

Using the field lizard as a model to study the impact of glyphosate on soil-dwelling vertebrates

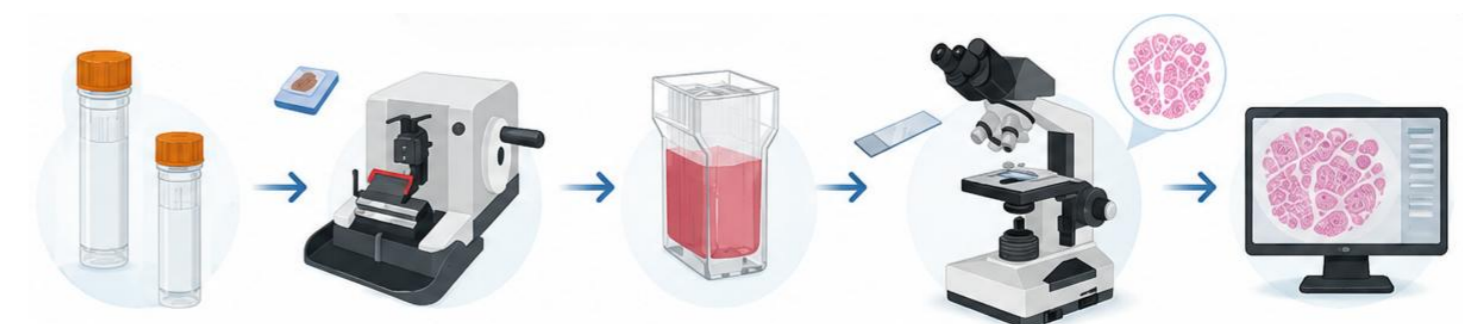
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INTRODUCTION & AIM

Podarcis siculus is the most widespread reptile in the Mediterranean region. Because it inhabits agricultural landscapes and urban parks, it is frequently exposed to environmental contaminants, including agricultural chemicals such as herbicides and pesticides, as well as pollutants deposited in the soil, such as heavy metals. These features make this lizard an excellent model for investigating the effects of soil contamination on terrestrial vertebrates. Our research focused on reproductive toxicity, as successful reproduction is essential for species survival. We examined the effects of glyphosate (Gly), a herbicide extensively used in agriculture, on both male and female gonads. Glyphosate is considered a potential endocrine-disrupting chemical, with evidence suggesting that it may interfere with endocrine regulation and consequently affect reproductive processes and gonadal function.

