Türk Tarım ve Doğa Bilimleri Dergisi 7(2): 301–307, 2020
https://doi.org/10.30910/turkjans.725770

TÜRK
TARIM ve DOĞA BİLİMLERİ
DERGİSİ

TURKISH
JOURNAL of AGRICULTURAL
and NATURAL SCIENCES

www.dergipark.gov.tr/turkjans

Araştırma Makalesi

Effects of Pesticides (Chlorpyrifos, Glyphosate) Applied to Soil on Earthworm Behaviors and Some Soil Biological Parameters

Cafer TÜRKMEN[1], Nurdan ÖZKAN[1], Yusuf ÖZÇELİK[1], Damla ÖZÇELİK[1], Osman TİRYAKİ[2*]
[1]Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition 17020, Çanakkale/TURKEY
[2] Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Plant Protection 17020, Çanakkale/TURKEY

*Corresponding author: osmantiryaki@yahoo.com

Received: 16.06.2019 Revised in Received: 05.02.2020 Accepted: 07.02.2020

Abstract

Pesticides are directly applied to soils or plants to improve yields. A significant portion of pesticides applied to plants also passes to soils. The number of works investigating the effects of pesticides on earthworms is quite limited. In this study, two doses of chlorpyrifos and glyphosate pesticides (recommended and double of recommended dose) were incorporated into the soil and served for the preference of earthworms (*Eisenia fetida*) in 2D (2-dimensions) terrarium. Changes in gallery areas, microorganism counts, catalase and urease enzyme activities were investigated. While the differences in gallery areas of untreated soil in both sides of 2D terrariums were not significant (p>0.05), the differences in gallery areas of chlorpyrifos-treated and untreated soils were significant (p=0.0298). Gallery areas at double dose of chlorpyrifos were greater than the control treatment (p=0.0323). The effects of glyphosate treatments on gallery areas were also insignificant (p>0.05). Increases were observed in microorganism counts with the recommended dose of chlorpyrifos (p=0.0074), but the differences were not significant at the double dose of chlorpyrifos. The double dose of glyphosate treatments significantly decreased microorganism counts (p=0.0273). While the double dose of chlorpyrifos significantly increased catalase enzyme activity (p=0.0041), the effects of chlorpyrifos and glyphosate treatments on urease enzyme activities were not significant (p>0.05). Here we demostrated that such as a method can also be used in soil quality, soil pollution, soil fertility and soil ecology studies which have gained a great significance for sustainable environment and agriculture.

Keywords: Pesticide, soil, earthworm, enzyme, microorganism

Toprağa Uygulanan Pestisitlerin (Chlorpyrifos, Glyphosate) Topraksolucanı Davranışlarına ve Bazı Toprak Biyolojik Özelliklerine Etkisi

Özet

Pestisitler verimi artırmak için doğrudan toprağa ya da bitkilere uygulanmaktadır. Bitkilere uygulanan pestisitlerin önemli bir kısmı da toprağa karışmaktadır. Pestisitlerin topraksolucanlarına etkisi konusunda ise az sayıda çalışma vardır. Bu çalışmada chlorpyrifos ve glyphosate pestisitlerinin ikişer dozu (önerilen doz ve önerilen dozun iki katı) toprağa katılarak 2D (2 boyutlu) düzenekte topraksolucanlarından *Eisenia fetida* türünün tercihlerine sunulmuştur. Düzeneklerdeki topraksolucanlarının topraklardaki galeri alanları, mikroorganizma sayıları, katalaz ve üreaz enzim aktiviteleri araştırılmıştır. Pestisit uygulanmayan topraklarda 2D düzeneğin her iki tarafında topraksolucanlarının açtığı galeri alanları arasında önemli fark bulunmazken (p>0.05), topraksolucanlarının chlorpyrifos uygulanmış toprak kısmı ile ilaçlanmamış kısmında açtığı galeriler arasında önemli fark gözlenmiştir (p=0.0298). Topraksolucanları chlorpyrifosun iki katı uygulanmış topraklarında kontrol tarafına göre daha çok galeri açmışlardır (p=0.0323). Galeri alanları bakımından glyphosate'in etkisi de önemsiz olmuştur (p>0.05). Mikroorganizma sayıları bakımından chlorpyrifosun önerilen dozunda artış olmuş (p=0.0074), chlorpyrifosun önerilen dozun iki katı uygulamasında kontrole göre fark görülmemiştir. Önerilen dozun iki katında glyphosate uygulamalarında mikroorganizma sayılarında önemli derece azalma olmuştur (p=0.0273). Katalaz enzim aktivitesi chlorpyrifosun iki kat uygulamasında önemli seviyede artmışken (p=0.0041), üreaz enzimi aktivitesi bakımından chlorpyrifos ve glyphosate uygulamalarının her iki dozlarında istatistiksel fark olmamıştır (p>0.05). Bu çalışma ile bu tür metotların, toprak kalitesi, kirliliği, verimliliği ve ekoloji çalışmalarında kullanılabileceği ortaya konulmuştur.

