Bulgarian Journal of Agricultural Science, 18 (No 5) 2012, 742-748
Agricultural Academy

HERBICIDES INFLUENCE THE COMMUNITY STRUCTURE OF THE SOIL MEZOFAUNA

I. VELCHEVA, S. PETROVA*, I. MOLLOV, G. GECHEVA and D. GEORGIEV
University of Plovdiv "Paisii Hilendarski", Faculty of Biology, Department of Ecology and Environmental Conservation, BG – 4000 Plovdiv, Bulgaria

Abstract

VELCHEVA, I., S. PETROVA, I. MOLLOV, G. GECHEVA and D. GEORGIEV, 2012. Herbicides influence the community structure of the soil mezofauna. *Bulg. J. Agric. Sci.,* 18: 742-748

A field bioassay was carried out to assess the impact of "NIRVANA" (active substances imazamox and pendimethalin) and "STOMP 330 EC" (active substance pendimethalin) herbicides residues to the soil mezofauna. The investigated herbicides caused changes in the community structure of the soil mezofauna. Highest negative impact (suppression of all studied ecological parameters) was observed until the 100-th day after treatment, and on the experimental plots treated with high concentrations of pendimethalin. The dynamic of the soil community structure was found to be related with those of the pesticides content.

Key words: pendimethalin, soil mezofauna, ecological characteristics

Introduction

Many pesticides used in the conventional agricultural practice are toxic for non-target organisms and may affect on beneficial for the agroecosystem invertebrates (Lee, 1985). The toxicity of pesticides to soil biota depends on the compound bioavailability, which is affected by the soil natural characteristics, and by the uptake routes of exposed organisms (Piola et al., 2009). Therefore, ecological studies can benefit from using experimental designs that improve data relevance for local exposure conditions in the agricultural lands (Rovira, 1994; Pankhurst, 1997; Yu et al., 2006). The soil cenoses consists of microorganisms such as bacteria, fungi, protozoans, yeasts and the autotrophic algae. The mezofauna is comprised by a variety of invertebrate animals mainly nematodes, mites, springtails, spiders, insects, and earthworms (Velcheva et al., 1997; Velcheva et al., 1999). The functions of such community, often referred as "soil food web", are complex and strongly depend on the residue of decomposition, the nutrient storage and release, the soil structure, and the degradation or immobilization of pesticides and other pollutants (Zwieten, 2004). For example, atrazine decreases the abundance of earthworms, wireworms, and springtails, whereas dalapon (2,2-dichloropropionic acid) and TCA (trichloroacetic acid) appeared to increase millipedes, springtails and mites (Freemark and Boutin, 1995). Certain herbicides may diminish or increase the numbers of both beneficial and harmful soil animals. Under certain cultural conditions, herbicides as well as insecticides could fit into an integrated control program and help to counter the two threats of insect resistance to insecticides and excessive insecticide residues in soil (Fox, 1964).

„STOMP 330 EC"(active substance pendimethalin 330 g.l^{-1}) and „NIRVANA"(active substances imazamox 16.7 g.l^{-1} + pendimethalin 250 g.l^{-1}) are selective soil herbicides against annual broadleaf weeds (BASF Chemical Company, 2009). Pendimethalin is

* *Corresponding author: University of Plovdiv "Paisii Hilendarski", Faculty of Biology, 4000 Plovdiv, Bulgaria, e-mail: slaveyapetrova@uni-plovdiv.bg*

a dinitroaniline herbicide used for selective control of weeds in crops such as corn (*Zea mays*), soybeans (*Glycine max*), peas (*Pisum sativum*), and several vegetable crops (Wilson et al., 1995). In Europe it is also approved for use in winter wheat (*Triticum aestivum*) and sunflower (*Helianthus annuus*).

Comparatively little work has been done on pendimethalin loss from soil, although soil degradation of dinitroaniline herbicides in general has been reviewed (Carlsen et al., 2006a, b). Persistence of pendimethalin is influenced by cultivation practices; soil temperature, moisture conditions, and soil type (Triantafyllidis et al., 2009). Degradation of the pendimethalin proceeds more rapidly under flooded, anaerobic conditions than under aerobic conditions, which is similar to other dinitroanilines (Walker and Welsh, 1991). Photodecomposition of pendimethalin can occur, although the rate decreases rapidly after the first 7 days of exposure on the soil surface. As much as 17% of applied pendimethalin has reportedly been lost by photodecomposition in 7 days (Walker & Brown, 1983; Zimdahl et al., 1984).

