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Additional Information

1   PHYTOREMEDIATION OF POLLUTED SOILS: RECENT PROGRESS AND DEVELOPMENTS

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3   **Spatio-temporal distribution of pyrethroids in soil in Mediterranean paddy fields**

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16

17 **Abstract**

18 *Purpose* The demand of rice by the increase in population in many countries has intensified the application

19 of pesticides and the use of poor quality water to irrigate fields. The terrestrial environment is one

20 compartment affected by these situations, where soil is working as a reservoir, retaining organic pollutants.

21 Therefore, it is necessary to develop methods to determine insecticides in soil and monitor susceptible areas

22 to be contaminated, applying adequate techniques to remediate them.

23 *Materials and methods* This study investigates the occurrence of ten pyrethroid insecticides (PYs) and its

24 spatio-temporal variance in soil at two different depths collected in two periods (before plow and during

25 rice production), in a paddy field área located in the Mediterranean coast. Pyrethroids were quantified using

26 gas chromatography–mass spectrometry (GC–MS) after ultrasound-assisted extraction with ethyl acetate.

27 The results obtained were assessed statistically using non-parametric methods, and significant statistical

28 differences ($p < 0.05$) in pyrethroids content with soil depth and proximity to wastewater treatment plants

29 were evaluated. Moreover, a geographic information system (GIS) was used to monitor the occurrence of

30 PYs in paddy fields and detect risk areas

31 *Results and discussion* Pyrethroids were detected at concentrations ≤ 57.0 ng g^{-1} before plow and ≤ 62.3 ng

32 g^{-1} during rice production, being resmethrin and cyfluthrin the compounds found at higher concentrations

33 in soil. PYs were detected mainly at the top soil and a GIS program was used to depict the obtained results,

34 showing that effluents from wastewater treatment plants (WWTPs) were the main sources of soil

35 contamination. No toxic effects were expected to soil organisms, but it is of concern that PYs may affect

36 aquatic organisms, which represents the worst case scenario.

37 *Conclusions* A methodology to determine pyrethroids in soil was developed to monitor a paddy field area.

38 The use of water from WWTPs to irrigate rice fields is one of the main pollution sources of pyrethoids. It

39 is a matter for concern that PYs may present toxic effects on aquatic organisms, as they can be desorbed

40 from soil. Phytoremediation may play an important role in this area, reducing the possible risk associated

41 to PYs levels in soil.

42

43 **Keywords** Gas chromatography-mass spectrometry • Geographical information system • Insecticides •

44 Paddy fields • Pyrethroids • Soil

1 Introduction

Rice is the cereal grain most widely consumed and it represents the third-highest worldwide production (FAO 2012). Its cultivation under hydric conditions is a very complex system due to water-soil interactions and anthropic interventions (Nawaz et al. 2013) such as gained land for cultivation and the elevated use of pesticides. Moreover, the high demand of water to keep the fields continuously flooded and the low rainfall in the Mediterranean areas make necessary the use of poor quality water such as regained water from WWTPs. Unfortunately, as some authors have pointed out, the treatment of this water will not satisfactory remove all contaminants (Alonso et al. 2012; Campo et al. 2013; Feo et al. 2010; Weston et al. 2013), then the spreading of contaminants, such as insecticides and biocides, through agricultural soils may take place (Arias-Estevez et al. 2008), where they can be considered pseudo-persistent due to their daily release into the environment.

Pyrethroid insecticides (PYs) were derived from chrysanthemic acid to obtain more stable compounds in the environment. They have been intensively used in agricultural, industrial and urban areas (Amweg et al. 2005; Aznar et al. 2014; Song et al. 2015), since they are a replacement of other banned pesticides, such as organochlorine and organophosphate pesticides. The occurrence of PYs is of concern because although they are retained in soil due to their hydrophobicity and low water solubility (see Table 1), PYs can be toxic to the aquatic life (Amweg et al. 2005; Song et al. 2015; Weston et al. 2005).