Anahtar Kelimeler: Pestisit, toprak, topraksolucanı, enzim, mikroorganizma

Introduction

Pesticides are either directly applied to soils or to plants. About 90-99.9% of the pesticides reach to the target organisms (Graham-Bryce, 1977; Tiryaki and Temur, 2010). The pesticides applied to the plants ultimately reach to the soil. Soil fertility was considered to largely depend on microorganisms providing nutrient cycle in soils (Pandey et al., 2000) and especially on earthworms so-called as "soil engineers" (Blouin et al., 2013; Dawood et al., 2017). Earthworms easily convert biodegradable material and organic waste into nutrient rich vermicast. Agro-chemicals and pesticides may improve crop yields significantly, but such practices seriously disturb the biodiversity of earthworms and other soil microorganisms (Datta et al., 2016).

By several researches have been conducted about the impacts of pesticides on high organisms (Jones et al., 1994) and about the toxic effects of pesticides on earthworms (Datta et al., 2016). But, the effects of pesticides on organisms, especially on soil microorganisms haven't been well elucidated, yet (Pelosi et al., 2014). Earthworms react against organic materials incorporated into the soils and their preferences of organic materials could reliable be identified in a short time without expensive and complex analyses (Türkmen et al., 2013).

Researchers (Givaudan et al., 2014) investigated detoxification and antioxidant enzyme activities in pesticide-treated lands with *Allolobophora chlorotica* and *Aporrectodea caliginosa* earthworm species. Araneda et al. (2016) reported that carboxylesterase enzyme activity of *Lumbricus terrestris* species decreased in summer.

In present study different from the earlier ones, two pesticides with chlorpyrifos and glyphosate active ingredients, commonly used in Turkey, were incorporated into the soils and conservation/orientation status of *Eisenia fetida* ISO (International Standardization Organization) earthworm, selected as biomarker, were investigated in 2D terrarium (ISO-International Standardization Organization, 2004). Microorganism counts and some soil enzyme activities were also investigated in this study.

Material and Method

Design of experimental apparatus (2D terrariums)

In this study, two different doses (application dose and double of application dose) of chlorpyrifos and glyphosate pesticides were homogeneously incorporated into air dried soil previously passed through 2 mm sieve (Table 1).

Table 1. Soil properties as adapted from Zambak et al., (2015).

Soil properties	Value	Assessment
pH (1:2.5 in water)	8.1	Mild alkaline
Sand (%)	56	
Silt (%)	28	Sandy-Loam (SL)
Clay (%)	16	
Organic Matter (%)	3.8	Adequate
N, ppm	0.66	Rich
P, ppm (Olsen)	7	20-25
K, ppm	150	200-320

These mixtures were placed into 2D (2 Dimensions; Din–A4/21.0x29.7x0.4 cm) terrariums, soil moisture levels were brought to field capacity with distilled water and 3 earthworms (*Eisenia fetida*) were placed into each one of these 2D terrariums (Fig.1). The systems were closed as specified by Evans (1947) and Fründ et al., (2008 and 2011).

Figure 1. Presentation of pesticide-applied soil on the top of 2D (2 Dimensions) glass apparatus.

