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COMMENT

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Glyphosate lessons: is biodegradation of pesticides a harmless process for biodiversity?

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Abstract

The historical perspective on the rapid biodegradation of pesticides as a mitigating factor in environmental risk assessment is reexamined through the example of glyphosate and its implications for freshwater biodiversity. Commonly employed standardized methods by national agencies for assessing the environmental risk of pesticides predominantly rely on single-species tests, overlooking the intricate nature of ecosystems. Glyphosate, one of the most widely used pesticides marketed for its purported rapid biodegradability, is often perceived as relatively innocuous. However, its degradation releases phosphorus into the environment, inducing a trophic state shift in water systems towards more eutrophic conditions, consequently affecting water quality. These findings highlight the cascading ecological repercussions of glyphosate biodegradation, driving the proliferation of specific aquatic organisms, such as picocyanobacteria and metaphyton, resulting in the alteration of ecosystem structure and dynamics. The study explores challenges posed by commercial pesticide formulations and investigates the consequences of pesticide interactions with specific anthropogenic factors. A case in point is the interaction of glyphosate with the invasive mussel *Limnoperna fortunei*, exacerbating the overall scenario. The ecological framework analyzed challenges the conventional notion that pesticide biodegradation is inherently a neutral or positive event. The results underscore the necessity of reassessing the role of biodegradation itself in environmental impact assessments for pesticides.

Keywords Pesticides, Biodegradation, Glyphosate, Pollution, Ecotoxicology, Environmental risk

Introduction

Historically, the environmental persistence of contaminants, particularly pesticides, has been considered a critical factor influencing their possible impacts and so their risk assessment [1]. Alarms arise when contaminants exhibit high persistence and/or biomagnification like the DDT [2], while relief comes with rapid dissipation, often through biodegradation. Recently, Maggi et al. [3] have estimated that the major degradation of pesticides, approximately 82%, occurs in the soil through biological mechanisms. This fact seems to be good news about their environmental risk. However, it is imperative to consider whether biodegradation of pesticides truly represents a harmless process for biodiversity. In this paper, we aim to comment why pesticide biodegradation could not be advantageous for the environment.

Generally, studies on the impact of pesticides and their degradation products have primarily focused on laboratory toxicological research, especially on single-species assays. Typically, these trials begin with tests on single-species cultures, overlooking studies of greater complexity such as those involving two or more species or communities with different trophic levels [4]. While these studies are significant, their extrapolation is limited when considering the actual ecological impacts on the environment. How do the direct impacts of both the active ingredients and/or their degradation products affect target and non-target populations in a natural

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system, where they interact with other populations (e.g., competition, predation) and the abiotic environment? It becomes apparent that indirect impacts in the natural environment can be substantial and may even surpass the direct ones. Most of the existing literature predominantly focuses on biodegradation from a bioremediation perspective or analyzes the toxicity of intermediate products, leaving relatively unexplored the ecological perspective of how pesticide biodegradation itself impacts various aspects of biodiversity.

Biodiversity decline is one of the major global threats in the context of global change. Recently, there has been increased emphasis on studying chemical pollution as a driver of biodiversity decline, underscoring the necessity of adopting a more ecosystem-centered approach for risk assessment. The significance of biodiversity extends beyond its intrinsic value; it serves as a vital source of natural contributions to people, often referred to as ecosystem services, making its preservation crucial. Therefore, there is a pressing need for comprehensive ecosystem studies for risk assessment to better understand and manage the intricate relationships between biodiversity, chemical pollution, and the provisioning of ecosystem services [5].

In the current landscape of global agriculture, pesticides play a pivotal role in pest management, crucial for obtaining crop yields. However, it is worth noting that in conventional industrial production systems, pesticides are not so much enhancing yields as they are necessary for any significant yield at all. The dependence on pesticides is so significant that crop production would be severely compromised without them, particularly due to the reliance of specific crop varieties on these chemicals [6, 7]. Among the most widely employed pesticides, glyphosate stands out as the primary herbicide integrated into the Roundup Ready® soybean—glyphosate technological package and other genetically modified crops. The widespread adoption of this herbicide has reshaped agricultural practices, particularly in soybean cultivation, prompting increased scientific scrutiny into its environmental and human health impacts [8, 9]. Glyphosate's presumed short half-life in soil and water has been viewed as a favorable characteristic, suggesting that it may not pose the same persistence risks as some older pesticide active principles. Nevertheless, the biodegradation of glyphosate reveals alarming negative implications for the environment and overall biodiversity [10, 11]. In particular, it is noteworthy that freshwater systems are heavily impacted by pesticides originating from agricultural activities within their watersheds serving as sinks for the runoff from such activities [12, 13]. The objective of this work is to examine the consequences of glyphosate biodegradation on freshwater systems and to utilize its example to advocate for a paradigm shift in environmental pesticide risk assessment, focusing specifically on the active ingredient glyphosate, irrespective of its various commercial formulations. Here, we discuss the assertion that biodegradability inherently reduces the environmental risk of any pesticide, particularly in terms of its impact on biodiversity and environmental health.

