THE IMPACT OF THE HERBICIDE GLYPHOSATE ON WATER SOURCES: A REVIEW

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ABSTRACT. Glyphosate is one of the most used herbicides worldwide. Its efficiency for weed control and cost make it a seemingly perfect choice in the agricultural area. Studies and monitoring reports show divergent results in what concerns the impact of glyphosate on water sources. Because of its nature the herbicide is prone to be found more in sediment and particulate matter than in water. Different compositions of the herbicide were found to pose certain negative impacts on species of amphibians, soil microbes and induce abnormalities in the activity of some enzymes in pregnant rats. In this review we will assess the literature in the last five years regarding this topic and portray the different findings.

Key words: *glyphosate, herbicide, review, water*

INTRODUCTION

The interest in assessing the negative effects of pesticides has been present since the 1960s. One of the forerunners of this environmental movement was Rachel Carson. The results of her research were published in a book titled *Silent Spring*, which have prompted policy changes regarding pesticide use. Along with the development of analytical techniques the ability to analyze complex matrices contaminated with pesticides has risen constantly (Ramos, 2012; Vuckovic et al., 2010).

Two of the major groups pertaining to pesticides are insecticides and herbicides. Glyphosate (figure1) is a nonselective, postemergent herbicide that first appeared on the market in 1971, studies revealing ecological risks associated with the aforementioned chemical (Solomon and Thompson, 2003). Although the mode of action of this herbicide is by inhibiting the activity of an enzyme important in the synthesis of proteins only in plants, deletrious effects on aquatic and terrestrial species have been confirmed (Contardo-Jara et al., 2009; Paganelli et al., 2010).
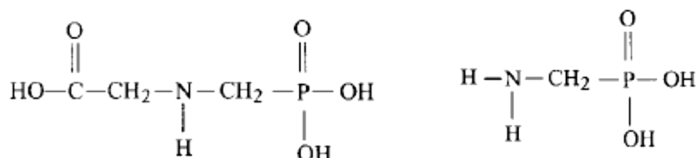
Fig. 1. *Structural forms of glyphosate and its metabolite AMPA (after Williams et al., 2000)*

In pure form the herbicide appears as a crystalline powder and is soluble in water, and due to its low vapor pressure it does not volatilize easily. Its toxicity is given by its persistence in the environment. The rate of degradation in soil and water differ. Depending on the types of soil the half-life can range between 4-19 days (Al-Rajab and Schiavon, 2010), whereas in the aquatic environment the range varies between 2.5-32 days (Mallat and Barcelo, 1998). Glyphosate can reach water sources through various ways, such as runoff - occuring during periods of rainfall, leaching - carrying the herbicide toward groundwater, and drift - settling after improper application of the herbicide on unintended areas of use (Zhou et al., 2010; Arronson et al, 2011). During degradation glyphosate transforms into aminomethyl phosphonic acid (AMPA), studies showing that both this metabolite and the herbicide itself can be more present in sediments than in water, being detected in almost 88.5% of samples from stream sediments (Aparicio et al., 2013). Furthermore, research has shown that the toxicity of the targeted compound is more deletrious when it pertains to glyphosate-based formulations, than stand-alone glyphosate, even though the adjuvants from formulations are said to be inert (Tsui and Chu, 2004; Benachour and Seralini, 2009; Lipok et al, 2010).

In the following chapters we will try to underline the major research results regarding the impact of glyphosate on water sources, focusing on the detrimental effects on aquatic living organisms, the different methods used for such studies and their limitations.

MATERIAL AND METHODS

High performance liquid chromatography (HPLC)

Scanning through the literature we can easily observe that the most often employed techniques for the analysis of glyphosate are the chromatographic techniques such as high performance liquid chromatography (HPLC). In older studies, high performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-ESI MS MS) was employed and showed satisfying detection levels in the range of 0.05 to 3 µg/L (Vreeken et al, 1998). HPLC has also been used in recent studies coupled with ultraviolet (UV) detection and on C18 columns having an analysis time of 15 minutes, but extra steps are needed to remove reagents used in the derivatization process, thus hampering a more swift analytical approach (Qian et al, 2009). While both UV detectors and fluorescence detectors are used to quantify glyphosate, the sensitivity of the fluorescence detector has proved to be superior

(Sundaram and Curry, 1997). In addition to the aforementioned detectors, HPLC is also coupled with a spectrophotometric module, which enables a faster detection (Kim et al., 2007).

