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7 **and glufosinate ammonium**

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Herbicides in River Water across the Northeastern Italy: Occurrence and Spatial Patterns of Glyphosate, Aminomethylphosphonic Acid and Glufosinate Ammonium

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ABSTRACT

Glyphosate and glufosinate ammonium are the active ingredients of commonly used herbicides. Active agricultural lands extend over ~45% of the Veneto region (Eastern Po Valley, Italy) and glyphosate and glufosinate ammonium are widely used. Consequently, surface waters can be potentially contaminated. This study investigates the occurrence of glyphosate and glufosinate ammonium as well as aminomethylphosphonic acid (AMPA, the degradation product of glyphosate) in river water of Veneto. Eighty-six samples were collected in 2015 at multiple sampling points across the region. Samples were analyzed for the 2 target herbicides, AMPA as well as for other variables, including water temperature, pH, dissolved oxygen, conductivity, hardness, BOD, COD, inorganic ions, total nitrogen, total phosphorus, total suspended solids, arsenic, and lead. The average concentrations (all samples) were 0.17, 0.18 and 0.10 $\mu\text{g L}^{-1}$ for glyphosate, AMPA, and glufosinate ammonium, respectively. The European upper tolerable level for pesticides (annual average 0.1 $\mu\text{g L}^{-1}$) was often exceeded. Chemometric analysis was therefore applied to: (i) investigate the relationships among water pollutants; (ii) detect the potential sources of water contamination; (iii) assess the effective water pollution of rivers by identifying river basins with anomalous pollution levels; and (iv) assess the spatial variability of detected sources. Factor analysis identified 4 factors interpreted as potential sources and processes (use of herbicides, leaching of fertilizers, urban/industrial discharges, and the biological activity on polluted or stagnant waters). A discriminant analysis revealed that the pollution from anthropogenic discharges is homogeneously present in Veneto, while biological activity and fertilizers present heterogeneous distributions. This study gives insights into the concentrations of herbicides in rivers flowing through a wide region that has heavy use of these chemicals in agriculture. The study also points out some hot-spots and suggests the future implementation of the current monitoring protocols and network.

Keywords: Rivers; Herbicides; Glyphosate; Glufosinate ammonium; Aminomethylphosphonic acid; Veneto

1. Introduction

The contamination of surface water is one of the most serious environmental issues currently addressed in research (Moore and Ramamoorthy 2012; Lapworth et al. 2012; Stuart et al. 2012; Meffe and de Bustamante 2014): water pollution was extensively associated with many adverse effects on human health and aquatic life (e.g., Schinegger et al. 2012; Zareitalabad et al. 2013; Khan et al. 2014; Richardson et al. 2015; Morrissey et al. 2015). In Europe, the protection of surface waters is regulated under the European Water Framework Directive (EC 2000), which aims to achieve good qualitative and quantitative status of all water bodies by 2015. However, its implementation has been –and still is– a major challenge for the Member States (Hering et al. 2010).

Since its commercial introduction in 1974, glyphosate (GLY, N-(phosphonomethyl)glycine) has become the main active ingredient of common herbicides and weed-killing formulations. GLY is used in agriculture and silviculture, as well as along roadways, railways and in urban areas. GLY is a water-soluble ($> 10 \text{ g L}^{-1}$ at 25°C), highly effective broad-spectrum, postemergence, non-selective herbicide. Its success was largely boosted by: (i) the absence of relevant harmful environmental effects except than for target organisms; (ii) the introduction of transgenic glyphosate-resistant crops around mid-90s; (iii) the apparent limited risk of leaching to groundwater due to its strong sorption in soil and a relatively fast microbial degradation to aminomethylphosphonic acid (AMPA), its primary metabolite (Baylis 2000; Cerdeira and Duke 2006; Duke and Powles 2008).

The use of GLY is controversial: its environmental implications, ecotoxicology and potential carcinogenicity are still debated. Its soil half-life is highly variable (2 to 215 days; Giesy et al. 2000; Battaglin et al. 2014a;b) as well as its mobility in soil, depending on soil properties, such as pH, mineralogy, texture, amount and chemical composition of organic matter, and soil tillage and erosion (Sørensen et al. 2006; Laitinen et al. 2006; Borggaard and Gimsing 2008; Todorovich et al. 2014). The half-life in aquatic environments ranges from 2 to 91 days (Vera et al. 2010). AMPA has a lower water solubility (5.8 g L^{-1} at 25°C) and a longer soil half-life (60 to 240 days), while the aquatic half-life is comparable to GLY (Giesy et al. 2000; Bergstrom et al. 2011).

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Despite the fast degradation in soils and the apparent low mobility, GLY adsorbed in soil particles may flow into surface and ground-waters due to leaching from crops and runoff. The use of GLY-based formulations near aquatic environments or spillage, volatilization, and atmospheric deposition after crop spraying are other potential pathways for water contamination. In addition, GLY has an extensive use in urban areas (Hanke et al. 2010): the pavement covering retards infiltration and promotes a rapid runoff to sewage systems. This way, GLY residues and AMPA are frequently found in sewer systems, in the environment (e.g., Battaglin et al. 2005;2009;2014a;b; Scribner et al. 2007; Crowe et al. 2011; Coupe et al. 2012; Glazier et al. 2012; Aparicio et al. 2013; Van Stempvoort et al. 2014; Struger et al. 2015; Poiger et al. 2017) and on food (Bøhn et al. 2014).

GLY essentially inhibits the enzyme 3-enol-pyruvyl-shikimate-5-phosphate synthase in chloroplasts and disrupts the biosynthesis of aromatic amino acids in the shikimate pathway. Since this process only exists in plants, bacteria, and fungi, its toxicity to animals is supposed negligible. However, the effects of various GLY formulations on nontarget organisms are amply discussed, especially for the degradation of microbial communities in soil (Lancaster et al. 2010; Zabaloy et al. 2012) and rivers (Pesce et al. 2009), the effects on phytoplankton (Saxon et al. 2011), periphyton (Vera et al. 2010), plants (Gomes et al. 2014), fishes (Çavaş and Könen 2007; do Carmo Langiano and Martinez 2008; Guilherme et al. 2012), amphibians (Wagner et al. 2013; Smalling et al. 2015; Battaglin et al. 2016) and vertebrates (Paganelli et al. 2010). In addition, the adverse effects on human health are still unclear (e.g., Mink et al. 2011; Myers et al. 2016). Although many studies report carcinogen/mutagen/reprotoxic effects (e.g., Benachour and Séralini 2008; Gasnier et al. 2009; Thongprakaisang et al. 2013), other studies do not show significantly increased risk (Mink et al. 2012; Kier and Kirkland 2013). Under this view, it is not completely clear if the possible toxic effects are driven by the active ingredient itself, its metabolites or other additives (surfactants) added to different herbicide formulations (e.g., Peixoto 2005; Mann et al. 2009; Lipok et al. 2010; Wagner et al. 2013; Mesnage et al. 2015).

Glufosinate ammonium (GLU, (RS)-2-Amino-4-(hydroxy(methyl)phosphonoyl)butanoic acid) is another widely-used active ingredient of non-selective herbicides (Duke 2014), and it is mostly used as a pre-harvest desiccant. GLU is highly soluble in water (solubility, ~1370 g L⁻¹) and has a half-life of ~1 week (range 3–42 days) (Siimes et al. 2006; Dinehart et al. 2009; Carpenter and Boutin 2010, and references therein). Similarly to GLY, the main sources of GLU in aquatic systems are related to surface runoff, spraying and leaching from treated crops. Many studies pointed out its

1 harmful effects on biota (e.g., Peltzer et al. 2013; Lajmanovich et al. 2014). Although toxicological
2 studies indicated that GLU is harmful to human beings at acute doses (Mao et al. 2012), its chronic
3 effects at low doses remain largely unclear (Calas et al. 2008).
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7 In Italy, the use of GLY has recently increased from 1473 to 2090 tons (+22%) between 2002 and
8 2011. However, northeastern regions of Italy have experienced even a faster trend: in Veneto (NE
9 Italy, Figure 1), the use of GLY has dramatically increased from 42 to 316 tons (+648%) in 2002/11
10 (APPA 2014). Although the use of GLU is more limited than GLY, its use across Italy has also
11 increased from 46 tons in 2002 to 79 tons in 2009, when it was temporarily banned from the market
12 in April 2010. However, the ban ended in April 2012, when more than 40% of GLU sold in Italy
13 was marketed in Veneto (APPA 2014). Since approx. 45% of the Veneto hosts active agricultural
14 lands mostly requiring irrigation, the surface waters of Veneto are strongly exposed to these
15 herbicides.
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25 In 2015, ARPAV (the Environmental Protection Agency of Veneto region, Italy) started a
26 monitoring program aiming to assess the water quality in major rivers across the Veneto. This study
27 investigates the concentrations and seasonality of GLY, AMPA and GLU measured over 1 year
28 (2015) as well as the levels of other water pollutants, nutrients and other physicochemical variables
29 in major rivers included in 8 main drainage basins. The study, the first carried out in the NE Italy,
30 aims to: (i) detect the concentrations of herbicides on a seasonal basis; (ii) identify rivers or
31 drainage basins with anomalous pollution levels (iii) investigate the relationships among water
32 pollutants; (iv) detect the potential sources of pollution; and (v) assess the spatial patterns of the
33 potential sources of water contamination.
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44 **2. Materials and methods**

45 *2.1 The Veneto region*

46 The Veneto (Figure 1) covers an area of $\sim 18 \cdot 10^3$ km² and hosts a population of $4.9 \cdot 10^6$ inhabitants.
47 The northwestern part is mainly occupied by mountains (Alps), with a low population density
48 mostly concentrated along narrow valleys. A wide southeastern alluvial plain accounts for most
49 (56%) of the territory and is affected by heavy anthropogenic pressures due to the presence of major
50 cities, industrial areas, and intensive farming. A belt of hilly environments is located between
51 mountains and the lowland: it hosts rural environments and farming, mostly vineyards and orchards.
52 Administrative and terrain relief, major river basins and land cover maps are provided in Figure 1a,
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1b, and 1c, respectively. The alluvial plain is composed of sandy to silty-clay materials deposited by major rivers: the northwestern plain is generally characterized by more permeable soils, while the central and southern plain host heavy soils and waterlogging with shallow groundwater levels (sometimes <2 m). The two areas are separated by a belt of springs called “risorgive”, which generate several streams. The soil permeability map is provided as supplementary material Figure S11. Soils in the plain areas are also characterized by low organic carbon content (Figure S11), especially where intensive agriculture is practiced. The low levels of soil organic matter limit the cation exchange capacity, lower the fertility and increase the potential mobility of contaminants, including herbicides.

