Distribution of pesticide residues in soil and uncertainty of sampling

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Abstract

Pesticide residues were determined in about 120 soil cores taken randomly from the top 15 cm layer of two sunflower fields about 30 days after pre-emergence herbicide treatments. Samples were extracted with acetone-ethyl acetate mixture and the residues were determined with GC-TSD. Residues of dimethenamid, pendimethalin and prometryn ranged from 0.005 mg/kg to 2.97 mg/kg. Their relative standard deviations (CV) were between 0.66 and 1.13. The relative frequency distributions of residues in soil cores were very similar to those observed in root and tuber vegetables grown in pesticide treated soils. Based on all available information, a typical CV of 1.00 was estimated for pesticide residues in primary soil samples

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(soil cores). The corresponding expectable relative uncertainty of sampling is 20% when composite samples of size 25 are taken. To obtain a reliable estimate of the average residues in the top 15 cm layer of soil of a field up to 8 independent replicate random samples should be taken. The obtain better estimate of the actual residue level of the sampled filed would be marginal if larger number of samples were taken.

Keywords: Pesticide residues in soil, distribution of pesticide residues, uncertainty of sampling

Introduction

The distribution of pesticide residues in / on treated objects has been extensively studied. The deposition of residues is affected by several factors such as, application technique, positioning of nozzles, growth stage and spatial arrangements of treated plants, microclimatic conditions.[1-5] Certain proportion of applied dose inevitably reaches the soil as a combined effect of factors mentioned above. [1, 6] Further on, heavy rain or sprinkling irrigation can wash off the residues from the treated surface.[1, 7, 8].

Around hundred-fold differences were found in various fruits (apple, banana, kiwi, orange, peach, pear, plum, tomato) being in various positions of the trees. [9-11] Similar variability was found in crops taking up the pesticide residues from soil following broadcast [12] or furrow application. [13]

Most of the studies on distribution of residues were performed by taking 80 to 130 samples from the treated areas. Each sample set provides one estimate of the true variability of

residues. Model experiment reported by Ambrus [14] revealed that a minimum of 300 samples should be taken from one field to get an estimate of the relative standard deviation (CV) describing the true variability of residues within about 3 percent. The large variability of CV values of residues ranging from 0.11 to 1.42 in sample sets of 100-130 crop units representing 182 crop-pesticide combinations [15,16] indicated the uncertainty of sampling. It was shown that one sample set may not provide reliable estimate of the true distribution of residues on the treated area. Farkas et al. reported [16] that the relative range of the expectable CV of residues in composite samples is independent from the CV of the residues in primary samples, and preferably minimum 4 replicate samples should be taken from each of 20 different fields to obtain the relative difference of CV values within 50%. Further on, their results confirmed that the central limit theorem describing the relationship between the variance of residues in primary samples (V_1) and composite samples (V_n) as a function of number of primary samples (n) is also applicable for strongly skewed continuous distribution:

$$V_n = \frac{V_1}{n} \quad (1)$$

The uncertainty of the measured residue comprises of four major components, [17] such as sampling (S_S), subsampling (S_{SS}), sample preparation (removing the parts from soil which are not analyzed e.g. plant remains, pebbles etc.), sample processing (comminution, homogenization of the bulk sample taken from the field) (S_{Sp}) and analysis of test portion (S_A) withdrawn from the homogenized analytical sample. The uncertainty of sample preparation cannot be quantified, but by carefully following the detailed standard operation procedure can be minimized. If the procedure is carried out correctly, the average concentration of the pesticide residue does not change during the above operations. Their contribution to the

combined uncertainty of the measured residues (CV_R) can be expressed with their relative standard deviation according to the general rule of propagation of random error: [18]

$$CV_R = \sqrt{CV_S^2 + CV_{SS}^2 + CV_{Sp}^2 + CV_A^2} \quad (2)$$

When subsampling is performed in the laboratory, the uncertainty of the laboratory phase of the analysis (CV_L) incorporates the subsampling together with sample processing and analysis:

$$CV_L = \sqrt{CV_{SS}^2 + CV_{Sp}^2 + CV_A^2} \quad (3)$$

The uncertainty of sampling, which cannot be directly determined, can be calculated as:

$$CV_S = \sqrt{CV_R^2 - CV_L^2} \quad (4)$$

Once the method is optimized and validated, the CV_L, representing the within laboratory reproducibility of the method, can be conveniently determined from the results of reanalyzes of retained test portions containing residues in well detectable concentration as part of the regular quality control of the laboratory. If the relative difference of the results of replicate measurements of one sample is

$$\Delta_i = \frac{|R_1 - R_2|}{\bar{R}} \quad (5)$$

and k samples were analyzed in replicates during the routine operation, the typical within laboratory reproducibility of the measurements can be calculated as:

$$CV_L = \frac{\sum \Delta_i}{1.128 \times k} \quad (6)$$

where the factor of 1.128, corresponding to duplicate measurements, is taken from range statistics. [19]