Studies of gallery area and enzyme activity

Gallery areas of the earthworms kept inside the incubator (+18 °C) for 3 days at dark were drawn over A4-size acetate surfaces with permanent-ink pens and surface areas were measured with the aid of root-scanning software (WinRHIZO Basic Pro-2007, Regent Instruments Inc., Quebec) after calibration the software for 1 cm^2 scanned area as specified by Gallagher et al (2015).

Samples of soil were taken from the 2D terrariums following the measurement of gallery areas. Then, aerobic mesophyll microorganism counts were performed in soil samples by Wollum (1982). Changes in catalase and urease enzyme activities of different doses were assessed through the comparisons with the control treatment

Statistical analysis

SAS statistical software was used for statistical analyses of experimental data (SAS Institute, 2009). Data were statistically analyzed with ANOVA in accordance with the following statistical model. Significant means were compared with the aid of Tukey's test.

Results and Discussion

Gallery areas

The differences in gallery areas in control soils without any pesticide treatments on both sides of the 2D terrarium were not found to significant (Fig. 2). This case was considered as a precondition for the assessment of the other parameters. Significant differences were observed in gallery areas opened in chlorpyrifos-treated soils with the recommended dose.

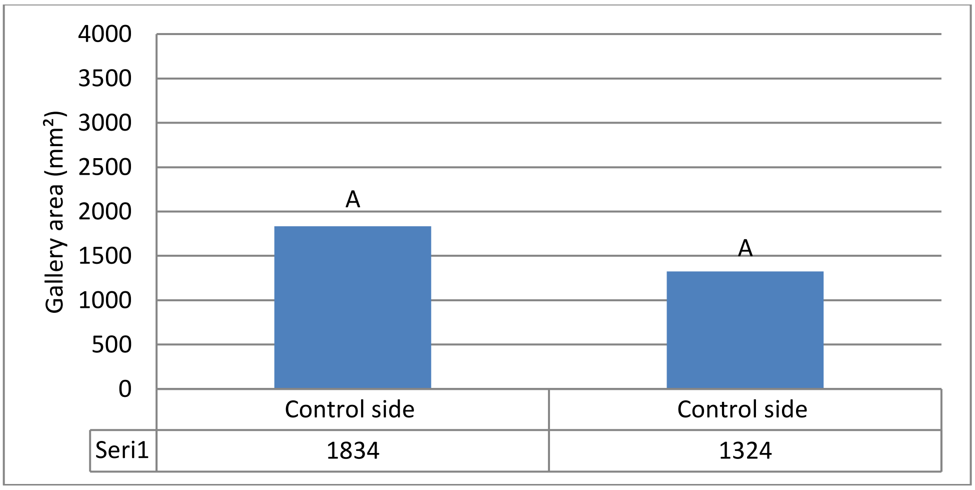
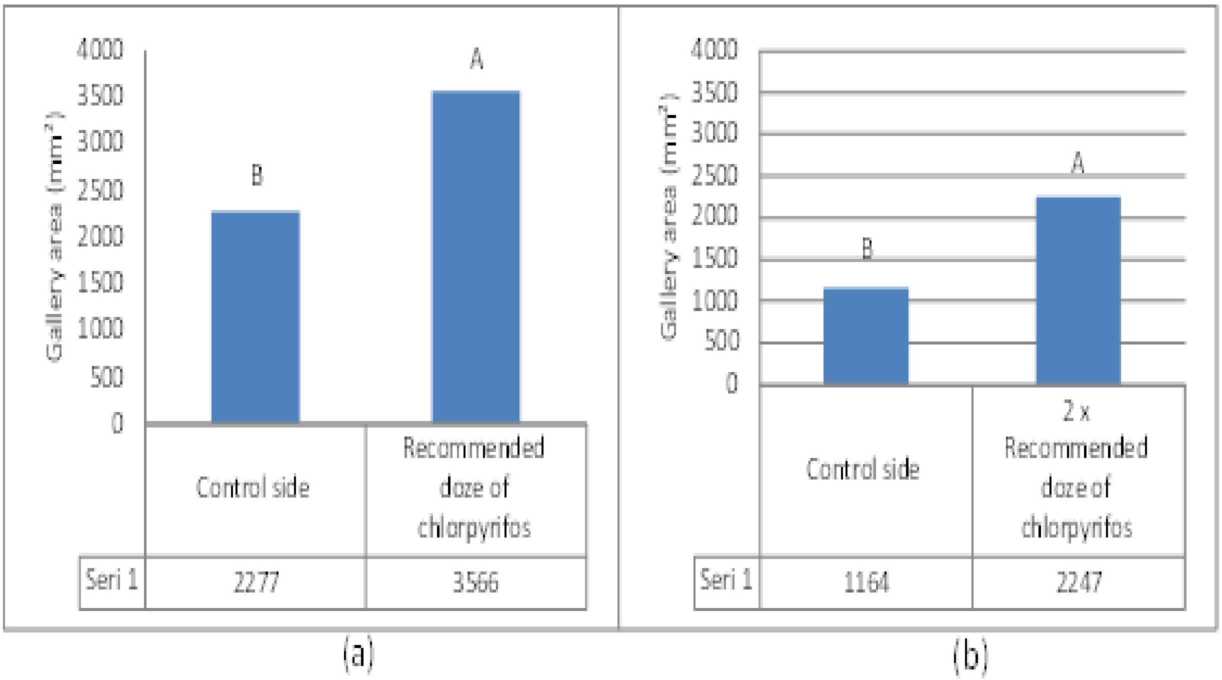