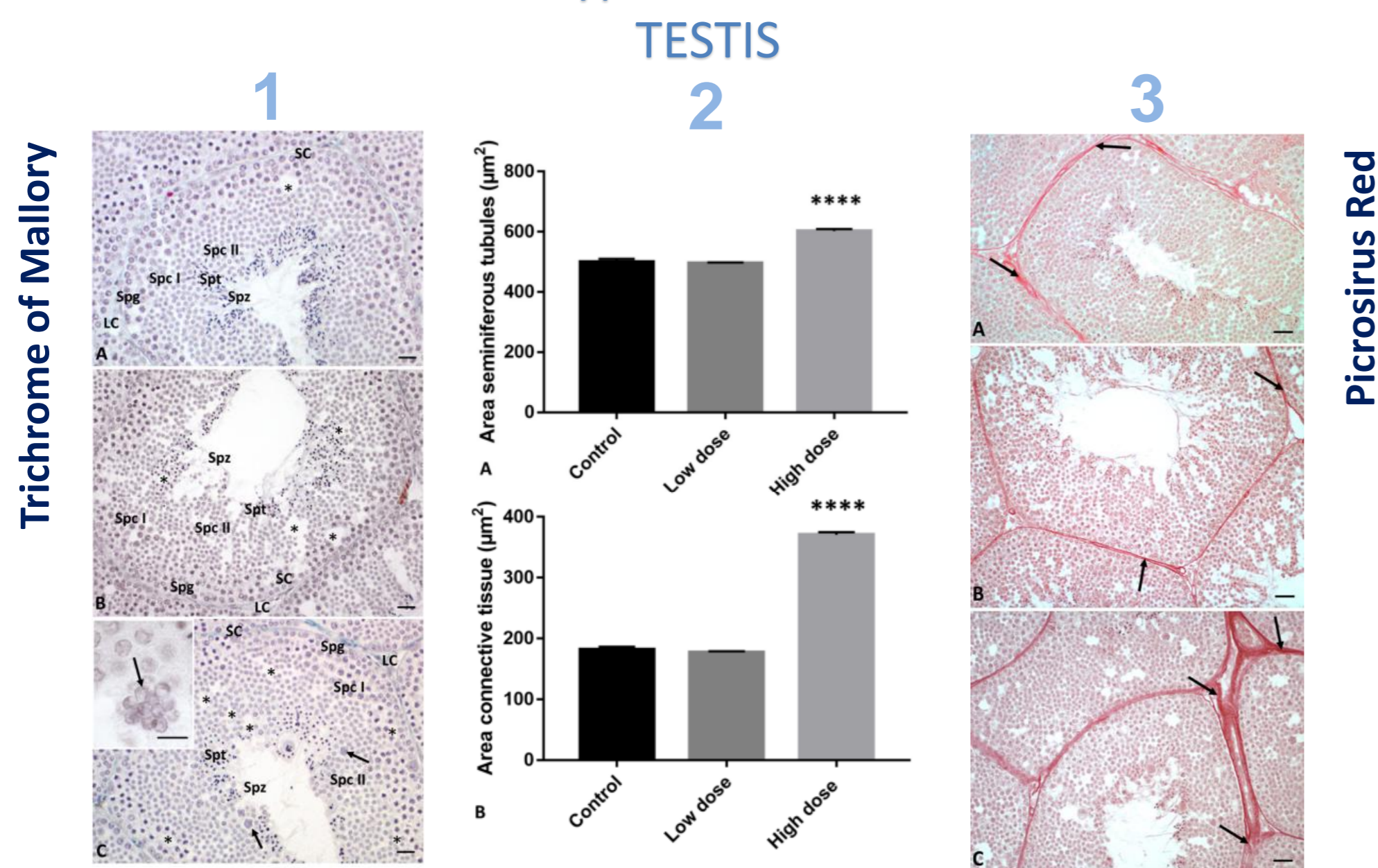
METHOD

The effects of glyphosate were evaluated in lizards orally exposed for 3 weeks to two herbicide concentrations (0.05 and 0.5 $\mu\text{g}/\text{kg}$). Control and treated gonadal tissues were examined using Mallory's trichrome and Picrosirius Red staining. In addition, immunohistochemical analyses were performed to assess the localization of Connexin 43 and Estrogen Receptors (ER α and ER β), while ER gene expression was investigated by Real-Time PCR.