The fate of residues in soil is widely studied as different tests are required for the assessment of the environmental behavior of residues before registration of a pesticide is granted. [20] For instance, samples are taken from the treated fields at various times after the application to determine the decline of residues, runoff from the treated fields and the potential of residues in follow crops. To correctly interpret the results of some environmental fate studies carried out on large scale test areas, the information on the uncertainty of sampling would be required.[21]

In contrast to the extensively-studied distribution of residues in treated plants, practically no information related to distribution of residues in soil of large fields is available.

The objectives of our work are to (a) determine the variability of residues in individual soil cores (primary samples) taken from the upper 15 cm layer of commercially treated fields; (b) demonstrate that, in the age of GC-MS/MS, LC-MS/MS techniques, simple gas chromatographic analyses of samples of known pesticide treatment history can still be used to obtain reliable results; (c) compare the distribution of residues in soil to those found in plants; (d) estimate the uncertainty of sampling of soil for determination of pesticide residues, and provide guidance for preparing sampling plans.

Materials and methods

120 ***Collection of soil core samples***

121

122 Two sunflower fields with different soil characteristics and known pesticide treatment
123 histories were selected in the northeast part of Hungary near Mezőkövesd and Hercegkút.
124 Both fields were treated according to the regular agricultural practice. Three active
125 ingredients: dimethenamid ((RS)-2-chloro-N-(2,4-dimethyl-3thienyl)-N-(2-methoxy-1-
126 methylethyl)acetamide), pendimethalin (N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine), and
127 prometryn (N^2,N^4-diisopropyl-6-methythio-1,3,5-triazine-2,4-diamine) were used as pre-
128 emergent herbicides and sprayed onto the soil surface. The details of the pesticide
129 applications and basic soil parameters are summarized in Tables 1 and 2, respectively.

130

131 The rectangular sampling sites of 100 x 100 m were selected in the middle of the fields. At
132 every 10 m along the four edges of the site white wooden sticks were placed to mark the
133 position. The random sampling positions were allocated with one meter accuracy based on the
134 X:Y coordinates drawn with MS Excel randbetween function. Six assistants and the project
135 leader took part in the sampling operations. Four assistants were moving along the edges of
136 the sampling site and stopped at the corresponding coordinate. Two assistants were taking the
137 samples from the imaginary crossing of the lines between the by-standers standing at the X:Y
138 coordinates at the edges of the field as illustrated in Figure 1. The persons taking the samples
139 carried with them a Garmin GPS navigation device and recorded the coordinates shown on it.
140 The accuracy of visual location of the sampling position was within the accuracy (±3 m) of
141 the navigation device.

142

Altogether 130-130 soil cores of 5 cm diameter down to 15 cm depth were taken from each sampling site (300-400 g/soil core) about four weeks after the pesticide treatments. The samples were stored in deep-freezer within 12 hours after sampling and kept frozen until their analysis. Untreated soil samples were taken from the nearby fields of similar soil characteristics. As an example, the positions of taking random samples and the approximate prometryn residues found in the primary soil cores are shown in Figure 2.

Preparation of soil samples

The soil cores were processed as described by Suszter et al. [22] Each sample was weighed, spread on a tray and the foreign materials, pebbles were removed, and the prepared soil was weighed again. The soil was pressed through a 5-mm sieve and transferred into the blender. The water content of the soil was adjusted to about 30-40 w/w % by adding distilled water. The amount of added water was recorded. The soil water mixture was let to stand for a few minutes and then it was homogenized. The consistency of the matrix was examined visually and, if required, more water was added to get a creamy soil pulp.

For checking the recoveries in each analytical batch, about 2 kg of blank, untreated soil was homogenized with sufficient amount of water in a blender. From the creamy soil pulp 20-20 g soil equivalents were measured in labeled polyethylene bags and stored in a freezer until they were used.

Analysis of samples

About hundred and twenty samples were analyzed with the validated method described in the preceding article,[23] and 10 samples were kept as reserve. The performance parameters of the method complied with the Codex GL[24] and the European Guidance Document [25].

