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**Monitoring and environmental risk assessment of pesticide residues
and some of their degradation products in natural waters of the
Spanish vineyard region included in the Denomination of Origin
Jumilla**

Eliseo Herrero-Hernández^{1,*}, Ana B. Simón-Egea², María J. Sánchez-Martín¹, M. Sonia Rodríguez-Cruz¹, M. Soledad Andrades²

¹ Instituto de Recursos Naturales y Agrobiología de Salamanca (IRNASA-CSIC), Cordel de Merinas 40-52, 37008 Salamanca, Spain

² Departamento de Agricultura y Alimentación. Universidad de La Rioja, 51 Madre de Dios, 26006 Logroño, Spain

* Corresponding author: elihh@usal.es (E. Herrero-Hernández)

1 **Abstract**

2 Water pollution by pesticides used in agriculture is currently a major concern
3 both in Spain and in Europe as a whole, prompting the need to evaluate water quality
4 and ecological risk in areas of intensive agriculture. This study involved monitoring
5 pesticide residues and certain degradation products in surface and ground waters of the
6 Jumilla Denomination of Origin (DO) vineyard area in Spain. Sixty-nine pesticides
7 were selected and evaluated at twenty-one sampling points using a multi-residue
8 analytical method, based on solid-phase extraction (SPE) and analysis by liquid
9 chromatography coupled with mass spectrometry (LC-MS), providing reliable results.
10 Twenty-six compounds from those selected were detected in the samples analyzed (11
11 insecticides including one degradation product, nine herbicides, and six fungicides) and
12 15 of them were found in concentrations over $0.1 \mu\text{g L}^{-1}$ (upper threshold established by
13 the EU for pesticides detected in waters for human consumption). Indoxacarb was
14 present in more than 70% of the samples, being the most frequently detected compound
15 in water samples. Some pesticides were ubiquitous in all the water samples.
16 Ecotoxicological risk indicators, toxic units (TUs) and risk quotients (RQs), for algae,
17 *Daphnia magna* and fish were calculated to estimate the environmental risk of the
18 presence of pesticides in waters. The compounds with the highest risk were the
19 herbicides pendimethalin, with RQ values > 1 for the three aquatic organisms, and
20 diflufenican, posing a high risk for algae and fish, and the insecticide chlorpyrifos, with
21 a high risk for *Daphnia magna* and fish. The $\sum\text{TU}_i$ determined for water at each
22 sampling point posed only a high risk for the three aquatic organisms in a sample. These
23 results are important for considering the selection of pesticides with less environmental
24 risk in intensive agricultural areas.

25

26 **Keywords:** Multi-residue analysis; Pesticides; Natural waters; Degradation products;
27 Vineyards

28

29 **1. Introduction**

30 Sustainability is the main focus of the European agricultural model, in which
31 agricultural production is one of the main pillars (Scherer et al., 2018). It is supported
32 by, among other production media, the application of agrochemicals. The use of these
33 compounds arises from the need to ensure the production of crops in sufficient quantity
34 and quality to satisfy the population's nutritional needs. Agriculture today is based on
35 obtaining maximum yields and profit, and to achieve these objectives conventional
36 agriculture undertakes a series of agronomic practices which frequently do not consider
37 the long-term effects of their use.

38 In 2017, Spain was the European country with the highest consumption of
39 pesticides, followed by France and Italy (MAPA, 2019). These compounds are used on
40 most crops (vegetables, olive and fruit trees, wheat, vineyards, etc.) in different forms
41 and at different times. This application could have an impact on water and soil
42 pollution, with a knock-on effect on human and animal health or the loss of
43 biodiversity. Water is a key feature of agriculture, being a crucial factor in plant growth,
44 but the use of groundwater for human consumption could be critical if pesticides and
45 nitrates reach the water by leaching processes (Pérez-Lucas et al., 2018). The
46 agricultural sector consumes a third of Europe's water reserves, with this percentage
47 being higher in Spain's case. Agriculture influences both the quantity and the quality of
48 water available for other uses. In some areas of Europe, pollution caused by the
49 pesticides and fertilizers used in agriculture is in itself one of the main causes of poor
50 water quality (EEA, 2018).

51 Surface and ground waters can become polluted by pesticides, with this process
52 being governed by the physicochemical characteristics of these compounds and the
53 environment in which they are applied, as well as by other external factors such as local

54 rainfall, wind and topography, which determine their dissipation or degradation (Perez-
55 Lucas et al., 2018). Modern pesticides are more effective and selective, using lower
56 doses. However, the environmental fate of these compounds is currently a major
57 concern because of the increasing number of pesticides detected in the waters of
58 different European countries, such as France (Cotton et al., 2016), the UK (Casado et
59 al., 2018), Italy (Triassi et al., 2019), Greece (Papadakis et al., 2018; Kapsi et al., 2019),
60 and Portugal (Palma et al., 2009). As well as in more or less developed countries all
61 over the world, such as Costa Rica (Carazo-Rojas et al., 2018), India (Mondal et al.,
62 2018), Chile (Climent et al., 2019), and Rwanda (Houbraken et al., 2017). These
63 pesticide residues could have an adverse impact on waters used for human consumption
64 or if they reach non-target aquatic organisms which are susceptible to their toxic effects.
65 The bioaccumulation of pesticide residues in waters through aquatic life on different
66 trophic levels could pose a risk for humans depending on food sources (Mondal et al.,
67 2018).

68 Accordingly, the European Community (EC) has established different directives
69 to protect water quality (Directive 2000/60/EC and Directive 2008/60/EC) (EC, 2000,
70 2008). Both have been amended by Directive 2013/39/EU in which more contaminants
71 and other issues have been added to the list of controlled substances (EC, 2013).
72 Furthermore, other directives on water for human consumption and on the protection of
73 groundwater against pollution and deterioration (Directive 98/83/EEC (EC, 1998) and
74 revised Directive 2006/118/EC (EC, 2006)) establish the individual threshold for a
75 pesticide ($0.1 \mu\text{g L}^{-1}$) and the total concentration of pesticides ($0.5 \mu\text{g L}^{-1}$) permitted in
76 waters. Other European guidelines also include assessing the risk pesticide residues
77 pose for aquatic organisms (EC, 2003, 2011). The indicators Toxic Units (TUs) and
78 Risk Quotients (RQs) for evaluating the ecological or exposure effects involving

79 pesticides at different trophic levels in the ecosystem have been reported of interest for
80 assessing the biological risks of different compounds (Ccanccapa et al., 2016a).

81 In Spain, agriculture plays a very important role in the economy, and the
82 environmental fate of the pesticides used is currently a major concern. In recent years,
83 several studies have reported the presence of pesticides and other substances in river
84 basins: Ebro (Ccanccapa et al., 2016a;), Júcar and Turia (Ccanccapa et al., 2016b), Júcar
85 (Pascual Aguilar et al., 2017), Turia (Carmona et al., 2017), Llobregat (Masiá et al.,
86 2015; Quintana et al., 2019), Guadalquivir (Hermosín et al., 2013; Masiá et al., 2013),
87 Guadiana (Palma et al., 2009) and Miño (Dagnac et al., 2012), and in the
88 Mediterranean's coastal waters (Köck-Schulmeyer et al., 2019). In some cases,
89 irrigation has also contaminated aquifers above the peak levels allowed in European
90 legislation for drinking water. However, the number of studies that include a large
91 amount of groundwater samples is scarce (Hildebrandt et al., 2007, 2008; Herrero-
92 Hernández et al. 2013, 2016, 2017).

93 Specifically, a large number of herbicides, insecticides and fungicides are used
94 annually in vineyard agricultural areas (AEPLA, 2015). Recent studies have estimated
95 that fungicides and bactericides are the main type of pesticides applied, accounting for
96 53% of the total amount used (MAPA, 2019). This is because wine is an important
97 economic sector in Spanish agriculture, with over a million ha of cultivated land and
98 more than 90 protected DO wine regions (MAPA, 2019). Some studies have been
99 conducted to evaluate the presence of these compounds in areas where vineyards are the
100 main activity, such as in the qualified DO Rioja (Herrero-Hernández et al. 2013, 2016,
101 2017), or in some areas of Galicia (Hildebrandt et al., 2008). However, these studies
102 have not been extended to other areas where viticulture is also an important agricultural
103 activity, such as DO Jumilla in the Murcia region (SE Spain). Additionally, this area is

104 located in a Spanish region with the fifth highest investment per hectare in crop
105 protection products, with a pesticide consumption of 27.8 kg ha⁻¹ in 2015 (MAPA,
106 2019).

107 Accordingly, the aims here were as follows: (i) to conduct a thorough
108 monitoring of natural waters in the DO Jumilla area, where vineyard cultivation is the
109 main activity, and evaluate possible pollution due to pesticides and their degradation
110 products, (ii) to estimate the local ecotoxicological risk towards the aquatic organisms
111 in three taxonomic groups (algae, *Daphnia magna*, and fish) using the TUs for each
112 sampling site and RQs for each pesticide. To the best of our knowledge, this is the first
113 study on the occurrence of pesticides and degradation products considering their
114 ecotoxicological risk in DO Jumilla, an area of intense anthropogenic activity.