How do we assess pesticides' environmental risk?

To assess pesticide environmental risk and establish guidance levels, governmental entities and national agencies worldwide typically rely on criteria from organizations such as the Environmental Protection Agency (EPA, USA) and the European Food Safety Authority (EFSA, European Union), usually considering the opinions of the World Health Organization´s (WHO) and the Food and Agriculture Organization (FAO), both from United Nations. Nevertheless, beyond their distinct focus and criteria, the different agencies and authorities in general use the same type of studies for the environmental risk assessment [14].

Globally, environmental pesticide risk assessment is based on two components: (1) the ecological effects and (2) the exposure characterization [15]. The first is based on pesticide's toxicity to several organisms and ecological entities (e.g., communities), using some habitual endpoints under different concentrations or levels. Usually, various standardized toxicity tests, including acute, subacute, and chronic tests, are conducted on key sentinel species measuring endpoints like No Observed Adverse Effect Concentration/Level (NOAEC/L), the concentration causing 50% lethality (LC50), and the concentration/dose producing 50% of the maximal response (EC50/ED50). Assuming that model species adequately represent natural community compositions and sensitivities, tests are conducted on species such as *Skeletonema costatum* [16], *Lemna minor* [17], *Anabaena flos-aquae* [18], *Pseudokirchneriella subcapitata* [19], and a freshwater diatom (*Navicula* sp.) [20] for assessing non-target aquatic plant phytotoxicity due to herbicides, while the cladoceran *Daphnia magna* [21] is standardized for invertebrates. However, the sensitivity of species to contaminants varies significantly, rendering reliance solely on a single non-target test species inadequate for assessing the ecotoxicological risk of pesticides in aquatic biota [22]. To address this limitation, Probabilistic Risk Assessment (PRA), grounded in species sensitivity distribution, has been proposed as a quantitative alternative for risk evaluation [23, 24]. Despite its merits, PRA may not comprehensively represent real-life aquatic ecosystem responses to pesticide exposure due to its inherent omission of various ecological interactions [25].

Another significant aspect in the environmental risk assessment of pesticides is the exposure characterization. Predicted environmental concentrations are estimated as the ambient concentration of a chemical in the environment. They are typically predicted using exposure models and available data on chemical use, fate, and behavior in the environment [26]. Elevated environmental persistence, typically quantified by long half-lives, is considered a substantial threat, as prolonged exposure periods for organisms heighten the potential for biomagnification [27]. In accordance with guidelines, ecological approaches are only pursued if the predicted environmental concentrations exceed the risk concentrations determined in single-species assays [4, 28]. In these cases, to capture a more ecological framework, ecotoxicological studies have transitioned from controlled laboratory conditions and single-species assays to outdoor mesocosm approaches [29]. However, a considerable number of pesticides have not undergone examination under such conditions, and most of them have been evaluated only as single active ingredients, presenting formidable challenges given the plethora of commercial formulations and environmental mixtures.

In essence, this comment highlights a significant concern: the quick biodegradation of active ingredients leads to reduced anticipated environmental concentrations, thus sidestepping additional ecological assessments. Nonetheless, it is crucial to acknowledge the potential ecological ramifications stemming from this rapid biodegradability itself.

What does glyphosate teach us?

Below this paradigm, the glyphosate's relatively low toxicity in single-species tests and its classification as low persistent pesticide, resulted in some of the highest environmental protection guideline levels among all pesticides. As it was stated above, standardized methods used by national agencies to assess pesticide risks have limitations due to the complexity of ecosystems. To address this, the case of glyphosate illustrates the need for multiscale analyses. Glyphosate, the world's most widely used herbicide, has been extensively studied in freshwater environments, utilizing microcosms and outdoor mesocosms to mimic ecosystem conditions [30–33].

