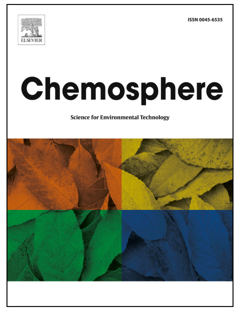
Journal Pre-proof



Regional evaluation of glyphosate pollution in the minor irrigation network

Stefano Gomarasca, Fabrizio Stefani, Emanuele Fasola, Caterina AM. La Porta, Stefano Bocchi

PII: S0045-6535(24)00572-1

DOI: https://doi.org/10.1016/j.chemosphere.2024.141679

Reference: CHEM 141679

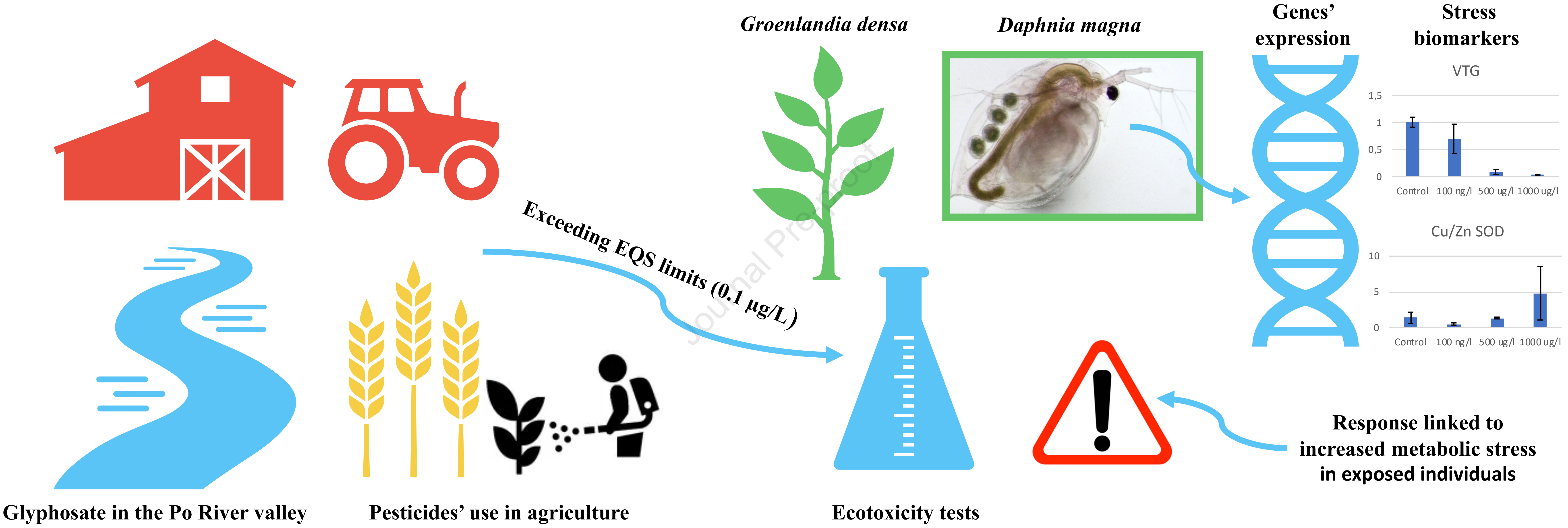
To appear in: *ECSN*

Received Date: 20 September 2023

Revised Date: 13 February 2024

Accepted Date: 8 March 2024

Please cite this article as: Gomarasca, S., Stefani, F., Fasola, E., La Porta, C.A., Bocchi, S., Regional evaluation of glyphosate pollution in the minor irrigation network, *Chemosphere* (2024), doi: https://doi.org/10.1016/j.chemosphere.2024.141679.


Groenlandia densa
Daphnia magna
Genes' expression
Stress biomarkers
VTG
1,5
1
0,5
0
Control
100 ng/l
500 ug/l
1000 ug/l
Cu/Zn SOD
10
5
0
Control
100 ng/l
500 ug/l
1000 ug/l
Exceeding EQS limits (0.1 µg/L)
Glyphosate in the Po River valley
Pesticides' use in agriculture
Ecotoxicity tests
Response linked to increased metabolic stress in exposed individuals

Regional evaluation of Glyphosate pollution in the minor irrigation network

Stefano Gomarasca[1], Fabrizio Stefani[2], Emanuele Fasola[2], Caterina AM La Porta[1], Stefano Bocchi[1*]

[1]- Dep. of Environmental Science and Policy (ESP), University of Milan, via Celoria 2, 20133 Milano, Italy; [*] Corresponding author

[2] -Water Research Institute-National Research Council (IRSA-CNR), Via del Mulino 19, 20861 Brugherio, MB, Italy

E-mail addresses: stefano.gomarasca@guest.unimi.it (Stefano Gomarasca), fabrizio.stefani@irsa.cnr.it (Fabrizio Stefani), emanuele.fasola@irsa.cnr.it (Emanuele Fasola), caterina.laporta@unimi.it (Caterina La Porta), stefano.bocchi@unimi.it (Stefano Bocchi)

Keywords: Glyphosate, AMPA, *Daphnia magna*, *Groenlandia densa*, *water pollution*, *water ecosystems*, *pesticide management*, *plant and animal resistance or sensitivity to pesticide, ecosystem health*

ABSTRACT

Due to its low cost, its ease of use and to the "mild action" declared for long time by the Control and Approval Agencies towards it, the herbicide Glyphosate, is one of the currently best-selling and most-used agricultural products worldwide. In this work, we evaluated the presence and spread of Glyphosate in the Po River Basin (Northern Italy), one of the regions with the most intensified agriculture in Europe and where, by now for decades, a strong and general loss of aquatic biodiversity is observed.

In order to carry out a more precise study of the real presence of this herbicide in the waters, samples were collected from the minor water network for two consecutive years, starting in 2022, at an interval time coinciding with those of the spring and summer crop treatments.

In contrast to the sampling strategies generally adopted by Environmental Protection Agencies, a more focused sampling strategy was adopted to highlight the possible high concentrations in minor watercourses in direct contact with cultivated fields. Finally, we investigated the possible consequences that the higher amounts of Glyphosate found in our monitoring activities can have on stress reactions in plant (*Groenlandia densa*) and animal (*Daphnia magna*)

In all the monitoring campaigns we detected exceeding European Environmental Quality Standard - EQS limits (0.1 µg/L) values. Furthermore, in some intensively agricultural areas, concentrations reached hundreds of µg/L, with the highest peaks during spring. In *G. densa* and *D. magna*, the exposition to increasing doses of herbicide showed a clear response linked to metabolic stress..

Overall, our results highlight how, after several decades of its use, the Glyphosate use efficiency is still too low, leading to economic losses for the farm and to strong impacts on ecosystem health. Current EU policy indications call for an agro-ecological approach necessary to find alternatives to chemical weed control, which farms can develop in different contexts in order to achieve the sustainability goals set by the Farm to Fork.

INTRODUCTION

The "green revolution" and the processes of intensification of cropping system cultivation have transformed and industrialized agriculture on a global scale from the end of the Second World War to today, leading to an indisputable increase in productivity (FAO, 2017). The increase of productivity was linked to the introduction of the so-called High Yielding Varieties (HYV) and to the use of synthetic chemicals including pesticides and fertilizers. The adoption of the industrial model and the use of these compounds have led over time to a progressive simplification of agricultural landscapes and, at the same time, to a relevant decrease in soil quality and agro-biodiversity.

In spite of the scientific community has highlighted for a long time the possible impact of pesticides used in agriculture on biological communities (via indirect or direct way), these products were used for decades, without fear, causing a lot of damage even on non-target species. Many publications have in fact demonstrated that pesticides weaken or, in some cases, wape out the community of useful organisms (soil microbiome, pollinator insects, mycorrhizal fungi) (Rani et al., 2021; Santovito et al, 2020; Sánchez-Bayo, 2021; Pagano et al., 2023), and that herbicides that are washed away from agricultural fields kill part of the spontaneous flora (Cedergreen and Streibig, 2005).

The decline in insect and wild plant communities had important implication on bird communities. For example, in Europe, over the last 40 years, 60% of bird species linked to agricultural areas disappeared (Rigal et al., 2023). At the same time, the widespread use of pesticides, and in particular herbicides, led to the appearance of resistance towards the used products (Heap, I. 2021). About that, there are a lot of data that links the use of the most widely used herbicide worldwide, Glyphosate, to the appearance of the greatest number of invasive weeds that are now insensitive to weed control activities (Heap, I. 2021).

Glyphosate, the focus of this article, is currently the most used herbicide in the world (Benbrook, 2016): about 600 to 750 thousand tons/year (with a prediction for 2025 is of 740 to 920 thousand tons/year) are actually used worldwide (Maggi et al., 2019).

Glyphosate was discovered in 1970 by Monsanto. The molecule is active against a broad spectrum of species belonging to the kingdoms of plants, fungi and microorganisms (Saunders and Pezeshki, 2015). It interferes with the shikimate synthesis pathway (which is not present in animals) and consequently causes the synthesis block of tyrosine, phenylalanine and tryptophan. Since the crucial role of this metabolic pathways, treated plants die in short time (Funke et al., 2006; van Bruggen et al., 2018; Meftaul et al., 2020). Since 1974 Glyphosate has been co-formulated with surfactants such as poly-oxyethylene amine (POEA) or isopropylamine salt (IPA). The best-known commercial formulation, Roundup, has been widely used throughout the world to effectively control weeds on soil, in water, in city parks, etc. (WHO, 1994; Franz et al., 1997). Most of its success is due to the fact that it is considered not toxic to animals. However, recent studies started to define Glyphosate as "potentially carcinogenic" (Guyton et al., 2015; IARC, 2018). In this contest it is therefore important to investigate further this crucial aspect (Saunders and Pezeshki 2015; Myers et al., 2016, van

Bruggen et al., 2021, Costas-Ferreira et al., 2022), considering that not only the active molecule can be toxic but also the surfactants contained in commercial products could be involved in increasing the toxicity as shown by (Annett et al., 2014). Moreover, it is known that Glyphosate, through bacterial action, is degraded into a stable metabolite, AMPA whose impact on the environment is still unknown. Both AMPA and Glyphosate are transported by water, percolate into groundwater or can reach the irrigation system where they could accumulate in the sediments (Saunders & Pezeshki, 2015; Bento et al., 2016).

In this connection, recent papers showed that both Glyphosate and AMPA residuals present in the waters can be assimilated by aquatic organisms via the food web, limiting their fitness (Kier and Kirkland, 2013; Kanissery et al., 2019).

These alarming premises lead us to affirm that in the next years, the research on the environmental impacts caused by pesticides will have to be increasingly intense and targeted. Between the complexity of pesticides mixture used in agriculture (Weisner et al., 2021), we recently focused on Glyphosate (and on its metabolite AMPA) since it is the most present pesticide detected in the Italian surface waters (ISPRA 2022). In this context, our group recently have described the level of contamination by pesticidesof the main rivers network of Lombardy, the Region with the richest industry and agriculture in Italy, finding levels of Glyphosate and AMPA above the legal limit for dozens of times (La Porta et al., 2021). We also showed a synergic effect between pollutants on the behaviour of common algae living this region (La Porta et al., 2021).

In the present paper, we further investigate the presence of Glyphosate in the Po Valley of Lombardy, one of the most fertile and also exploited and anthropized areas of Europe. One of its most important characteristics of this territory is the richness of water. In addition to the great rivers system it is crossed by a dense network of canals and ditches which develop for thousands of km that carry water for the fields irrigation (Pierik et al., 2016). These waters, which densely run through the entire Plain, bring, on one side, life to the agricultural system but, on the other hand, collect the products that are used in agriculture including nutrients and pesticides.

In the present paper we monitored the Glyphosate and AMPA concentrations in this area to answer at these questions: a) what is the dimension of Glyphosate contamination in the minor water network, the water system that is not monitored by Environmental Protection Agencies, because it is not considered by the European Environmental Regulations Laws, b) are there relevant ecotoxicologically time windows? c) is this related to the treatment phase suggested by the agronomic calendars? d) does the Glyphosate concentration found in the ditches water impact on the metabolism of representative species living this territory? To tackle these goals we evaluated the Glyphosate contamination in the minor waters systems of Po Valley by adopting a sampling design able to consider adequate temporal and spatial scales. We then assessed if the same concentrations of Glyphosate found in the environment could induce stress responses in *Groenlandia densa* (L.) Fourr and in *Daphnia magna* Straus .