115

116 **2. Materials and methods**

117 *2.1. The study area*

118 The wine-producing area in DO Jumilla covers around 25000 hectares of
119 vineyards, of which 40% are located within Jumilla's municipal boundaries, with the
120 rest being in the province of Albacete, involving almost 2000 winegrowers (Fig. 1). The
121 geographical area is located in the southeast of Spain, in a transition zone between the
122 Murcia coast and the Castilla-La Mancha plateau. Its terrain consists of a series of
123 mountain ranges, separated by valleys, ravines, woodlands and plains, located at heights
124 between 400 m (southern part) and 800 m (northern part), where the cultivation soils are
125 located. The soils have a high pH and low salinity, and they are generally brown,
126 consisting of brown limestone and limestone with low organic matter content. They
127 have a loam or loam-sandy texture, high water capacity, and medium permeability

128 (Consejo Regulador de Jumilla, 2019). There is only one permanent water course,
129 namely, the Mundo River, which flows through Hellín. There are now few natural
130 springs because wells and pumping have dried them. However, DO Jumilla has
131 numerous aquifers of noteworthy extent. Some of them as El Molar are considered
132 aquifers in a sustainable situation without overexploitation, but others are overexploited
133 aquifers as Ascoy-Sopalmo or Jumilla-Villena, and their situation could not be
134 maintained indefinitely over time (Molina and García Aróstegui, 2009; CHS, 2017).
135 Figure 1 shows the map of the area of study including the names of the hydrological
136 units or aquifers and the sampling sites.

137 Despite the Mediterranean's relative proximity, the climate has clearly
138 continental features, with a marked semi-arid nature. Rainfall is one of the main
139 climatic challenges in the area; the rainfall pattern is very irregular, with long periods of
140 drought. Precipitation occurs mostly in the spring and autumn. The rains in April-May
141 and October-November account for 50% of the whole year, with an average annual
142 rainfall of 300 mm. The rains are often torrential, so the water is not absorbed by the
143 soil, running off in torrents and down dried-up river beds (*ramblas*). The average annual
144 temperature is relatively high, 16°C, with a significant seasonal variation; 40°C can be
145 reached in summer, while in winter minimums of close to -10°C can sometimes be
146 recorded (Consejo Regulador de Jumilla, 2019).

147 2.2. *Sampling network and pesticides selected*

148 The spatial sampling network involved 21 sites throughout the region. Twenty of
149 these samples corresponded to groundwater and one to surface water from the Mundo
150 River (Fig. 1). The water samples were collected between December 2016 and January
151 2017 in the different areas of DO Jumilla (Table S1 in Supplementary Material):
152 thirteen samples correspond to groundwater from private wells with different depths

153 (from 80 to 490 m), and seven correspond to public sources. The sampling points were
154 both excavated wells and natural sources in vineyard areas. Details of the sample
155 collection and of their characteristics are shown in Supplementary Material and Table
156 S1.

157 The study selected 69 pesticides (20 herbicides, 14 insecticides, and 23
158 fungicides, as well as 12 of their degradation products) from among those most used in
159 the area in recent years, according to data provided by public agencies, product
160 distributors, and local farmers. In addition we considered of interest to include in the
161 study some banned pesticides in Spain, which had been previously detected in other
162 areas to expand the information obtained (Table S2 in Supplementary Material). The
163 pesticides selected (fungicides, herbicides, and insecticides), which belong to several
164 chemical classes, are listed in Table S2 (Supplementary Material). The analytical
165 standards of pesticides and some of their degradation products (minimum purity > 98%)
166 were supplied by Sigma Aldrich Química S.A. (Madrid, Spain).

167 *2.3. Analytical determination of pesticides*

168 The concentration of pesticides in the aqueous phase was determined in the
169 samples preconcentrated using the multi-residue methodology proposed by Herrero-
170 Hernández et al. (2013), albeit slightly modified to include the new pesticides used in
171 this region. Oasis HLB cartridges were used as SPE sorbents and analysis of pesticides
172 by LC–MS was carried out using a Waters (Milford, MA, USA) system with an ESI
173 interface. Details of preconcentration samples and pesticide analytical method are
174 included in Supplementary Material and Table S3.

175 *2.4. Environmental risk assessment*

176 TUs were used for the ecotoxicological risk assessment of each compound
177 determined, and were calculated according to the European guidelines (EC, 2003) in at
178 least three representative taxa (algae, *Daphnia magna*, and fish) of three trophic levels
179 in the ecosystem. The TU_i is based on each compound's acute toxicity values
180 (Ccanccapa et al., 2016a), and was calculated by dividing the measured environmental
181 concentration (MEC) of each compound (MEC_i) in each water sample by the effective
182 or lethal concentration required to produce 50% of the effect on individuals (EC_{50_i} or
183 LC_{50_i}) when exposed to the substance concerned, TU_i (algae; *Daphnia magna*; fish) =
184 MEC_i/EC_{50_i} or LC_{50_i} . The specific toxic stress of each site (TU_{site}) was calculated by
185 adding up all the individual TU_i of each compound detected at that site (Ccanccapa et
186 al., 2016a).

187 The pesticides' Risk Quotient (RQ) for aquatic organisms was evaluated as the
188 ratio of (MECs) divided by predicted no-effect concentration (PNEC) ($RQ =$
189 $MEC/PNEC$). The median and maximum measured concentrations for each pesticide
190 were used, respectively, as median and maximum MECs for the determination of
191 RQ_{mean} and RQ_{max} as indicators of the general and worst scenarios, respectively
192 (Thomatou et al., 2013). An assessment factor (AF) of 100 was applied to calculate the
193 PNEC when long-term or chronic toxicity data were available from the lowest no
194 observed effect concentration (NOEC) as indicated by the European Commission (EC,
195 2003), or an AF of 1000 when only short-term or acute toxicity data were available
196 from the EC_{50} or LC_{50} concentration (Ccanccapa et al., 2016a), or from the EC_{50} or
197 LC_{50} concentration when only short-term or acute toxicity data were available divided
198 by an AF of 1000 (Ccanccapa et al., 2016a). This AF is an arbitrary factor for
199 considering the inherent uncertainty in the laboratory toxicity data obtained. Four
200 different levels of risk can be established depending on the RQ value: minimal risk (RQ

201 < 0.01), low risk (0.01 < RQ < 0.1), medium risk (0.1 < RQ < 1), and high risk (RQ > 1)
202 (Liu et al., 2015).

203 The acute EC₅₀ for *Daphnia magna* at 48 h, and for algae at 72 h and LC₅₀ for
204 fish at 96 h, as well as the chronic NOEC for algae at 96 h and for fish and *Daphnia*
205 *magna* at 21 days were obtained from the Pesticide Properties Data Base (PPDB, 2019).
206 The PPDB (2019) considers the effect of immobilization for *Daphnia magna*, growth
207 inhibition for algae (unknown species) and survival for fish (*Oncorhynchus mikiss*
208 mostly) in the EC50 values of each one of these organism.

209

210 **3. Results and Discussion**

211 *3.1. Occurrence of pesticide residues in natural waters*

212 The pollution of natural waters by 69 pesticides and some of their degradation
213 products was evaluated in the DO Jumilla wine region by an optimized SPE-LC-MS
214 method. A total of twenty-six different pesticides (11 insecticides, 9 herbicides and 6
215 fungicides) were detected in one or more of the samples, and 15 were detected in
216 concentrations over 0.1 µg L⁻¹. The distribution of the concentrations detected for each
217 pesticide is included in Fig. S1 and Table 1 compile the median and maximum
218 concentrations together with the number of samples in which each compound was
219 found. The most ubiquitous compounds were the insecticides indoxacarb and
220 pirimicarb, and the compounds found in the highest concentration were the insecticides
221 diazinon and λ-cyhalothrin. These compounds are characterized by a GUS
222 (Groundwater Ubiquity Score) index ranging from very low to medium. A GUS index
223 is used as indicator of the potential pesticide leachability to groundwater, and it allows
224 classifying pesticides as leachable (GUS > 2.8), non-leachable (GUS < 1.8), and in

225 transition ($1.8 < \text{GUS} > 2.8$). However, a negative significant correlation ($p < 0.05$) was
226 observed here between the total concentrations of pesticides detected and the GUS
227 index of individual pesticides. Furthermore, a significant correlation was observed
228 between total concentrations of pesticides and their hydrophobicity expressed by log
229 K_{ow} . These results indicate that the presence of pesticides in groundwater could not be
230 explained by these compounds' properties. Other factors could be more relevant for
231 explaining pesticide residues, such as the type of soil where the compounds are applied
232 or the environmental conditions in the study area.