Figure 2. Gallery areas on both sides of 2D terrarium in control treatment (without pesticide treatment).

A similar difference was also supported by greater gallery areas opened by the earthworms in chlorpyrifos-treated soils with the double of recommended dose than the control treatment (Fig. 3). With regard to gallery areas, the effects of glyphosate were not found to be significant (Zambak et al., 2015).



Figue 3. Gallery areas in chlorpyrifos treated soils with the recommended dose (a) and double of the chlorpyrifos recommended dose (b).

Casabé et al. (2007), in a study with ISO N 281 (ISO-International Standardization Organization 2004) method, reported decreased cocoon and juvenile earthworm counts soils 10 days after the glyphosate treatments and indicated that earthworm avoided in glyphosate treatments. Additionally, other researchers (Lowe et al., 2016) carried out a study with *Eisenia fetida* and 500 and 1000 mg kg^{-1} glyphosate doses for 56 days and reported 20-40% mortality in 14 days and total mortality at the end of the tests. Differences from the present findings were mainly because of the treatment method (2D system) and incubation duration. About 100 g soil was used in 2D system and earthworms were not allowed to leave the system. Thus, they might have got into stress in a closed system and thus opened larger galleries. Such outcomes should be validated with field and laboratory research carried out with the other pesticides and earthworm species.

As compared to the control treatment, increases were observed in microorganism counts at the recommended dose of chlorpyrifos (p=0.0074). However, a significant difference was not observed from the control treatment at double of the recommended dose of chlorpyrifos. Considering the effects of glyphosate treatments on microorganism counts, it was observed as compared to control treatment that double of recommended dose decreased microorganism counts and these decreases were found to be significant (Fig.4).

