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EXPOSIÇÃO AGUDA E CRÔNICA AO HERBICIDA À BASE DE GLIFOSATO EM ESPÉCIES GRILOS (ORTHOPTERA: GRYLLOIDEA): IMPLICAÇÕES SOBRE OS EFEITOS OXIDATIVOS A ABORDAGENS ECOLÓGICAS

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Dissertação apresentada ao Programa de Pós-graduação em Conservação e Manejo de Recursos Naturais, Mestrado, do Centro de Ciências Biológicas e da Saúde, da Universidade Estadual do Oeste do Paraná.

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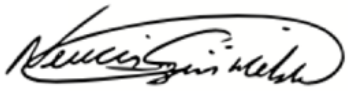

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Aos meus guerreiros pais Edaid e Ledonir, pelo amor que nunca envelhece e sabedoria que nunca acaba;

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“Água em escassez bem na nossa vez
Assim não resta nem ‘as barata’
Injustos fazem leis e o que resta ‘pr'ocês’?
Escolher qual veneno te mata”

(Passarinhos – Emicida)

SUMÁRIO

RESUMO

O Brasil é um importante produtor agrícola mundial e, para aumentar a produção, tem feito uso extensivo de herbicidas à base de glifosato (HBG), superando recordes de consumo e vendas. Este herbicida é reconhecido por seu potencial de causar danos ambientais ao contaminar ambientes aquáticos, terrestres e atmosféricos, afetando organismos não-alvo, ao promover a geração de compostos oxidantes que prejudicam os principais grupos biomoleculares. A quantificação da atividade de enzimas antioxidantes (GST, GR, GPx e CAT), enzimas colinérgicas (ChE) e lipoperoxidação (LPO) foram empregadas como indicadores para avaliar a os efeitos da exposição do HBG em insetos não-alvo de duas espécies da ordem Orthoptera: A espécie de grilo florestal *Eidmanacris meridionalis* pode ser considerada um biomarcador sensível, enquanto os grilos de campo *Gryllus* (*Gryllus*) *assimilis* podem representar biomarcadores tolerantes. A avaliação dos efeitos da exposição aguda às crescentes concentrações 0.00; 0.0135 0.054; 0.108; 0.216 e 0.864 $mg.L^{-1}$ de HBG, em espécies de grilos de diferentes habitats, demonstrou que concentrações elevadas resultaram em aumento da resposta de defesa antioxidante e sistema colinérgico em grilos florestais (*E. meridionalis*), demonstrando suscetibilidade à contaminação que pode causar uma possível redução da população. Em contraste, as defesas antioxidantes e o sistema colinérgico permaneceram estáveis em grilos de campo (*G.* (*Gryllus*) *assimilis*), indicando sua adaptabilidade em ambientes com aplicação de HBG. Além disso, ao avaliar os efeitos da exposição crônica de 0.864 $mg.L^{-1}$ de HBG, ao longo da ontogenia de *G*. (*Gryllus*) *assimilis*, os resultados revelaram alterações nos sistemas ao longo dos estágios de desenvolvimento. Particularmente, a atividade da CAT apresentou um aumento significativo durante a fase ninfal, a GST apresentou um aumento em resposta ao HBG, principalmente na fase adulta e durante a senescência, e houve um aumento considerável das enzimas ChE. A eficácia dessas enzimas no controle da peroxidação lipídica demonstra ainda mais a adaptabilidade dessa espécie à contaminação ambiental. Este estudo reforça a importância de estudar espécies diferentes, destacando que recomendações baseadas apenas em uma espécie dentro de um táxon específico podem não ser suficientes para garantir a conservação da biodiversidade. Essas descobertas enfatizam os efeitos de longo prazo da poluição agroquímica e a importância de práticas sustentáveis, regulamentações eficazes e métodos alternativos de controle de ervas daninhas.

PALAVRAS-CHAVE: Contaminação ambiental, herbicidas, ecotoxicologia, *Eidmanacris meridionalis, Gryllus* (*Gryllus*) *assimilis.*

ACUTE AND CHRONIC EXPOSURE TO GLYPHOSATE-BASED HERBICIDE IN CRICKET SPECIES (ORTHOPTERA: GRYLLOIDEA): IMPLICATIONS ON OXIDATIVE EFFECTS FOR ECOLOGICAL APPROACHES

ABSTRACT

Brazil is a significant global agricultural producer, and to enhance production, it has extensively utilized glyphosate-based herbicides (GBH), surpassing consumption and sales records. This herbicide is renowned for its potential to cause environmental damage by contaminating aquatic, terrestrial, and atmospheric environments, affecting non-target organisms and promoting the generation of oxidizing compounds that harm essential biomolecular groups. The quantification of antioxidant enzyme activity (GST, GR, GPx, and CAT), cholinergic enzymes (ChE), and lipid peroxidation (LPO) were employed as indicators to evaluate the effects of acute and chronic exposure to GBH in non-target insects of the Orthoptera order. Thus, the effects of acute and chronic exposure were evaluated using two cricket species: the forest crickets *Eidmanacris meridionalis* can be considerate a sensitive biomarker, while the field crickets *Gryllus* (*Gryllus*) *assimilis,* a tolerant biomarker. Assessing the effects of acute exposure to increasing concentrations of GBH (0.00; 0.01 0.05; 0.10; 0.21 e 0.86 $mg.L^{-1}$) in crickets from different habitats demonstrated that elevated concentrations resulted in an increased antioxidant defense response and cholinergic system inhibition in forest crickets, indicating their susceptibility to contamination, which may potentially lead to population reduction. In contrast, antioxidant defenses and the cholinergic system remained stable in field crickets, indicating their adaptability to environments with GBH application. Furthermore, evaluating the effects of chronic exposure to 0.864 $mg.L^{-1}$ of GBH throughout the ontogeny of *G*. (*Gryllus*) *assimilis* revealed alterations in the systems across developmental stages. Specifically, CAT activity exhibited a significant increase during the nymphal phase, while GST showed an increase in response to GBH, primarily during adulthood and during senescence, and there was a considerable increase in ChE enzymes. The efficacy of these enzymes in controlling lipid peroxidation further demonstrates the adaptability of this species to environmental contamination. This study underscores the importance of studying different species, highlighting that recommendations based solely on one species within a specific taxon may not be sufficient to ensure biodiversity conservation. These findings emphasize the long-term effects of agrochemical pollution and the significance of sustainable practices, effective regulations, and alternative weed control methods.

KEYWORDS: Environmental contamination, herbicides, ecotoxicology, *Eidmanacris meridionalis, Gryllus* (*Gryllus*) *assimilis.*

INTRODUÇÃO

O Brasil é uma das potências do setor agropecuário mundial (Zalles *et al.*, 2019), e estima-se que a área cultivada seja de 76,6 milhões de hectares para a safra 2022/23 (CONAB, 2022). Em consequência, o país tem liberado agrotóxicos considerados perigosos ao meio ambiente, para combater pragas e doenças associadas ao cultivo (ANVISA, 2020). Um dos ingredientes ativos mais comercializados no país é o Glifosato (IBAMA, 2023), utilizado como desfolhante e no controle de ervas daninhas (Cerdeira & Duke, 2006). Apesar de eficaz, o uso excessivo e indiscriminado provoca a contaminação do ar, solo e lençol freático (Moraes & Rossi, 2010), podendo causar alterações celulares, comportamentais e morfológicas em diferentes espécies animais, incluindo vertebrados terrestres (Benedetti *et al.*, 2004), peixes (Kreutz *et al.*, 2010), anfíbios (Gandhi & Cecala, 2016) e artrópodes, como crustáceos (Hansen & Roslev, 2016) aranhas (Benamú *et al.*, 2010), coleópteros (Michalková & Pekár, 2009) e abelhas (Battisti *et al.*, 2021).

Para avaliar o efeito de HBG nos organismos as enzimas do sistema antioxidante podem ser usadas como biomarcadores de defesa celular (Cotinguiba *et al.*, 2013). Algumas enzimas dependem de glutationa reduzida (GSH), uma molécula que age como um doador de elétrons, reduzindo outras moléculas oxidantes, desempenhando um papel importante na eliminação de peróxidos, hidroperóxidos e outras substâncias tóxicas para as células (Marí *et al.*, 2020). Ao oxidar a GSH em glutationa dissulfeto (GSSG), a enzima glutationa peroxidase (GPx) hidrolisa peróxido de hidrogênio (H_2O_2) formado por processos oxidativos danosos para as células, enquanto a glutationa redutase (GR) ajuda a regenerar a GSH a partir de GSSG, tornando novamente disponível. A glutationa s-transferase (GST) usa o GSH como substrato para desintoxicar as células e ajudar a reduzir ou eliminar xenobióticos (Huber *et al.*, 2008). Além das enzimas do ciclo das glutationas, a enzima catalase (CAT) decompõe o H_2O_2, em água e oxigênio, tornando-o eliminável pelo organismo (da Silva & Ferrari, 2011).

Outro método para avaliar a toxicidade em organismos não-alvos é mensurar a atividade do sistema colinérgico, que é responsável por funções básicas como o controle do movimento nos animais (Mesulam *et al.*, 2002). As enzimas colinesterases, incluindo acetilcolinesterase (AChE) e butirilcolinesterase (BChE), estão presentes nas fendas sinápticas e hidrolisam o neurotransmissor acetilcolina

(Araújo *et al.*, 2016), no entanto, a BChE também decompõe a butirilcolina e atua como um agente de detoxificação celular (Ha *et al.*, 2019). A exposição a diferentes tipos de substâncias tóxicas, tais como pesticidas organofosforados e carbamatos, podem inibir ou induzir a atividade dessas enzimas, tornando-as um biomarcador comum de contaminação ambiental (Sobjak *et al.*, 2017; Olisah & Adams, 2020).

Esses biomarcadores podem ser avaliados em diferentes organismos, tais como os grilos, que são importantes bioindicadores da qualidade de ambientes impactados, devido às suas características que os tornam sensíveis às mudanças no hábitat, apresentando múltiplas respostas regulatórias (Masaki, 1996). Esses animais podem ser usados para detectar poluentes químicos em sistemas terrestres (Yoshimura *et al.*, 2005), uma vez que algumas espécies são altamente sensíveis à intoxicação (Piechowicz & Grodzicki, 2014) enquanto outras, apresentam extrema tolerância à exposição (Cripps *et al.*, 1988).

Eidmanacris meridionalis Desutter-Grandcolas (1995) (Phalangopsidae) é um grilo potencial bioindicador encontrado na Mata Atlântica do sul do Brasil (Cigliano *et al.*, 2023), que habita ambientes úmidos protegidos e está ativo principalmente à noite (Desutter-Grandcolas, 1993). Devido ao seu corpo pouco quitinizado, pernas alongadas e asas reduzidas, esses insetos são sensíveis à dessecação e absorção cutânea de substâncias tóxicas (Kumar, 2000; Prado & Fontanetti, 2005). O uso de HBGs em matrizes agrícolas circundantes aos corpos d'agua e florestas úmidas pode resultar na degradação microbiana do herbicida em metabólitos altamente tóxicos (Lutri *et al.*, 2020), que permanecem nos tecidos das plantas (Botten *et al.*, 2021) podendo causar danos oxidativos e neurotóxicos nos animais destes locais através da alimentação (Lima *et al.*, 2020).

O grilo de campo *Gryllus* (*Gryllus*) *assimilis* (Fabricius, 1775) (Gryllidae) é amplamente distribuído na América, bem como em regiões da África, Europa e Ásia (Cigliano *et al.*, 2023). Eles habitam locais abertos e secos, com vegetação rasteira e pastagens com solos arenosos (Zoltán Kenyeres, 2006). Caracterizam-se por terem um corpo bastante quitinizado e asas longas, o que os torna menos sensíveis à dessecação (Masson *et al.*, 2020). Esta espécie pode ser considerada um bioindicador tolerante, sendo que vivem em ambientes antropizados e possuem uma adaptação extremamente plástica às condições ambientais locais (Harrison, 1979), além de um mecanismo imunológico bem desenvolvido com 492 genes que regulam a defesa celular (Hussain *et al.*, 2020). São reconhecidos mundialmente

como pragas agrícolas em várias culturas, especialmente durante a fase de germinação das plantas (Andaló *et al.*, 2018; Júnior *et al.*, 2019).

Animais de diferentes origens evolutivas apresentam respostas distintas na atividade do sistema de defesa e na tolerância aos xenobióticos (Rainio *et al.*, 2019) e avaliando espécies não-alvo de grilos florestais e de campo, buscamos contribuir com estudos de ecotoxicologia ao investigar os efeitos dos herbicidas à base de Glifosato. A proposta foi desvendar os mecanismos de defesa exercidos por essas espécies quando expostas ao composto e fechar lacunas sobre os malefícios do glifosato para o meio ambiente, fornecendo subsídios para estratégias de controle de pragas mais ecológicas.

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CHAPTER 1

Oxidative and neurotoxic effects of glyphosate-based herbicide exposure on two cricket species (Orthoptera: Grylloidea)

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Highlights

- The use of glyphosate-based herbicides may favor the resistance or disruption of crickets, depending on the population evolutionary origin.
- The forest and field cricket species, *Eidmanacris meridionalis* and *Gryllus* (*Gryllus*) *assimilis* respectively, are two populations that shows divergence in pesticide tolerance.
- When the forest specie lives near to a conventional agriculture it is more susceptible to antioxidant system changes and increase of cholinergic enzymes activity.
- The field specie shows physiologic homeostasis indicating an adaptive plasticity in contaminated environments.

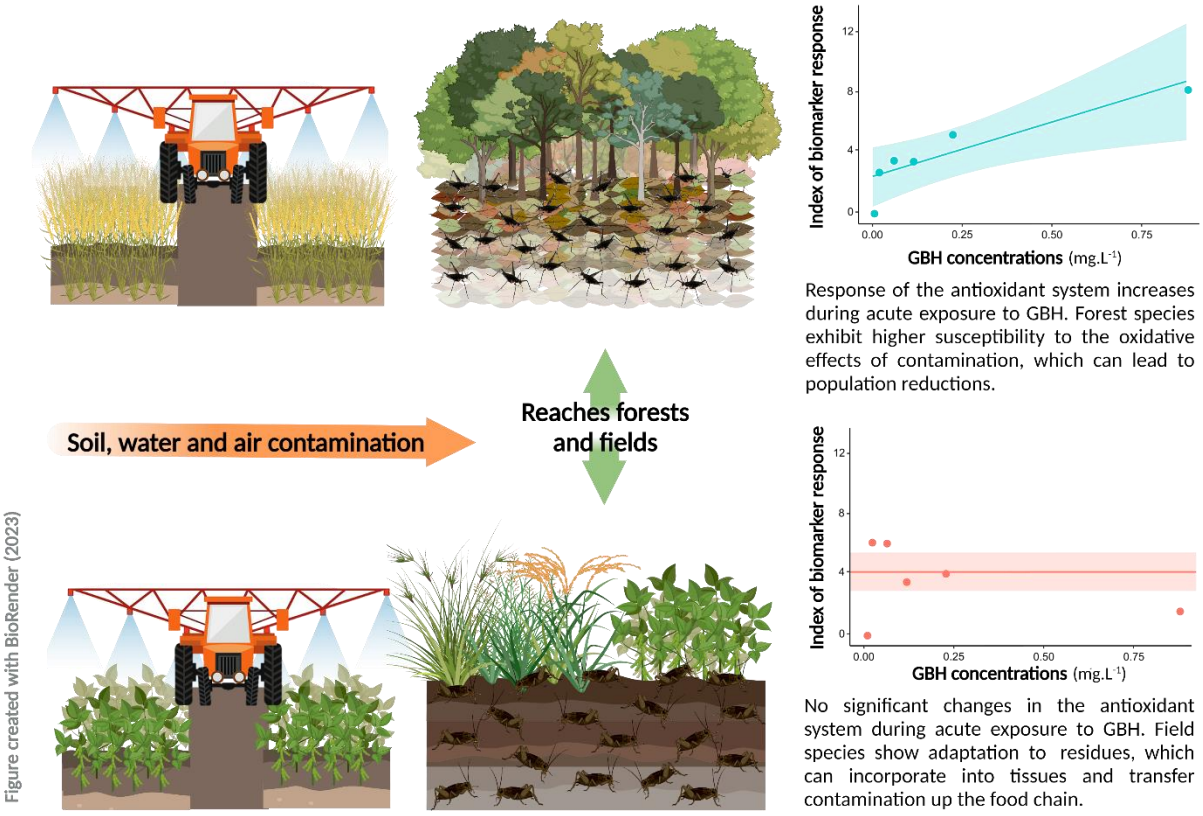
Abstract

Brazil has become a major agricultural producer and one of the largest consumers of pesticides in the world, including glyphosate-based herbicides (GBH), widely used in crops. This herbicide is known to cause environmental damage through the contamination of aquatic, terrestrial and atmospheric environments, reaching non-target organisms and favoring the generation of oxidizing compounds that cause damage to the main groups of biomolecules. Quantifications of antioxidant defense enzymes, lipid peroxidation and

cholinergic activity were used as indicators to assess GBH toxicity in non-target insects of the order Orthoptera. Evaluating the effects of chronic exposure to increasing concentrations of GBH in species of crickets from different habitats, showed that the increase in GBH concentrations would also lead to increases of the response of the antioxidant defenses and inhibition of the cholinergic system of forest crickets *E. meridionalis*. In contrast, the antioxidant defenses and the cholinergic system remained stable in the field cricket *G.* (*Gryllus*) *assimilis*. This work highlights the importance of studying species that are dissimilar and thus showing that recommendations based only on one species of each specific taxon may not be enough to secure biodiversity. The field cricket is a species that has been in contact with GHB most of their life through the direct application of this pesticide in their ecosystems.