However, in contrast to the data of PYs levels documented in aquatic ecosystems, information on the levels of these insecticides in soil ecosystems is scarce. Given the universal dependence on hydric soils for rice production and their high ecological value, their maintenance in good environmental conditions is crucial. Hence, it is necessary to monitor their presence regularly and evaluate their potential risk to the environment (Huang et al. 2015).

The aim of this work was to monitor and assess the occurrence and distribution of PYs in soil samples collected from paddy fields in a Mediterranean region at different depths (0-40 and 40-60 cm) and during two campaigns (plow and rice production periods). To determine PYs in soil, a method based on ultrasound assisted extraction and gas chromatography-mass spectrometry (GC-MS) was developed. In addition, a geographical information system (GIS) was used to assess the main sources of pollution as well as to identify and indicate areas where PYs may be toxic. To the best of our knowledge, this is the first time that these insecticides are studied and monitored in soil at different depths in paddy fields.

74

2 Materials and methods

2.1 Site description

The study was carried out in Albufera of Valencia, a Natural Park located in the Spanish eastern coast (Fig. 1). This area is a wetland composed of three distinct environments: the lake, the marsh area where rice is cultivated and the sand barrier. The area was formed due to sedimentary contributions of the Turia and Júcar Rivers closing a gulf in the Mediterranean Sea. In the 18th century the lake had an area of 300 km^2, but nowadays the lake's area is 23 km^2, being currently the largest freshwater lake in Spain. The lake's area reduction was caused by two main processes: the natural process of silting (sediments from both rivers over the years) and anthropogenic processes to gain land to produce rice over the last century (Pascual-Aguilar et al. 2015). This area is usually flooded and soils are classified as hydric due to rice production management and the presence of the water table near the soil surface. Following the Soil Taxonomy classification (Soil Survey Staff, 2014), soils are defined as Entisols and Aridisols (Moreno-Ramón et al. 2015). These soils are carbonated, saline and show a moderate surface organic carbon content due to the rice management (incorporation of post-harvest residues).

The area studied is ruled by the rice production cycle (Fig. 2). It starts with a period of fallow when the lake reaches its maximum level, flooding part of the rice fields (November - January). In January, the gates connecting with the Mediterranean Sea are opened and the fields are drained reaching the lake its normal water level. From the end of February till May, paddy fields are dried, so they can be plowed and prepared prior to sowing. In May, the rice growing season starts and water flows around the whole park and the paddy fields are flooded again. In September, the period of harvest starts and paddy fields are drained to allow harvest by the heavy machinery, and the rice cultivation cycle will start again. Water inputs come from the Júcar and Turia Rivers that run south and north in the area of study, respectively. Due to the shortage of fresh water during summer, water from two WWTPs located 6-8 Km from the lake (Fig. 1) is used to irrigate rice fields.

99

2.2 Standards and reagents

101   Ethyl acetate (EtAc) and Florisil (magnesium silicate adsorbent, 150-250 μm, 60-100 mesh for

102   chromatography) were purchased from Scharlab (Barcelona, Spain). Sodium sulfate (purity $\geq$ 99 %) was

103   obtained from Aldrich (Steinheim, Germany).

104   Insecticides resmethrin (RESM), bifenthrin (BIFE), fenpropathrin (FENP), λ- cyhalothrin (CYHA),

105   permethrin (PERM), cyfluthrin (CYFL), α-cypermethrin (CYPE), τ-fluvalinate (FLUV), esfenvalerate

106   (ESFE) and deltamethrin (DELT) (purity 99 %) were supplied by Riedel-de Haën (Seelze, Germany),

107   whereas the surrogate standard trans-permethrin-D6 (purity >99 %) was supplied by Symta (Madrid,

108   Spain). The list of investigated compounds is shown in Table 1 along with their physicochemical properties.

109   Individual stock solutions of each compound at 500 μg mL^{-1} were prepared in EtAc and stored in the

110   darkness at 4 ºC up to 8 weeks. A mixed stock solution of 1000 ng mL^{-1} containing all analytes was prepared

111   by dilution with EtAc of the individual stock solutions. A working mixture solution at 200 ng mL^{-1} was

112   prepared weekly by dilution with EtAc of the mixed stock solution. A solution containing the surrogate

113   standard was prepared in EtAc at the same concentration as the working mixture solution.