Matrix matched calibration mixtures containing dimethenamid (DI), pendimethalin (PE) and prometryn (PR) were prepared in 8 different concentrations (½LOQ – 150*LOQ ranged about 28-8000 ng/mL in case of DI and PE, and 15-4000 ng/mL in case of PR) in ethyl acetate. Chlorpyrifos (300 ng/mL) was added to each calibration solution as internal standard (ISTD). The samples were analyzed in sample sets. One set consisted of one system suitability mixture (SST) [26], one reagent blank and blank soil sample, 8 calibration solutions (from 0.5*LOQ up to 150*LOQ), ten soil samples containing field incurred residues, one extract of a retained test portions of a sample analyzed earlier, and one spiked sample at the LOQ or 20*LOQ or 100*LOQ level. The order of injection was randomized. Figures 3 and 4 illustrate the separation of compounds and the selectivity of the detection.

Internal quality control

The concurrent recoveries obtained during the analyses of samples are summarized in Table 3.

To estimate the long-term within laboratory reproducibility (CV_L), replicate test portions were taken from some of the samples and their residue contents were measured on different days. For this experiment 20-20 g soil equivalents from the homogenized treated soils were withdrawn into a labeled PE bag and stored in a freezer until the replicate analysis.

The long-term reproducibility was calculated with Equations 5 and 6. The results are summarized in Table 4.

Results and discussion

Based on the binominal theorem n=119 samples would cover the 97.5th percentile (βp) of the expected residues with 95% probability level (βt). [14]

$$1-\beta_t=\left(\beta_p\right)^n \quad n=\frac{\log(1-\beta_t)}{log\beta_p} \tag{7}$$

It is recognized that larger number of samples would have provided better coverage of variability of residues, but the laboratory capacity did not allow the analyses of more samples. Further on, most of the experiments carried out with plant samples [12, 14, 15] included the analyses of about 100-130 primary samples, which made the comparison of the results easier. The residues determined in individual soil cores are summarized in Table 5. The spread of residues in soil cores (CV_{distr}), excluding the contribution of the variability of analysis, can be calculated from the variances of CV_R, and the reproducibility CV_L values (Table 4).

$$CV_{distr}=\sqrt{CV_R^2-CV_L^2} \tag{8}$$

The contribution of within field variability of residues (CV_{distr}) to the variability of detected residues CV_R (calculated from the corresponding variances as V_{distr}/V_R%) ranged between 95-99%, which indicates that the contribution of the variability (uncertainty) of analytical results to that of measured residues in soil cores is negligible. Therefore, the sampling uncertainty can be directly calculated from the measured residues applying Equation 1.

The relative frequency distribution of normalized residues (residues measured in soil cores taken from one field are divided with their average value) found in samples taken from the Mezőkövesd field is shown in Figure 5. The pattern is same as found in case of carrot samples

taken from treated fields in another study reported earlier. [12, 15] For comparison, the relative frequency of linuron residues in carrot is also included in Figure 5.

The applicability of central limit theorem for pesticide residues present in cores of treated soil was tested by drawing 10000 random samples of sizes 10 and 25 with replacement [26]. The results, summarized in Table 6, show that the difference (Δ_{CV}%) in the relative standard deviations of residues in composite samples obtained with random sampling (CV_R) and the theoretically expected ones (CV_{Rth}) based on Equation 1 are less than 1.2%. The difference in the average residues in primary samples and the corresponding averages of calculated residues in composite samples (CV_{AVE}%) are less than 0.4%. The averages of CV_{Rsoil} and $CV_{Rrootveg}$ values from the five primary soil datasets and from 14 datasets of the residues in carrot and potato[15] are 88% and 99%, respectively. Farkas and co-workers (16) estimated a $CV_{Rrootveg}$ of 1.03 for primary samples of root and tuber vegetables based on 256 supervised trials. The $CV_{Rrootveg}$ values encompass the CV_{Rsoil} values indicating that the results obtained from different sources are in good agreement.

Conclusions and recommendations

The performance parameters of analytical method including long-term reproducibility developed and validated for determination of pesticide residues with GC-TSD are within the corresponding criteria specified by the Guidance documents for analytical quality control (24, 25). Our results indicate that gas chromatographic elution and detection may be reliably used, in combination with appropriate internal quality control,[27] for the analyses of pesticide residues especially in samples of known pesticide treatment history.