The studied selective herbicides probably have additional impact on non-target organisms, particularly on invertebrates, which are strongly related to the soil fertility. Since, there are no sufficient data on the impact of these herbicides on the mezofauna, we aimed to: 1) track the degradation of the herbicides in the soil, and 2) study the impact of these herbicides on the community structure of the soil mezofauna.

Material and Methods

The study was conducted in 2006-2007 on the test-fields of the Agricultural University, Plovdiv (Bulgaria). Three sampling plots were chosen, as follows:

1 – plot treated with “NIRVANA” herbicide in concentration 400 $ml.da^{-1}$;

2 – plot treated with “NIRVANA” herbicide in concentration 700 $ml.da^{-1}$;

3 – plot treated with “STOMP 330 EC” herbicide in concentration 400 $ml.da^{-1}$.

All sampling plots were sown with sunflower plants (Rhymes hybrid, Limagrain Company). Herbicides were applied at the 3-5^{-th} leaf phase of the crops (BASF Chemical Company, 2009).

A composite soil sample of each experimental plot, prepared from 3 different sub-samples, was taken immediately after treatment and the main soil characteristics were examined.

Mechanical content and structure were determined after Totev et al. (1991) in the Laboratory of Ecology, University of Plovdiv “Paisii Hilendarski”. Based on texture analysis the soil was classified according to FAO system. Particle size of soil was determined by aerometric method (ISO 11277), while the pH value of the aqueous soil extracts (1:5, w/v) were measured in triplicate with a pH-Electrode SenTix 41 by a pHoto-Flex/Set (Germany) at 20°C (ISO 10390).

The chemical analyses were conducted in the Research Laboratory of Agricultural University, Plovdiv. The concentrations of K^+, P^{5+}, Na^+, Ca^{2+} ions were determined using atomic absorption spectrometry (AAS). Humus content was calculated after dichromate oxidation in the presence of concentrated sulphuric acid (ISO 14235) and the total nitrogen content was measured by Kjeldahl method (ISO 11261).

During the experiment, parallel soil samples for quantifying herbicide residues into the soil and to assessing the soil community structure were collected seasonally, as follows: at the 4-th (Sample 1, end of spring 2006), 22-th (Sample 2, summer 2006), 126-th (Sample 3, autumn 2006) and 313-th (Sample 4, spring 2007) day after treatment. At each experimental plot were processed three sections with dimensions 0.5/0.5/0.3 m. Sampling was made according to the field methodology of Guilyarov (1987).

The analyses of herbicides concentrations were conducted in Research Laboratory of Agricultural University by capillary gas chromatography. The results were compared with maximal permitted values in Bulgaria for pesticide residues in soils (Regulation norm 3, 2008).

The collected soil invertebrates were preserved in 70% ethanol. Species determination to the lowest possible taxon (family) according Fauna Europea (2011) and ecological evaluations were made in the Laboratory of Ecology, University of Plovdiv “Paisii Hilendarski”, using a reference collection.

To characterize the soil mezofauna the following ecological characteristics were used (Magurran, 1988):

- Density: the number of the individuals of one taxon relative to 1 m^3 of soil;
- Abundance: the ratio between the number of individuals of one taxon and the total number of individuals of all taxa.;
- Frequency: the number of individuals, which represent the percentage proportion of the taxon in the community;
- Consistency: the percentage ratio between the number of samples, containing certain taxon and total number of samples;
- Reciprocal value of the Simpson's diversity index;
- Simpson's equitability index.

Ecological parameters were calculated using the computer software "BioDiversityPro" (McAleece et al., 1997). Data were statistically processed using χ^2-test, correlation analysis (Pearson correlation index with logarithmically transformed data) and cluster analysis (Fowler et al., 1998). For all statistical analyses, the STATISTICA 7.0 statistical package was used (StatSoft Inc., 2004).