Keywords: Toxicity, pesticides, *Eidmanacris meridionalis*, *Gryllus* (*Gryllus*) *assimilis*, environmental contamination

Graphical abstract



1. Introduction

In the past five decades, Brazil has emerged as a prominent agricultural producer and exporter, ranking among the global leaders in soybean, sugarcane, cotton, and corn production (Alves et al., 2008; Zalles et al., 2019). To maintain high levels of production and marketing in the country, glyphosate-based herbicides (GBH) are extensively used to

control weeds in various cultivars, particularly in genetically modified crops that are resistant to these products (Tauhata et al., 2020). In 2021, a total of 720.87 thousand tons of active pesticide ingredients were marketed, with 219.58 thousand tons of Glyphosate (IBAMA, 2023).

One of the issues associated with the continuous and excessive use of glyphosate-based herbicides (GBH) is their detrimental impact on non-target organisms. Exposure to GBH can induce the generation of reactive oxygen species (ROS), including superoxide ion ($O2^{\bullet}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$) (Cogo et al., 2009; Rondón-Barragán et al., 2012), thereby increasing the toxicity to animal cells (Cattani et al., 2014) and promoting oxidative stress (Su et al., 2019). This oxidative stress condition leads to impaired ion exchange selectivity, release of organelle hydrolytic content, and the formation of cytotoxic by-products, ultimately resulting in cell death (Ghezzi, 2020).

To assess the possible impact of GBH in organisms, different biomarkers have already been used (Cotinguiba et al., 2013; Khare et al., 2019; Marí et al., 2020; Sanchez-Hernandez, 2011). Commonly used biomarkers include the enzyme catalase (CAT) that dismutes H_2O_2, formed by oxidative processes, into water and oxygen (da Silva and Ferrari, 2011). In the antioxidant system, the glutathione cycle, whose enzymes are dependent on reduced glutathione (GSH), have an important action in detoxifying peroxides, hydroperoxides and other xenobiotics (Marí et al., 2020). Glutathione peroxidase (GPx) promotes the dismutation of H_2O_2 by oxidizing GSH to glutathione disulfide (GSSG), while glutathione reductase (GR) catalyzes the regeneration of GSH from GSSG. As for the glutathione s-transferases (GST) use GSH as a substrate to detoxify cells, promoting the decrease or elimination of xenobiotics (Huber et al., 2008). Exposure to glyphosate-based herbicides can lead to an imbalance between the activity of the antioxidant system and the level of reactive oxygen species (ROS), resulting in oxidative stress, as reported in various animals such as rats, fish, gastropods, crustaceans, and human cells (Benedetti et al., 2004; Demirci et al., 2020; Gasnier et al., 2009; Hansen and Roslev, 2016; Langiano and Martinez, 2008). Another method of assessing toxicity is the activity of the cholinergic system, which is responsible for essential functions such as cortical organization of movement in organisms (Mesulam et al., 2002). Cholinesterase enzymes (e.g., acethylcholinesterase – AChE, propionylcholinesterase – PChE and butyrylcholinesterase – BChE) are present in the synaptic clefts and hydrolyze the neurotransmitter acetylcholine, allowing the neuron to return to its resting state (Araújo et al., 2016). Cholinesterases is often used as a biomarker of environmental exposure, generally evaluated in bioindicator species affected by

carbamates (Gambi et al., 2007) and organophosphates (Olisah and Adams, 2020). There are studies with non-target species that, when exposed to GBH, have ChE induction as a response (Gard and Hooper, 1993), while others show inhibition (Sobjak et al., 2017).

Crickets are susceptible to changes in their habitats (Masaki, 1996), and therefore are considered important indicators of environmental quality, especially in impacted areas (Wink et al., 2005). These organisms have been used to assess the effects of exposure to radioactive substances (Gustavino et al., 2014), quantify bioaccumulation by metals (Pyatt et al., 2002), detect sulfur emissions (Hoffmann et al., 2002) and determine the toxic effects of cadmium (Lijun et al., 2005). This indicates that some Grylloidea species are highly sensitive to damage (Piechowicz and Grodzicki, 2014), while others have good survival capacity against contamination according to physiological needs and developmental stages (Cripps et al., 1988). Apart from the mentioned examples, the species *Eidmanacris meridionalis*, Desutter-Grandcolas (1995) (Phalangopsidae) e *Gryllus* (*Gryllus*) *assimilis* (Fabricius, 1775) (Gryllidae) can be considerate good bioindicators. The first species is a Neotropical cricket found in conserved areas of the Atlantic Forest in southern Brazil (et al., 2023). These crickets exhibit a straminicolous habitat preference, thriving in protected and humid environments due to their lower levels of chitin in their bodies and elongated legs. Their reduced wings makes them susceptible to desiccation (Prado and Fontanetti, 2005), prompting them to seek shelter in small natural cavities during the day and exhibit activity exclusively at night (de Campos et al., 2021). Consequently, they can be considered a species with heightened sensitivity to xenobiotics. On the other hand, the field crickets *Gryllus* (*Gryllus*) *assimilis*, which is considered a pest of cultivars such as soybeans and corn (Andaló et al., 2018; Masson et al., 2020; Ribeiro et al., 2022). This species occurs widely in the American continent from Canada to Argentina and regions of Africa, Europe, and Asia (Cigliano et al., 2023). In addition to attacking the plantations, they inhabit open and drier places with low vegetation, heaths, and pastures with sandy soils (Zoltán Kenyeres, 2006). These species of crickets have a more chitinized bodies and long wings, therefore, it has characteristics and high population abundance, which suggest that it is more tolerant and resistant to exposure to chemical elements such as pesticides.

Understanding the effects of exposure to GBH in non-target species such as orthopterans, which have different degrees of sensitivity to environmental impacts, is a way to explore new knowledge regarding the ecotoxicological damage caused by the wide application of this pesticide in rural areas close to conservation units. In this work, we

evaluated the effects of acute exposure to different concentrations of GBH in *E. meridionalis* and *G.* (*Gryllus*) *assimilis*.

2. Material and methods

2.1 Obtaining and maintaining specimens

Adults of *E. meridionalis* were collected in the Iguaçu National Park, Foz do Iguaçu municipality, Paraná state, on the Macuco Safari trail (25°39'04"S; 54°26'17"W) and Cataratas trail (25°41'04"S; 54° 26'23"O), through active nocturnal collections, in October 2021. The fertilized females were kept individually in a transparent ventilated container (13 cm high x 13 cm diameter) and deposited their eggs in the bottoms of plastic cups filled with moistened cotton. The eggs were relocated to another container of the same size and kept moist until hatching, allowing the obtention of nymphs of this species. The nymphs of *G.* (*Gryllus*) *assimilis* (two weeks old) were purchased in a specialized commercial establishment in a single batch.

The nymphs of both species were placed separately in transparent and ventilated plastic boxes (32 cm high x 48 cm long x 30 cm wide) and kept in a climate-controlled room at the Laboratório de Orthoptera of the Universidade Estadual do Oeste do Paraná (UNIOESTE), Cascavel *campus*, with temperature between 24 ± 2°C, with a humidity of 57 ± 7% and a 12h light/dark photoperiod. Water was regularly provided to the cotton-filled containers, to maintain humidity levels, and food (fish and chicken feed) was offered *ad libitum*. Crickets were kept under these conditions for 15 days for acclimatization purposes until the beginning of the experiments.

2.2 Experimental design

A total of 90 nymphs of each species were used for the experiments. These organisms were divided into transparent rectangular boxes (35 cm x 25 cm x 10 cm), with six boxes for each species and 15 nymphs per box. The nymphs were kept under the same conditions of temperature, humidity, photoperiod and feeding described previously. Each box was considered as a GBH treatment (Figure 1).

The GBH (Gli-Up ® 72 WG) was purchased from a certified commercial establishment. The commercial formula was diluted into a stock solution of 0.1 $g.L^{-1}$ of GBH. From this stock solution, a geometric progression was established with the following dilutions for the treatments: T1: control (water without contaminants), T2: 0.0135 $mg.L^{-1}$,

T3: 0.054 mg.L^{-1}, T4: 0.108 mg.L^{-1}, T5: 0.216 mg.L^{-1}, and T6: 0.864 mg.L^{-1} of GBH. The midpoint equivalent to 0.108 mg L^{-1} of GBH was established by Pereira et al. (2018), who described the impact of transgenic soy and GBH on the soil arthropod community. In each box, cotton wool was soaked with 25 mL of each concentration of GBH, and these solutions were offered for 96 hours. Therefore, exposure was performed by ingestion of water (Figure 1).

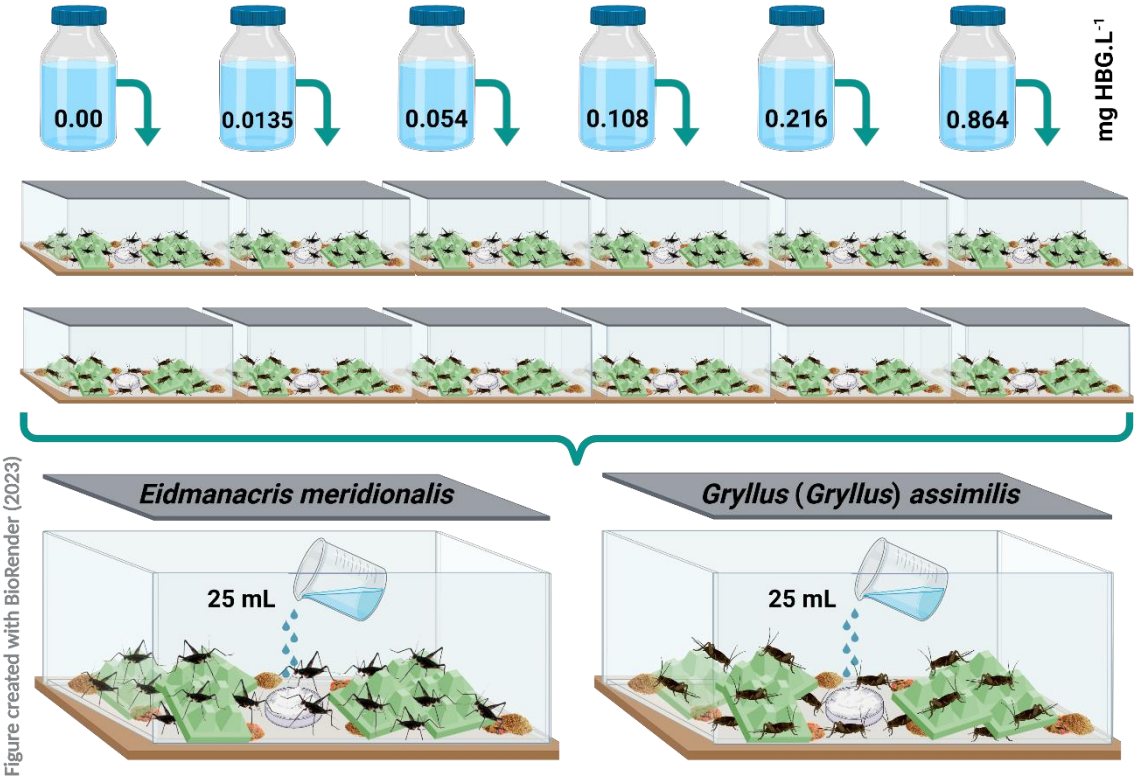


Figure 1. *Eidmanacris meridionalis* (n = 90) and *Gryllus* (*Gryllus*) *assimilis* (n = 90) received 25 mL of each concentration of GBH (n = 15): 0; 0.0135; 0.054; 0.108; 0.216 and 0.864 mg.L^{-1}. Treatments were evaluated every five hours.

Mortality was recorded every five hours, and during the experiment, with the dead animals being removed from the boxes. At the end of the experiment, the surviving individuals were euthanized by freezing and kept at -80ºC for further analysis.

2.3 Laboratory analysis

The euthanized crickets were weighed on an analytical scale and placed in a microtube, homogenized in a 50 mM tris-HCl buffer, pH 7.4, and centrifuged at 10.600 g for 10 min at 4ºC. Protein quantification of the samples was determined by the Bradford

(1976) method in triplicate, using bovine serum albumin as a standard. The samples were normalized to 1 mg protein.mL^{-1} and, therefore, each mass added per microtube represented a sampling unit in the remainder of the experiment.

2.3.1 Analysis of the antioxidant defense system

Catalase activity (CAT) was accompanied by a decrease in absorbance at 240 nm (Aebi, 1984), based on the principle of peroxide dismutation, whose molar extinction coefficient is 40 M^{-1}.cm^{-1}. The triplicates were pipetted with 200 µL of solution in a quartz microplate with 96 wells at 25°C, and the results of the catalase enzyme activity were expressed in mmol.min^{-1}.mg of protein^{-1}.

The activity of glutathione peroxidase (GPx) was evaluated according to the technique of Flohé and Günzler (1984). In triplicate, enzymatic activity was accompanied by a decrease in NADPH absorbance at 340 nm. The reaction system was composed of phosphate buffer 100 mmol.L^{-1} (pH 7.0) EDTA 1 mmol.L^{-1}, GSH 2 mmol.L^{-1}, NADPH 0.15 mmol.L^{-1}, purified glutationa reductase 0.2 U, t-butyl hydroperoxide 0.5 mmol.L^{-1} and 50 µg prot.mL^{-1} of the sample. The reaction was initiated by adding t-butyl hydroperoxide and monitored for one minute. The unit was expressed in nmol.min^{-1}.mg of protein^{-1}.

The glutathione reductase (GR) was evaluated through the reduction of glutathione disulfide (GSSG), whose absorbance decrease is measured at 340 nm (Sies et al., 1979). The assay was performed in triplicate in a microplate with 20 µL of sample and 140 µL of reaction medium (0.138 mM NADPH, 3.81 mM GSSG and 3.75 mM EDTA). The reaction was set up at 22°C, the NADPH molar extinction coefficient was 6.22 mM^{-1}.cm^{-1}, and the unit was expressed in nmol.min^{-1}.mg of protein^{-1}.

The enzyme glutathione *s*-transferase (GST) catalyze the conjugation of reduced GSH with the synthetic substrate CDNB, producing a conjugate detected at 340 nm (Habig et al., 1976), in which the enzymatic activity is proportional to the rate of production of the conjugated compound. The assay was performed in triplicate, in a microplate, and the reaction medium had 0.94 mM of CDNB and 0.94 mM of GSH and 20 µL of biological material. The reading was done at 22°C, the molar extinction coefficient of the GSH/CDNB conjugate was 9.6 mM^{-1}.cm$^{-1,}$ and the unit was expressed in nmol.min^{-1}.mg of protein^{-1}.

2.3.2 Oxidative stress analysis

The determination of the lipid peroxidation (LPO) reaction was carried out to indirectly quantify the peroxides, thus reflecting the intensity of lipid peroxidation. The

TBARS method (Buege and Aust, 1978) was performed, by comparing absorbance and the standard curve of Malondialdehyde (MDA), the main by-product of cellular lipid peroxidation. The reaction was read at 22°C, after 60 minutes of incubation at 60°C, at an absorbance of 535 nm. The results of lipid peroxidation were expressed in □mol of MDA.mg of protein^{-1}.