239 The variability of residues being present in the experimental fields (CV_{Rsoil}) was within the
240 $CV_{Rrootveg}$ range of carrot and potato primary samples indicating that similar variability can be
241 expected in soil cores and root vegetables grown in treated soil. Because underestimation of
242 the uncertainty of the results of soil sampling may lead to erroneous conclusions, it is
243 recommended to use the rounded relative standard deviation of 100% for describing the
244 variability of residues in soil cores taken from the top 15 cm soil layer, until further more
245 robust data obtained directly from treated soils will be available. The uncertainty of the
246 residues measured in composite soil samples can be calculated with Equation 1 based on the
247 number of soil cores taken. Since the uncertainty of measured residues in composite samples
248 inversely proportional to the square root of number of soil cores, it may only be slightly
249 reduced by taking lager number of soil cores over 25 (CV_{25}=20%; CV_{30}=18%; CV_{50}=14%)
250 and the processing of larger samples may be difficult in typical residue laboratory and could
251 increase the CV_{Sp} and the combined uncertainty of the results (CV_R) as well. A sample size of
252 25, also recommended by ISO Standard 10381-1:2002 [28] seems to be a good practical
253 compromise.
254
255 For the sampling area of 100× 100 m, the sticks placed at each 10m provided a practical
256 solution. However, if samples are to be taken from a large area of several hectares this method
257 cannot be applied. Once the sampling target is precisely defined, an imaginary rectangular
258 coordinate system should be overlaid on it, the zero point permanently marked, and the
259 sampling positions defined by the X:Y coordinates should be randomly selected including the
260 entire sampling target, but excluding those points which are outside the sampling target as
261 shown in Figure 6. The sampling positions should be identified based on the GPS coordinates.
262 Nowadays GPS devices with ± 1m accuracy exist at reasonable cost. One of the advantages of

using GPS devices is that the repeated sampling, if necessary, from the same sampling position is possible.

Concerning the number of composite samples of size 25 to be taken there is no optimum, however over 8 independent replicate samples the gain becomes marginal. The optimum can be calculated, on a case-by-case basis, taking also into account the cost of sampling and analysis. [29]

Acknowledgement

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FIGURE CAPTIONS

Figure 1. Location of sampling position based on randomly selected coordinates. (position of sampling assistants standing at the positions of X=48, Y=23 coordinates, ◆ sampling position

Figure 2. Sampling positions with approximate concentration of prometryn residues (upper chart) in soil cores taken from the Mezőkövesd sampling site

Figure 3. Overlaid chromatogram of a reagent blank (blue), a field treated soil sample (red) and a blank sample fortified at F_1 level (brown)

Figure 4. Overlaid chromatogram of a blank soil(red), a field treated soil sample (blue) and a blank sample fortified at F_2 level (brown)

Figure 5. Relative frequency distribution of normalized residues detected in Mezőkövesd field, and linuron residues in carrot.

Figure 6. Sampling target (indicated with gray color) placed in a coordinate system.

TABLE CAPTIONS

Table 1. Summary of pesticide applications on the experimental sunflower fields

Table 2. Summary of soil parameters

Table 3. Summary of recoveries and their relative standard deviations

Table 4. Long-term reproducibility of determination of pesticide residues in soil samples

Table 5. Characteristic of residue distributions

Table 6. Examples for the CV values of residues in composite samples drawn with random sampling with replacement from the primary residue populations in individual soil cores.

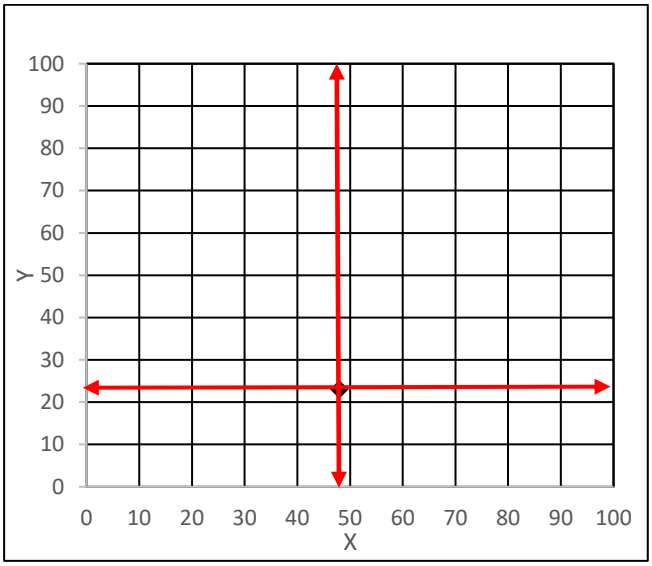

Figure 1. Location of sampling position based on randomly selected coordinates.