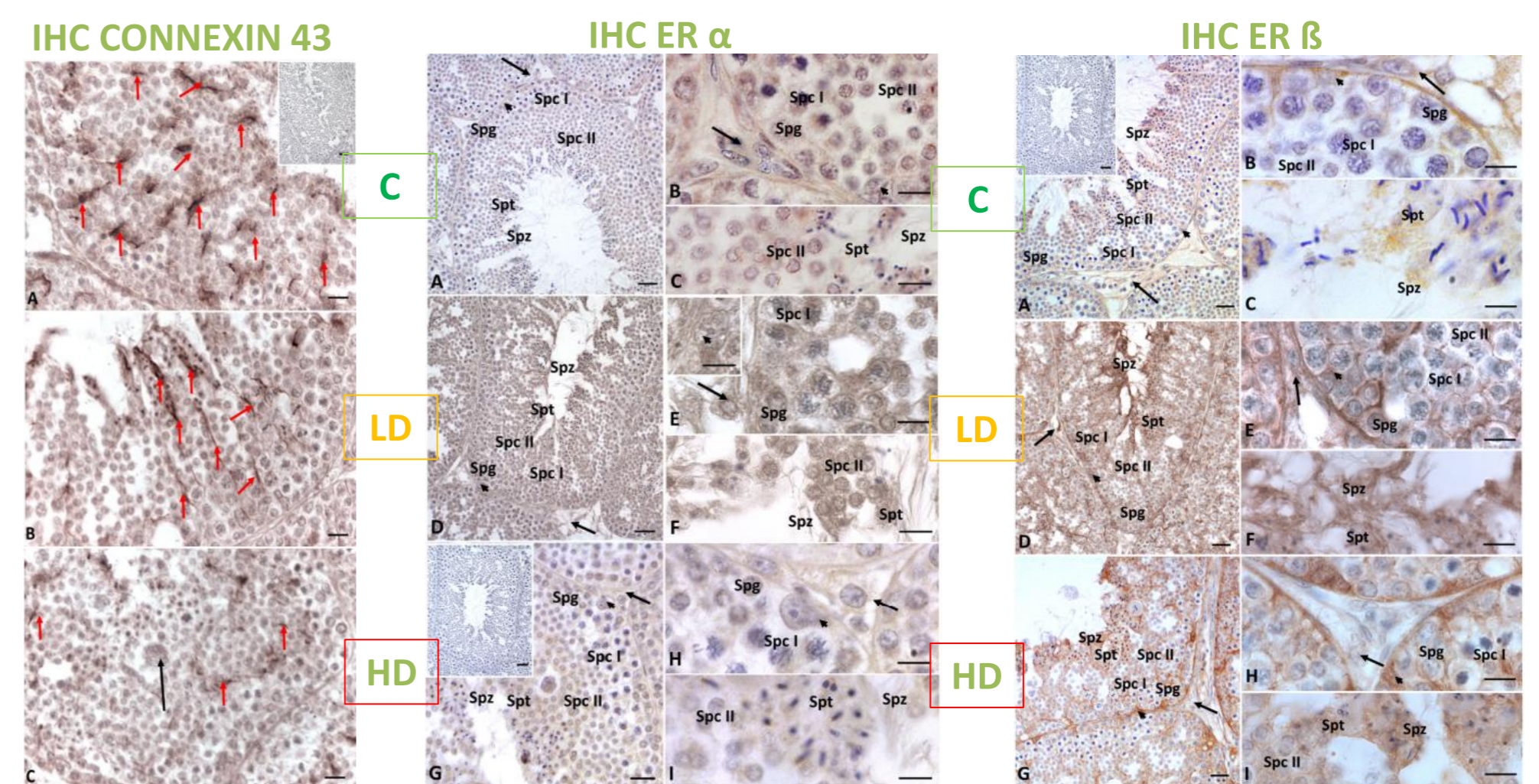


RESULTS & DISCUSSION

Results Glyphosate Treatments:

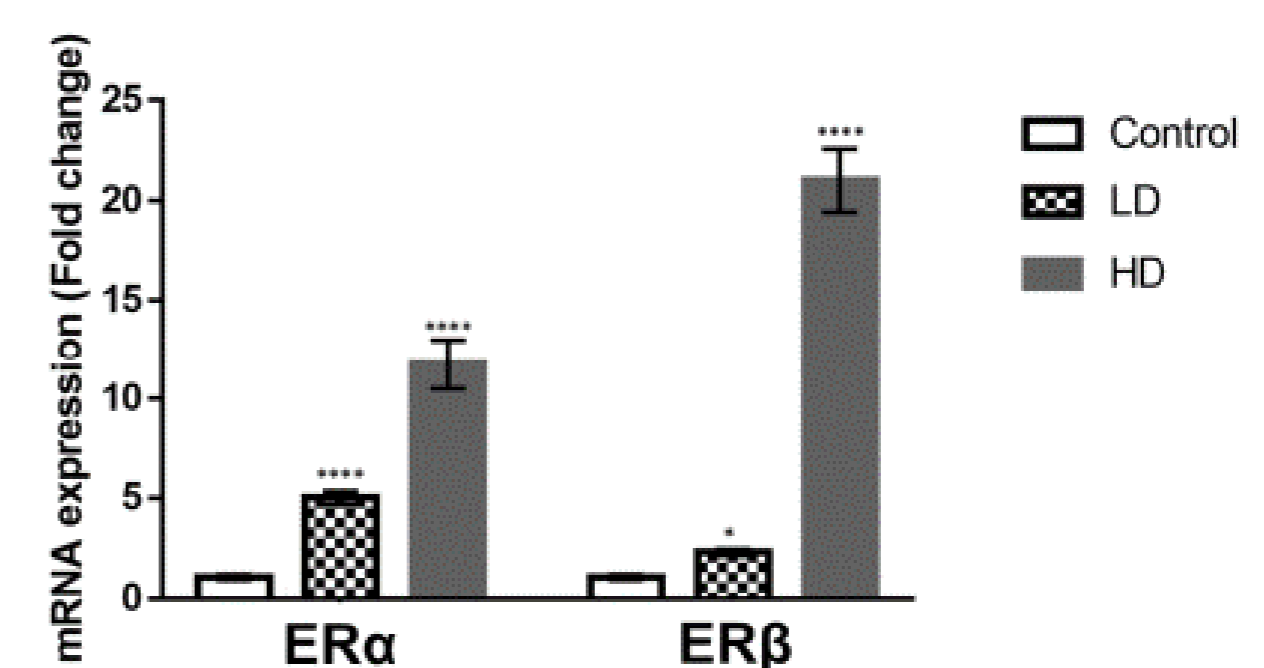


Trichrome staining showed impaired spermatogenesis in Gly-treated males. The low dose resulted in gaps in the germinal epithelium and few spermatozoa in the lumen (1B); the higher dose induced a further reduction in the number of spermatozoa, a greater number of lacunae and the aggregation of germ cells to form rosettes (1C). This morphological finding was confirmed by measuring the area of the seminiferous tubules (2). Picrosirius Red staining showed increased connective tissue and, with the higher dose, the presence of fibrosis in the peritubular space (3).



Immunohistochemical data showed that Gly-treatment, in a dose-dependent manner, reduced the distribution in the seminiferous tubules of Connexin 43, a connection protein between Sertoli cells and germ cells, whereas it did not affect the distribution of steroidogenic enzymes. Increased distribution of ERs, particularly of ER β , was also observed. This result was confirmed by real-time PCR analysis that showed a dose-dependent increase in the expression of both ERs, particularly for ER β .

REAL TIME PCR



CONCLUSION

The qualitative degradation of soil resulting from anthropogenic contamination represents a significant ecological pressure on terrestrial ecosystems, leading to a progressive loss of biodiversity and disrupting the functioning of trophic networks. The bioaccumulation of contaminants across different trophic levels may exert deleterious effects on terrestrial vertebrates, impairing their physiology, reproductive capacity, and developmental success. In this context, the evidence obtained in *Podarcis siculus* suggests that exposure to contaminated soils can significantly affect reproductive processes and organismal development, with potential long-term consequences for population viability and the persistence of species within natural ecosystems.

FUTURE WORK

- Transgenerational effects and epigenetics:** Investigate whether the reproductive damage induced by glyphosate is transmitted to subsequent generations (F1 and F2) through DNA methylation and other non-mutational epigenetic mechanisms.
- Co-exposure and synergistic effects:** Evaluate the real-world environmental impact by analyzing the "cocktail effect," specifically focusing on the co-exposure of glyphosate with other persistent soil contaminants, such as heavy metals (e.g., cadmium and lead).
- Model validation via transcriptomics:** Utilize advanced techniques like RNA-Seq on wild populations to definitively validate *Podarcis siculus* as a sensitive bioindicator for terrestrial vertebrate health and environmental monitoring.