2.2 Sampling

The sampling sites are shown in Figure 1; their characteristics are listed in Table 1. Sites were selected along 24 major rivers or streams flowing across 8 main drainage basins, named Adige, Brenta, Canalbianco, Livenza, Piave, Po, Sile and the drainage basin of the Lagoon of Venice (DBLV) (Figure 1b). This latter basin needs special care: it hosts several streams and small rivers flowing directly into a large (~500 km²-wide) coastal lagoon with high pollution and nutrient levels (e.g., Zirino et al. 2016). Three more samples were collected close to springs in the “risorgive” area. Each site was sampled during one year with different frequency (1-5 samples per site): the total number of samples is reported in Table 1. Water was collected near the center of the river or, wherever not possible, at points having flowing water stream (i.e. no samples were collected on stagnant water conditions). Samples were stored in pre-cleaned HDPE bottles and in the dark at +4°C to prevent sample degradation, photochemical reactions and were analyzed within 6 days (ISO 2014). During the sampling, water temperature was also measured, as well as pH (method APAT-CNR-IRSA-2060) and dissolved oxygen (method APAT CNR-IRSA 4120).

2.3 Experimental

GLY, AMPA and GLU were analyzed following the method ISO 16308:2014 (Hanke et al. 2008; ISO 2014). Briefly, the compounds are derivatized using 9-fluorenylmethylchloroformate (FMOC-Cl) in order to lower their polarity and increase the retention of compound in a separation on a reverse phase column as well as to improve the mass spectrometric detection. The derivatized sample is then purified by liquid/liquid extraction and then concentrated by solid phase extraction (SPE). The analysis is performed by high performance liquid chromatography coupled with tandem mass spectrometry via an electrospray source (HPLC–ESI–MS/MS; Hanke et al. 2008), using

1 matrix-matched calibration. Calibration of the instrument was performed for every analytical batch;
2 limit of quantification (LOQs) was 0.05 $\mu\text{g L}^{-1}$.
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5 Physicochemical characteristics of water and chemical species were also analyzed using well
6 established analytical protocols (methods are reported within brackets): water conductivity by
7 conductivity-meter (EPA Method 120.1; EPA 1982); hardness by titration with EDTA (APAT CNR
8 IRSA 2040); total suspended solids (TSS) by gravimetric determination (APAT CNR-IRSA 2090);
9 biochemical oxygen demand by respirometric method (5-day BOD, method APHA 5210D);
10 chemical oxygen demand (COD) using ready-to-use small tube tests (ISO 15705:2002; ISO 2002);
11 cations (Na^+ , Mg^{2+} , K^+ , Ca^{2+} , APAT CNR-IRSA 3030) and anions (F^- , EPA 300.1; Cl^- and sulfate,
12 APAT-CNR-RSA 4020) by ion chromatography; N-NH_4^+ (APHA 4500-NH₃-H) by flow injection
13 analysis, total Kjeldahl using oxidative digestion with peroxodisulfate (TKN, APHA 4500-N-B); N-NO_2^-
14 and N-NO_3^- by spectrometric detection (UNI-EN-ISO 13395:1996; ISO 1996); P-PO_4^{3-} by
15 Mo-blue spectrophotometric method (APAT-CNR-IRSA 4110); total phosphorus by oxidation and
16 Mo-blue spectrophotometric method (TP, APAT-CNR-IRSA 4060); arsenic by hydride generation
17 (APAT CNR-IRSA 3080A); and lead by FAAS (APAT CNR-IRSA 3230).
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31 *2.4 QA/QC and data handling*

32 Method performance for GLY, AMPA and GLU was tested by participation to LGC proficiency
33 test AQ 492/2015 (LGC Aquacheck 2017) obtaining a satisfactory Z-score ($Z < 2$) among
34 laboratories of European countries and confirming a good accuracy of the adopted analytical
35 protocol. At least two water samples for each batch were spiked with target compounds and then
36 included in the analytical procedure: recoveries were in the range of 80-100%. Precision was
37 evaluated through analysis of replicated spiked water samples: results showed relative standard
38 deviations $< 30\%$. In this study, all the samples analyzed for herbicides were used for descriptive
39 statistics, but only samples also analyzed for the remaining chemical and physical variables were
40 further used for explorative analysis. Data below the LOQ were set as LOQ/2.
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51 *2.5 Chemometrics*

52 The water samples were collected in rivers with different characteristics and/or affected by different
53 anthropogenic pressures. Rivers also flow over different soil and rock types. This way, the chemical
54 and physical characteristics of water may change according to the strength of natural/anthropogenic
55 sources, the occurrence of biochemical processes in water, the soil characteristics, the flow rate, the
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1 closeness to point sources, the spatial distribution of diffuse sources, etc. A factor analysis (FA) was
2 therefore performed to investigate the inter-variable relationships and to identify the most probable
3 sources of water contamination or the ongoing biochemical processes. The principal aim of FA is to
4 reduce the dimensionality of the dataset and to detect the main hidden processes/sources driving
5 most of the variance of the original dataset.
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10 Most of the species analyzed in this study are not normally distributed (Shapiro–Wilks test), with
11 most of the variables exhibiting positive skewness. In addition, most variables have large
12 differences in the units, i.e. the variables exhibits a striking difference in the amount of variability.
13 For these reasons, nonparametric tests and correlations are used. Since factor analysis is affected by
14 data distribution and data scale (e.g., Reimann et al. 2002), a series of data transformations were
15 applied to obtaining a robust dataset. Firstly, a Box-Cox transformation (Box and Cox 1964) was
16 applied to approach normal distributions; thus, a standardization (mean zero and unit variance) was
17 applied to scale the data and overcome differences in variation ranges.
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27 In a second step, a discriminant analysis (DA) was applied to the factor score matrix to study the
28 spatial distribution of identified factors, i.e., to verify whether the sites in a drainage basin are
29 isolated or characterized by a general homogeneity of the sources/processes. DA is typically applied
30 to detect variables which significantly explain differences between two or more groups (drainage
31 basins, in this case). The results of the test of univariate equality of group means can classify
32 variables (factors, in this case) as not discriminant or discriminant: high Wilks' Λ (>0.9) and
33 significance >0.3 identify not discriminant variables, i.e., homogeneously present in all drainage
34 basins. On the contrary, significances below 0.05 identify discriminant variables, i.e., having a
35 heterogeneous distribution over the study area.
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45 **3. Results and Discussion**

46 Figure 2, summarizes the results as boxplots. The annual average and min-max concentrations are
47 reported in Table 1 for each river. The maximum level of GLY permitted in the United States for
48 drinking water is $700 \mu\text{g L}^{-1}$ (maximum contaminant level and goal) and is based on toxicity tests
49 (EPA 2017). In Europe, the upper tolerable level for all the pesticides in drinking water is
50 administratively set to $0.1 \mu\text{g L}^{-1}$ (EC 1998). This regulatory limit is applied to annual average
51 concentrations. The average concentrations across the Veneto (all seasons, all sites) were 0.17, 0.18
52 and $0.10 \mu\text{g L}^{-1}$ for GLY, AMPA and GLU, respectively. The threshold of $0.1 \mu\text{g L}^{-1}$ was often
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1 exceeded in single samples (26%, 37% and 22% of all analyzed samples, respectively); however,
2 only 11, 14 and 7 sites breached the annual upper tolerable level computed over multiple samples
3 for GLY, AMPA and GLU, respectively. The higher annual average concentrations of GLY were
4 recorded on Musoncello ($0.72 \mu\text{g L}^{-1}$), followed by some sites along Livenza (Cervada, $0.49 \mu\text{g}$
5 L^{-1} ; Livenza $0.45 \mu\text{g L}^{-1}$) and Canalbianco (Nuovo Adigetto $0.4 \mu\text{g L}^{-1}$), while AMPA was higher
6 on Teva ($0.77 \mu\text{g L}^{-1}$), Musoncello ($0.48 \mu\text{g L}^{-1}$), and Livenza ($0.55 \mu\text{g L}^{-1}$). The river Musoncello
7 was also affected by the higher annual concentrations of GLU ($0.72 \mu\text{g L}^{-1}$), followed by Teva
8 ($0.42 \mu\text{g L}^{-1}$). Musoncello presents, therefore, the higher annual average concentrations of
9 herbicides: it is affected by substantial loads from the urban sewer of Castelfranco Veneto (~33,000
10 inhabitants) and then flows through agricultural areas by also touching other towns (Resana).
11 Finally, it joins the Dese River and, then, flows into the Lagoon of Venice. Therefore, further
12 investigations and/or sampling campaigns are suggested for those polluted rivers in order to better
13 monitor the sources of herbicides. In addition, more sites should be placed close to the outlets to
14 quantify the load of herbicides flowing into the Lagoon of Venice.
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27 The design of our sampling campaign is not sufficient to accurately represent seasonal
28 concentrations or to identify peak concentrations at single rivers, which can be missed even with a
29 weekly sampling intervals (Mahler et al. 2016). However, the analysis of seasonal differences for
30 the whole set of data (all rivers together) may still give indications of some processes or potential
31 sources. The Kruskal-Wallis analysis of variance by ranks was applied as a global non-parametric
32 test for depicting statistically significant seasonal variations of analyzed variables. The null
33 hypothesis is rejected for $p < 0.05$, meaning that concentrations are statistically different among
34 seasons. Results are shown in Table SII: only water temperature, pH, and concentrations of GLY
35 and AMPA were statistically different.
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45 Water temperature followed a typical seasonal pattern ranging from $5\text{-}10^\circ\text{C}$ in winter and $15\text{-}30^\circ\text{C}$
46 in summer; such pattern reflects the variations in the ambient air temperature. The pH ranged from
47 7 to 8.5. The seasonality of pH is the mirror image of water temperature: the less alkaline values in
48 summer are likely due to the increase of the biological activity in water. The herbicides were rarely
49 detected during spring, while the higher median concentrations for GLY and AMPA were measured
50 in summer, followed by autumn and winter. This pattern is likely related to the seasonality of crops.
51 Glyphosate is typically applied after crops and weeds have emerged from the soil (Battaglin et al.
52 2014a), but it can be applied more than once during the growing season: this way, in Northern Italy
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1 crops and orchards are mostly treated in late-spring and summer when unwanted plants grow faster.
2 However, herbicides are also applied to vineyards until mid-autumn (grape-harvest). In addition,
3 residues of herbicides are supposed to remain in the soil for weeks (half-life for GLY and GLU are
4 2–91 and 3–42 days, respectively), therefore surface runoff and draining to groundwater may
5 continue for months after treatment. Similar patterns were found in surface waters in Switzerland
6 (Poiger et al. 2017) and rural/urban catchments in Canada (Struger et al. 2015).
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10 11 12 3.1 Correlations among variables 13