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sampling position

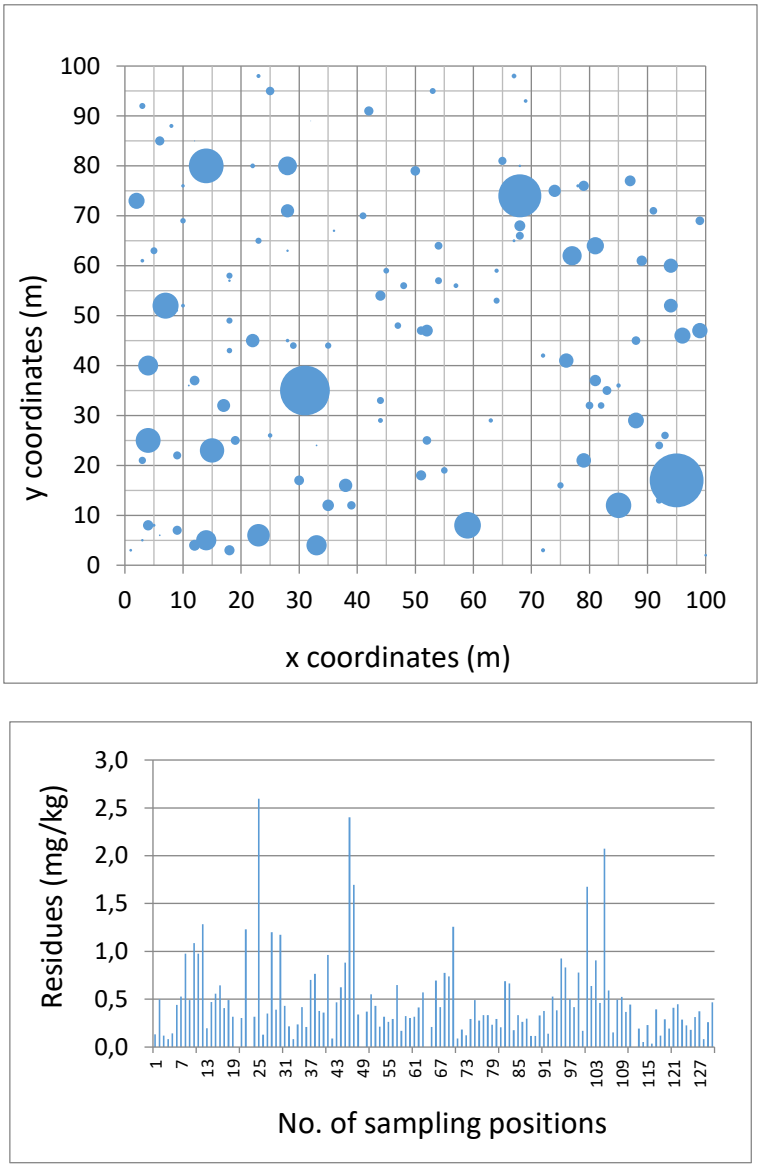

Figure 2. Sampling positions with approximate concentration of promethrin residues (upper chart) in soil cores taken from the Mezőkövesd sampling site

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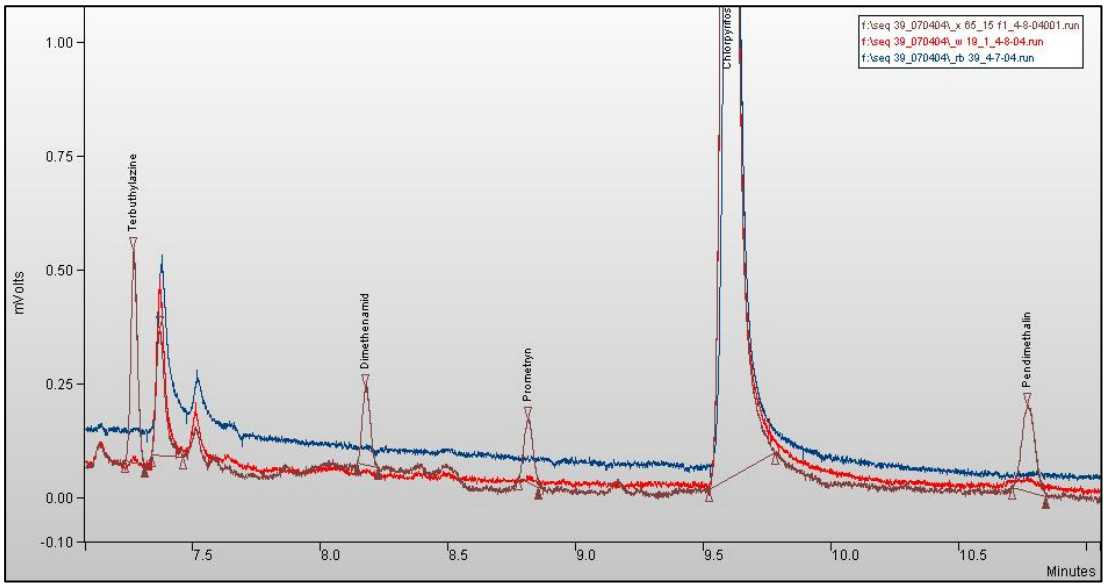

