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Introduction

Groundwater Ecotoxicology and Chemistry

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INTRODUCTION

Ecotoxicology is the study of the effects and fate of contaminant substances on ecosystems. It aims to determine if there is an ecological risk by comparing the concentration that produces toxic effects with the environmental concentration of these chemicals. Although, René Truhaut was de facto recognized as the originator of this area of study, the pioneering contributions of Jean-Michel Jouany, Truhaut's assistant, in conceptualizing the discipline and defining its objectives are now fully acknowledged (Di Marzio & Sáenz, 2013; Vasseur et al., 2021). Jouany envisioned ecotoxicology as closely linked to ecology, aiming to understand the influence of stress factors on the relationships between organisms and their habitats. This vision has been realized through a substantial body of ecotoxicological research in marine, freshwater, and terrestrial ecosystems from the poles to the tropics. It is likely that, some 50 years ago, Jouany did not envision that we would be discussing the ecotoxicology of groundwater environments. Groundwater ecosystems are crucial to our planet's health, and ecotoxicological knowledge is needed to ensure the protection of the ecosystems and their functions; however, ecotoxicological knowledge of the subsurface lags far behind that of surface ecosystems.

Aquifers and the groundwater ecosystems within them provide approximately 30% of the world's freshwater and support diverse biological communities (Gleeson et al., 2012; Saccò et al., 2024). Subterranean waters play an essential role in sustaining agriculture, industry, and human consumption, with nearly 2.5 billion people depending on groundwater for their daily water needs (Morris et al., 2003). They are the most widespread land-based ecosystem on earth. Yet, despite their significance, groundwater ecosystems are "out of sight and out of mind," resulting in omission from environmental policies and insufficient public awareness and legislative protection against contamination (Boulton et al., 2023).

The biodiversity of groundwaters is different from that occurring in surface environments, particularly in terms of morphological, behavioral, and physiological traits (Hose et al., 2022) and responses to stressors (Di Lorenzo et al., 2023; Di Marzio et al., 2009, 2013). Groundwater ecosystems host a wide variety of life forms, including bacteria; fungi; invertebrates, such as crustaceans and insects; and occasionally vertebrates. Many of these organisms are specially adapted to the dark, nutrient-poor conditions of subterranean habitats (Marmonier et al., 2023). Microbial species play key roles in biogeochemical cycles, including the transformation of nitrogen, sulfur, and carbon compounds (Fillinger et al., 2023; Griebler & Lueders, 2009), while invertebrates maintain water flow in the aquifer and control microbial communities (Hose et al., 2023). The presence and activity of these organisms help maintain the quality and availability of groundwater, making them essential for ecosystem services that humans rely on (Hose et al., 2023).

The fauna of groundwaters is collectively referred to as *stygofauna*, which is an homage to the Greek Styx, who was a goddess and a river in the underworld that bordered Hades, the resting place of the dead. Styx was the daughter of Oceanus, the Titan and great river that encircled the earth and a brother to the Potamoi, over 3000 gods of rivers and streams. These family ties show that even the ancient Greeks recognized the significant connections between surface and subterranean waters. The gods would swear to Zeus "by the inviolable water of Styx," referring to the absolute and sacrosanct integrity of Styx, which could not be compromised. However, more recent history has shown that groundwaters can be violated, contaminated, and overexploited and their ecological integrity compromised as a result (Kretschmer et al., 2023). Rather than being "inviolable," they are indeed among the most fragile and threatened natural environments of the world (Kurwadkar et al., 2020).

As global awareness of, interest in, and threats to groundwater ecosystems increase, we felt it timely to review the state of knowledge on the pathways and impacts of stressors into and on groundwater ecosystems, through a special issue of this journal focused on groundwater ecotoxicology and chemistry. We are pleased to provide readers with a series of papers that progress significantly the understanding of contaminants and their impacts in the aquatic subsurface.

THREATS TO GROUNDWATER ECOSYSTEMS AND NEW KNOWLEDGE

Despite their importance, groundwater ecosystems are under significant threat from various human activities. Industrial pollution, agricultural runoff, and improper waste disposal introduce contaminants such as nitrates, heavy metals, pesticides, and

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pharmaceuticals into aquifers (Castaño‐Sánchez et al., 2020). These pollutants can have detrimental effects on groundwater quality and the health of subterranean organisms (Foster & Chilton, 2003). In addition, overextraction of groundwater for human use can lead to reduced water levels, habitat loss, and decreased biodiversity (Gleeson et al., 2012; Kurwadkar et al., 2020). Protecting groundwater ecosystems from these many stressors requires comprehensive management strategies that integrate scientific understanding with policy measures (Griebler et al., 2023). This includes monitoring groundwater quality, regulating pollutant sources, and promoting sustainable water‐use practices. Conservation efforts must also address the need for habitat protection and restoration, ensuring that groundwater‐dependent species and processes are maintained (Boulton et al., 2023; Morris et al., 2003).