233 Two or more of the pesticides were found in concentrations over $0.1 \mu\text{g L}^{-1}$ in
234 most of the samples analyzed. The FA-1 sample had only one pesticide (linuron) in a
235 concentration of more than $0.1 \mu\text{g L}^{-1}$, although eight pesticides were detected in this
236 sample (Fig. 2). Furthermore, only the superficial water sample (HE-2) satisfied the
237 other criteria for drinking waters, and presented a sum of pesticides of $0.407 \mu\text{g L}^{-1}$
238 (below the limit of $0.5 \mu\text{g L}^{-1}$ established in European legislation). Four samples had ten
239 or more pesticides: 12 in FA-2, 11 in J-3, and 10 each in F-4 and MA-2 (Fig. 2).
240 However, no relationship can be established between the number of pesticides and the
241 depth of the groundwater; while F-4 level is 1.5 m deep, the rest of the samples were
242 taken at depths of 100 and 180 meters. The deepest water samples do not record the
243 highest total concentrations of pesticides. A negative relationship between the depth of
244 the groundwater and the total content of pesticides was not established, as reported for
245 the vineyards of La Rioja (Herrero-Hernandez et al., 2013). The presence of pesticides
246 in deep groundwater could therefore be explained by the mainly sandy texture of soils
247 located in the study area, as previously indicated.

248 If we consider the type of pesticide, insecticides were the compounds most
249 frequently detected (82 times in concentrations over the LOD, representing 49% of the

250 total pesticides detected), and the compounds with the highest total concentration (14.02
251 $\mu\text{g L}^{-1}$) in the area (Fig. S2). Meanwhile, herbicides were detected 50 times (30% of the
252 total pesticides detected, and at concentrations up to $8.87 \mu\text{g L}^{-1}$), and fungicides were
253 the compounds least detected (34 times, 21% of the total pesticides detected), and were
254 found in the lowest total concentration ($2.60 \mu\text{g L}^{-1}$) (Fig. S2). These results are
255 different from those found in the Spanish qualified DO Rioja region (Herrero-
256 Hernández et al., 2013), where fungicides were the most common compounds found in
257 surface and ground waters, and insecticides were the compounds found least. However,
258 these results are consistent with the climate conditions in both regions; while the
259 number of rainfall episodes is higher in DO Rioja, they are relatively rare in DO
260 Jumilla, and reveal the importance of the weather for determining the type of pesticides
261 to be applied in each region. Insecticides were also the type of pesticide most frequently
262 found in other countries, such as India (Mondal et al., 2018), where chlorpyrifos was
263 found in concentrations over $0.1 \mu\text{g L}^{-1}$ in 25-31% of the samples studied.

264 Pendimethalin and diflufenican were detected in almost 50% of the samples
265 analyzed, with concentrations over the limit established by European legislation ($0.1 \mu\text{g}$
266 L^{-1}), especially in the case of pendimethalin, which exceeds this limit in eight of the
267 samples (Fig. S1 and Table 1), reaching a maximum concentration of $0.526 \mu\text{g L}^{-1}$. This
268 compound has even been found in air samples in Spain's Mar Menor area (Carratalá et
269 al., 2017), and its presence may be due to its frequent use in the vineyard areas where
270 the sampling points are located. Moreover, pendimethalin was one of the compounds
271 most frequently detected in waters from the River Louros (Kapsi et al., 2019) and Lake
272 Amvrakia (Thomatou et al., 2013) in Greece. However, it was not the herbicide detected
273 in the highest concentration, as other herbicides, such as terbuthylazine, were detected
274 with a maximum concentration of $0.760 \mu\text{g L}^{-1}$, despite appearing in only seven

275 samples. These results are consistent with those reported by the authors in DO Rioja
276 (Herrero-Hernández et al., 2013, 2017), where terbuthylazine was one of the
277 compounds more widely found in the waters. Other herbicides, such as chlorotoluron,
278 linuron, lenacil, flufenacet, metolachlor, and oxyfluorfen, were detected in a lower
279 number of samples; only lenacil and metolachlor did not exceed the legal limit of $0.1 \mu\text{g L}^{-1}$
280 L^{-1} . None of the other herbicides included in the study, such as DEA, diuron, atrazine,
281 etc., were detected in any of the samples.

282 In the case of insecticides, the most ubiquitous compound was indoxacarb, found
283 in 80% of the samples (Fig. S1 and Table 1), due to its frequent use in this area, and in
284 some cases its concentration exceeded $0.1 \mu\text{g L}^{-1}$. Other compounds, such as pirimicarb,
285 acephate, methoxyfenozide and the degradation product chlorpyrifos oxon were found
286 in fewer than 50% of the samples, but in no case was the legal limit exceeded ($0.1 \mu\text{g L}^{-1}$
287 L^{-1}). On the other hand, insecticides such as cypermethrin, λ -cyhalothrin, diazinon,
288 diazoxon, chlorpyrifos, and hexythiazox recorded concentrations over that limit in most
289 of the samples in which they were found. Diazinon was the insecticide found in the
290 highest concentration ($1.049 \mu\text{g L}^{-1}$), although it was detected in only 20% of the
291 samples at concentrations over $0.1 \mu\text{g L}^{-1}$, as well as its metabolite diazoxon, which
292 exceeds that limit in 28% of the samples. This insecticide is considered non-leachable,
293 but is frequently found in groundwater. The same occurs with chlorpyrifos and its
294 metabolite chlorpyrifos oxon, detected in 30% of the samples, with chlorpyrifos always
295 being found in concentrations over $0.1 \mu\text{g L}^{-1}$. Chlorpyrifos is one of the compounds
296 more frequently detected in water samples in South-East Spain (Masia et al., 2013;
297 Ccancapa et al., 2016b), and even in air samples in the Mar Menor area (Carratalá et
298 al., 2017). None of the other insecticides, such as dimethoate, imidacloprid,
299 methamidophos, etc., were detected.

300 The fungicides included in this study were found in few samples (Fig. S1 and
301 Table 1) with only six of the twenty-three samples being detected. Carbendazim was the
302 most ubiquitous fungicide, present in more than 60% of the samples, with a maximum
303 concentration of $0.238 \mu\text{g L}^{-1}$. Only the fungicides carbendazim and bupirimate were
304 detected in concentrations over the limit of $0.1 \mu\text{g L}^{-1}$ in five and three samples,
305 respectively. Other fungicides present in more than 25% of the samples were
306 fenbuconazole and kresoxim-methyl, which were always found in concentrations below
307 $0.1 \mu\text{g L}^{-1}$. Finally, metalaxyl and boscalid were detected only in one sample. These
308 results are quite different from those obtained by the authors in DO Rioja, where
309 metalaxyl was one of the most ubiquitous compounds, together with tebuconazole,
310 which has not been detected in DO Jumilla (Herrero-Hernández et al., 2013, 2016).
311 None of the other compounds were detected in any of the samples.

312

313 *3.2. Ecotoxicological risk assessment*

314 TU and RQ approaches were used for prioritizing sampling sites and
315 ecotoxicological assessment according to the contamination level for flagging acute and
316 chronic risk.

317 *3.2.1. Acute risk*

318 The $\sum\text{TU}_i$ for a site was used for helping to estimate the toxic effects of the
319 combination of pesticide residues detected by adding up each single compound's TU.
320 The $\sum\text{TU}_i$ determined for water samples is included in Table 2. Only the HE-3 site
321 recorded TU_{site} values higher than 1 (high risk) for the three aquatic organisms. Another
322 six sampling points recorded values > 1 for two of the aquatic organisms, and a further
323 seven sampling points recorded values > 1 for one of the aquatic organisms, evidencing

324 acute risk due to water pollution. By contrast, only the HE-1 site recorded values < 0.1
325 (low risk) for the three aquatic organisms. Six sampling points recorded TU_{site} values $<$
326 1 for the three aquatic organisms, suggesting a low-to-medium risk of water pollution
327 affecting algae and/or aquatic invertebrates, and/or fish. Eleven of the sampling sites
328 recorded values > 1 for *Daphnia magna*, seven for fish, and only two for algae. Our
329 results indicate that specific sites in this area are exposed to acute toxicity due to a
330 combination of pesticides. Moreover, the results indicate that a high risk for *Daphnia*
331 *magna* could be due to the pesticides detected in waters in 53% of the points sampled,
332 followed by fish and algae, where the risk affected 38% and 9% of the sampling points,
333 respectively (Fig. 3). This revealed *Daphnia magna*'s sensitivity as a representative
334 organism of aquatic insects and other invertebrates in the zooplankton category to the
335 global mixture of pesticides compared with algae in the study area.