MATERIALS AND METHODS

The study area and identification of water sampling stations

Territorial framing

The Po Valley is a vast region of Northern Italy. It stretches from the foothills of the Alps in the north to the Apennine Mountains in the south and is characterized by flat landscape and extensive network of rivers and waterways. It is of great importance both at the national level and at a broader context of the European Union (EU). Its exceptionally fertile soils, favorable climate, and agricultural organization allowed development and birth of most important italian agrofood system, producing cereals (mainly rice and corn during summer or barley and wheat during winter) fruits, vegetables, wine grapes, and livestock products such as meat and milk. The diverse ecosystems of Po Valley provide habitat for various plant and animal species. In particular, wetlands, rivers, springs (fontanili) and the minor irrigation network support a rich array of wildlife, making the Lombardy “wet biotopes” important ecosystems for biodiversity conservation. These complex of Po Valley waterways served also as crucial resources for irrigation, transportation, and energy production, the extensive canal and ditch systems, representing the minor irrigation network, have historically facilitated the social, economic and agricultural development.

Sampling campaign

The sampling campaign was focused on the intermediate portion of the Po river basin, a representative area for the two most prevalent spring-summer crops, corn (*Zea mais* L.) and rice (*Oryza sativa* L.). The sampling activity began in 2021, collecting water from three irrigation ditch systems that feed rice fields in the province of Novara (41, 42, 43 stations Fig. 1). It then continued during 2022 on the total of 46 stations identified in the Po Valley area belonging to the Provinces of Novara, Pavia, Milan, Lodi, Bergamo and Cremona, in such a way as to be able to cover the two major types of agricultural crops (Fig. 1).

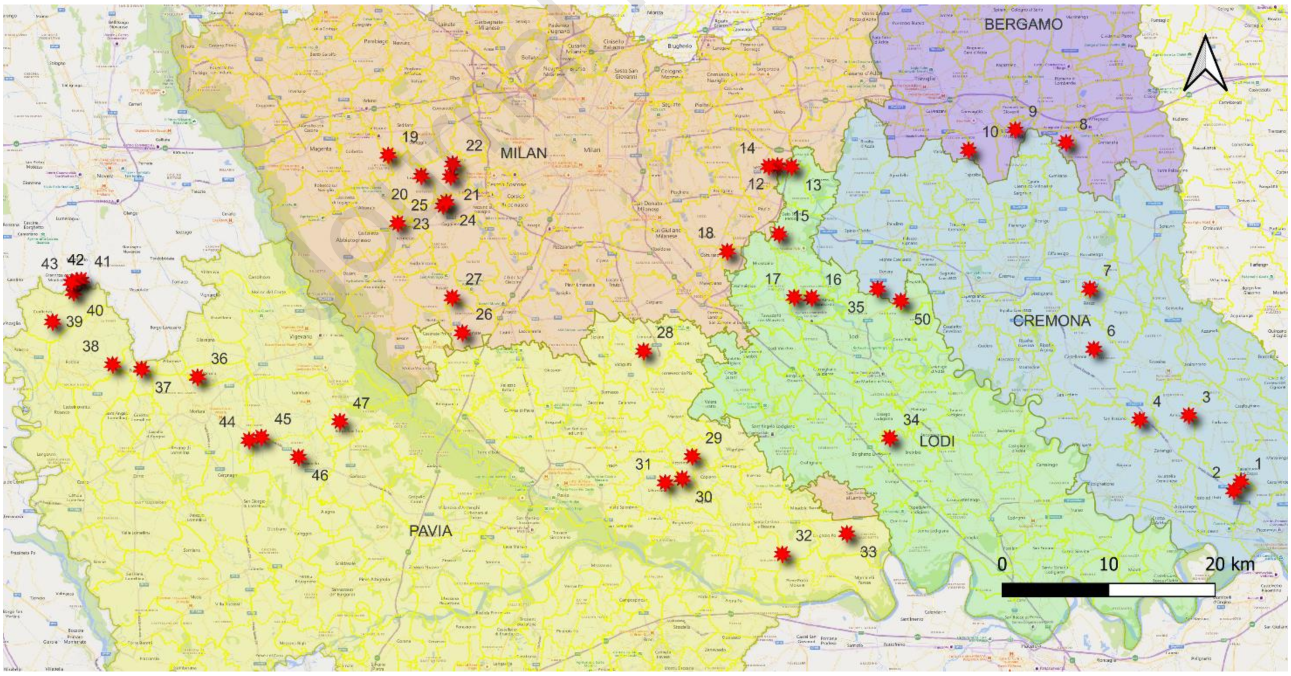


Figure 1) Position of the 46 sampling point (as red stars) along the Po Plain area of Lombardy. On the left the Novara (NO) and Pavia (PV) Provinces (yellow), on the center those of Pavia (PV) (yellow), Milan (MI) (orange) and Lodi (LO) (green), on the right those of Bergamo (BG) (violet), Lodi (LO) (green) and Cremona (CR) (sky blue).

NO stations: 41, 42 and 43; PV stations: 29, 30, 31, 32, 33, 36, 37, 38, 39, 40, 44, 45, 46 and 47; MI stations: 12, 14, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 and 28; LO stations: 13, 15, 16, 17 and 34; BG stations: 8, 9 and 10; CR stations: 1, 2, 3, 4, 6, 7, 35 and 50. The missing numbers correspond to some stations identified in the first part of the project, but below not considered due to lack of sufficient water

The samplings collected in 2021 were carried out in June, July and September, while in 2022 the samplings were carried out in two periods: the first from mid-April to May 5, the second from 12 to 15 June. It is important to highlight that the spring of 2022 was very dry and in April, only few water management consortiums were authorized to derive water from main rivers. Therefore, only the ditches of the Provinces of Cremona and Bergamo had running water while the rest of the samplings (Lodi, Milan, Pavia and Novara Provinces) were carried out during May when irrigation had started in all the Po Valley.

The stations were sufficiently distant from each other so that they are uncorrelated respect to water origin (although in some cases, far upstream, they could have a common origin). The samplings were carried out in the days after rain events, to better highlight the "runoff" phenomena related to the Glyphosate treatments. At the sampling points, water was collected with a clean PVC bucket. Water was then filtered through a 100 mesh/cm^2 nylon filter to eliminate suspended particulate. Finally, the sample was poured into a 1 liter polyethylene bottle, previously washed with distilled water. The samples were kept in the dark at 0C° till the chemical analysis.

Glyphosate and AMPA concentrations

AMPA and Glyphosate are detected from water samples with “dilute-and-shoot” procedure. Samples are diluted 1:1 with a solution of 99% methanol and 1% acetic acid. Isotope labeled analogues of the compounds are used as internal standards (ILISs) to correct for volumetric variations, matrix effects and other errors. So, they were added and the extracted samples were injected into a UPLC system coupled with a Triple Quadrupole mass spectrometer (reference method for Products of Vegetable Origin, cod. 1.3 - CVUA EU RL-SRM QuPPe - Quick Polar Pesticides Method - Vers. 12 (2021) (www.eurl-pesticides.eu, 2023)). The quantification is carried out by means of a 5-point calibration curve with internal standard.

The instrumentation used is the following:

UPLC system: Waters ACQUITY I-Class with FTN autosampler (Waters, Milford, MA, USA) Chromatography: Thermo Hypercarb 2.1 x 100 mm 5 µm (P/N 35005-102130) @40°C (Thermo Fisher (Waltham, MA, USA). Water/methanol with 1% acetic acid gradient @ 0,2ml/min.

Mass spectrometer: Sciex 6500+ LC-MS/MS Triple quadrupole system with heated electrospray ionisation in negative ionisation mode and SelexION ion mobility device. (Sciex, Framingham, MA, USA).

Water conductivity

Conductivity was measured with the HQ Hach multiparameter probe.

Groenlandia densa growth condition and its exposure to Glyphosate

To test the potential hazard of the Glyphosate concentrations found during the sampling campaigns, we used a native species characteristic of the Lombardy water: the *Groenlandia densa*.

Groenlandia densa (L.) Fourr. is widespread in the minor water network for at least twenty years but a recent surveys showed a strong decline throughout the whole Po Plain (personal observations, Šegota et al, 2019). *G. densa* is a plant that lives completely submerged, presents stolons and the leaves are characterized by a very simplified structure (three layers of cells with no impermeable epidermis. Through the leaf, *Groenlandia* absorbs nutrients but also polluting molecules directly from the water.

We used plant derived from two populations living in different sites characterized by different environmental conditions and contamination pressure. The first station corresponds to a small, practically "uncontaminated", mountain basin, the Alpe Cova lake (45°56' 29, 2'' N; 9° 25' 03, 73''.

Population B), which is located at 1301m of altitude (Pasturo, LC). The second one is located on the Tormo canal, near Monte Cremasco (CR) flows at an altitude of 84 m in full agricultural area (45°23' 00, 85" N; 9° 33' 25, 23" *Population A)*. The Tormo system is a minor river characterized by a high naturalistic value, rich in vegetation and fish fauna. For these reasons, since 2004, it has been subject to local protection (Rio Tormo Inter-Municipal Park). The plants collected from the two sites were immediately transferred to the lab of the Milan Department of Environmental Science and Policy (DESP), and grown in 50-litre tanks, with a weak but constant flow of drinking water. Washed sand and gravel were taken from a spring not contaminated by Glyphosate, and placed at the bottom of the tank. In order to allow a good "acclimatization", the plants were grown at room temperature, in full daily light for three months (from July to September).

At the end of September the tips of each branch were taken from the acclimatization tanks (the end tuft plus 4 well-spaced nodes). The tips used in the experimentation were all very similar in size and development. The harvested plants' tips were placed in 50 ml Falcon pvc test tubes containing 30 ml of water and Glyphosate (as Roundup) at various experimental concentrations (50, 25, 10, 1, 0.5 mg/lt of Glyphosate). Since the type and the concentration of surfactants present in Roundup are not repoted in the label, we used a solution of TritonX-100 (Sigma) as vehicle . The concentration of Triton X-100 was formulated empirically (3 µl/lt) by comparing the amount of bubbles that formed after stirring the solution (3 µl/lt of Triton 100 had the same quantity of bubbles of the solution with Glyphosate 50 mg/lt). To maintain the same concentration of Glyphosate throughout the experiment the solution was completely replaced every 5 days. The test tubes were placed for 3 weeks in a glass greenhouse at the DESP (night at 10 to 15°C, day at 20 to 25°C, 14 hours of light and 10 of darkness.

Lipid peroxidation

As a marker of stress, we observed the level of lipid peroxidation. Lipid peroxidation was determined by using the thiobarbituric acid reactive substances (TBARS) method (Heath & Packer, 1968).

Briefly, 1 g of apical tissue, after treatment with Glyphosate, was homogenized in 5 mL of trichloroacetic acid (TCA) 0.1% w/v and centrifuged at 4500×g for 10 min at room temperature. 1 mL of the supernatant was mixed with 4 ml of 20% (w/v) TCA, 25 µl of 0.5% thiobarbituric acid (TBA). After vortexing the mixture was heated at 95 °C (30 min) in a water bath and then cooled on ice. The samples are then analyzed spectrophotometrically both at 532 nm (specific for the TBA/TBARS complex) and 600 nm for non-specific turbidity index. The TBARS concentration (C) expressed in malondialdehyde (MDA) equivalents (nmol/g f.w.) was calculated using the molar extinction coefficient of MDA (ε=155 mM^{-1} cm^{-1}) (Heath and Packer 1968) according to the following formula:

$$C = (Abs532\ Abs600) * \varepsilon$$

Biochemical response of Daphnia magna under exposition to Roundup

A Daphnia magna reared strain was donated by the Civic Aquarium of Milan. The small crustaceans were housed for one month in a 20-litre tank containing mineral bottled water (Mg 31 mg/L, Ca 96 mg/L, Na 3.7 mg/L, conductivity 578mS/cm). The tank has been placed in a thermostated chamber at 20°C, with 16h of light and 8 hours of dark. Animals were fed daily with *Chlorella* algae (OECD, 2012).

For each tested concentrations (100, 500 and 1,000 µg/L), plus the control, twenty-four young *D. magna* individuals, aged less than 24 hours, were transferred to four 120 ml polypropylene beakers (6 specimens for each of the four beaker). Each beaker was stored without aeration, containing 100 ml of dilution water control or contaminated water. We replaced test medium with fresh solution each 24 h. The experiment duration was of 96h. For each concentration triplicate individual experiments has been carried out. The beakers were maintained in a thermostated chamber at 20°C, with a circle night/light of 8/16 hours.