114

115   2.3 Apparatus

116   *2.3.1 Extraction equipment*

117   Glass columns (20 mL) of 10 cm x 20 mm i.d., Afora, Spain, and Whatman No.1 filter paper circles of 2

118   cm diameter (Whatman, Maidstone, UK) were used.

119   An ultrasonic water bath (Raypa, Barcelona, Spain) was used in the extraction step. A vacuum manifold

120   (Supelco, Visiprep, Madrid) was employed to collect the extracts.

121

122   *2.3.2 Detection equipment*

123   Gas chromatography-mass spectrometry (GC-MS) analysis was performed with an Agilent 6890

124   (Waldbronn, Germany) gas chromatograph equipped with a mass spectrometric detector, Model HP 5977A.

125   The operating conditions are summarized in Table S1.

126 The target and qualifier abundances were determined by injection of standards under the same

127 chromatographic conditions using full-scan with the mass/charge ratio ranging from 50 to 400 m/z. The

128 compounds were confirmed by their retention times, the identification of target and qualifier ions and the

129 determination of qualifier to target ratios. Retention times must be within ± 0.1 min of the expected time

130 and qualifier-to-target ratios within a 20 % range for positive confirmation. The quantification was

131 accomplished by calibration with the surrogate standard at 10 ng g^{-1}. To reduce possible memory effects of

132 the column, prior to the analysis of samples, the inlet was flushed by heating at 300 ºC for 30 min and

133 procedural blanks were analyzed after every four samples.

134

135 2.4 Samples

136 *2.4.1 Sample collection*

137 Soils from thirty-three sites were sampled in rice fields at two different depths (0–40 and 40–60 cm).

138 Sampling points were located with a virtual reference station (Leica GPS 1200) that supplied the universal

139 transverse mercator (UTM) coordinates for the geostatistical treatment (Tables S2–S3, Electronic

140 supplementary material).

141 The first layer (0–40 cm) of soil is the plow surface in which rice crop residues are incorporated. On the

142 other hand, the deeper layer of soil (40–60 cm) remains unchanged and is usually saturated by the presence

143 of a saline water table. A stainless steel Eijkelkamp auger was used for soil sampling according to a

144 stratified sampling design. After soils were sampled, they were transported to the laboratory, where they

145 were air dried at room temperature (21 °C) in darkness to avoid PYs photodegradation (Katagi 2004), sieved

146 through a 2-mm mesh, thoroughly mixed, and kept frozen (−18 °C) in glass containers until analysis.

147 Two sampling campaigns were carried out. The first campaign at the end of February, before plow period,

148 when fields are dried to prepare them to produce rice and the second sampling was in July when the fields

149 are flooded (Fig. 2).

150

151 *2.4.2 Physical-chemical properties of soil samples*

152   Soil properties may affect insecticides behavior (transport, persistence, leaching, etc.) and, therefore, they

153   were determined. Ganulometric fractions of soil (sand, silt, clay) were determined for each sample

154   following the Bouyoucos method. Soil pH was measured in a 1:2.5 (soil/distilled water) extract shaken for

155   15 min and measured after 2 h. Soil organic carbon was analyzed by the ignition method and carbonate

156   content by Bernard calcimeter method. Finally, soil salinity was measured by the electrical conductivity

157   (EC) 1:5 (soil/distilled water) (Tables S2-S3). All the methodologies described in this paper have been

158   carried out according Soil Survey Staff (2009).

159

160   *2.4.3 Insecticides analyses in soil*

161   Extraction of PYs from soil was carried out by ultrasound assisted extraction as one of the most favorable

162   techniques to extract the target compounds (Albaseer et al. 2010). Briefly, 1 g of sieved soil was placed in

163   a glass column containing 1 g sodium sulfate and 1.5 g of Florisil over a paper filter and a frit.