336 3.2.2. Chronic risk

337 The best options for assessing chronic risk are passive samplers or other on-line
338 sampling techniques, which are expected to provide more reliable results. Nevertheless,
339 one way of describing the continuous exposure assumed during chronic toxicity risk
340 assessment is to calculate the RQ using the median and maximum concentrations as a
341 worst case scenario (RQ_{mean} and RQ_{max}). Table 3 shows the PNEC values and RQ_{mean}
342 and RQ_{max} determined for the pesticides detected based on median and maximum values
343 for algae, *Daphnia magna*, and fish. Whenever possible, NOEC values rather than EC
344 values were used to produce the corresponding PNECs. In general, fungicide RQs were
345 lower than insecticide RQs, and both were lower than RQs derived from herbicides
346 using both median and maximum concentrations of each compound in the area.
347 Fungicides recorded the lowest RQ values, and only carbendazim presented
348 unacceptable risk for *Daphnia magna* and fish ($RQs > 1$), mainly due to a combination

349 of the relatively high concentrations found and a moderate PNEC value for these two
350 organisms. Kresoxim-methyl recorded a medium risk in all cases. All the other
351 fungicides recorded a minimum or low risk consistent with the lower detection of these
352 compounds in this area.

353 Pendimethalin was the only compound that recorded RQs higher than 1 for both
354 median and maximum concentrations for the three aquatic organisms. However, other
355 pesticides recorded higher RQ values, such as the herbicides diflufenican, flufenacet,
356 and terbuthylazine ($RQ_{\max} = 503, 154, \text{ and } 63.3$, respectively) and the insecticides
357 chlorpyrifos and cypermethrin ($RQ_{\max} = 124 \text{ and } 1400$, respectively), probably due to a
358 relatively low value of PNEC that was always < 0.01 (and especially low in the case of
359 cypermethrin). Several of these compounds record unacceptable RQs for two of the
360 aquatic organisms, as in the case of the herbicides diflufenican and terbuthylazine and
361 the insecticide chlorpyrifos, or for one of the aquatic organisms, such as the herbicides
362 chlorotoluron, linuron, flufenacet, and oxyfluorfen, or the insecticides pirimicarb,
363 chlorpyrifos oxon, diazinon, hexythiazox, and λ -cyhalothrin.

364 The pesticides detected with a minimum risk ($RQs < 0.01$) and a low one ($0.01 <$
365 $RQs < 0.1$) for all the aquatic organisms at both median and maximum concentrations,
366 according to the RQ approach, were the insecticides acephate (minimum) and
367 methoxyfenozide (low), the fungicides metalaxyl (minimum), and fenbuconazole (low),
368 and the herbicide metolachlor (low).

369

370 **4. Conclusions**

371 The analysis of 21 natural waters, corresponding mostly to groundwater, located
372 in the DO Jumilla wine region revealed the presence of ten or more compounds in four

373 of the samples analyzed. Some of the compounds studied were detected at all the
374 sampling points, despite the depth of some of the wells, reaching down to 400 m. The
375 herbicides diflufenican and pendimethalin, the insecticides pirimicarb, indoxacarb, and
376 λ -cyhalothrin, and the fungicide kresoxim-methyl were detected in more than half of the
377 samples analyzed. The fungicides were found less in water samples than the other
378 groups of pesticides, and this may be due to their unusual or low application in this area,
379 with climatic conditions that do not favor the onset of fungal diseases. The compounds
380 found correspond to the pesticide market survey previously conducted in the area, with
381 the compounds more frequently found corresponding to those with a high degree of
382 application. On the other hand, the ecotoxicological risk assessment derived from the
383 presence of pesticides revealed the existence at most of the sampling points of an acute
384 toxicity risk for some of the aquatic organisms considered, and only one sampling point
385 recorded a low risk for all three organisms. As far as pesticides are concerned, several
386 compounds among those detected pose a chronic risk, which is unacceptable for some
387 of the aquatic organisms, with pendimethalin recording a hazard risk for all of them.
388 The results indicate that fungicides are the compounds with a lower chronic risk and, in
389 general, *Daphnia magna* and fish were the most sensitive aquatic organisms for
390 assessing the ecotoxicological risk due to the combination of pesticide residues present
391 in the water samples.

392

393 **Conflicts of Interest**

394 The authors declare no conflict of interest.

395

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400

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562

563

564 **Figure captions**

565 **Fig. 1.** Map of the DO Jumilla wine-growing region in Spain, indicating the sampling
566 points. Source: MAPAMA (2009), MITECO (2011, 2014) and IGN (2014).

567

568 **Fig. 2.** Total concentration and number of pesticides detected in each sampling point.

569

570 **Fig. 3.** Distribution of the sampling points as a function of the risk level expressed by
571 ΣTU_i for the three taxonomic levels selected: algae, *Daphnia magna* and fish.

572

Table 1. Percentage of positive samples with concentrations below and over 0.1 $\mu\text{g L}^{-1}$ and average and maximum concentrations for the pesticides detected in the samples analyzed.

Pesticide	Positive samples		Concentration ($\mu\text{g L}^{-1}$)	
	C < 0.1	C > 0.1	Average \pm SD	C _{max}
Fungicides				
Carbendazim	7	5	0.097 \pm 0.061	0.238
Metalaxyl	1	-	0.017	0.017
Boscalid	1	-	0.081	0.081
Fenbuconazole	6	-	0.068 \pm 0.008	0.073
Kresoxim-methyl	8	-	0.033 \pm 0.005	0.040
Bupirimate	3	3	0.112 \pm 0.036	0.166
Insecticides				
Acephate	1	-	0.088	0.088
Pirimicarb	11	-	0.013 \pm 0.001	0.015
Chlorpyrifos	-	5	0.136 \pm 0.028	0.173
Chlorpyrifos-oxon	5	-	0.028 \pm 0.002	0.030
Methoxyfenozide	7	-	0.065 \pm 0.004	0.070
Cypermethrin	2	5	0.257 \pm 0.148	0.420
Diazinon	1	5	0.604 \pm 0.403	1.049
Diazoxon	2	6	0.222 \pm 0.147	0.528
Indoxacarb	14	2	0.081 \pm 0.026	0.145
Hexythiazox	2	5	0.099 \pm 0.026	0.129
λ -Cihalothrin	2	7	0.369 \pm 0.281	0.940
Herbicides				
Terbuthylazine	3	4	0.258 \pm 0.260	0.760
Chlorotoluron	2	1	0.137 \pm 0.104	0.257
Linuron	1	1	0.208 \pm 0.212	0.358
Lenacil	3	-	0.048 \pm 0.001	0.049
Flufenacet	2	1	0.070 \pm 0.036	0.104
Metolachlor	4	-	0.054 \pm 0.009	0.061
Diflufenican	6	6	0.177 \pm 0.156	0.503
Pendimethalin	3	8	0.255 \pm 0.148	0.526
Oxyfluorfen	2	3	0.147 \pm 0.089	0.279

Table 2. Sum of toxic units ($\sum TU_i$) for all detected pesticides in the different sampling points for different aquatic organisms.

Sample	Algae	<i>Daphnia magna</i>	Fish
ALB-1	0.886	0.247	0.422
FA-1	0.067	0.002	0.001
FA-2	0.678	2.439	1.276
F-1	0.369	1.738	0.061
F-2	0.183	3.717	2.899
F-3	0.333	1.802	0.009
F-4	0.280	0.962	1.203
F-5	0.640	0.292	0.003
HE-1	0.095	0.002	0.002
HE-2	0.066	1.092	0.006
HE-3	1.978	3.508	2.861
J-1	2.056	0.136	0.006
J-2	0.012	4.203	4.685
J-3	0.047	0.914	0.002
J-4	0.286	2.350	0.219
J-5	0.970	0.006	0.005
MA-1	0.059	2.074	1.755
MA-2	0.018	1.732	0.008
O-1	0.425	0.472	0.029
TOB-1	0.096	0.209	0.354
TOB-2	0.036	1.781	1.281

Table 3. Predicted no-effect concentration (PNEC) values and Risk Quotients (RQ) for the three aquatic organisms calculated with the mean and maximum (max) concentrations for each detected pesticide in the area of study.