14 The relationship between the analyzed variables was preliminarily investigated through the
15 correlation analysis (Table 2). The Spearman correlation ρ was chosen because of the different
16 nature, scales and units used by variables and the non-normal distribution of data. Water
17 conductivity showed strong ($\rho > 0.6$), significant ($p < 0.05$) positive correlations with most ions and
18 hardness: a higher conductivity involves higher concentrations of ions, including carbonate and
19 bicarbonate concentrations (not directly measured) and, consequently, increases water hardness (Ca
20 and Mg). The mountain chains (Alps and Prealps) are mainly composed of sequences of
21 sedimentary rocks (mainly limestone and dolomite) on metamorphic basements with magmatic
22 extrusions. Springs of major rivers (e.g., Piave, Brenta, Adige) are located in the Alps, while other
23 rivers flow (Livenza) or join tributaries (e.g., Brenta) flowing from karstic systems (Capraro et al.
24 2011). Other major rivers (e.g., Bacchiglione, Dese, Sile, Zero) born in the “risorgive” area from
25 springs fed by aquifers catching water across the Prealps area. This way, the dissolution of
26 limestones (and, in a minor extent, Dolomites) releases Ca and Mg.
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40 Among other ions, sodium and potassium are strongly correlated with chlorides; they reach the
41 higher concentrations close to river outlets and, thus, this relationship may also reflect the saltwater
42 intrusion. Among the nutrients, ammonium is strongly correlated with orthophosphates, but only
43 weakly ($0.4 < \rho < 0.6$) correlated with nitrates. The high correlations between TKN and nitrate and
44 between TP and orthophosphate reflect the dominant fraction of such species upon N- and P-
45 compounds, respectively.
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52 The correlation between chloride and arsenic is expected: generally, high As concentrations
53 correlated well with increased salinity in surface waters (Smedley and Kinniburgh 2002). Under
54 oxidizing conditions and pH values commonly recorded in the monitored rivers, most of the arsenic
55 is expected in the As(V) state, presumably as HAsO_4^{2-} ; however, As(III) can be favored by the
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1 biological reduction in anoxic waters, i.e. mostly during summer. In Veneto, high concentration of
2 As are commonly found in: (i) the Brenta river valley (average 20 and 24 mg kg⁻¹ in topsoil and
3 subsoil, respectively; Ungaro et al. 2008); (ii) groundwater (> 400 µg L⁻¹; Carraro et al. 2013;2015),
4 and (iii) river waters. The relatively high levels of As are mainly linked to lithogenic enrichment
5 (e.g., volcanic and metamorphic rocks are present in some regions of the Alps) or anthropogenic
6 contamination. A study on the levels of heavy metals in bryophytes along the Brenta River (Nimis
7 et al. 2002) showed a widespread contamination by As. This latter study related the high
8 concentrations of arsenic to the discharge of pesticides from agricultural activities.
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16 GLY and GLU exhibit a moderate correlation (0.53) and are also well correlated with AMPA (0.64
17 and 0.44, respectively). However, they are not well correlated ($\rho < 0.4$) with any other variable.
18 AMPA is the main metabolite of GLY but is also formed by the degradation of phosphonic acids in
19 detergents, such as EDTMP and DTPMP (Skark et al. 1998; Jaworska et al. 2002; Nowack 2003;
20 Lesueur et al. 2005). Finally, AMPA degrades to inorganic phosphate, ammonium, and CO₂
21 (Borggaard and Gimsing 2008). Under this view, it may play a role in the levels of total phosphorus
22 in aquatic systems (Vera et al. 2010). AMPA exhibits a strong correlation with GLY ($\rho = 0.64$), a
23 poor correlation with orthophosphate ($\rho = 0.26$) and it is uncorrelated to TP. This result suggests that
24 GLY degradation is the dominant source of AMPA in river waters of Veneto. However, the lack of
25 a clear correlation with P-compounds may be masked by the strong input of P-containing species
26 from other sources, e.g., fertilization, urban and industrial discharges.
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38 *3.2 Potential sources of river contamination*

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40 The transformed dataset (Box-Cox/standardized) was used as input for a Varimax rotated FA. A
41 first attempt was made by including all the species. However, a pre-selection of variables to be
42 processed in FA was subsequently performed to ensure robust and reliable results and to exclude
43 chemically redundant species: (i) some variables (COD, Na⁺, K⁺, Pb) were excluded because their
44 high percentage of missing data (>25%); (ii) missing data for other variables were substituted with
45 the variable median; (iii) hardness was preferred to Mg²⁺ and Ca²⁺ because of their high correlation
46 and the lower number of missing data; (iv) total phosphorous and TKN were excluded because their
47 strong correlations with orthophosphates and the sum of N-species, respectively; (v) nitrite was
48 excluded because the high associated uncertainty due to its relatively unstable oxidation state; (vi)
49 dissolved O₂ was converted from percent saturation to water concentration by considering the
50 correction factors for water conductivity, water temperature and barometric pressure (USGS
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1 DOTABLES; U.S. Geological Survey 2011); (vii) hydrogen ion activity $[H^+]$ (mEq L⁻¹) was
2 calculated from pH to obtain a linear variable. Four factors with eigenvalues >1 were extracted,
3 accounting for ~70% of total variance. A 4-factor solution was also suggested by parallel analysis, a
4 method for determining the number of components or factors to retain from FA (Hayton et al.
5 2004).
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10 Along with the factor loadings (Table 3), an $n \times m$ factor score matrix is also extracted: it is
11 composed of n cases (samples collected) and m new variables proportional to the daily source
12 impact. The factor scores were then mapped on a seasonal basis (Figure 3): although a quantitative
13 analysis is not possible due to the data transformation, the factor scores may be mapped to show the
14 spatial/temporal gradients of extracted factors; they are, therefore, useful to qualitatively interpret
15 the data.
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23 *Factor 1* (23% of variance) mainly represents the analyzed ions and, in particular, all the nutrients.
24 It is primarily composed (loading >0.6) of anions (chloride, sulfate, nitrate), ammonium and,
25 secondarily (0.35 < loadings < 0.6), orthophosphate. Consequently, the factor also exhibits high
26 loading of hardness (directly linked to Ca and Mg) and water conductivity (0.83), which reflects the
27 ionic activity. Ammonium, nitrate and phosphate are the main components of NPK fertilizers;
28 ammonium also derives from fertilizers made by ammonia, anhydrous ammonium nitrate as well as
29 from urea. Nitrate may enter water bodies directly as the result of runoff of fertilizers containing
30 nitrate, from atmospheric deposition and/or urban/industrial discharges. The oxidation of more
31 reduced N-containing compounds, including nitrite, ammonia, and organic nitrogen compounds
32 (e.g., amino acids, urea) is another source of nitrate: this process occurs in both soil and water
33 following the biological activity of ammonia-oxidizing bacteria (e.g., Nitrosomonas) and the
34 oxidation of nitrite driven by Nitrobacter and Nitrospira. Sulfate is a ubiquitous ion in natural
35 waters and can be released by a variety of natural (e.g., dissolution of minerals) and anthropogenic
36 (e.g., fertilizers, mine drainage, urban and industrial runoff) sources. Chloride can also derive from
37 similar anthropogenic sources (wastewaters, fertilizers). In winter, chloride may also derive from
38 the runoff of deicing salts in roads. The use of fertilizers is the most probable source, also according
39 to some insights: (i) fertilizers are used throughout the year and factor scores are not statistically
40 significant different among seasons (analysis of variance, ANOVA, at $p < 0.05$); (ii) maps of factor
41 scores (Figure 3) show that high scores are recorded in the sites across the lowland, where crops are
42 present; and (iii) although K^+ was excluded from the FA due to the missing data, it shows high
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1 correlations with most of the ions, conductivity (Table 2) and with the factor scores for factor 1; (iv)
2 potassium chloride and potassium sulfate are two main species in modern fertilizers.
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5 Since an excessive loading of nutrients (considering all nitrogen species and phosphorus) is a major
6 threat to water quality and may cause eutrophication (e.g., Cherry et al. 2008; Withers and Jarvie
7 2008; Carey and Migliaccio 2009), rivers showing high scores of factor 1 should be investigated in
8 more detail to find the most impacting point and diffuse sources. Since eutrophication is a major
9 threat for the Lagoon of Venice (e.g., Masiol et al. 2014; Zirino et al. 2016), rivers flowing through
10 DBLV and having high scores for factor 1 deserve future investigations.
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17 *Factor 2* (19% of variance) is made up of arsenic, orthophosphate, total suspended solids and,
18 secondarily, chloride and ammonium. Under this view, it can be related to a pollution source. For
19 example, phosphate may be released from various anthropogenic sources, including urban and
20 industrial sewage discharges (e.g., Withers and Jarvie 2008), while arsenic may be linked to several
21 anthropogenic point sources (e.g., Smedley and Kinniburgh 2002). However, high concentrations of
22 arsenic can be also related to the high pedo-geochemical background concentrations, e.g. above 20
23 mg kg⁻¹ in the Brenta River basin (Giandon et al. 2000;2004; Ungaro et al. 2008) and in
24 groundwater (Carraro et al. 2013;2015).
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34 Factor 2 also shows a strong negative loading with dissolved oxygen (-0.67), which is indicative of
35 an ongoing aerobic activity. Low levels of dissolved oxygen are generally indicative of oligotrophic
36 water conditions, which likely occurs during the warmer season, i.e. when higher water temperature
37 and lower river flow rates are expected. Under this view, statistically significant (ANOVA at
38 $p < 0.05$) inter-seasonal differences were found: the application of a multiple comparison post hoc
39 test (Tukey HSD; Tukey 1949) reported significantly (95% confidence level) higher factor scores in
40 summer and autumn than in winter. Such seasonal pattern confirms that the lower dissolved oxygen
41 is likely driven by a stronger aerobic activity during the warmer season, as well as the more
42 stagnant waters in summer due to low flow rates (ARPAV 2017). The high loading of TSS further
43 confirms this hypothesis, as the turbidity and the presence of colloids generally increase in more
44 stagnant waters. The poor correlation of factor 2 with BOD (0.23) further suggests that the amount
45 of biodegradable organic material is not a limiting factor for the aerobic activity or may indicate
46 that the biological activity has depleted most of the organic material (i.e. the source does not
47 represent a fresh input to the river).
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2 *Factor 3* (15%) only links GLY, AMPA and GLU. The absence of high loadings with any other
3 analyzed species indicates that the contamination of herbicides is uncorrelated with other pollution
4 sources. The higher scores are found in summer > autumn > winter, and sites in the province of
5 Treviso (TV, Figure 1) generally show the higher factor scores throughout the year. Relatively high
6 scores are also recorded during summer in the two more northern sites (Piave drainage basin),
7 which generally show the lower scores for the remaining factors (Figure 3). These rivers (Anfella
8 and Val di Frari) flow in mountain areas and, therefore, are not likely affected by a load of
9 herbicides from agriculture or silviculture. These sites represent an anomaly that should be
10 investigated in more detail.
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20 *Factor 4* (12%) links BOD, dissolved oxygen and, secondarily, ammonium; it also shows a negative
21 correlation with the activity of H⁺, i.e. it is linked to the more alkaline waters. No statistically
22 significant inter-seasonal differences are found, i.e. it is almost constant all the year.
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25 This factor depicts waters with high loads of organic matter (BOD), but it also represents waters
26 with high primary production and/or affected by low aerobic activity (high loading of dissolved O₂).
27 A possible interpretation is the fresh release of anthropogenic discharges of nutrients and effluents
28 and the consequent increased photosynthetic activity. Figure 3 shows pretty similar factor scores
29 recorded during the year at the sites across the plain area of the region; however, a significant
30 increase of the scores was found for the more northerly sites. This increase could be related to the
31 expected boost of anthropogenic effluents (sometimes without efficient sewer systems) following to
32 the summer touristic season in the mountain.
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42 *3.3 Spatial distribution of sources*

