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Use of *Eisenia andrei* as Bioindicator of Soil Toxicity of Agrochemicals

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***Abstract* – Given the importance of soil as a source of water and nutrients for plants and other organisms, pollution of the soil ecosystem can have important consequences for all forms of life and the quality of food, water and the atmosphere. The indiscriminate use and undue disposal by most agricultural properties can increase the accumulation of agrochemicals in the soil causing contamination. The ecological niche of the earthworms characterizes them as very important organisms in the soil and as environmental bioindicators. The objective of this study was to evaluate the sensitivity of *Eisenia andrei* by means of leakage test in soils contaminated by two agrochemicals. The experiment was composed of four treatments: T1 (Wood soil + Herbicide), T2 (Wood soil + Insecticide), T3 (Cultivated soil + Herbicide), T4 (Cultivated soil + Insecticide), in a randomized block design and factorial scheme. About 2000 g of soil were placed in polypropylene trays lined with parchment paper, whose dimensions were 44 x 28.5 x 10.4 cm, filled to the height of 4 cm of soil layer. The agrochemicals used were: glyphosate and organophosphate. After 72 hours of addition of the agrochemicals, 6 adult individuals were added. After the 48-hour period, according to NBR ISO 17512-1, the evaluation was carried out. Through the analysis of variance and multivariate, it was found that organophosphate acts under individuals, regardless of soil, while glyphosate tends to have interaction with the soil type evaluated.**

Keywords* – Ecotoxicology, Glyphosate, Organophosphorus, Edaphic Fauna, *Oligochaeta.

I. Introduction

The soil, as a living and functional compartment, has been constantly debated and its attention turned to agricultural needs. This attention is especially given to the intensive and indiscriminate use of agrochemicals, which have caused environmental damage, not only to the soil and plants, but also to other living organisms that constitute it as microorganisms and the constituents of edaphic fauna.

Contamination of the soil causes a high social impact, as it does not only affect agricultural production, taking its consequences to all levels of the population. The indiscriminate use of agrochemicals, with the consequent contamination of soils, affects the momentary elimination of edaphic individuals, such as the constituents of the edaphic mesofauna, causing it to reach several levels of the trophic chain [1]. Depending on, this elimination of organisms can cause death of those beneficial to the environment, such as biological indicators of pests and agricultural diseases, saprophytic organisms, among others.

For the proper functioning of the soil, in its biological aspects, it is necessary that all the organisms that inhabit it live in harmony. This harmony will be given according to the physical and chemical characteristics of the soil, which undergo direct influence of the management employed. Among the characteristics of the soil, its chemical composition affects all life forms found in it. The main factor that changes the chemical characteristics of the soil in agricultural areas is the use of fertilizers, chemical or organic [2]. In this way, the unbridled application of agrochemicals in agriculture exerts influence on the different forms of life present there.

Soil quality assessment can be inferred through the presence and/or absence of specific species or groups of species, which are directly related to environmental conditions (soil type, water availability, management, vegetal cover, among others). Among these organisms used as bioindicators of soil quality, it can be mention the edaphic macrofauna, which comprises organisms that are characterized by being able to create specific structures, which allow their mobility in the soil, forming holes, tunnels and galleries [2].

A group of organisms currently applied to soil quality studies are worms belonging to the *Oligochaeta* subclass, being important organisms in terrestrial ecosystems [3], since they perform specific functions such as nutrient cycling. Its locomotive habits promote the formation of tunnels, facilitating the locomotion of other individuals in the soil extract, besides improving the water distribution through the profile of the same [4], as well as providing better aeration.

Ecotoxicology is a science aimed at studying the effects of pollutants on organisms and how these interactions in their habitats [5]. Descriptions and research suggestions of organisms through the use of soil invertebrates, such as earthworms of the species *Eisenia andrei* and *E. fetida*. According to Velásquez *et al*. [6] they are sensitive to environmental changes and an important biological indicator.

Among the chemicals inputs applied to agricultural crops there are the glyphosate and organophosphorus groups, which are used in various agricultural crops, however, although there are restrictions on their use, bad administered applications occur, which affect environmental accidents. Glyphosate is an organic, non-selective, post-emergent herbicide classified as Class IV-Low toxicity, although it is also classified as hazardous to the environment. Organophosphates are insecticides, however, their classification may range from highly toxic to low toxicity, depending on their formulation and use. Although widely used in the agricultural and non-agricultural areas these molecules cause in several cases of intoxication every year.