Analyte	Algae			<i>Daphnia magna</i>			Fish		
	PNEC ^a µg L ⁻¹	RQ _{mean}	RQ _{max}	PNEC ^b µg L ⁻¹	RQ _{mean}	RQ _{max}	PNEC ^c µg L ⁻¹	RQ _{mean}	RQ _{max}
Fungicides									
Carbendazim	7.7	0.013	0.031	0.015	6.47	15.9	0.032	3.03	7.44
Metalaxyl	100	0.000	0.000	546.5	0.000	0.000	30	0.001	0.001
Boscalid	3.75	0.022	0.022	13	0.006	0.006	1.25	0.065	0.065
Fenbuconazole	1.8	0.038	0.041	0.78	0.087	0.094	3.2	0.021	0.023
Kresoxim-methyl	0.049	0.663	0.816	0.32	0.102	0.125	0.13	0.250	0.308
Bupirimate	1.6	0.070	0.104	5.6	0.020	0.030	3	0.037	0.055
Insecticides									
Acephate	1511.5	0.000	0.000	430	0.000	0.000	47	0.002	0.002
Pirimicarb	500	0.000	0.000	0.009	1.41	1.67	180	0.000	0.000
Chlorpyrifos	0.43	0.316	0.402	0.046	2.95	3.76	0.0014	97.0	124
Chlorpyrifos-oxon	0.43	0.065	0.070	0.046	0.609	0.652	0.0014	20.0	21.4
Methoxyfenozide	3.4	0.019	0.021	3.9	0.017	0.018	2.4	0.027	0.029
Cypermethrin	13	0.020	0.032	0.0004	644	1050	0.0003	858	
Diazinon	100	0.006	0.010	0.0056	108	187	7	0.086	0.150
Diazoxon	--	--	--	--	--	--	--	--	--
Indoxacarb	0.11	0.740	1.32	0.42	0.194	0.345	1.5	0.054	0.097
Hexythiazox	4	0.025	0.032	0.061	1.63	2.11	0.4	0.249	0.323
λ-Cihalothrin	3.1	0.119	0.303	3	0.123	0.313	0.0025	148	376
Herbicides									
Terbuthylazine	0.012	21.5	63.3	0.19	1.36	4.00	0.9	0.286	0.844
Chlorotoluron	0.01	35.6	73.0	167	0.002	0.004	4	0.089	0.182
Linuron	0.1	2.08	3.58	1.8	0.116	0.199	1	0.208	0.358
Lenacil	0.1	0.480	0.490	4.8	0.010	0.010	23	0.002	0.002
Flufenacet	0.00204	80.7	154	32.6	0.005	0.010	2	0.082	0.158
Metolachlor	57.1	0.001	0.001	7.07	0.008	0.009	3.9	0.014	0.016
Diflufenican	0.001	177	503	0.52	0.341	0.967	0.15	1.18	3.35
Pendimethalin	0.03	8.51	17.5	0.145	1.76	3.63	0.06	4.25	8.77
Oxifluorfen	20	0.007	0.014	0.13	1.13	2.15	0.38	0.386	0.734

^a Calculated with the chronic 96/72h NOEC in algae; ^b Calculated with the chronic 96/72h NOEC in *Daphnia magna*; ^c Calculated with the chronic 21 days NOEC in fish.