Results and Discussion

Soil characteristics and herbicide degradation dynamics

The soil in the investigated area was defined as Humo-Fluvisols, medium sandy-loam, so it can be defined as moderate (Regulation norm 3, 2008). High humus content (30.9-40.04 $g.kg^{-1}$), light to medium alkaline pH (7.65-7.75), and low salt content (0.5-0.8 $g.kg^{-1}$) were measured. Our results showed high content of total N, K^+ and P^{5+} (Table 1). Data obtained were similar in the three experimental plots, so the subsequent analyses were focused only on pesticides residues. It was observed that under identical conditions of soil moisture and temperature, effect of soil type on pendimethalin persistence was rather small (Zimdahl et al., 1984).

Herbicide break down dynamics also were monitored. A clear tendency to their decrease with time expired was established (Table 2). At the 126-th day after treatment (Sample 3) the measured concentrations were beyond the maximal permitted content (<0.1 $mg.kg^{-1}$) according to the Regulation norm 3 (2008).

Ecological characteristics

The taxa composition as well as its density, seasonal dynamics, abundance and frequency of the recorded taxa is presented in Table 3.

It is notable that we observed a negative correlation between the residual amounts of all herbicide test-variants in the soil and the abundance of the taxa recorded. For "STOMP 330 EC" the Pearson's index showed a value of -0.912 ($P<0.05$) and index of determination was 0.831, which means that the observed increase of the abundance in the taxa is due to the reduction of the herbicide in the soil and there is only 16.89% chance that other factors may be responsible. For "NIRVANA"-400

Table 1
Main soil characteristics in the experimental plots

	Depth, m	Humus content, $g.kg^{-1}$	Humus reserve, $t.da^{-1}$	pH	Total N, %	P, mg.kg	K, mg.kg
Plot 1	0-0.3	39.6	14.26	7.75	0.24	845	610
Plot 2	0-0.3	39.2	14.11	7.75	0.23	853	603
Plot 3	0-0.3	40.4	14.30	7.65	0.25	867	646

Table 2
Herbicides concentrations in the soil, mg.kg

	Sample 1, $mg.kg^{-1}$	Sample 2, $mg.kg^{-1}$	Sample 3, $mg.kg^{-1}$	Sample 4, $mg.kg^{-1}$
"STOMP 330 EC" – 400 $ml.da^{-1}$	0.359	0.299	0.045	0.020
"NIRVANA" – 400 $ml.da^{-1}$	0.321	0.292	0.031	0.010
"NIRVANA" – 700 $ml.da^{-1}$	0.503	0.488	0.113	0.051

Table 3
Composition, abundance, density, frequency and consistency of the registered taxa in the sample fields treated with the studied herbicides during the four studied seasons

