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Genotoxicity of Agricultural Soils after one year of Conversion Period and under Conventional Agriculture

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ABSTRACT: Agricultural soils are often contaminated with genotoxic chemicals. Hence, transition to sustainable agriculture requires a conversion period. We performed a comparative evaluation of the soil genotoxicity in a field after one year of conversion and in a field under conventional agriculture, not located close to sources of pollution. Soil samples were taken from 0-20 cm and 20-40 cm depth. *Allium cepa*-test system was used for the cytogenetic analysis. The higher mitotic index and lower frequency of chromosome aberrations in the samples from the field in conversion period compared with the field under conventional agriculture are indicative of a decrease of the soil genotoxicity. A slight increase in the frequency of aberrant cells and a substantial decrease in the mitotic index in 20-40 cm soil layer from both fields were observed. Regarding the field in conversion this might be due to the presence of agrochemicals used in the previous years, and indicates the necessity of longer conversion period. The results showed that *Allium cepa*-test might be was used for monitoring of genotoxic pollution of the soils without preliminary extraction of the chemicals they contain. @ JASEM

Pollution of the agricultural areas as a result of used agrochemicals turns into a global problem for contemporary mankind. The genotoxic compounds in soil can affect human health in various ways (De Souza Bueno et al., 2002; Šiviková and Dianovský, 2000). Sustainable agriculture provides protection of the environment and requires a conversion period, during which no agrochemicals are used. Some agrochemicals can persist in the soil for several years, contaminating crops that are supposed to be chemical-free. Soil condition can be defined by different methods depending on the functional aspect of the investigation. Bioassays provide a means of assessing the toxicity of complex mixtures like soil, without prior knowledge about their chemical composition (Watanabe and Hirayama, 2001). A number of studies have shown that higher plant tests are suitable for detecting the genotoxic potential of pollutants (Chang et al, 1997; Cotelle et al., 1999). Among plant test systems, *Allium cepa* is one of the most commonly used species (Fiskesjo, 1985; Grant, 1999).

The objective of this study was to perform a comparative evaluation of the genotoxicity of two soils – one from a field after the first year of conversion period to sustainable agriculture and the other – from a field, treated by the methods of conventional agriculture. Both fields are not located close to sources of pollution and were treated in the previous years in a similar way by agrochemicals.

MATERIALS AND METHODS

Soil samples were taken from 0-20 cm and 20-40 cm depth: 1) agricultural soil 1 (A1-20 and A1-40) – from field after one year of conversion period; leaf fertilizer (19:19:19:1+microelements) was used; 2) agricultural soil 2 (A2-20 and A2-40) – from field cultivated by conventional methods; oxyfluorfen (90 ml/dka), acetochlor (220 ml/dka) and quizalofop-p-ethyl (200 ml/dka) were used. Some investigations on soil genotoxicity are based on soil extracts but the extracted components cannot always represent the real mixture of chemicals and the extracts with organic solvents and water might show different results (Bordelon et al., 1996). So, in our study we choose to test water:soil suspension (1:25). *Allium cepa* L. bulbs with approximate size 2.5 cm were used as test objects. The outer scales of the bulbs and the old roots were removed and the bulbs with new roots (1.5 cm length) were put into water:soil suspension for 72 hours at 25±1ºC in thermostat. Microscope preparations were produced as described by Rank (2003). From each soil sample 6 bulb roots were fixed in a Clarke's fixative (95% ethanol: acetic acid glacial, 3:1) for 90 min, hydrolysed in 3N HCl for 8 min and in 45% acetic acid for 30 min, and stained for 90 min in 4% acetocarmine. The terminal root tips (1–2 mm) were squashed in 45% CH_3COOH. Mitotic index was determined as a ratio between the number of cells in mitosis and the total number of analysed cells. The index of each phase of mitotic division was calculated as a ratio between the cell number in the respective period and the number of dividing cells. The frequency of aberrant cells was calculated as percentage of the total number of analysed cells. The data were statistically analysed for their significance by Student's t-test.

RESULTS AND DISCUSSION

We established a higher mitotic index in samples from A1 in comparison with A2 (Table 1). These results prove a higher degree of pollution of A2 as the mitotic index shows the total cytotoxic influence of the environment. A number of studies also established a decreased intensity of cell division under the influence of various pollutants in

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the environment (Fiskesjo, 1997; Smaka-Kincl et al., 1996; Staykova et al., 2005). The mitotic index in the samples from 20-40 cm was lower compared with the samples from 0-20 cm in both fields. This is indicative of a stronger cytotoxic influence in the deeper soil layer. The analysis of the phase indices (Table 1) showed differences in the mitotic phase distribution mainly in the samples from 20-40 cm depth. Such interference in the cell cycle kinetics may also indicate cytotoxic influence (Amin, 2002).