Gas chromatography (GC)

Due to the herbicide's low volatilization analysis through gas chromatography (GC) is not as often employed as HPLC (Sánchez-Bayo et al., 2010). A landmark study has researched the mobility and adsorption characteristics of glyphosate, concluding that it can bind to soil in a similar way as organophosphates compounds (Sprankle et al., 1975). Therefore, numerous studies assess glyphosate in soil, due to its ability to easily bind to soil particles coupled with different detectors, such as a nitrogen-phosphorus detector (NPD) (Druart et al., 2011; Hu et al., 2008). In a case of glyphosate poisoning, human serum was analysed by GC and coupled with mass-spectrometry (MS) the method being valided for both the herbicide and its metabolite AMPA with good recoveries >73% (Megumi et al., 2008).

Admittedly, there are also other techniques employed for the analysis of glyphosate, such as capillary electrophoresis (CE), immunoassays (ELISA), ion chromatography (IC), which start to become more and more prevalent (Lee et al., 2013; Guo et al., 2005, Marques et al., 2009

Derivatization

Analyses are complicated by the fact that glyphosate usually needs to be derivatized due to its lack of chromophores, therefore reagents are needed in order to enable flourescence, so they can be detected. Derivatization accomplishes the following objectives:

- Enhancement of the detectability of non-absorbing or non-fluorescent analytes;
- Sensitivity enhancement of "problematic" analytical techniques such as, capillary electrophoresis, while, in some cases, derivatization has been reported to improve the sensitivity even in mass spectrometric detection;
- Stabilization of samples after collection, with low molecular weight;
- Alteration of the properties of the analytes to ensure compatibility with selected analytical techniques such as, separation of derivatized polar amino acids with reversed phase liquid chromatography or of non-volatile compounds by gas chromatography (Zacharis and Tzanavaras, 2013).

The most commonly used reagents in the derivatization process are 9-fluorenylmethylchloroformiate (FMOC), Trifluorethanol (TFE) and Trifluoracethic anhidride (TFAA) (Alexa et al., 2008; Freuze et al., 2007; Waiman et al., 2012).

IMPACT ON WATER SOURCES

Although soils are prone to its contamination, due to its ability to bind, water sources can also get contaminated through different processes such as leaching, runoff and discharge of sewage systems.

The main source of herbicides in the water matrix is usually agriculture, but recent studies have indicated that urban sources are also significant, the herbicide being used on roads or railways (Coupe et al., 2012; Botta et al., 2009). Even though the E.U. has a maximum residue level (MLR) set at 0.1 ng/ml in drinking water regarding glyphosate in what concerns concentrations in groundwater there is a lack of policy, but the issue is being recently reviwed. In urban areas concentrations of glyphosate were found to be ranging between 75-90 µg/L, while in agricultural basins ranging between 0.5- 4 µg/L (Botta et al., 2009; Aparicio et al., 2013). In table 1 we can observe the concentrations of glyphosate through Europe.

Table 1. *Concentrations of glyphosate in surface waters in European countries (Horth and Blackmore, 2009)*