Therefore, the objective of this study was to evaluate the sensitivity of earthworms of the *E. andrei* species to two agrochemical molecules by means of leak test.

II. Materials and Methods

In order to conduct the experiment, soil sampling was carried out in two different environments (sugarcane cultivation area - *Saccharum officinarum* L., and an area of Atlantic forest preserved with minimal anthropogenic action, with all its characteristic vegetation) (9 And 29'45 "south latitude, 35° and 49'54" longitude and 165 m altitude), characterized by the same type of soil, being a yellow red latosol [9]. The earthworms of the *E. andrei* species used in this study are grown in organic compost, fed with vegetables.

Then 2000g of soil was weighed (referring to its unsaturated wet weight) and packed in polypropylene trays covered with parchment paper with the following dimensions: 44 x 28.5 x 10.4 cm. After conditioning the soil in the trays, this comprised a layer of 4 cm in height. After the addition of the soil in all the trays, these were temporarily divided with a strip of paper, in order to demarcate the application area of the agrochemicals, and the free area for escape of the individuals.

For the addition of agrochemicals, doses equivalent to those used in the field were calculated (for glyphosate, the recommended dose for *Brachiaria* spp. 2.50 L/ha^{-1} was used and organophosphate with the recommended dose for *Spodoptera frugiperda* of 0.4-0, 6 L/ha^{-1}).

After doses calculation and area demarcation to be applied and free area to escape the worms, the agrochemicals were applied by spraying, using disposable sprayers. Then, 72 hours after spraying, in each tray, six worms were added and covered with PVC film and conditioned on 12 hours photoperiod at environment temperature. The evaluation of the individuals leakage was performed 48h after incubation, as proposed by NBR ISO 17512-1 [7], where the number of individuals migrated from the pole of the tray in which the application of the agrochemicals to the application free pole was counted.

The experimental design was in randomized blocks, in a 2x2 factorial scheme. The treatments corresponded to: T1 (Woodforest soil + Herbicide), T2 (Wood soil + Insecticide), T3 (cultivaded Soil + Herbicide), T4 (Cultivaded Soil + Insecticide). The collected data were expressed as leakage index (LI) percentage by means of the formula:

$$LI(\%) = (N \times 100)/6$$

Where:

LI(%) = Individuals leakage index expressed as a percentage,

N = Number of individuals who migrated

The calculated and tabulated data were submitted to analysis of variance ($p \leq 0.05$) using the Sisvar Software [8]. In order to discuss the interaction of individuals' leakage according to the soil samples and the molecules used, the data were submitted to multivariate Analysis of Principal Components (PCA) using XLStat® Software, with significance ($p \leq 0.01$ and 0.05).

III. Results and Discussion

By means of the analysis of variance, it was verified that the effect of the exposure of the individuals to the tested agrochemicals was statistically significant ($p \leq 0.01$), being observed that there is dependence between the provenances of the soil as a function of the agrochemicals tested, that is, the soil alone, does not interfere with the mobility of organisms, which are strongly influenced by the type of chemical compound to which they are exposed.

Thus, it was verified that there is a positive interaction between the soil origin and the type of agrochemical, as shown in Table 1. Thus, it is possible to note that when evaluating the individual factors, the organophosphate is responsible for the higher LI in this experiment.

Table 1. Index of leakage (%) of earthworms (*E. andrei*) exposed to two types of agrochemicals evaluated by behavioral leakage test

Factor	LI (%)*
Glifosate	45.00 a
Organofosforate	73.35 b
Cultivated Soil	51.67 a
Wood Soil	66.68 a

*Means followed by the same letter do not differ statistically from each other by the Tukey test ($p \leq 0.01$). LI – Leakage Index.

The largest LI were found for the organophosphorus compound, which was independent of the origin of the sampled soil. As for glyphosate, a higher leakage rate was observed when in interaction with soil from the forest (Table 2).

Table 2. Worm Leakage index (%) (*E. andrei*) exposed to two types of agrochemicals evaluated in the leakage test, regarding the interaction between agrochemical and soil sampling source

Factor		LI (%)*
Wood soil	Glifosate	63.34 b
	Organofosforate	70.02 a
Cultivated soil	Glifosate	26.66 c
	Organofosforate	76.68 a

*Means followed by the same letter do not differ statistically from each other by the Tukey test ($p \leq 0.01$). LI – Leakage Index.