At the end of the experiment, sixteen whole animals for each test (four *Daphnias* from each of the four beaker) were placed in a single vials and immediately frozen in liquid nitrogen, and preserved at -80°C until processing. RNA extraction was performed using the Aurum Total RNA Mini Kit (Biorad), following manufacturer's instructions. Initially, the frozen animals were fragmented with liquid nitrogen using a glass tube with teflon pestle, then the total amount of the obtained tissue was resuspended in the kit's extraction buffer. RNA expression of eight genes were analysed (Table 1 reports the primer sequences and the biological function of each tested gene). Beta-actin was used as housekeeping gene.

Reverse transcription was performed using the High-Capacity cDNA Reverse Transcription Kit (Life Technologies). Subsequently Real Time qPCR reactions were carried out on a Step-One-Plus, Applied Biosystems thermocycler in 12 µl of a solution containing: 1X mix PowerUp™ SYBR™ Green Master Mix (Life Technologies), 0.5 µl of the 10 mM primer solution and 1 µl of cDNA diluted 1:3. The thermal cycle includes: 1) 95°C for 20 s; 2) 40 cycles with 95°C for 3s and 30s at the annealing temperature of the primers. Each reaction was performed in triplicate. An analysis of the melting temperature was performed to verify the specificity of the amplicon. The relative expression of the treated versus control genes was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

Biological function and gene identity		Gene	Primer sequences (5' - 3')	T_m (°C)	Reference
Detoxification	cytochrome P450 4	cyp4	GCGGTCCTCAGTAGCAATAAA GCTACCTGTGCTCGTCAATAG	60	Blewett et al., 2017
	glutathione-S-transferase	**G**st	CGTCAATCTTATGGGAGGAGAAC GAATCCCGAGTCATCCAAAGTAG	60	Blewett et al., 2017
Oxidative stress responses	Cu/Zn-superoxide dismutase	Cu/Zn sod	TGCCGTCGTCTGCTGCTTTGTT TCCGTTGCTGAATACATCGCCGAAT	60	Cui et al., 2017
	catalase	**C**at	CTGTTGGCGGAGAAAGCGGTTCA ATCTGGTGTTCCACGGTCGGAGAA	60	Cui et al., 2017

	Glutathione peroxidase	GPx	TGCCGTCGTCTGCTGCTTTGTT TCCGTTGCTGAATACATCGCCGAAT	60	Cui et al., 2017
Methabolism and growth	Fatty acid biosynthesis D	fabD	GCCAACTACCTGTATCCTGAATG GTGGAACGCTCCGCTAACT	60	Seyoum & Pradhan, 2019
	Ecdysone receptor trascription factor	EcR	AGTCCGTCAGACGAGCATTC GGACGGTCCATTAATGTCAAG	60	Hannas et al., 2011
Reproduction	Vitellogenine	vtg2	CGTCCGCCACTGGTTGGGTC GGGGCAGCCAAGACAGAGCG	60	Seyoum & Pradhan, 2019
Reference	Actin	**A**ct	CCTCCACCTCTTTGGAGAAAT CAAGAATGAGGGCTGGAAGAG	60	Cui et al., 2017

Table 1: molecular targets chosen for *D. magna* qPCR analysis of gene transcription. Primers employed for qPCR reactions are reported, together with the melting temperature and the associated references and biological function of each gene.

Statistics

Statistical analyses were processed with Past Statistics® and Statistica v. 8.0 (StatSoft, Tulsa, OK, USA).

For Glyphosate and AMPA values obtained by spectrometer analysis, we evaluated the statistical uncertainty value on the basis of the error propagation theory, in accordance with the Horwitz – Thompson methods (Horwitz W., 1997; Thompson M., 2000).

Statistical analyses of gene expression were done using Statistica v. 8.0. Normality and homogeneity of variance for gene expression data (n = 3 replicates for treatment) were verified using Shapiro-Wilk's test and Levene's test, respectively ($p > 0.05$). Significant differences among treatment transcripts were assessed by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). All data are represented as mean ± standard error of the mean (SEM)

RESULTS

Levels of Glyphosate and AMPA in the minor water of Po Valley

Table 2 shows the results obtained from sampling in 2021. In June 2021, we detected high concentrations of Glyphosate and AMPA. The quantities of the two molecules exceeded thousands of times the European Environmental Quality Standard - EQS (0.1 μg/L) in all the samples analyzed. In water samples taken in July and September, Glyphosate and AMPA concentrations were found to be below European detection limits (LOQ 0.05 μg/L) or very close to it.

date of collection	08/06/2021				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
AMPA (μg/l)	401±143	372±138	501±171	652±216	897±288
glyphosate (μg/l)	85±38	104±46	112±49	231±93	94±41
date of collection	30/07/2021				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
AMPA (μg/l)	<0.05	0.13±0.03	<0.05	0.19±0.05	<0.05
glyphosate (μg/l)	<0.05	0.05±0.01	<0.05	0.08±0.02	0.06±0.02
date of collection	14/09/2021				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
AMPA (μg/l)	0.61±0.12	0.99±0.25	<0.05	0.20±0.05	<0.05
glyphosate (μg/l)	<0.05	<0.05	<0.05	<0.05	<0.05

Table 2 – Levels (μg/L) of Glyphosate and AMPA detected in the samples taken and analysed in June, July and September 2021 campaigns. Samples 1, 2 and 4 correspond to the stations 41, 42, 43 of map reported in Figure 1). Samples 3 and 5 are other ditches of the country area that are not shown in Figure 1. LOQ 0.05 μg/lt is the detection limit. Error was calculated according with Horwitz – Thompson.

In Table 3 are reported the results of the samples taken during the campaigns of 2022 (station map reported in Figure 1). Values exceeding the EQS, with concentrations reaching hundreds of μg/L, were often found. The highest values of Glyphosate and AMPA were found in the province of Cremona, in the earliest spring (April-May 2022).

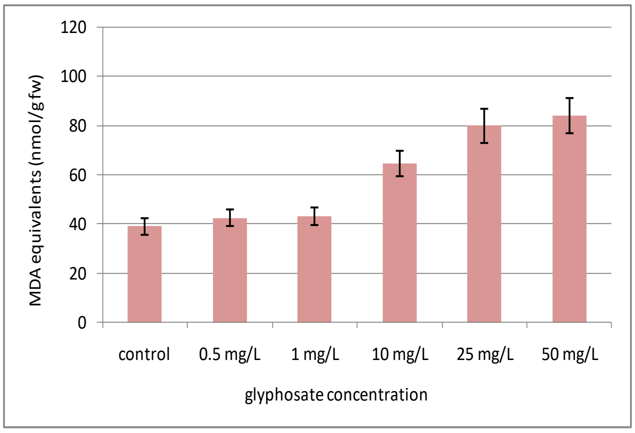
	April-May sampling					samplings of 12-14-15 June				
	conduc	glypho.	+/-	AMPA	+/-	conduc	glypho.	+/-	AMPA	+/-
Prov.	µScm^{-1}	µg/L	µg/L	µg/L	µg/L	µScm^{-1}	µg/L	µg/L	µg/L	µg/L
CR**	485	275	107	37	16	468	nr	-	0.97	0.43
CR**	628	372	138	28	12	374	nr	-	3	1.3
CR**	706	194	79	17	7	350	nr	-	nr	-
CR**	428	4.3	1.9	2.2	1	280	nr	-	1.6	0.7
CR**	819	21	9	4.4	1.9	344	nr	-	nr	-
CR**	728	83	37	12	5	400	nr	-	nr	-
CR**	545	179	74	13	6	552	nr	-	nr	-
CR	530	2.3	1	4.2	1.8	543	nr	-	nr	-
BG**	820	nr		nr		777	nr	-	nr	-
BG**	740	nr		nr		736	nr	-	nr	-
BG**	697	nr		nr		704	nr	-	nr	-
MI*	568	19	8	16	7	578	nr	-	3.5	1.5
MI*	635	nr		11	5	621	nr	-	nr	-
MI*	721	49	22	19	8	585	nr	-	nr	-
MI*	450	104	46	22	10	222	nr	-	nr	-
MI*	450	0.58	0.26	7.5	3.3	460	nr	-	nr	-
MI*	528	nr		2.1	0.9	563	nr	-	nr	-
MI*	687	0.19	0.08	3.7	1.6	678	nr	-	nr	-
MI*	520	49	22	22	10	546	nr	-	nr	-
MI*	614	49	22	21	9	626	nr	-	nr	-
MI*	585	61	27	34	15	580	nr	-	nr	-
MI*	264	74	33	40	18	210	0.11	0.05	4.9	2.2
MI*	329	44	19	45	20	366	nr	-	nr	-
MI	-	-	-	-	-	626	0.1	0.04	5.9	2.6
LO*	692	nr		6.9	3	623	nr	-	0.24	0.11
LO*	306	32	14	10	4	257	nr	-	0.13	0.06
LO*	529	29	13	5.7	2.5	481	nr	-	nr	-
LO*	515	31	14	25	11	234	nr	-	0.2	0.09
LO	297	6.8	3	11	5	225	nr	-	nr	-
PV	455	11	5	14	6	610	nr	-	1.1	0.5
PV	583	16	7	8.3	3.7	320	nr	-	0.37	0.16
PV	412	2.5	1.1	25	11	475	nr	-	0.5	0.22
PV	1170	2.9	1.3	11	5	890	nr	-	3.2	1.4
PV	790	5.2	2.3	19	8	717	nr	-	5.2	2.3
PV	222	13	6	15	7	317	nr	-	4.3	1.9
PV	243	7.3	3.2	9.7	4.3	357	nr	-	3.7	1.6
PV	269	2	0.9	15	7	407	nr	-	2.8	1.2
PV	207	2.9	1.3	14	6	295	nr	-	3.5	1.5
PV	268	15	7	17	7	306	nr	-	5.3	2.3
PV	310	6.1	2.7	22	10	391	nr	-	0.62	0.27
PV	238	8.1	3.6	14	6	283	nr	-	nr	-
PV	216	7.2	3.2	8.6	3.8	244	nr	-	nr	-
PV	256	2.8	1.2	11	5	251	nr	-	nr	-
NO	251	7.7	3.4	13	6	296	nr	-	nr	-
NO	390	9.6	4.2	15	7	270	nr	-	5.5	2.4
NO	170	12	5	12	5	273	nr	-	nr	-

Table 3 – Concentrations of Glyphosate and AMPA (µg/L) in the irrigation network of the Lombardy plain. The April and May samplings were carried out in two different blocks: CR**, BG** on 14 April, MI*, LO* between 5 and 6 May, MI, PV, LO, CR, NO between 9 and May 10, 2022 (station map, Fig. 1). The different Provinces are identified by different background colors. The high Glyphosate and AMPA values are highlighted in red (very high), green (medium high) and blue when values are less than 10 µg/L. Error was calculated according Horwitz–Thompson.

The conductivity parameters have been used as "tracing" of waters origin and as signal of any spills from urban or industrial sewage systems. In the Po Valley the irrigation system is powered by two typologies of water. One is derived from the rivers and the other from the underground aquifer through the network of spring names "Fontanili" (Bischetti et al., 2012). The first are characterized by conductivity values that vary from 200 to 400 µS/cm, while the second from 450 to 750-800 µS/cm. The results reported in Table 3 highlight this provenance quite clearly, although in some stations an increase or decrease in conductivity levels is visible over the seasons, highlighting the possible combination of the two kind of waters (dependent on the amount of water required by farmers). Finally, it should be underlined how a canal of Pavia (PV) Province present a probable spill from sewer pipe (in spring - 1170 µS/cm, in summer - 890 µS/cm). Overall, however, it is clear that conductivity is not a parameter linked to the presence or absence of Gyphosate or AMPA (Table 3).

Stress response of Groenlandia densa to Glyphosate

G. densa obtained from the two different stations, the" uncontaminated" one, of the Alpe Cova lake (population 2) and the "contaminated" one of Rio Tormo, Monte Cremasco (CR) (179mg/l of Glyphosate at 9 May 2022) (population 1), were exposed to different concentration of Glyphosate as described in Materials and Methods. The results presented in Figures 2 and those obtained from ANOVA (supplementary material) highlight that in populations 1 and 2 MDA equivalents do not appear to be significantly different from the control up to 1mg/l of Glyphosate. However, from 10 mg/l of pesticide, a significant increase in MDA equivalents is evident. The data of ANOVA (supplementary material) and those presented in Figure 2 highlight a different response as of 10 mg/l of Glyphosate by the two populations. While in population 2 (Fig. 2-b) the maximum peak is observed at 10mg/l of Glyphosate and then a decline in response is observed, in population 1 (Fig. 2-a) a parallel trend is observed between the increase of Glyphosate and the production of MDA equivalents.