Country / Substance	Date	No. sites	No. samples	Detected (samples)		Samples ≥ 0.1 µg l^{-1}		Max. Conc. µg l^{-1}	LoQ (LoD) µg l^{-1}
				No.	%	No.	%		
Austria									
AMPA	2001-02	?	345	≥90	≥26	90	26	3.4	?
Belgium									
Glyphosate (Flanders)	2006	105	≥ 1260 ≤ 2520	83 (s)	79 (s)	?	?	<10	?
Glyphosate (Wallonia)	2001 -06	26	531	≥ 429	≥ 81	429	81	1.3	≤ 0.1
AMPA (Flanders)	2006	105	≥ 1260 ≤ 2520	97 (s)	92 (s)	?	?	<10	?
Finland									
Glyphosate	2002-09	3	26	3	11.5	2	7.7	0.46	0.1
AMPA	2002-09	3	26	3	11.5	1	3.8	0.22	0.05
France									
Glyphosate	97-06	2493	39166	12680	32	10495	27	50	0.05-0.2
AMPA	98-06	2217	28963	15983	55	14583	50	48.9	0.05-0.1
Germany (Baden-Württemberg, Rheinland-Pfalz, Thüringen & River Rhine combined)									
Glyphosate	1997-08	104	1176	264	22	96	8	4.7	0.02-1.5
AMPA	1997-08	65	660	449	68	392	59	3.6	0.05-0.5
Ireland									
Glyphosate	2007-09	256	1700	98	5.8	≥1	≥ 0.06	1.8	(0.08)
Italy (Lombardia Region)									
Glyphosate	2005-07	150	714	142	20	13	1.8	11.0	(0.1)
Norway									
Glyphosate	97-06	11	80	74	92.5	≤ 57	≤ 71	0.93	(0.01)
AMPA	97-06	11	80	74	92.5	≤ 48	≤ 60	0.54	(0.01)
Slovak Republic									
Glyphosate	2006-08	40	330	105	31.8	49	14.8	3.6	(0.05)
Spain *									
Glyphosate	2006-08	115	748	96	7.4	80	11	15.3	0.003-0.1
Sweden									
Gly	2000-08	≥ 21	881	218	24.7	≥8	≥0.9	13.0	<0.1
AMPA	2000-08	≥ 21	868	118	13.6	≥7	≥0.8	4.0	<0.1
The Netherlands									
Glyphosate	2006	300	1384	215 (s)	72 (s)	109 (s)	36 (s)	>1.0	<0.1
AMPA	2006	305	1410	305 (s)	100 (s)	≥ 50 (s)	≥16 (s)	>8.0	?
UK									
Glyphosate	93-07	≥92	2809	297	10.6	297	10.6	8.8	0.1
Total									
Glyphosate	93-09	≥ 3716	≥ 50805	≥ 14704	~29	≥ 11700	~23	50	0.003-0.2
AMPA	97-09	≥ 2728	≥ 33612	≥ 17119	~50	≥ 15172	~45	48.9	0.05-0.5

LOD - *limit of detection,* **LoQ** - *limit of quantification,* **?** - *no information,* **(s)** - *sites (number of samples not known), - not relevant, * data from sites with known quality problems*

There can be noted a gap, a scarcity of studies regarding leaching of glyphosate in groundwater, even though there are a few researches that signal its presence (Van Stempvoort et al., 2014; Crowe et al., 2011). Data can be also contradictory and controversial, mentioning that it has a low potential to leach into groundwater, also not responding well to photodegradation (Manassero et al., 2010; NPIC, Glyphosate Technical Factsheet). Studies have proved that gyphosate can pose risks toward an array of aquatic organisms, ranging from benthic algae to bivales and amphibians (Edge et al., 2013; Mottier et al., 2013). Moreover, the literature indicates cases in which adjuvants in the glyphosate based formulations are more toxic than glyphosate itself, causing distress to algal strains (Lipok et al., 2010; Sihtmae et al., 2013).

Studies have been numerous and at times divergent. Vendrell et al (2009), established that glyphosate does not pose risks for the alge from a Spanish lake due to low concentrations, while Perez et al (2011) has concluded that even at concentrations ranging from >0.1 mg/L and <1 mg/L, algae and aquatic plants can be affected. Negative effects may be not only due to the singular impact of this agent, studies showing how the toxicity of glyphosate and AMPA can act as an additional stressor. Amphibians are an especially sensitive species because they can absorb water borne chemicals through their skin. Relyea (2005) conducted a study in which he exposed species of anurans to direct overspray observing lethal effects on a high percentage of the exposed individuals. Edge and his team (2013) concluded that Roundup WeatherMax (a glyphosate formulation used in agriculture) does not cause negative effect on juvenile amphibians under field exposure.