164   Soil samples were extracted twice for 15 min in an ultrasonic water bath with 5 mL EtAc and an additional

165   1 mL was used to wash the glass material. The combined extracts were collected in 10 mL graduated tubes

166   using a multiport vacuum manifold, concentrated to 0.1 mL using a gentle stream of air and analyzed by

167   GC-MS. To counteract matrix effects a surrogate standard was used.

168

169   2.5 Method validation and quality control

170   In order to evaluate the method developed for the detection of insecticides in soil, different quality

171   parameters were studied: recoveries, reproducibility, linearity and sensitivity.

172   For the recovery studies, samples were previously fortified with a mixture of the different analytes to reach

173   final concentrations of 10 and 2 ng g^{-1} and the labeled surrogate standard at 10 ng g^{-1}. They were kept at

174   room temperature overnight to allow solvent evaporation. The recoveries obtained for all the studied

175   compounds were satisfactory, ranging from 75 to 107 % (Table 2). The precision of the analytical

176   procedure, expressed as relative standard deviations (RSD, %) of the analysis of four replicates, ranged

177   between 1 and 11% (Table 2).

178 Limits of detection (LODs) and quantification (LOQs) of the developed method were determined using ten

179 replicates of soil extracts, spiked at 1 ng g^{-1}. The equation to calculate the LOD was the following: LOD =

180 $t_{99} \times$ S, where t_{99} is the Students's value for a 99% confidence level and n-1 degrees of freedom and S is the

181 standard deviation of the replicate analyses. The LOQ was calculated as 10 times the standard deviation of

182 the results of the replicate analysis used to determine LOD. Low limits were obtained due to the high

183 selectivity and sensitivity of GC–MS. As shown in Table 2, LODs ranged from 0.1 to 0.4 ng g^{-1} and LOQs

184 from 0.3 to 1.2 ng g^{-1} allowing the detection of insecticides at trace levels in soil samples.

185 A multipoint calibration curve with five standard solutions at different concentration levels (from 1 to 100

186 ng g^{-1}), appropriate to the levels found in soil samples, was used. The surrogate standard was added at the

187 concentration of 10 ng g^{-1} for all levels.

188

189 2.6 Software

190 Standard statistical analyses were carried out with SPSS statistical program (Mann-Whitney and Spearman

191 correlation test) to determine the levels of insecticides in soil. The use of non-parametric methods was

192 confirmed by the outcome of Shapiro-Wilk test, which did not show a normal distribution. The compounds

193 included in the statistical analysis were those with detection rates higher than 70 %. To create the matrix, a

194 pretreatment of the data was necessary. Values below quantification limit were converted in numerical

195 results, by adding a value of half their limit of quantification.

196 Cartography was performed by the Bayesian maximum entropy method (BME) (Fig. 3) which allowed a

197 complete stochastic description of those non-sampling areas (Money et al. 2009). BME maps showed gentle

198 transitions between the different mapping units which reflected the normal behavior of continuous variables

199 like water contaminants. The software used was ARCGIS 9.3 with a BMEGUI module.

200

201 **3 Results and discussion**

202 3.1 Spatial and temporal distribution of PYs in soil

203 In general, soils sampled had an electrical conductivity of 0.72-0.95 dS m^{-1} and many of them were

204 calcareous. The maximum values registered in the EC$_{1/5}$ (2.89 dS m^{-1}) revealed that there was soil

205 salinization in the area (Table 3). Regarding particle size, 39 % of samples were classified as silty clay,

206 followed by 30 % of samples classified as clay loam according USDA textural classes.

207 The developed method was applied to the analysis of PYs in soils from paddy fields collected in two

208 periods, before plow and during rice production. Table 4 summarizes the overall results obtained, showing

209 the range of concentrations found and the detection frequencies for each compound. The complete set of

210 concentration values are shown in Tables S4-S7.