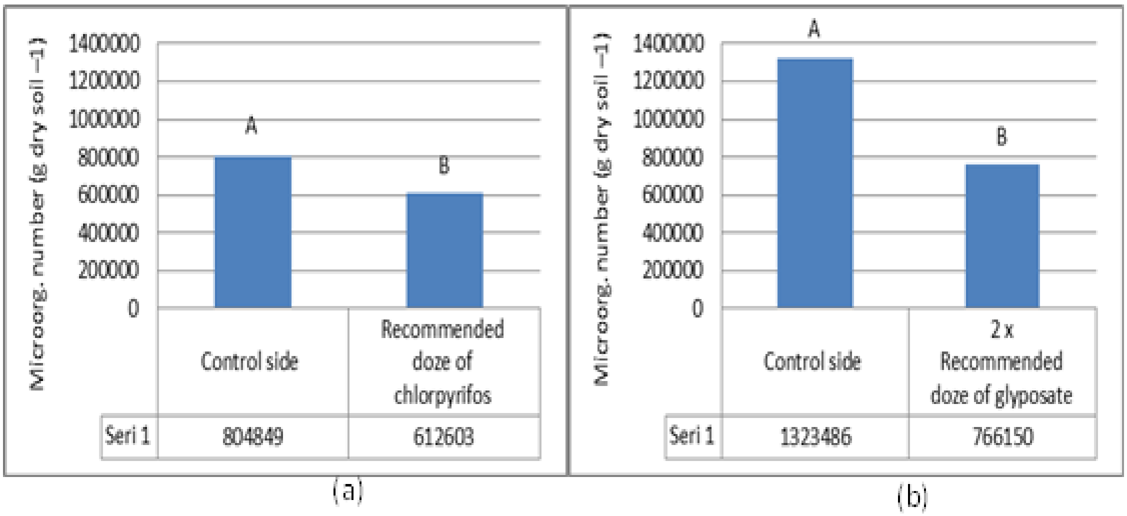


Figure 4. Microorganism counts in chlorpyrifos-treated soils with recommended dose (a) and with double of the glyphosate recommended dose (b).

Such a case was also supported by the study of Van Hoesel et al., (2017) with *Lumbricus terrestris* species indicating insignificant differences in microbial load of herbicide applied soils including glyphosate.

Changes in enzyme activities

Catalase enzyme activity significantly increased with the double dose of chlorpyrifos (Fig. 5). Such a case can be explained significantly increased gallery areas (in other words, earthworm activities) at both the recommended and double dose of chlorpyrifos. It can be mentioned a negative relationship between microorganism counts and gallery areas (Fig. 3, 4 and 5). Earthworms secrete mucous/coelom to protect themselves (Zhenjun, 2011). Such a case then triggered defense mechanisms against oxidative stress conditions and thus increased catalase enzyme activities (Dick, 1994).

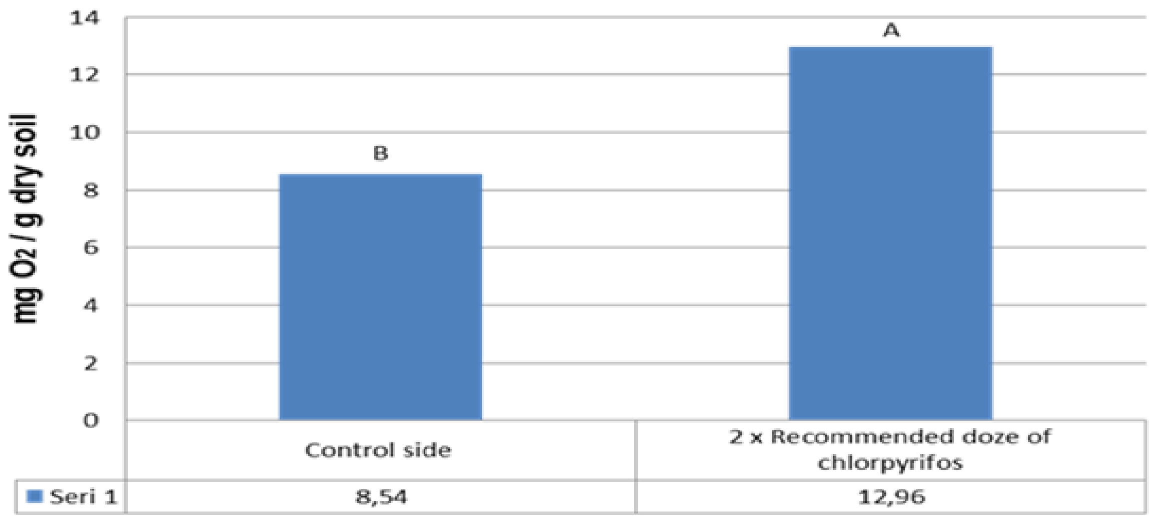


Figure 5. Changes in catalase enzyme activities of treated soils with the double of chlorpyrifos recommended dose.