This compendium of papers reflects that diversity of chemical threats and pathways for contamination of groundwater ecosystems. Agriculture is the major global user of groundwaters for irrigation and the primary user of agrochemicals such as pesticides (Food and Agriculture Organization of the United Nations, 2022, pp. 1990–2020). It is not surprising then that pesticides are common contaminants of groundwaters (Castaño‐Sánchez et al., 2020; Kurwadkar et al., 2020). The ability of pesticides to move (leach) through the soil profile into groundwaters is assessed using a variety of leaching indices, the efficacy of which has been reviewed in this issue (Araya et al., 2024). This is an important and timely evaluation because these indices are a key tool for protecting groundwaters from pesticide contamination.

Many pesticide leaching indices are based on simple physicochemical properties of the active ingredients and the attributes of the soil to which they are applied (Araya et al., 2024). The simplicity of many indices limits their predictive ability and utility, particularly for polar pesticides. The omission of important (i.e., equally or more mobile or toxic) by‐products in the estimation of these indices means that the risk posed by the parent compounds may be underestimated. However, in many cases, environmental decisions must be made with limited available data; and in this case, the leaching metrics provide a valuable "stopgap" until further knowledge is available. Like all metrics used in ecological risk assessments, there is a need to validate the results based on local conditions, such as soil types, pesticide use regimes, and climate.

As Araya et al. (2024) highlight, pesticides have potential to leach through soils and contaminate groundwaters and pose an ecological risk where concentrations in groundwater are present above levels that are toxic to groundwater biota. Estimates, such as those from leaching metrics or from empirical measurements, mean that the "contamination" side of the risk‐assessment process is often well characterized; but the impacts of those contaminants, specifically to groundwater ecosystems, remain poorly known, and where known, such data are often disparately located and hard to find. With this in mind, Groote‐Woortmann et al. (2024) consolidated the available ecotoxicological data for groundwater fauna in their STYGOTOX database.

Given the global distribution of aquifers, their societal importance, and the role of groundwater biota in the delivery of clean groundwater, it is surprising that risk assessments for groundwater lag far behind those of surface ecosystems; the vision of Louray for all ecosystems is not yet fully realized. Nevertheless, there is a growing body of ecotoxicological research on groundwater fauna, which has accelerated over the last two decades. Groote‐Woortmann et al. (2024) captured 46 studies, providing 472 toxicity endpoints for groundwater species, including groundwater specialist and nonspecialist inhabiting taxa. The database includes data for 43 stressors, covering (among other types) pesticides, metals, salinity, pharmaceuticals, and organic compounds. Toxicity data were available for 19 different pesticide compounds, which reflects only a small proportion of the many different biocide compounds in use globally and highlights the dearth of knowledge on the mobility, fate, and effects of pesticides and other contaminants in groundwater. This dearth of knowledge for so many contaminants highlights the ongoing need for simple metrics or indices, to inform risk assessments in such a data‐deficient space.

The general dominance of crustaceans in groundwater ecosystems (Marmonier et al., 2023) is reflected in the available ecotoxicological data, with crustaceans accounting for the majority of test taxa. Also included were Oligochaeta, fungi, and mites (Acarina). The new ecotoxicological data presented in this issue by Adams et al. (2024) and Di Cicco et al. (2024) are from tests also focusing on Crustacea (Copepoda) and will be included in regular updates to the STYGOTOX database. In most cases, organisms for testing were derived from field collections, which reflects the challenges with culturing groundwater organisms because of their typical traits of long life spans and low metabolic and reproductive rates (Di Lorenzo et al., 2019; Hose et al., 2022).

Metal contamination of groundwaters has occurred historically as a consequence of mining and leaching of wastewaters. In recent decades, the global demand for energy, particularly gas, has led to exploitation of unconventional gas resources. The extraction of gas held tightly in geological formations requires drilling and hydraulic fracturing (*fracking*), which are aided by fluids containing complex mixtures of additives, including metals, such as barium (Ba). Adams et al. (2024) evaluate the toxicity of Ba to stygobitic (groundwater specialist) copepods and, in doing so, tackle the challenging geochemistry of Ba, which is insoluble in the presence of sulfates. The study provides an extensive data set, testing the sensitivity of copepods in the presence of dissolved Ba and precipitated, insoluble $BaSO_4$. Not surprisingly, the Ba, present as insoluble $BaSO_4$, had little effect on the copepods, even as a precipitated floc. However, soluble Ba was highly toxic in a range of test waters. While providing new data for an important contaminant, the study shows that synthetic diluent waters can be used for testing stygofauna, meaning that organisms do not need to be tested in water from their collection site, which makes it possible to test and compare the impact under more standardized conditions.