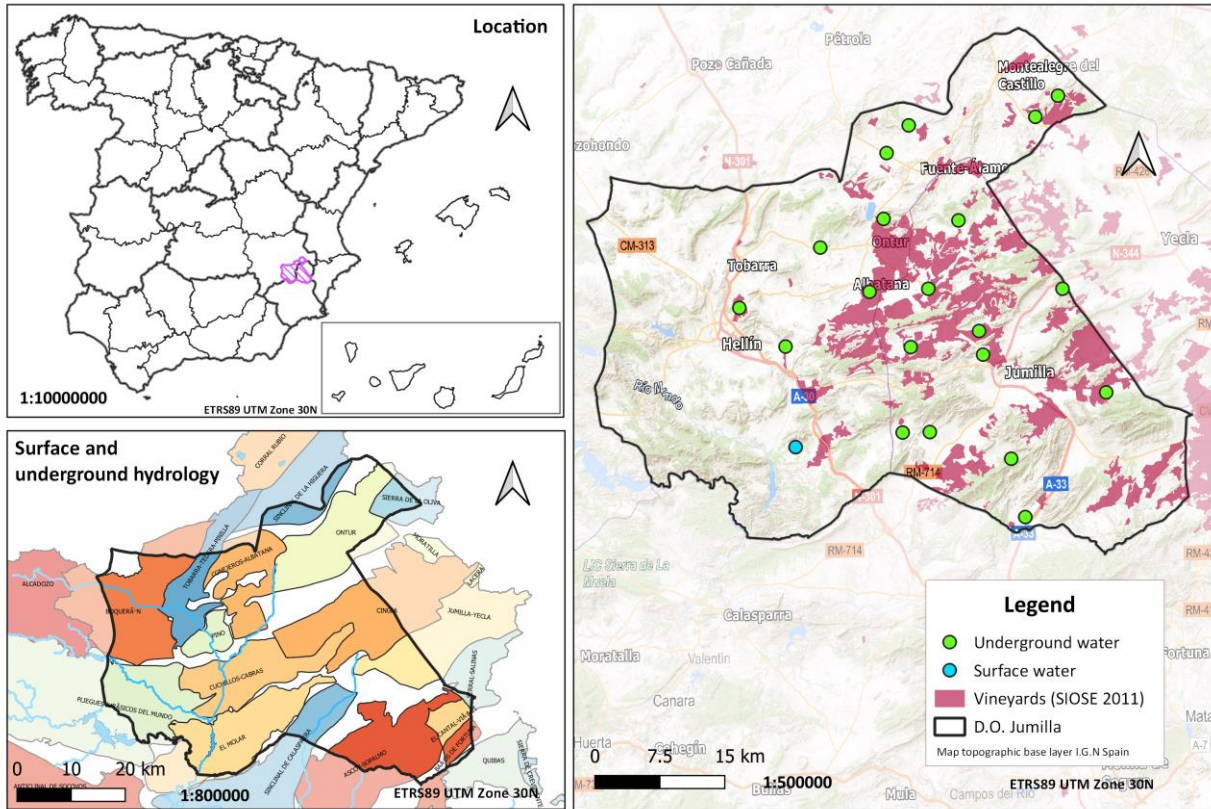


Figure 1

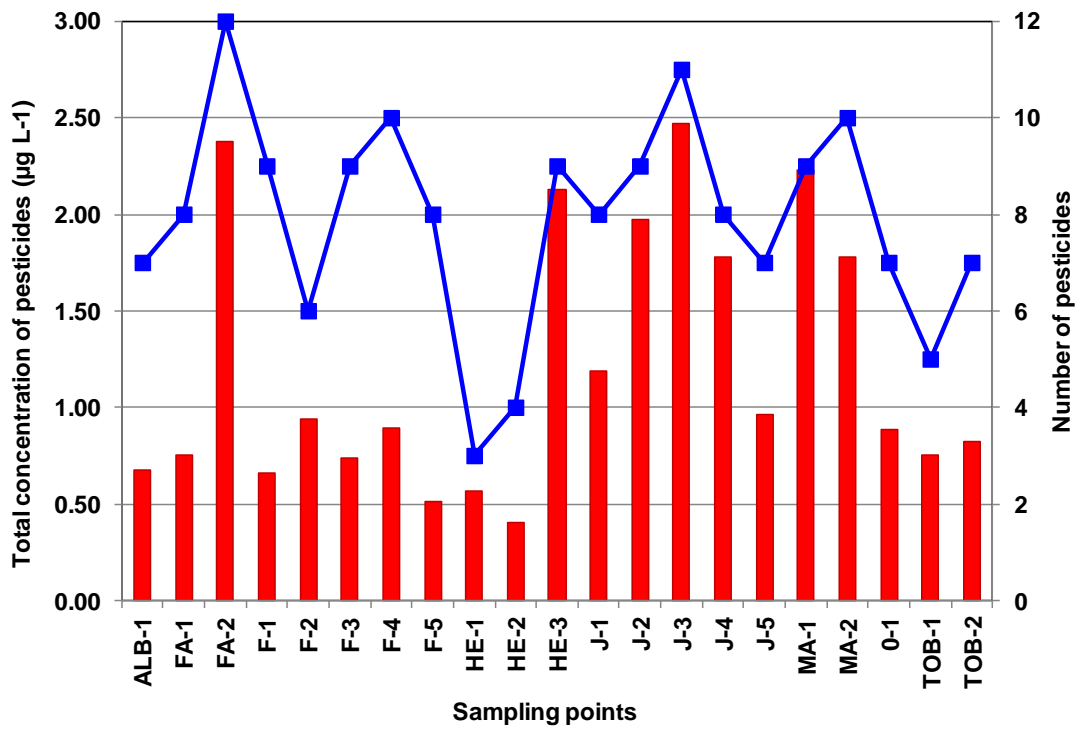


Figure 2

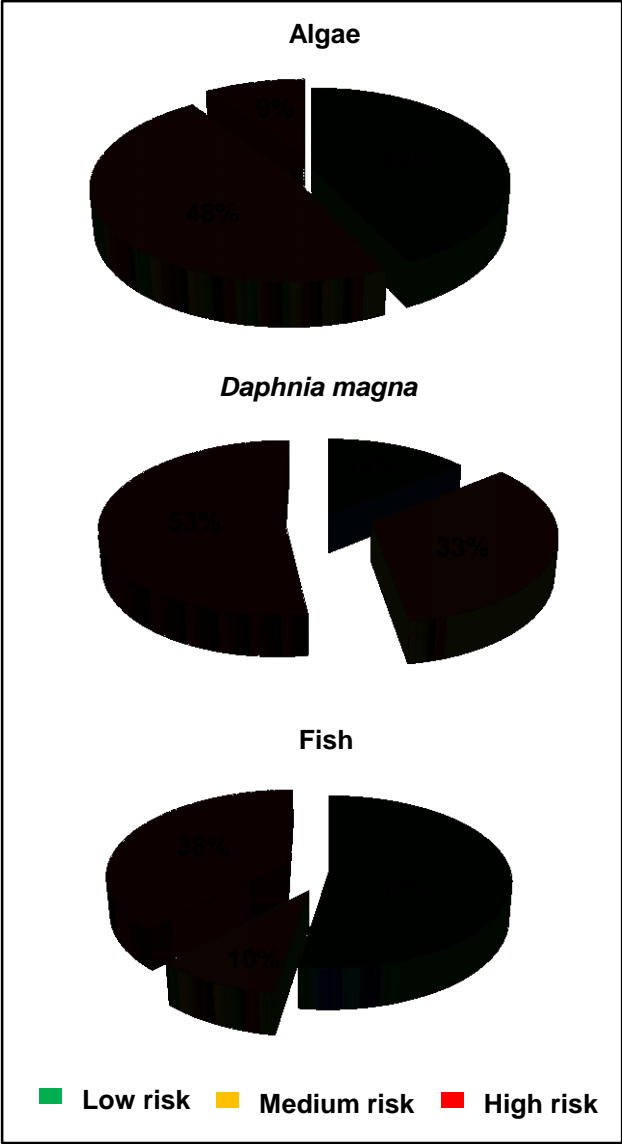


Figure 3

**Monitoring and environmental risk assessment of pesticide residues
and some of their degradation products in natural waters of the
Spanish vineyard region included in the Denomination of Origin
Jumilla**

Eliseo Herrero-Hernández^{1,*}, Ana B. Simón-Egea², María J. Sánchez-Martín¹, M. Sonia Rodríguez-Cruz¹, M. Soledad Andrades²

1 Instituto de Recursos Naturales y Agrobiología de Salamanca (IRNASA-CSIC),
Cordel de Merinas 40-52, 37008 Salamanca, Spain

2 Departamento de Agricultura y Alimentación. Universidad de La Rioja, 51 Madre de
Dios, 26006 Logroño, Spain

* Corresponding author: E-mail address: elihh@usal.es (E. Herrero-Hernández)

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Tables 3

Figures 2

Material and methods

Sample collection

Samples were collected manually in the case of springs and the river, or by pumping in the case of wells. They were collected in duplicate in 1 L amber glass bottles to avoid alterations caused by light, and transported to the laboratory in refrigerated boxes. The samples' physicochemical parameters (pH, electrical conductivity, and dissolved oxygen) were determined immediately using a portable measuring device (Crison PH 25+, CM 35+ and OXI 45+), and are included in Table S1. Within four days, the samples were filtered through nitrocellulose filters with 0.45 μm pore size membranes (Millipore) and kept refrigerated at 4°C in the dark before extracting. The extracts were analyzed within two weeks of collection.

Table S1. Characteristics of the sampling points monitored in the studied region.

Sampling point	Water type	Water depth (m)	Water characteristics		Physicochemical characteristics			
			Crops cultivated	Watering	pH	CE ($\mu\text{S}/\text{cm}$)	Dissolved O ₂ (mg/L)	Dissolved O ₂ (%)
ALB-1	Groundwater	490	Vineyard, olives	Yes	7.28	2120	7.61	86.4
FA-1	Groundwater	80	Vineyard	Yes	7.49	1310	7.13	80.0
FA-2	Groundwater	100	Vineyard, almonds	Yes	7.72	194	6.46	71.9
F-1	Groundwater	2	Vineyard	No	7.64	694	8.30	90.1
F-2	Groundwater	27	Vineyard	No	7.53	617	8.63	95.7
F-3	Groundwater	4	Vineyard, almonds	No	7.58	587	7.68	84.3
F-4	Groundwater	1.5	Vineyard, almonds	Yes	7.59	1973	8.05	88.7
F-5	Groundwater	3	Vineyard, almonds	No	7.80	922	8.36	91.2
HE-1	Groundwater	200	Vineyard, fruits	Yes	7.87	1085	8.88	99.2
HE-2	Surface water	-	Vineyard, cereals	Yes	7.89	1200	9.05	100
HE-3	Groundwater	150	Vineyard, almonds	Yes	7.72	1799	7.53	84.0
J-1	Groundwater	120	Vineyard, cherries	Yes	7.29	686	7.53	85.7
J-2	Groundwater	200	Vineyard, almonds	Yes	7.29	993	8.66	96.8
J-3	Groundwater	180	Vineyard, fruits	Yes	7.28	1340	8.43	94.4
J-4	Groundwater	230	Vineyard, almonds	Yes	7.31	710	8.45	94.4
J-5	Groundwater	330	Vineyard, almonds	Yes	7.40	2003	8.48	94.5
MA-1	Groundwater	150	Vineyard	Yes	7.60	481	8.02	89.3
MA-2	Groundwater	180	Vineyard	Yes	7.48	475	8.07	90.2
O-1	Groundwater	Spring	Vineyard, almonds	No	7.91	1387	8.62	95.3
TOB-1	Groundwater	150	Vineyard	Yes	7.48	2140	8.43	91.3
TOB-2	Groundwater	Spring	Vineyard, almonds	Yes	7.68	588	8.23	90.8

CE, Electric conductivity

Analytical methodology

Stock standard solutions (1000 or 500 $\mu\text{g mL}^{-1}$) for each of the selected analytes (Table S2) and an intermediate standard solution at a concentration of 10 $\mu\text{g mL}^{-1}$ of all the target analytes were prepared in methanol and stored in the dark at 4 °C. The intermediate standard solution was used as spiking solution for the aqueous calibration standards. The organic solvents (acetonitrile, methanol, and acetone) were of HPLC grade, and supplied by Fischer Scientific (Loughborough, UK), being used as received. Ultra-high quality (UHQ) water was obtained with a Milli-Q water purification system (Millipore, Milford, MA, USA).

In order to assess the concentration of pesticides in the aqueous phase, the samples and the standards were preconcentrated using the multi-residue methodology with Oasis HLB cartridges. A volume of 500 mL of water was preconcentrated in triplicate with a Gilson Minipuls 2 HP 8 peristaltic pump at a constant flow of 7 mL min^{-1} . Each cartridge was previously conditioned with 5 mL of acetone, 5 mL of acetonitrile, and 10 mL of UHQ water. After the passage of the sample, the cartridges were vacuum-dried under an air stream (at -20 mm Hg) for five minutes. Elution was performed with 4 mL of acetone and 4 mL of acetonitrile. The solvent was evaporated to dryness under a nitrogen stream at 37°C, and the residue was redissolved in 500 μL of methanol/water mixture (1:1) for analysis by LC-MS.

LC-MS analysis was carried out using a Waters (Milford, MA, USA) system with an ESI interface. The compounds were separated in a Luna PFP2 analytical column. The mobile phase was methanol (solvent A) – 5 mM ammonium formate at pH = 5 (solvent B). The elution gradient was as follows: the mobile phase started with 65% of methanol, which was increased linearly to 75% in 5 min, then raised to 100% in 8 min. and kept constant for 6 min. Finally, it was returned to the initial state in 1 min.

The column was equilibrated for 5 min, and the flow rate was 0.3 mL min⁻¹. The volume injected was 20 µL.

Optimization of the preconcentration procedure was carried out in a previous work, where SPE sorbent and elution conditions were optimized (Herrero-Hernández et al., 2013). Quantification was performed by external calibration using matrix-matched standards, which were managed in a similar way to collected water samples. Calibration curves were obtained by plotting analyte peak areas (obtained from the total ion chromatogram (TIC) in SIM mode versus concentration. The proposed methodology was validated for each of the compounds by studying different analytical parameters as, the accuracy (average recovery), precision (reproducibility and repeatability) at the level of concentration established by EU legislation, the linearity parameters and the limits of detection (LOD) and quantification (LOQ) of the complete method.

The accuracy and precision of the proposed method were determined by recovery experiments and the relative standard deviations (RSD) of the signals or peak areas obtained for each analyte corresponding to five groundwater samples spiked with 0.1 µg L⁻¹ for each analyte. To estimate linearity, LOD and LOQ, eight matrix-matched standards were spiked with all the analytes in the 0.05-1.50 µg L⁻¹ concentration range. The LODs and LOQs were estimated as the analyte concentration with a signal-to-noise ratio of 3 and 10, respectively. Quality control parameters of the proposed methodology are summarized in Table S3.

Table S2. Common names, uses and physicochemical properties of pesticides and degradation products selected for the study. Letters in brackets correspond to degradation products (Degr. Prod.) of pesticides, with the same letters as superscript.

