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Environmental Risk Assessment of Glyphosate and Aminomethylphosphonic Acid (AMPA) in Portuguese Groundwater Ecosystems

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Abstract: The aim of the present study was to assess the risk related to the exposure of groundwater ecosystems to herbicide glyphosate and its non-relevant metabolite aminomethylphosphonic acid (AMPA) based on the quotient between measured concentrations gathered from the Water Resources Information System of Portugal, and groundwater quality standards set in legislation and estimated from environmental quality standards in surface waters. Glyphosate was analyzed in 103 groundwater samples collected from 80 wells located in 21 aquifer systems from the 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, and 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. 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 4.69 and 4.24 µg/L for glyphosate and AMPA, respectively. The results of this study demonstrate that it is extremely important to raise awareness and offer 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. There is also an urgent need to carry out ecotoxicological tests with further groundwater species from different functional groups in order to obtain a quality standard that accurately represents the groundwater communities.

Keywords: AMPA; glyphosate; groundwater; groundwater quality standards; Portugal

Citation: Inês, S.; Ana, L.; Silva, E. Environmental Risk Assessment of Glyphosate and Aminomethylphosphonic Acid (AMPA) in Portuguese Groundwater Ecosystems. *Environments* **2024**, *11*, 258. https://doi.org/10.3390/environments11110258

Academic Editor: Chin H. Wu

Received: 7 August 2024
Revised: 2 November 2024
Accepted: 15 November 2024
Published: 19 November 2024

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 ecosystem services related to water purification and nutrient turnover. Many of the groundwater-dwelling organisms (stygofauna) are endemic and very specialized to a life in eternal 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 and 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 the legal limits at between 4% and 11% of groundwater monitoring sites in Europe, from 2013 to 2021 [5]. Given the great difficulty of restoring 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], in 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 an environmental quality standard set for each individually [10]. Through the groundwater watch list process, 17 countries provided groundwater data on the non-relevant metabolite compounds for review. The data indicate that non-relevant metabolites were widely detected in European groundwater above the 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 performed 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 surrogates 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 that we should 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 (the most frequently used herbicide in Portugal [17]) and non-relevant metabolite AMPA constitute new substances added to the lists of pollutants that need to be controlled, this study aimed to provide potential scenarios of environmental risk induced by them in Portuguese groundwaters. The approach followed was based on the relationship 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]. The implications of our findings for the classification of the chemical status of groundwater bodies in Portugal are discussed.

2. Materials and Methods

2.1. Study Area

The present study aimed to assess the quality of the groundwater ecosystems in mainland Portugal located on the southwest of the Iberian Peninsula occupying a total area of 88,889 km^2. Different edaphoclimatic conditions influence the unequal groundwater distribution in the Portuguese territory, as well the storage and water transmission conditions.

There are porous, karstic, and fractured hydrogeological aquifers, and groundwater linked to surface water in various forms. Considering its geological characteristics, mainland Portugal is divided into four main hydrogeological units, each one containing several aquifer systems [19–21].

Igneous and metamorphic rocks compose the Hercynean Massif unit. No aquifers have been identified in this area due to its low permeability and specific yield values, with the exception of some carbonate aquifers in the Alentejo region. In the Western unit, the majority of the aquifer systems are of sedimentary and karstic types, and few are multi-layer systems with groundwater interchange between aquifers due to leakage mechanisms. The first ones may be confined or unconfined and may locally be artesian. The Meridional unit is composed of carbonated aquifer systems with some karstification development. The Tagus-Sado multi-layer system is the most important aquifer system of the Iberian Peninsula, supplying drinking water, agriculture, and industry, as well as being the deepest one and the most productive. It is included in the Tagus-Sado unit covering a large sedimentary basin of about 8000 km^2. Flowing artesian behavior can occur in some areas, as well common water exchanges due to links between small alluvial aquifers and watercourses [19–21].

2.2. Groundwater Data

A search in the groundwater monitoring network of the Water Resources Information System of Portugal [18] was performed for glyphosate and AMPA concentration values. A total of 103 and 63 groundwater samples analyzed for glyphosate and AMPA were collected from 80 and 63 sites, respectively (Figure 1). 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 belong 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 center, and the Ribeiras do Algarve, Guadiana, and Arade basins in the south (Table 1). Information on the monitoring sites is given in Table S1, Supporting Information. Sampling was carried out during April–June 2019, September–December 2020, and April–June 2021.

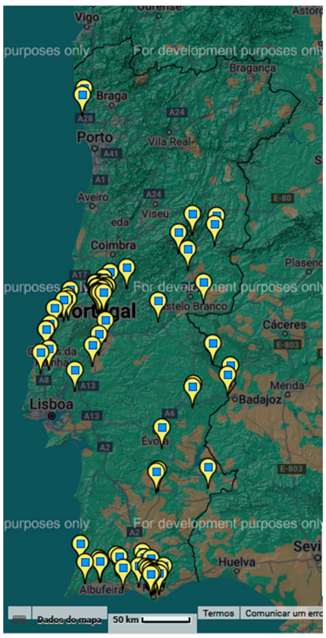

Figure 1. Locations of the 80 sites sampled in mainland Portugal.