The final study in the compendium further extends the range of contaminants tested using groundwater organisms by providing novel toxicity data from exposing surface‐dwelling and groundwater specialist copepods to tetrachloroethylene. Tetrachloroethylene is a common contaminant of groundwater across the world (Liu et al., 2020), making these data internationally relevant. Perhaps most valuably, this research develops and demonstrates the utility of sublethal response measures for groundwater fauna, which the authors link to the provision of ecosystem services and ecological functions. By comparing surface‐dwelling and obligate subterranean species, this research also contributes to the ongoing discussion of the relative sensitivities of groundwater and surface water to contaminants and whether toxicity data for surface‐water taxa are an appropriate surrogate for use in risk assessments (see Di Lorenzo et al., 2021, 2023; Di Marzio et al., 2009; Hose, 2005, 2007; Humphreys, 2007). In this case, the greater sensitivity of the surface‐dwelling copepod makes it a potentially conservative and useful surrogate for the groundwater species.

FUTURE DIRECTIONS

The outcomes of the research presented in this special series have significantly advanced science and begun to fill important knowledge gaps. Toxicity data for Ba and tetracholorethylene specifically for specialist groundwater taxa expand the limited database with previously untested compounds. Importantly, these newly tested compounds are common contaminants of groundwaters (Liu et al., 2020) and are thus valuable to progressing ecological risk assessments. However, data are absent for many other contaminants, and there is much more work to be done. Even for those compounds for which there are toxicity data for groundwater taxa, the range of taxa tested remains limited and generally insufficient for robust risk assessments or for deriving environmental quality guidelines, where multiple taxa across different phyla are required. Expanding the suite of contaminants (including mixtures) and the range of taxa tested should be priorities for advancing risk assessments for groundwater ecosystems (Adams et al., 2024; Di Cicco et al., 2024; Groote‐Woortmann et al., 2024). Groote‐Woortmann et al. (2024) highlight the absence of toxicity data for subterranean vertebrates, which are often iconic and culturally significant, and warrant targeted conservation (Boulton et al., 2023).

The toxicity tests reported in these papers, including most of those in the STYGOTOX database, were done using field‐collected animals. The challenges of culturing groundwater taxa under laboratory conditions are well documented (Di Lorenzo et al., 2019; Di Marzio et al., 2009), although there has been some recent progress in this regard (Rütz et al., 2023). Nevertheless, further research leading to the establishment of cultures of groundwater taxa, from crustaceans to microbes, would enable a range of experimental research, not just in ecotoxicology, and could provide a step‐change in understanding the functioning of groundwater ecosystems and the impacts of environmental change.

While the discipline of groundwater ecotoxicology and chemistry remains deficient in empirical data, the continued use of summary metrics and surrogate data is vital. Araya et al. (2024) show that this is the case for assessments of groundwater leaching and recommend that, where indices are used, they are used as an initial screening tool that is supported by further validation and investigation. Di Cicco et al. (2024) show that the study species *Bryocamptus zschokkei* was more sensitive to tetracholorethylene than the stygobitic *Moraria* sp. and may be a useful substitute in future studies, but further work is needed to reliably extrapolate to other contaminants.

CONCLUSION

Groundwater ecosystems are critically important but are increasingly threatened by human activities. Industrial pollution, agricultural runoff, and improper waste disposal introduce harmful contaminants into aquifers, affecting water quality and subterranean organisms. Overextraction of groundwater further exacerbates these issues by lowering water levels and reducing biodiversity. Effective protection of groundwater ecosystems necessitates comprehensive management strategies, including monitoring water quality, regulating pollutants, and promoting sustainable water‐use practices. In addition, habitat protection and restoration are essential for maintaining groundwater‐dependent species and ecological processes.

The diverse chemical threats to groundwater ecosystems, especially from agriculture, are significant. Pesticides, common groundwater contaminants, are assessed using leaching indices, which, despite their limitations, serve as valuable tools in environmental risk assessments. However, there remains a gap in knowledge about the impacts of these contaminants on groundwater ecosystems. The STYGOTOX database consolidates ecotoxicological data, highlighting the need for more research on various pollutants and their effects on groundwater biota.

Efforts to protect these ecosystems are hampered by gaps in reporting and testing. The major points of improvement in groundwater toxicity testing include the need for validity and compliance information (e.g., percentage of mortality in controls), detailed test media characteristics (pH, hardness, dissolved oxygen), chemical quality, and outcomes from range‐finding tests (Di Lorenzo et al., 2019; Di Marzio et al., 2009, 2013, 2018). Broader data on stressors and a larger number of taxa are also required. Future toxicity tests should include greater taxonomic detail, exposure durations beyond 96 h, and studies investigating the toxicity of mixtures and multiple stressors.

In summary, protecting groundwater ecosystems from contamination and overuse is crucial due to their vital ecological services and the increasing pressures from human activities. The papers presented in this special series provide valuable new knowledge to inform and improve the assessment of risks of contaminants in groundwaters, but further research and improved management strategies are essential for safeguarding these often-overlooked components of the natural world.

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