

**Environmental Risk Assessment of glyphosate and
AMPA in Portuguese Groundwater Ecosystems**

**Avaliação do Risco Ambiental de glifosato e AMPA em
Ecossistemas de Águas Subterrâneas em Portugal Continental**

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Dissertação para a obtenção do Grau de Mestre em
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que nunca desistam de lutar pelos vossos sonhos.*

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Resumo

O objetivo do presente estudo foi avaliar o risco relacionado com a exposição dos ecossistemas aquáticos subterrâneos ao glifosato e ao seu metabolito AMPA com base na abordagem do risco entre as concentrações medidas recolhidas do Sistema Nacional de Informação de Recursos Hídricos e as normas de qualidade das águas subterrâneas estabelecidas na nova proposta legislativa e estimadas a partir de normas de qualidade ambiental para as águas de superfície.

O glifosato foi analisado em 103 amostras de água subterrânea colhidas em 80 locais em 21 sistemas aquíferos de quatro unidades hidrogeológicas de Portugal Continental, entre 2019 e 2021. Foi detetado em 14% do total de amostras, porém apenas 10% apresentaram níveis de concentração acima de 0.1 µg/L, norma de qualidade para as águas subterrâneas estabelecida na nova proposta legislativa, embora nenhum desses valores tenha ultrapassado o valor de 8.67 µg/L estimado da norma de qualidade ambiental proposta para o glifosato. Em comparação, o AMPA foi detetado em apenas 5% das 63 amostras de águas subterrâneas, em quatro poços. Em ambos, a concentração máxima foi quantificado num poço localizado no sistema aquífero O25 - Torres Vedras, da Orla Ocidental, com 4.690 µg/L e 4.240 µg/L para glifosato e AMPA, respetivamente.

Os resultados do presente estudo demonstram que é importante sensibilizar e formar os agricultores sobre a utilização sustentável de produtos fitofarmacêuticos e boas práticas agrícolas, a fim de prevenir a contaminação das águas subterrâneas e melhorar a sua qualidade. Adicionalmente, o ensino agrícola relacionado com a fitofarmacologia desempenha um papel fundamental na formação avançada de especialistas para a redução dos riscos da utilização de produtos fitofarmacêuticos.

Há também uma necessidade urgente de realizar testes ecotoxicológicos com outras espécies de águas subterrâneas pertencentes a diferentes grupos funcionais para obter uma norma de qualidade que represente verdadeiramente as comunidades de águas subterrâneas.

Palavras-chave: Águas subterrâneas; Glifosato; AMPA; Portugal; Normas de qualidade para as águas subterrâneas.

Abstract

The aim of the present study was to assess the risk related to the exposure of the groundwater ecosystems to glyphosate and its metabolite AMPA based on the risk approach between measured concentrations retrieved from the Water Resources Information System of Portugal, and groundwater quality standards set in new legislation and estimated from environmental quality standards in surface water.

Glyphosate was analysed in 103 groundwater samples collected from 80 wells located in 21 aquifer systems from four hydrogeological units of mainland Portugal, between 2019 and 2021. It was detected in 14% of the total samples, however only 10% presented concentration levels above 0.1 µg/L, the groundwater quality standard set in the new legislative proposal, although none of these values exceeded the value of 8.67 µg/L estimated from the environmental quality standard proposed for glyphosate. In comparison, AMPA was detected in only 5% of 63 groundwater samples, in four dug wells. In both compounds, the maximum concentration level was quantified in a dug well located in the O25 - Torres Vedras aquifer system, from the Western unit, with 4.690 µg/L 4.240 µg/L for glyphosate and AMPA, respectively.

The results of this study demonstrate that it is extremely important to raise awareness and training to farmers on the sustainable use of plant protection products and good agricultural practices, in order to prevent groundwater contamination and improve its quality. In addition, agricultural education related to pesticide science plays a fundamental role in the advanced training of specialists to reduce the risks of using plant protection products.

There is also an urgent need to carry out ecotoxicological tests with further groundwater species belonging to different functional groups to obtain a quality standard that truly represents the groundwater communities.

Keywords: Glyphosate; AMPA; Groundwater; Portugal; Groundwater quality standards.

Resumo Alargado

As águas subterrâneas são um dos recursos naturais com maior importância para o Homem, dado que é a maior reserva de água doce disponível mundialmente e a maior fonte de água potável, sendo predominantemente utilizada para abastecimento público e para a agricultura [1-4].

Contudo, a qualidade das mesmas tem vindo a diminuir consideravelmente devido a várias causas como as alterações climáticas, a contaminação ambiental e a pressão antropogénica, que contribuem para o aumento da recolha excessiva de água e para o aumento da contaminação da mesma por poluentes provenientes da atividade humana, nomeadamente da indústria, da extração mineira, das descargas domésticas e da prática agrícola [3,5,6].

Para fazer face ao supracitado, a União Europeia definiu um quadro de política ambiental que visa a monitorização da qualidade das águas subterrâneas através da gestão integrada das mesmas para o bem-estar humano, para garantir o seu desenvolvimento sustentável e proteger os ecossistemas naturais dependentes, dentro do quadro das seguintes Diretivas e Regulamento Europeus: (i) Diretiva 2000/60/CE (Diretiva Quadro da Água); (ii) Diretiva 2006/118/CE (Diretiva das Águas Subterrâneas); (iii) Diretiva 98/83/CE (Diretiva da Água destinada ao consumo humano); (iv) Diretiva 2008/105/CE (Normas de Qualidade Ambiental); (v) Regulamento Europeu (CE) n.º 1107/2009 [3,7,8].

Adicionalmente, a Comissão Europeia tem vindo a desenvolver novas estratégias nos últimos anos, nomeadamente a atualização das normas de qualidade para algumas substâncias, bem como a criação de um grupo de trabalho para as águas subterrâneas, a *Groundwater Working Group*. Este grupo de trabalho estabelece uma colaboração entre a Comissão, os Estados-Membros e as partes interessadas, com o objetivo de encontrar novas abordagens mais eficientes e eficazes, denominada por Estratégia de Implementação Comum [3,8,9]. As novas normas de qualidade das águas subterrâneas foram avaliadas pelo Comité Científico dos Riscos para a Saúde, Ambientais e Emergentes (SCHEER), que concluiu que a utilização de uma norma de qualidade não superior a 0.1 µg/L nas águas subterrâneas protege o ecossistema das mesmas [10,11]. Contudo, esta norma de qualidade tem como base a vasta literatura existente sobre a avaliação da toxicidade dos poluentes nos organismos aquáticos das águas superficiais, em vez dos organismos aquáticos presentes nas águas subterrâneas (denominados por estigofauna) [6,12].