Table 1. 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
Hercynean Massif						
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
Meridional unit						
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 1, in relation to the Hercynean Massif unit, 21 groundwater samples were collected from five aquifer systems located in five river basins in the north, center, and south of Portugal, with 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 was 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. The determination of glyphosate-based pesticides is performed using a pre-column derivatization method followed by concentration on solid-phase extraction columns with the W-PESLMSD1 method. Separation and detection of pesticides are carried out using liquid chromatography coupled with mass spectrometric detection. The intensity of the fragmentation of the parent ion to the daughter ion is monitored after applying collision energy (multiple reaction monitoring, MRM). For result confirmation, two MRM transitions are monitored for each analyte to ensure the method complies with the Commission Decision 2002/657/EC [22]. A positive result is considered when the ratio of the transitions corresponds to the ratio of transitions in the standard. Quantification is performed with an external standard, and the recovery rate is corrected for a portion of the analytes to internal standards and the remaining analytes to the fortified matrix. This test was performed under accreditation ISO 21458:2008 [23]. The lowest concentrations of glyphosate and AMPA that can be measured with certainty (limit of quantification, LOQ) are 0.03 and 0.05 µg/L, respectively [18]. Pollutants and indicators such as nitrate (Groundwater Quality Standard 50 mg/L, [24]), conductivity (Threshold Value 2500 µS/cm, [25]), pH (Sorensen scale) (Threshold Value 5.5-9, [25]), ammoniacal nitrogen (Threshold Value 0.5 mg/L NH4, [25]), and chloride (Threshold Value 250 mg/L, [25]) were also measured, and the results are presented in Table S2, Supporting Information.

2.3. Risk Characterization of Glyphosate and Aminomethylphosphonic Acid (AMPA) in Groundwater Ecosystems

Potential scenarios of environmental risk induced by glyphosate in Portuguese groundwaters were depicted using two different methodologies. One scenario was based on the groundwater quality standard for active substances in pesticides, including their relevant metabolites and degradation and reaction products (0.1 µg/L; [3,10,26]). The other scenario was based on the European Medicine Agency guidelines [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 from 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 µg/L according to [10], then the $PNEC_{GW}$ is 8.67 µg/L (Table 2).

Table 2. Groundwater quality standards for glyphosate and aminomethylphosphonic acid (AMPA) (µg/L).

Pesticide Compound	Legislation	Estimated
Glyphosate	0.1 [3,10,26]	8.67 [10,16]
AMPA	1 [10]	-

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 µg/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 2). AMPA does not meet the criteria for "relevant metabolites" or that for "metabolites of no concern", but has been identified in many groundwater bodies across the European Union [11], so it is was considered in this study a non-relevant metabolite (Table 2).

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 2). There is an environmental risk when the measured concentration values are higher than the quality standard values.

3. Results and Discussion

3.1. Risk Assessment of Glyphosate in Portuguese Groundwater Ecosystems

As shown in Tables 3 and S3, Supporting Information, glyphosate was analyzed 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 [3,10,26], and 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].

Table 3. Glyphosate occurrence in groundwater sampled at 80 sites on mainland Portugal, from April 2019 to June 2021.

Hydrogeological Unit/ Aquifer System	Groundwater Samples Analyzed	No. (and %) of Groundwater Samples with Concentration Levels in µg/L					Median (and Max.) Concentration (µg/L)
		<LOQ	≥LOQ–<0.05	≥0.05–<0.1	≥0.1–<8.67	≥8.67	
Hercynean Massif							
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)**
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	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)**
Western unit							
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)**
Tagus-Sado basin							
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 highest detection frequencies were found in the Tagus-Sado basin (75%), followed by the Hercynean 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 3). This shallow aquifer system consists of alluvial deposits and alluvial terrace deposits, containing alternate layers of fine and coarse sand or silt sandstones, clays, and silts, occasionally intercalated by gravel and by a deposit of this with sand at the base. The Tagus river influences groundwater flow powerfully. The hydraulic potential influences whether the Almonda and Alviela tributaries recharge or discharge the alluvial aquifer system. Most of the holes implemented in the system capture the sands and gravel layers of the alluvium and terraces as they have the highest transmissivity values [27]. In the other hydrogeological units, glyphosate was detected in the A0-Maciço Antigo Indiferenciado (23%) and M5–Querença-Silves (20%) aquifer systems (Table 3).

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 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, with the last one for domestic use with a depth of 8.9 m. 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 [28], dug wells have shallow water-table depths, leading to less groundwater protection from surface contaminant sources. Additionally, irrigation wells tend to be located nearer to agricultural fields, where plant protection products are applied.