However, increased catalase enzyme activity may not be sufficient to prevent a reduction in microorganism counts. Ekberli and Kizilkaya (2006) carried out a study with *L. terrestris*, control soils, casts and soils around the galleries and reported higher catalase enzyme activity for surrounding soils than the other soils. However, as indicated also by Van Hoesel et al (2017), tests carried out with a single earthworm species and single pesticide may not reveal the actual cases in soils. Urease enzyme activity was also investigate in this study since the soil was rich in nitrogen (Table 1), but significant differences were not observed at both doses of chlorpyrifos and glyphosate treatments.

The enzymes and the other variables investigated in the present study have not been included together in previous work. Sanchez-Hernandez et al. (2014), in a previous study with *Lumbricus terrestris* earthworms, applied 4 mg/kg chlorpyrifos on to soils and reported carboxylesterase enzyme activity 2 and 6 days after the treatments respectively as between 40-72% and between 37-53%.

Conclusion

It can be concluded base on present results that earthworms reacted against the materials incorporated into the soils and such reactions could be measured through calculating the gallery areas in a 2D terrariums (Fründ et al., 2008 and 2011; Türkmen et al., 2013). With this specific characteristic of the earthworms, their tendency in different soils and to different materials could be determined. Such a method can also be used in soil quality, soil pollution, soil fertility and soil ecology studies which have gained a great significance for sustainable environment and agriculture (Lowe et al., 2016).

There is no need for expensive and complex devices and tools in this method and an assessment of positive/negative or well/poor could be made about soil parameters.

References

Araneda, A.D., Undurraga, P., Lopez, D., Saez, K. and Barra, R. 2016. Use of earthworms as a pesticide exposure indicator in soils under conventional and organic management. *Chilean Journal of Agricultural Research,* 76 (3): 356-362.

Blouin, M., Hodson, M.E., Delgado, E.A., Baker, G., Brussaard, L., Butt, K.R., Dai, J., Dendooven, L., Peres, G., Tondoh, J.E., Cluzeau, D. and Brun, J.J. 2013. A review of earthworm impact on soil function and ecosystem services. *European Journal of Soil Science*, 64: 161–182.

Casabé, N., Piola, L., Fuchs, J., Oneto, M.L., Pamparato, L., Basack, S., Gimenez, R., Massaro, R., Papa, J.C. and Kesten, E. 2007. Ecotoxicological assessment of the effects of glyphosate and chlorpyrifos in an Argentine soya field. *J Soil Sed*., 7: 232–239.

Datta, S., Singh, J., Singh, S. and Singh, J. 2016. Earthworms, pesticides and sustainable agriculture: A review. *Environmental Science and Pollution Research International,* 23 (9): 8227-8243.

Dawood, M., Wahid, A., Hashmi, M.Z., Mukhtar, S. and Malik, Z. 2017. Use of earthworms in biomonitoring of soil xenobiotics. In: Hashmi M., Kumar V., Varma A. (eds) Xenobiotics in the Soil Environment. *Soil Biology,* 49: 73-88.

Dick, R.P. 1994. Soil enzyme activities as indicators of soil quality in; J.W. Doran, D.C. Coleman, D.F. Bezdicek, B.A. Stewart (Eds.), Defining soil quality for a sustainable environment, *Soil Science Society of America*, Madison 107-124.

Ekberli, I., and Kizilkaya, R. 2006. Catalase enzyme and its kinetic parameters in earthworm *L. terrestris* casts and surrounding soil. *Asian J. Chem*., 18: 2321–2328.

Evans, A.C. 1947. A method of studying the burrowing activities of earthworms. *Ann Mag Nat Hist.,* 14: 643–650.

Fründ, H.C., Graefe U. and Tischer, S. 2011. Earthworms as Bioindicators of Soil Quality. In: Karaca A. (ed.) Biology of Earthworms. Springer Berlin Heidelberg, *Soil Biology,* 24: 261-278.

