kvocient (qCO_2); přístupnost

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INFORMACE

Doporučení pro vyjadřování výsledků agrochemických rozborů rostlin, půd, hnojiv a potřeby hnojení

Komise výživy rostlin odboru rostlinné výroby ČAZV se na svém plenárním jednání dne 21. 3. 2001 zabývala problematikou vyjadřování výsledků chemických analýz rostlin, půd a hnojiv, dále používanými jednotkami a celkovou úrovní a znalostí v této oblasti. Byla konstatována značná různorodost, nejednotnost a rozdílná úroveň, a tím i mnoho nepřesností a rozdílný výklad uváděných hodnot, zvláště u pracovníků, kteří se danou problematikou bezprostředně nezabývají. Proto předkládáme doporučení, která usnadní orientaci a jednotný výklad.

1. Obsah živin v rostlinách a půdách. U všech živin (včetně rizikových prvků) uvádět jejich obsah výhradně v čistých prvcích – tedy N, P, K, Ca, Mg, S, Cl, Cu, Mo, Ni, Cr, Cd apod. Také ostatní údaje, týkající se rostlin a půd, jako je odběr, potřeba a dávky živin, uvádět jen v čistých prvcích, např. odběr živin pšenicí při výnosu zrna 5 t na ha činí 120 kg N, 22 kg P, 88 kg K, 20 kg Ca a 11 kg Mg. Podobně dávky živin, např. pro hnojení cukrovky bylo použito dávky 90 kg N, 35 kg P, 120 kg K a 1 kg B na ha. U jednotlivých živin lze uvádět např. k cukrovce byla použita dávka 120 kg K na ha ($K.ha^{-1}$) v 60% draselné soli, dávka 0,8 t Ca na ha ve formě uhličitanu vápenatého.

Také pro uvádění obsahů živin a dávek živin ve statkových hnojivech je účelné používat čistých prvků i u P, K, Ca i Mg.

2. Obsah živin v hnojivech. Pro uvádění obsahů živin v hnojivech je nutné respektovat zákon č. 156/1998 Sb. o hnojivech, pomocných půdních látkách, pomocných rostlinných přípravcích a substrátech a agrochemickém zkoušení zemědělských půd ve znění zákona č. 308 /2000 Sb. a vyhlášek souvisejících s těmito zákony, tedy především vyhlášky č. 474/2000 Sb. Ministerstva zemědělství ze dne 13. 12. 2000 o stanovení požadavků na hnojiva, která stanovuje, že obsah živiny v jednosložkových hnojivech musí být uveden v procentech hmotnosti jako celé číslo, nebo na jedno desetinné místo, a to dusík jako N, fosfor, draslík, vápník a hořčík v oxidech, tedy P_2O_5, K_2O, CaO a MgO. U fosforu, draslíku, vápníku a hořčíku se může uvést vedle obsahu v oxidu i obsah prvku, nejlépe v závorce. Pro vzájemné přepočty z oxidu na prvky a naopak je možné použít těchto přepočítávacích koeficientů:

P_2O_5 na P – 0,44
K_2O na K – 0,83
CaO na Ca – 0,71
MgO na Mg – 0,60

Uvádění obsahu živin v minerálních hnojivech v oxidech má své opodstatnění v dlouhodobě zavedené praxi a také v komerčně propagační strategii. Např. trojitý superfosfát, obsahující 40 % P_2O_5 obsahuje 17,4 % P. Je tedy zřejmé, že z propagačního hlediska není údaj v prvku žádoucí, i když je obsah vlastní účinné složky stejný.

Pro vícesložková hnojiva je nutné používat toto pořadí živin: N, P_2O_5 (P), K_2O (K). Např. pro vícesložkové hnojivo Lovofert 12–19–19, dříve označované jako NPK 1, by bylo vhodné označení 12–19 (8,4)–19 (15,8).