2.3.3 Cholinesterase activity

The analysis of the cholinesterase (ChE) activity was performed using the method described by Ellman et al. (1961), modified for microplate (de Assis, 1998), whose principle is the measurement of thiocholine production when acetylcholine is hydrolyzed. The reaction was performed in triplicate, in 300 µL of a solution containing 0.05 mM 5,5'-Dithiobis-2-nitrobenzoic acid (DTNB) and 1.5 mM acetylcholine (ATC). ChE activity was calculated in relation to protein concentration (mg.mL^{-1}), using the molar extinction coefficient of DTNB (1.36 mM^{-1}.cm^{-1}). Protein quantification was calculated from the crude sample, and reading was performed at 22°C. The results were expressed in nmol.min^{-1} .mg protein^{-1}.

2.4 Statistical analyzes

The biomarker variables were analyzed using polynomial linear model, with Gaussian distribution. In the construction of the models, the response variables of the scatter plots were the values of the biomarkers (CAT, GPx, GR, GST, LPO and ChE), the predictor variable was represented by the concentrations of the glyphosate-based herbicide (0, 0.0135, 0.054, 0.108, 0.216, 0.864 mg.L^{-1}).

From the biomarkers' variables, the Integrated Biomarker Response Index - IBR, second version (Beliaeff and Burgeot, 2002; Sanchez et al., 2013), was calculated from the ratio between the individual values of the biomarkers (X_i) and the average of the values of the control group (X_0), followed by logarithmization: $Y_i = \log (X_i/X_0)$. The means (μ) and standard deviations (σ) of Y_i were calculated, and the values were standardized in the formula: $Z_i = (Y_i - \mu) / \sigma$. To create a baseline centered at 0 and represent biomarker variation according to this line, the mean Z_i of treatments was subtracted from the mean of the reference biomarker data (Z_0), and the value was considered a deviation index (A), which determined the response value of each biomarker: $A = Z_i – Z_0$. The significant data from (A) were represented in radar graphs, indicating the deviation of the investigated biomarker in

relation to the control for each concentration of GBH. The controls were considered as the central reference, so that positive values in relation to the reference indicate biomarker induction and negative values indicate inhibition. The sum of the values of (A) results in the IBR that was, analyzed regarding the relationship with the concentrations of GBH and the species through a generalized linear model (GLM), with Poisson distribution and z statistics and represented in a graph of dispersion. All analyzes were performed in the R program, version 4.2 (R Core Team, 2022).

3. Results

During exposure to the GBH treatments, four nymphs of *E. meridionalis* (one in 0, 0.0135, 0.054, 0.216 mg.L) and two of *G.* (*Gryllus*) *assimilis* died (one in 0.216 and other in 0.864 $mg.L^{-1}$), representing less than 10% of mortality.

3.1 Antioxidant defense system

For *Eidmanacris meridionalis*, the catalase activity (CAT) was higher for the groups exposed to GBH ($F_{2,33} = 53.41$; $p < 0.0001$), with significant inductions of enzymatic activity at concentrations (T2: A = -0.144; T3: *A* = 0.753; T4: *A* = 1.063; T5: *A* = 1.430 e T6: *A* = 1.674). As for *G.* (*Gryllus*) *assimilis*, CAT activity was not significantly affected by increasing GBH concentrations ($F_{2,43} = 1.48$; $p = 0.239$) (Figure 2). The enzyme glutathione peroxidase (GPx) activity was inhibited by the increase in GBH concentrations in *E. meridionalis* ($F_{2,32} = 3.648$; $p = 0.0374$) and *G.* (*Gryllus*) *assimilis* ($F_{2,41} = 4{,}756$; $p = 0.01389$). *Gryllus*, however, presents a slight induction in the first two concentrations followed by inhibition in the others (T2: A = 0.715; T3: A = 1.084) (Figure 2).

Glutathione reductase (GR) activity was lower in *E. meridionalis* ($F_{1,30} = 3{,}987$; $p = 0.05498$) with increasing GBH concentrations, corroborating with the high induction at 0.0135 mg $GBH.L^{-1}$ (A = 0.477) and subsequent reduction to 0.864 mg $GBH.L^{-1}$ (A = -0.659). For *G.* (*Gryllus*) *assimilis*, GR activity did not change significantly ($F_{1,44} = 0{,}755$; $p = 0.3896$) (Figure 2). The glutathione s-transferase (GST) activity was lower with increasing GBH concentrations in both *E. meridionalis* ($F_{2,33} = 4{,}407$; $p = 0.02012$) and *G.* (*Gryllus*) *assimilis* ($F_{1,44} = 4{,}016$; $p = 0.05$). For both species, there was progressive inhibition of GST activity throughout the treatments (Figure 2).

3.2 Oxidative stress and Cholinesterase activity

Increased concentrations of GBH did not influence the Lipid peroxidation rate (LPO) in *E. meridionalis:* ($F_{1,26}$ = 0,5782; p = 0.4538) and *G.* (*Gryllus*) *assimilis* ($F_{1,44}$ = 0.723; *p* = 0.3998) (Figure 3). The cholinesterase (ChE) enzyme was higher in *E. meridionalis* ($F_{2,14}$ = 8.858; p = 0.01417) with induction, especially at the concentrations 0.054; 0.108; 0.216, and 0.864 mg/L^{-1} (A = 0.610; 0.724; 1.061; 1.142). The ChE was not influenced by GBH concentrations in *G.* (*Gryllus*) *assimilis* ($F_{2,43}$ = 0,5613; p = 0.5746) (Figure 3).

3.3 Integrated Antioxidant System Biomarker Response

Antioxidant system enzymes considered significant in the statistical analyzes were included in the Integrated Biomarker Response assessment. In this analysis, GBH concentrations promoted a significant effect on IBR (*z* = 2.935; *p* = 0.0033), as well as regarding species (*z* = 2.062; p = 0.0392) and the interaction of factors (*z* = -2.524; p = 0.0116). For *Eidmanacris meridionalis* the IBR increased with increasing concentrations of GBH, while for *G.* (*Gryllus*) *assimilis* the IBR remained unchanged (Figure 4).

4. Discussion

Ecotoxicological studies with low concentrations and traces of contaminants are important, since, in the long term, these contaminants promote alterations in antioxidant enzymes (Villatte and Bachmann, 2002), resulting in alterations throughout non-target species generations due to negative effects on physiology and inducing phenotypic differentiation (Hu et al., 2021). Considering the uncertainties surrounding the effects of GBH on invertebrates, this investigation aimed to evaluate the effects of acute exposure to varying concentrations of GBH on two different species of orthopterans.

In *E. meridionalis*, the enzymatic activity of catalase (CAT) was glyphosate-dependent concentration. This suggests that GBH can increase the production of hydrogen peroxide (H_2O_2), inducing an increase in CAT, an enzyme of the antioxidant system has protective activity against oxidative damage in this species. Increased CAT was also observed in the silkworm *Bombyx mori* and the fruit fly *Drosophila* melanogaster during acute exposure to GBH 2975 mg.L and 5 mg.L, respectively (de Aguiar et al., 2016; Feng et al., 2022). Other pesticides can also increase CAT activity, as observed in gonads of the grasshopper *Spathosternum prasiniferum* exposed to the insecticide Azadiractina (Manna et

al., 2020) Oxya chinensis exposed to organophosphate insecticides Phoxim, malathion and chlorpyrifos (Wu et al., 2011a; Wu et al., 2011b).

Catalase and glutathione peroxidase have complementary roles in hydrogen peroxide detoxication, in which catalase is more important at high fluxes of H_2O_2, whereas GPx deals with lower 'physiological' levels of H_2O_2 generation (Halliwell and Gutteridge, 2015). *E. meridionalis* showed an activity reduction of glutathione peroxidase (GPx) throughout GBH treatments. This inhibition also can be observed *L. acuta* polychaete exposed to 5.35 mg.L for 96 h (Tarouco et al., 2017).

Another reason for the low GPx activity could be related to the low GR activity on *E. meridionalis*, which is responsible for reducing GSSG to GSH. Reductions in GR were also observed in other animals treated with GBH, such as in *Melanopsis praemorsa* snails for acute exposure with 4.65 mg.L (Demirci et al., 2020), *Bulinus truncatus* snails after two weeks of exposure (Bakry et al., 2015). The inhibition of GR activity could be due to the change in the availability of NADPH in the cell (Wu et al., 2011a), which may be used by the CAT (Halliwell and Gutteridge, 2015) or due the decrease in G6PDH activity (Xu et al., 2017).

The enzymatic activity of glutathione s-transferase (GST) in *E. meridionalis* and *G. (Gryllus) assimilis* decreased significantly in the treatments with higher concentration of GBH, indicating that there was consumption of the initially available GSH followed by an inability to maintain an adequate GSH level or due to GR enzyme inhibition. This means that GBH can alter the enzymatic activities of the glutathione cycle, causing failures in the process of biotransformation and elimination of toxic compounds, as observed in the fish *Anabas testudineus* and *Heteropneustes fossilis* as well (Samanta et al., 2014).

Regarding the oxidative state, the homogeneity of Lipid peroxidation (LPO) between treatments of *E. meridionalis* may be related to the action of the Catalase enzyme of the antioxidant system on the reactive oxygen species (ROS) produced by low concentrations of GBH. This effect was observed in the fly *Hermetia illucens*, which showed an increase in CAT and decrease LPO when exposed to increasing concentrations of the organophosphate Malathion (Abdelfattah and El-Bassiony, 2022).

In *G. (Gryllus) assimilis*, the absence of LPO reaction together with the inactivity of response of the CAT, GPx, GR enzymes, and reduction of GST activity, suggest that the exposed concentration did not alter the antioxidant system. Nevertheless, the short-term exposure considered in this study does not allow us to definitively exclude potential long-term effects on survival.

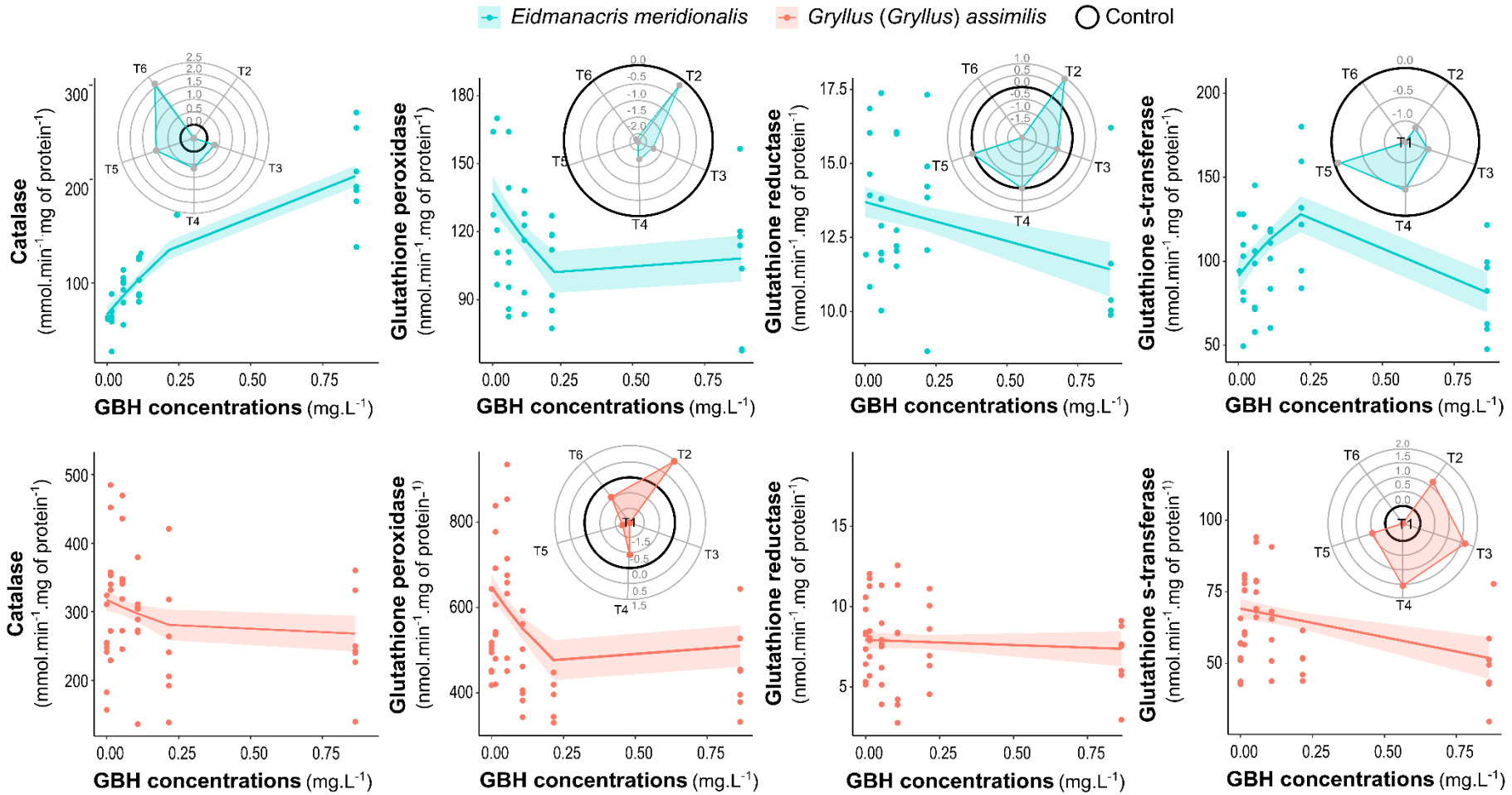


Fig. 3. Activity of antioxidant system enzymes (CAT, GPx, GR and GST) of *Eidmanacris meridionalis* and *Gryllus* (*Gryllus*) *assimilis* when exposed to GBH. Radar graph were incorporated into the graphs when there was statistical significance.

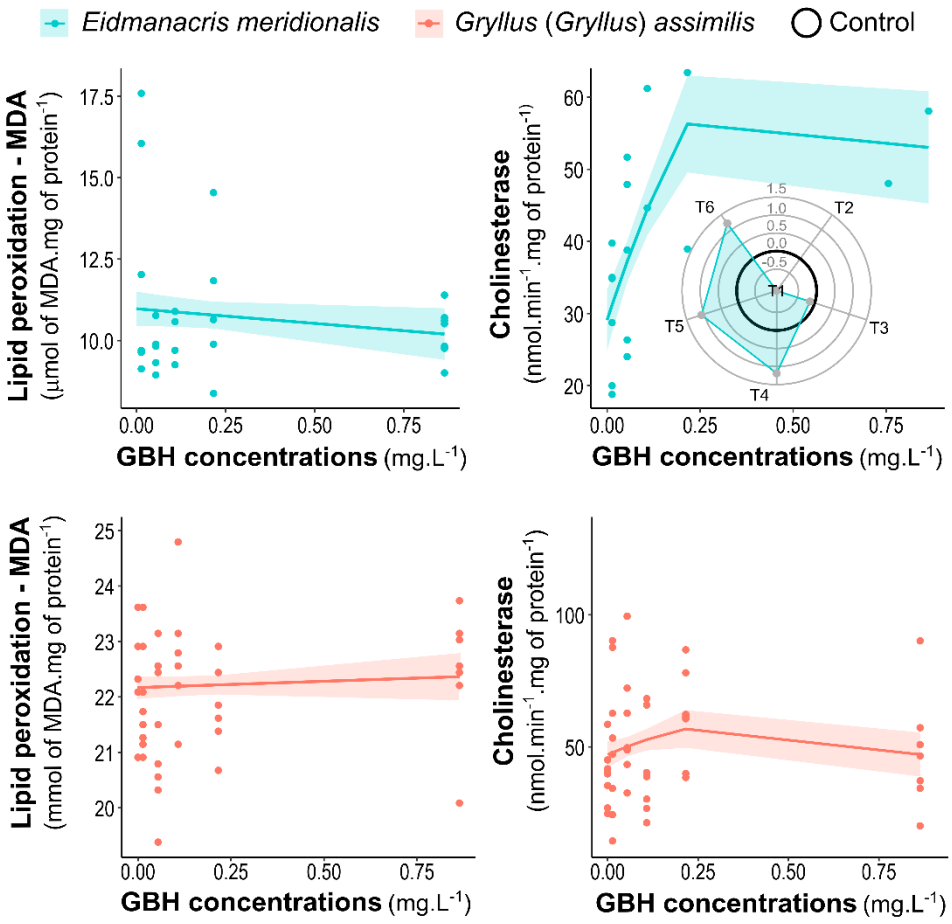


Figure 3. Effect of increased GBH concentrations on lipid peroxidation rates (LPO) and cholinesterase activity (ChE) of *Eidmanacris meridionalis* and *G.* (*Gryllus*) *assimilis*. Radar graph were incorporated into the graphs when there was statistical significance.