43 The factor scores were used in DA as independent variables; three areas including 5 drainage basins
44 having similar characteristics were selected as grouping variable: (1) Brenta and Bacchiglione, i.e.
45 rivers flowing in the center and southern part of Veneto; (2) DBLV and Sile, rivers mostly flowing
46 from “risorgive” springs; (3) Piave and Livenza, rivers flowing in the northern part of Veneto with
47 sources located in the Alps, but also having heavy contributions from “risorgive”. Samples
48 collected in Po, Canalbianco and Adige were excluded from DA due to the low number of sites and
49 samples. The test of univariate equality of group means (Table 4) shows that only factor 4 is not
50 discriminant, having the highest Wilks’ λ (0.98) and presenting a significance of 0.5. This result
51 indicates that the pollution due to the fresh release of anthropogenic discharges (mostly attributable
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2 to urban or industrial sewage effluents) is homogeneously present in all the study area. Since the
3 outputs from urban or industrial sewage effluents are expected to be constant through the year, this
4 result confirms the interpretation of factor 4.
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7 On the contrary, factors 1, 2 and 3 are highly discriminant (significance <0.05), i.e. they present
8 heterogeneous distributions over the 3 groups of rivers. Two discriminating functions (Table 4)
9 were also extracted and interpreted by analyzing their correlations with the input variables (factors):
10 the first function only presents weak correlations with factor 1 (fertilizers/salinity) and 2 (biological
11 activity and arsenic), while the second one presents the largest absolute correlation with the factors
12 2 and 3 (herbicides). Figure 4 shows the bi-dimensional scatterplot of sample scores into the planes
13 defined by the discriminant functions. The plot shows that the samples in the 3 groups of rivers are
14 generally well differentiated under the discriminant function 1 (weakly correlated with factors 1 and
15 2), with higher scores for samples collected in the southern area (Brenta-Bacchiglione) and lower
16 for the samples collected to the north (Piave-Livenza). On the contrary, group centroids are not well
17 separated along the discriminant function 2.
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28 **4. Conclusions**

29 This study is the first one investigating the occurrence of glyphosate, glufosinate ammonium and
30 AMPA in river water of the NE Italy. The main findings of this study can be summarized as
31 follows:
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- 36 1) The contamination of herbicides is a critical issue in Veneto: GLY, AMPA and GLU
37 frequently exceeded the European upper tolerable levels for pesticides (annual average $0.1 \mu\text{g}$
38 L^{-1}) during 2015. However, this tolerable level is based on political consensus, not
39 ecotoxicological significance and it is very low if compared to the maximum level of GLY
40 permitted in the United States ($700 \mu\text{g} \text{L}^{-1}$) based on toxicity tests;
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42 2) GLY and AMPA showed statistically different seasonal concentrations, with higher medians
43 in summer and autumn and lower in spring. This seasonal pattern agrees with the use of
44 herbicides in agriculture and silviculture;
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46 3) The River Musoncello was affected by the higher annual average concentrations of GLY and
47 GLU. This river catches substantial loads from urban sewer of some large towns and should be
48 investigated in more detail in future studies;
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50 4) The correlation and factor analyses pointed out the inter-species relationships. Four factors
51 were extracted and interpreted as possible sources/processes affecting the water quality of
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1 rivers. Herbicides were identified by a single factor. Two more factors were linked to possible
2 sources: the leaching of fertilizers and the urban/industrial discharges. Another factor was
3 attributed to the biological activity on polluted or stagnant waters;
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5 5) A discriminant analysis was performed on the factor scores and over 3 areas representative
6 of 5 drainage basins. Results revealed that the anthropogenic discharges (mostly attributable to
7 urban or industrial sewage effluents) are homogeneously present over all the study area, while
8 biological activity and fertilizers present heterogeneous distributions. However, a clear spatial
9 gradient was not detected.
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11 This study shows that the extensive use of herbicides in Veneto affects the water
12 quality of major rivers and poses the basis for further investigations. The study also gives
13 important insights upon the most impacting pollution sources of river water. The large
14 uncertainty raised by the current scientific literature on the possible adverse health effects of
15 glyphosate and glufosinate ammonium does not make possible to assess a potential
16 increased risk for human health. Reducing the use of herbicides across hydrologically
17 contributing areas, close to streams and rivers potentially affected by fast runoff and/or soil
18 erosion is, therefore, the most simple and effective approach to mitigate a diffuse herbicide
19 pollution in Veneto.
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31 **Disclaimer**

32 The views and the conclusion expressed in this paper are exclusive of the authors and may not
33 reflect those of ARPAV. This study is not intended to replace any official report and/or data
34 released by ARPAV; these latter data should be considered as a reference for administrative and
35 regulatory purposes.
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51 **References**

52 Aparicio, VC, De Gerónimo, E, Marino, D, Primost, J, Carriquiriborde, P and Costa, JL (2013)
53 Environmental fate of glyphosate and aminomethylphosphonic acid in surface waters and
54 soil of agricultural basins. *Chemosphere* 93(9):1866-1873
55
56

57 ARPAV (2017) Rapporto sulla risorsa idrica del Veneto [Report on water resources of the Veneto].
58 <http://www.arpaveneto.it/temi-ambientali/idrologia/file-e-allegati/rapporti-e->
59
60
61
62
63
64
65

documenti/idrologia-regionale/idrologia-regionale-rapporti-sulla-risorsa-
idrica/2015/rapporti-risorsa-idrica-2015. Accessed March 2017 [in Italian]

- 1
2
3 Battaglin, WA, Kolpin, DW, Scribner, EA, Kuivila, KM, Sandstrom, MW (2005) Glyphosate, other
4 herbicides, and transformation products in midwestern streams:2002. *J Am Water Resour*
5 *As* 41(2):323-332
6
7 Battaglin, WA, Rice, KC, Focazio, MJ, Salmons, S, Barry, RX (2009) The occurrence of
8 glyphosate, atrazine, and other pesticides in vernal pools and adjacent streams in
9 Washington, DC, Maryland, Iowa, and Wyoming:2005–2006. *Environ Monit Assess*
10 155(1-4):281-307
11
12
13 Battaglin, WA, Meyer, MT, Kuivila, KM and Dietze, JE:2014a Glyphosate and its degradation
14 product AMPA occur frequently and widely in US soils, surface water, groundwater, and
15 precipitation. *J Am Water Resour As* 50(2):275-290
16
17
18 Battaglin, WA, Meyer, MT, Kuivila, K, Dietze, JE:2014b Glyphosate and AMPA in US streams,
19 groundwater, precipitation and soils. *J Am Water Resour As* 50(2):275-290
20
21
22 Battaglin, WA, Smalling, KL, Anderson, C, Calhoun, D, Chestnut, T, Muths, E (2016) Potential
23 interactions among disease, pesticides, water quality and adjacent land cover in amphibian
24 habitats in the United States. *Sci Total Environ* 566:320-332
25
26
27 Baylis AD (2000) Why glyphosate is a global herbicide: strengths, weaknesses and prospects. *Pest*
28 *Manag Sci* 56:299–308
29
30
31 Benachour, N and Séralini, GE (2008) Glyphosate formulations induce apoptosis and necrosis in
32 human umbilical, embryonic, and placental cells. *Chem Res Toxicol* 22(1):97-105
33
34
35 Bergstrom, L, E Borjesson, and J Stenstrom (2011) Laboratory and Lysimeter Studies of
36 Glyphosate and Aminomethylphosphonic Acid in a Sand and a Clay Soil. *J Environ Qual*
37 40:98-108
38
39
40 Bøhn, T, Cuhra, M, Traavik, T, Sanden, M, Fagan, J and Primicerio, R (2014) Compositional
41 differences in soybeans on the market: glyphosate accumulates in Roundup Ready GM
42 soybeans. *Food Chem* 153:207-215
43
44
45 Borggaard, OK and Gimsing, AL (2008) Fate of glyphosate in soil and the possibility of leaching to
46 ground and surface waters: a review. *Pest Manag Sci* 64(4):441-456
47
48
49 Box GEP, Cox DR:1964 An analysis of transformations. *J Roy Statist Soc B* 26, 211–252
50
51 Calas, AG, Richard, O, Mème, S, Beloeil, JC, Doan, BT, Gefflaut, T, Mème, W, Crusio, WE,
52 Pichon, J and Montécot, C (2008) Chronic exposure to glufosinate-ammonium induces
53 spatial memory impairments, hippocampal MRI modifications and glutamine synthetase
54 activation in mice. *Neurotoxicology* 29(4):740-747
55
56
57 Capraro, F, Bizzotto, A, Masiol, M, Pavoni, B (2011) Chemical analyses of spring waters and factor
58 analysis to monitor the functioning of a karstic system The role of precipitations regimen
59 and anthropic pressures. *J Environ Monit* 13:2543-2549
60
61
62
63
64
65

- 1 Carey, RO and Migliaccio, KW (2009) Contribution of wastewater treatment plant effluents to
2 nutrient dynamics in aquatic systems: a review. *Environ Manage* 44(2):205-217
3
- 4 Carraro, A, Fabbri, P, Giaretta, A, Peruzzo, L, Tateo, F and Tellini, F (2013) Arsenic anomalies in
5 shallow Venetian Plain (Northeast Italy) groundwater. *Environ Earth Sci* 70(7):3067-3084
6
7
- 8 Carraro, A, Fabbri, P, Giaretta, A, Peruzzo, L, Tateo, F and Tellini, F (2015) Effects of redox
9 conditions on the control of arsenic mobility in shallow alluvial aquifers on the Venetian
10 Plain (Italy). *Sci Total Environ* 532:581-594
11
12
- 13 Carpenter, D and Boutin, C (2010) Sublethal effects of the herbicide glufosinate ammonium on
14 crops and wild plants: short-term effects compared to vegetative recovery and plant
15 reproduction. *Ecotoxicology* 19(7):1322-1336
16
17
- 18 Çavaş, T and Könen, S (2007) Detection of cytogenetic and DNA damage in peripheral
19 erythrocytes of goldfish (*Carassius auratus*) exposed to a glyphosate formulation using the
20 micronucleus test and the comet assay. *Mutagenesis* 22(4):263-268
21
22
- 23 Cerdeira, AL, Duke, SO (2006) The current status and environmental impacts of glyphosate-
24 resistant crops: a review. *J Environ Qual* 35:1633–1658
25
26
- 27 Cherry, KA, Shepherd, M, Withers, PJA and Mooney, SJ (2008) Assessing the effectiveness of
28 actions to mitigate nutrient loss from agriculture: A review of methods. *Sci Total Environ*
29 406(1):1-23
30
- 31 Coupe, RH, Kalkhoff, SJ, Capel, PD and Gregoire, C (2012) Fate and transport of glyphosate and
32 aminomethylphosphonic acid in surface waters of agricultural basins. *Pest Manag Sci*
33 68(1):16-30
34
35
- 36 Crowe, AS, Leclerc, N, Struger, J, Brown, S (2011) Application of a glyphosate-based herbicide to
37 *Phragmites australis*: Impact on groundwater and near-shore lake water at a beach on
38 Georgian Bay. *J Great Lakes Res* 37(4):616-624
39
40
- 41 Dinehart, SK, Smith, LM, McMurry, ST, Anderson, TA, Smith, PN and Haukos, DA (2009)
42 Toxicity of a glufosinate-and several glyphosate-based herbicides to juvenile amphibians
43 from the Southern High Plains, USA. *Sci Total Environ* 407(3):1065-1071
44
45
- 46 do Carmo Langiano, V, Martinez, CB (2008) Toxicity and effects of a glyphosate-based herbicide
47 on the Neotropical fish *Prochilodus lineatus*. *Comp Biochem Physiol C Toxicol Pharmacol*
48 147(2):222-231
49
50
- 51 Duke, SO and Powles, SB (2008) Glyphosate: a once-in-a-century herbicide. *Pest Manag Sci*
52 64(4):319-325
53
54
- 55 Duke, SO (2014) Biotechnology: herbicide-resistant crops In: Van Alfen NK (Ed), *Encyclopedia of*
56 *Agriculture and Food Systems*, 2nd volume: 94-116, Elsevier, Amsterdam, The
57 Netherlands
58
59
60
61
62
63
64
65