Taxa	Sample 1				Sample 2				Sample 3				Sample 4				C
	N	D	P	F	N	D	P	F	N	D	P	F	N	D	P	F	
"STOMP 330 EC" – 400 ml.da^{-1}																	
Oligochaeta, Lumbricidae	0	0.00	0.00	0.0%	4	17.78	0.57	57.1%	89	395.55	0.93	92.7%	11	48.89	0.579	57.9%	75%
Insecta, Coleoptera, Carabidae (larvae)	0	0.00	0.00	0.0%	0	0.00	0.00	0.0%	0	0.00	0.00	0.0%	3	13.33	0.158	15.8%	25%
Insecta, Coleoptera, Elateridae (larvae)	4	17.78	0.50	50.0%	0	0.00	0.00	0.0%	1	4.44	0.01	1.0%	0	0.00	0.000	0.0%	50%
Insecta, Diptera, Empididae (larvae)	3	13.33	0.38	37.5%	0	0.00	0.00	0.0%	5	22.22	0.05	5.2%	2	8.88	0.105	10.5%	75%
Myriapoda, Juliformia, Julidae	0	0.00	0.00	0.0%	1	4.44	0.14	14.3%	0	0.00	0.00	0.0%	0	0.00	0.000	0.0%	25%
Myriapoda, Geophilomorpha, Geophilidae	1	4.44	0.13	12.5%	2	8.89	0.29	28.6%	1	4.44	0.01	1.0%	3	13.33	0.158	15.8%	100%
"NIRVANA" – 400 ml.da^{-1}																	
Oligochaeta, Lumbricidae	1	4.44	0.14	14.3%	8	35.55	0.38	38.1%	75	333.33	0.87	92.7%	16	71.11	0.356	35.6%	100%
Insecta, Coleoptera, Carabidae (larvae)	1	4.44	0.14	14.3%	0	0.00	0.00	0.0%	0	0.00	0.00	0.0%	24	106.7	0.533	53.3%	50%
Insecta, Coleoptera, Elateridae (larvae)	3	13.33	0.43	42.9%	2	8.89	0.10	9.5%	0	0.00	0.00	1.0%	0	0.00	0.000	0.0%	50%
Insecta, Diptera, Empididae (larvae)	2	8.88	0.29	28.6%	2	8.89	0.10	9.5%	10	44.44	0.12	5.2%	0	0.00	0.000	0.0%	75%
Myriapoda, Juliformia, Julidae	0	0.00	0.00	0.0%	1	4.44	0.05	4.8%	0	0.00	0.00	0.0%	0	0.00	0.000	0.0%	25%
Myriapoda, Geophilomorpha, Geophilidae	0	0.00	0.00	0.0%	8	8.89	0.38	38.1%	1	4.44	0.01	1.0%	5	22.22	0.111	11.1%	75%
"NIRVANA" – 700 ml.da^{-1}																	
Oligochaeta, Lumbricidae	0	0.00	0.00	0.0%	5	22.22	0.28	27.8%	98	435.55	0.88	87.5%	9	40.0	0.529	52.9%	75%
Insecta, Coleoptera, Carabidae (larvae)	0	0.00	0.00	0.0%	2	8.89	0.11	11.1%	0	0.00	0.00	0.0%	4	17.8	0.235	23.5%	50%
Insecta, Coleoptera, Elateridae (larvae)	1	4.44	0.50	50.0%	0	0.00	0.00	0.0%	0	0.00	0.00	0.0%	0	0.00	0.000	0.0%	25%
Insecta, Diptera, Empididae (larvae)	1	4.44	0.50	50.0%	4	17.78	0.22	22.2%	13	57.78	0.12	11.6%	0	0.00	0.000	0.0%	75%
Myriapoda, Juliformia, Julidae	0	0.00	0.00	0.0%	0	0.00	0.00	0.0%	0	4.44	0.00	0.0%	0	0.00	0.000	0.0%	0%
Myriapoda, Geophilomorpha, Geophilidae	0	0.00	0.00	0.0%	7	31.11	0.39	38.9%	1	0.00	0.01	0.9%	4	17.8	0.235	23.5%	75%

Legend: N – numbers; D – abundance, ind./m^3; P – abundance; F – frequency; C – consistency.

ml.da^{-1} the values were: $R=-0.806$ ($P<0.05$); $R^2=0.65$ and "NIRVANA"-700 ml.da^{-1} showed the lowest values: $R=-0.488$ ($P<0.05$); $R^2=0.239$. We observed an increase in the density of the earthworms (Lumbricidae) and the larvae of the Empididae flies during the four studied seasons in the samples of "STOMP 330 EC" ($\chi^2=168.15$, df=3, $P<0.05$) treatment and these of "NIRVANA"-400 ml.da^{-1} ($\chi^2=90.33$, df=3, $P<0.05$). These results could be related also and to the registered high humus content and to the significant soil macro porosity respectively. The samples treated with "NIRVANA"-700 ml.da^{-1} showed also a slight increase in the density of Geophilidae ($\chi^2=204.32$, df=3, $P<0.05$). The rest of the taxa showed similar values in density in all seasons.