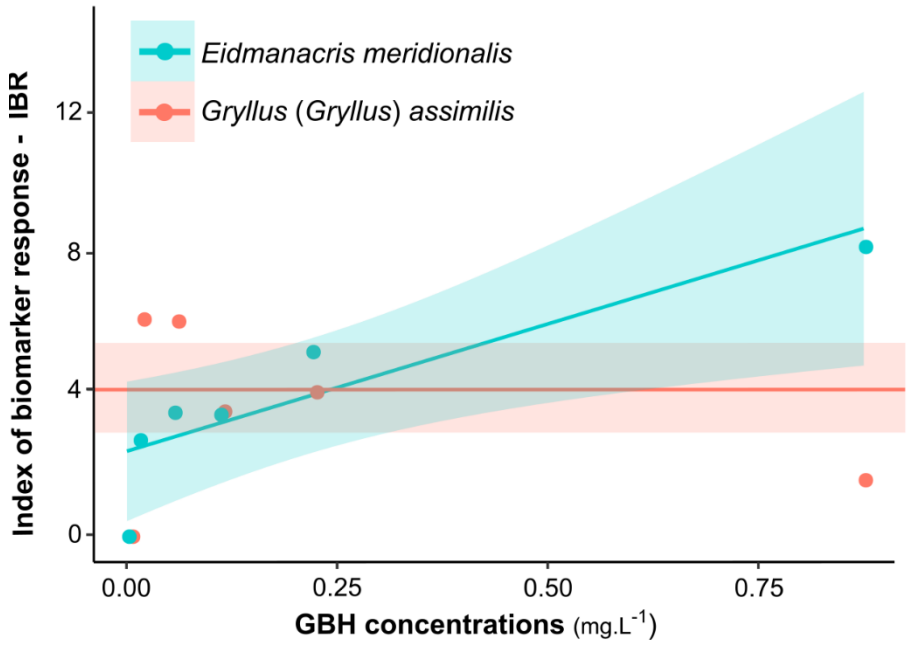


Figure 5. Integrated Biomarker Response for different GBH treatments.

With regard to neurotoxicity in arthropods, genetic polymorphisms in cholinesterases (ChE) have been regularly reported and experiments suggest the presence of different forms of ChE in several species of invertebrates (Villatte and Bachmann, 2002). Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) enzymes are forms of ChE that hydrolyze choline esters, with AChE being more specific in the hydrolysis of Acetylcholine (ACh) in cholinergic synapses and neuromuscular junctions (Stepankova and Komers, 2008), while BChE presents a wide scope of substrates and hydrolytic effects (Ha et al., 2019).

The induction of *E. meridionalis* ChE activity at higher concentrations of GBH may be related to the catalytic properties of butyrylcholinesterase, generally more activated in synthetic substrates (Stepankova and Komers, 2008), including pesticides (Raveh et al., 1993). The increased activity of BChE as a form of cellular detoxification may explain the reduction of GST for this species. In *G.* (*Gryllus*) *assimilis*, the constant activity of ChE corroborates its physiological maintenance of homeostasis even in contaminated environments. It is suggested that this fact is an adaptive process, as reported for *Drosophila melanogaster*, in which changes in amino acids in the ChE gene altered the sensitivity of the enzyme when the animals were exposed to organophosphates and carbamates, which made it possible to increase its rate of survival (Villatte and Bachmann, 2002). Upon analyzing the biomarker datasets, it is evident that the concentrations employed to assess *E.meridionalis* and *G.* (*Gryllus*) *assimilis*, which have shown sublethal effects, are 5 to 3443 times lower than those reported in the studies discussed herein.

The physiological reaction is an integrated response of numerous metabolic reactions, being a latent variable for the Integrated Biomarker Response Index (Sanchez et al., 2013). This study utilized the IBR index to integrate the responses of six selected biomarkers (CAT, ChE, GPx, GR, GST, and LPO) for evaluating the toxicity of glyphosate in crickets. The results showed an increase in the IBR index indicating the dose-dependent induction of *E. meridionalis* antioxidant activity during exposure. These findings are consistent with those on the shrimp *Macrobrachium nipponensis* (Hong et al., 2018) and *M. potiuna* (Melo et al., 2019) exposed to GBH, both showed concentration-dependent responses for each biomarker. On the other hand, the IBR value in G. (*Gryllus*) *assimilis* did not show enzymatic activity of the antioxidant defense system, as well as the absence of lipid peroxidation and unchanged cholinergic system. The species seems adapted to environments with application of GBH, attesting that interaction between its effective niche and natural selection allowed the guarantee of remaining in a contaminated environment, without

significant metabolic alterations that lead to loss of health. The transcriptome of insects of the genus *Gryllus* provides evidence for their survival through a well-developed immune mechanism with 492 genes that regulate cellular defense, including recognition receptors, signal modulators, signal transducers and effectors, which contribute to increased resistance of the population to invaders (Hussain et al., 2020).

The changes observed in the antioxidant defense system promote metabolic changes in species exposed to xenobiotics. Thus, some morphological traits of *E. meridionalis* make the species a bioindicator susceptible to poisoning, as the less chitinized body can facilitate the cutaneous absorption of toxic substances, since chitin is highly hydrophobic and insoluble (Kumar, 2000). In addition, humid habitats, such as neotropical forests, might favor the microbial degradation of glyphosate into the metabolite aminomethylphosphonic acid (AMPA), an element that is more toxic than its precursor (Lutri et al., 2020). This fact can occur especially in the interior portion of the Atlantic Forest, which is surrounded by agricultural matrices, with a large application of GBH (Borges et al., 2021; Florido et al., 2022).

The soil of the Atlantic Forest in the region of Santa Tereza do Oeste, Paraná state, one of the habitats of *E. meridionalis*, has GBH concentrations of up to 10 mg.kg-1 of soil (Pereira et al., 2020), and this exposure can trigger a collapse in biomarker activities, increasing the possibility of oxidative and neurotoxic damage, as the defense system loses its ability to respond properly, making the body more vulnerable, interfering with vital functions, negatively affecting the reproduction and development of species and by consequence reducing population sizes (Marc et al., 2005; Zebral et al., 2018).

Contaminations can indirectly alter arthropod populations and species compositions, leading to unknown consequences for ecosystem services (Cerdeira and Duke, 2006; Fuchs et al., 2021). In this case, GBH tends to disrupt populations of *E. meridionalis* in forest environments or favor the increase in resistance of the pest population of *G.* (*Gryllus*) *assimilis*. It is suggested that this fact can promote an ecological imbalance, causing chain reactions that directly affect the functioning of the ecosystem (Verma, 2018). Orthoptera are part of the nutrition of animals such as birds (Fasola and Cardarelli, 2015), reptiles (Sluys et al., 2001) and mammals (Kok and Louw, 2000) and due to biomagnification, contaminants can be transferred along the food web, generating diverse and often neglected effects, resulting in ecological problems due to contamination in the trophic chain (Torretta et al., 2018).

5. Perspective and Conclusion

Small concentrations of GBH can be dispersed in water and soil, accumulate in forest litter, and are sufficient to contribute to the production of ROS, promote neurotoxic effects and activate the antioxidant defense system of non-target individuals. Commercial formulations still have synergistic effects between their components, being more toxic than the isolated compound, including for animals, with consequences for non-target species close to crops, such as an increase in the activity of the antioxidant system and cholinergic defense, as seen in *Eidmanacris meridionalis*.

Animals with different evolutionary origins show different responses in the activity of the defense system and tolerance to xenobiotics (Rainio et al., 2019), and this was evident when comparing the antioxidant and cholinergic systems, as well as the installation of oxidative stress in both species of crickets studied. Therefore, the different Orthoptera genera can be used as objects of studies aiming the development of a pollution gradient since *E. meridionalis* was more sensitive and *G.* (*Gryllus*) *assimilis* more tolerant to contact with pesticides.

Important actions can be taken to reduce the environmental impact caused by the intense application of GBH, such as the use of forest biosecurity for forecasting and monitoring weeds, vegetation mapping (Gupta and Sankaran, 2021) and deforestation (Araújo & Vieira, 2019), implementation of natural pesticides (Grzywacz et al., 2014) and compliance with safety legislation for the use of agrochemicals (Brasil, 1989). After diagnosing environmental contamination by GBHs, it is essential to apply remediation techniques, including the use of waste by-products from industry and agriculture to produce chemical adsorbents (Herath et al., 2016; Jiang et al., 2018), the bioremediation (Masotti et al., 2021), phycoremediation (Priyadharshini et al., 2021) and the use of biotechnology to remove pesticides (Espinoza-Montero et al., 2020). To reduce the damage caused to crops by insect pests such as some species of the genus *Gryllus*, the alternatives used can be biological control (Grzywacz et al., 2014), fungal applications (Gad and Nada, 2020) and entomopathogenic nematodes (Andaló et al., 2018) and also the use of bioinsecticides (Singh et al., 2019)

The rampant use of GBH seems to be a silent enemy that generates apparently subtle responses, but which can cause chronic harmful effects in the environment (Druille et al., 2016; Gill et al., 2017; Van Bruggen et al., 2018). The unregulated application combined with the intensified process of environmental impact, can increase the toxicity of the compound, reduce the longevity of the insects due to the drop in the feeding rate, fixation of

body mass and growth rate, resulting in smaller and contaminated adults, which can prevent them of providing fundamental ecosystem services, harming environmental conditions and life on the planet (Stahlschmidt et al., 2022).

Therefore, actions to monitor the application of pesticides are necessary, as well as remediation of contaminated environments to reduce the deleterious environmental impact caused by the intense application of GBH.

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CHAPTER 2

Ecotoxicological effects of a glyphosate-based herbicide on Gryllus (Gryllus) assimilis (Orthoptera: Gryllidae) ontogeny: A study on oxidative stress, antioxidant and cholinergic systems

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Highlights

- In *Gryllus (Gryllus) assimilis*, exposure to GBH can alter the defense systems and promote adaptation to contaminated environments.
- The tolerance increase of non-target species can contribute to pest insect proliferation, food chain contamination, and escalating use of GBH concentrations.
- Stricter regulations on GBH use, promotion of alternative approaches, and encouragement of sustainable farming practices.

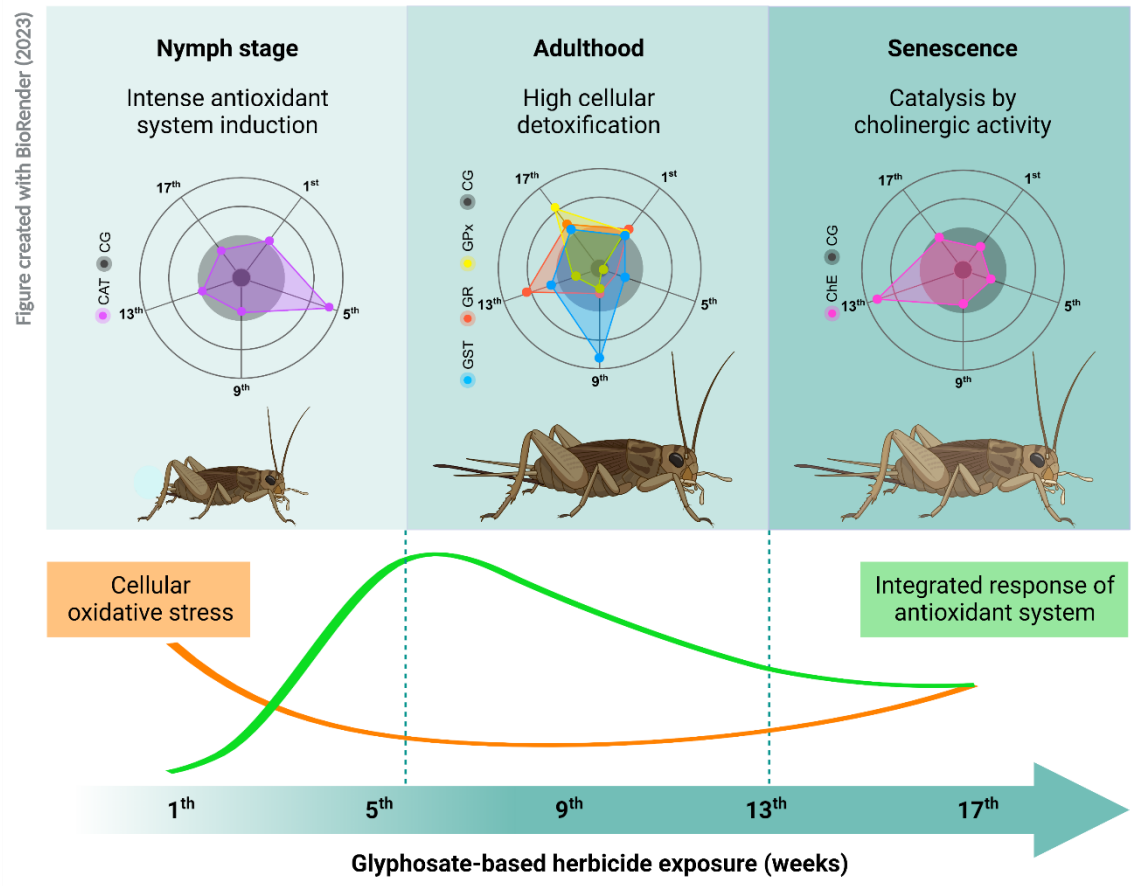
Abstract

Brazil is an important global agricultural producer and to increase production the country has extensively used glyphosate-based herbicides (GBH), surpassing consumption and sales records. Consequently, concerns have arisen regarding the potential impact of GBH on ecosystems and non-target organisms. Thus, the effects of GBH exposure were evaluated throughout the cricket *Gryllus* (*Gryllus*) *assimilis* ontogeny, with five developmental stages. Each period contained 3 control and 3 treated boxes, with 15 crickets each, resulting in 90 insects at a time. The control groups received water, while the treated ones were continuously exposed to GBH (0.864 mg.GBH.L-1), with the solutions changed every 48 hours. After each exposure time the crickets' group were euthanized to assess the activity of antioxidant enzymes (GST, GR, GPx, and CAT), cholinergic enzymes (ChE), and lipid peroxidation (LPO). The results revealed changes in the systems throughout different developmental phases. Specifically, CAT activity exhibited a significant increase during the

nymphal phase, associated with the dismutation of hydrogen peroxide. The GST increased GBH, indicating its role in cellular detoxification, particularly during adulthood. In the senescence stage there was a considerable rise in ChE enzymes, suggesting their involvement in both, choline esters breakdown and potential pesticide detoxification. The action of these enzymes to effectively control lipid peroxidation shows the adaptability of this species to environmental contamination. These findings underscore the long-term effects of agrochemical pollution and emphasize the importance of sustainable practices, effective regulations, and alternative weed control methods.

Keywords: Field-cricket, herbicide, development, resistance

Graphical abstract



1. Introduction

The agricultural industry is essential to the global economy and ith its extensive land area and favorable climate, Brazil has emerged as one of the world's leading agricultural producers (Zalles et al., 2019). However, to increase its production, the country broke

records in the consumption of highly dangerous pesticides, which can have harmful effects on the environment (Brasil, 2019).

Widely commercialized in the country (IBAMA, 2023), glyphosate is a modified glycine used as defoliant to control weeds (Cerdeira and Duke, 2006), and despite its effectiveness, the use can lead to contamination of the soil, air, and water, causing adverse effects on non-target organisms (Mesnage et al., 2015; Vázquez et al., 2021). Glyphosate-based herbicides (GBH) can be more toxic than the isolated active principle due to the interaction between its chemical compounds (Van Bruggen et al., 2018) and several studies have linked this pesticide to adverse effects on human health, including teratogenic, tumorigenic, and hepatorenal effects (Mesnage et al., 2015) and reproductive hormone disorders (Thongprakaisang et al., 2013).