A Comissão procedeu a uma revisão da lista de substâncias prioritárias do anexo X da Diretiva 2000/60/CE, assim como a uma revisão das listas de substâncias

constantes dos anexos I e II da Diretiva 2006/118/CE, tendo concluído que, à luz dos novos conhecimentos científicos, é adequado alterar essas listas através da inclusão de novas substâncias, do estabelecimento de normas de qualidade ambiental ou de normas de qualidade das águas subterrâneas para as substâncias agora acrescentadas, da revisão das normas de qualidade ambiental de algumas substâncias já incluídas em consonância com o progresso científico e do estabelecimento de normas de qualidade ambiental para o biota para algumas substâncias já incluídas e outras agora acrescentadas.

O presente estudo pretendeu avaliar a qualidade das águas subterrâneas em Portugal Continental, tendo em conta as concentrações medidas do herbicida glifosato e do seu principal metabolito, ácido aminometilfosfónico (AMPA), em amostras de águas subterrâneas entre 2019 e 2021, em Portugal, e recolhidas do Sistema Nacional de Informação de Recursos Hídricos (SNIRH). Nesta avaliação do risco ambiental foi considerada a recente norma de qualidade para as águas subterrâneas do grupo dos metabolitos de pesticidas não-relevantes, aditado ao anexo I da Diretiva 2006/118/CE relativa à proteção das águas subterrâneas contra a poluição e a deterioração, assim como a norma de qualidade estabelecida e estimada para o glifosato, a partir da norma de qualidade ambiental para as águas de superfície. As Diretivas 2000/60/CE, 2006/118/CE e 2008/105/CE devem ser alteradas em conformidade na nova proposta.

O herbicida glifosato foi selecionado dado que é uma das substâncias ativas mais utilizadas em produtos fitofarmacêuticos a nível mundial para controlo de infestantes, devido à sua ação sistémica de amplo espectro [13-15], assim como uma das substâncias mais discutidas atualmente e que pode ser futuramente incluída na lista de substâncias prioritárias proposta pela Comissão, ou seja, com normas de qualidade ambiental definidas apenas para a mesma [8]. Considerou-se ainda, para o presente estudo, a avaliação do metabolito AMPA, resultante de processos de degradação que o glifosato sofre quando entra em contacto com o solo [15-17]. Esta substância pertence ao grupo dos metabolitos não-relevantes (nrMs) [18,19], aditadas ao anexo I, sendo para cada uma estabelecidas normas de qualidade das águas subterrâneas [20-22].

Quanto à área em estudo, esta é caracterizada pela heterogeneidade da paisagem ao longo de aproximadamente 92212 Km² [23], facto que contribui para uma boa diversidade hidrogeológica [5,19,24,25], e divide-se em quatro unidades hidrogeológicas, de acordo com as suas características geológicas [5,26-28]: (i) Maciço Antigo; (ii) Orla Ocidental; (iii) Bacia do Tejo-Sado; (iv) Orla Meridional. Cada uma destas unidades incorpora um conjunto de sistemas aquíferos dos 62 existentes em Portugal Continental [26,27].

O Maciço Antigo é a maior unidade hidrogeológica, contudo possui apenas 11 sistemas aquíferos. Comparativamente, a Orla Ocidental, que apenas abrange parte da zona Oeste do país, possui 28 sistemas aquíferos. A Bacia do Tejo-Sado é a unidade hidrogeológica com menos sistemas aquíferos (cinco) e localiza-se na bacia dos rios Tejo e Sado, uma das nove zonas consideradas vulneráveis de Portugal Continental [29]. Relativamente à Orla Meridional, esta unidade hidrogeológica situa-se no litoral Algarvio e possui o segundo maior número de sistemas aquíferos (18) [26].

Os valores de glifosato e AMPA recolhidos do SNIRH corresponderam a 103 e 63 amostras de águas subterrâneas provenientes de 80 e 63 captações, respetivamente, de diversos tipos e finalidades (furo vertical, furo horizontal, poços e nascentes; abastecimento público e doméstico, agricultura (rega), monitorização, sem dados disponíveis [26]), localizadas em 21 sistemas aquíferos pertencentes às quatro unidades hidrogeológicas supracitadas, entre abril de 2019 e junho de 2021. Recolheram-se 21 amostras em cinco sistemas aquíferos do Maciço Antigo, 24 amostras em nove da Orla Meridional, 54 e 14 amostras (para o glifosato e o AMPA, respetivamente) em seis da Orla Ocidental e quatro amostras em apenas um sistema da Bacia do Tejo-Sado.

O glifosato foi detetado em 14% do total de 103 amostras, sendo que em 10% foram apresentados níveis máximos de concentração iguais ou superiores a 0.1 µg/L, a norma de qualidade da água subterrânea estabelecida; contudo, não foi detetado nenhum valor que excedesse 8.67 µg/L, a norma de qualidade ambiental estimada com base na norma de qualidade ambiental-média anual estabelecida em águas de superfície para o glifosato. As mais elevadas frequências de deteção foram encontradas nas unidades hidrogeológicas da Bacia do Tejo-Sado (75%), seguido pelo Maciço Antigo (14%), Orla Ocidental (13%) e Orla Meridional (4%). Estes níveis máximos de concentração foram observados nas quatro unidades hidrogeológicas, sendo maioritariamente na Orla Ocidental, em cerca de 56% das amostras, seguida da Bacia do Tejo-Sado com 22%. Foi no sistema aquífero O25 - Torres Vedras, situado na Orla Ocidental, que se verificou o maior nível de concentração desta substância (4.690 µg/L). Foram em cinco furos verticais (três para rega na agricultura, dois para abastecimento público e um sem dados) e quatro poços (um doméstico e três sem dados) que se detetaram os níveis de concentração de glifosato iguais ou superiores a 0.1 µg/L, assim como numa nascente para abastecimento público.

Relativamente ao AMPA, este metabolito não foi detetado com valor superior a 5 µg/L, a norma de qualidade ambiental estimada com base na norma de qualidade ambiental-média anual estabelecida em águas de superfície para o AMPA. As únicas quatro deteções positivas foram encontradas em poços, por ordem decrescente da sua

concentração, nos sistemas aquíferos O25 - Torres Vedras (4.24 µg/L), T7 - Aluviões do Tejo (1.91 µg/L), O33 - Caldas da Rainha-Nazaré (0.454 µg/L) e A0 - Maciço Antigo Indiferenciado (0.05 µg/L). Nestas amostras foram simultaneamente quantificadas concentrações de glifosato, em valores superiores à do AMPA, à exceção da amostra recolhida no sistema aquífero do O25 – Torres Vedras.