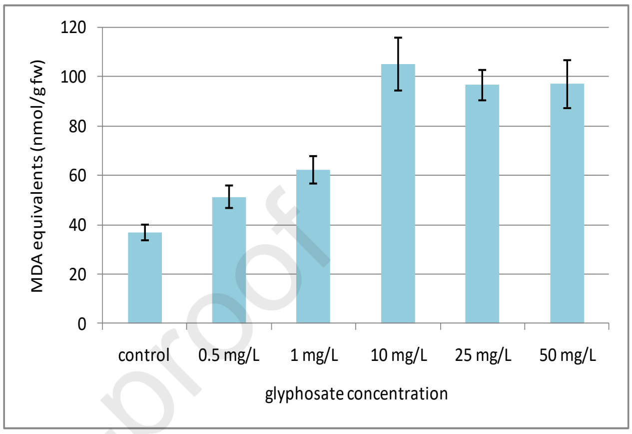


Figure 2-a

Figure 2-b

Figures 2: Quantification of MDA equivalent expressed as nmol/g fw according to Materials and Method section. Panel a) response of the Rio Tormo *Groenlandia densa* population; panel b) response of the Alpe Cova Lake *Groenlandia densa* population. Control plants were treated with TitonX-100 according to Materials and Methods section. The equivalents of MDA correspond to the total amount of oxidized phospholipids. The bars reports the mean of three independent experiments; +/- SE.

Stress response to Glyphosate of Daphnia magna

Analysis of gene expression in *D. magna* showed significant responses, respect to controls, for all the processes investigated: detoxification, oxidative stress contrast, metabolism and growth and reproduction (fig. 3).

Regarding detoxification pathways, expression of cytochrome P450 4 (*cyp4*) gene resulted invariant to Glyphosate concentration up to 100 µg/L, with a slight (but not significant) inhibition at 500 µg/L. Conversely, glutathione-S-transferase (*gst*) gene showed a marked activation at the lowest concentration followed by a rapid decrease.

The response to oxidative stress was similarly modulated for glutathione peroxidase (*GPx*) and catalase (*cat*), while an opposite trend was observed for Cu/Zn-superoxide dismutase (*Cu/Zn sod*). In these two last cases, however, high variability between the test replicates (of the lowest and highest tested concentrations respectively for *cat* and *Cu/Zn sod*) affected the significance of the observed trends.

Metabolism and growth markers resulted as well inhibited at the highest concentration, with particular regard to the fatty acid biosynthesis D (*fabD*) gene marker, while the same trend, although visible, was not significantly clear for the ecdysone receptor transcription factor gene (*Ecr*).

Finally, possible effects on reproduction may be indicated by the significant inhibition of vitellogenine (*vtg*) gene transcription starting from 200 µg/L onward.

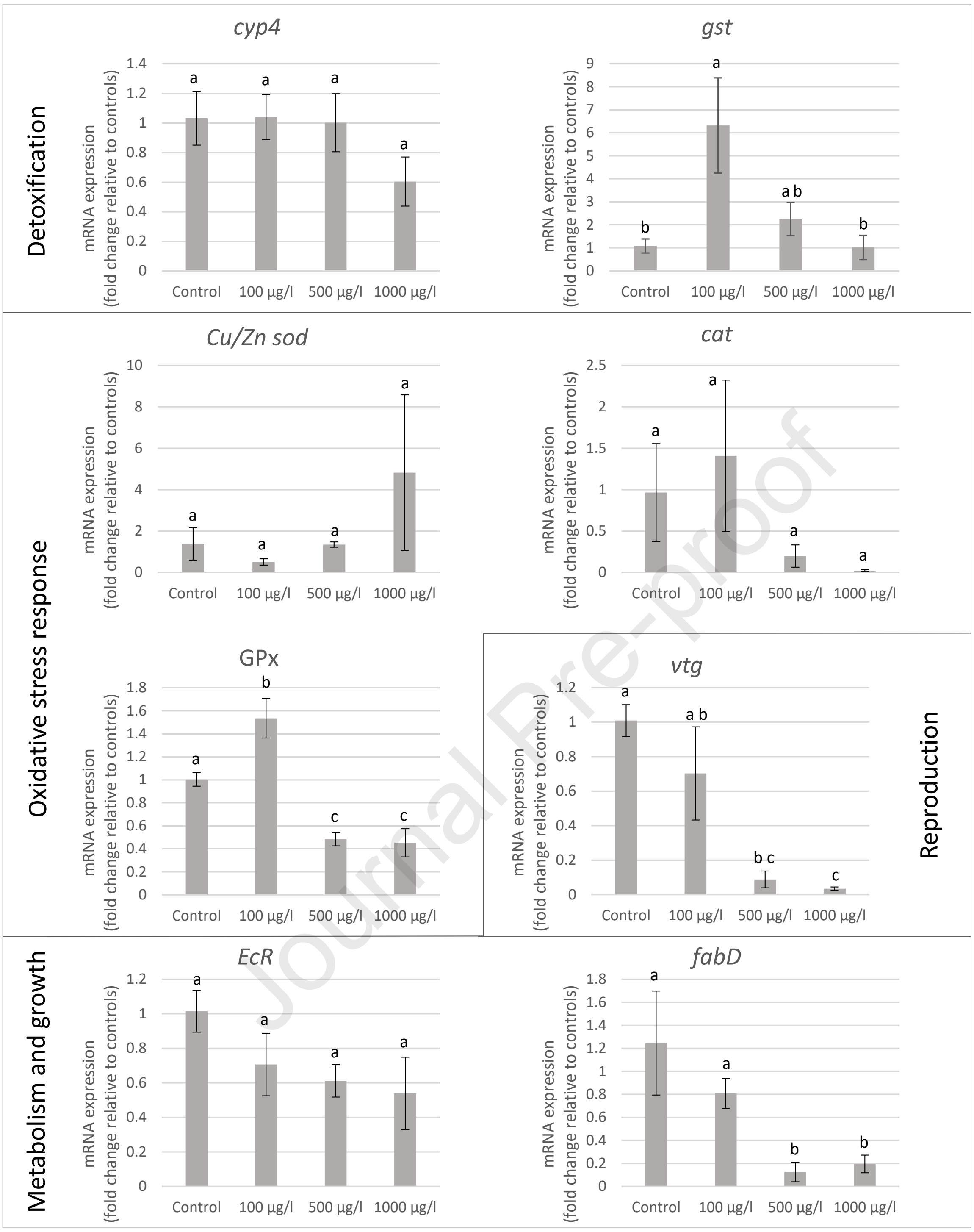


Figure 3 –mRNA gene expression of genes related to detoxification, oxidative stress response, metabolism and growth and reproduction in *D. magna* exposed to Roundup at different nominal concentrations of Glyphosate. Data are expressed as transcripts fold changes relative to the control expression levels, after normalization against β-actin. Data are shown as mean ± SEM (n = 3). For each gene, exposure groups with different letters are significantly different from each other (Tukey's test; $p < 0.05$)

DISCUSSION and CONCLUSION

As part of the Green Deal, Europe is aiming to the full environmental sustainability. Zero climate impact and sustainable growth are the political objectives that EU wants to pursue by 2050. Agriculture is one of the areas on which many resources have been allocated and on which Europe has many expectations. The strategies dictated by the Common Agricultural Policy (C.A.P.) are many and varied, starting from the "Farm to Fork" Strategy, arriving to the reduction of 30 percent in the pesticides use. Reduction that obviously includes the lower and more efficient use, or even the elimination, of all most toxic and dangerous products.

Glyphosate is certainly one of them, both because of its current heavy use and because of its "border-line" position at the level of public and scientific opinions. Although EFSA has extended its use on European territory at least until December 2023, justifying its decision to the lack of certain and useful information on its dangerousness, several research, including the presented in this paper, present new data that will allow the Monitoring Agencies to have a greater focus on the real and/or potential problems that this molecule has and could have on the environment and, consequently, also on humans.

In the present paper, we studied the spatial and temporal distribution of Glyphosate and AMPA in the minor water system, that is, in the irrigation network that on the one hand gives water to fields for irrigation of cultivated plants, but that on the other receives water from agricultural lands, more or less enriched with pesticides and nutrients.

We focused our attention on this water network because it is closely linked to the source of pollution (the agricultural sector, but not only...) that uses pesticides in significant quantities (when it is conventional agriculture), but also because it is not monitored by the Regional Agencies for Environmental Protection (ARPA). In fact, ARPAs, according to EU and national directives, monitor only the main rivers that receive pollutants from their wide watershed. Once in the river, due to the large amount of the present water, pesticides are consistently diluted changing their original amount and underestimating their potential ecological impact.

Our research considered the minor water systems of the lowland Lombardy Provinces (Northern Italy), where conventional intensive agriculture is the most common practice and where this type of agriculture is spread over about 70 percent of the territory (the rest is mostly urbanized).

With a sampling strategy capable of highlighting any relevant concentrations due to the intensive use of the herbicide over time, the research revealed extremely high values of Glyphosate and AMPA (with exceedances of thousands of times the EQS value), well beyond those identified by other research conducted in the same area (Di Guardo and Finizio, 2018, ISPRA, 2022), USA or France (Coupe et al, 2012). It is also true that high values of Glyphosate and AMPA have been found in other circumstances and in other water systems in both the USA and Europe, some times linked to the concomitant presence of resistant crops (Battaglin, et al., 2014; Silva, et al., 2018; Szekacs and Darvas, 2018).

The high concentrations of Glyphosate and AMPA in Lombardy's waters are certainly a worrying fact. Certainly there are many causes, although there are some clear evidence such as these linked to the amount of use and of sale of products containing Glyphosate, which in Italy ranks only after sulfur-based products (ISPRA, 2022). Certainly, some causes are also linked to the its use in agriculture. Very high amounts of Glyphosate and AMPA, reveled in the 2021 and 2022 campaigns, indicate a very low Glyphosate use efficiency (GUE). Low GUE values result on the one hand is damage to the environment and ecosystem health and on the other hand, in a deterioration of the farm which invests economic resources but that it does not use them efficiently.

For most post-emergence applications in glyphosate-resistant crops, the concentration of glyphosate used per hectare varies from 1800g to 2000g/ha of acid active molecule (from 2200g to 2360g of Glyphosate potassium salt). With these doses the farmer is supposed to assure efficiency of use and effectiveness of weed control so that the goal of flora management is achieved without having negative impacts on farm economics and the water/soil environment.

Treatment efficacy is high the younger the weeds are, so generally the farmer distributes before spring-summer crop sowings, such as corn and rice, in order to reduce initial competition. To limit potential non target effects of applications it is important to distribute the recommended amount, but even more crucial is the timing and method of distribution. Double passes over the same strip of land, distributions in field strips too close to waterways made just before heavy rains, overdosing set to control weeds no longer in their early stages of development, errors in dilution of the active ingredient can result in surface water pollution. Despite the high affinity of glyphosate for soil particles and its consequent low mobility (Sprankle et al., 1975; Vereecken et al., 2005). In effect it has been detected in a wide spectrum of water bodies (groundwater and surface water such as springs, rivers and lakes). Glyphosate can reach surface waters through either runoff and soil leaching or more rarely through a direct application into water (e.g., to control aquatic weeds) (EPA, 2019; Borggaard, 2008). In groundwater, herbicide can occur due to karst phenomena, as reported in intensive agricultural areas, but it rarely reaches high levels in groundwater because it remains bind to soil particles (Borggaard, 2008). Yang et al. (Yang et al., 2015), for example, reported that up to 14% of applied glyphosate can reach the water bodies due to runoff, while the European Food Safety Authority (EFSA) (EFSA, 2015) has calculated a dissipation half-life (DT50) of 13.8–301 days in river.

However, it is also true that concentrations higher than EQS levels are limited in time, limited within well-defined time intervals and which correspond to the spring periods foreseen by the agronomic calendars.

Ecotoxicological relevance on *Groenlandia densa* and *Daphnia magna*

Toxicological tests were done with Roundup (Glyphosate plus adjuvants) because it is the most widely used Glyphosate-based product in Italy. Using Glyphosate concentrations similar to those found in the Lombardy irrigation network, we obtained interesting results that, overall highlight a whole range of stress-related responses. It is likely that, at least in part, the effect of Roundup is given, in addition to the presence of Glyphosate, by the surfactants that are used as adjuvants (Annett et al, 2014), although in *G. densa*, the controls samples had been treated with relatively high *Tween20* concentrations (reportable to the effect of the adjuvants of the highest concentration of Roundup used in the experiment). The eperiments conducted on *G. densa* revealed a different trend in responses. A greater susceptibility by plants that, in all likelihood, have never been in contact with Glyphosate (Alpe Cova pond) and some resistance by plants that come from contaminating lowland agricultural environments (Rio Tormo, 179 µg/L of Glyphosate, 13µg/L of AMPA). At first glance, this response could be considered an important adaptive advantage. However some indications highlight how the changes of plant metabolism necessary to become resistant, especially if the response mechanisms is of constitutive type (Calow, 1998; Hua et al., 2013; Semlitsch et al., 2000), can lead to a loss of competitiveness with respect to the other species present in the community, in particular when the selective pressure determined by the presence of the pesticide is missing, i.e. for the most of the time (Vila Aiub et al., 2019). This data could, perhaps, justify why this species has been greatly reduced in favor of others typical of the same environments (e.g., *Nasturtium officinalis* L., *Veronica anagallis aquatica* L., *Callitriche* ssp.).