Continuous and excessive use of GBH also has been associated with toxic effects in animals, including liver histological changes and leakage of intracellular enzymes in terrestrial vertebrates (Benedetti et al., 2004), decreased intracoelomic cells and phagocytes in fish (Kreutz et al., 2010), and sublethal effects on the natural behavior of amphibians (Gandhi and Cecala, 2016). The actions also extend to arthropods, where negative effects on prey consumption, web building, fecundity, fertility, and progeny have been observed in spiders (Benamú et al., 2010) and reduced mobility and speed in crustaceans (Hansen and Roslev, 2016). In bees, studies have shown that even when used in doses and concentrations recommended, GBH negatively affects survival, development, and behavior (Battisti et al., 2021).

The toxicity caused by GBH can be explained by the increase in the production of reactive oxygen species (ROS), including superoxide ion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) (Cogo et al., 2009). The excess of ROS tends to lead to cellular oxidative stress, as they react with unsaturated fatty acids, causing lipid peroxidation (LPO) of plasma membranes (Rondón-Barragán et al., 2012), culminating in the accumulation of cytotoxic products and cell death (Ghezzi, 2020).

The evaluation of the effects of glyphosate-based herbicides (GBH) on organisms can be performed by quantifying antioxidant system enzymes, which are considered biomarkers of cellular defense (Cotinguiba et al., 2013). Some of these enzymes rely on reduced glutathione (GSH), a molecule that acts, reducing other oxidizing molecules and playing a critical role in eliminating toxic substances for cells (Marí et al., 2020). Glutathione s-transferase (GST) utilizes GSH as a substrate to detoxify cells and assist in the reduction or elimination of xenobiotics. Glutathione peroxidase (GPx), by oxidizing

GSH to glutathione disulfide (GSSG), is capable of hydrolyzing hydrogen peroxide (H_2O_2) formed by harmful oxidative processes, while glutathione reductase (GR) contributes to GSH regeneration from GSSG (Huber et al., 2008). In addition to the glutathione cycle enzymes, the catalase (CAT) enzyme also decomposes H_2O_2 into water and oxygen, making it excretable (da Silva and Ferrari, 2011).

An alternative method to detect the toxicity of GBH in living organisms is through the analysis of cholinergic system activity, which is responsible for fundamental functions such as movement control in animals (Mesulam et al., 2002). The cholinesterase enzymes, including acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), are present in synaptic clefts and are responsible for hydrolyzing the neurotransmitter acetylcholine. Moreover, BChE also has the ability to decompose butyrylcholine and act as a cellular detoxification agent (Araújo et al., 2016; Ha et al., 2019). Exposure to various types of toxic substances, such as organophosphate and carbamate pesticides, can inhibit or induce the activity of these enzymes, making them a common biomarker of environmental contamination (Olisah and Adams, 2020; Sobjak et al., 2017).

Several organisms can be used as biomarkers, with crickets being particularly valuable for assessing the quality of impacted environments due to their sensitivity to habitat changes, as well as the diversity of their life cycles and regulatory responses (Masaki, 1996). Crickets are also useful for detecting chemical pollutants in terrestrial systems (Yoshimura et al., 2005).

The field cricket species *Gryllus* (*Gryllus*) *assimilis* (Fabricius, 1775) (Orthoptera: Gryllidae) is widely distributed across the Americas, Africa, Europe, and Asia (Cigliano et al., 2023). They inhabit open and dry areas, with low vegetation and sandy soils (Zoltán Kenyeres, 2006). These crickets are characterized by a heavily chitinized body and long wings, rendering them less sensitive to desiccation (Masson et al., 2020). It can be considered a tolerant bioindicator because they exhibit a plastic adaptation to anthropized environments and local conditions (Harrison, 1979), and are globally recognized as agricultural pests in several crops (Marsaro-Júnior et al., 2019).

Understanding the effects of exposure to GBH on non-target species, such as orthopterans, is a way to explore new knowledge about the ecotoxicological and ecosystem damage promoted by the widespread use of this pesticide. In this study, we evaluated the effects of chronic exposure to a glyphosate-based herbicide (GBH) on *Gryllus* (*Gryllus*) *assimilis* testing the hypothesis that GBH concentration would lead to

an increase in the activity of the antioxidant defense system and cholinergic activity, in addition to promoting greater oxidative stress in the life stages of this insect.

2. Material and methods

2.1 Obtaining and maintaining specimens

The two weeks old nymphs of *Gryllus* (*Gryllus*) *assimilis* were acquired in a single batch, from a specialized commercial establishment and subsequently transported to the Laboratório de Orthoptera at Universidade Estadual do Oeste do Paraná. The insects were placed in transparent and ventilated plastic boxes (32 cm high x 48 cm long x 30 cm wide) and kept in a climate-controlled room at 24±2°C, 57±7% humidity and 12 hours' light/dark. Water, in cotton-filled containers, and food (flake feed for fish Alcon® Basic and wheat bran feed for chickens) were offered *ad libitum*, while egg cartons were used as shelter. All crickets were kept under these conditions for 7 days for acclimatization.

2.2 Experimental design

The glyphosate-based herbicide (Gli-Up ® 72 WG) was purchased from a certified commercial establishment and the product was diluted in a stock solution of 0.1 g of GBH.L^{-1} in water, that was used to establish the treatment whose concentration was 0.864 mg GBH.L^{-1}. The concentration used in our study follows a geometric progression based on the findings that examined the impact of transgenic soy and glyphosate-based herbicides (GBH - 1.080 g/ha, considering the first soil mm) on soil surface arthropods (Pereira et al., 2018).

Acclimatized nymphs with four weeks' old were used for the experiments (n = 450) and then placed in 30 transparent rectangular boxes (35 cm x 25 cm x 10 cm). Each box was considered a sampling unit, housing 15 nymphs. Fifteen boxes were used for the Control Group (CG) while the remaining 15 boxes were designated as the treated group (GBH).

To evaluate the effects caused by GBH exposure over time, five exposure periods were analyzed, through the development of crickets, defined as T1 – 1^{st} week, T2 – 5^{th} week, T3 – 9^{th} week, T4 – 13^{th} week, and T5 – 17^{th} week. For each period, 3 control units (CG) received 25 mL of water directly on the cotton-filled containers, while 3 treatments were constantly exposed to the same amount of treatment solution (GBH), characterizing oral exposure through water. Cottons and food were changed every 48 h. The cricket was kept under the same conditions of temperature, humidity, photoperiod, and feeding as

described previously (figure 1). After each exposure period, the crickets were euthanized by freezing at -20°C.

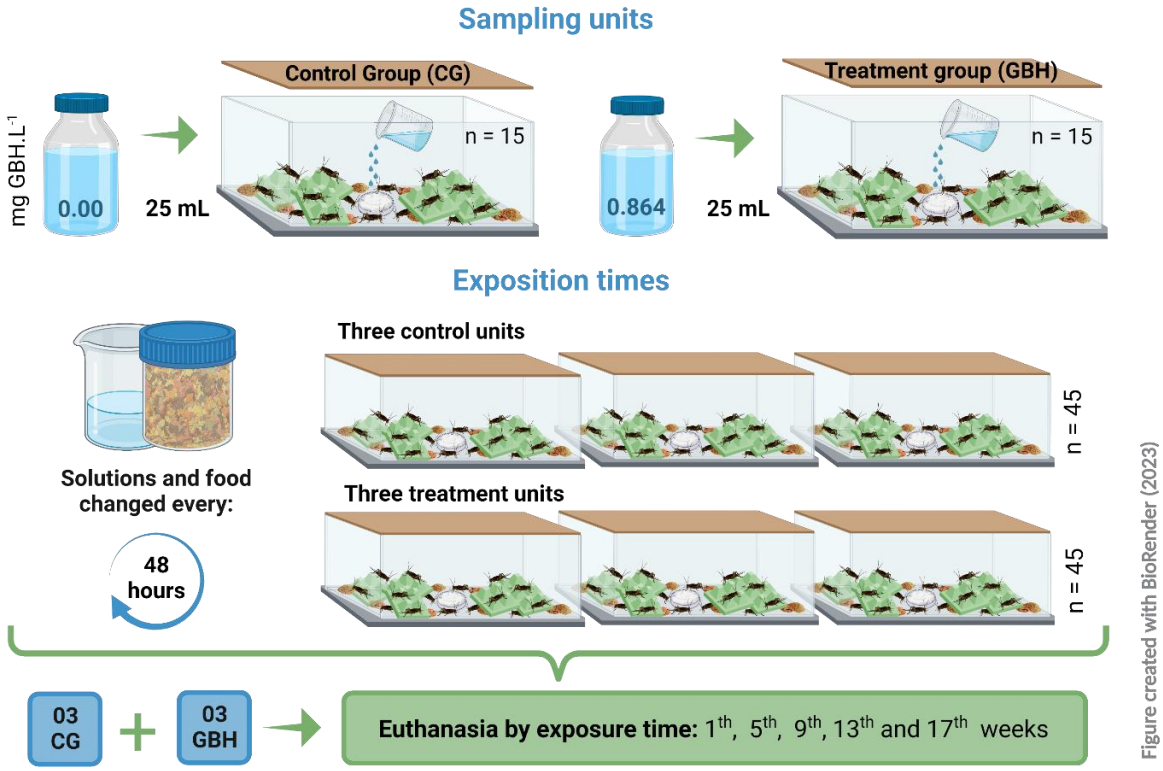


Figure 1. Nymphs 5 weeks old separated in 15 per box. For each exposure time, 3 control boxes (water) and 3 treatment boxes (0.86 mg GBH.L) were analyzed.

2.3 Laboratory analysis

The euthanized crickets were placed in a microtube, homogenized in a 50 mM tris-HCl buffer, pH 7.4, and centrifuged at 10.600 g for 10 min at 4ºC. Protein quantification of the samples was determined by the Bradford (1976) method in triplicate, using bovine serum albumin as a standard and were normalized to 1 mg protein.mL^{-1}.

2.3.1 Antioxidant defense system

Assays was conducted in triplicate in a microplate. The enzyme glutathione s-transferase (GST) catalyzes the conjugation of reduced glutathione (GSH) with the synthetic substrate CDNB, resulting in the formation of a conjugate that can be detected at 340 nm (Habig et al., 1976). The results were expressed in nmol.min^{-1}.mg of protein^{-1}.

The activity of glutathione reductase (GR) was determined by measuring the reduction of glutathione disulfide (GSSG) from NADPH oxidation, which was monitored

by a decrease in absorbance at 340 nm (Sies et al., 1979). The results were expressed in nmol.min^{-1}.mg of protein^{-1}.

Glutathione peroxidase (GPx) was accompanied by a decrease in NADPH absorbance at 340 nm (Flohé and Günzler, 1984). The reaction was observed for one minute and expressed in nmol.min^{-1}.mg of protein^{-1}.

Catalase activity (CAT) was monitored at decreasing absorbance at 240 nm (Aebi, 1984), based on the principle of peroxide dismutation. The the results were expressed in mmol.min^{-1}.mg of protein^{-1}.

2.3.3 Cholinergic system

The Cholinesterase (ChE) enzyme activity was analyzed using the Ellman (1961) method, which was modified for microplate use by de Assis (1998). This method measures the production of thiocholine upon hydrolysis of Acetylcholine and was conducted with a solution comprising DTNB and Acetylcholine. The results were expressed in nmol.min^{-1}.mg of protein^{-1}.

2.3.2 Cellular oxidative stress

This analysis reflects the intensity of cellular oxidative stress through the quantification of the lipid peroxidation reaction (LPO) (Lushchak et al., 2009). The measurement of thiobarbituric acid reactive substances method (TBARS) (Buege and Aust, 1978) was employed to compare the absorbance of a standard curve of Malondialdehyde (MDA). The reaction was read after 60 minutes of incubation at 60°C, at an absorbance of 535 nm and the results were expressed in µmol of MDA.mg of protein^{-1}.

2.4 Statistical analyzes

As our design was done in blocks and as it was not possible to work with only males or only females, we analyzed the biomarker variables using Generalized Linear Mixed-effects Models (GLMER), including the factors “sampling unit” and “sex” as random effects. In the model construction the response variables were the values of the biomarkers (CAT, GPx, GR, GST, ChE and LPO), while the predictor variables were represented by the time of exposure (weeks) and treatment (GBH and control). Models were adjusted with Gamma distribution (Cox, 1983) because it showed better adequacy of the residues.

We evaluated nonlinearity for each complete model by adjusting Generalized Additive Models (GAMs) with Gamma distribution, using the "mgcv" package (Wood et al., 2015), including a smoother for the continuous explanatory variable. The smooth function is a curve adjusted to the data that can vary from linear, in which case the estimated number of degrees of freedom (edf) is equal to one, to curvilinear, in which case the edf is higher than one. We used the "gam.check" procedure to evaluate if the adjusted GAM's basis dimension (k) was adequate. We used the F test (procedure ANOVA of the adjusted GAM model) to evaluate if the adjusted curves presented edf higher than 1, plotting the adjusted GAM curves to evaluate if they presented non-linear shape (Wood et al., 2015). We compared the models with and without smoothers using ANOVA. If there were significant differences, we choose the model with lowest Akaike Information Criterion (AIC). Significance of the explanatory variables was evaluated by deletion of non-significant terms, beginning with interaction terms (Crawley, 2013). Therefore, complete models were simplified until the minimum adequate model was achieved for each response variable.

The calculation of the Integrated Biomarker Response Index (IBR), second version was calculated according to the methodology proposed by Sanchez et al., (2013), calculating the deviation index (A). Data from (A) were plotted on radar graphs to represent the induction or inhibition of each investigated biomarker with regard to the control, for each developmental time throughout GBH exposure. The sum of the values of (A) results in the IBR that were analyzed through a Generalized Linear Model (GLM), assuming the Gamma distribution and Z statistics and represented in a graph of dispersion. All analyzes were performed in the R program, version 4.2 (R Core Team, 2022).

3. Results

During the exposure period, as the animals matured, it was possible to discern their sex based on ovipositor emergence in females. The imaginal molting (last before adulthood) occurred from the 5^{th} week of exposure, and from the 13^{th} week until the end of the experiment the animals were already old. There was no significant relationship between, groups (CG and GBH) and exposure times ($p > 0.3452$).

Regarding the antioxidant defense system, initially, glutathione s-transferase (GST) activity was higher in CG but after the 5^{th} week, the GBH became higher and both

groups showing a little decrease between the 13th and 17th week (AIC = 1030.381; F = 17.332; p < 0.001). Overall, GST activity (GR) increased for groups across exposure weeks (Figure 2). In the GBH, glutathione reductase significantly decreases until the 5th week but tends to increase from the 9th week of exposure, overlapping CG (AIC = 479.54; F = 6.669; p < 0.001; Figure 2). It was observed that glutathione peroxidase (GPx) was less active in the GBH compared to CG for the first nine weeks of exposure (AIC = 1426.60; F = 8.037; p < 0.001; Figure 2). In the last week, GPx activity increased for the treated group, surpassing the control. The enzyme catalase (CAT) exhibited an increase in activity from the beginning of the exposure until approximately the 13th week, followed by a reduction until the end of the treatment. There was CAT activity difference just about exposure times (AIC = 1458.902; F = 40.997; p < 0.001; Figure 2). As for the cholinergic system, the GBH showed significantly higher activity from the 9th week compared to the CG until the end of the exposure period (AIC = 894.55; F = 6.0224; p < 0.001; Figure 2).

In the evaluation of oxidative stress by quantifying lipid peroxidation (LPO), the reaction decreases for all groups during the first 5 weeks, but GBH shows lower values compared to CG (AIC = 255.391; F = 4.744; p = 0.0031). After this period, LPO increases, and the interaction reverses, with higher peroxidation observed in the GBH compared to CG until the end of the exposure period (Figure 2).

3.1 Integrated Biomarker Response on cricket ontogeny

Concerning the antioxidant defense system, the deviation index indicates that in the initial results about the 1st week, there was no representative alteration of the enzymes (CAT: A = 0.0703; GR: A = 0.2042; GPx: A = -0.0118; GST: A = -0.05427). In the 5th week, we observed CAT induction (A = 1.675), and the inhibition of GR, GPx and GST (A = -0.905, -1.368, and -0.605, respectively). By the 9th week, CAT was inhibited (A = -0.369), GPx and GR remained persistently inhibited (A = -0.797 and -0.632, respectively), while GST was induced (A = 1.572527). After 13 weeks, GR exhibited induction (A = 1.151), remained inhibited CAT and GPx (A = -0.085 and -0.633, respectively), while GST returned to levels similar to the control, persisting until the end of the experiment (A13th = 0,275; A17th = 0.209). In the 17th week, GPx was little induced (A = 0.919), and CAT remained inhibited (A = -0.3214). The integrated response of antioxidant defense system biomarkers showed a significant increase in integrated biomarkers from the first week, peaking during the 5th week of GBH exposure, and decreasing thereafter (Figure 3).