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 in which productivity varies from moderate to high [29]. According to Carreta et al. [30], flow of pollutants to groundwater via preferential transport can be noteworthy in well-structured soils due to the inter-aggregate solute flow path (in dynamic regions). Groundwater quality problems were registered in these aquifers due to their overload with pollutants derived from agriculture and livestock, or from urban origins [29]. 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 [31], 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 banks due to the great amount of this herbicide applied on the land, jointly with the capability of surface water bodies being connective to groundwater aquifers, could present an additional probable pathway for groundwater contamination, as acknowledged by the Pesticides Peer Review Experts' TC 81 [32]. On the other hand, glyphosate can contaminate groundwater via point sources, such as the cleaning/filling of the sprayer and/or the management of remnants, including tank left-overs, on the farmyard (i.e., on hard/concrete surfaces). The analysis of regional patterns of glyphosate occurrence and their relation to its use is not possible because glyphosate is registered in Portugal for a wide of agricultural crops and also for non-cultivated zones [33].

3.2. Risk Assessment of Aminomethylphosphonic Acid (AMPA) in Portuguese Groundwater Ecosystems

As shown in Tables 4 and S4, Supporting Information, AMPA was analyzed 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 and degradation and reaction products [3,10,26]. However, two groundwater samples (with values equal to 1.91 and 4.24 µg/L) 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].

Table 4. Aminomethylphosphonic (AMPA) occurrence in groundwater sampled at 63 sites on mainland Portugal, from April 2019 to June 2021.

Hydrogeological Unit/ Aquifer System	Groundwater Samples Analyzed	No. (and %) of Groundwater Samples with Concentration Levels in µg/L					Median (and Max.) Concentration (µg/L)
		<LOQ	≥LOQ–<0.05	≥0.05–<0.1	≥0.1–<1	≥1	
Hercynean 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)**

The highest detection frequencies were found in the Tagus-Sado (25%) basin, followed by the Western (14%) and Hercynean 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 4).

AMPA reached the maximum concentration levels in the same three dug wells where the 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 are no data available, according to [18]), and one for domestic supply in the T7–Aluviões do Tejo aquifer system, where the AMPA concentration was higher than the glyphosate concentration. This could indicate persistence or a long distance of this hydrological setting from the glyphosate source application, as suggested by [30]. Certainly, as glyphosate degrades, the AMPA ratio increases in the long term because this metabolite usually presents a longer residence time and greater mobility than glyphosate [34–36]. In several of the studies that Carreta et al. [30] examined, glyphosate was detected less frequently and at lower concentrations than AMPA. The presence of this metabolite in groundwater is not exclusive due to glyphosate degradation, as it can also originate from amino polyphosphate sources.

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 occurs 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 when using a deterministic approach based on reliable chronic experimental toxicity data on that taxonomic group (1500 µg/L; [37]). In any case, it is the authors' opinion that the value set for non-relevant metabolites should be 0.1 µg/L.

However, it is our opinion that 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] 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 insecticide 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 0.1 µg/L. Ecotoxicity testing with stygobiotic species in the context of groundwater risk assessment is highly recommended, as suggested by [14].

Although this is not the case with glyphosate, there are priority substances whose average annual environmental quality standards 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), which 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 and degradation and reaction products of pesticides [38]. 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 [38].

Our opinion on 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 [39] and the Commission's Scientific Committee on Health, Environmental, and Emerging Risks (SCHEER) [40,41].

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, with concentrations equal to or greater than the groundwater quality standards (0.1 and 1 μg/L, respectively). In the Western unit, specifically in the O25–Torres Vedras aquifer system, glyphosate (4.69 μg/L) was detected with the highest concentration along with AMPA (4.24 μg/L) in a groundwater sample collected from a dug well for which no data are available to indicate its type of use. This well is located in the middle of a village (Maxial), so it is assumed that the finding may have resulted from point contamination, such as incorrect disposal of packaging of glyphosate-based plant protection products, with the user not following the instructions on the labels of these products and good agricultural practices. It is essential to emphasize the importance of raising awareness through training and education in this regard, as well as the use of alternative methods of control (direct or indirect), such as non-chemical active substances and conservation tillage. Furthermore, edapho-climatic conditions and their vulnerability, as well as agricultural practices, can also influence the leaching of glyphosate into groundwater. Therefore, understanding the processes of glyphosate and AMPA mobility in the soil–sediment–water compartments is also essential for risk management, namely for the advance of measures to reduce groundwater contamination.

We must also point out that insufficient information was collected to conclude on the impact to groundwater biodiversity through the effects on sygofauna. In addition, it should be noted that there is a lack of a quality standard that fully represents these organisms (that is not the case with the one currently used, based on aquatic organisms from surface water), which leaves the main providers of ecosystem services in groundwater unprotected.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/environments11110258/s1, Table S1: Information on the monitoring sites; Table S2: Statistics of some pollutants and indicators in groundwater sampled at 80 sites on mainland Portugal, from April 2019 to June 2021; Table S3: Statistics of glyphosate (μg/L) from April 2019 to June 2021. Table S4: Statistics of AMPA (μg/L) from April 2019 to June 2021.

Author Contributions: Conceptualization, E.S.; investigation, E.S., S.I. and L.A.; resources, E.S.; writing—original draft preparation, E.S. and S.I.; writing—review and editing, E.S.; supervision, E.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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