211 Before plow period (March), when there is no water flowing through the rice fields, six out of the ten PYs

212 studied were detected (Table 4). RESM, CYFL, CYPE and ESFE were the compounds more often detected,

213 up to 70 % of the analyzed samples, with levels up to 57 ng g^{-1} in the case of ESFE near to an area of

214 discharge of the North WWTP (Fig. 3). BIFE, PERM, FLUV and DELT were not detected in any of the

215 studied samples and CYHA was quantified only in one sample. However, during rice production (July),

216 when freshwater flows through the fields, the soil sampled presented a higher detection rate (almost 100

217 %) of RESM, BIFE, FENP, CYFL, CYPE and ESFE, being seven PYs detected, up to 62.3 ng g^{-1} for RESM

218 nearby the area close to the North WWTP (see Table S6).

219 The presence of PYs could be explained by their application to local crops as well as their non-efficient

220 removal during WWTPs processes (Campo et al. 2013; Weston et al. 2013). During rice production period,

221 when there is not enough freshwater to keep fields flooded to produce rice properly, the use of regained

222 water from WWTPs is required, and as a result some of these compounds are introduced into the

223 environment increasing the contamination of soil, which is an important reservoir. The outcome of non-

224 parametric statistical analyses (Table 5) showed that water source had a clear influence over PYs levels in

225 the area, particularly for CYFL and ESFE. Fig. 3 depicts their distribution in the area showing that the main

226 sources were the WWTPs. The contamination of CYFL and ESFE decreased along the park indicating that

227 the marsh area may act as a buffer, retaining the contamination before reaching the lake. Further work needs

228 to be done to assess the main paths of pollutant dissipation; plants in the marsh area *Arundo donax, Typha*

229 *angustifolia*, etc (typical plants used in phytoremediation) and *Oryza sativa* (rice) may play an important

230 role reducing the concentration of contaminants and improving the environmental conditions of the area

231 studied.

232

233   3.2 Distribution in depth

234   Soil organic carbon showed a decrease in depth due to the rice management in the area, because straw is

235   incorporated in soil after the harvest at first 40 cm increasing its content in the top soil. The average content

236   was around 31 g Kg^{-1} of soil. On the contrary, the soluble organic carbon showed an increase in depth, and

237   this trend can be explained due to the hydric characteristics of soils. Soluble compounds were accumulated

238   in depth because at 40-60 cm there was a permanent water table. In the upper parts, the water table can be

239   intermittent depending on the crop management period.

240   In general, the target compounds tend to be found in the first 40 cm of soil, where higher content of organic

241   matter is present. However, PYs concentrations against depth showed that CYFL, BIFE, FENP, CYHA and

242   ESFE (Table 5) did not present that trend whereas RESM and CYPE show significant statistical differences

243   (ρ value < 0.001). RESM, which presents the highest water solubility of the studied family of insecticides

244   (Table 1), may be translocated deeply under hydric conditions and accumulated at the second layer studied

245   (40-60 cm). On the other hand, the low solubility in water of CYPE and their application during rice

246   production to eradicate common armyworm, may explain the accumulation of this pesticide in the top layer.

247   Comparing the maps generated by GIS depicted in Fig. 3, it can be observed that CYFL and ESFE

248   contamination on top soil matched the highest points of pollution at deep soil, which are nearby WWTPs

249   discharge (see Fig. 1). On the other hand, the adsorption of PYs is higher in organic matter and mineral

250   particles with a large surface area (Zhou et al. 1995). Moreover, wetland soils due to natural conditions and

251   paddy soils due to the rice management tend to accumulate organic matter in the surface layers. Thus, the

252   presence of PYs in the area studied may be explained by the content of clay and organic matter in soils,

253   where PYs can be bounded making more unlikely their degradation.

254   The correlation between distance to the WWTP and pesticide levels showed a significant statistical

255   relationship. It should be noted that RESM, CYFL, CYPE and ESFE showed higher concentration in the

256   locations near North WWTP discharge. The Spearman coefficient between CYFL and distance showed a

257   high-moderate correlation (r = 0.42 p < 0.001), whereas the rest of the data set showed a low-moderate

258   correlation grade (Table 5).