Some considerations about the possible effects on *D. magna* may be advanced looking at the overall scenario of gene expression alterations.

Admitting that exposure to a contaminant has occurred, detoxification pathways are the first processes which may contrast the potential adverse effects to organisms. In this regard, the *cyp4* enzyme is part of the detoxification phase I, producing the first oxidation of xenobiotics and reducing the lipophilicity of xenobiotics. Inhibition of these transcription was notable, but not significant in our study. Otherwise, it has been clearly demonstrated with regard to exposures more intense than those tested in this study (200 mg/L - Le et al., 2010). Inhibition of *cyp* family genes in *Daphnia* has been also observed following exposure to various other contaminants, for example diclofenac (Liu et al., 2017).

After the first oxidation in phase I, the contaminant can be excreted or proceed to further detoxification through other enzymes, such as *gst*. This enzyme intervenes in the detoxification phase II and conjugates the xenobiotics with glutathione (*GSH*), reducing their lipophilicity, and therefore making them more easily eliminated by excretion. Interestingly, a significant hormetic response has been found in *gst* expression (β shaped curves), with induction of expression at the lowest tested concentration (100 µg/L) and inhibition at the two higher concentrations. While hormetic response to contaminants exposure may be common (Calabrese and Baldwin, 2002; Zhang and Lin, 2020), it has not been described for *gst* in *D. magna* so far. For example, *D. magna* exposed to diclofenac, showed progressive inhibition as the dose increased (Liu et al., 2017). On the other hand, there was no evidence of altered *gst* expression in *D. magna* exposed to prochloraz (Salesa et al., 2022).

Hormesis has recently been considered as a general model to respond to a wide set of stressors (Agathokleous, 2018), including contaminants (Sebastiano et al., 2022), but also general ecological factors, such as population density (Saitanis and Agathokleous, 2019). There are growing evidence that sublethal effects showing an hormetic dynamics have been largely overlooked so far in ecotoxicological assessments (Sebastiano et al., 2022; Zhang and Lin, 2020).

Other two monitored markers showed an hormetic response, both belong to the biochemical pathways contrasting oxidative stress (i.e. *cat* and *GPx,* although not significantly for *cat*). *GPx* has the function to oxidize peroxides, using *GSH* as an electron donor. Together with *GST,* it reduces lipid peroxides to alcohols, through the oxidation of *GSH* to *GSSG*. Catalase dissociates hydrogen peroxide, that were created as a byproduct of superoxide dismutases (*SODs*) and *GPx* activity. *GPx* levels often increase as a result of catalase inhibition, so that peroxide levels in the organisms may be under control. This is not the case in the present study, as the two markers showed correlated responses. Hence, in this case, the enzymatic inhibition at high concentrations may have resulted in damages at cellular level, such as the peroxidation of membrane lipids (Regoli and Giuliani, 2014). Differently, *Cu/Zn sod* showed a different response respect to the others above discussed markers, which however resulted not significant respect to control. *Cu/Zn sod* is an enzyme responsible for the removal of the O2-

ions, which are present in the cytosol of all eukaryotic cells. Its activity produces hydrogen peroxide, so it must be followed by catalase or by glutathione peroxidase activity, since these enzymes are specifically dedicated to the removal of reactive oxygen species. The apparent increased transcription of *Cu/Zn sod* must be evaluated in parallel with the trends for *cat* and *GPx*, which however appear in opposition. This could imply a decompensation of the response chain, with the occurrence of possible oxidative stress cascade (Aksakal, 2020; Salesa et al., 2022). However, it is important to remember that gene transcription may not be directly correlated with real enzymatic activity, and also that the same enzymes can be implied in other biochemical pathways.

Overall, the fact that therefore most of the contrast mechanisms of oxidative stress, in particular of peroxidases, appeared inhibited at the highest concentrations of the test suggests however possible harmful effects on exposed populations.

FabD is related to the metabolism and synthesis of fatty acids (Seyoum and Pradhan, 2019). Its expression is not an indication of detoxification processes, but it can indicate the downstream effects of this process. It is linked to the synthesis of fatty acids, such as those of the membrane, and to the management of energy resources. In this study, *fabD* inhibition at the highest concentration may indicate a preferential allocation of energy resources towards the processes of detoxification and contrast of oxidative stress.

EcR is a nuclear receptor that regulates the development and/or progression of moults in arthropods; it also contributes to various reproductive processes (Hannas et al., 2011). In this case, a slight progressive inhibition of the expression of this gene has been observed, but resulted not significant. This is in contrast to what found, for instance, under both chemical exposure and thermal changes (Liu et al., 2017).

Differently, *vtg* showed a strong progressive inhibition correlated to the intensity of the exposure. Vitellogenine is a marker often used to evaluate effects related to reproduction, including endocrine interference, as it stimulates and regulates egg production; regulating the transport of lipids and other essential compounds towards the forming oocyte (Hannas et al., 2011). The same trend of inhibition for *vtg* was found in *D. magna* exposed to diclofenac (Liu et al., 2017); on the other hand, the fungicide *prochloraz* did not cause alteration of the vitellogenin expression in *D. magna* (Salesa et al., 2022). The results seem to indicate, as well, a possible overcome of the compensatory capacity of the organisms, especially from 500 µg/L upward, with possible implications for the reproductive fitness of the exposed populations.

Agroecological implications.

The pervasive presence of Glyphosate and AMPA, as well as of many other molecules certainly present in the same period, but also in subsequent moments, can negatively influence all forms of sustainable agriculture which seeks with great difficulty and many efforts to significantly reduce the use of synthetic chemicals.

After decades of use of Glyphosate, the impact exceeding the levels considered safe shows that the knowledge of the herbicide is not sufficient to ensure an efficiency of use necessary to avoid damage to the environment, to the health of the farmer, to the economic balance of the farm.

A second point of reflection consists in the discrepancy between the toxicity tests required by the European and national laws to do a new molecule registration, towards people, animals, plants and the real and complex response when we move from "model" tests and species to ecosystem. To understand the real impacts on the environment environmental monitoring campaigns at different scales (from the soil, to the waters, to target and non-target species, to declining species because particularly sensitive) would indeed required. Only through this approach, albeit challenging, we will be able to have a "vision" more relevant to reality, and therefore more truthful. The systems approach should also be considered, thus evaluating the spectrum of the possible responses at different scales, from fields to farm, to territory. This "procedure" would be able to highlight the real risks connected to the mixtures of products that can form in the environment (cocktail effect) or to the high concentrations of molecules that can be locally and temporarily determined, for various reasons, within agricultural production areas.

Regarding the monitoring and control of pesticides spread in the environment, it should be pointed out that, to date, the Environmental Protection Agencies seriously and rigorously apply the protocols assigned to them to evaluate the presence and toxicity risks of molecules such as Glyphosate and AMPA. However, this does not seem to be sufficient to achieve a complete, organic and territorial-scale assessment of the multiple and synergistic impacts that these molecules can have on human health, animal, plant and microbial communities. Agencies assess, in accordance with legislation, only the most relevant water systems, that for inland waters are the lakes and the major rivers. In contrast, the minor water network, which is capillary, has a wide presence over the territory and is the first to intercept and collect any kind of pollutant (especially those of agricultural origin) is left out.

Another point to consider is that Glyphosate has been used for about 50 years in all the countryside of the world, often without a valid and real alternative offered by the market. The same product has by now determined conditions of global contamination such as to alter the quality of waters with many consequences on the organisms that come into contact with the molecule. From recent studies conducted in France, the human organism is also widely affected (Grau et al., 2022).

In light of what has been reported so far, appears increasingly clear that the strategy dictated by the industrial model according to which the end life of a product (in this case a pesticide) determines its replacement (and often its (re)birth) with another product which probably also has an equally impactful impact, should be abandoned. It is clear that this "consumerist" strategy no longer adapts to the current needs of integral sustainability pursued by the Agricultural Policy of the European Community (C.A.P.).

A valid alternative to this model could be the one proposed by agroecology. It will be necessary to start from an analysis of the problems (e.g. the presence of unwanted species) found both at the field (scouting) and farm scale. By knowing the problem in a profound and holistic way, strategies can be identified to manage agronomic problems in the best possible way, i.e. with the lowest economic, social and ecological expense, but with the greatest profit for the farm and the territory.

In fact, agroecology bases its action strategies on the best agronomic tradition combined with modern agroecological innovation. Crop rotations, efficient use of chemical products, introduction of low impact active products as biostimulants are perhaps among the most important ones. But water protection and training are also two essential prerogatives of agroecology. The defense of the minor water bodies is implemented by reducing the exposure of water bodies to the dispersion of sprayed products, to drainage and run-off (through the provision of grassy or tree-lined strips - hedges and rows). Training, information, communication with the agricular operators (real stakeholders), but also with the citizens (general stakeholders) are other fundamental elements of the agroecological strategy. A correct and impartial communication seems therefore to be the key element. Targeted disinformation is in fact an aggravating factor that increases the confusion and uncertainty in communication and therefore in correct knowledge. Recently, a series of scientific review papers have been published which highlight how valid research papers, presented in equally good scientific journals, have been rejected without any reason by Ufficial Control Agencies such as USEPA or EFSA (Mie and Rudén, 2022). In this way a state of the “data uncertainty” and confusion tends to be created. A disorientation condition that in some cases is fueled by the action of industrial lobbies which, following commercial logic in defense of their product, contrast the information taken from the independent scientific literature by creating pages on the internet dedicated to some of their products, making criticisms to the "unfriendly research institutions" and of Control Agencies or to the National and European legislation, accused of being too restrictive (Glyphosate Renewal Group (GRG), 2023).

It is evident how different interests can lead to contrasts of ideas, information and knowledge.

Public institutions, committed to the defense and care of common goods, including the health of ecosystems and plant and animal species as well as man, assume a dual inalienable function: on the one hand, they have the task of indicating the main sources of risk for global health associated with the use of certain substances/technologies, on the other they are called to propose innovative practices within alternative models that are fully sustainable.

References

Agathokleous, E., 2018. Environmental hormesis, a fundamental non-monotonic biological phenomenon with implications in ecotoxicology and environmental safety. Ecotoxicol. Environ. Saf. 148, 1042–1053. https://doi.org/10.1016/j.ecoenv.2017.12.003

Aksakal, F.I., 2020. Evaluation of boscalid toxicity on Daphnia magna by using antioxidant enzyme activities, the expression of genes related to antioxidant and detoxification systems, and life-history parameters. Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol. 237, 108830. https://doi.org/10.1016/j.cbpc.2020.108830

Annett, R., Habibi, H.R., Hontela, A., 2014. Impact of Glyphosate and Glyphosate-based herbicides on the freshwater environment. J. Appl. Toxicol. 34, 458–479. https://doi.org/https://doi.org/10.1002/jat.2997

Battaglin, W.A., Meyer, M.T., Kuivila, K.M., Dietze, J.E., 2014. Glyphosate and its degradation product AMPA occur frequently and widely in U.S. soils, surface water, groundwater, and precipitation. Journal of the American Water Resources Association (JAWRA). 50 (2), 257-290. https://doi.org/10.1111/jawr.12159

Benbrook, C.M., 2016. Trends in Glyphosate herbicide use in the United States and globally. Environ. Sci. Eur. 28:3, 1-15. doi 10.1186/s12302-016-0070-0

Bento, C.P.M., Yang, X., Gort, G., Xue, S., van Dam, R., Zomer, P., Mol, H.G.J., Ritsema, C.J., Geissen, V., 2016. Exsistence of Glyphosate and aminomethylphosphonic acid in loess soil under different combinations of temperature, soil moisture and light/darkness. Sci. Total Environ. 572, 301–311

Bischetti G., Fumagalli N., Gomarasca S., Pillitteri T., Piantanida E., Senes G. , Negri G., Marziali L., Toccolini A., 1012. Tutela e valorizzazione dei fontanili del territorio lombardo. Progetto Fonte Quaderni della Ricerca n° 144 , Regione Lombardia, D.G. Agricoltura ed. https://www.cartografia.regione.lombardia.it/metadata/fontanili/QdR_n.144_FonTe.pdf (accessed on 29 -01-2024)

Blewett, T.A., Delompré, P.L.M., He, Y., Folkerts, E.J., Flynn, S.L., Alessi, D.S., Goss, G.G., 2017. Sublethal and Reproductive Effects of Acute and Chronic Exposure to Flowback and Produced Water from Hydraulic Fracturing on the Water Flea Daphnia magna. Environ. Sci. Technol. 51, 3032–3039. https://doi.org/10.1021/acs.est.6b05179

Borggaard, O.K., Gimsing, A.L., 2008. Fate of Glyphosate in soil and the possibility of leaching to ground and surface waters: A review. Pest. Manag. Sci. 64, 441–456.