Glyphosate is marketed for its purported rapid biodegradability, touted as a property that renders it relatively innocuous. This attribute, emphasizing the efficient breakdown of glyphosate in environmental conditions, contributes to its promotion as a seemingly environmentally friendly herbicide. Glyphosate is a low-molecular-weight phosphonate; upon introduction into water bodies, the fate of glyphosate, like that of any other pesticide, is subject to various factors. The original molecule may be absorbed, complexed, precipitated, or undergo degradation processes such as photolysis, oxidation, hydrolysis or biodegradation [34, 35]. The primary degradation products of glyphosate include aminomethylphosphonic acid (AMPA) and sarcosine [35]. It is noteworthy that documented records suggest the toxicity of these degradation products may surpass that of the original active ingredient [36].

This insight is derived from laboratory studies conducted in monospecific-scale microcosms. In the environment, the ultimate degradation of glyphosate results in the release of phosphorus into the surrounding environment, leading to a shift in the trophic state of water systems towards more eutrophic scenarios with consequential significant changes in the structure and functioning of the ecosystem. Hérbert et al. [37] estimated that the current phosphorus (P) load derived from glyphosate into hydrological basins worldwide is now reaching levels comparable to those from fertilizers. In the USA, it increased from 1.6 kg P/km^2 in 1993 to 9.4 kg P/km^2 in 2014, with values frequently exceeding 20 kg P/km^2 in areas planted with glyphosate-resistant crops. Pérez et al. [30] used 25 m^3 outdoor mesocosms to evaluate the effect on aquatic systems of the glyphosate-based formulation Roundup®, demonstrating significant changes in the microbial community structure and function. They demonstrated that the swift biodegradation of glyphosate played a pivotal role yielding diverse effects on autotrophic microbial communities. Glyphosate is known to play as an antibiotic and probably contributes to the direct mortality of some specific algal and cyanobacteria species. Nevertheless, its presence did not result in a reduction in the specific richness of larger phytoplankton (micro + nano-phytoplankton) but did lead to a decrease in their abundance, indicating both the mortality of some organisms and a physiological response to herbicide-induced stress, reducing reproduction. In contrast, picocyanobacteria exhibited a 40-fold increase in abundance compared to controls. This phenomenon could be attributed to several factors. Firstly, picocyanobacteria demonstrate the capacity to utilize phosphorus from glyphosate as a nutrient; certain picocyanobacteria possess the ability to break down the phosphonate bond of glyphosate [38]. Secondly, the observed increase could be a result of reduced competition due to the diminished abundance of larger algae, indicating trophic modifications within the community. Alternatively, it could be a combination of both factors acting simultaneously. The introduction of phosphorus into the aquatic system via glyphosate appears to be primarily targeting the biomass of picocyanobacteria.

While possessing the shikimic acid pathway, surviving autotrophic organisms exhibit diverse responses to glyphosate exposure. These responses often involve physiological adaptations such as modifications in gene expression that leads on the synthesis of protective substances [39], or an overproduction of the enzyme EPSP (target of glyphosate in the shikimic metabolic pathway of synthesis of aromatic amino acids). Organisms that withstand glyphosate through these adaptive mechanisms can capitalize on the bio-available phosphorus released from bacterial biodegradation of glyphosate. Phosphorus differential exploitation triggers ecosystem-wide changes, culminating in a significant surge in picocyanobacteria abundance, ultimately leading to a substantial enhancement in the overall phytoplankton primary production and turbidity of water bodies [30, 31].

The key findings resulting from micro- and mesocosm trials, carried out in both laboratory and outdoor conditions, were validated through in situ studies at the landscape scale [40, 41]. The surveys spanned Pampa and Patagonia lakes indicate significant impacts of glyphosate-based herbicides on freshwater microbial communities. Authors found evidence that supports the increased picocyanobacteria abundance in response to long-term glyphosate-based agricultural practices, highlighting the consequential effects on microbial ecosystems.

An ecological study of the environmental risk of a pesticide should also analyze the characteristics of the ecosystems it impacts. The resilience of each system to pesticide exposure is crucial, as their vulnerability varies based on the characteristics of their constituent communities, which ultimately determine their recovery capability [42]. We observed this phenomenon through various tests on clear freshwater systems (low turbidity, mesotrophic) and turbid systems (high organic turbidity, eutrophic), each with distinct planktonic community compositions, comparing the effects using glyphosate [43] and 2,4-D [44]. In both approaches, the impact of pesticides was scenario-dependent, with clear systems exhibiting greater resilience than turbid systems.