- 1 EC (European Commission), 1998 Directive 1998/83/EC of 3 November 1998 on the quality of
2 water intended for human consumption. Official J Europ Comm 5(98), L330
- 3 EC (European Commission) (2000) Directive 2000/60/EC of the European Parliament and of the
4 Council of 23 October 2000 establishing a framework for Community action in the field of
5 water policy. Official J Europ Union L 327 :22/12/2000 P 0001 – 0073
- 6
7
- 8 EEA (European Environment Agency) (2017) CORINE Land Cover Database 2012.
9 <http://landcopernicuseu/pan-european/corine-land-cover/clc-2012>. Accessed November
10 2017
- 11
12
- 13 EPA (US Environmental Protection Agency):1982 Method 1201: Conductance (Specific
14 Conductance, $\mu\text{mhos}/25^{\circ}\text{C}$) by Conductivity Meter.
15 https://www.epa.gov/sites/production/files/2015-08/documents/method_120-1_1982.pdf.
16 Accessed March 2017
- 17
18
- 19 EPA (US Environmental Protection Agency) (2017) National Primary Drinking Water Regulations.
20 [https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-](https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations)
21 [regulations](https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations). Accessed November 2017
- 22
23
- 24 Gasnier, C, Dumont, C, Benachour, N, Clair, E, Chagnon, MC and Séralini, GE (2009) Glyphosate-
25 based herbicides are toxic and endocrine disruptors in human cell lines. *Toxicology*
26 262(3):184-191
- 27
28
- 29 Giandon, P, Vinci, I, Fantinato, L (2000) Heavy metal concentration in the soils of the basin
30 draining the Venice Lagoon. *Bollettino della Società Italiana di Scienza del Suolo* 49 (1–
31 2):359–366 [in Italian]
- 32
33
- 34 Giandon, P, Cappellin, R, Ragazzi, F, Vinci, I (2004) Confronto tra il livello naturale e il livello
35 antropico dei metalli pesanti nei suoli della pianura veneta in relazione al materiale di
36 partenza. *Bollettino della Società Italiana di Scienza del Suolo* 53 (1–2):540–544 [in
37 Italian]
- 38
39
- 40 Glozier, NE, Struger, J, Cessna, AJ, Gledhill, M, Rondeau, M, Ernst, WR, Sekela, MA, Cagampan,
41 SJ, Sverko, E, Murphy, C, Murray, JL (2012) Occurrence of glyphosate and acidic
42 herbicides in select urban rivers and streams in Canada (2007). *Environ Sci Pollut Res*
43 19(3):821-834
- 44
45
- 46 Guilherme, S, Gaivão, I, Santos, MA and Pacheco, M (2012) DNA damage in fish (*Anguilla*
47 *anguilla*) exposed to a glyphosate-based herbicide—elucidation of organ-specificity and the
48 role of oxidative stress. *Mutat Res-Gen Tox En* 743(1):1-9
- 49
50
- 51 Giesy JP, Dobson S and Solomon KR (2000) Ecotoxicological risk assessment for Roundup c
52 herbicide. *Rev Environ Contam Toxicol* 167:35–120
- 53
54
- 55 Gomes, MP, Smedbol, E, Chalifour, A, Hénault-Ethier, L, Labrecque, M, Lepage, L, Lucotte, M
56 and Juneau, P (2014) Alteration of plant physiology by glyphosate and its by-product
57 aminomethylphosphonic acid: an overview. *J Exp Bot* 65(17):4691-4703
- 58
59
60
61
62
63
64
65

- 1 Hanke, I, Singer, H and Hollender, J (2008) Ultratrace-level determination of glyphosate,
2 aminomethylphosphonic acid and glufosinate in natural waters by solid-phase extraction
3 followed by liquid chromatography–tandem mass spectrometry: performance tuning of
4 derivatization, enrichment and detection. *Anal Bioanal Chem* 391(6):2265-2276
5
- 6 Hanke I, Wittmer I, Bischofberger S, Stamm C and Singer H (2010) Relevance of urban glyphosate
7 use for surface water quality. *Chemosphere* 81:422–429
8
- 9 Hayton, JC, Allen, DG, Scarpello, V (2004) Factor retention decisions in exploratory factor
10 analysis: A tutorial on parallel analysis. *Organ Res Meth* 7(2):191-205
11
- 12 Hering, D, Borja, A, Carstensen, J, Carvalho, L, Elliott, M, Feld, CK, Heiskanen, AS, Johnson, RK,
13 Moe, J, Pont, D and Solheim, AL (2010) The European Water Framework Directive at the
14 age of 10: a critical review of the achievements with recommendations for the future. *Sci*
15 *Total Environ* 408(19):4007-4019
16
17
- 18 ISO:1996 Water quality - Determination of nitrite nitrogen and nitrate nitrogen and the sum of both
19 by flow analysis (CFA and FIA) and spectrometric detection.
20 http://www.iso.org/iso/catalogue_detail.htm?csnumber=21870. Accessed February 2017
21
22
- 23 ISO (2002) Water quality–Determination of the chemical oxygen demand index (ST-COD)–Small-
24 scale-sealed-tube method. http://www.iso.org/iso/catalogue_detail.htm?csnumber=28778.
25 Accessed February 2017
26
27
- 28 ISO (2014) Water quality - Determination of glyphosate and AMPA - Method using high
29 performance liquid chromatography (HPLC) with tandem mass spectrometric detection.
30 http://www.iso.org/iso/catalogue_detail.htm?csnumber=56140. Accessed February 2017
31
32
33
- 34 Jaworska, J, Van Genderen-Takken, H, Hanstveit, A, van de Plassche, E and Feijtel, T (2002)
35 Environmental risk assessment of phosphonates, used in domestic laundry and cleaning
36 agents in the Netherlands. *Chemosphere* 47(6):655-665
37
38
- 39 Khan, MB, Masiol, M, Hofer, A and Pavoni, B (2014) Harmful Elements in Estuarine and Coastal
40 Systems. In *PHEs, Environment and Human Health* (37-83), Springer, The Netherlands
41
42
- 43 Kier, LD and Kirkland, DJ (2013) Review of genotoxicity studies of glyphosate and glyphosate-
44 based formulations. *CRC Cr Rev Toxicol* 43(4):283-315
45
46
- 47 Laitinen P, Siimes K, Eronen L, Rämö S, Welling L, Oinonen S, et al 2006 Fate of the herbicides
48 glyphosate, glufosinate ammonium, phenmedipham, ethofumesate and metamilon in two
49 Finnish arable soils. *Pest Manag Sci* 62:473–491
50
- 51 Lajmanovich, RC, Cabagna-Zenklusen, MC, Attademo, AM, Junges, CM, Peltzer, PM, Bassó, A
52 and Lorenzatti, E (2014) Induction of micronuclei and nuclear abnormalities in tadpoles of
53 the common toad (*Rhinella arenarum*) treated with the herbicides Liberty® and
54 glufosinate-ammonium. *Mutat Res-Gen Tox En* 769:7-12
55
56
57
58
59
60
61
62
63
64
65

- 1 Lancaster, SH, Hollister, EB, Senseman, SA and Gentry, TJ (2010) Effects of repeated glyphosate
2 applications on soil microbial community composition and the mineralization of
3 glyphosate. *Pest Manag Sci* 66(1):59-64
- 4 Lapworth, DJ, Baran, N, Stuart, ME and Ward, RS (2012) Emerging organic contaminants in
5 groundwater: a review of sources, fate and occurrence. *Environ Pollut* 163:287-303
- 6
7
8 LGC Aquacheck (2017) Standards Proficiency Testing PT AQ492 LGC Standards Proficiency
9 Testing, Bury, Lancashire, UK. <https://uslgstandardscom/US/en/proficiency-testing>.
10 Accessed November 2017
- 11
12
13 Lesueur, C, Pfeffer, M and Fuerhacker, M (2005) Photodegradation of phosphonates in water.
14 *Chemosphere* 59(5):685-691
- 15
16
17 Lipok, J, Studnik, H and Gruyaert, S (2010) The toxicity of Roundup® 360 SL formulation and its
18 main constituents: glyphosate and isopropylamine towards non-target water
19 photoautotrophs. *Ecotox Environ Safe* 73(7):1681-1688
- 20
21
22 Mahler, BJ, Van Metre, PC, Burley, TE, Loftin, KA, Meyer, MT, and Nowell, LH:2016,
23 Similarities and differences in occurrence and temporal fluctuations in glyphosate and
24 atrazine in small Midwestern streams (USA) during the 2013 growing season. *Sci Total*
25 *Environ* 579:149-158
- 26
27
28 Mann, RM, Hyne, RV, Choung, CB and Wilson, SP (2009) Amphibians and agricultural chemicals:
29 review of the risks in a complex environment. *Environ Pollut* 157(11):2903-2927
- 30
31
32 Masiol, M, Facca, C, Visin, F, Sfriso, A and Pavoni, B (2014) Interannual heavy element and
33 nutrient concentration trends in the top sediments of Venice Lagoon (Italy). *Marine*
34 *Pollution Bulletin* 89(1):49-58
- 35
36
37 Mao, YC, Hung, DZ, Wu, ML, Tsai, WJ, Wang, LM, Ger, J, Deng, JF and Yang, CC (2012) Acute
38 human glufosinate-containing herbicide poisoning. *Clin Toxicol* 50(5):396-402
- 39
40
41 Meffe, R and de Bustamante, I (2014) Emerging organic contaminants in surface water and
42 groundwater: a first overview of the situation in Italy. *Sci Total Environ* 481:280-295
- 43
44
45 Mesnage, R, Defarge, N, De Vendomois, JS and Seralini, GE (2015) Potential toxic effects of
46 glyphosate and its commercial formulations below regulatory limits. *Food Chem Toxicol*
47 84:133-153
- 48
49
50 Mink, PJ, Mandel, JS, Lundin, JI and Scurman, BK (2011) Epidemiologic studies of glyphosate
51 and non-cancer health outcomes: a review. *Regul Toxicol Pharm* 61(2):172-184
- 52
53
54 Mink, PJ, Mandel, JS, Scurman, BK and Lundin, JI (2012) Epidemiologic studies of glyphosate
55 and cancer: a review. *Regul Toxicol Pharm* 63(3):440-452
- 56
57
58 Moore, JW and Ramamoorthy, S (2012) Heavy metals in natural waters: applied monitoring and
59 impact assessment. Springer Science & Business Media