Pesticide	Use	Water Solubility ¹ (mg L ⁻¹)	Log K _{ow} ²	GUS Index ³	DT ₅₀ ⁴ (days)
Methamidophos*	Insecticide	200000	-0.79	2.41	3.5
Acephate*	Insecticide	790000	-0.85	1.76	3
Pyrimidinol (d)	Degr. Prod.	-	0.7	6.81	-
Imidacloprid	Insecticide	610	0.57	3.76	191
Dimethoate*	Insecticide	39800	0.704	1.05	2.6
Pirimicarb	Insecticide	3100	2.48	2.52	34.3
Diazoxon (j)	Degr. Prod.	-	3.09	3.25	142
Methidathion*	Insecticide	240	2.57	1.4	10
Methoxyfenozide	Insecticide	3.3	3.72	3.02	146
Azinphos-methyl*	Insecticide	28	2.96	1.42	10
Chlorpyrifos-oxon (l)	Degr. Prod.	-	-	-	-
Diazinon* ^{d,j}	Insecticide	60	3.69	1.14	9.1
Indoxacarb	Insecticide	200	4.6	0.13	5
Chlorpyrifos ^l	Insecticide	1.05	4.7	0.17	76
Hexythiazox	Acaricida	0.1	2.67	0.03	30
Cypermethrin	Insecticide	0.009	5.3	-1.66	60
λ-Cyhalothrin	Insecticide	0.005	7	-3.28	175
CGA-62826 (a)	Degr. Prod.	-	-	-	31.2
CGA 92370 (h)	Degr. Prod.	-	-	-	
Carbendazim*	Fungicide	8	1.48	2.64	40
Cymoxanil	Fungicide	780	0.67	-0.37	0.7
Flutriafol	Fungicide	95	2.3	5.29	1358
Metalaxyl ^{a,h}	Fungicide	8400	2.5	3.02	45
Cyproconazole	Fungicide	93	3.18	3.52	150
Nuarimol*	Fungicide	26	2.5	2.43	78
Azoxystrobin	Fungicide	6	3.95	3.84	131
Boscalid	Fungicide	4.6	2.96	2.66	200
Iprovalicarb	Fungicide	17.8	3.2	2.35	10.5
Myclobutanil	Fungicide	132	2.94	3.54	365
Triadimenol*	Fungicide	72	3.18	3.75	250
Pyrimethanil	Fungicide	121	2.84	2.65	55
Dimethomorph	Fungicide	28.95	2.63	2.56	57
Penconazole	Fungicide	73	3.7	1.51	117
Fluopyram	Fungicide	16	3.3	3.87	309
Fenbuconazole	Fungicide	2.47	3.79	0.77	60
Kresoxim-methyl	Fungicide	2	3.4	1.82	16

Iprodione*	Fungicide	6.8	3.0	0.58	36.2
Benalaxyl	Fungicide	28.6	3.54	0.51	33.2
Tebuconazole	Fungicide	36	3.7	2	63
Cyprodinil	Fungicide	13	4	1.2	37
Bupirimate	Fungicide	13.06	3.68	1.47	79
Trifloxystrobin	Fungicide	0.61	4.5	0.53	0.34
Flazasulfuron	Herbicide	2100	-0.06	2.34	41.2
DIHA (b)	Degr. Prod.	-	-	-	-
DEHA (c)	Degr. Prod.	-	-	-	-
DIA (e)	Degr. Prod.	670	-	-	-
Chloridazon	Herbicide	422	1.19	2.54	31
HA (f)	Degr. Prod.	-	-	-	-
Metamitron	Herbicide	1770	0.85	3.09	30
DEA (g)	Degr. Prod.	3200	-	3.54	-
Metribuzin	Herbicide	1165	1.65	2.57	11.5
Lenacil	Herbicide	2.9	1.69	4.25	179
Atrazine* ^{b.c.e.f.g}	Herbicide	35	1.75	2.11	42
Chlorotoluron	Herbicide	74	2.28	4.19	63.6
Fluometuron	Herbicide	111	1.7	2.73	86
Metobromuron	Herbicide	330	-	-	
Diuron	Herbicide	35.6	2.87	1.83	75.5
Terbuthylazine ^{b.e.i.k}	Herbicide	6.6	3.4	3.07	75.1
Terbutryn*	Herbicide	25	3.66	2.4	74
Ethofumesate	Herbicide	50	2.7	3.38	21.6
Linuron*	Herbicide	63.8	3.0	2.03	57.6
Metolachlor	Herbicide	530	3.4	3.32	90
Flufenacet	Herbicide	56	3.2	2.23	40
Diflufenican	Herbicide	0.05	4.2	1.51	180
Diclofop-methyl*	Herbicide	0.39	4.8	0.0	1
Pendimethalin	Herbicide	0.33	5.4	-0.32	182.3
Oxyfluorfen	Herbicide	0.116	4.5	0.26	138
HTbz (i)	Degr. Prod.	7.19	-	4.59	-
DETbz (k)	Degr. Prod.	327.1	-	3.54	-

¹Solubility in water at 20 °C. ²logK_{ow}, octanol/water partition coefficient at pH 7 and 20 °C. ³DT50, Half-life in soil. ⁴GUS index, indicator of potential pollution that allows classify pesticides in leaching (GUS > 2.8), non-leaching (GUS < 1.8) and transition (1.8 < GUS < 2.8). (PPDB. Pesticide Properties DataBase, 2019). *Compounds nowadays banned included in community list of active substances approved, excluded and under community evaluation, low risk substances, substances candidates for substitution and list of basic substances (<https://www.mapa.gob.es/>)

Table S3. Quality control parameters of the SPE-LC-MS method applied to the analysis of pesticides in surface and ground waters. Letters in brackets correspond to degradation products of pesticides, with the same letters as superscript.

Pesticide	m/z ¹	V cone ²	t _R ³ (min)	Recovery %	RSD ⁴ (%)	r ² (0.05-1.50) ⁵ μg L ⁻¹	LOD ⁶ μg L ⁻¹	LOQ ⁷ μg L ⁻¹
Methamidophos	142	20	6.9	24.3	6	0.985	0.088	0.159
Acephate	184.1	15	7.1	18.1	12	0.997	0.058	0.121
Pyrimidinol (d)	153.2	25	8.6	79.4	4	0.990	0.024	0.059
Imidacloprid	256.2	15	9.3	95.0	18	0.984	0.027	0.08
Dimethoate	230.2	15	10.3	84.8	16	0.993	0.012	0.032
Pirimicarb	239.2	20	16.9	14.4	14	0.993	0.076	0.245
Diazoxon (j)	289	25	17.1	91.2	6	0.998	0.011	0.036
Methidathion	303	15	19.6	15.8	7	0.998	0.031	0.086
Methoxyfenozide	369.3	20	19.7	80.7	10	0.994	0.023	0.062
Azinphos-methyl	318	10	20.1	73.0	4	0.998	0.054	0.134
Chlorpyrifos-oxon (l)	334	20	22.4	90.8	12	0.991	0.028	0.068
Diazinon ^{d,j}	305.2	20	25.8	60.6	25	0.995	0.022	0.049
Indoxacarb	528	20	29.9	67.0	8	0.999	0.038	0.084
Chlorpyrifos ^l	350.1	20	30	49.8	27	0.988	0.033	0.064
Hexythiazox	353	20	30.5	49.	18	0.993	0.025	0.067
Cypermethrin	416.2	20	32.3	19.6	18	0.983	0.083	0.185
λ-Cihalothrin	450	20	32.4	25.3	28	0.984	0.065	0.156
CGA-62826 (a)	266.2	25	5.5	93.4	12.6	0.991	0.026	0.062
CGA 92370 (h)	194.2	25	11.2	88.0	14	0.996	0.021	0.064
Carbendazim	192.2	35	12	15.4	10	0.999	0.016	0.046
Cymoxanil	199.2	35	12.9	35.1	22	0.989	0.024	0.071
Flutriafol	302.2	20	14.7	96.7	16	0.997	0.022	0.062
Metalaxy ^{l,a,h}	280.2	25	16.3	102.7	5	0.993	0.011	0.037
Cyproconazole	292.2	20	17.8	70.9	19	0.998	0.027	0.071
Nuarimol	315.1	30	18	87.8	12	0.998	0.019	0.044
Azoxystrobin	404.2	25	18.2	68.4	13	0.993	0.013	0.035
Boscalid	344	25	18.8	85.8	2	0.994	0.026	0.071
Iprovalicarb	321.3	20	19.1	92.7	23	0.999	0.018	0.045
Myclobutanil	289.1	25	19.2	80.1	6	0.990	0.012	0.037
Triadimenol	296.2	15	19.8	77.6	11	0.990	0.019	0.038
Pyrimethanil	200.2	35	20	81.6	15	0.989	0.011	0.037
Dimethomorph	388.2	25	21.1	73.9	15	0.995	0.017	0.063
Penconazole	284.2	20	22.4	82.6	4	0.996	0.017	0.062
Fluopyram	397	30	22.7	93.1	13	0.996	0.014	0.037
Fenbuconazole	337	25	23.1	91.3	16	0.995	0.021	0.062
Kresoxim-methyl	314.4	15	23.9	74.4	7	0.996	0.026	0.056