Moreover, compounding the complexities delineated hitherto, lies in the utilization of commercial formulations. In the instance of glyphosate, akin to all pesticides, the application in the field comprises a blend encompassing the active ingredient alongside assorted additives and adjuvants, exhibiting variations among commercial brands and even within the same brand across different batches. This intricate variability exacerbates the difficulty in evaluating its impacts. Evidence of this is found in the study conducted by Sabio and García et al. [45], wherein they compared the impacts of five distinct glyphosate-based herbicides, including the monoisopropylamine salt of glyphosate (GIPA), on microbial communities in natural shallow lakes. They observed significantly different effects on the structure of phytoplankton based on the specific glyphosate-based herbicide used, even when the herbicides shared similar active ingredients. Meanwhile, Lipok et al. [33] compared the toxicity of pure glyphosate, GIPA, and isopropylamine salt on various species of algae and cyanobacteria, finding that predominantly isopropylamine alone was more toxic than glyphosate. In general, the composition of additives and coadjuvants in commercial formulations remains largely unknown while salt-compositions are disclosed. These components, in addition to their intrinsic toxicity, may potentially introduce nutrients into the system. Gattás et al. [46] performed a comparative analysis of nutrient levels in water after applying both a commercial glyphosate formulation and the active ingredient. Their study revealed a notable increase in ammonium concentration in the commercial formulation compared to the pure active ingredient.

Finally, in the broader context of global change, the confluence of simultaneous anthropogenic drivers may result in synergistic effects. This is exemplified by the introduction of glyphosate into a system inhabited by the invasive mussel *Limnoperna fortunei*, which arrived in South America in the 1990s and is now widely distributed throughout the entire estuary basin of the Río de la Plata. Experimental evidence demonstrates that the invasive mussel reduces the half-life of glyphosate by a factor of four, implying a pronounced acceleration of its biodegradation [47]. In experimental outdoor mesocosm conditions, it has been demonstrated that the acceleration of glyphosate biodegradation mediated by the potent degradation capabilities of mussel resulted in the massive development of microalgae forming macroscopic mats (metaphyton) [48] which often float and accumulate in large masses along the shores and eddies of aquatic systems. This community, in turn, represents the fate of phosphorus introduced into the system via glyphosate. These findings underscore the cascading ecological consequences of accelerated glyphosate biodegradation, highlighting the shift towards the proliferation of specific aquatic organisms and the subsequent alteration of the structure and dynamics within the ecosystem.

What insights does glyphosate offer? It highlights that the biodegradation of any pesticide in the environment involves significant and intricate variations that must be ecologically considered on an ecosystem scale. This understanding has been made possible by the extensive research conducted on glyphosate, the world's most applied and studied pesticide, allowing for a comprehensive examination of its effects and contributing to a broader awareness of the complex ecological dynamics associated with pesticide use. All the instances demonstrated by glyphosate contribute to the understanding that it is necessary to conduct ecosystem-level approaches to determine responses and reduce uncertainty regarding the true impact of pesticides on the environment.

Conclusions

The example of glyphosate illustrates that biological degradation is not neutral; rather, organisms involved in the biodegradation process may be favored and have the potential to alter the entire ecosystem based on their roles and behavior. These effects, whether in soil or aquatic environments, could propagate trophically and/or persist over time, potentially altering the overall quality of an ecosystem. It is crucial that environmental risk assessment methods take this factor into account and develop strategies to gain a comprehensive understanding of the actual impact of pesticide biodegradation on the environment. This issue must be supplemented by considering the implications of the fact that what is actually applied in the field is composed of commercial formulations, so the active ingredient plus additives and adjuvants, forming mixtures with varied chemical characteristics, which do not undergo any risk analysis. We understand the urgency of integrating such considerations into risk assessment protocols. Our primary suggestion is to emphasize the need for a comprehensive review of existing risk assessment, highlighting the various aspects currently overlooked that contribute to the significant environmental and public health issues associated with pesticides. It is imperative that risk analyses be approached with a more ecological focus. The example of glyphosate should serve as an alarming signal, as the dominant toxicological perspective in ecotoxicology has consistently underestimated the impacts of pesticides, considered the world's most significant environmental contaminant, over the last two decades. Our intention is to contribute evidence aimed at raising awareness among the scientific community and decision-makers about the necessity of incorporating more ecological approaches on pesticide risk evaluation.

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VLL and HNP conceived and wrote equally this work based on the last 15 years of H.P. research.

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