259

260   3.3 Toxicity to soil organisms

261   The toxicity of PYs to soil organisms, earthworms and other non-target soil organisms, is very low, with

262   LC50 > 1,000,000 ng g^{-1} for *Eisenia fetida*, lower than those reported for other insecticides (European

263   Commission 2002, 2004 and 2005). Thus, the concentrations found in this field-based study indicate

264   negligible toxic effects for terrestrial organisms. However, invertebrates have been found to be the species

265   most sensitive to PYs, presenting very low LC50s (Amweg et al. 2005). These invertebrates are present in

266   aquatic and semiaquatic habitats and are an important food supply for fish and insectivorous birds, and the

267   alteration of invertebrates population could break the ecological equilibrium of the area.

268   Thus, due to the hydromorphic condition of the soils studied (Fig. 2), PYs in soil can be desorbed and

269   aquatic organisms should be also taken into account in this study. The maximum equilibrium concentration

270   expected in water can be calculated from the soil adsorption coefficient Kd, using Koc values from Table

271   1 and % OC of soil.

272   [Koc = Kd x 100 / % OC]

273   Table 6 shows the toxic effects of PYs in three aquatic trophic levels. In algae, the EC_{50} is high for all PYs

274   (*Scenedesmus subspicatus* EC_{50} (72 h) >1 x 10^7 ng L^{-1} for CYFL) (European Commission, 2002) and no

275   toxic effect is expected. As shown in Table 6, the no observed effect concentration (NOEC) data of PYs

276   for fish is higher than the equilibrium concentration in water in the studied area. Thus, these PYs

277   concentrations would not produce toxicity effects to fish. On the other hand, the NOEC data for

278   invertebrates is closer to those equilibrium concentrations in water as aquatic invertebrates are the most

279   sensitive organism to PYs (Maund et al. 2002). Moreover, the equilibrium values of BIFE in water are

280   higher than the NOEC, which means that some toxic effects may be produced to the invertebrate community

281   in the area studied.

282   The areas in which the concentration of BIFE in soil may present harmful effects for aquatic invertebrates

283   are those with levels higher than 10.1 ng g^{-1}, which corresponds to an equilibrium concentration in water

284   of the NOEC value (Table 5). In order to identify the area to mitigate contamination, a GIS program was

285   used (Fig. 4). The increase of BIFE contamination in this area could be explained by its enrichment during

286   transport by runoff, as Gan et al. (2005) pointed out, resulting in progressively higher pesticide levels in

287   the soil downstream from the source.

288   Nowadays, phytoremediation may be a good management practice to mitigate contamination as it has been

289   proven to work in wetlands (Moore et al. 2009, Mahabali and Spanoghe. 2014).

290

291   **4 Conclusions**

292   PYs were monitored in hydric soils at two depths collected in two seasons in a paddy field area within the

293   Natural Park of Albufera to assess their occurrence in the environment. During the period before plow,

294   RESM, CYFL, CYPE and ESFE were the compounds detected more often, up to 70 % of detection rate,

295   but at lower concentrations than during the second sampling period (rice production), when soils sampled

296   presented a higher rate of detection (almost 100 %) of RESM, BIFE, FENP, CYFL, CYPE and ESFE. The

297   results provided in this field-based study combined with GIS showed that water from WWTPs and field

298   application are the main sources of soil contamination by these insecticides. It was a matter of concern that

299   the levels of BIFE may cause harmful effects on the aquatic invertebrates within the area monitored, and

300   an area where BIFE levels may present a risk was highlighted. Phytoremediation can be applied to reduce

301   this risk but further work needs to be done to assess how phytoremediation should be performed to be

302   effective in situ.