In the first sample (four days after the treatment) we observed very low density or none for the earthworms. Avoidance behavior is an ecologically relevant endpoint, directly related to the energy budget of the worms, and indirectly to the soil structure. Exposure to pesticides that alters earthworm behavior can induce migratory behavior, which can lead to modifications in population abundance or biomass and to changes in species diversity. Although avoidance tests with earthworms have been considered as suitable screening tools for the assessment of potentially contaminated soils (Da Luz et al., 2004; Sousa et al., 2008), our results showed that the avoidance behavior was not sufficiently sensitive endpoint for assessment of the effects of pendimethalin on the earthworms. It seems, however, that this taxon is more sensitive to the presence of selected herbicides in the soil, as Krogh et al. (2007) also show it, but it is also influenced by the soil humidity. We observed the highest density in Lumbricidae in the autumn sample (126-th day), due to the high rainfall in this month. Similar to our results Velcheva et al. (1997) recorded the lowest density of earthworms in the summer, due to the low humidity and high temperatures and maximum in the autumn and spring sample. According to Velcheva et al. (1999) the Lumbricidae family is highly sensitive to the presence of pesticides in the soil and this taxa could be used in the ecological monitoring of contaminated with pesticides soils.

The larvae of the Carabid beetles showed very low density and slight increase at the end of the study period – the spring sample. Since some of the species from this family are herbivorous, perhaps this taxon is sensitive to some herbicides. On the contrary – the other Coleoptera family – Elateridae (larvae) showed highest density in the beginning of the experiment (first sample) and decrease of density during the other seasons. It is known that these beetles are pests feeding on decaying vegetation and the roots of plants, and often causing damage to agricultural crops such as potato, strawberry, corn, and wheat (William and Howard, 2001; Vernon et al., 2008). Perhaps this family is more sensitive to the used herbicides and was eradicated from all sample fields almost immediately after the treatment. This is more evident in the sample from experimental plot treated with "NIRVANA"-700 ml.da^{-1}.

The centipedes (Myriapoda) did not show any specific trends. The Julidae family is completely absent from the "NIRVANA"-700 ml.da^{-1} sample and presented with only one specimen in the second sample in the plots treated with the other two test-variants. With some exceptions the Geophilidae family shows very close values during all studied seasons.

According to the consistency index, there are three constant taxa during whole study in all experimental plots – Lumbricidae, Empididae (larvae) and Geophilidae. There is one additional taxon for "STOMP 330 EC" (Elateridae-larvae) and "NIRVANA"-700 ml.da^{-1} (Carabidae-larvae) and both are additional taxa in the samples treated with "NIRVANA"-400 ml.da^{-1}. The Julidae family should be considered as accidental taxon in all studied communities.

The cluster analysis showed very high similarity in the structure of the edaphocenoses from all sample fields treated with the three test-variants. The plot treated with "NIRVANA"-400 ml.da^{-1} differentiates from the others with about 85% similarity. It seems that "STOMP 330 EC" and "NIRVANA"-700 ml.da^{-1} have an analogous effect on the mezobiontic fauna, since the data from these plots groups in a cluster at about 90% similarity. This is maybe due to the fact that the active ingredient in both herbicides is pendimethalin. "NIRVANA" contains another chemical called imazamox. It seems that the concentration of pendimethalin in experimental plots treated with "NIRVANA"-700 ml.da^{-1} and "STOMP 330 EC"-400 ml.da^{-1} are similar, thus the similar effect they have on the mezobiontic fauna.

Table 4
Diversity indices of the mezofauna

Sample	Simpson's Diversity Index (1/D)	Simpson's Equitability (E)	Berger-Parker (d)
"STOMP 330 EC" – 400 ml.da^{-1}			
Sample 1	3.111	0.789	0.5
Sample 2	3	0.79	0.571
Sample 3	1.161	0.23	0.927
Sample 4	2.758	0.726	0.579
"NIRVANA" – 400 ml.da^{-1}			
Sample 1	5.25	0.921	0.429
Sample 2	3.033	0.778	0.381
Sample 3	1.054	0.126	0.872
Sample 4	2.071	0.702	0.533
"NIRVANA" – 700 ml.da^{-1}			
Sample 1	2	1	0.333
Sample 2	3.882	0.892	0.389
Sample 3	1.041	0.102	0.875
Sample 4	2.167	0.756	0.529

The Simpson's diversity index shows a decreasing trend with the advancing of the seasons (Table 4). The lowest value of herbicides content is registered in the autumn sample, due to the high abundance of the earthworms. The Simpson's equitability index and the Berger-Parker index show that the edaphocenosis is monodominant in this season and polydominant in the rest of the seasons. Probably, it is due to both low herbicide content and high soil moisture as a result from the high rainfall. Velcheva et al. (1997) also registered a decrease of the Simpson's diversity index for the mezofaunal communities in fields treated with pesticides.