Table 1: Mitotic index and phase indices of root tip cells of *Allium cepa*, grown in agricultural soil 1 (from field in conversion period) and agricultural soil 2 (from field under conventional agriculture)

Sample	Number of cells analysed	Number of dividing cells	MI% (±SE)	Prophase PhI% (±SE)	Metaphase PhI% (±SE)	Anaphase PhI% (±SE)	Telophase PhI% (±SE)
A1-20	8750	677	7.7 [b] (±0.37)	56.13 (±5.38)	11.97 (±2.67)	10.64 (±1.68)	21.26 (±3.50)
A1-40	8890	520	5.9 [a, b] (±0.23)	53.27 (± 3.04)	16.73 (±2.39)	9.04 (±1.18)	20.96 (± 3.74)
A2-20	9800	673	6.9 [c] (±0.33)	53.05 (±3.76)	12.33 (±1.78)	11.00 (±1.55)	23.63 (±2.29)
A2-40	10370	497	4.8 [a, c] (±0.07)	31.59 (± 3.90)	20.72 (±3.09)	16.70 (±1.90)	30.99 (± 3.1)

Data are expressed as means ± SE (standard error) in six root meristems, analysed per treatment;
MI – mitotic index; PhI - phase index; [b] – P≤0.01, [a, c] – P≤0.001;
A1-20 – agricultural soil from depth to 20 cm from field in conversion period;
A1-40 – agricultural soil from depth of 20-40 cm from field in conversion period;
A2-20 – agricultural soil from depth to 20 cm from field under conventional agriculture;
A2-40 – agricultural soil from depth of 20-40 cm from field under conventional agriculture.

Table 2: Types and frequency of aberrant root tip cells of *Allium cepa,* grown in agricultural soil 1 (from field in conversion period) and agricultural soil 2 (from field under conventional agriculture)

Sample	No of analysed cells	Abnormalities, % of analysed cells						Total abnormalities % (±SE)
		Anaphase bridges and chromosome fragments	Anaphases with vagrant chromosomes	Telophases with chromosome fragments	Telophases with vagrant chromosomes	Multipolar anaphases	Cells with micronuclei	
A1-20	8750	0.25	0.11	0.03	0.08	0.08	0.10	0.66 [a] (0.06)
A1-40	8890	0.20	0.21	0.03	0.05	0.05	0.30	0.84 [b] (0.07)
A2-20	9800	0.39	0.18	0.10	0.13	0.01	0.24	1.04 [a] (±0.09)
A2-40	10370	0.31	0.24	0.06	0.20	0.01	0.47	1.30 [b] (±0.11)

Data are expressed as means ± SE (standard error) in six root meristems, analysed per treatment; [a, b] – P≤0.01;
A1-20 – agricultural soil from depth to 20 cm from field in conversion period;
A1-40 – agricultural soil from depth of 20-40 cm from field in conversion period;
A2-20 – agricultural soil from depth to 20 cm from field under conventional agriculture;
A2-40 – agricultural soil from depth of 20-40 cm from field under conventional agriculture.

The results regarding the type and frequency of aberrant cells in mitotic and interphase root tip cells of *Allium cepa* are given in Table 2. The percent of aberrant cells in both layers from A2 was significantly higher compared with A1. Both investigated fields are not situated near arterial roads or factories, therefore the agrochemicals used in the present and in the previous years are the main source of pollution. These results confirm data of other authors about presence of genotoxic components in the soil as a result of used agrochemicals (Brown et al., 1985; Sivanesan et al., 2004). The established trend towards an increase in the frequency of aberrant cells in the samples from 20–40 cm in both fields showed that the agrochemicals accumulated in the deeper soil

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layer. Regarding the field in conversion this might be due to the presence of agrochemicals used in the previous years.

Anaphase/telophase fragments and bridges were the most frequent kinds of aberrations and anaphases/telophases with vagrant chromosomes were the second. Chromosome bridges and fragments occur as a result of chromosome breakage and vagrant chromosomes show abnormalities in the mitotic spindle (Grant, 1978; Rank, 2003). The occurrence of abnormal anaphases in *Allium cepa* root tips is indicative of the presence of compounds that caused inhibition of spindle formation. The induction of micronuclei in interphase cells is the manifestation of chromosome breakage and disruption of spindle apparatus (Grover and Kaur, 1999). In our study, the frequency of cells with micronuclei was highest in A2-40 and smallest in A1-20.

The results revealed that *Allium*-test might be used for cytogenetic monitoring of soils without preliminary extraction of the chemicals they contain. The present study proves the positive effect of one-year conversion period, but a slight increase in the frequency of aberrant cells and a substantial decrease in the mitotic index in 20-40 cm soil layer indicate the necessity of longer conversion period.

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