A deteção do maior nível de concentração, tanto de glifosato como de AMPA, no sistema aquífero O25 - Torres Vedras verificou-se num poço cuja finalidade é desconhecida. Este poço localiza-se no meio de uma aldeia, pelo que se estima que possa ter decorrido de uma contaminação pontual, como por exemplo uma incorreta eliminação de embalagens de produtos fitofarmacêuticos à base de glifosato, não tendo o seu utilizador seguido as instruções nos rótulos destes produtos e as boas práticas agrícolas. Acresce afirmar que as condições edafo-climáticas e a sua vulnerabilidade, assim como as práticas agrícolas, podem conduzir à lixiviação do glifosato para as águas subterrâneas.

Face ao exposto, para evitar a contaminação das águas subterrâneas é fundamental a sensibilização e formação dos agricultores para o uso sustentável dos produtos fitofarmacêuticos bem como das boas práticas agrícolas. Paralelamente, é essencial compreender a estigofauna e desenvolver mais estudos sobre os efeitos das substâncias ativas nestes ecossistemas por forma a estabelecer uma norma de qualidade para as águas subterrâneas mais adequada e representativa para as mesmas.

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List of Abbreviations

Abbreviation	Designation
AA-EQS	Annual Average value of Environmental Quality Standards
AA-EQS_{ISW}	Annual Average value of Environmental Quality Standards of inland surface waters
AF	Assessment Factor
AGG	Assessment Group on Glyphosate
AMPA	Aminomethylphosphonic Acid
CIS	Common Implementation Strategy
DT₅₀	Half Dissipation Time
DWD	Drinking Water Directive
EC	European Commission
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EPSP	Enolpyruvyl Shikimate Phosphate
EQS	Environmental Quality Standards
EQSD	Environmental Quality Standards Directive
EQS_{GW}	Environmental Quality Standards of groundwaters
EU	European Union
FOCUS	FORum for the Co-ordination of pesticide fate models and their Use
FW	Freshwaters
GAP	Good Agricultural Practice
GUS	Groundwater Ubiquity Score
GW	Groundwater
GWD	Groundwater Directive
GWG	Groundwater Working Group
GWWL	Voluntary Groundwater Watch List
HRAC	Herbicide Resistance Action Committee
HU	Hydrogeological Units
IARC	International Agency for Research on Cancer
ISW	Inland Surface Waters
Koc	Water Partition Coefficient
LC-MS/MS	Liquid Chromatography coupled to tandem Mass Spectrometry
LOQ	Limit of Quantification
MEC_{GW}	Measured Environmental Concentrations in groundwater

NrM	Non-relevant Metabolite
PAFF Committee	Standing Committee on Plants, Animals, Food and Feed
PFAS	Perfluoroalkyl and Polyfluoroalkyl Substances
PNEC	Predicted No Effect Concentration
PNEC_{FW}	Predicted No Effect Concentration in freshwaters
PPP	Plant Protection Products
QS_{GW}	Groundwater Quality Standards
SNIRH	Sistema Nacional de Informação de Recursos Hídricos
WFD	Water Framework Directive

1. Introduction

Groundwater stores almost one third of total global freshwater resources and, in the European Union, supplies 65% of drinking water and 25% of water for agricultural irrigation [1]. In addition, groundwater is also an ecosystem hosting a vast diversity of microbial and metazoan species playing a key role in ecosystems services related to water purification and nutrient turn-over. Many of the groundwater-dwelling organisms (stygofauna) are endemic and highly specialized to a life in permanent darkness [2].

The Groundwater Directive [3] provides the detailed procedures for meeting the Water Framework Directive's [4] environmental objectives for groundwater quality. It sets European Union-wide groundwater quality standards for nitrates (50 mg/L) and active substances in pesticides, including their relevant metabolites, degradation and reaction products (0.1 µg/L and, for total, 0.5 µg/L) in Annex I, requires Member States to set threshold values for substances of national concern, including, where relevant, those listed in Annex II, and requires measures to be taken to prevent or limit the input of pollutants into groundwater.

Based on country data reported to the European Environment Agency, pesticides were found to exceed legal limits at between 4% and 11% of groundwater monitoring sites in Europe, from 2013 to 2021 [5]. Given the great difficulty to restore contaminated groundwater bodies, as well as indications that groundwater ecosystems are more vulnerable to stressors than many other freshwater ecosystems due to slower biological and physical degradation processes, they need to be treated with a corresponding level of care [6].

In order to revise the watch lists of surface water and groundwater pollutants to be monitored and controlled to protect freshwater bodies, a proposal was tabled by the Commission in October 2022 [7,8] alignment with the European Green Deal's zero pollution ambition of having an environment free of harmful pollution by 2050. The Council adopted its negotiating mandate on the revisions of the Water Framework Directive [4], Groundwater Directive [3] and Environmental Quality Standards Directive (Surface Water Directive) [9] on 19 June 2024. The list of water pollutants was updated by adding new pollutants and related quality standards for some per- and poly-fluorinated alkyl substances (PFAS), pharmaceuticals and pesticides [10].

Glyphosate was one of the 23 individual substances added to the priority substance list with environmental quality standard set for each individually [10]. Through the groundwater watch list process, 17 countries provided groundwater data on the non-relevant metabolite's compounds for review. The data indicate that non-relevant metabolites were widely detected in European groundwater above limits of quantification. The non-relevant metabolite monitoring results show 16 substances were detected in four or more participating countries and at 10 or more sites in each of these countries. Aminomethylphosphonic acid (AMPA)

fulfilled the criteria for addition to the list facilitating the 6-yearly review of Groundwater Directive Annexes I and II [11].

The environmental risk assessment of pesticides in groundwater with the generic value of 0.1 µg/L is questionable since this value was not based in ecotoxicological data with stygofauna. In fact, only a small number of studies with pesticides have been done using aquatic subterranean organisms [12,13] since they are often naturally present in low numbers, difficult to collect, and difficult to culture under laboratory conditions [14,15]. This scarcity of ecotoxicological data for subterranean fauna has led to the use of sensitivity data for surface species as surrogate in the development of environmental quality and protection thresholds. The new guideline from the European Medicines Agency on the environmental risk assessment of medicinal products for human use stipulates to calculate the predicted no-effect concentration for the groundwater compartment based on the predicted no-effect concentration for the surface water compartment and an additional assessment factor [16].

Since glyphosate is the most frequently used herbicide in Portugal [17] and along with non-relevant metabolite AMPA constitute new substances added to the lists of pollutants that need to be controlled, this study aims to provide potential scenarios of environmental risk induced by them in Portuguese groundwaters. The approach followed is based on the relation between measured environmental concentrations gathered from the Water Resources Information System of Portugal [18] and groundwater quality standards set in legislation and estimated from environmental quality standards in surface waters [10]. Implications of these findings for the classification of the chemical status of groundwater bodies in Portugal are discussed.