Kartalska et al. (2009) studied the impact of the same concentrations of "NIRVANA" and "STOMP 330 EC" herbicides on soil microorganisms. Significant changes in the soil microflora were observed. Similar to our results, these authors proved that for the complete degradation of pesticides more than 100 days were necessary. On the end of the experiment (105-th day after treatment), the decrease of pesticides residues was lead up to the restoration of the microbiologic equilibrium in the soil.

Conclusions

The investigated herbicides ("NIRVANA" and "STOMP 330 EC") caused changes in the community structure of the soil mesofauna. The influence was specific for each taxon and was in relation with some additional factors, affecting the soil mezofauna. The determination coefficient showed the prevailing negative effect of "NIRVANA" and "STOMP 330 EC" to the other factors. The dynamic of the soil community structure was found to be related with those of the herbicides content. High negative impact (suppression of all studied ecological parameters) was observed till the 100-th day after treatment, and on the experimental plots treated with high concentrations of pendimethalin. At the end of the experiment (with decreasing the content of the herbicides), the stability of the communities increased. Our results indicate that the degradation of the investigated herbicides in the soil is relatively fast and they do not have a sustained negative impact on soil community structure, and hence on soil functions themselves.

References

BASF Chemical Company, 2009. http://www.basf.com/group/corporate/en/ (Accessed on : 15.XII.2009)

Carlsen, S., N. Spliid and B. Svensmark, 2006a. Drift of 10 herbicides after tractor spray application. 2. Primary drift (droplet drift). *Chemosphere,* **64**: 778-78.

Carlsen, S., N. Spliid and B. Svensmark, 2006b. Drift of 10 herbicides after tractor spray application. 1. Secondary drift (evaporation). *Chemosphere,* **64**: 778-78.

Da Luz, T., R. Ribeiro and J. Sousa, 2004. Avoidance tests with collembolan and earthworms as early screening tools for site-specific assessment of polluted soils. *Environmental Toxicology and Chemistry*, **23**: 2188-2193.

Fauna Europaea, 2009. Fauna Europaea version 2, http://www.faunaeur.org (Accessed on : 15.XII.2009).

Fowler, J., L. Cohen and P. Jarvis, 1998. Practical statistics for field biology. Chichester: John Wiley & Sons, 259 pp.

Fox, C., 1964. The effects of five herbicides on the numbers of certain invertebrate animals in grassland soil. *Canadian Journal of Plant Science,* 44: 405-409.

Freemark, K. and C. Boutin, 1995. Impacts of agricultural herbicide use on terrestrial wildlife in temperate landscapes: A review with special reference to North America. *Agriculture, Ecosystems & Environment,* **52**(2-3): 67-91.

Guilyarov, M.S., 1987. Quantative methods in soil zoology. Nauka, Moscow, Russia, 179 pp. (Ru).

Kartalska, Y., N. Kuzmanov and K. Sapundjieva, 2009. Influence of the herbicides Stomp and Nirvana on soil microflora. *Scientific studies of University of Russe,* **48** (Series 1.1): 13-16. (Bg).

Krogh, P., B. Griffiths, D. Demšar, M. Bohanec, M. Debeljak, M. Andersen, C. Sausse, A. Birch, S. Caul, M. Holmstrup, L.-H. Heckmann, J. Cortet, B. Griffiths, 2007. Responses by earthworms to reduced tillage in herbicide tolerant maize and *Bt* maize cropping systems. *Pedobiologia,* 3: 219-227.

Lee, K.E., 1985. Earthworms: their ecology and relationships with soils and land use. Academic Press, Australia.

Magurran, A., 1988. Ecological Diversity and its Measurement. Princeton University Press, Princeton, NJ, 179 pp.