Fründ, H.C., Wallrabenstein, H., Leibner, S. and Blohm, R. 2008. Developing a soil quality test with 2D terraria and Aporrectodea caliginosa–Berichte der DBG, http://www.dbges.de 1-4.

Gallagher, F.J., Caplan, J.S., Krumins, J.A. and Grabosky, J.C. 2015. Root Growth Responses to Soil Amendment in an Urban Brownfield. *Ecological Restoration,* 33 (1): 10-13.

Givaudan, N., Binet, F., Le Bot, B., and Wiegand, C. 2014. Earthworm tolerance to residual agricultural pesticide contamination: field and experimental assessment of detoxification capabilities. *Environmental Pollution,* 192: 9-18.

Graham-Bryce, I.J. 1977. Ecological effects of pesticides. Edited by: Perring, F.H. and Mellanby, K., London: *Academic Press* 47–60.

ISO-International Standardization Organization 2004. Soil quality – Avoidance test for testing the quality of soils and the toxicity of chemicals – Test with earthworms (*Eisenia fetida*), ISO N 281 draft protocol, Geneva, Switzerland.

Jones, C.G., Lawton, J.H., and Shachak, M. 1994. Organisms as ecosystem engineers. In Ecosystem management (pp. 130-147). Springer, New York, NY.

Lowe, C.N., Butt, K.R. and Cheynier, K.Y-M. 2016. Assessment of avoidance behaviour by earthworms (*Lumbricus rubellus* and *Octolasion cyaneum*) in linear pollution gradients. *Ecotox. Environ. Safe* (124): 324–328.

Pandey, K.D., Shukla, P.N., Giri, D.D., and Kashyap, A.K. 2000. Cyanobacteria in alkaline soil and the effect of cyanobacteria inoculation with pyrite amendments on their reclamation. Biology and fertility of soils, 41(6): 451-457.

Pelosi, C., Barot, S., Capowiez, Y., Hedde, M. and Vandenbulcke, F. 2014. Pesticides and earthworms, Agron. *Sustain. Dev*., 34: 199–228.

Sanchez-Hernandez, J.C., Morcillo, S.M., del Pino, J.N. and Ruiz, P. 2014. Earthworm activity increases pesticide-sensitive esterases in soil. *Soil Biol. and Biochem.,* 75: 186-196.

SAS Institute. 2009. Using JMP Student Edition for Windows and Macintosh: The User's Guide to Statistics with JMP Student Edition. SAS institute.

Tiryaki, O., and Temur, C. 2010. The fate of pesticide in the environment. *Journal of Biological and Environmental Sciences,* 4 (10): 29-38.

Türkmen, C., Temel, E., Çatal, G., Sinecen, M. and Mısırlıoğlu, M. 2013. Effect of some waste and soil regulators on worm behavior in soil. COMU, *Journal of Agriculture Faculty,* 1(1): 79–86.

Van Hoesel, W., Tiefenbacher, A., König, N., Dorn, V.M., Hagenguth, J.F., Prah, U. and Lagerlöf, J. 2017. Single and combined effects of pesticide seed dressings and herbicides on earthworms, Soil Microorganisms, and Litter Decomposition. *Frontiers in Plant Science Feb*., 21: 8-215.

Wollum, A.G. 1982. Cultural methods for soil microorganisms. In Methods of Soil Analysis: Chemical and Microbial Properties, 2nd Edn. ed. Page, A.L., Miller, R.H. and Keeny, D.R., Madison-WI-USA, *Society of Agronomy and SSSA*. 718–802.

Zambak, Ş., Ünsal, B. and Tiryaki, O. 2015. Effects of chlorpyrifos and glyphosate pesticides on behaviour of earthworm in the soil. 6th. International Scientific Agriculture Symposium “Agrosym 2015” Jahorina, 15-18 October 2015, Bosnia and Herzegovina, *Book of Proceedings* 1558-1563.

Zhenjun, S. 2011. Antimicrobial vermipeptides: From methods to characteristics. In; Karaca, A. (ed.) Biology of Earthworms, *Soil Biol*., 24 Springer-Verlag, Berlin, 1-18.