2. Materials and Methods

2.1. Environmental fate, behavior and ecotoxicology of glyphosate and AMPA

In terms of chemical characteristics, glyphosate is a small molecule with three polar functional groups (carboxyl, amino, and phosphonate groups), and behaves as an amphoteric molecule. It is highly polar and soluble in water (> 100 g/L at 20 °C), and is strongly adsorbed onto variable-charge surface sites in the soil, particularly iron and aluminum oxides. Glyphosate is highly persistent, with a DT₅₀ value (time for the dissipation of 50% of the initial concentration) in the soil that equals 161.1 days (Table 1; [19,20]).

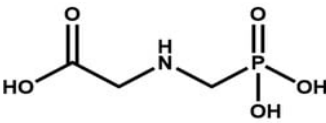
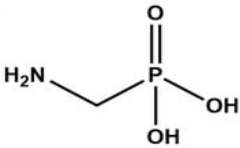
Glyphosate in the environment is primarily degraded by microorganisms, which use it as a source of carbon and phosphorus. In anaerobic soil incubations glyphosate was stable compared to aerobic incubation conditions [20]. The enzymatic reactions involved in microbial degradation lead to the cleavage of either the C-N or C-P bond, resulting in the formation of

aminomethylphosphonic acid (AMPA) or sarcosine, respectively, as metabolic products. AMPA is the main metabolite of glyphosate and is an aminophosphonate with a primary amine group. AMPA is polar and highly soluble in water (1467 g/L at 20 °C) and is more persistent in the environment than glyphosate ($DT_{50} = 1040$ days). Owing to these characteristics, AMPA tends to accumulate in soil. Similar to glyphosate, the adsorption of AMPA is associated with the presence of amorphous aluminum and iron oxides. According to Carretta *et al.* (2022) [21], AMPA has the same high adsorption range as glyphosate, and they seem to be sorbed to the same extent without any apparent competition for adsorption sites. Nevertheless, it seems that glyphosate and AMPA might not sorb the same way onto the soil, despite their similar atomic compositions.

N-(phosphonomethyl)-glycine), commonly referred to as glyphosate, is the active substance phosphonomethyl derivative of the natural amino acid glycine used in plant protection products to control weeds. It exerts its herbicidal activity by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase of the shikimate metabolic pathway. This leads to a blockage of the biosynthesis of essential aromatic amino acids and consequently to plant death [22]. The shikimate metabolic pathway is present in all plants and thus glyphosate acts as a non-selective broad-spectrum herbicide. It is the most frequently used herbicide both worldwide and in the European Union and it has been used for several decades. Glyphosate-based pesticides are used as herbicides in agriculture, horticulture and in some non-cultivated areas, such as urban areas or along roadways and railway. They are typically applied before crops are sown to control weeds and therefore facilitate better growth of crops by eliminating competing plants [23].

The vulnerability of groundwater to contamination by pesticides via soil is determined by a combination of factors, including the characteristics of the pesticides themselves (e.g., adsorption, degradation, and solubility) and site-related factors such as climate, land use, and soil and hydrogeological conditions. Given the strong tendency to adsorb to soil components, glyphosate and AMPA have been commonly considered to have a low groundwater leaching potential [24].

Table 1. Physical and chemical properties of glyphosate and AMPA.

	Glyphosate	AMPA
Structural formula		
Molar mass (g/mol)	169.10	111.04
Melting point (°C)	189.5	-
Solubility at 20°C (pH 7) g/L	> 100 g/L	1466.561
Vapor pressure at 25 °C (Pa)	1.31×10^{-5}	-
Partition coefficient Log Pow at 25°C (at pH buffers at 7)	-6.28	-1.63
DT₅₀ soil (d)	161.1	1040
DT₅₀ water/sediment/system (d)	143.3	93.1
Koc/Kom (mL/g)	4348/2522	2541/1474
GUS leaching potential index^a	0.29 (improbable leacher)	1.56 (improbable leacher)

^a If GUS > 2.8: leacher (L); if 1.8 < GUS < 2.8: transition (T); if GUS < 1.8: improbable leacher (IL) [24].

2.2. Study area

The present study aims to assess the quality of the groundwater ecosystems in Portugal, at Continental level. Portugal is a country in Southern Europe, occupying a total area of 92212 Km². The mainland is located on the southwest of the Iberian Peninsula, bordering Spain to the north and east, and the Atlantic Ocean to the west and south. Groundwater is unequally distributed in the Portuguese territory due to differences in edaphoclimatic conditions. From an hydrogeological point of view there are porous, karstic and fractured aquifers, which govern the conditions of storage and transmission of water. Groundwater is linked to surface water in many different forms. According to the geological characteristics, mainland Portugal is divided into four main hydrogeological units, each one containing several aquifer systems [25-27].

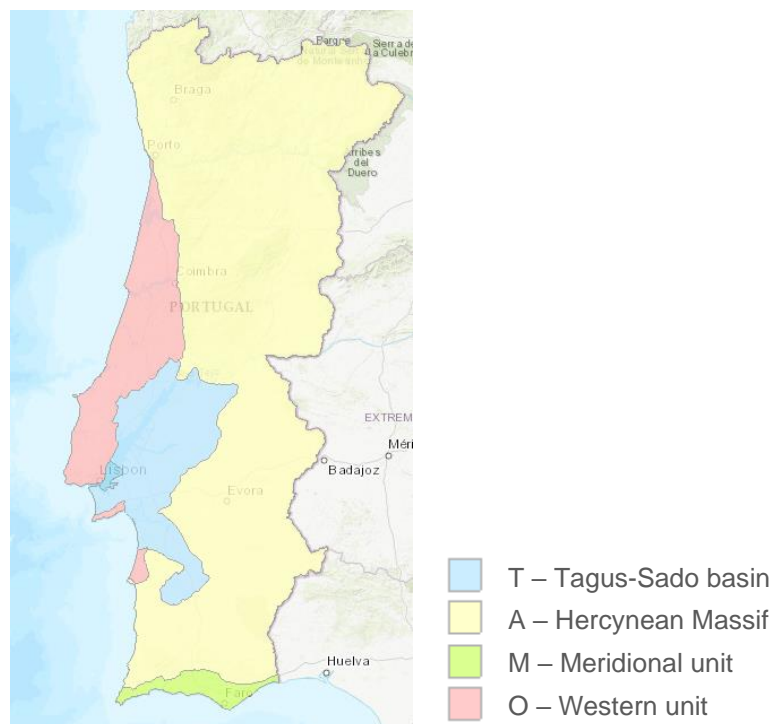


Figure 1. Main hydrogeological unities (Source: [18]).