303

306

307   **References**

308   http://faostat.fao.org/site/339/default.aspx, Accessed 8 June of 2015

309   Albaseer SS, Rao RN, Swamy YV, Mukkanti K (2010) An overview of sample preparation and extraction

310   of synthetic pyrethroids from water, sediment and soil. J Chromatogr A 1217(35):5537-5554

311   Alonso MB, Feo ML, Corcellas C, Vidal LG, Bertozzi CP, Marigo J, Secchi ER, Bassoi M, Azevedo AF,

312   Dorneles PR, Torres JPM, Lailson-Brito J, Malm O, Eljarrat E, Barcelo D (2012) Pyrethroids: A new

313   threat to marine mammals?. Environ Int 47:99-106

314   Amweg EL, Weston DP, Ureda NM (2005) Use and toxicity of pyrethroid pesticides in the Central Valley,

315   California, USA. Environ Toxicol Chem 24(4):966-972

316   Arias-Estevez M, Lopez-Periago E, Martinez-Carballo E, Simal-Gandara J, Mejuto JC, Garcia-Rio L

317   (2008) The mobility and degradation of pesticides in soils and the pollution of groundwater resources.

318   Agri Eco Environ 123(4):247-260

319   Aznar R, Albero B, Sanchez-Brunete C, Miguel E, Tadeo JL (2014) Multiresidue Analysis of Insecticides

320   and Other Selected Environmental Contaminants in Poultry Manure by Gas Chromatography/Mass

321   Spectrometry. J AOAC Int 97(4):978-986

322   Campo J, Masia A, Blasco C, Pico Y (2013) Occurrence and removal efficiency of pesticides in sewage

323   treatment plants of four Mediterranean River Basins. J Hazard Mater 263:146-157

324   European Commission (2002) Review report for the active substance Cyfluthrin, 6843/VI/97-final

325   European Commission (2004) Review report for the active substance α-Cypermethrin, SANCO/4335/2000-

326   final

327   European Commission (2005) Review report for the active substance Esfenvalerate, 6846/VI/97-final

328   Feo ML, Ginebreda A, Eljarrat E, Barcelo D (2010) Presence of pyrethroid pesticides in water and

329   sediments of Ebro River Delta. J Hydrol 393(3-4):156-162

330   Fojut TL, Palumbo AJ, Tjeerdema RS (2012) Aquatic life water quality criteria derived via the UC Davis

331   method: II. Pyrethroid Insecticides. Rev Env Contam Toxicol 216:51-103

332   Gan J, Lee SJ, Liu WP, Haver DL, KAbashima JN (2005) Distribution and persistence of pyrethroids in

333   runoff sediments. J Environ Qual 34:836-841

334   Hill IR (1985) Aquatic organisms and pyrethroids. Pestic Sci 27:429-465

335   Huang LM, Thompson A, Zhang GL, Chen LM, Han GZ, Gong ZT (2015) The use of chronosequences in

336   studies of paddy soil evolution: A review. Geoderma 237:199-210

337   Katagi T (2004) Photodegradation of pesticides on plant and soil surfaces Rev Env Contam Toxicol 182:1-

338   189

339   Laskowski DA (2002) Physical and chemical properties of pyrethroids. Rev Environ Contam Toxicol

340   174:49–170

341   Mahabali S, Spagnoghe P (2014) Mitigation of two insecticides by wetlands plants: feasibility study for the

342   treatment of agricultural runoff in Suriname (South America). Water Air Soil Pollut 225:1771

343 Maund SJ, Hamer MJ, Lane MCG, Farrelly E, Rapley JH, Goggin UM, Gentle WE (2002) Partitioning,
344   bioavailability, and toxicity of the pyrethroid insecticide cypermethrin in sediments. Environ Toxicol
345   Chem 21(1):9-15

346 Maund SJ, Campbell PJ, Giddings JM, Hamer MJ, Henry K, Pilling ED, Warinton JS, Wheeler JR (2012)
347   Ecotoxicology of synthetic pyrethroids. Top Curr Chem 314:137-65

348 Money E, Carter GP, Serre ML (2009) Using river distances in the space/time estimation of dissolved
349   oxygen along two impaired river networks in New Jersey. Water Res 43(7):1948-1958