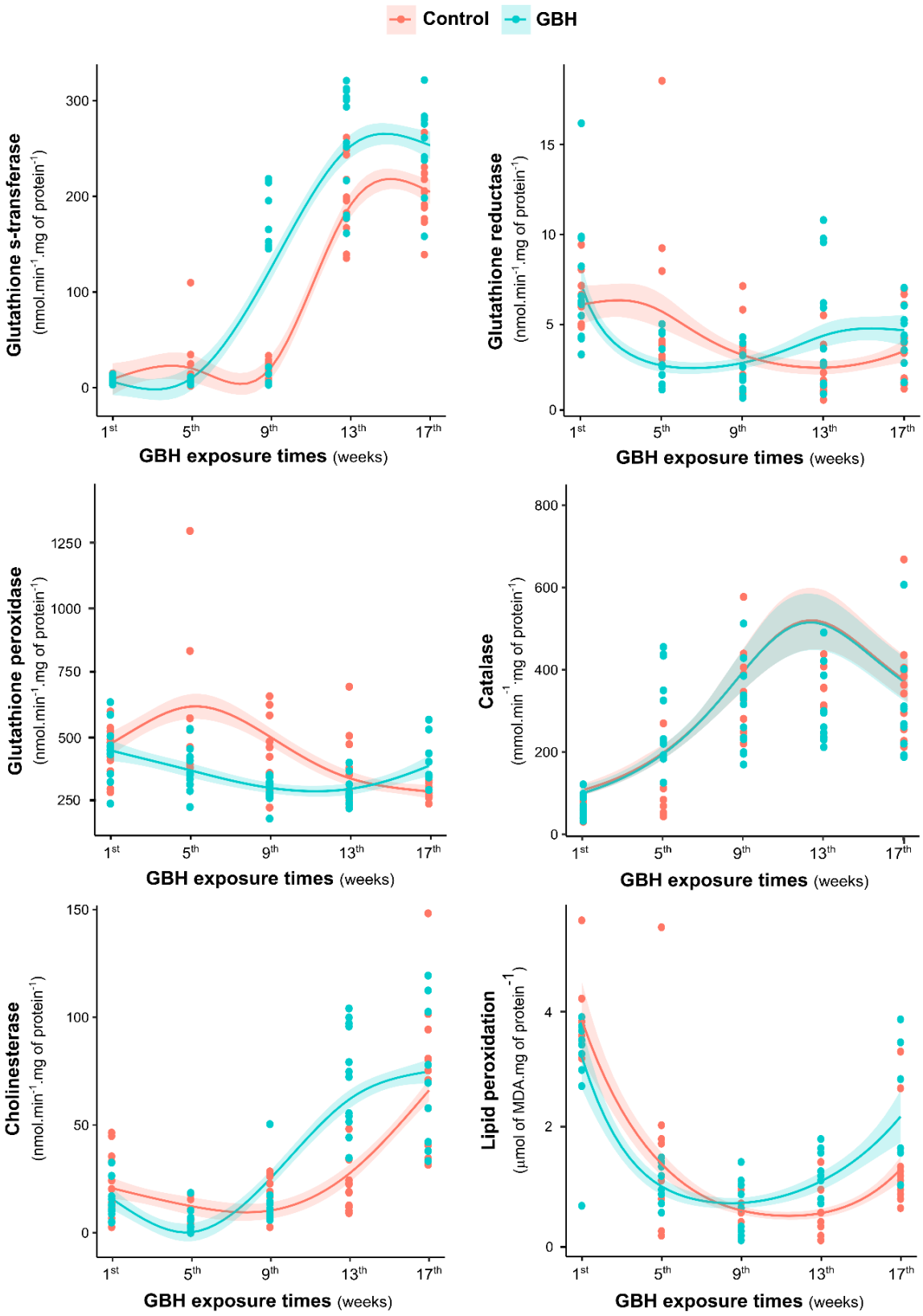


Figure 2. Activities of enzymes of the antioxidant defense system (GST, GR, GPX and CAT), activity of enzymes of the cholinergic system (ChE) and lipid peroxidation reaction (quantification of malondialdehyde), over 17 weeks of the control group and exposure to GBH.

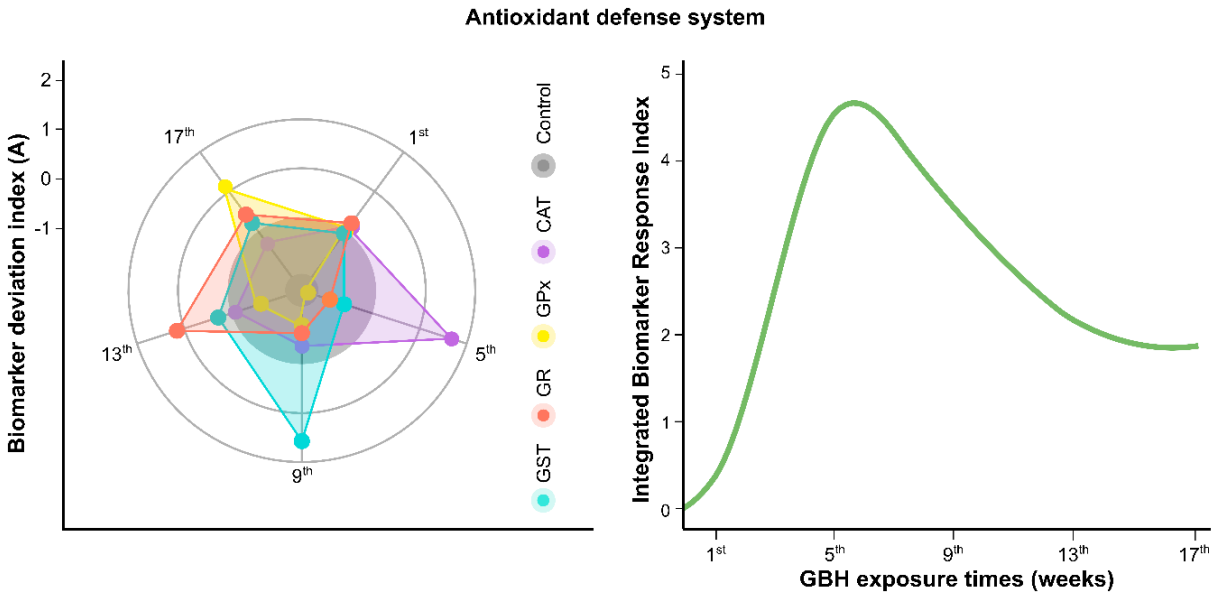


Figure 3. Deviation of biomarkers of the antioxidant defense system, where the central reference value is the control(CG), and positive values of biomarkers indicate enzyme induction, while negative values indicate inhibition. The sum of the values of (A) results in the Integrated Biomarker Response index (IBR), for each developmental point (weeks) over the course of GBH exposure.

The deviation index showed that cholinesterase was consistently inhibited and induced only in the 13th week (A = 1,647). Lipid peroxidation reaction was induced from the 13th week (A = 1,476) until the end of the experiment (Figure 4).

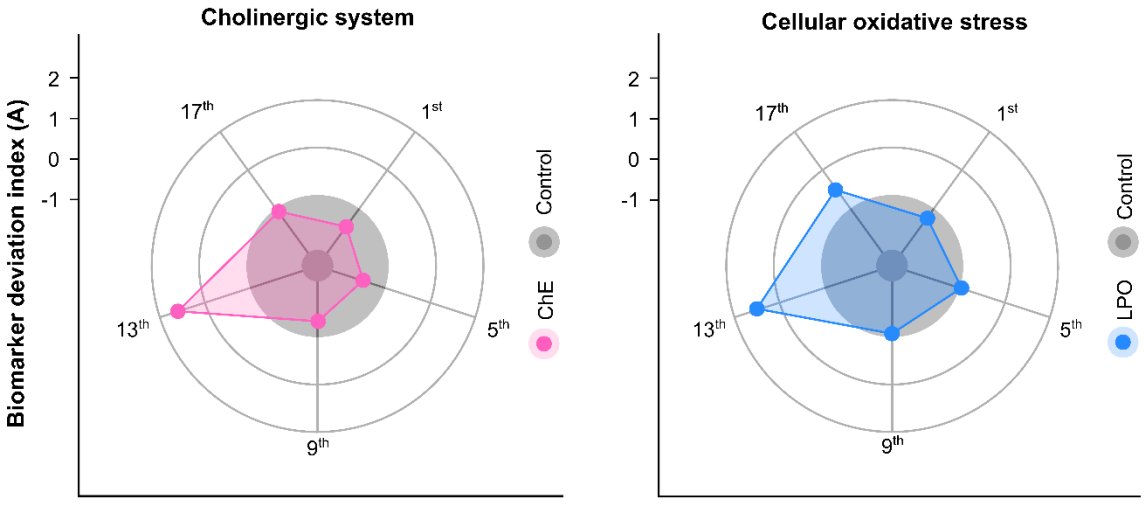


Figure 4. Deviation of biomarkers of the cholinergic system and cellular oxidative stress, where the central reference value represents the control (CG). Positive values of biomarkers indicate ChE or LPO induction, while negative values indicate activities and reactions inhibition.

4. Discussion

The transformation in *Gryllus* systems is influenced by the specific nutrient requirements associated with life history, and the distinct patterns observed in different age and sex groups (Limberger et al., 2021), with evident physiological compensatory mechanisms under conditions of environmental stress (Boggs, 2009). Therefore, exposure to xenobiotics, such as GBH, can generate physiological changes during ontogenetic development, as described below.

4.1 Cricket antioxidant system response to chronic GBH exposure

The concentration used in our study is next of the glyphosate residual values reported by Ferreira and collaborators (2023), which ranges from 0.000024 to 0.6638 mg/L (original value in mg/kg soil), and the antioxidant defense system and the cholinergic system were altered throughout the *Gryllus* (*Gryllus*) *assimilis* development, with changes in enzymatic activities according to the exposure time to GBH and the physiological requirements of each life stage.

In the nymphal stage, there is intense induction of the antioxidant system in response to reactive oxygen species (ROS). Although catalase (CAT) did not differ between CG and GBH groups, the increase in its activity reflects the organism's need to break down hydrogen peroxide to control the lipid peroxidation (Khare et al., 2019), being more expressive during the nymphal stage. This enzyme was also more relevant in the larval stage of the fly *Drosophila melanogaster*, however, it was increased by exposure to a GBH 10 g/L (Strilbytska et al., 2022), indicating that in different insect species the CAT activation can reduce levels of oxidative damage and extend lifespan (Giraldo et al., 2021).

Embryonic and nymphal *Gryllus* development is characterized by an elevated metabolism of ecdysteroids (molting hormones), cytochrome P450s (enzymes involved in substrate oxidation) (Berdan et al., 2016). These processes, together with the increase in CAT can indicate a phase of cellular defense and elimination of toxic compounds associated with cellular transformations occurring during molting.

In *G.* (*Gryllus*) *assimilis* adult and during the senescence period, the activation profile of the antioxidant and cholinergic systems changes, mainly inducing cellular detoxification reactions. According to Stahlschmidt et al. (2015), the natural action of the immune system of *Gryllus* adults produces ROS, and at the same time there is an increase in the production of the antioxidant reduced glutathione (GSH), which allows protection against ROS damage and tolerance to exposure. to xenobiotics.

The increase in glutathione s-transferase (GST) activity throughout the herbicide exposure demonstrated its active role in cellular detoxification, especially during the adulthood (9^{th} week). This indicates that the herbicide can cause a toxic effect that needs to be controlled by the enzyme, as observed in bees, where an increase in both head and gut GST activities was observed in GBH 10 μg/L (Pal et al., 2022). The decrease in GST activity between the 13^{th} and 17^{th} week may be related to tissues aging (Hazelton and Lang, 1980), and the activity of glutathione reductase (GR) and glutathione peroxidase (GPx).

During the adult stage, the increase in GR activity in GBH groups indicates a greater demand for maintaining a sufficient supply of GSH, which is utilized by GST and GPx for detoxification processes (Couto et al., 2016). Akin to our observations, in three species of amphibians exposed to concentrations below 51.2 mg.L of GBH occurred a significant increase in the levels of GST and GR (Güngördü, 2013) and this finding highlights that enzymatic activity can be altered in polluted environments. GPx activity remained inhibited among the animals exposed to GBH between the 5th and 13th week of development. This enzymatic behavior can be an indicative of a potential accumulation of hydrogen peroxides, reflected in the increase of lipoperoxidation (LPO). GPx inhibition also can be observed *L. acuta* polychaete exposed to 5.35 mg.L (Tarouco et al., 2017). Over *G.* (*Gryllus*) *assimilis* aging, GPx was induced in the 17^{th} week and also actually reduced lipoperoxidation. In control groups, we highlight that the combination of GR and GPx activities shows an increasing trend during the juvenile phase, indicating the need for GSH replenishment to break down peroxides generated from molting processes in the nymphal phase (Huber et al., 2008; White and Ewer, 2014). However, a subsequent reduction in these activities was observed, which may reflect the senescence process in these insects (Michalkova et al., 2014)

In an integrative assessment of biochemical responses, the Index of biomarker response (IBR) reveals that the antioxidant system was activated, particularly during the first five weeks of exposure to GBH, a phase in which the insects are undergoing imaginal

ecdysis and the beginning of the adult stage. During this period, the ROS production occurs naturally due to the various cellular transformations that insects undergo during early development (Eleftherianos et al., 2021), but it can also be potentiated by contamination with xenobiotics (Bailey et al., 2018).

4.2 Damages caused by chronic GBH exposure on crickets

As shown in these results, we found that there was chronic damage from exposure to GBH. However, it is important to highlight changes that are natural with aging and changes that result from exposure to pesticides. During aging, *G.* (*Gryllus*) *assimilis* exhibits immunosenescence, characterized by a decrease in melanotic nodules and subsequent cutaneous protection, an increase in granulocytes indicative of infection, a decrease in plasmatocytes responsible for antibody production, and the presence of damaged hemocytes, indicating the deterioration of immune functions(Park et al., 2011).

In our results, the exponential increase in cholinesterase enzymes (ChE) is also a characteristic of the age-related development in animals (Gard and Hooper, 1993), as observed through measurements of enzyme activity and integrated values (A). This enzymatic activity is likely associated with the catalytic properties of cholinesterases (Villatte and Bachmann, 2002). Specifically, acetylcholinesterase (AChE) is particularly efficient at breaking down acetylcholine (ACh) at cholinergic synapses (Stepankova and Komers, 2008), while butyrylcholinesterase (BChE) in addition to choline esters, also exhibits hydrolytic effects on other substrates such as drugs (Ha et al., 2019).

The increase in *G.* (*Gryllus*) *assimilis* ChE, more expressive in the GBH group, may be attributed to both the elevated catalysis of ACh in the synaptic cleft during the aging process (Güngördü, 2013; Santana et al., 2021), and the action of BChE, which tends to be more activated when exposed to external substrates (Pohanka, 2013; Stepankova and Komers, 2008), including pesticides (Raveh et al., 1993). It is possible that in the 13th week of treatment there was an increase in BChE activity as a detoxification mechanism, since in the same period there is a reduction in GST activity.

The decline in LPO reaction until the 9th week, followed by an exponential increase throughout the rest of the treatment, may be a result of the aging process (Hazelton and Lang, 1980). In the adult stage, LPO was higher in treated groups, indicating that in addition to senescence, exposure to GBH may intensify oxidative stress.

The increased activity of GST, GR, GPx, CAT, and ChE during adulthood, may reflect this oxidation and the need for elimination of toxic cellular compounds.

4.3 Ecological implications related to the excessive use of GBH

Insects represent the majority of animal biodiversity (Tihelka et al., 2021) and provide indispensable ecosystem services, such as high percentage of plant pollination (Ollerton et al., 2011), nutrient recycling in soil through the action of detritivores and decomposers and serving as the base to the food chain (Goulson, 2019). However, some species have adverse effects on the human economy as they act as disease vectors and agricultural pests and to control the population of these insects, increasing amounts of pesticides have been used (Liebhold, 2012). The exposure of insects to these xenobiotics has contributed to the development of resistance mechanisms at the metabolic and cellular levels, involving cellular detoxifications, redox reactions, and transcriptional adaptive responses, that can be transmitted to future generations as an epigenetic process (Lu et al., 2021).