Calabrese, E.J., Baldwin, L.A., 2002. Defining hormesis. Hum. Exp. Toxicol. 21, 91–97. https://doi.org/10.1191/0960327102ht217oa

Calow, P.,1998. Ecological Risk Assessment: Risk for What? How Do We Decide? Ecotoxicology and ironmental Safety. 40 (1–2), 15-18

Cedergreen, N., Streibig, J. C., 2005. The toxicity of herbicides to non-target aquatic plants and algae: assessment of predictive factors and hazard. Pest Management Science. 61, 1152-1160. https://doi.org/10.1002/ps.117

Costas-Ferreira, C., Duran, R., Faro, L. R. F., 2022. Toxic Effects of Glyphosate on the Nervous System: A Systematic Review. Int. J. Mol. Sci. 23(9), 4605. doi: 10.3390/ijms23094605

Cui, F., Chai, T., Qian, L., Wang, C., 2017. Effects of three diamides (chlorantraniliprole, cyantraniliprole and flubendiamide) on life history, embryonic development and oxidative stress biomarkers of *Daphnia magna*. Chemosphere 169, 107–116. https://doi.org/10.1016/j.chemosphere.2016.11.073

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

Di Guardo A., Finizio A. 2018. A new methodology to identify surface water bodies at risk by using pesticide monitoring data: The Glyphosate case study in Lombardy Region (Italy). Science of the Total Environment 610–611 (2018) 421–429

EFSA, 2015. Conclusion on the peer review of the pesticide risk assessment of the active substance Glyphosate: Peer review of the pesticide risk assessment of the active substance Glyphosate. EFSA J., 13, 4302.

EPA, United States Environmental Protection Agency Washington, D.C., 20460., 2019. Glyphosate-Response to Comments Usage and Benefits–Final. Available online: https://www.epa.gov/sites/production/files/2019-04/documents/glyphosate-response-comments-usage-benefits-final.pdf (accessed on 03-02-2024)

FAO., 2017. The future of food and agriculture – Trends and challenges. Rome. ISBN 978-92-5-109551-5

Franz, J.E., Mao, M.K., Sikorski, J.A., 1997. Glyphosate: A Unique Global Herbicide; American Chemical Society: Washington, DC, USA,; p. 615

Funke, T., Han, H., Healy-Fried, M.L., Fischer, M., Schönbrunn, E., 2006. Molecular basis for the herbicide resistance of Roundup Ready crops. Proc. Nat. Acad. Sci. USA. 103, 13010–13015

Glyphosate Renewal Group (GRG), 2023. https://www.glyphosate.eu/authorisation-process/setting-environmental-quality-standard-for-glyphosate/grg-statement-on-the-setting-eqs-for-glyphosate/ (accessed on 07 -02-2024)

Grau, D., Grau, N.,Gascuel, Q., Paroissin, C., Stratonovitch, C., Lairon, C., Devault, D.A., Di Cristofaro, J., 2022. Quantifable urine Glyphosate levels detected in 99% of the French population, with higher values in men, in younger people, and in farmers. Environmental Science and Pollution Research. 29, 32882–32893

Guyton, K.Z., Loomis, D., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., Scoccianti, C., Mattock, H., Straif, K., 2015. International Agency for Research on Cancer, Monograph Working Group, IARC, Lyon, France. Carcinogenicity of tetrachlorvinphos, arathion, malathion, diazinon, and glyphosate. Lancet Oncol. 5, 490–491

Hannas, B.R., Wang, Y.H., Thomson, S., Kwon, G., Li, H., LeBlanc, G.A., 2011. Regulation and dysregulation of vitellogenin mRNA accumulation in daphnids (*Daphnia magna*). Aquat. Toxicol. 101, 351–357. https://doi.org/10.1016/j.aquatox.2010.11.006

Heap, I., 2021. The International Herbicide-Resistant Weed Database. www.weedscience.org, (accessed on 26 -07-2023)

Heath, R. L., and Packer, L., 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of biochemistry and biophysics. 125 (1), 189-198

Horwitz, W., 1997. A Heuristic Derivation of the Horwitz Curve. Anal. Chem. 69 (4), 789-790. doi:10.1021/ac9608376

Hua, J., Morehouse, N.I., Relyea, R., 2013. Pesticide tolerance in amphibians: Induced tolerance in susceptible populations, constitutive tolerance in tolerant populations. Evol. Appl. 6, 1028–1040. https://doi.org/10.1111/eva.12083

IARC (International Agency for Research on Cancer), 2018. IRAC Response to Critisms of the Monographs and the Glyphosate Evaluation. Available online: https://www.iarc.who.int/wp-content/uploads/2018/07/IARC_response_to_criticisms_of_the_Monographs_and_the_glyphosate_evaluation.pdf (accessed on 26 -06-2023)

ISPRA, 2022. Rapporto Nazionale Pesticidi nelle Acque. https://www.isprambiente.gov.it/files2022/pubblicazioni/rapporti/rapporto_371_2022.pdf (accessed on 28 -09-2023)

Kanissery, R., Gairhe, B., Kadyampakeni, D., Batuman, O., Alferez, F., 2019. Glyphosate: Its Environmental Persistence and Impact on Crop Health and Nutrition. Plants. 8, 499. doi:10.3390/plants8110499

Kier, L.D., Kirkland, D.J., 2013. Review of genotoxicity studies of Glyphosate and Glyphosate-based formulations. Crit. Rev. Toxicol. 43, 283–315

La Porta, C., Fumagalli, M., Gomarasca, S., Lionetti, M., Zapperi, S., Bocchi, S., 2021. Synergistic Effects of Contaminants in Lombardy Waters. Scientific Reports. 11, 13888 https://doi.org/10.1038/s41598-021-93321-6

Le, T.H., Lim, E.S., Lee, S. K., Choi, Y.W., Kim, Y.H., Min, J., 2010. Effects of Glyphosate and methidathion on the expression of the Dhb, Vtg, Arnt, CYP4 and CYP314 in *Daphnia magna*. Chemosphere. 79 (1), 67-71, https://doi.org/10.1016/j.chemosphere.2009.12.067

Liu, Y., Wang, L., Pan, B., Wang, C., Bao, S., Nie, X., 2017. Toxic effects of diclofenac on life history parameters and the expression of detoxification-related genes in *Daphnia magna*. Aquat. Toxicol. 183, 104–113. https://doi.org/10.1016/j.aquatox.2016.12.020

Livak, K.J. and Schmittgen, T. D., 2001. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-ΔΔCT Method. Methods. 25 (4): 402–8. https://doi.org/10.1006/meth.2001.1262

Maggi, F., Tang, F.H.M., la Cecilia, D., McBratney, A., 2019. PEST-CHEM-GRIDS, global gridded maps of the top 20 crop-specific pesticide application rates from 2015 to 2025. Scientific data. 6 (1), 1–20

Meftaul, I.M., Venkateswarlu, K., Dharmarajan, R., Annamalai, P., Asaduzzaman, Parven, A., Megharaj, M., 2020. Controversies over human health and ecological impacts of Glyphosate:

Is it to be banned in modern agriculture? Environ. Pollut. 263, 114372. https://doi.org/10.1016/j.envpol.2020.114372 0269

Mie A., Rudén C., 2022. What you don’t know can still hurt you - underreporting in EU pesticide regulation. Environmental Health. 21(1) -79. doi: 10.1186/s12940-022-00891-7

Myers, J.P., Antoniou, M.N., Blumberg, B., Carroll, L., Colborn, T., Everett, L.G., Hansen, M., Landrigan, P.J., Lanphear, B.P., Mesnage, R., et al., 2016. Concerns over use of Glyphosate-based herbicides and risks associated with exposures: A consensus statement. Environ. Health. 2016, 15-19

OECD Guidelines for the Testing of Chemicals, Section 2. Test N° 202, 2004. *Daphnia s*p. Acute Immobilisation Test. https://doi.org/10.1787/9789264069947-en., Test N° 211, 2012. *Daphnia magna* Reproduction Test. https://doi.org/10.1787/9789264185203-en

Pagano, M., Kyriakides, M. and Kuyper, T.W., 2023. Effects of Pesticides on the Arbuscular Mycorrhizal Symbiosis Agrochemicals. 2(2), 337-354; https://doi.org/10.3390/agrochemicals2020020

Pierik, M., Dell’Acqua, M., Confalonieri, R., Bocchi, S., Gomarasca, S., 2016. Designing ecological corridors in a fragmented landscape: A fuzzy approach to circuit connectivity analysis. Ecological Indicators. 67, 807 - 820

Rani, L., Thapa, K., Kanojia, N., Sharma, N., Singh, S., Singh, Grewal A. S., Lal Srivastav, A., Kaushal, J., 2021. An extensive review on the consequences of chemical pesticides on human health and environment Journal of cleaner production. Journal of cleaner production. 283 - 124657 https://doi.org/10.1016/j.jclepro.2020.124657

Regoli, F., Giuliani, M.E., 2014. Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. Marine Environmental Research. 93, 106-117. https://doi.org/10.1016/j.marenvres.2013.07.006 (accessed on 28 -09-2023)

Rigal, S., Dakos, V., Alonso, H., Devictor, V., 2023. Farmland practices are driving bird population decline across Europe. PNAS. 120 (21), 2216573120; https://doi.org/10.1073/pnas.2216573120

Santovito, A., Audisio, M., Bonelli, S., 2020. A micronucleus assay detects genotoxic effects of herbicide exposure in a protected butterfly species. Ecotoxicology. 29(9), 1390-1398. doi: 10.1007/s10646-020-02276-3

Saitanis, C.J., Agathokleous, E., 2019. Stress response and population dynamics: Is Allee effect hormesis? Sci. Total Environ. 682, 623–628. https://doi.org/10.1016/j.scitotenv.2019.05.212

Salesa, B., Torres-Gavilá, J., Ferrando-Rodrigo, M.D., Sancho, E., 2022. Gene expression study alerted to possible impairment in Daphnia magna individuals as a consequence of exposure to sublethal concentrations of prochloraz. Chemosphere 308. https://doi.org/10.1016/j.chemosphere.2022.136040

Sánchez-Bayo, F., 2021. Indirect Effect of Pesticides on Insects and Other Arthropods. Toxics. 9 (8), 177. https://doi.org/10.3390/toxics9080177

Saunders, L.E. and Pezeshki, R., 2015. Glyphosate in Runoff Waters and in the Root-Zone: A Review. Toxics. 3(4), 462-480. https://doi.org/10.3390/toxics3040462

Sebastiano, M., Messina, S., Marasco, V., Costantini, D., 2022. Hormesis in ecotoxicological studies: A critical evolutionary perspective. Curr. Opin. Toxicol. 29, 25–30. https://doi.org/10.1016/j.cotox.2022.01.002

Šegota, V., Zlatković, B., Vukov, D., Alegro, A., Koletić, N., Vuković, N., Rimac, A., 2019. Status assessment of the rare aquatic plant *Groenlandia densa* (L.) Fourr. (Potamogetonaceae) in the Western Balkans, Botany Letters, doi:10.1080/23818107.2019.1585285

Semlitsch, R. D., Bridges , C. M., Welch , A. M. 2000. Genetic variation and a fitness tradeoff in the tolerance of gray treefrog (*Hyla versicolor*) tadpoles to the insecticide carbaryl. Oecologia.. 125 (2), 179–185. ISSN 0029-8549. doi: 10.1007/s004420000443.

Seyoum, A., Pradhan, A., 2019. Effect of phthalates on development, reproduction, fat metabolism and lifespan in *Daphnia magna*. Sci. Total Environ. 654, 969–977. https://doi.org/10.1016/j.scitotenv.2018.11.158

Silva, V., Montanarella, L., Jones, A., Fernández-Ugalde, O., Mol, H.G.J., Ritsema, C.J., Geissen, V., 2018. Distribution of glyphosate and aminomethylphosphonic acid (AMPA) in agricultural topsoils of the European Union. Sci. Total Environ. 621, 1352–1359

Sprankle, P., Meggitt, W.F., Penner, D., 1975. Adsorption, mobility, and microbial degradation of glyphosate in the soil. Weed Sci. 23, 229–234.