McAleece, N., P. Lambshead, G. Paterson and J. Gage, 1997. BioDiversity Professional London (UK), Oban (Scotland). The Natural History Museum, The Scottish Association for Marine Sciences. Software, http://www.sams.ac.uk/research/software.

Pankhurst, C.E. 1997. Biodiversity of soil organisms as an indicator of soil health. In: Biological indicators of soil health. Editors Pankhurst CE, Double BM, Gupta VVSR, pp. 297-324. CAB International Wallingford.

Piola, L., J. Fuchs, M.L. Oneto, S. Basack, R. Gimenez, R. Massaro, J.C. Papa, E. Kesten and N. Casabe, 2009. Biomarkers for the assessment of chlorpyrifos effects on earthworms in an Argentine wheat field. *Pesquisa Agropecuária Brasileira, Brasilia,* **44**(8): 874-880.

Regulation norm 3, 2008. About permitted level of harmful substances in soils. www.eea.government.bg (Accessed on 12.08.2008)

Rovira, A.D., 1994. The effect of farming practices on the soil biota. In: C.E. Pankhurst (Editor), Soil biota: Management in sustainable farming. CSIRO Australia. pp 81-87.

Sousa, A., R. Pereira, S.C. Antines, A. Cachada, E. Pereira, A.C. Duarte and F. Goncalves, 2008. Validation of avoidance assays for the screening assessment of soils under different anthropogenic disturbances. *Ecotoxicology and Environmental Safety,* **71**: 661-670.

StatSoft, Inc. 2004. STATISTICA (data analysis software system), version 7. www.statsoft.com.

Totev, T., P. Gribachev, H. Nechev and N. Artinova, 1991. A field guide on soil analyses. Zemizdat, Sofia, pp. 179.

Triantafyllidis, V., D. Hela, G. Salachas, P. Dimopoulos and T. Albanis, 2009. Soil dissipation and runoff losses of the herbicide pendimethalin in tobacco field. *Water, Air and Soil Pollution,* **201**: 253-264.

Velcheva, I., P. Kostadinova and V. Popov, 1997. Research on some ecological properties of soil mezobiota in treated and untreated with pesticides plots. *Higher School of Agriculture – Plovdiv, Scientific Works,* **XLII**(3): 53-60.

Velcheva, I., P. Kostadinova and V. Popov, 1999. Ecological monitoring of the soil mezobiota in apple orchard under biological and integrated agriculture. *Higher School of Agriculture – Plovdiv, Scientific Works,* **XLIV**(2): 39-46.

Vernon, R., W. van Herk, J. Tolman, H. Saavedra, M. Clodius and B. Gage, 2008. Transitional sublethal and lethal effects of insecticides after dermal exposures to five economic species of wireworms (Coleoptera: Elateridae). *Journal of Economic Entomology,* **101**(2): 365-374.

Walker, A. and P. Brown, 1983. Spatial variability in herbicide degradation rates and residues in soil. *Crop protection,* **2**: 17-25.

Walker, A. and S. Welsh, 1991. Enhanced degradation of soil-applied herbicides. *Weed Researches,* **31**: 49-57.

William, E. and J. Howard, 2001. The biology and management of wireworms (*Agriotes* spp.) on potato with particular reference to the U.K. *Agricultural and Forest Entomology,* **3**(2): 85-98.

Wilson, R.G., P.J. Shea and D.R. Tupy, 1995. Dinitroaniline herbicide carry over to sugarbeet. *Nebraska Agricultural Research Division,* **32**(4): 201-213.

Yu, Y.L., X.M. Wu, S.N. Li, H. Fang, H.Y. Zhan and J.Q. Yu. 2006. An exploration of the relationship between adsorption and bioavailability of pesticides in soil to earthworm. *Environmental Pollution,* **141**: 428-433.

Zimdahl, R.L., P. Catizone and A.C. Butcher, 1984. Degradation of pendimethalin in soil. *Weed Science,* **32**: 408-412.

Zwieten, L.V., 2004. Impacts of pesticides on soil biota. In: Soil Biology in Agriculture, Proceeding of workshop on current research into soil biology in agriculture, 11-12 August 2004, pp. 72-79.

Received August, 2, 2011; accepted for printing July, 2, 2012.