The Hercyean Massif unit is mainly composed by igneous and metamorphic rocks. Due to their low permeability and specific yield values only some carbonate aquifers in the Alentejo region have been identified in this large region. In the Western unit there are sedimentary and karstic aquifers, which may be confined or unconfined and may locally be artesian. A few aquifers are multi-layer systems, where leakage mechanisms can occur, and provide groundwater interchange between aquifers. The aquifer systems in the Meridional unit are carbonated ones with some karstification development. The Tagus-Sado unit includes the most important aquifer system of the Iberian Peninsula, the Tagus-Sado aquifer system, which covers a large sedimentary basin of about 8000 km². This is a multi-layer system, the deepest aquifer being the most productive one. In some areas the system displays a flowing artesian behavior. This aquifer system is a unique source of water supply for drinking, agricultural and industrial supply. There are also some small alluvial aquifers which have strong links with watercourses with frequent water exchanges [25-27].

2.3. Groundwater data

A search in the groundwater monitoring network of the Water Resources Information System of Portugal [18] was performed to glyphosate and AMPA concentration values. A total of 103 and 63 groundwater samples analysed for glyphosate and AMPA were collected from 80 and 63 sites, respectively (Figure 2).

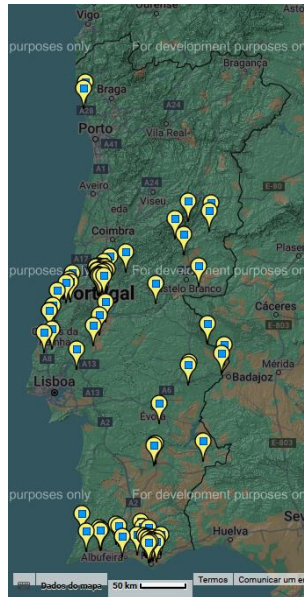


Figure 2. Location of the 80 sites sampled in the mainland Portugal (Source: [18]).

From the 80 sites, 75% are vertical drilled wells mainly used for public supply and agricultural irrigation, with depths varying between 17 and 207 m. They are belonging to 21 of the 62 existing aquifer systems and seven river basins in Portugal: the Cávado/Ribeiras Costeiras basin in the north, the Ribeiras do Oeste basin in the west, the Tejo and Sado basins in the central and the Ribeiras do Algarve, Guadiana and Arade basins in the south (Table 2). Sampling was carried out during April-June 2019, September-December 2020 and April-June 2021.

Table 2. Number of sites and groundwater samples collected in the Portuguese river basins and aquifer systems, from April 2019 to June 2021.

Hydrogeological unit / Aquifer system	River basin	Drilled well (Vertical)	Drilled well (Horizontal)	Dug well	Spring	Groundwater samples
<i>Hercynian Massif</i>						
A0 - Maciço Antigo Indiferenciado	Tejo	3	1	3	1	13
	Guadiana	2	0	0	0	
	Ribeiras do Algarve	0	0	1	0	
	Cávado/Ribeiras Costeiras	0	0	2	0	
A4 - Estremoz – Cano	Guadiana	2	0	0	0	2
A9 - Gabros de Beja	Sado	0	0	3	0	3
A10 - Moura – Ficalho	Guadiana	1	0	0	0	1
A11 - Elvas – Campo Maior	Guadiana	1	0	1	0	2
<i>Meridional unit</i>						
M2 - Almádena – Odeáxere	Ribeiras do Algarve	1	0	0	0	1
M3 - Mexilhoeira Grande – Portimão	Ribeiras do Algarve	1	0	1	0	3
	Arade	1	0	0	0	
M4 - Ferragudo – Albufeira	Ribeiras do Algarve	1	0	0	0	1
M5 - Querença – Silves	Ribeiras do Algarve	3	0	0	2	5
M7 - Quarteira	Ribeiras do Algarve	1	0	0	0	1
M9 - Almansil – Medronhal	Ribeiras do Algarve	4	0	0	0	4
M10 - S. João da Venda-Quelfes	Ribeiras do Algarve	3	0	0	0	3
M12 - Campina de Faro	Ribeiras do Algarve	5	0	0	0	5
M13 - Peral - Moncarapacho	Ribeiras do Algarve	1	0	0	0	1

Western unit							
O11 - Sicó – Alvaiázere	Tejo	1	0	0	0	1	
O15 - Ourém	Tejo	20*/3**	0	0	0	43*/3**	
O18 - Maceira	Ribeiras do Oeste	1	0	0	0	1	
O19 - Alpedriz	Ribeiras do Oeste	2	0	0	0	2	
O25 - Torres Vedras	Ribeiras do Oeste	0	0	2	0	2	
O33 - Caldas Da Rainha – Nazaré	Ribeiras do Oeste	3	0	2	0	5	
Tagus- Sado basin							
T7 - Aluviões do Tejo	Tejo	3	0	1	0	4	

* For glyphosate; ** For AMPA

As described in Table 2, in relation to the Hercynian Massif unit, 21 groundwater samples were collected from five aquifer systems located in five river basins placed in the north, central and south of Portugal, with more predominance in the latter. For the Meridional unit, 24 groundwater samples were collected from nine aquifer systems located in two river basins in the Southern Portugal. The largest sampling was conducted in the Western unit, with 54 and 14 groundwater samples collected from 31 and 14 sites for glyphosate and AMPA, respectively, belonging to six aquifer systems in the Tagus river basin. The remaining four groundwater samples were taken from one single aquifer system belonging to the Tagus-Sado unit, located also in the Tagus river basin.

If possible, water should be pumped for approximately five minutes from the well to purge the pipes in order to obtain a representative groundwater sample. Groundwater samples were collected in 150 mL single-use plastic bottles and then kept stored in the dark under cool conditions (use of special thermo boxes) until arrival at the Analysis Laboratory of the Instituto Superior Técnico (LAIST), where they were stored in a refrigerator until analysis at a temperature that did not exceed 5°C. Glyphosate based pesticides were determined through liquid chromatography-tandem mass spectrometry (LC-MS/MS) with derivatization, following the W-PESLMSD1 method. The lowest concentration of glyphosate and AMPA that can be measured with certainty (limit of quantification, LOQ) is 0.03 and 0.05 µg/L, respectively [18]. Field parameters (electrical conductivity and pH) were also measured indicating that the samples were representative of the aquifer conditions.

2.4. Risk characterization of glyphosate and AMPA in groundwater ecosystems

Potential scenarios of environmental risk induced by glyphosate in Portuguese groundwaters were depicted using two different methodologies. One scenario based on the groundwater quality standard for active substances in pesticides, including their relevant metabolites, degradation and reaction products (0.1 µg/L [3,10,28]). The other scenario was based on the European Medicine Agency guideline [16], which suggest using the predicted no-effect concentration for the surface water compartment (PNEC_{SW}) as a proxy of the

predicted no-effect concentration for the groundwater compartment ($PNEC_{GW}$). Groundwater ecosystems are fundamentally different to surface water ecosystems and therefore may be more vulnerable as they lack the ability to recover from perturbations. Consequently, an additional assessment factor of 10 should be applied to extrapolate the $PNEC_{GW}$ from the $PNEC_{SW}$. Since the annual average-environmental quality standard for glyphosate in surface waters is equal to 86.7 $\mu\text{g/L}$ according to Proposal for a Directive of the European Parliament and of the Council amending Directive 2000/60/EC establishing a framework for Community action in the field of water policy, Directive 2006/118/EC on the protection of groundwater against pollution and deterioration and Directive 2008/105/EC on environmental quality standards in the field of water policy [10], then the $PNEC_{GW}$ is 8.67 $\mu\text{g/L}$ (Table 3).