Binding on sediments, glyphosate can also pose risks to the periphyton communities from streams. Numerous studies indicate this divergent nature between field and laboratory studies. While in the laboratory, single concentrations of glyphosate may not pose significant deleterious effects, in real exposure studies, due to multiple stressors benthic communities can suffer significantly (Magbanua et al, 2013).

CONCLUSIONS

Glyphosate has a high polarity and as said previously said high water solubility, which has made its analysis extremely difficult. Even though GC can offer good selectivity, sensitivity and it is economical the tedious process of derivatization counterbalances the aforementioned benefits. Most methods require pre-column or post-colum derivatization and are not fast and selective enough in a market where the demand for fast and easy analysis is constantly rising. Unlike another herbicide of interest named atrazine, studies that reveal the occurence of glyphosate in the environment are just recently becoming higher in number.

Even though the use of glyphosate generates benefits, recent studies draw attention on new aspects that should continue to be constantly monitorized by the newest analytical methods available. In addition, glyphosate based formulations are diverse encompassing different adjuvants, continuous studies being required in order to fully assess their deletrious impact.

The results pointed out that although laboratory experiments are a significant starting point, in order to completely understand the deletrious effects on water source and aquatic ecosystems of glyphosate studies must be undertaken in natural systems, as well.

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REFERENCES

Alexa E., Hafner M., Negrea M., Lazureanu A., 2008, *HPLC and GC Determination of Glyphosate and Aminomethylphosphonic Acid (AMPA) in Water Samples*, Proceedings. 43rd Croatian and 3rd International Symposium on Agriculture. Opatija. Croatia, pp. 100-105.

Al-Rajab A.J., Schiavon M., 2010, Degradation of 14C-glyphosate and aminomethylphosphonic acid (AMPA) in three agricultural soils. *J Environ Sci (China),* **22** (9), pp. 1374-1380.

Aparicio V.C., De Gerónimo E., Damian Primost M.J., Carriquiriborde P., Costa J.L., 2013, Environmental fate of glyphosate and aminomethylphosphonic acid in surface waters and soil of agricultural basins. *Chemosphere,* **93**, pp.1866-1873.

Benachour N., Seralini G.E., 2009, Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. *Chem Res Toxicol.*, **22**(1), pp. 97-105.

Botta F., Lavison G., Couturier G., Alliot F., Moreau-Guigon E., Fauchon N., Guery B., Chevreuil M., Blanchoud H., 2009, Transfer of glyphosate and its degradate AMPA to surface waters through urban sewerage systems*,* **77**(1), pp. 133-139

Contardo-Jara V., Klingelmann E., Wiegand C., 2009, Bioaccumulation of glyphosate and its formulation Roundup Ultra in Lumbriculus variegatus and its effects on biotransformation and antioxidant enzymes. *Environ. Pollut*. **157**, pp. 57–63.

Crowe A.S., Leclerc N., Struger J., Brown S., 2011, Application of a glyphosate-based herbicide to *Phragmites australis:* Impact on groundwater and near-shore lake water at a beach on Georgian Bay. *Journal of Great Lakes Research*, **37**(4), pp. 616-624.

Coupe R.H., Kalkhoff S.J., Capel P.D. and Gregoire C., 2012, Fate and transport of glyphosate and aminomethylphosphonic acid in surface waters of agricultural basins. *Pest. Manag. Sci*., **68**, pp. 16–30.

Druart C., Delhomme O., de Vaufleury A., Ntcho E., Millet M., 2011, Optimization of extraction procedure and chromatographic separation of glyphosate, glufosinate and aminomethyl-phosphonic acid in soil. *Anal Bioanal Chem* **399**, pp. 1725–1732.

Edge C.B., Gahl M.K., Thompson D.G., Houlahan J.E., 2013, Laboratory and field exposure of two species of juvenile amphibians to a glyphosate-based herbicide and *Batrachochytrium dendrobatidis, Science of the Total Environment*, **444**, pp.145–152.

Freuze I., Jadas-Hecart A., Royer A., Communal P.-Y., 2007, Influence of complexation phenomena with multivalent cations on the analysis of glyphosate and aminomethyl phosphonic acid in water. *Journal of Chromatography A*, **1175**, pp. 197–206.