Székács, A., Darvas, B., 2018. Re-registration challenges of glyphosate in the European Union. Frontiers in Environmental Science. 6, 78. https://doi.org/10.3389/fenvs.2018.00078

Thompson, M., 2000. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing. Analyst. 125, 385-386. doi:10.1039/b000282h

van Bruggen, A.H.C., He, M.M., Shin, K., Mai, V., Jeong, K.C., Finckh, M.R., Morris, J.G., 2018. Environmental and health effects of the herbicide Glyphosate. Sci. Total Environ. 616–617, 255–268

van Bruggen, A.H.C. , Finckh, M.R., He, M., Ritsema, C.J., Harkes, P., Knuth, D., Geissen, V., 2021. Indirect Effects of the Herbicide Glyphosate on Plant, Animal and Human Health Through its Effects on Microbial Communities. Sec. Toxicology, Pollution and the Environment. 9. https://doi.org/10.3389/fenvs.2021.763917

Vereecken, H., 2005. Mobility and leaching of Glyphosate: A review. Pest. Manag. Sci. 61, 1139–1151.

Vila-Aiub, M.M, Yu, Q., Powles, S.B., 2019.Do plants pay a fitness cost to be resistant to **G**lyphosate? New Pytologyst. 223 (2), 532-547. https://doi.org/10.1111/nph.15733

Weisner, O., Frische, T., Liebmann, L., Reemtsma, T., Roß-Nickoll, M., Schäfer, R. B., et al., 2021. Risk from pesticide mixtures – the gap between risk assessment and reality. Sci. of Total Environ. 796, 149017

WHO (World Health Organization), 1994. Glyphosate/Published under the Joint Sponsorship of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. World Health Organization & International Programme on Chemical Safety. Available online: https://apps.who.int/iris/handle/10665/40044 (accessed on 26-01-2024)

www.eurl-pesticides.eu, 2023. https://www.eurl-pesticides.eu/userfiles/file/EurlSRM/EurlSrm_meth_QuPPe_PO_V12_1.pdf (accessed on 07-02-2024)

Yang, X., Wang, F., Bento, C., Xue, S., Gai, L., van Dam, R., Mol, H., Ritsema, C.J., Geissen, V., 2015. Short-term transport of Glyphosate with erosion in Chinese loess soil–a flume experiment. Sci. Total Environ. 512–513, 406–414

Zhang, X., Lin, Z., 2020. Hormesis-induced gap between the guidelines and reality in ecological risk assessment. Chemosphere 243, 125348. https://doi.org/10.1016/j.chemosphere.2019.125348

Table 2- Regional evaluation of Glyphosate pollution in the minor irrigation network

date of collection	6/8/2021				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
AMPA (µg/l)	401±143	372±138	501±171	652±216	897±288
glyphosate (µg/l)	85±38	104±46	112±49	231±93	94±41
date of collection	7/30/2021				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
AMPA (µg/l)	<0.05	0.13±0.03	<0.05	0.19±0.05	<0.05
glyphosate (µg/l)	<0.05	0.05±0.01	<0.05	0.08±0.02	0.06±0.02
date of collection	9/14/2021				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
AMPA (µg/l)	0.61±0.12	0.99±0.25	<0.05	0.20±0.05	<0.05
glyphosate (µg/l)	<0.05	<0.05	<0.05	<0.05	<0.05

L'errore è dato da un valore d'incertezza probabilistico.
Non è un valore reale, ma un fattore d'incertezza calcolato attraverso il metodo Horwitz – Thompson
Il metodo Horwitz – Thompson svincola l'incertezza di misura dal metodo ma la rende dipendente esclusivamente dal risultato (dalla concentrazione riscontrata).

			campionamenti aprile-maggio				
stazione	prov.	ult. inform.	conduc	GLIFO	+/-	AMPA	+/-
1	CR		485	275	107	37	16
2	CR		628	372	138	28	12
3	CR		706	194	79	17	7
4	CR		428	4.3	1.9	2.2	1
6	CR		819	21	9	4.4	1.9
7	CR		728	83	37	12	5
8	BG		820	nr		nr	
9	BG		740	nr		nr	
10	BG		697	nr		nr	
12	MI	font 4 pon	568	19	8	16	7
13	LO	fontanile	692	nr		6.9	3
14	MI	fontanile	635	nr		11	5
15	LO		306	32	14	10	4
16	LO	Lodi	529	29	13	5.7	2.5
17	LO		515	31	14	25	11
18	MI	Addetta	721	49	22	19	8
19	MI	Cisliano	450	104	46	22	10
20	MI	Cisliano	450	0.58	0.3	7.5	3.3
21	MI	Cusago	528	nr		2.1	0.9
22	MI	Cusago	687	0.19	0.1	3.7	1.6
23	MI	sud Cisliar	520	49	22	22	10
24	MI	sud Cisliar	614	49	22	21	9
25	MI	Abbiategr.	585	61	27	34	15
26	MI	Vernate	264	74	33	40	18
27	MI	Rosate	329	44	19	45	20
stazione	prov.	ult. inform.	conduc	GLIFO	+/-	AMPA	+/-
28	MI						
29	PV		455	11	5	14	6
30	PV		583	16	7	8.3	3.7
31	PV		412	2.5	1.1	25	11
32	PV		1170	2.9	1.3	11	5
33	PV		790	5.2	2.3	19	8
34	LO		297	6.8	3	11	5
35	CR	font Dove	530	2.3	1	4.2	1.8
36	PV		222	13	6	15	7
37	PV		243	7.3	3.2	9.7	4.3
38	PV		269	2	0.9	15	7
39	PV		207	2.9	1.3	14	6
40	PV		268	15	7	17	7
41	NO		251	7.7	3.4	13	6
42	NO		390	9.6	4.2	15	7
43	NO		170	12	5	12	5
44	PV		310	6.1	2.7	22	10
45	PV		238	8.1	3.6	14	6
46	PV		216	7.2	3.2	8.6	3.8
47	PV		256	2.8	1.2	11	5
50	CR	Tormo	545	179	74	13	6

campionamenti	data
CR-BG	14-Apr
MI-LO	5-May
MI	6-May
MI-PV-LO-CR	9-May
PV-NO	10-May

campionamenti del 12-14-15 giugno				
conduc	GLIFO	+/-	AMPA	+/-
468	nr		0.97	0.43
374	nr		3	1.3
350	nr		nr	
280	nr		1.6	0.7
344	nr		nr	
400	nr		nr	
777	nr		nr	
736	nr		nr	
704	nr		nr	
578	nr		3.5	1.5
623	nr		0.24	0.11
621	nr		nr	
257	nr		0.13	0.06
481	nr		nr	
234	nr		0.2	0.09
585	nr		nr	
222	nr		nr	
460	nr		nr	
563	nr		nr	
678	nr		nr	
546	nr		nr	
626	nr		nr	
580	nr		nr	
210	0.11	0.05	4.9	2.2
366	nr		nr	
626	0.1	0.04	5.9	2.6
610	nr		1.1	0.5
320	nr		0.37	0.16
475	nr		0.5	0.22
890	nr		3.2	1.4
717	nr		5.2	2.3
225	nr		nr	
543	nr		nr	
317	nr		4.3	1.9
357	nr		3.7	1.6
407	nr		2.8	1.2
295	nr		3.5	1.5
306	nr		5.3	2.3
296	nr		nr	
270	nr		5.5	2.4
273	nr		nr	
391	nr		0.62	0.27
283	nr		nr	
244	nr		nr	
251	nr		nr	
552	nr		nr	

	campionamenti del 12-14-15 giugno

campionamenti	data

campionamenti del 12-14-15 giugno				
conduc	GLIFO	+/-	AMPA	+/-

	campionamenti aprile-maggio					
stazione	prov.	conduc	GLIFO	+/-	AMPA	+/-
1	CR	485	275	107	37	16
2	CR	628	372	138	28	12
3	CR	706	194	79	17	7
4	CR	428	4.3	1.9	2.2	1
6	CR	819	21	9	4.4	1.9
7	CR	728	83	37	12	5
8	BG	820	nr		nr	
9	BG	740	nr		nr	
10	BG	697	nr		nr	
12	MI	568	19	8	16	7
13	LO	692	nr		6.9	3
14	MI	635	nr		11	5
15	LO	306	32	14	10	4
16	LO	529	29	13	5.7	2.5
17	LO	515	31	14	25	11
18	MI	721	49	22	19	8
19	MI	450	104	46	22	10
20	MI	450	0.58	0.26	7.5	3.3
21	MI	528	nr		2.1	0.9
22	MI	687	0.19	0.08	3.7	1.6
23	MI	520	49	22	22	10
24	MI	614	49	22	21	9
25	MI	585	61	27	34	15
26	MI	264	74	33	40	18
27	MI	329	44	19	45	20
28	MI					
29	PV	455	11	5	14	6
30	PV	583	16	7	8.3	3.7
31	PV	412	2.5	1.1	25	11
32	PV	1170	2.9	1.3	11	5
33	PV	790	5.2	2.3	19	8
34	LO	297	6.8	3	11	5
35	CR	530	2.3	1	4.2	1.8
36	PV	222	13	6	15	7
37	PV	243	7.3	3.2	9.7	4.3
38	PV	269	2	0.9	15	7
39	PV	207	2.9	1.3	14	6
40	PV	268	15	7	17	7
41	NO	251	7.7	3.4	13	6
42	NO	390	9.6	4.2	15	7
43	NO	170	12	5	12	5
44	PV	310	6.1	2.7	22	10
45	PV	238	8.1	3.6	14	6
46	PV	216	7.2	3.2	8.6	3.8
47	PV	256	2.8	1.2	11	5
50	CR	545	179	74	13	6

campionamenti	data
CR-BG	14-Apr
MI-LO	5-May
MI	6-May
MI-PV-LO-CR	9-May
PV-NO	10-May

campionamenti del 12-14-15 giugno				
conduc	GLIFO	+/-	AMPA	+/-
468	nr		0.97	0.43
374	nr		3	1.3
350	nr		nr	
280	nr		1.6	0.7
344	nr		nr	
400	nr		nr	
777	nr		nr	
736	nr		nr	
704	nr		nr	
578	nr		3.5	1.5
623	nr		0.24	0.11
621	nr		nr	
257	nr		0.13	0.06
481	nr		nr	
234	nr		0.2	0.09
585	nr		nr	
222	nr		nr	
460	nr		nr	
563	nr		nr	
678	nr		nr	
546	nr		nr	
626	nr		nr	
580	nr		nr	
210	0.11	0.05	4.9	2.2
366	nr		nr	
626	0.1	0.04	5.9	2.6
610	nr		1.1	0.5
320	nr		0.37	0.16
475	nr		0.5	0.22
890	nr		3.2	1.4
717	nr		5.2	2.3
225	nr		nr	
543	nr		nr	
317	nr		4.3	1.9
357	nr		3.7	1.6
407	nr		2.8	1.2
295	nr		3.5	1.5
306	nr		5.3	2.3
296	nr		nr	
270	nr		5.5	2.4
273	nr		nr	
391	nr		0.62	0.27
283	nr		nr	
244	nr		nr	
251	nr		nr	
552	nr		nr	

stazion	prov.	conduc	GLIFO	+/-	AMPA	+/-
1	CR	468	nr		0.97	0.4
2	CR	374	nr		3	1.3
3	CR	350	nr		nr	
4	CR	280	nr		1.6	0.7
6	CR	344	nr		nr	
7	CR	400	nr		nr	
8	BG	777	nr		nr	
9	BG	736	nr		nr	
10	BG	704	nr		nr	
12	MI	578	nr		3.5	1.5
13	LO	623	nr		0.24	0.1
14	MI	621	nr		nr	
15	LO	257	nr		0.13	0.1
16	LO	481	nr		nr	
17	LO	234	nr		0.2	0.1
18	MI	585	nr		nr	
19	MI	222	nr		nr	
20	MI	460	nr		nr	
21	MI	563	nr		nr	
22	MI	678	nr		nr	
23	MI	546	nr		nr	
24	MI	626	nr		nr	
25	MI	580	nr		nr	
26	MI	210	0.11	0.1	4.9	2.2
27	MI	366	nr		nr	
	prov.	conduc	GLIFO	+/-	AMPA	+/-
28	MI	626	0.1	0	5.9	2.6
29	PV	610	nr		1.1	0.5
30	PV	320	nr		0.37	0.2
31	PV	475	nr		0.5	0.2
32	PV	890	nr		3.2	1.4
33	PV	717	nr		5.2	2.3
34	LO	225	nr		nr	
35	CR	543	nr		nr	
36	PV	317	nr		4.3	1.9
37	PV	357	nr		3.7	1.6
38	PV	407	nr		2.8	1.2
39	PV	295	nr		3.5	1.5
40	PV	306	nr		5.3	2.3
41	NO	296	nr		nr	
42	NO	270	nr		5.5	2.4
43	NO	273	nr		nr	
44	PV	391	nr		0.62	0.3
45	PV	283	nr		nr	
46	PV	244	nr		nr	
47	PV	251	nr		nr	
50	CR	552	nr		nr	