The negotiating mandate on the directive which will amend the Water Framework Directive, the Groundwater Directive and the Directive on Environmental Quality Standards simplified the quality standards for non-relevant metabolites of pesticides in relation to the Commission’s proposal, setting out a unique value equal to 1 $\mu\text{g/L}$ for individual ones. The Council also added the obligation for the Commission to establish a list of known pesticides, indicating if they are relevant or not (Table 3). AMPA does not meet the criteria for “relevant metabolites” or that for “metabolites of no concern”, however has been identified in many groundwater bodies across the European Union [11], so it is has been considered in this study a non-relevant metabolite (Table 3).

The risk quotient for the groundwater compartment is determined using the measured environmental concentrations for glyphosate and AMPA gathered from [18] and groundwater quality standards (Table 3). There is an environmental risk when the measured concentration values are higher than the quality standard values.

Table 3. Groundwater quality standards for glyphosate and aminomethylphosphonic acid (AMPA) ($\mu\text{g/L}$).

Pesticide compound	Legislation	Estimated
Glyphosate	0.1 [3,10,28]	8.67
AMPA	1 [10]	-

3. Results and Discussion

3.1. Risk assessment of glyphosate in Portuguese groundwater ecosystems

As shown in Table 4, glyphosate was analysed in 103 groundwater samples collected from 80 sites located in 21 aquifer systems from four hydrogeological units. It was detected in 14% of the total samples, however only 10% presented concentration levels above 0.1 µg/L, the groundwater quality standard set in legislation [3,10,28], although none of these values exceeded the value of 8.67 µg/L estimated from the annual average-environmental quality standard proposed for glyphosate in surface waters [10,16].

The highest detection frequencies were found in the Tagus-Sado basin (75%), followed by the Hercynian Massif (14%), Western (13%) and Meridional (4%) units. The Western was the hydrogeological unit where glyphosate was detected in more aquifer systems: O11 – Sicó – Alvaiázere (100%), O25 - Torres Vedras (100%), O33 - Caldas da Rainha – Nazaré (60%) and O15 - Ourém (2%). The detection frequency of 75% in the Tagus-Sado basin was found in the unique aquifer system sampled, the T7 - Aluviões do Tejo (Table 4). This shallow aquifer system is consisting of alluvial deposits and alluvial terrace deposits, containing alternate layers of sand (fine and coarse) or more or less silt sandstones, clays and silts, sometimes intercalated by gravel and, at the base, by a deposit of sand with gravel. The Tagus river influenced powerfully the groundwater flow. The hydraulic potential influences if Almonda and Alviela tributaries recharge or discharge the alluvial aquifer system. Most of the holes implemented in the system capture in the sands and gravel layers of the alluvium and terraces as they have the highest transmissivity values [29]. In the other hydrogeological units, glyphosate was detected in the A0 - Maciço Antigo Indiferenciado (23%) and M5 – Querença - Silves (20%) aquifer systems (Table 4).

In fact, from 80 sites, glyphosate was quantified in concentration levels above 0.1 µg/L in six vertical drilled wells and four dug wells. With regard to the vertical drilled wells, three are irrigation wells, i.e. used in agricultural practice in the areas where area located the O33 – Caldas da Rainha - Nazaré and the T7 – Aluviões do Tejo aquifer systems. The remaining two vertical drilled wells are used for public supply (A0 – Maciço Antigo Indiferenciado and O11 – Sicó - Alvaiázere) and one belongs to the wells with no information available (M5 – Querença – Silves). Among the four dug wells with glyphosate concentration levels above 0.1 µg/L, two are located in the O33 – Caldas da Rainha – Nazaré, and the other two in the O25 - Torres Vedras and the T7 – Aluviões do Tejo aquifer systems being the last one for domestic use with a depth of 8.9 meters. Maximum concentration levels of 4.69 µg/L, 1.03 µg/L and 0.618 µg/L were reached in three of these dug wells. As shown by several studies reviewed by Barbash and Resek (1997) [30], dug wells have shallow water-table depths, leading to less groundwater

isolation from surface contaminant sources. Furthermore, irrigation wells tend to be located closer to agricultural fields, where pesticides are most commonly applied.

Table 4. Detection frequencies observed for glyphosate analysed in 103 groundwater samples collected from 80 sites of mainland Portugal, from April 2019 to June 2021.

Hydrogeological unit / Aquifer system	Groundwater samples analysed	Groundwater samples with concentration levels (µg/L)										Median (Maximum) concentration (µg/L)
		<LOQ	%	≥LOQ- <0.05	%	≥0.05- <0.1	%	≥0.1- <8.67	%	≥8.67	%	
<i>Hercynian Massif</i>												
A0 – Maciço Antigo Indiferenciado	13	10	77	1	8	1	8	1	8	0	0	0.06 (0.35)
A4 – Estremoz – Cano	2	2	100	0	0	0	0	0	0	0	0	-
A9 – Gabros de Beja	3	3	100	0	0	0	0	0	0	0	0	-
A10 – Moura – Ficalho	1	1	100	0	0	0	0	0	0	0	0	-
A11 – Elvas – Campo Maior	2	2	100	0	0	0	0	0	0	0	0	-
Total	21	18	86	1	5	1	5	1	5	0	0	0.05 (0.35)
<i>Meridional unit</i>												
M2 – Almádena – Odeáxere	1	1	100	0	0	0	0	0	0	0	0	-
M3 – Mexilhoeira Grande – Portimão	3	3	100	0	0	0	0	0	0	0	0	-
M4 – Ferragudo – Albufeira	1	1	100	0	0	0	0	0	0	0	0	-
M5 – Querença – Silves	5	4	80	0	0	0	0	1	20	0	0	0.11 (0.44)
M7 – Quarteira	1	1	100	0	0	0	0	0	0	0	0	-
M9 – Almansil – Medronhal	4	4	100	0	0	0	0	0	0	0	0	-
M10 – S. João da Venda – Quelfes	3	3	100	0	0	0	0	0	0	0	0	-
M12 – Campina de Faro	5	5	100	0	0	0	0	0	0	0	0	-
M13 – Peral – Moncarapacho	1	1	100	0	0	0	0	0	0	0	0	-
Total	24	23	96	0	0	0	0	1	4	0	0	0.05 (0.44)
<i>Western unit</i>												
O11 – Sicó – Alvaiázere	1	0	0	0	0	0	0	1	100	0	0	0.21 (0.21)
O15 – Ourém	43	42	98	1	2	0	0	0	0	0	0	0.03 (0.04)
O18 – Maceira	1	1	100	0	0	0	0	0	0	0	0	-
O19 – Alpedriz	2	2	100	0	0	0	0	0	0	0	0	-
O25 – Torres Vedras	2	0	0	1	50	0	0	1	50	0	0	2.36 (4.69)
O33 – Caldas Da Rainha – Nazaré	5	2	40	0	0	0	0	3	60	0	0	0.19 (0.618)
Total	54	47	87	2	4	0	0	5	9	0	0	0.13 (4.69)
<i>Tagus-Sado basin</i>												
T7 – Aluviões do Tejo	4	1	25	0	0	0	0	3	75	0	0	0.34 (1.03)
Total	4	1	25	0	0	0	0	3	75	0	0	0.34 (1.03)
TOTAL	103	89	86	3	3	1	1	10	10	0	0	0.10 (4.69)