Guo Z.X., Cai Q., Yang Z., 2005, Determination of glyphosate and phosphate in water by IC— inductively coupled plasma mass spectrometry detection. *Journal of Chromatography A*, **1100**(2), pp. 160-167

Horth H., Blackmore K., 2009, *Survey of glyphosate and AMPA in groundwaters and surface waters in Europe*, Report no. UC8073.2.

Hu J.-Y., Chen C.-L., Li J.-Z., 2008, A Simple Method for the Determination of Glyphosate Residues in Soil by Capillary Gas Chromatography with Nitrogen Phosphorus. *Journal of Analytical Chemistry*, **63**(4), pp. 371–375.

Kim M., Stripeikis J., Inon F., Tudino M., 2007, A simplified approach to the determination of *N*-nitroso glyphosate in technical glyphosate using HPLC with post-derivatization and colorimetric detection, *Talanta*, **72**, pp. 1054–1058

Lee H.U., Jung D.U., Lee H.J., Song Y.S., Park C., Kim S.W., 2013, Detection of glyphosate by quantitative analysis of fluorescence and single DNA using DNA-labeled fluorescent magnetic core–shell nanoparticles. *Sensors and Actuators B*, **177**, pp. 879–886.

Lipok J., Studnik H., Gruyaer S., 2010, The toxicity of Roundups 360 SL formulation and its main constituents: Glyphosate and isopropylamine towards non-target water photoautotrophs. *Ecotoxicology and Environmental Safety,* **73**, pp. 1681–1688.

Magbanua F.S.,Townsend C.R., Hageman K.J., Lange K., Lear G., Lewis G.D., Matthae C.D., 2013, Understanding the combined influence of fine sediment and glyphosate herbicide on stream periphyton communities. *Water research*, **47**, pp. 5110-5120.

Mallat E., Barceló D., 1998, Analysis and degradation study of glyphosate and of aminomethylphosphonic acid in natural waters by means of polymeric and ion-exchange solid-phase extraction columns followed by ion chromatography–post-column derivatization with fluorescence detection. *Journal of Chromatography A*, **823**(1–2), pp. 129–136.

Manassero A., Passalia C., Negro A.C., Cassano A.E., Zalazar C.S., 2010, Glyphosate degradation in water employing the H_2O_2/UVC process. *Water Research*, **44**(13), pp. 3875-3882.

Marques M.N., Passos E.A., da Silva M.T.S., Correia F.O., Santos A.M.O., Gomes S.S., Alves J.P.H., 2009, Determination of Glyphosate In Water Samples by IC. *Journal of Chromatographic Science*, **47**(9), pp. 822-824.

Megumi M., Takeshi S., Kazuki A., Hiroyuki O., Isotoshi Y., Sadaki I., 2008, Determination of glyphosate, glyphosate metabolites, and glufosinate in human serum by gas chromatography–mass spectrometry. *Journal of Chromatography B: Analytical Technologies in the Biomedical & Life Sciences*, **875**(2), pp. 509-514.

Mottier A., Kientz-Bouchartb V., Serpentinia A., Lebela J.M., Jhac A.N., Costil K., 2013, Effects of glyphosate-based herbicides on embryo-larval development and metamorphosis in the Pacific oyster, Crassostrea gigas. *Aquatic Toxicology*, **128–129**, pp. 67–78.

Paganelli A., Gnazzo V., Acosta H., López S.L., Carrasco A.E., 2010, Glyphosate Based Herbicides Produce Teratogenic Effects on Vertebrates by Impairing Retinoic Acid Signaling. *Chem. Res. Toxicol.*, **23**, pp. 1586–1595.

Perez L.G., Solange M., Miranda L., 2011, Effects of herbicide glyphosate and glyphosate-based formulations on aquatic ecosystems. *Herbicides and Environment* ISBN 978-953-307-476-4, Publisher: InTech.

Qian K., Tang T., Shi T., Wang F., Li J., Cao Y., 2009, Residue determination of glyphosate in environmental water samples with high-performance liquid chromatography and UV detection after derivatization with 4-chloro-3,5-dinitrobenzotrifluoride, *Analytica Chimica Acta*, **635**(2), pp. 222–226.