410

411 **Figure 3.** Overlaid chromatogram of a reagent blank (blue), a field treated soil sample

412 (red) and a blank sample fortified at F_1 level (brown)

413

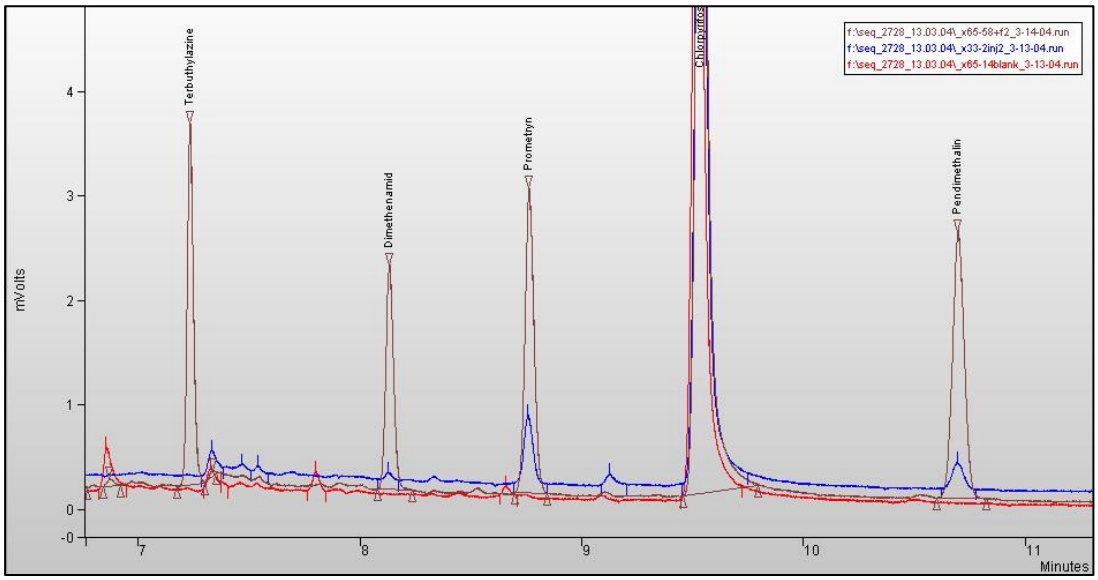

Figure 4. Overlaid chromatogram of a blank soil(red), a field treated soil sample (blue) and a blank sample fortified at F_2 level (brown)