- 1 Morrissey, CA, Mineau, P, Devries, JH, Sanchez-Bayo, F, Liess, M, Cavallaro, MC and Liber, K
2 (2015) Neonicotinoid contamination of global surface waters and associated risk to aquatic
3 invertebrates: a review. *Environ Int* 74:291-303
- 4 Myers, JP, Antoniou, MN, Blumberg, B, Carroll, L, Colborn, T, Everett, LG, Hansen, M,
5 Landrigan, PJ, Lanphear, BP, Mesnage, R and Vandenberg, LN (2016) Concerns over use
6 of glyphosate-based herbicides and risks associated with exposures: a consensus statement.
7 *Environ Health* 15(1), p19
- 8
9
10 Nimis, PL, Fumagalli, F, Bizzotto, A, Codogno, M and Skert, N (2002) Bryophytes as indicators of
11 trace metal pollution in the River Brenta (NE Italy). *Sci Total Environ* 286(1):233-242
- 12
13
14 Nowack, B (2003) Environmental chemistry of phosphonates. *Water Res* 37(11):2533-2546
- 15
16
17 Paganelli, A, Gnazzo, V, Acosta, H, López, SL and Carrasco, AE (2010) Glyphosate-based
18 herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling.
19 *Chem Res Toxicol* 23(10):1586-1595
- 20
21
22 Peixoto, F (2005) Comparative effects of the Roundup and glyphosate on mitochondrial oxidative
23 phosphorylation. *Chemosphere* 61(8):1115-1122
- 24
25
26 Peltzer, PM, Junges, CM, Attademo, AM, Bassó, A, Grenón, P and Lajmanovich, RC (2013)
27 Cholinesterase activities and behavioral changes in *Hypsiboas pulchellus* (Anura: Hylidae)
28 tadpoles exposed to glufosinate ammonium herbicide. *Ecotoxicology* 22(7):1165-1173
- 29
30
31 Pesce, S, Batisson, I, Bardot, C, Fajon, C, Portelli, C, Montuelle, B and Bohatier, J (2009) Response
32 of spring and summer riverine microbial communities following glyphosate exposure.
33 *Ecotox Environ Safe* 72(7):1905-1912
- 34
35
36 Poiger, T, Buerge, IJ, Bächli, A, Müller, MD and Balmer, ME (2017) Occurrence of the herbicide
37 glyphosate and its metabolite AMPA in surface waters in Switzerland determined with on-
38 line solid phase extraction LC-MS/MS. *Environ Sci Pollut Res* 24(2):1588–1596
- 39
40
41 Reimann, C, Filzmoser, P and Garrett, RG (2002) Factor analysis applied to regional geochemical
42 data: problems and possibilities. *Appl Geochem* 17(3):185-206
- 43
44
45 Richardson, SD and Kimura, SY (2015) Water analysis: emerging contaminants and current issues.
46 *Anal Chem* 88(1):546-582
- 47
48
49 Saxton, MA, Morrow, EA, Bourbonniere, RA and Wilhelm, SW (2011) Glyphosate influence on
50 phytoplankton community structure in Lake Erie. *J Great Lakes Res* 37(4):683-690
- 51
52
53 Schinegger, R, Trautwein, C, Melcher, A and Schmutz, S (2012) Multiple human pressures and
54 their spatial patterns in European running waters. *Water Environ J* 26(2):261-273
- 55
56
57 Schulte-Hermann, R, Wogan, GN, Berry, C, Brown, NA, Czeizel, A, Giavini, E, Holmes, LB,
58 Kroes, R, Nau, H, Neubert, D and Oesch, F (2006) Analysis of reproductive toxicity and
59 classification of glufosinate-ammonium. *Reg Toxicol Pharm* 44(3):1-76
- 60
61
62
63
64
65

- 1 Scribner, EA, Battaglin, WA, Gilliom, RJ, Meyer, MT (2007) Concentrations of glyphosate, its
2 degradation product, aminomethylphosphonic acid, and glufosinate in ground-and surface-
3 water, rainfall, and soil samples collected in the United States:2001-06 (No 2007-5122),
4 Geological Survey (US)
- 5
- 6 Skark, C, Zullei-Seibert, N, Schöttler, U and Schlett, C:1998 The occurrence of glyphosate in
7 surface water International. *J Environ Anal Chem* 70(1-4):93-104
- 8
- 9
- 10 Siimes, K, Rämö, S, Welling, L, Nikunen, U and Laitinen, P (2006) Comparison of the behaviour
11 of three herbicides in a field experiment under bare soil conditions. *Agr Water Manage*
12 84(1):53-64
- 13
- 14 Smalling, KL, Reeves, R, Muths, E, Vandever, M, Battaglin, WA, Hladik, ML, Pierce, CL (2015)
15 Pesticide concentrations in frog tissue and wetland habitats in a landscape dominated by
16 agriculture. *Sci Total Environ* 502:80-90
- 17
- 18
- 19 Smedley, PL and Kinniburgh, DG (2002) A review of the source, behaviour and distribution of
20 arsenic in natural waters. *Appl Geochem* 17(5):517-568
- 21
- 22
- 23 Sørensen SR, Schultz A, Jacobsen OS and Aamand J (2006) Sorption, desorption and
24 mineralisation of the herbicides glyphosate and MCPA in samples from two Danish soil
25 and subsurface profiles. *Environ Pollut* 141:184–194
- 26
- 27
- 28 Stuart, M, Lapworth, D, Crane, E and Hart, A (2012) Review of risk from potential emerging
29 contaminants in UK groundwater. *Sci Total Environ* 416:1-21
- 30
- 31 Struger, J, Van Stempvoort, DR and Brown, SJ (2015) Sources of aminomethylphosphonic acid
32 (AMPA) in urban and rural catchments in Ontario, Canada: Glyphosate or phosphonates in
33 wastewater? *Environ Pollut* 204:289-297
- 34
- 35
- 36 Thongprakaisang, S, Thiantanawat, A, Rangkadilok, N, Suriyo, T and Satayavivad, J (2013)
37 Glyphosate induces human breast cancer cells growth via estrogen receptors. *Food Chem*
38 *Toxicol* 59:129-136
- 39
- 40
- 41
- 42 Todorovic, GR, Rampazzo, N, Mentler, A, Blum, WE, Eder, A, Strauss, P (2014) Influence of soil
43 tillage and erosion on the dispersion of glyphosate and aminomethylphosphonic acid in
44 agricultural soils. *Int Agrophys* 28(1):93-100
- 45
- 46
- 47 Tukey, J:1949 Comparing Individual Means in the Analysis of Variance. *Biometrics* 5(2):99–114
- 48
- 49
- 50 Ungaro, F, Ragazzi, F, Cappellin, R and Giandon, P (2008) Arsenic concentration in the soils of the
51 Brenta Plain (Northern Italy): mapping the probability of exceeding contamination
52 thresholds. *J Geochem Expl* 96(2):117-131
- 53
- 54
- 55 US Geological Survey:2011, Change to solubility equations for oxygen in water: Office of Water
56 Quality Technical Memorandum 201103.
57 <https://water.usgs.gov/admin/memo/QW/qw1103pdf>. Accessed November 2017
- 58
- 59
- 60
- 61
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26
27
28
29
30
31
32
33
34
35
- Van Stempvoort, DR, Roy, JW, Brown, SJ and Bickerton, G (2014) Residues of the herbicide glyphosate in riparian groundwater in urban catchments. *Chemosphere* 95:455-463
- Vera, MS, Lagomarsino, L, Sylvester, M, Pérez, GL, Rodríguez, P, Mugni, H, Sinistro, R, Ferraro, M, Bonetto, C, Zagarese, H and Pizarro, H (2010) New evidences of Roundup®(glyphosate formulation) impact on the periphyton community and the water quality of freshwater ecosystems. *Ecotoxicology* 19(4):710-721
- Wagner, N, Reichenbecher, W, Teichmann, H, Tappeser, B and Lötters, S (2013) Questions concerning the potential impact of glyphosate- based herbicides on amphibians. *Environ Toxicol Chem* 32(8):1688-1700
- Withers, PJA and Jarvie, HP (2008) Delivery and cycling of phosphorus in rivers: a review. *Sci Total Environ* 400(1):379-395
- Zabaloy, MC, Gómez, E, Garland, JL and Gómez, MA (2012) Assessment of microbial community function and structure in soil microcosms exposed to glyphosate. *Appl Soil Ecol* 61:333-339
- Zareitalabad, P, Siemens, J, Hamer, M and Amelung, W (2013) Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in surface waters, sediments, soils and wastewater—a review on concentrations and distribution coefficients. *Chemosphere* 91(6):725-732
- Zirino, A, Elwany, H, Facca, C, Neira, C and Mendoza, G (2016) Nitrogen to phosphorus ratio in the Venice (Italy) Lagoon (2001–2010) and its relation to macroalgae. *Marine Chem* 180:33-41

36 **Table Captions**

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Table 1. Characteristics of the sampling sites and average (min-max) concentrations of target compounds. GLY= Glyphosate; GLU= Glufosinate Ammonium; AMPA= Aminomethylphosphonic Acid. Provinces (see Figure 1) are: BL= Belluno; TV= Treviso; VE= Venice; PD= Padua; RO= Rovigo. LOD= limit of detection.

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Table 2. Spearman's correlations among variables. Only correlations statistically significant at $p < 0.05$ are shown.

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Table 3. Results of factor analysis (Varimax rotated solution). Variables with factor loadings (>0.6) are in bold; factor loadings less than 0.35 are not shown; variables are ordered for decreasing absolute loadings. Var (%): percentage of variance explained by each factor; Cum.Var. (%): cumulative variance.

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Table 4. Results of the discriminant analysis: test of equality of group mean (left) and structure matrix (right) with extracted discriminant functions. Largest absolute correlation between each variable and any discriminant function are in bold.

Figure Captions

Figure 1. Map of the Veneto Region: administrative borders and terrain relief (left); main rivers and drainage basins (center); land use and cover from CORINE Land Cover 2012 data (right) (EEA, 2017). Provinces (left map) are: BL= Belluno; TV= Treviso; VI= Vicenza; VR= Verona; VE= Venice; PD= Padua; RO= Rovigo.

Figure 2. Seasonal distributions of the analyzed variables. Data are aggregated to show data collected at all sites during the four seasons. Boxplot lines= medians, boxes= 25th-75th percentile ranges, whiskers= ± 1.5 *inter-quartile ranges. Outliers and extremes not shown.

Figure 3. Maps of factor scores for the four factors extracted.

Figure 4. Discriminant scores scatterplot. Group centroids are shown as gray crosses.