The O25 - Torres Vedras, O33 – Caldas da Rainha – Nazaré and T7 – Aluviões do Tejo are porous aquifer systems while O11 – Sicó – Alvaiázere and M5 – Querença – Silves are fissured including karstified aquifers which productivity varies from moderate to high [31]. According to Carreta *et al.* (2022) [21], transport of pollutants to groundwater can be significant in well-structured soils that are rich in macropores, where it generally occurs via preferential flow. Groundwater quality problems were registered in these aquifers due to their overload with pollutants derived from agriculture and livestock, or from urban origin [31]. The T7 – Aluviões do Tejo aquifer system belongs to the Tagus vulnerable zone, in accordance with the Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources [32], which contribute to the leaching of pollutants through the soil and unsaturated zone into the groundwater. This transport process with pesticides is mainly influenced by edapho-climatic conditions, pesticide properties, and management practices. The infiltration of glyphosate via bank due to the large proportion of land treated with this pesticide, jointly with the connectivity of surface water bodies to groundwater aquifers could be another possible pathway for contamination of groundwater, as acknowledged by the experts at the Pesticides Peer Review Experts Teleconferences [33]. Groundwater contamination by glyphosate can also occur via point sources, such as the cleaning of the sprayers, the filling of the sprayer and/or the management of effluents on the farmyard (i.e. on hard/concrete surfaces). The analysis of regional patterns of glyphosate occurrence and their relation to its use it is not possible because it is registered in Portugal for a wide of agricultural crops and also to non-cultivated areas [34].

3.2. Risk assessment of AMPA in Portuguese groundwater ecosystems

As shown in Table 5, AMPA was analysed in 63 groundwater samples collected from 63 sites located in 21 aquifer systems from four hydrogeological units. It was detected in 6% of the total samples, however only 5% presented concentration levels above 0.1 µg/L, the groundwater quality standard set for active substances in pesticides, including their relevant metabolites, degradation and reaction products [3,10,28]. However, there are two groundwater samples (with values equal to 1.91 and 4.24 µg/L) that exceeded the value of 1 µg/L estimated from the groundwater quality standard proposed for non-relevant metabolites set in the new legislative proposal [10].

The highest detection frequencies were found in the Tagus-Sado (25%) basin, followed by Western (14%) and Hercynian Massif (5%) units. The Western was the hydrogeological unit where AMPA was detected in more aquifer systems: O25 – Torres Vedras (50%) and O33 – Caldas da Rainha – Nazaré (20%). The detection frequency of 25% in the Tagus-Sado

hydrogeological unit was found in the unique aquifer system sampled, the T7 – Aluviões do Tejo (Table 5).

AMPA reach maximum concentration levels in the same three dug wells where maximum glyphosate concentration levels were quantified in the O25 - Torres Vedras (4.24 µg/L), T7 - Aluviões do Tejo (1.91 µg/L) and O33 – Caldas da Rainha-Nazaré (0.454 µg/L), two dug wells for which the purpose is unknown (there is no data available, according to Agência Portuguesa do Ambiente [18]), and one for domestic supply in the T7 – Aluviões do Tejo aquifer system, where AMPA concentration was higher than glyphosate concentration. This could indicate the long residence time or long distance of this hydrological setting from the glyphosate source application, as suggested by Carreta *et al.* (2022) [21]. Indeed, as glyphosate degrades, the AMPA ratio increases over time because AMPA is generally more persistent and mobile than glyphosate [19,24,35]. In several of the studies that Carreta *et al.* (2022) [21] examined, AMPA was detected more frequently and at higher concentrations than glyphosate. The presence of AMPA in groundwater is not exclusive due to glyphosate degradation, as it can also originate from amino polyphosphates sources.

Table 5. Detection frequencies observed for aminomethylphosphonic (AMPA) analysed in 63 groundwater samples collected from 63 sites of mainland Portugal, from April 2019 to June 2021.

Hydrogeological unit / Aquifer system	Groundwater samples analysed	Groundwater samples with concentration levels (µg/L)										Median (Maximum) concentration (µg/L)
		<LOQ	%	≥LOQ- <0.05	%	≥0.05- <0.1	%	≥0.1- <1	%	≥1	%	
Hercynian Massif												
A0 – Maciço Antigo Indiferenciado	13	12	92	1	8	0	0	0	0	0	0	0.05 (0.05)
A4 – Estremoz – Cano	2	2	100	0	0	0	0	0	0	0	0	-
A9 – Gabros de Beja	3	3	100	0	0	0	0	0	0	0	0	-
A10 – Moura – Ficalho	1	1	100	0	0	0	0	0	0	0	0	-
A11 – Elvas – Campo Maior	2	2	100	0	0	0	0	0	0	0	0	-
Total	21	20	95	1	5	0	0	0	0	0	0	0.05 (0.05)
Meridional unit												
M2 – Almádena – Odeáxere	1	1	100	0	0	0	0	0	0	0	0	-
M3 – Mexilhoeira Grande – Portimão	3	3	100	0	0	0	0	0	0	0	0	-
M4 – Ferragudo – Albufeira	1	1	100	0	0	0	0	0	0	0	0	-
M5 – Querença – Silves	5	5	100	0	0	0	0	0	0	0	0	-
M7 – Quarteira	1	1	100	0	0	0	0	0	0	0	0	-
M9 – Almansil – Medronhal	4	4	100	0	0	0	0	0	0	0	0	-
M10 – S. João da Venda – Quelfes	3	3	100	0	0	0	0	0	0	0	0	-
M12 – Campina de Faro	5	5	100	0	0	0	0	0	0	0	0	-
M13 – Peral – Moncarapacho	1	1	100	0	0	0	0	0	0	0	0	-
Total	24	24	100	0	0	0	0	0	0	0	0	-
Western unit												
O11 – Sicó – Alvaiázere	1	1	100	0	0	0	0	0	0	0	0	-
O15 – Ourém	3	3	100	0	0	0	0	0	0	0	0	-
O18 – Maceira	1	1	100	0	0	0	0	0	0	0	0	-
O19 – Alpedriz	2	2	100	0	0	0	0	0	0	0	0	-
O25 – Torres Vedras	2	1	50	0	0	0	0	0	0	1	50	2.14 (4.24)