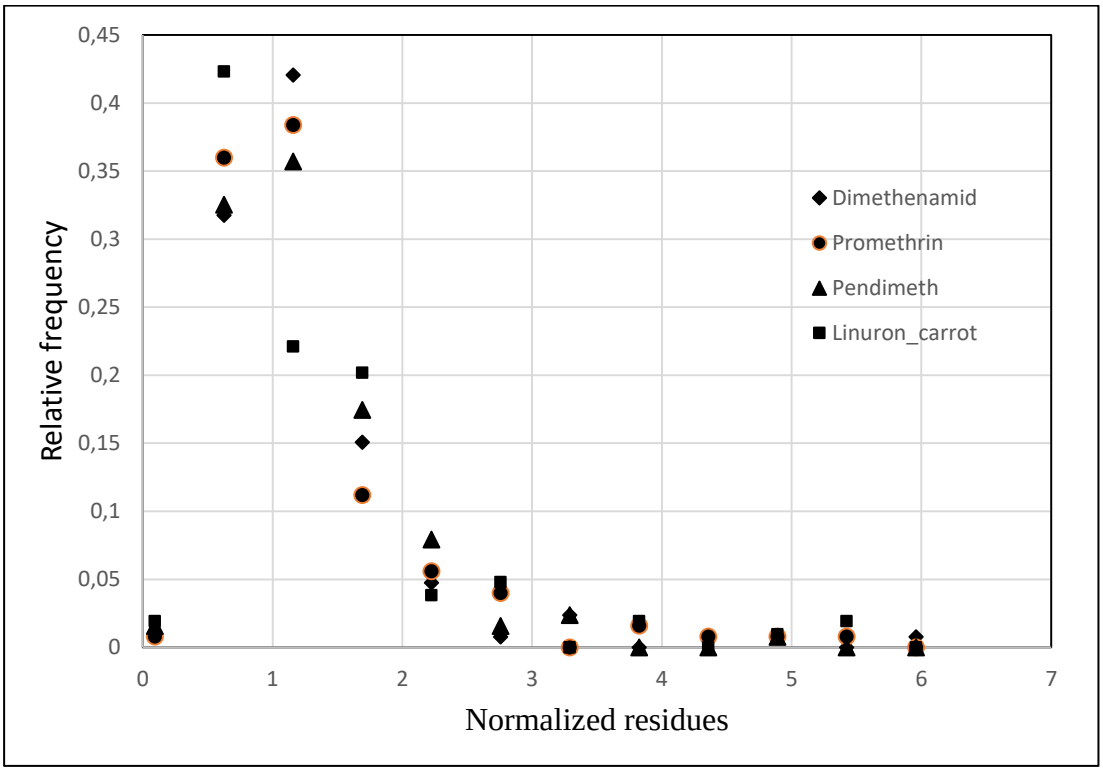

Figure 5. Relative frequency distribution of normalized residues detected in Mezőkövesd field, and linuron residues in carrot.

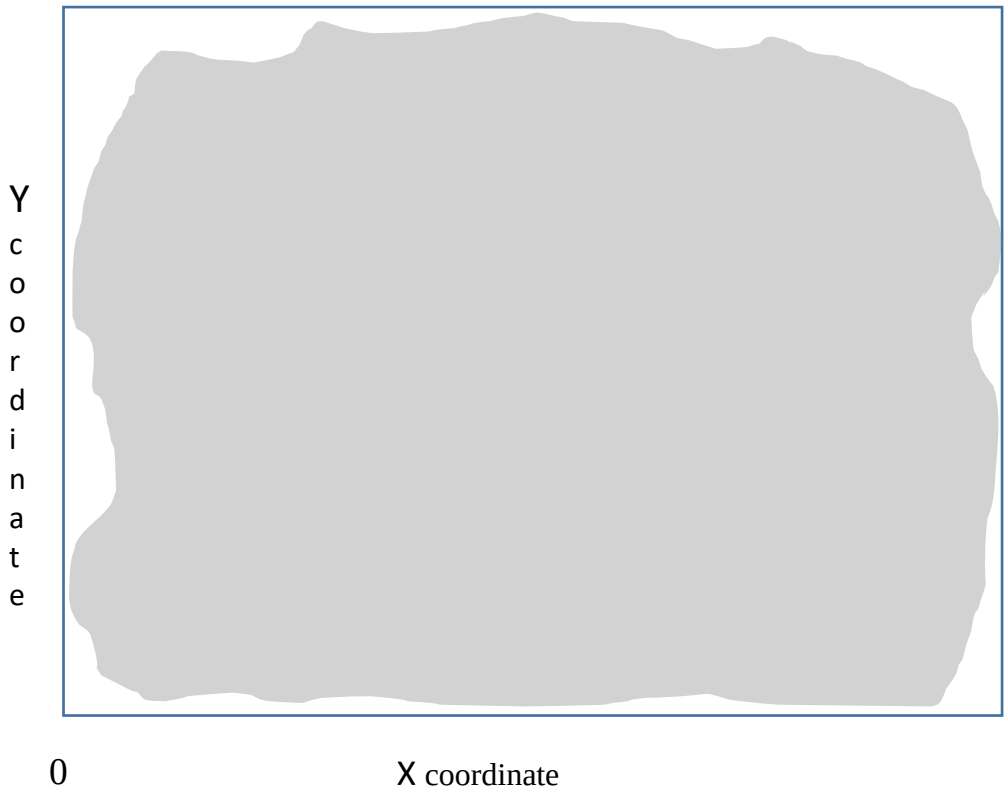

Figure 6. Sampling target (indicated with grey colour) placed in a coordinate system.

