

Oral Communication 16

Exposure to a glyphosate-based herbicide induces avoidance behavior and impairs coelomocyte viability in *Eisenia andrei* earthworms

Diovana Gelati de Batista^{1,*}, Juliana Furlanetto Pinheiro¹, Isadora Sulzbacher Ourique¹, Bethina Barz Basso¹, Maria Eduarda Todendi de Bragas², Lucas Machado Sulzbacher¹, Pauline Brendler Goettens Fiorin¹ and Thiago Gomes Heck¹

¹ Regional University of Northwestern Rio Grande do Sul, Rua do Comércio 3000, Ijuí, RS, Brazil, CEP 98700-000

² 25 de Julho State Technical School, Ijuí, RS, Brazil, CEP 98700-000

* Correspondence: diovana.g.batista@gmail.com

Abstract

Background: Glyphosate-based herbicides (GBH) are the most widely used agrochemicals [1]. Earthworms are key soil organisms used as bioindicators and alternative experimental models for studying the immune system [2,3]. **Objective:** We tested whether agronomic dosages of GBH induce avoidance behavior and alter the immunological profile of earthworms *Eisenia andrei*. **Methods:** Adult earthworms (0.318 ± 0.007 g) were divided into four groups and exposed for 48h: Control group (native soil), GBH1.5, GBH3, and GBH6 groups (native soil with GBH at concentrations equivalent to 1.5, 3.0, and 6.0 L/ha, respectively). Under these conditions, we applied the Avoidance Behavior Test (% of animals that escape from contaminated areas) and Acute Toxicity Test. We used glyphosate (Roundup®, Original DI, Monsanto, 44.5% w/v active ingredient) or water (control) in each experimental unit (n=6; 6 animals/experimental unit, 6 replicates each, in a box with 600g of soil, 95% of dystrophic red latosol:5% organic matter). The coelomocytes were collected by a non-invasive method [4]. **Results:** The highest concentration (GBH6) induced avoidance behavior in earthworms (% avoidance = GBH6 = 83.3 ± 18.2 , $p=0.01$) without modification in the immune profile. Furthermore, there was a reduction in cell viability of the coelomocytes obtained from the GBH6 ($p=0.001$) and also GBH3 ($p=0.01$) groups, when the animals had no option to avoid the contaminated area (CTRL: $75.7 \pm 18.9\%$; GBH1.5: $63.7 \pm 22\%$; GBH3: $56.7 \pm 29.6\%$; and GBH6: $56.0 \pm 21.4\%$). **Conclusion:** The presence of GBH in the soil at a typical agronomic dose (3.0 L/ha) or higher (6.0 L/ha) threatens the immune defense of earthworms and may lead to the loss of the ecological function of soil.

Keywords: glyphosate; behavior; immune cells; environmental pollution

Acknowledgments

This study was partially financed by the Coordination for the Improvement of Higher Education Personnel (CAPES) in Brazil, under grant 001, and by The Brazilian National Council for Scientific and Technological Development (CNPq) under grants 307926/2022-2 and 405546/2023-8 to TGH.

References

1. Van Bruggen, A.H.C. et al. Environmental and health effects of the herbicide glyphosate. *Sci Total Environ* 2018, 616-617, p. 255-268.
2. Liu, T. et al. Earthworms Coordinate Soil Biota to Improve Multiple Ecosystem Functions. *Curr Biol* 2019, 29, p. 3420-3429.
3. Teixeira, C. F. et al. Safety indicators of a novel multi supplement based on guarana, selenium, and L-carnitine: Evidence from human and red earthworm immune cells. *Food Chem Toxicol* 2021, 150, p. 112066.
4. Eyambe, G. S. et al. A non-invasive technique for sequential collection of earthworm (*Lumbricus terrestris*) leukocytes during subchronic immunotoxicity studies. *Lab Anim* 1991, 25, p. 61-67.



In *Scientific Letters*, works are published under a CC-BY license (Creative Commons Attribution 4.0 International License at <https://creativecommons.org/licenses/by/4.0/>), the most open license available. The users can share (copy and redistribute the material in any medium or format) and adapt (remix, transform, and build upon the material for any purpose, even commercially), as long as they give appropriate credit, provide a link to the license, and indicate if changes were made (read the full text of the license terms and conditions of use at <https://creativecommons.org/licenses/by/4.0/legalcode>).