During the nymphal stage of *Gryllus* (*Gryllus*) *assimilis*, the increase in GST activity, along with CAT, appears to be sufficient to regulate the decrease in lipid peroxidation (LPO) during the youth and early adulthood. This suggests that the species may be adapted to environments with GBH application, indicating that the natural selection process has ensured the ability to control xenobiotic exposure with only minor alterations to the defense system. According to Hussain and collaborators (2020), transcriptomic analysis of *Gryllus* genus provides evidence for their survival through a well-developed immune mechanism involving hundreds of genes regulating cellular defense. The plastic adaptability of this insects to local environmental conditions can be attributed to their specific developmental, physiological, and reproductive strategies, which likely enable *G*. (*Gryllus*) *assimilis* to survive in anthropized environments (Harrison, 1979), leading to various negative interactions, such as pest outbreaks in gardens and lawns (Salvadori et al., 2007), plantations, gardens, and young trees (Masson et al., 2020).

Increased exposure to different xenobiotics can favor the population growth of tolerant and resistant insect species (Nauen et al., 2022), creating opportunities for higher concentrations of GBH to be applied (Myers et al., 2016). If tolerant species become more abundant, they may compete with others for resources and habitat and occupy vacant niches left by declining species (Sánchez-Bayo and Wyckhuys, 2019), and the dispersal

of contaminated individuals can result in the flow of contaminants among ecosystems (Augusto et al., 2022).

Considering that soil invertebrates can contain glyphosate residues in their tissues (Hernández-Gutiérrez et al., 2021), orthopterans serve as the foundation of the food chain, being consumed by birds, reptiles, and mammals (Fasola and Cardarelli, 2015; Kok and Louw, 2000; Sluys et al., 2001). The risk of contamination of other animals through consumption increases, and potential glyphosate residues can result in oxidative damage and trigger broader ecological issues, due to the transfer of GBH across different trophic levels (Bundschuh et al., 2022).

The combination of pesticide pollution and climate changes has the potential to induce alterations in species composition and population declines of insects worldwide (Outhwaite et al., 2022). This may occur because different temperatures can alter pesticide toxicity in insects according to their physiological mechanisms (Yang et al., 2021). Consequently, some species show reduced sensitivity and expand their distribution and generations, while others may encounter limitations in their capacity for adaptive plasticity when exposed to this combined set of environmental changes (Skendžić et al., 2021).

4.4 Strategies to minimize GBH exposure and preserve ecosystems

The risks associated with pesticide contamination can be minimized through efforts towards environmental sustainability, as well as the preservation and protection of non-target organisms and preserved areas. When it comes to pesticide pollution, it is essential to follow product instructions and implement laws on good agricultural practices. This includes appropriate transportation and storage to prevent leaks or spills, and the responsible disposal of packaging and waste (Brasil, 1989). The preference for using biopesticides can also be considered an effective alternative for pest management, linked to the reduction of environmental contamination (Ebadollahi, 2020; Grzywacz et al., 2014).

When it is not possible to avoid pollution from GBHs, remediation techniques need to be employed, including the use of residual by products from industry and agriculture for the production of chemical adsorbents (Jiang et al., 2018), bioremediation (Masotti et al., 2021), phytoremediation (Priyadharshini et al., 2021), and biotechnologies for pesticide removal (Espinoza-Montero et al., 2020).

To mitigate the damage caused by pest insects like *Gryllus* in various crops, additional protective measures can be implemented, such as the use of biological control methods involving natural predators, parasitoids, nematodes, bacteria, fungi, and viruses (Kumari et al., 2022), monitoring of pest populations followed by predictions of damage intensity (Speight, 2016), management of pest insect habitats (Akter et al., 2019), and physical control methods (Thakur et al., 2021).

The unregulated application of GBH combined with intensified environmental impacts reduces the longevity of non-target insects and generates responses that are still poorly understood, which can cause chronic detrimental effects to the environment (Druille et al., 2016; Gill et al., 2017; Van Bruggen et al., 2018). The replacement of conventional agricultural practices with more sustainable and ecologically-based activities is needed to slow down or reverse current trends, enable the recovery of declining insect populations, and maintain the essential ecosystem services they provide (Stahlschmidt et al., 2022).

5. Conclusions

The country's reliance on glyphosate-based herbicides raises concerns about crop, non-target organisms, and human health. Exposure can alter the antioxidant system and cholinergic enzymes in *Gryllus* (*Gryllus*) *assimilis*, indicating potential adaptation to contaminated environments, which may result in long-term effects on ecosystem functioning. To address these challenges, prioritizing environmental sustainability and human well-being is crucial. This involves adopting responsible farming practices, implementing effective regulations, and exploring alternative weed control methods and insect pests. More research is needed to understand the long-term effects of glyphosate-based herbicides on organisms and ecosystems, including cumulative effects, mechanisms of action, and interactions with other environmental stressors. These insights can support the development of strategies, regulations, and guidelines for the responsible use of herbicides.

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CONCLUSÃO

O uso generalizado de herbicidas à base de glifosato (HBG) tem contribuído para a contaminação ambiental de ecossistemas aquáticos, terrestres e atmosféricos e, por consequência, atingindo organismos não-alvo. Por meio da quantificação da atividade de enzimas antioxidantes e colinérgicas, além da reação de lipoperoxidação, é possível avaliar a toxicidade do HBG em insetos não-alvo, como os grilos (Orthoptera).

A avaliação da exposição aguda a concentrações crescentes de HBG em grilos de duas espécies de diferentes habitats, revelaram respostas elevadas nas defesas antioxidantes e no sistema colinérgico dos grilos florestais da espécie *Eidmanacris meridionalis*, potencialmente resultando em redução populacional. Em contraste, as defesas antioxidantes e o sistema colinérgico dos grilos do campo *Gryllus* (*Gryllus*) *assimilis* permaneceram estáveis, indicando sua adaptabilidade à exposição ao HBG em seus habitats naturais. Nesse caso, o grilo florestal se apresentou como um biomarcador sensível, enquanto o grilo de campo um biomarcador tolerante frente à exposição ao herbicida.

Ao examinar os efeitos da exposição crônica a uma concentração de HBG ao longo da ontogenia de *G*. (*Gryllus*) *assimilis*, foi possível observar alterações nas atividades enzimáticas em diferentes estágios de desenvolvimento. Houve aumento significativo do sistema de defesa durante a fase ninfal, associada principalmente à enzima catalase, assim como observado para *E. meridionalis.* Sugere-se, portanto, que para ninfas de grilos a CAT cumpre uma importante ação antioxidante durante a dismutação de peróxido de hidrogênio. Na fase adulta, as atividades de detoxificação celular aumentam através da ação das enzimas glutationas. Durante a senescência, o aumento considerável nos níveis das colinesterases sugere, além do próprio processo de envelhecimento, o potencial de ChE para desintoxicação de pesticidas. A ação conjunta dos sistemas e sua eficácia no controle da lipoperoxidação destaca ainda mais a adaptabilidade de *G*. (*Gryllus*) *assimilis* à contaminação ambiental.

Podemos sugerir que grilos, no geral, apresentam diferentes comportamentos, funções fisiológicas, morfologia e eventos de desenvolvimento resultantes de contextos ecológicos que permitem uma ampla gama de respostas frente às contaminações ambientais. Este estudo ressalta a importância de estudar diferentes espécies para entender suas variadas

respostas à exposição a pesticidas, contribuindo no desenvolvimento de um gradiente de poluição. Essas descobertas enfatizam os efeitos de longo prazo da poluição agroquímica e a necessidade métodos alternativos de controle de ervas daninhas.

Para reduzir os impactos ambientais adversos da HBG e proteger a biodiversidade, é imprescindível adotar abordagens que considerem as complexas interações entre agroquímicos e organismos não-alvo, priorizar práticas agrícolas sustentáveis e implementar estruturas regulatórias robustas, principalmente em matrizes agrícolas próximas a unidades de conservação. Estudos mais detalhados deste herbicida em diferentes condições ambientais são importantes para um correto uso, diminuindo assim os danos ambientais diretos ou indiretos que este produto possa causar.

ANEXO

CHEMOSPHERE

AUTHOR INFORMATION PACK

TABLE OF CONTENTSDescription

ISSN: 0045-6535

DESCRIPTION

Chemosphere is an international journal designed for the publication of original communications as well as review articles on chemicals in the environment. Chemosphere, as a multidisciplinary journal, offers maximum dissemination of investigations related to all aspects of the identification, quantification, behavior, fate, toxicology, treatment, and remediation of chemicals in the bio-, hydro-, litho- and atmosphere.

Chemosphere will publish:

- Original communications (Research Papers) describing important new discoveries or furtherdevelopments in important fields of investigation
- Review Articles, mainly of new developing areas
- Short communications
- Letters to the Editor
- Special, themed issues on relevant topics

All papers should demonstrate a high level of novelty, originality and uniqueness. The following sections and subject fields are included:

Environmental Chemistry

This section will publish manuscripts dealing with fundamental processes in the environment that are related to the analysis, behavior, fate, and alteration of organic and inorganic contaminants focused on the dynamics of contaminants in environmental compartments such as water, soil, sediment, particulate matter, organisms, dust and indoor/outdoor air. Only studies that are of significance to an international audience, include sites of particular global interest, or lend themselves to interpretationat the global level should be submitted.

Topics of specific interest include, but not limited to, are:

- All aspects of emerging contaminants, such as pharmaceuticals, pesticides, flame retardants, otherindustrial chemicals, persistent organic pollutants, endocrine disruptors, etc.
- All aspects of trace metals, organometals, metalloids (e.g., arsenic) and radionuclides

- Environmental fate studies including transport, biodegradation, bio-accumulation and/or deposition,atmospheric (photo)chemical processes, hydrolysis, adsorption/desorption
- Transformation and mineralisation of chemicals (e.g., by bio- and photo degradation, redoxprocesses and hydrolysis)
- Novel environmental analytical methods including case studiesEnvironmental modelling and quantitative structure-activity relationships to study fate and environmental dynamics
- Monitoring studies presenting new strategies, report of novel contaminants, findings orinterpretations of interest for an international readership.
- Passive sampling
- Non-target and suspect screening (e.g. effect-directed analysis)
- Natural marine toxins

The following studies are not considered for publication: studies on (micro)organisms (unless chemicals are clearly involved), monitoring studies based on standard methodology, and/or onlyof regional importance, studies dealing only with nutrients in agricultural ecosystems, pesticide application studies, plant physiology studies, studies on improvement of crops and purely analytical methodology studies. As regards papers on air pollution, we focus on contaminants in air, particulate matter studies and also consider papers on NO_X, SO_X or ozone.

Toxicology and Risk Assessment

The section on Environmental Toxicology and Risk Assessment covers all aspects of toxicology, i.e., the science of adverse effects of chemicals on living organisms including humans, and the scientific risk assessment.

Topics of specific interest include, but not limited to, are:
- Adverse effects of chemicals in environmental, aquatic and terrestrial, organisms
- Similar studies in experimental organisms (under laboratory conditions)
- Epidemiological studies on effects of chemicals in humans
- Biochemical studies related to mechanisms of adverse effects
- Toxicokinetics and metabolic studies on chemicals related to adverse effects
- Development and validation of testing methods based on living organisms or biological materials
- Nanopolymers, nanocomposites, and microplastics in the environment
- Adaptation
- Human biomonitoring
- Elucidation of mechanisms of toxic effects
- DNA and protein adducts
- In vitro assays and omics techniques
- Phytotoxicity

Not considered are, e.g., studies that report only concentrations of chemicals in the environment, living organisms, food or other materials etc. and studies on biochemical effects of chemicals non-relevant to toxicology.

Treatment and Remediation

This section deals with papers about technologies that manage and/or reduce environmental contaminants, including reuse and recycling processes. The technology must be beyond a basic laboratory study or have obvious implications for current or potential treatment or remediation technologies and, for example, for any advanced oxidation process, the intermediates and/or the extent of mineralization of the targeted compound(s) and wastes must be quantified.

Topics of specific interest include, but not limited to, are:
- Advanced water and wastewater treatment processes and sludge management
- Produced water
- Drinking water
- Incineration
- Remediation including bio/phytoremediation employing new strategies

- Hydraulic fracturing
- Use of biochar amended soil to bind (e.g., herbicides)
- Nanotechnology
- Advanced oxidation processes
- Photolysis/photocatalysis and electrochemical and photo-assisted electrochemical methods
- Sonolysis/sonocatalysis
- Mechanochemical destruction (MCD)
- Natural treatment systems (riverbank filtration and aquifer recharge/recovery)
- Characterization of natural and effluent organic matter
- Technologies for recycle/reuse (e.g., of microbial fuel cell techniques)
- Gasification/pyrolysis for biomass-to-energy and energy recovery from waste streams

Not considered are studies that focus on the synthesis of new materials to be used in waste water purification or remediation. Studies focusing on the removal of single contaminants are often less interesting for publication.

AUDIENCE

Environmental scientists, chemical engineers, biologists, toxicologists.

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Nanotechnology

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Environmental chemistry, Biogeochemistry, Bioaccumulation, Persistent organic pollutants, Chemicals of emerging concern, Chemical inventories, Mercury, Polycyclic aromatic compounds,Arctic, Marine mammals, Fish

Associate Editors Environmental

Chemistry

Xinde Cao, Shanghai Jiao Tong University - Fahua Campus, Shanghai, China
Soil remediation, contaminant removal from water, flue gas, metal speciation, metals in soil, bioavailability and human health, arsenic, phosphate, development, application of solid waste-derived environmentally functional materials, biochar, recycling, groundwater
Ralf Ebinghaus, Helmholtz-Zentrum Hereon, Geesthacht, Germany
Atmosphere, particles, mercury, emerging contaminants, PFAS, ,
Milena Horvat, Jozef Stefan Institute, Ljubljana, Slovenia
Radionuclides, essential and potentially toxic elements metals, speciation, mercury, environment and health, biogeochemistry, marine environment, metrology contaminated sites, food contaminants, ,
Magali Houde, Montreal, Canada
POPs, PFAS, flame retardants, emerging compounds, Bioaccumulation/ biomagnification, Toxicogenomics, Arctic Marine mammals
Petra Krystek, University of Siegen, Siegen, Germany
Trace elements, (nano)materials, speciation, (hyphenated) analytical techniques, method development, validation, environment, health, exposure, water emissions, sustainability
Lena Q. Ma, Zhejiang University College of Environmental and Resource Sciences, Hangzhou, China Biogeochemistry of trace metals, Phytoremediation and hyperaccumulation, Bioavailability andbioaccessibility, Human health and food safety, Plant metal uptake, Rhizosphere chemistry, Rootexudates, Microbial transformation of metals, Metal transports in plants, Metal detoxification in plantsand microbes
Keith Maruya
Emerging contaminants, persistent organic pollutants, mass spectrometry, passive sampling, bioanalytical screening, contaminated sediments, bioaccumulation, trophic transfer
Volker Matthias, Helmholtz Centre Hereon Institute of Coastal Environmental Chemistry, Geesthacht, Germany Atmospheric chemistry, aerosol particles, air pollution, numerical modeling, emissions (both natural and anthropogenic), NOx, SOx
Derek Muir, Environment and Climate Change Canada, Aquatic Contaminants Research Division, Burlington, Ontario, Canada
Environmental chemistry, Biogeochemistry, Bioaccumulation, Persistent organic pollutants, Chemicals of emerging concern, Chemical inventories, Mercury, Polycyclic aromatic compounds,Arctic, Marine mammals, Fish
Patryk Oleszczuk, Maria Curie-Sklodowska University, Lublin, Poland
Biochar; organic contaminants; heavy metals; polycyclic aromatic hydrocarbons; nanoparticles; sewage sludge; ecotoxicology; remediation
Myrto Petreas, California, Department of Toxic Substances Control Berkeley Laboratory, Berkeley, California,United States of America
Levels, trends, dl-POPs, BFRs, analytical methods, bioaccumulation, biomonitoring, exposure assessment, emission, production, generation

Toxicology and Risk Assessment

Michael Bank, Institute of Marine Research, Bergen, Norway
Mercury, microplastics, ocean health, seafood safety, ecotoxicology, isotopic niches, Bayesian modeling, contaminants
Andreas Gies, Federal Environmental Agency Berlin City Campus, Berlin, Germany
Bioassays, human biomonitoring, ecotoxicology, epidemiology, indoor air, oil fracking and drilling
Giulia Guerriero, University of Naples Federico II, Napoli, Italy
antioxidative physiological defence, steroids and steroid receptors, antioxidants under steroidcontrol, non-invasive environmental monitoring, remediation assessment, biodiversity conservation microassays, reproductive health assessment, reprotoxicity monitoring
Jian-Ying Hu, Peking University College of Urban and Environmental Sciences, Beijing, China
Bioassays, human biomonitoring, effects of EDCs on reproduction and development, health risk assessment, ecological risk assessment, Biomagnification in aqueous food-web, epidemiology, and drinking water quality.
Nynke Kramer, Wageningen University & Research, Wageningen, Netherlands

Toxicokinetics, Next generation risk assessment, In vitro toxicology, PBPK modelling, Toxicology, Pharmacokinetics, Chemical safety assessment, Ecotoxicology

James Lazorchak, United States Environmental Protection Agency, Washington, District of Columbia, United States of America

Effect of EDCs on fish populations; Estrogenicity of WWTP discharge; G expression; Toxicity effects on a population; Invasive toxic algae; DNA and protein adducts; Impact of coal; Mineral, gas and oil extraction; Pharmaceuticals; Water quality criteria, Ecotoxicology, Cyanotoxins, Microplastics

Alvine Mehinto, Southern California Coastal Water Research Project, Costa Mesa, California, United States of America

Cell bioassays, Transcriptomics, Ecotoxicology, Fish physiology, Ecological risk assessment, Emerging contaminants, Microplastics

Marisa Passos, European Commission Joint Research Centre Ispra, Ispra, Italy

Ecotoxicology, toxic effects of environmental contaminants on aquatic organisms, bioaccumulation studies (phyto-/zoo- plankton, bivalves, zebrafish embryos), nanotoxicity, in vitro cytotoxicity, FET assay, nano-/micro- plastic particles, nanomaterials, nanoparticles, EDCs.