443 **Table 1.** Summary of pesticide applications on the experimental sunflower fields

Site	Active substance	Trade name, formulation	Dosage, g a.i./ha	DLA
Hercegkút	Dimethenamid	FRONTIER 900 EC	1440	27
	Prometryn	GESAGARD 500 FW	1000	
Mezőkövesd	Dimethenamid	WING EC	1000	30
	Pendimethalin	WING EC	1000	
	Prometryn	PROMETREX 500 SC	1000	

444 Formulations: EC: emulsifiable concentrate; FW: smoke pellets; SC suspension concentrate. ;
445 a.i. active ingredient; DLA: days between last application and sampling
446

447

448 **Table 2**. Summary of soil parameters

Site	Soil type	Organic matter [%]	pH	Sand %	Silt %	Clay %
Hercegkút	Ramann-type brown forest soil	3.14	6.41	33.8	41.6	24.6
Mezőkövesd	Brown forest soil with clay illuviation	2.4	6.8	36.0	26.5	37.5

449 The measurements were carried at the Soil Testing Laboratory of Agricultural Service
450 Institute of Fejér County, Hungary.

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455 **Table 3.** Summary of recoveries (R%) and their relative standard deviations (CV_A)

Spike levels	Dimethenamid			Pendimethalin			Prometryn		
mg/kg	Q (%)	CV_A	n	Q (%)	CV_A	n	Q(%)	CV_A	n
F_1: LOQ: 0.01-0.02	86.4	0.19	6	97.2	0.02	4	82.2	0.06	6
F_2:20*LOQ: 0.2-0.4	74.5	0.09	8	75.5	0.11	8	77.0	0.07	8
F_3:100*LOQ:	88.9	0.08	6	87.1	0.12	6	86.4	0.07	6
Combined F_1- F_3:	82.4	0.15	20	84.2	0.14	18	81.4	0.08	20

456 F_1, F_2 and F_3: fortification levels; LOQ: Limit of quantitation; Q: recovery; CV_A: coefficient
457 of variation; n: number of replicate tests; Combined: the reported values were calculated from
458 all recoveries obtained at 3 spike levels.
459

460 **Table 4**. Long-term reproducibility of determination of pesticide residues in soil samples

	k	CV_L
Dimethenamid all*	25	0.260
Pendimethalin	16	0.191
Prometryn all*	28	0.176

461 * measured in samples taken from both fields

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465 **Table 5.** Characteristic of residue distributions

	Mezőkövesd			Hercegkút	
	Dimethenamid	Prometryn	Pendimethalin	Prometryn	Dimethenamid
Ave	0.498	0.495	0.143	0.108	0.267
CV_R	0.83	0.88	0.69	0.87	1.14
R_{min}	0.046	0.035	0.010	0.005	0.010
R_{max}	2.97	2.60	0.644	0.836	2.44
CV_{distr}	0.81	0.86	0.66	0.85	1.13

466 CV_R: relative standard deviation of residues measured in soil cores (rounded values);

467 CV_{distr}: within field distribution of residues in randomly taken 120 soil cores (rounded values)

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Table 6. Examples for the CV values of residues in composite samples drawn with random sampling with replacement from the primary residue populations in individual soil cores.

	n	R_{ave}	CV_R	CV_{Rth}	Δ_{CV}%	Δ_{AVE}%
Dimethenamid	1	0.498	0.829			
	10	0.499	0.262	0.262	0.28	0.15
	25	0.500	0.167	0.166	0.45	0.36
Prometryn	1	0.495	0.877			
	10	0.495	0.278	0.277	0.06	0.08
	25	0.494	0.175	0.175	-0.28	0.30
Pendimetanil	1	0.143	0.688			
	10	0.143	0.216	0.217	0.59	0.15
	25	0.143	0.136	0.138	1.17	0.02

R_{ave}: average residues in primary and 10000 composite samples

CV_R: relative standard deviation of residues found in primary (soil cores) and composite samples

CV_{Rth}: the theoretical relative standard deviation of residues calculated based on equation 1

Δ_{CV}%: percentage difference between the CV_{Rth} and CV_R values relative to CV_{Rth}

Δ_{AVE}%: percentage difference between the average of residues in composite samples and the average of primary samples relative to that of primary samples