Formu živiny u dusíku je vhodné vyjadřovat takto:
– dusík dusitanový (nitritový) $N\text{-}NO_2^-$
– dusík dusičnanový (nitrátový) $N\text{-}NO_3^-$
– dusík amonný $N\text{-}NH_4^+$

Bude vhodné postupně přecházet na udávání všech obsahů v prvcích, např. dusičnan hořečnatý $Mg(NO_3)_2$ obsahuje 10 % N a 8,4 % Mg ve vodě rozpustného – podle dosavadní praxe je nutné napsat, že dusičnan hořečnatý obsahuje vedle 10 % N ještě 14 % MgO, tedy hořčík ve vodě rozpustný vyjádřený jako MgO (oxid hořečnatý je však ve vodě nerozpustný).

3. Musí být samozřejmostí používat jednotek SI. Komise však doporučuje při vyjadřování výsledků **přednostně používat relativních jednotek, především % a ppm**, kdy není nutné uvádět absolutní jednotky – běžně známá hodnota % je setina základu – 1 % = 1.10^{-2} a ppm je miliontina základu (part per million) – 1 ppm = 1.10^{-6}.

Např. obsah N v zrnu pšenice je jednodušší uvádět jako 2,01 % N než 2,01 g N.100 g^{-1} nebo 20,1 g N.1000 g^{-1}, případně 20,1 g $N.kg^{-1}$. Z hlediska psaní by bylo praktičtější napsat lomítko – 20,1 g N/kg, případně slovně 20,1 g N v 1 kg.

Podobně nižší obsahy, většinou pod 0,1 % je vhodnější uvádět v ppm. Např. obsah 5,5 ppm Cu je jednodušší než 5,5 μg $Cu.g^{-1}$, nebo 5,5 mg $Cu.kg^{-1}$, případně 5,5 g $Cu.t^{-1}$. Jistě je jednodušší napsat, že listy obilnin mají obsahovat v sušině 15–25 ppm Mn, než že listy obilnin mají obsahovat 15–25 mg $Mn.kg^{-1}$ sušiny.

Pokud je to možné, je vhodné dodržet v tabulkách stejné jednotky. Není přípustné např. obsah N uvádět v procentech a obsah C v mg na 100 g apod., případně v textu procenta a v tabulkách nebo grafech hmotnostní jednotky.

Prof. Ing. Václav Vaněk, CSc.
předseda komise výživy rostlin ČAZV

REJSTŘÍK – INDEX

INFORMACE – INFORMATION

KRÁTKÉ SDĚLENÍ – SHORT COMMUNICATION

Z VĚDECKÉHO ŽIVOTA – FROM THE SPHERE OF SCIENCE

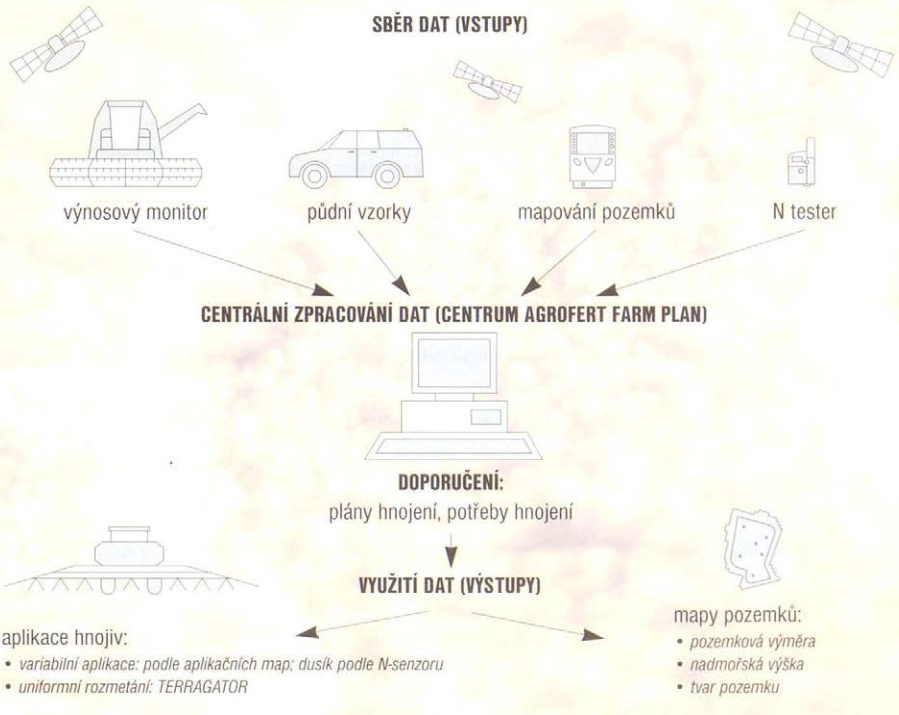
RECENZE – REVIEW

Jedním ze znaků moderní fungující společnosti je přínos a uplatňování nových myšlenek a technologií v praxi, a proto tým odborníků AGROFERT HOLDING, a. s., sestavil ucelený komplex služeb, řadících se do systému precizního zemědělství, nabízený pod obchodním názvem:

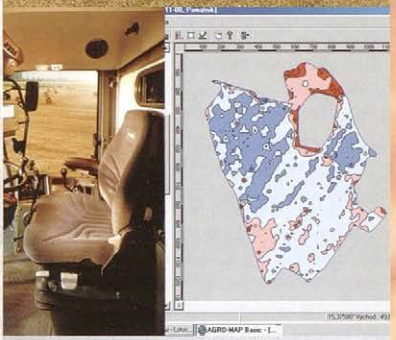
AGROFERT FARM PLAN

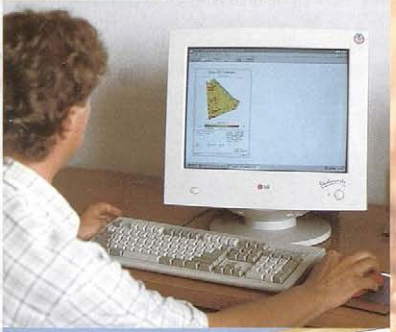
Snahou bylo jednak lépe a efektivněji využít stávající technické vybavení a lidský potenciál sdružený ve skupině, ale také zpřístupnit tyto moderní technologie a postupy co nejširší skupině zákazníků. Velkoplošné intenzivní zemědělství s sebou přineslo, mimo jiné, rozdílnou úroveň půdních vlastností v rámci jednoho honu (zásoba živin, výnos atd.). Ale teprve s nástupem moderních, především IT technologií (internet, GPS signál) je možno tohoto faktoru cíleně využít.

Struktura fungování systému precizního hospodaření AGROFERT FARM PLAN



KOMPLETNÍ NABÍDKU A PODROBNÉ PŘEDSTAVENÍ VÁŠ DODAVATEL





AGROFERT HOLDING, a.s.

Vám v rámci systému FARM PLAN nabízí:

- **PLÁNY HNOJENÍ**
  – pomoc při využití AZP – PC program stanoví teoretickou potřebu živin a potřebu hnojiv
- **MAPOVÁNÍ HRANIC POZEMKŮ**
  – využití satelitní technologie (DGPS) – přesná výměra, nadmořská výška a tvar pozemku
- **N TESTER**
  – stanovení potřeby N hnojení na základě zjištění chlorofylu (on-line)
- **ODBĚRY A ZPRACOVÁNÍ PŮDNÍCH VZORKŮ**
  – přesné zaznamenání souřadnic odběrového místa (DGPS) a mapy půdní zásobenosti
- **CÍLENÉ ODBĚRY PŮDNÍCH VZORKŮ**
  – bodů na pozemku pro každoroční kontrolu výživy rostlin a účinku agrotechnických zásahů
- **MONITORING VÝNOSŮ**
  – zpracování dat, tvorba výnosových map
- **ZPRACOVÁNÍ DOPORUČENÍ**
  – pro variabilní aplikaci hnojiv, tvorba aplikačních map
- **VARIABILNÍ APLIKACE PRŮMYSLOVÝCH HNOJIV**
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VÝZNAM A PŘÍNOS:

- zvýšení efektivity rostlinné výroby
- napomáhá ke snižování zátěže životního prostředí
- usnadňuje rozhodování o optimalizaci vstupů a výstupů do RV
- zvyšuje komfort a úroveň zemědělského hospodaření
- zvyšuje kvalitu a efektivitu aplikace hnojiv
- nabízí rychlý přístup k využití moderních technologií a myšlenek
- nabízené postupy ověřuje v praxi a spolu s vědeckou sférou pracuje na jejich dalším rozvoji

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ROSTLINNÁ VÝROBA PLANT PRODUCTION

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