CR-BG	14-Apr
MI-LO	5-May
MI	6-May
MI-PV-LO-CR	9-May
PV-NO	10-May

468	nr		0.97	0.43
374	nr		3	1.3
350	nr		nr	
280	nr		1.6	0.7
344	nr		nr	
400	nr		nr	
777	nr		nr	
736	nr		nr	
704	nr		nr	
578	nr		3.5	1.5
623	nr		0.24	0.11
621	nr		nr	
257	nr		0.13	0.06
481	nr		nr	
234	nr		0.2	0.09
585	nr		nr	
222	nr		nr	
460	nr		nr	
563	nr		nr	
678	nr		nr	
546	nr		nr	
626	nr		nr	
580	nr		nr	
210	0.11	0.05	4.9	2.2
366	nr		nr	
626	0.1	0.04	5.9	2.6
610	nr		1.1	0.5
320	nr		0.37	0.16
475	nr		0.5	0.22
890	nr		3.2	1.4
717	nr		5.2	2.3
225	nr		nr	
543	nr		nr	
317	nr		4.3	1.9
357	nr		3.7	1.6
407	nr		2.8	1.2
295	nr		3.5	1.5
306	nr		5.3	2.3
296	nr		nr	
270	nr		5.5	2.4
273	nr		nr	
391	nr		0.62	0.27
283	nr		nr	
244	nr		nr	
251	nr		nr	
552	nr		nr	

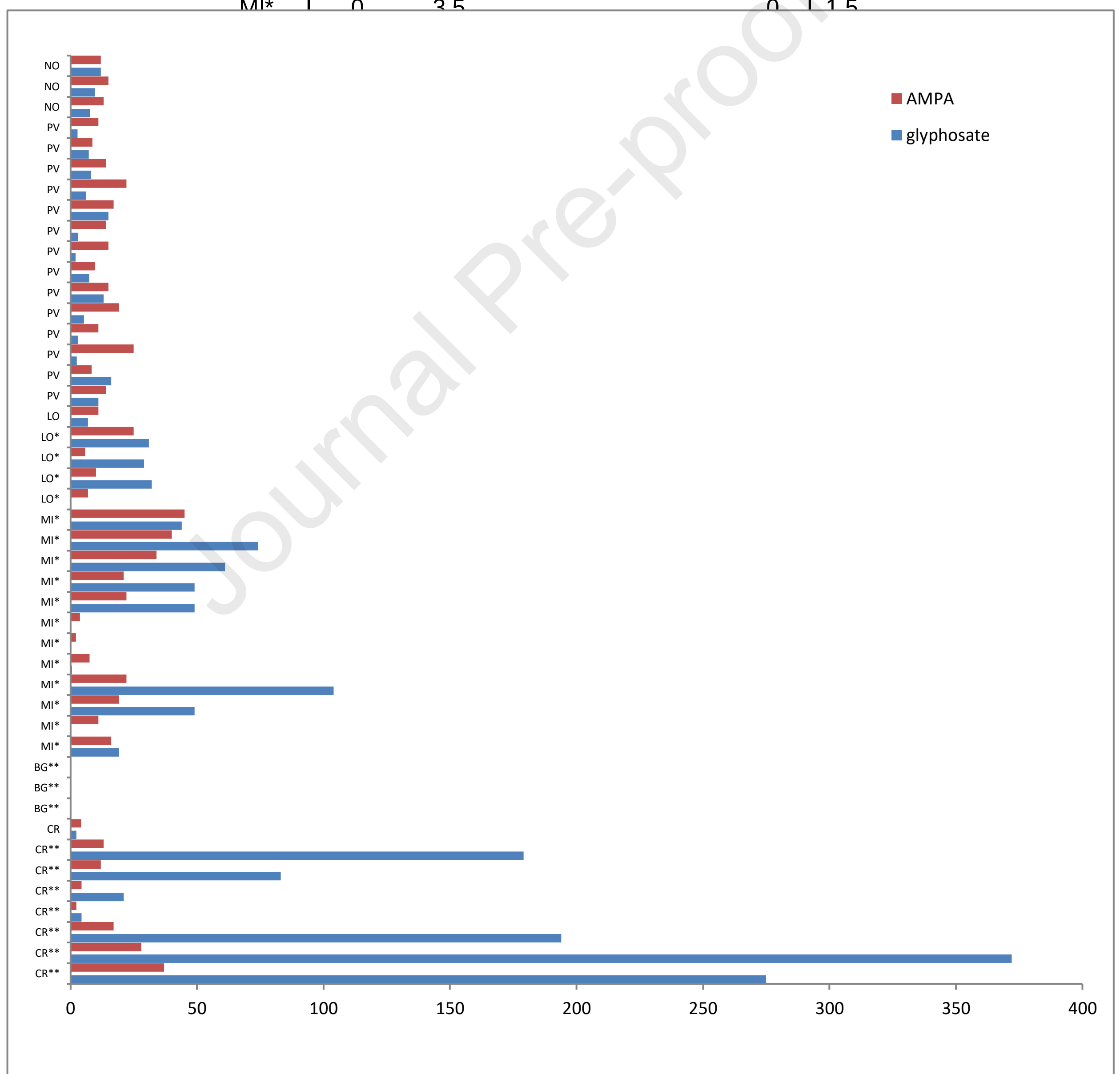
Table 3- Regional evaluation of Glyphosate pollution in the minor irrigation network

	April-May sampling					samplings of 12-14-15 June				
	conduc	glypho.	+/-	AMPA	+/-	conduc	glypho.	+/-	AMPA	+/-
Prov.	µScm-1	µg/L	µg/L	µg/L	µg/L	µScm-1	µg/L	µg/L	µg/L	µg/L
CR**	485	275	107	37	16	468	nr	-	0.97	0.43
CR**	628	372	138	28	12	374	nr	-	3	1.3
CR**	706	194	79	17	7	350	nr	-	nr	-
CR**	428	4.3	1.9	2.2	1	280	nr	-	1.6	0.7
CR**	819	21	9	4.4	1.9	344	nr	-	nr	-
CR**	728	83	37	12	5	400	nr	-	nr	-
CR**	545	179	74	13	6	552	nr	-	nr	-
CR	530	2.3	1	4.2	1.8	543	nr	-	nr	-
BG**	820	nr		nr		777	nr	-	nr	-
BG**	740	nr		nr		736	nr	-	nr	-
BG**	697	nr		nr		704	nr	-	nr	-
MI*	568	19	8	16	7	578	nr	-	3.5	1.5
MI*	635	nr		11	5	621	nr	-	nr	-
MI*	721	49	22	19	8	585	nr	-	nr	-
MI*	450	104	46	22	10	222	nr	-	nr	-
MI*	450	0.58	0.26	7.5	3.3	460	nr	-	nr	-
MI*	528	nr		2.1	0.9	563	nr	-	nr	-
MI*	687	0.19	0.08	3.7	1.6	678	nr	-	nr	-
MI*	520	49	22	22	10	546	nr	-	nr	-
MI*	614	49	22	21	9	626	nr	-	nr	-
MI*	585	61	27	34	15	580	nr	-	nr	-
MI*	264	74	33	40	18	210	0.11	0.05	4.9	2.2
MI*	329	44	19	45	20	366	nr	-	nr	-
MI	-	-	-	-	-	626	0.1	0.04	5.9	2.6
LO*	692	nr		6.9	3	623	nr	-	0.24	0.11
LO*	306	32	14	10	4	257	nr	-	0.13	0.06
LO*	529	29	13	5.7	2.5	481	nr	-	nr	-
LO*	515	31	14	25	11	234	nr	-	0.2	0.09
LO	297	6.8	3	11	5	225	nr	-	nr	-

PV	455	11	5	14	6	610	nr	-	1.1	0.5
PV	583	16	7	8.3	3.7	320	nr	-	0.37	0.16
PV	412	2.5	1.1	25	11	475	nr	-	0.5	0.22
PV	1170	2.9	1.3	11	5	890	nr	-	3.2	1.4
PV	790	5.2	2.3	19	8	717	nr	-	5.2	2.3
PV	222	13	6	15	7	317	nr	-	4.3	1.9
PV	243	7.3	3.2	9.7	4.3	357	nr	-	3.7	1.6
PV	269	2	0.9	15	7	407	nr	-	2.8	1.2
PV	207	2.9	1.3	14	6	295	nr	-	3.5	1.5
PV	268	15	7	17	7	306	nr	-	5.3	2.3
PV	310	6.1	2.7	22	10	391	nr	-	0.62	0.27
PV	238	8.1	3.6	14	6	283	nr	-	nr	-
PV	216	7.2	3.2	8.6	3.8	244	nr	-	nr	-
PV	256	2.8	1.2	11	5	251	nr	-	nr	-
NO	251	7.7	3.4	13	6	296	nr	-	nr	-
NO	390	9.6	4.2	15	7	270	nr	-	5.5	2.4
NO	170	12	5	12	5	273	nr	-	nr	-

			+/-	+/-
Prov.	lyphosat	AMPA	µg/L	µg/L
CR**	275	37	107	16
CR**	372	28	138	12
CR**	194	17	79	7
CR**	4.3	2.2	1.9	1
CR**	21	4.4	9	1.9
CR**	83	12	37	5
CR**	179	13	74	6
CR	2.3	4.2	1	1.8
BG**	0	0		
BG**	0	0		
BG**	0	0		
MI*	19	16	8	7
MI*	0	11		5
MI*	49	19	22	8
MI*	104	22	46	10
MI*	0.58	7.5	0.26	3.3
MI*	0	2.1		0.9
MI*	0.19	3.7	0.08	1.6
MI*	49	22	22	10
MI*	49	21	22	9
MI*	61	34	27	15
MI*	74	40	33	18
MI*	44	45	19	20
LO*	0	6.9		3
LO*	32	10	14	4
LO*	29	5.7	13	2.5
LO*	31	25	14	11
LO	6.8	11	3	5
PV	11	14	5	6
PV	16	8.3	7	3.7
PV	2.5	25	1.1	11
PV	2.9	11	1.3	5
PV	5.2	19	2.3	8
PV	13	15	6	7
PV	7.3	9.7	3.2	4.3
PV	2	15	0.9	7
PV	2.9	14	1.3	6
PV	15	17	7	7
PV	6.1	22	2.7	10
PV	8.1	14	3.6	6
PV	7.2	8.6	3.2	3.8
PV	2.8	11	1.2	5
NO	7.7	13	3.4	6
NO	9.6	15	4.2	7
NO	12	12	5	5

	glypho.	AMPA	+/-	+/-
Prov.	µg/L	µg/L	µg/L	µg/L
CR**	0	0.97	0	0.43
CR**	0	3	0	1.3
CR**	0	0	0	0
CR**	0	1.6	0	0.7
CR**	0	0	0	0
CR**	0	0	0	0
CR**	0	0	0	0
CR	0	0	0	0
BG**	0	0	0	0
BG**	0	0	0	0
BG**	0	0	0	0
MI*	0	3.5	0	1.5
NO	0	0	0	0
NO	0	5.5	0	2.4
NO	0	0	0	0



UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE E POLITICHE AMBIENTALI
DEPARTMENT OF ENVIRONMENTAL SCIENCE AND POLICY

Highlights

1) The herbicide Glyphosate is one of the currently best-selling and most-used agricultural products worldwide
2) Its impacts on the ecosystem and human health are yet to be further investigated considering the levels of pollution possible in certain territorial contexts
3) Sampling to establish the spread is generally carried out in the main watercourses and does not follow agronomic crop treatment calendars, leading to a possible underestimation of the problem
4) Studies of health impacts on organisms should be updated considering the concentrations actually recorded in minor watercourses in contact with cultivated fields

Dipartimento di Scienze e Politiche Ambientali - Department of Environmental Science and Policy
via Celoria, 2 - 20133 Milano - tel: 02 503 16475 / 16471 fax: 02 503 16486