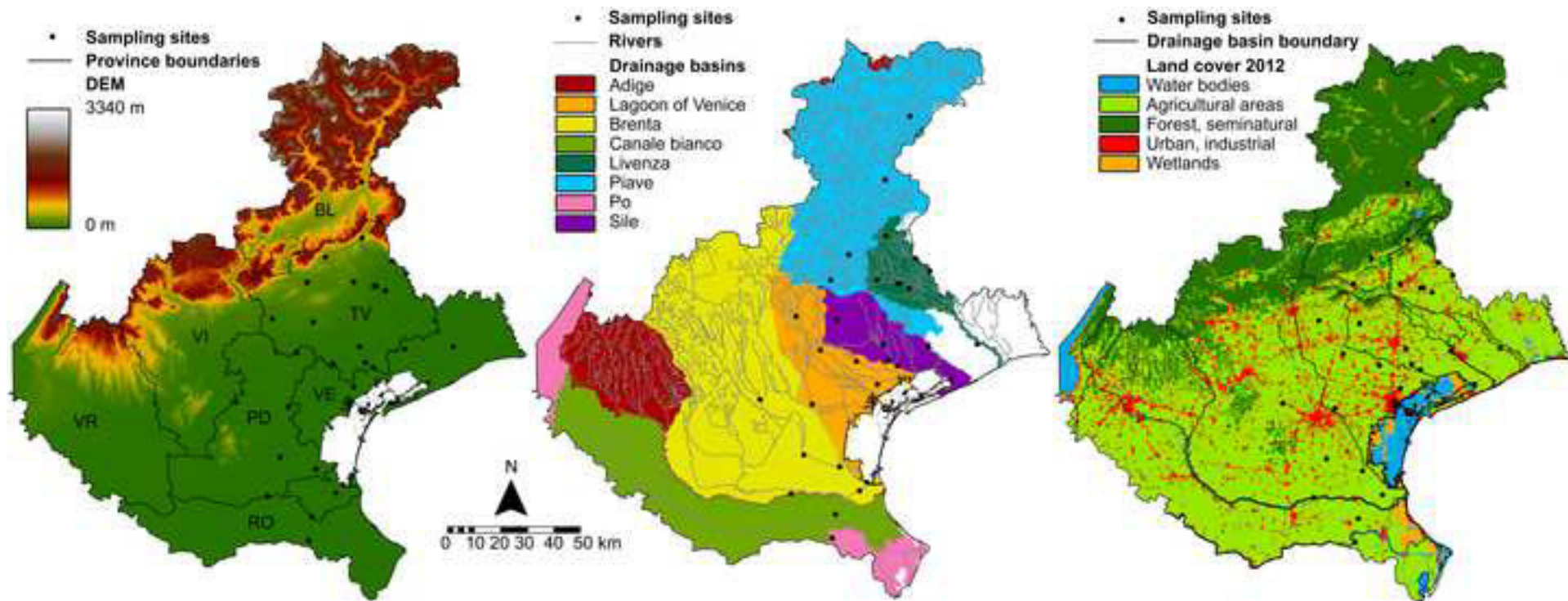


Figure 2

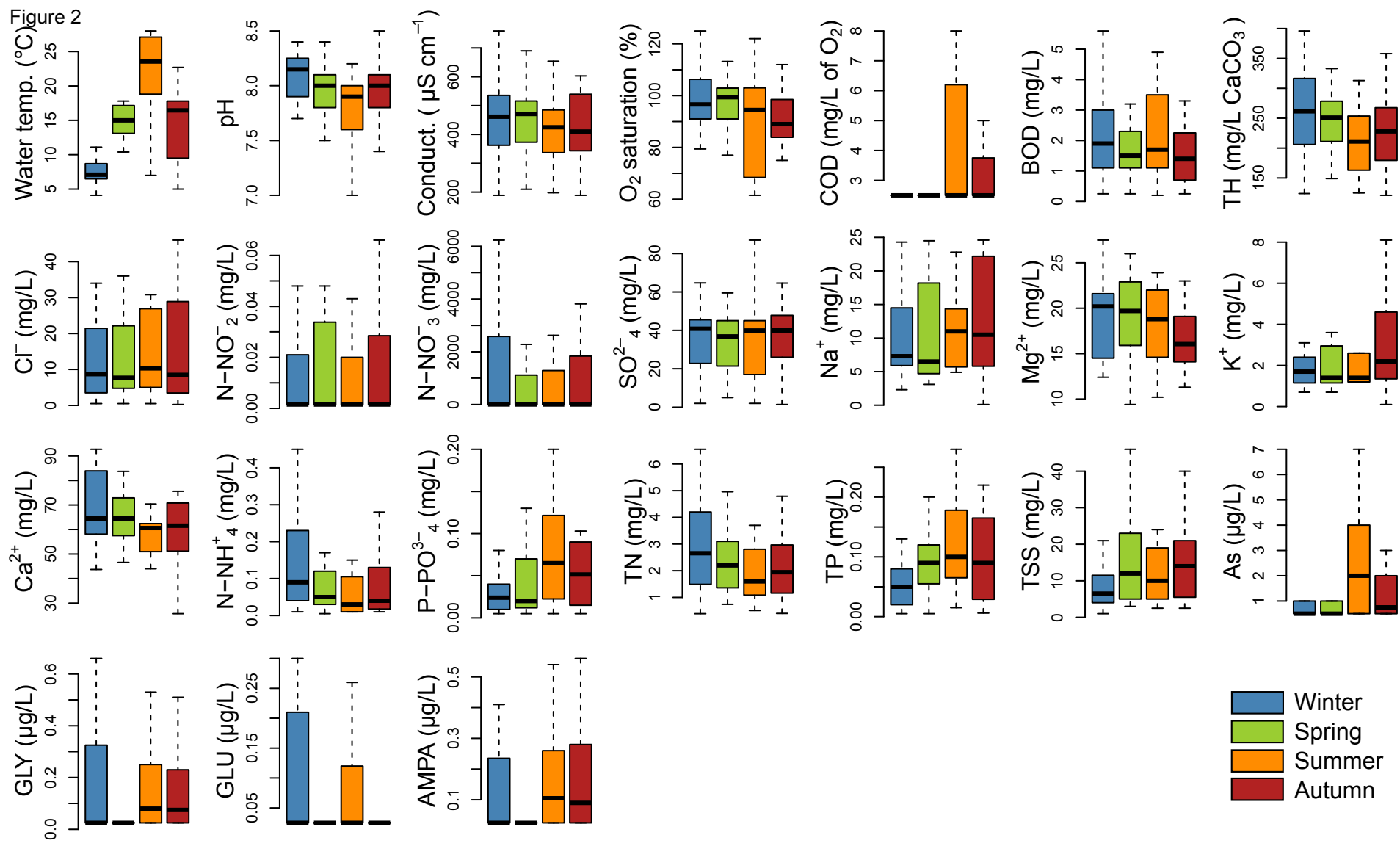


Figure 3

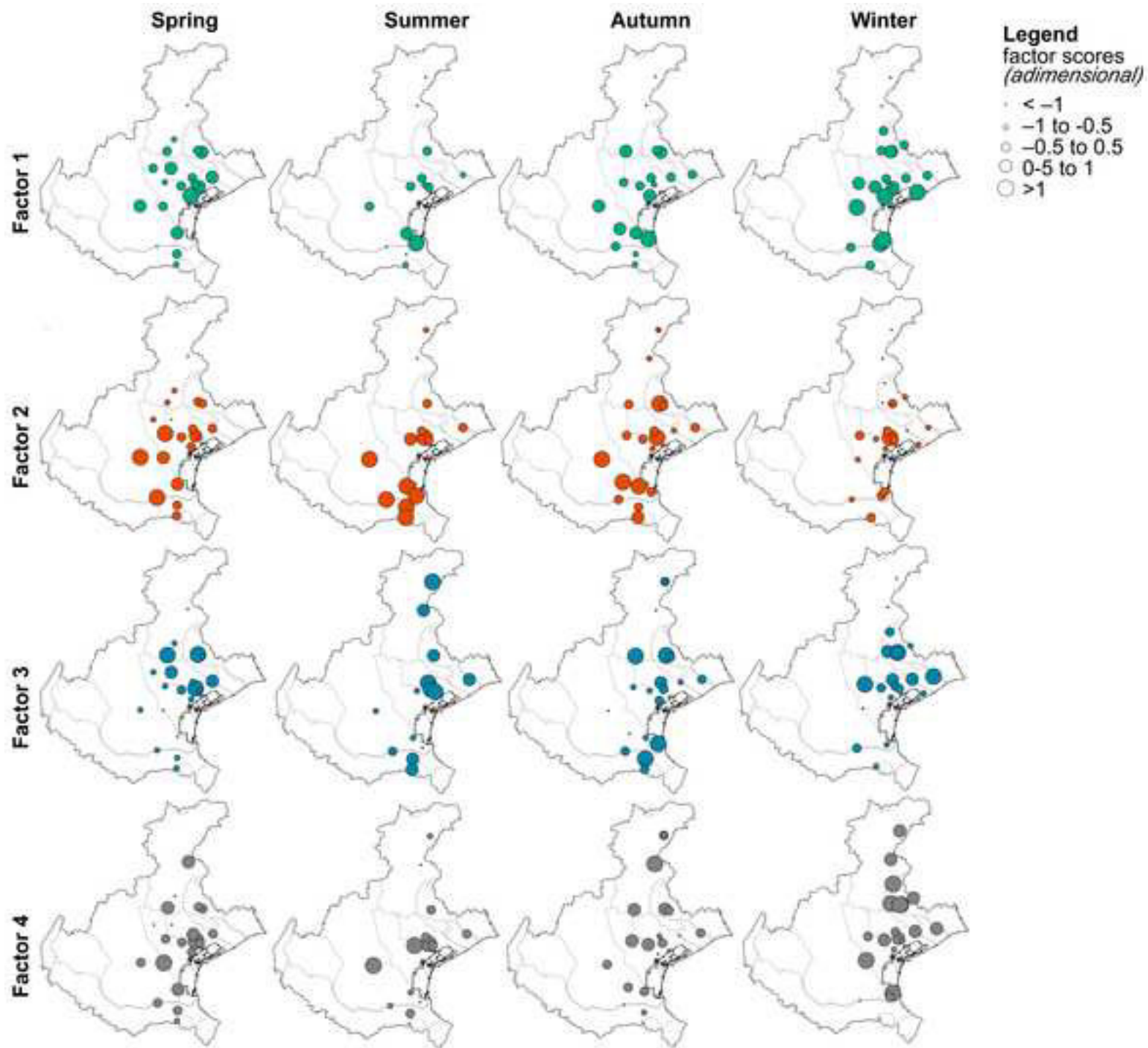


Figure 4

Canonical Discriminant Functions

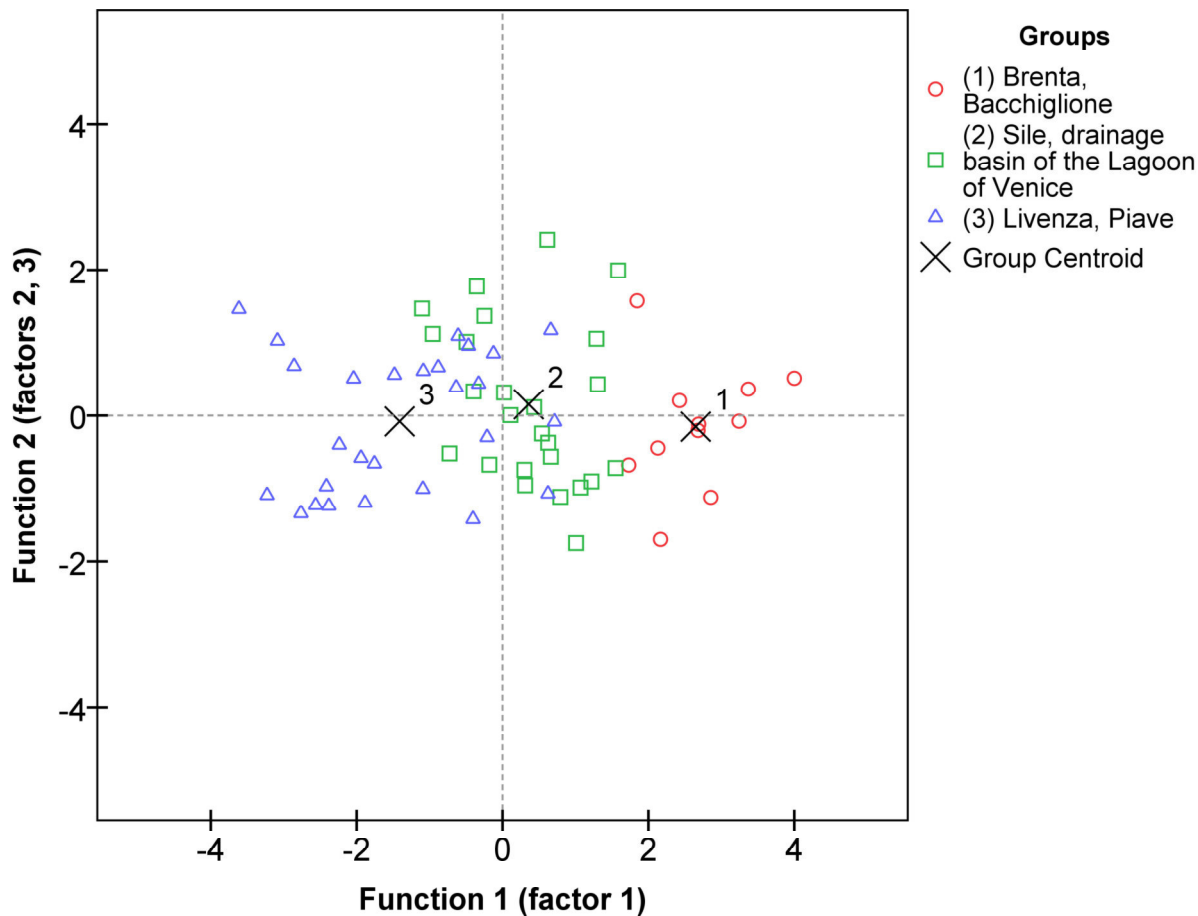


Table 1. Characteristics of the sampling sites and average (min-max) concentrations of target compounds.

Main drainage basin	Secondary drainage basin	River name	Site no.	Province	No. samples	GLY		AMPA		GLU	
						$\mu\text{g L}^{-1}$		$\mu\text{g L}^{-1}$		$\mu\text{g L}^{-1}$	
						Mean	(min-max)	Mean	(min-max)	Mean	(min-max)
Adige		Adige	206	PD	4	0.04	(<LOD–0.1)	0.17	(<LOD–0.23)	0.07	(<LOD–0.22)
Brenta		Brenta	436	VE	3	0.29	(<LOD–0.83)	0.13	(<LOD–0.35)	0.13	(<LOD–0.33)
Brenta	Bacchiglione	Bacchiglione	181	PD	3	<LOD	—	<LOD	—	<LOD	—
Brenta	Bacchiglione	Cagnola	175	PD	2	<LOD	—	<LOD	—	<LOD	—
Brenta	Bacchiglione	Tesinella	112	PD	3	0.04	(<LOD–0.06)	<LOD	—	<LOD	—
Brenta	Gorzone	Gorzone	437	VE	2	<LOD	—	<LOD	—	<LOD	—
Canalbianco		N. Adigetto	223	RO	4	0.40	(<LOD–1.4)	0.36	(<LOD–0.75)	0.18	(<LOD–0.55)
Livenza	Monticano	Monticano	620;1147	TV	5	0.04	(<LOD–0.1)	0.31	(<LOD–0.83)	0.08	(<LOD–0.3)
Livenza	Monticano	Cervada	621	TV	4	0.49	(0.07–1.3)	0.28	(0.07–0.56)	0.11	(<LOD–0.3)
Livenza	Monticano	Crevada	6008	TV	1	0.45	(0.45–0.45)	<LOD	—	0.30	(0.3–0.3)
Livenza		Livenza	72;453	TV, VE	4	0.34	(<LOD–0.55)	0.42	(<LOD–1.4)	0.05	(<LOD–0.11)
Livenza		Meschio	23;236	TV	2	0.11	(<LOD–0.2)	<LOD	—	0.11	(<LOD–0.2)
Piave		Anfella	409	BL	4	0.06	(<LOD–0.1)	0.04	(<LOD–0.07)	0.07	(<LOD–0.22)
Piave		Piave	65	VE	5	0.17	(<LOD–0.66)	0.28	(<LOD–1.2)	0.06	(<LOD–0.12)
Piave		Teva	6013	TV	2	0.31	(0.11–0.51)	0.77	(0.72–0.82)	0.42	(<LOD–0.82)
Piave		Val di Frari	420	BL	4	0.06	(<LOD–0.17)	<LOD	—	0.05	(<LOD–0.12)
Po		Po	227	RO	4	0.05	(<LOD–0.08)	0.22	(<LOD–0.54)	<LOD	—
Sile		Bigonzo	6033	TV	4	0.32	(<LOD–0.7)	0.16	(0.09–0.27)	0.08	(<LOD–0.14)
Sile		C.U.A.I.	351	VE	3	0.10	(<LOD–0.26)	0.06	(<LOD–0.14)	<LOD	—
Sile		Melma	333	TV	4	0.13	(<LOD–0.37)	0.12	(<LOD–0.26)	0.10	(<LOD–0.26)
Sile		Sile	238;329	TV, VE	5	0.07	(<LOD–0.25)	0.10	(<LOD–0.25)	0.09	(<LOD–0.25)
Lagoon of Venice		Musoncello	1127	TV	3	0.72	(<LOD–2.1)	0.48	(<LOD–1.4)	0.72	(<LOD–2.1)
Lagoon of Venice		Tergola	117	PD	3	<LOD	—	<LOD	—	<LOD	—
Lagoon of Venice		Zero	488	TV	5	0.04	(<LOD–0.06)	0.03	(<LOD–0.05)	0.03	(<LOD–0.05)
Extra samples		“Risorgive”	NA	TV	3	<LOD	—	0.05	(<LOD–0.1)	0.05	(<LOD–0.1)

Table 2

Table 2. Spearman's correlations among variables. Only correlations statistically significant at $p < 0.05$ are shown.

	Water temp.	pH	Conduct.	Diss. O ₂	COD	BOD	Hardness	Cl ⁻	N-NO ₂ ⁻	N-NO ₃ ⁻	SO ₄ ²⁻	Na ⁺	Mg ²⁺	K ⁺	Ca ²⁺	N-HN ₄ ⁺	P-PO ₄ ³⁻	Total N	Total P	TSS	As	GLY	GLU	AMPA	