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Thus, the interaction between glyphosate and soil from wood soil is evidenced by the PCA through Pearson correlation, where it shows the dependence of these factors along axis 1, this axis being strongly expressive regarding the interaction of factors, explaining 57.35% of the data variability, being stronger than axis 2, which explains 46.65% (Figure 1). This explanation is verified in the graphical representation through linear correlations between the sets of explanatory variables and descriptors of the data samples.

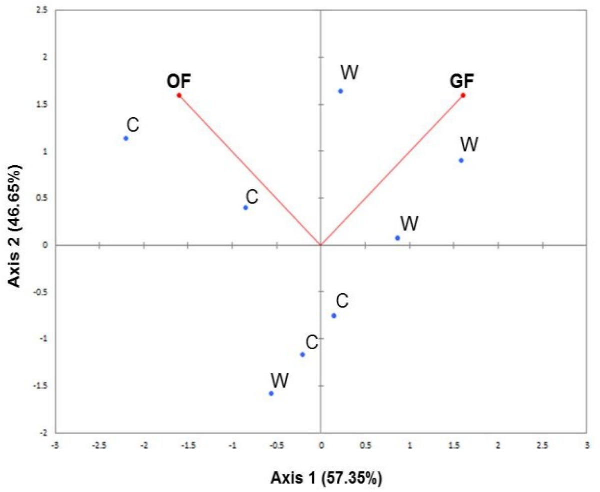

Fig. 1. (PCA) Principal component analysis ordering by four factors Pearson's correlation (n) that influence the mobility of worms (E. andrei), exposed to two factors two agrochemicals types. Dispersion points correspond to the soil according to their sampling origin. Points with lines correspond to the studied agrochemicals, depending on the soil. GF (Glyphosate), OF (Organofosforate), W (Wood soil), C (Cultivated soil).

Ecotoxicity studies are intended not only to demonstrate the living organisms sensitivity to harmful substances. These studies are also able to infer about the levels of contamination of degraded or degraded environments, whether terrestrial or aquatic environments.

Although more artificial, laboratory-conducted trials generate direct and specific results of the effect of a particular contaminant on a population and allow the manipulation of several factors in order to predict possible impacts under field conditions [10]. Among the organisms usually employed in these tests, earthworms and colembole are the most commonly used, especially when testing temperate climate conditions.

Environmental degradation is a reality that has reached alarming proportions since the late nineteenth century, whether for the cultivation of agricultural crops and pastures or for the expansion and creation of urban areas [2]. Soil degradation can arise from several points and levels, and can start from anthropic actions causing erosion, compaction, pollution; By the release of harmful substances; Agriculture, through physical, chemical or biological actions. In this way, it is highly important to study the ecotoxicity of degraded environments, mainly by the use of marker organisms.

In this way, some studies, although still scarce, have already been carried out in the field and laboratory in order to evaluate the sensitivity of edaphic organisms as a function of substances harmful to the environment.

Based on the results presented by [11] using collmbole leakage tests, it is concluded that the presence of cypermethrin negatively affects the behavior of this species, as it is capable of and promotes the The individuals escape at concentrations of 7.5 mg kg^{-1} in the dystrophic Red Latosol, while for the tropical artificial soil, leakage can be observed from 22.5 mg kg^{-1}.

As it was observed in this study, the use of organophosphate, a molecule used as an insecticide in agriculture and in the control of urban pests, showed a greater leakage of individuals, proving to be more toxic to *E. andrei* worms compared to glyphosate. This fact can be verified by the type of organisms that are the target of the organophosphates, since it is used for the control of insects, specifically in the larval stages of development, biologically this molecule will be more toxic to soil edaphic organisms than glyphosate, used as a systemic action herbicide.

Soil fauna is sensitive to changes in the environment, such as the management and cultivation practices, which depending on the intensity of the impact promoted to the environment, can have a direct effect on soil populations [2].

Due to the sensitivity of the organisms that compose the edaphic fauna of the soils, these become highly susceptible to the action of toxic substances. An example is the use of insecticides, which by many are not selective, end up reaching a multitude of non-target organisms, causing an imbalance in populations. This mortality of non-target organisms can lead to a lack of food for organisms that are predators of agricultural pests, decreasing the population of the latter as favoring the appearance of potential pests, by the absence of its natural predator.

Regarding the motility of the agrochemicals in the soil extract, the finding of a greater migration of earthworms from the treatments that received wood soil is related to the fact that, due to receiving less or no anthropic action, it favors a greater movement of the molecules, making With which the dispersion is greater and consequently the individuals have more contact with this molecule than with the organophosphorus.