Iprodione	330	25	24.5	15.8	19	0.976	0.041	0.112
Benalaxyl	326.2	25	24.7	84.0	3	0.996	0.012	0.036
Tebuconazole	308	25	24.9	92.1	14	0.998	0.015	0.05
Cyprodinil	226.2	40	25.8	64.1	13	0.994	0.028	0.082
Bupirimate	317	30	26.2	76.2	25	0.996	0.029	0.076
Trifloxystrobin	409.1	20	28.8	74.9	6	0.995	0.018	0.041
Flazasulfuron	408.2	20	6.6	50.6	17	0.991	0.038	0.094
DIHA (b)	156.1	25	6.9	18.2	18	0.990	0.041	0.106
DEHA (c)	170.1	25	7.4	25.9	19	0.989	0.038	0.097
DIA (e)	174.2	25	9.1	63.7	9	0.996	0.021	0.064
Chloridazon	222.1	30	10	84.9	14	0.999	0.026	0.071
HA (f)	198.1	25	10.5	66.8	17	0.992	0.042	0.109
Metamitron	203.2	25	10.5	27.0	8	0.986	0.015	0.04
DEA (g)	188.1	25	10.7	87.8	8	0.992	0.013	0.034
Metribuzin	215.1	20	13.7	82.6	6	0.997	0.031	0.079
HTbz (i)	212.2	25	12.8	82.7	7	0.996	0.011	0.038
DETbz (k)	202.2	20	13.5	100.4	9	0.998	0.024	0.058
Lenacil	235.2	15	15.3	90.9	9	0.996	0.015	0.038
Atrazine ^{b,c,e,f,g}	216.1	30	16	60.1	11	0.990	0.021	0.057
Chlorotoluron	213	20	16.3	72.2	7	0.995	0.026	0.066
Fluometuron	233.2	20	16.7	97.1	12	0.999	0.028	0.073
Metobromuron	259.1	20	17	67.9	12	0.997	0.019	0.037
Diuron	233.2	25	18.5	90.7	20	0.995	0.016	0.043
Terbutylazine ^{b,e,i,k}	230.2	25	18.7	73.0	10	0.993	0.023	0.055
Terbutryn	241	25	19.2	78.5	13	0.995	0.018	0.042
Ethofumesate	287.2	20	19.2	88.7	19	0.998	0.024	0.063
Linuron	250.1	20	21.5	87.9	13	0.983	0.021	0.037
Metolachlor	284.2	15	21.7	92.4	9	0.999	0.017	0.052
Flufenacet	364		26	50.6	24	0.994	0.02	0.054
Diflufenican	395		29.7	59.1	21	0.994	0.087	0.204
Diclofop-methyl	341.2	20	30.4	59.2	21	0.991	0.019	0.032
Pendimethalin	282	25	31.8	21.0	14	0.993	0.026	0.068
Oxyfluorfen	362	20	31.8	94.5	10	0.996	0.013	0.048

¹Mass/charge relation. ²Volume of cone. ³t_R, retention time. ⁴Standard relative deviation, calculated from the replicated analysis (n = 5) of spiked (0.1 µg L⁻¹) groundwater samples. ⁵Linear calibration range. ⁶Limit of Detection. ⁷Limit of Quantitation.

Results

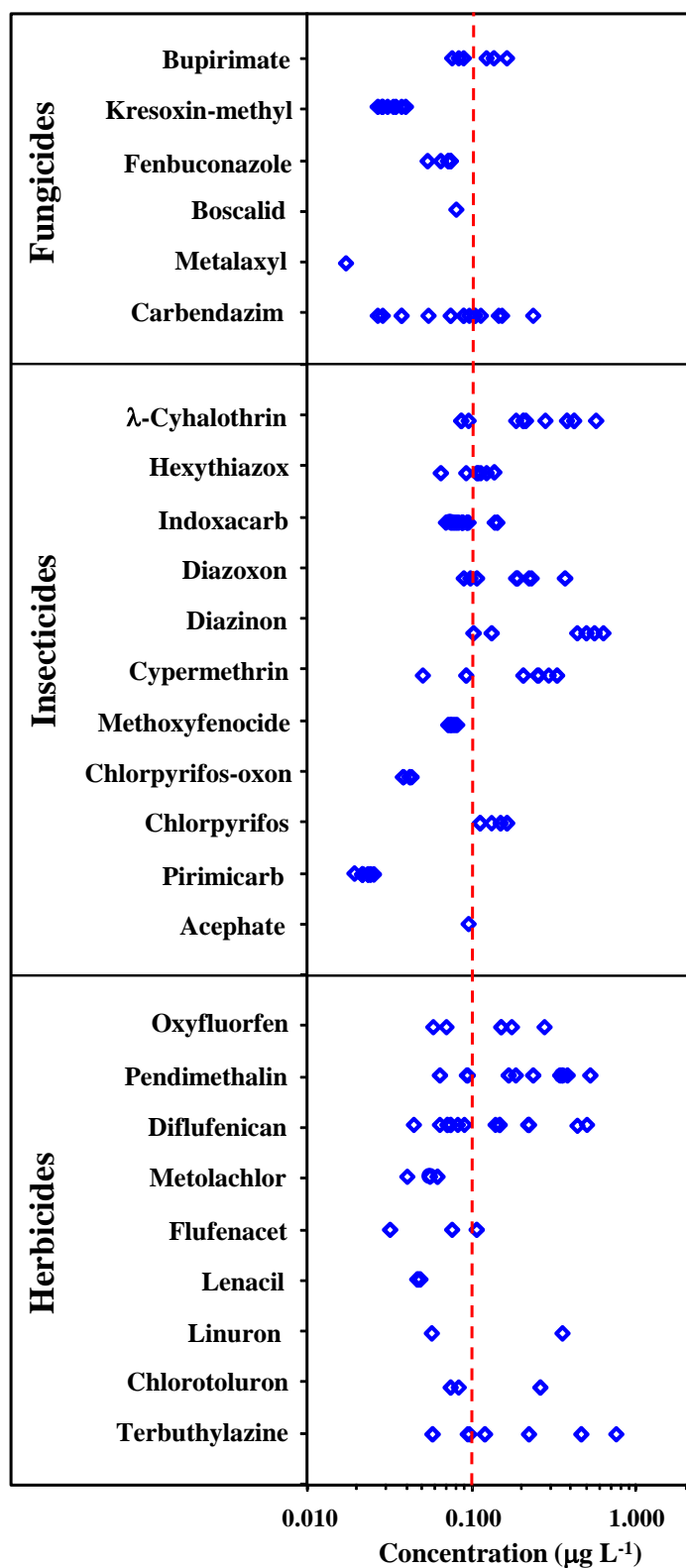


Figure S1. Concentrations of fungicides, insecticides and herbicides detected in the surface and groundwater samples (logarithmic scale).

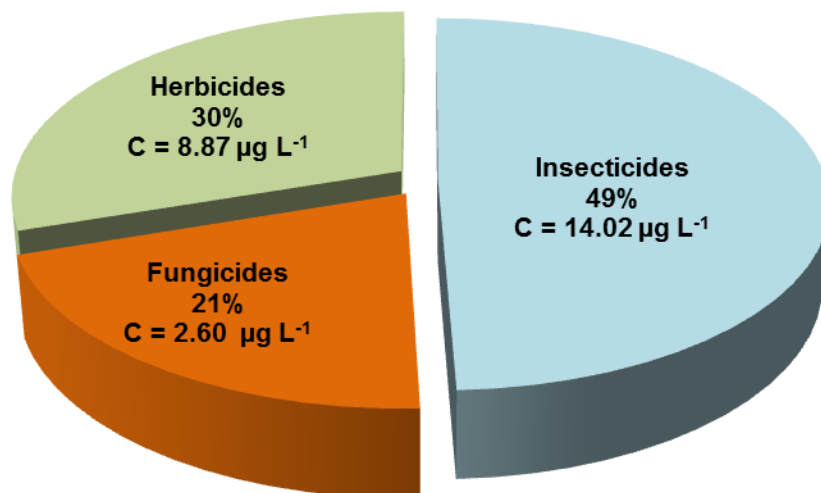


Figure S2. Distribution of samples according to type of pesticide detected with indication of the total concentration of fungicides, insecticides and herbicides.

References

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Highlights

- 69 pesticides at 21 sampling points of a Spanish vineyard area were evaluated.
- Twenty-six pesticides were detected, 15 of them in concentrations over $0.1 \mu\text{g L}^{-1}$.
- Insecticides were the most frequently detected pesticides.
- Acute toxicity risk for some aquatic organisms was revealed at most of the samples.
- Some compounds detected pose a chronic risk unacceptable in several trophic levels.