Willie Peijnenburg, Leiden University Institute of Environmental Sciences, Leiden, Netherlands

Risk assessment, Ecological risk assessment, Environmental fate and effect assessment, Nanoparticles, Bioavailability, Metals, Organics, Quantitative structure-activity relationships (QSARs), Transformation of chemical substances, Biodegradation, Abiotic transformations, Pollutants, fate, toxicity, LCA, Persistent chemicals, Modeling, Predictive toxicology, Soil pollution, Water pollution, Nanomaterials, Microplastics

Frederik-Jan van Schooten, Maastricht University, Maastricht, Netherlands

Treatment and Remediation

Enric Brillas, University of Barcelona, Barcelona, Spain

Anodic oxidation; Electrochemical advanced oxidation processes; Electro-Fenton; Photoelectrocatalysis; Photoelectro-Fenton

Teresa J. Cutright, University of Akron, Akron, Ohio, United States of America

bioremediation, phytoremediation, environmental engineering

Sergi Garcia-Segura, Arizona State University, Tempe, Arizona, United States of America

Water treatment, Persistent organic pollutants, Electrochemically driven processes, Electrochemical Advanced Oxidation Processes, Electrochemical oxidation Electro-Fenton, Photoelectrocatalysis, Electrochemical management of nitrogen cycle, Photocatalysis, Nanotechnology

Jun Huang, Tsinghua University, Beijing, China

Advanced oxidation processes (AOPs), advanced reduction process (ARPs), photochemistry, mechanochemistry, per- and polyfluoroalkyl substances (PFAS)

Am Jang, Sungkyunkwan University College of Natural Science, Suwon, South Korea

Resources Recovery, FO, RO, Water Reuse, Foulng

Hyunook Kim, University of Seoul, Department of Environmental Engineering, Seoul, South Korea

Biological removal of organic compounds, nutrient removal, analysis and degradation of trace organics, odorants from water/wastewater

Yongmei Li, Tongji University, Shanghai, China

Removal and recovery of phosphorus; biological nitrogen removal; anaerobic fermentation of biosolids; fate and attenuation of emerging organic contaminants

Tsair-Fuh Lin, National Cheng Kung University, Tainan, Taiwan

identification, treatment, and process modeling relevant to cyanobacteria, taste and odor compounds and cyanotoxins present in reservoirs and water treatment plants, monitoring and treatment of arsenic and chlorinated hydrocarbons in ground water

Grzegorz Lisak, Nanyang Technological University, Singapore, Singapore

Waste to energy, Waste to materials, Circular economy, Waste management, Soil remediation, Municipal solid wastes, Gasification, Pyrolysis, Application of waste derived materials, Waste upcycling and recycling

Yu Liu, Nanyang Technological University, Singapore, Singapore

Energy- and carbon-neutral municipal wastewater reclamation, Aerobic and anaerobic membrane bioreactors, Integrated mainstream anammox processes, Circular economy-driven energy and resource recovery from biosolids, Biofilms and microbial granulation

Junfeng Niu, North China Electric Power University, Beijing, China

Electrochemical wastewater treatment, Electrochemical oxidation, Photochemistry, Removal of emerging contaminants from (waste)water, Physicochemical wastewater treatment processes, Advanced wastewater treatment

Adalberto Noyola, National Autonomous University of Mexico, Ciudad de México, Mexico

Biological wastewater treatment, anaerobic process for wastewater and sludge treatment, biological nitrogen removal, biofiltration of odorous gases, control of GHG emissions from wastewater treatment facilities

Chang Min Park, Kyungpook National University, Daegu, South Korea

Sustainable development of multifunctional nanostructures, Environmental remediation, Fate processes of engineered and Natural nanomaterials
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Environmental biotechnology, biodegradation, microbial electrochemical technology, biological wastewater treatment, bioremediation
Xiangru Zhang, Treatment and Remediation, Chemosphere, Hong Kong University of Science and TechnologyDepartment of Civil and Environmental Engineering, Kowloon, Hong Kong, China
Water treatment, drinking water, emerging compounds, disinfection byproducts

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disinfection by-products, fate of micropollutants in the aquatic environment and water treatment processes, ozonation, advanced oxidation
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Persistent Organic Pollutants and Dioxins
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Jiarui Han, The Hong Kong University of Science and Technology, Department of Civil and Environmental Engineering, Kowloon, Hong Kong, China
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Persistent Organic Pollutants and their environmental fate, transport and passive sampling methods
Muhammad Zaffar Hashmi, COMSATS University Islamabad, Islamabad, Pakistan
Treatment Technologies for PCBs and Arsenic, Toxicology, Antibiotics and Antimicrobial Resistance, Microplastic pollution, E-waste Pollution
Ron Hoogenboom, Wageningen University, Wageningen, Netherlands
dioxins, PCBs, transfer, bioassay, PFASs, analysis, risk assessment, exposure assessment **Guanghui Hua**, South Dakota State University, Department of Civil and Environmental Engineering, Brookings, United States of America
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Arctic, emerging contaminants
Sarit Kaserzon, University of Queensland, Brisbane, Queensland, Australia
High-resolution mass spectrometry for targeted and non-target chemicals, Passive sampling, Occurrence and fate of emerging contaminants, Biomonitoring, Environmental chemistry
Martine Leermakers, VUB University, Brussel, Belgium
Trace metals, organometals, metalloids, radionuclides, analytical techniques, geochemical cycling,

metals and human health, gel diffusion techniques , for in situ trace metal speciation DGT (diffusive gradients in thin films) and DET (diffusive equilibrium in thin films), mining

Stefan van Leeuwen, Wageningen University, Wageningen, Netherlands

PFASs; BFRs; dioxins/PCBs; Fish; Exposure assessment; Human biomonitoring; Analytical techniques; Interlaboratory studies; Effect-directed analysis; Food safety

Pim Leonards, VU University Amsterdam Institute for Environmental Studies, Amsterdam, Netherlands

metabolomics, analytical chemistry, flame retardants, dust

Domen Lestan, University of Ljubljana, Ljubljana, Slovenia

Soil washing, phytoextraction, immobilisation of toxic elements, metals in soil, metals bioavailability and bioaccessibility, soil functioning, soil ecosystem services

Hong-Bo Li, Nanjing University, Nanjing, China

Metal bioavailability and bioaccessibility in soil, dust, food, human metal exposure and health risk, in vivo and in vitro methods, factors influencing metal bioavailability, strategies to decrease metal bioavailability

Xing-Fang Li, University of Alberta, Edmonton, Alberta, Canada

HPLC-MS, Water disinfection byproducts, Toxicology, Water Disinfection byproducts, Analytical and Environmental Toxicology

Heng Liang, Harbin Institute of Technology, School of Environment, Haerbin, China

Membrane-based water treatment process, Membrane fouling, Drinking water treatment, Water reuse, Advanced oxidation

Kai Liu, Westlake University Division of Environment and Resources, Hangzhou, China

Advanced Oxidation Process (AOP), Metal-Organic Frameworks (MOFs), Photoelectrochemical Water Splitting, Water Harvesting, Emerging Contaminants

Zekun Liu, University of California Riverside, Riverside, California, United States of America

PFAS degradation, UV-based reduction

Jian Lu, Yantai Institute of Coastal Zone Research, Yantai, China

Emerging contaminants; Endocrine -disrupting chemicals; Antibiotics and heavy metals; Antibiotic resistance genes; Microplastics; Biodegradation; Persistent organic pollutants; Fate and transport; Coastal and marine pollution; Aquaculture and pollution

Zhi Luo, Huazhong Agriculture University College of Fisheries, Wuhan, China

Aquatic Animals, Molecular Toxicology, Molecular Nutrition, Risk Assessment of Potential Toxicants

Xiang-Zhou Meng, Tongji University, Shanghai, China

persistent organic pollutants; wastewater; sewage sludge; soil; occurrence; fate; risk assessment **William Mitch**, Stanford University, Department of Civil and Environmental Engineering, Stanford, California, United States of America

Environmental organic chemistry, disinfection byproduct formation mechanisms, nitrosamines, other research interests include the formation of nitrosamines formed by the reaction of NOx with amines used to capture CO2 from power plants, the effect of halides on the natural (i.e., sunlight)or engineered (i.e., advanced oxidation) photodegradation of contaminants, and the reductive transformation of contaminants sorbed to black carbons.

Hyo-Bang Moon, Hanyang University, Seongdong-gu, South Korea

Persistent organic pollutant, Emerging contaminant, Bioaccumulation, Human biomonitoring, Non-target analysis

Catherine Munschy, French Research Institute for the Exploitation of the Sea Atlantic Centre, Nantes, FrancePersistent Organic Pollutants, Contaminants of Emerging Concern, Marine Ecosystems, Trophic Webs, Bioaccumulation, Biomagnification, Top predator fish

Hai Tran Nguyen, DuyTan University Institute of Fundamental Science and Application, Da Nang, Viet Nam

Adsorption, nanomaterial, water treatment, water pollution, waste management

Pongsak Noophan, Kasetsart University, Bangkok, Thailand

biological treatment processes

Guillermo Quijano, National Autonomous University of Mexico Research Laboratory of Advanced Water Treatment Processes, Querétaro, Mexico

Biogas, Desulfurization, Greenhouse Gases, Wastewater Treatment, Hydrogenotrophic Processes, Volatile Metil Siloxanes, Volatile Organic Pollutants, Anoxic Denitrifying Processes, Renewable Energy, Microalgae

Gerhard Rimkus, Intertek Caleb Brett Germany GmbH, Hamburg, Germany

Synthetic fragrances and personal care products in the environment; Bioaccumulation and metabolism in biota like fish, seals, birds etc.; Bioaccumulation in human tissue/breast milk; Analysis of contaminants in biota and food samples; Residues and contaminants in food, EU food legislation

Paolo Roccaro, University of Catania, Catania, Italy

Water quality, removal of micro-pollutants from water, Formation and control of disinfection by-products (DBP) in water, Removal and control of contaminants of emerging concern (CEC) in water and wastewaters systems, Wastewater treatment and reuse, Monitoring and modeling trace contaminants in water by spectroscopy, soil and aquifer remediation, Water quality management in

precision farming, Waste valorization, Human exposure to airborne contaminants

Rosaria Sciarrillo, University of Sannio, Benevento, Italy

Thyroid, neurotox, nonylphenol effects, western blotting, phytoremediation, marine sediment, reptiles
Virender K Sharma, Texas A&M University, College Station, Texas, United States of America
Advanced Oxidation Processes, Ferrate, Nanomaterials, Engineered and Natural Nanoparticles, Disinfection Byproducts, Remediation, Environmental Persistence Free Radicals
Liguo Shen, Zhejiang Normal University, Jinhua, China
Membrane technologies, water treatment, antifouling modification, membrane fouling mechanism, environmental remediation
Reyes Sierra-Alvarez, The University of Arizona, Tucson, Arizona, United States of America
Bioremediation, water and wastewater treatment, microbial toxicity, PFAS, emerging contaminants, engineered nanomaterials
Shane Snyder, The University of Arizona, Tucson, Arizona, United States of America
Drinking water, hydraulic fracturing, produced water, water treatment processes (particularly advanced oxidation), use of cellular bioassays for characterizing complex mixtures of contaminants **Gwonhwa Song**, Korea University, Seongbuk-gu, South Korea
Ecotoxicology, Reproductive Biology, Zebrafish
Athanasios S. Stasinakis, University of the Aegean, Department of Environment, Mytilini, Greece Wastewater treatment and valorization, Sludge management, Emerging contaminants, Aquatic pollution, Biodegradation
Werner Tirler, Eco Research Srl, Bolzano, Italy
air pollution, dioxins
Ngoc Han Tran, National University of Singapore, Singapore, Singapore
Environmental analytical chemistry, Emerging contaminants, Transformation of emerging contaminants, High-resolution mass spectrometry for targeted and non-target analyses, Occurrence and fate of emerging contaminants
Dan Tsang, The Hong Kong Polytechnic University, Department of Civil and Environmental Engineering, Hong Kong, Hong Kong
Green chemistry/engineering, Soil/sediment remediation, Engineered biochar, Wastevalorization, Resource recovery, Wastewater/stormwater treatment, Catalytic conversion/ degradation, Pollutant transport, Environmental pollution | Sustainable urban development, Contaminated land and water, Waste management (food, wood, Green remediation, Wastewater treatment, CO2 adsorption
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Bioremediation, Biodegradation of hydrocarbons, Biosorption of heavy metals, Treatment of industrial effluents, Solid waste management
David Volz, University of California Riverside, Riverside, California, United States of America
Developmental toxicology, Exposure science, Risk assessment, Molecular toxicology **Hongtao Wang**, Tongji University, Shanghai, China
Sludge conditioning, Advanced oxidation processes, Removal of heavy metals from aqueous phase **Qilin Wang**, University of Technology Sydney, Faculty of Engineering and Information Technology, Broadway,Australia
Anaerobic digestion technologies, Wastewater treatment technologies, Sludge and waste treatment technologies, Biological nutrient removal, Aerobic digestion, Microplastics, Antimicrobial resistance, Greenhouse gas, Algae, Biochar, Fermentation, Bioenergy
Zhi Wang, Chinese Academy of Sciences Institute of Geodesy and Geophysics, Wuhan, China
Ecotoxicology, Toxic algae bloom and microcystins, Antibiotics and antibiotic resistance genes, Pollutant removal/phytoremediation, Emerging contaminants
Zongsu Wei, Aarhus University Centre for Water Technology, Aarhus, Denmark
Advanced oxidation, photocatalysis, emerging contaminants, aquatic chemistry, water and wastewater treatments, ,
Ping Xiang, Southwest Forestry University, Kunming, China
Indoor pollution and human health, Mechanistic toxicology, Novel flame retardants, Bioavailability and intestinal cell absorption, Soil pollution and food safety
Lingtian Xie, South China Normal University, School of Environment, Environmental Research Institute, Guangzhou, China
The impacts of temperature and pollutants on the functional integrity of the aquatic ecosystems, Trophic transfer of pollutants in aquatic ecosystems, The effects of emerging contaminants inaquatic organisms, Endocrine disruption chemicals, The evolution of resistance to contaminants **Zeyu Yang**, Environment Canada Emergencies Science and Technology Division, Ottawa, Canada
Organic contaminants; Oil fingerprinting; Fate and behavior of oil and organic contaminants; Analytical method development; Bioavailability assessment of organic contaminants; Passive sampling technologies; Polycyclic aromatic hydrocarbons; Petroleum biomarkers; Naphthenic acids Chromatography
Xin Yu, Xiamen University, Xiamen, China
Drinking water, health-related water microbiology, water and wastewater treatment, bloom algae

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INTRODUCTION

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