Regarding the methodology addressed in this study, (NBR ISO 17512-1), it is able to infer about the sensitivity of the organisms studied, being able to be correlated to the field conditions. As suggested by Baretta *et al.* [2], it is important to carry out studies within soil ecotoxicology with test organisms, because this line of research, in addition to being still lacking in data, has the potential to assess the risks and environmental impacts of residues by methods standardized, through the use of soil quality indicator organisms. In this context, the application of data evaluation and validation techniques is also a fundamental aspect of the research, such as the use of multivariate analysis methods [2], [12].

Based on the data found in the present study, it is important to implement ecotoxicology-related research through the use of bioindicators, such as laboratory studies to test the sensitivity of these organisms, in order to promote information that will be applied to the knowledge of toxicity of Substances that are harmful to the environment in all its aspects, not only in agricultural ecosystems, but also in all biomes for the knowledge and preservation of biodiversity.

IV. Conclusions

Cultivated soils are more sensitive to organophosphate contamination compared to forest soils that do not have anthropic activity. *E. andrei* is specie that has importance in ecotoxicological studies.

References

[1] E. P. Odum, "*Ecology*." Rio de Janeiro: Guanabara, 1983. 434p.

[2] D. Baretta, J. C. P. Santos, J. C. Segat, E. V. Geremia, L. C. L. Oliveira Filho & M. V. Alves. Edaphyc fauna and soil quality. In: "*Topics in Soil Science*", O. Klauberg Filho, A. L. Mafra & L. C. Gatiboni, Eds. Viçosa: Brazilian Soil Sience Society, 2007, pp. 141-81.

[3] M. L. Bartz, G. G. Brown, J. O. Fernandes, P. Curmi, J. Dorioz & R. Ralisch. "Earthworm communities in organic and conventi--onal coffee cultivation." *Pesq. Agropec. Bras. 44(8),* 2009, pp. 928-33. doi: http: // dx.doi.org/10.1590/S0100-204X200900080 0019

[4] E. Blanchart, A. Albrecht, G. G. Brown, T. Decaens, A. Duboisset, P. Lavelle, L. Mariani & E. Roose. "Effects of tropical endogenic earthworms on soil erosion." *Agric. Ecosyst. Environ. 104(2)*, 2004, 303-315. doi: https://doi.org/10.1016/ j.agee. 2004.01.031

[5] P. A. Zagatto & E. Bertoletti. "Aquatic Ecotoxicology – principi--es e applications." São Carlos: RIMA, São Carlos. 2008, 478p.

[6] E. Velásquez, S. J. Fonte, S. Barot, M. Grimaldi, T. Desjardins & P. Lavelle P. "Soil macrofauna-mediated impacts of plant species composition on soil functioning in Amazonian pastures." *Applied Soil Ecol. 56(5)*, 2012, pp.43-50. doi: https://doi.org/ 10.1016/j.apsoil.2012.01.008

[7] Brazilian Association of Technical Standards. ABNT NBR ISO 17512-1 Soil Quality - "Leak test to assess soil quality and effects of chemicals on behavior. Part 1: Assay with earthworms (*Eisenia fetida* e *Eisenia andrei*)." Rio de Janeiro. 2011.

[8] D. F. Ferreira. "Sisvar - variance analysis system for balanced data." Lavras: UFLA, 2014.

[9] Brazilian Agricultural Research Corporation. "Brazilian system of soil classification." (4.ed). Brasília, Distrito Federal. 2014.

[10] W. Didden & J. Rombke. "Enchytraeids as indicator organisms for chemical stress in terrestrial ecosystems." *Ecotoxicol. Environm. Saf. 50(1)*, 2001, pp.25-43. doi: 10.1006/ eesa.2001.2075

[11] T. Zortéa, D. Baretta, J. C. Segat, A. P. Maccari, C. R. D. M. Baretta & A. S. da Silva. "Leakage behavior of exposed clones on soils contaminated with cypermethrin." *Sci. Agr. 16(4)*, 2015, pp. 49-58. doi: http://dx.doi.org/10.5380/rsa.v16i4.47842

[12] D. Baretta, A. L. Mafra, J. C. P. Santos, C. V. T. do Amarante and I. Bertol, "Multivariate analysis of soil fauna in different systems of soil preparation and cultivation." *Pesq. Agropec. Bras. 41(11)*, 2006, pp. 1675-1679. Available: https://seer.sct.embrapa.br/index.php/pab/article/view/7331/4376