O33 – Caldas Da Rainha – Nazaré	5	4	80	0	0	0	0	1	20	0	0	0.13 (0.454)
Total	14	12	86	0	0	0	0	1	7	1	7	0.38 (4.24)
Tagus-Sado basin												
T7 – Aluviões do Tejo	4	3	75	0	0	0	0	0	0	1	25	0.51 (1.91)
Total	4	3	75	0	0	0	0	0	0	1	25	0.51 (1.91)
TOTAL	63	59	94	1	2	0	0	1	2	2	3	0.15 (4.24)

3.3. Implications for the classification of the chemical status of groundwater bodies in Portugal

This study demonstrates that exposure of glyphosate and its non-relevant metabolite AMPA occur in Portuguese groundwaters at concentrations greater than 0.1 µg/L. This value is maintained for active substances in pesticides in the new proposal [10] for amending the Water Framework Directive, the Groundwater Directive, and the Directive on Environmental Quality Standards. In the case of glyphosate, determining a groundwater quality standard from the annual average-environmental quality standard for surface waters was not relevant, because the estimated value was greater than 0.1 µg/L if considering the annual average equal to 86.7 µg/L for freshwater not used for abstraction and preparation of drinking water.

In the case of the non-relevant metabolite AMPA, considering that it is a “data-rich” chemical and does not demonstrate high (eco)toxicity, the groundwater quality standard (1 µg/L) is not very high compared to the determined value using a deterministic approach based on reliable chronic experimental toxicity data on that taxonomic group (1500 µg/L [36]). In any case, the setting value for non-relevant metabolites should be 0.1 µg/L.

However, the value of 0.1 µg/L is no longer justifiable, since the performance of analytical methodologies has markedly improved over the course of the past 40 years. Furthermore, it is not guaranteed to be sufficiently protective for human health, surface-dwelling species and groundwater-living organisms. Groote-Woortmann *et al.* (2024) [12] have compiled the toxicity data on stygofauna and other aquatic subterranean organisms in one (eco)toxicological database. Pesticides were the second most tested compound group, with 83 endpoints identified across 19 compounds and obtained from eight studies, with most studies testing multiple pesticides. Even though the active substance aldicarb did not have the most endpoints, it is the only pesticide that was tested in more than two studies. There are at least nine pesticides with effect (lethal) concentration values below than 0.1 µg/L. Ecotoxicity testing with stygobiotic species in the framework of groundwater environmental risk assessment is highly recommended, as suggested by Di Lorenzo *et al.* (2019) [14].

Although this is not the case with glyphosate, there are priority substances whose average annual-environmental quality standard for priority substances in surface waters are

below 0.1 µg/L some of that driven by human health concerns (drinking water consumption, seafood consumption), but that simply supports the notion that a generic threshold of 0.1 µg/L needs to be carefully justified.

The Threshold of Toxicological Concern is a science-based tool for prioritizing chemicals, when hazard (toxicological) data are incomplete and human exposure can be estimated as low, such as metabolites, degradation and reaction products of pesticides [37]. In this case, that approach could be applied in order to demonstrate if a 0.1 µg/L groundwater quality standard for non-relevant metabolites would be sufficiently protective for human health, as suggested by Thomas Backhaus (2023) [38].

The EU Commission's proposal for amending the Water Framework Directive, the Groundwater Directive, and the Directive on Environmental Quality Standards corroborates commentaries, opinions and reviews provided by Thomas Backhaus (2023) [38] and the Commission's Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) [39,40].

4. Conclusions

The results obtained in this study show that glyphosate and its non-relevant metabolite AMPA occurred in Portuguese groundwater ecosystems, between 2019 and 2021. That parent compound was analysed in 103 samples collected from 80 sites belonging to the 21 aquifer systems under study. However, only 10% of these samples showed concentrations equal to or greater than 0.1 µg/L, the groundwater quality standard set for active substances of pesticides. Tagus-Sado basin was the hydrogeological unit that present the highest detection frequency with concentration levels above 0.1 µg/L, although was in the Western unit that these values were registered in more aquifer systems (O11 – Sicó-Alvaiázere, O25 - Torres Vedras and O33 - Caldas da Rainha-Nazaré). It was also in the Western unit, specifically in the O25 – Torres Vedras aquifer system, that the maximum concentration level of glyphosate was detected in a groundwater sample collected from a dug well located in the middle of a village (Maxial), for which no data is available to indicate its type of use.

With regard to AMPA, considered in this study a non-relevant metabolite of glyphosate, it was analysed in 63 samples collected from 63 sites belonging to the 21 aquifer systems under study. However, only 5% of these samples demonstrated concentrations equal or greater than 0.1 µg/L of which 3% above 1 µg/L, the groundwater quality standard for that group of water pollutants. These concentrations were only observed in the Tagus-Sado and Western units, with the former registering approximately 25% of the samples (T7 - Aluviões do Tejo) and the latter 14% (O25 - Torres Vedras and O33 - Caldas da Rainha-Nazaré). It was

also at O25 - Torres Vedras aquifer system that AMPA (4.24 µg/L) was detected with the highest concentration along with glyphosate (4.69 µg/L).

The information presented in this study highlights the presence of detectable groundwater contamination in some aquifer systems of Portugal, with concentrations found to exceed the groundwater quality standard, 0.1 µg/L, which does not seem to be a value sufficiently protective to human health and the ecosystem. In fact, insufficient information was provided to draw a firm conclusion on the impact to groundwater biodiversity via effects on stygofauna. In addition, it is acknowledged the lack of a quality standard that fully represents these organisms than the one currently used (based on aquatic organisms from surface water) and do not leave the main providers of ecosystem services in groundwater unprotected. Therefore, understanding the processes controlling the mobilization of glyphosate and AMPA in the soil-sediment-water environments is also essential for risk management and for the development of measures to mitigate groundwater contamination. It is essential to emphasize the importance of raising awareness about good agricultural practices and training farmers in this regard, in order to further reduce the contamination of groundwater by glyphosate and AMPA, as well as the possible contamination by other active substances currently approved in Portugal. Compliance with the mitigation measures indicated on the labels of plant protection products to mitigate diffuse and point contaminations, as well as the use of alternative methods of control (direct or indirect), such as conservation tillage, are measures that contribute to reducing groundwater contamination.

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