Water temp.	1																								
pH		1																							
Conductivity			1																						
Diss. O₂	-0.24	0.56		1																					
COD	0.31		0.4		1																				
BOD		0.33	0.28	0.26	0.25	1																			
Hardness			0.8		0.26		1																		
Cl⁻	0.36		0.78	-0.3	0.49	0.25	0.44	1																	
N-NO₂⁻			0.3		0.46		0.25		1																
N-NO₃⁻			0.6		0.27		0.62	0.44	0.69	1															
SO₄²⁻			0.38				0.3	0.3		0.26	1														
Na⁺			0.46					0.96				1													
Mg²⁺			0.71	0.42			0.67						1												
K⁺			0.71				0.4	0.82				0.81		1											
Ca²⁺		0.34	0.79	0.56			0.93			0.52			0.44	0.43	1										
N-HN₄⁺			0.66			0.4	0.54	0.52	0.29	0.48	0.28		0.4			1									
P-PO₄³⁻	0.42		0.55				0.32	0.57		0.31	0.3	0.5		0.64		0.64	1								
Total N			0.71		0.3		0.72	0.57	0.44	0.88	0.27		0.36	0.38	0.57	0.61	0.44	1							
Total P	0.41		0.53					0.59		0.28		0.53		0.58		0.54	0.87	0.41	1						
TSS	0.32		0.29		0.32			0.3	0.42	0.35						0.47	0.52	0.3	0.53	1					
As	0.44		0.28	-0.3	0.5			0.6	0.38			0.48			-0.6	0.27	0.48		0.47	0.45	1				
GLY						0.26								0.38									1		
GLU				0.25									0.38		0.38								0.53	1	
AMPA												0.34		0.48			0.26						0.64	0.44	1

Table 3. Results of factor analysis (Varimax rotated solution). Variables with factor loadings (>0.6) are in bold; factor loadings less than 0.35 are not shown; variables are ordered for decreasing absolute loadings. Var (%): percentage of variance explained by each factor; Cum.Var. (%): cumulative variance.

Factor 1	Factor 2	Factor 3	Factor 4
Fertilizers/salinity	Biological activity/arsenic	Herbicides	Urban/industrial discharges
Hardness (0.85)	As (0.81)	GLY (0.85)	BOD (0.77)
Conductivity (0.81)	P-PO₄³⁻ (0.69)	GLU (0.83)	H⁺ activity (-0.73)
SO₄²⁻ (0.71)	TSS (0.67)	AMPA (0.77)	Dissolved O ₂ (0.55)
Cl⁻ (0.69)	Dissolved O₂ (-0.67)		N-NH ₄ ⁺ (0.4)
N-NO₃⁻ (0.67)	Cl ⁻ (0.56)		
N-NH₄⁺ (0.62)	N-NH ₄ ⁺ (0.37)		
P-PO ₄ ³⁻ (0.41)			
Var.= 23%	Var.= 19%	Var.= 15%	Var.= 12%
Cum. Var.= 23%	Cum. Var.= 42%	Cum. Var.= 56%	Cum. Var.= 69%

Table 4. Results of the discriminant analysis: test of equality of group mean (left) and structure matrix (right) with extracted discriminant functions. Largest absolute correlation between each variable and any discriminant function are in bold.

	Tests of Equality of Group Means			Structure matrix	
	Wilks' Lambda	F	Sig.	Function 1	Function 2
Fertilizers/salinity	0.704	12.59	0.000	0.43	-0.18
Biological activity/arsenic	0.602	19.86	0.000	0.54	0.63
Herbicides	0.891	3.67	0.031	-0.22	-0.78
Urban/industrial discharges	0.980	0.63	0.538	-0.09	-0.28