Ramos L., 2012, Critical overview of selected contemporary sample preparation techniques. *J Chromatogr A.*, **1221**, pp. 84-98.

Relyea R.A., 2005, The lethal impacts of Roundup and predatory stress on six species of North American tadpoles. *Archives of Environmental Contamination and Toxicology,* **48**, pp. 351-357.

Sánchez-Bayo F., Hyne R.V., Desseille K.L., 2010, An amperometric method for the detection of amitrole, glyphosate and its aminomethyl-phosphonic acid metabolite in environmental waters using passive samplers. *Analytica Chimica Acta,* **675**, pp. 125–131.

Sihtmae M., Blinova I., Kunnis-Beres K., Kanarbik L., Heinlaan M., Kahru A., 2013, Ecotoxicological effects of different glyphosate formulations. *Applied Soil Ecology*, **72**, pp. 215– 224.

Solomon K.R., Thompson D.G., 2003, Ecological Risk Assessment for Aquatic Organisms from Over-Water Uses of Glyphosate. *Journal of Toxicology and Environmental Health*, Part B, **6**, pp. 289–324.

Sundaram K.M.S., Curry J., 1997, A Comparison of UV and Fluorescence Detectors in the Liquid Chromatographic Analysis of Glyphosate Deposits After Post-Column Derivatization. *Journal of Liquid Chromatography & Related Technologies*, **20**, pp. 511-524.

Sprankle P., Meggit M.F., Penner D., 1975, Adsorption, Mobility, and Microbial Degradation of Glyphosate in the Soil. *Weed Science*, **23**(3), pp. 229-234.

Tsui M.T.K., Chu L.M., 2004, Comparative toxicity of glyphosate-based herbicides: aqueous and sediment porewater exposures. *Arch Environ Contam Toxicol,* **46**, pp. 316– 323.

Vuckovic D., Zhang X., Cudjoe E., Pawliszyn J., 2010, Solid-phase microextraction in bioanalysis: New devices and directions. *Journal of Chromatography A*, **1217**, pp. 4041–4060.

Van Stempvoort D.R., Roy J.W., Bickerton G., 2014**,** Residues of the herbicide glyphosate in riparian groundwater in urban catchments. *Chemosphere*, **95**, pp. 455-463.

Vendrell E., Gomez de Barreda Ferra D., Sabater C., Carrasco J.M., 2009, Effect of glyphosate on growth of four freshwater species of phytoplankton: a microplate bioassay. *Bulletin of Environmental Contamination and Toxicology* **82**(5), pp. 538-542.

Vreeken R.J., Speksnijder P., Bobeldijk-Pastorova I., Noij Th.H.M.,1998, Selective analysis of the herbicides glyphosate and aminomethylphosphonic acid in water by on-line solid-phase extraction–high-performance liquid chromatography–electrospray ionization mass spectrometry. *Journal of Chromatography A*, **794**(1–2), pp. 187–199.

Waiman V.C., Avena J.M., Garrido M., Band B.F., Zanini G.P., 2012, A simple and rapid spectrophotometric method to quantify the herbicide glyphosate in aqueous media. Application to adsorption isotherms on soils and goethite, *Geoderma,* **170**, pp. 154–158.

Williams G., M., Kroes R., Munro I.C., 2000, Safety Evaluation and Risk Assessment of the Herbicide Roundup and Its Active Ingredient, Glyphosate, for Humans Regulatory. *Toxicology and Pharmacology*, **31**, pp. 117–165.

Zacharis K.C., Tzanavaras P.D., 2013, Liquid chromatography coupled to on-line post column derivatization for the determination of organic compounds: A review on instrumentation and chemistries. *Analytica Chimica Acta,* **798**, pp. 1– 24.

Zhou Y., Wang Y., Hunkeler D., Zwahlen F., Boillat J., 2010, Differential Transport of Atrazine and Glyphosate in Undisturbed Sandy Soil Column. *J. Soil and Sediment Contamination: An Int. Journal*, **19**(3), pp. 365-377.

 ***National Pesticide Information Center, Glyphosate Technical Fact Sheet Accessed 15 nov 2013.