350 Moore MT, Cooper CM, Smith S, Jr Cullum RF, Knight SS, Locke MA, Bennett ER (2009) Mitigation of
351   two pyrethroid insecticides in Mississippi Delta constructed wetland. Environ Pollut 157:250-256

352 Moreno-Ramón H, Marqués-Mateu A, Ibáñez-Asensio S, Gisbert JM (2015) Wetland soils under rice
353   management and seawater intrusion: characterization and classification. Spa J Soil Sci 5(2):111-129

354 Nawaz MF, Bourrie G, Trolard F, Mouret JC, Henry P (2013) Effects of agronomic practices on the
355   physico-chemical properties of soil waters in rice culture. Turk J Agric For 37(2):195-202

356 Oros DR, Werner I (2005) Pyrethroid Insecticides: An Analysis of Use Patterns, Distributions, Potential
357   Toxicity and Fate in the Sacramento-San Joaquin Delta and Central Valley. White Paper for the
358   Interagency

359 Pascual-Aguilar J, Andreu V, Gimeno-Garcia E, Pico Y (2015) Current anthropogenic pressures on agro-
360   ecological protected coastal wetlands. Sci Total Environ 03:190-199

361 Soil Survey Staff. 2009. Soil Survey Field and Laboratory Methods Manual. Soil Survey Investigations
362   Report No. 51, Version 1.0. Burt, R., eds. U.S. Department of Agriculture, Natural Resources
363   Conservation Service. Washington. USA. pp 407

364 Soil Survey Staff. 2014. Keys to Soil Taxonomy, 12th ed. USDA-Natural Resources Conservation Service.
365   Washington. USA. pp 372

366 Song Y, Kai J, Song X, Zhang W, Li L (2015) Long-term toxic effects of deltamethrin and fenvalerate in
367   soil. J Hazard Mater 289:158-164

368 Weston DP, Holmes RW, You J, Lydy MJ (2005) Aquatic toxicity due to residential use of pyrethroid
369   insecticides. Environ Sci Technol 39(24):9778-9784

370 Weston DP, Ramil HL, Lydy MJ (2013) Pyrethroid insecticides in municipal wastewater. Environ Toxicol
371   Chem 32(11):2460-2468

372   Zhou JL, Rowland S, Mantoura RFC (1995) Partition of synthetic pyrethroid insecticides between dissolved

373   and particulate phases. Water Res 29:1023–103

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Table 1 Properties of the target compounds and abbreviations

Chemical	Abbreviation	Log Kow	Log Koc	Solubility (mg L^{-1})	Soil aerobic half-life (days)	Soil anaerobic half-life (days)
Resmethrin	RESM	5.4[a]	5[e]	<1[c]	-	-
Bifenthrin	BIFE	6.0[a]	5.4[b]	0.1[a]	96.3[b]	425[b]
Fenpropathrin	FENP	6.0[a]	5[e]	0.014[a]	22[d]	276[d]
λ-Cyhalothrin	CYHA	6.9[a]	5.5[b]	0.003[a]	42.6[b]	-
Permethrin	PERM	6.5[a]	5.4[b]	-	39.5[b]	197[b]
Cyfluthrin	CYFL	5.9[a]	5.1[b]	0.002[a]	11.5[b]	33.6[b]
α-Cypermethrin	CYPE	6.6[a]	5.5[b]	0.004[a]	27.6[b]	55[b]
τ-Fluvalinate	FLUV	4.3[a]	-	0.002[a]	-	-
Esfenvalerate	ESFE	4.0[a]	5.4[b]	0.0002[a]	38.6[b]	90.4[b]
Deltamethrin	DELT	6.1[a]	-	<0.002[a]	24[d]	29[d]

[a]: Oros DR and Werner I, 2005

[b]: Laskowskin DA, 2002

[c]: http://www.inchem.org/documents/pds/pds/pest83_e.htm#1.3.2

[d]: http://www.cdpr.ca.gov/docs/registration/reevaluation/chemicals/environmental_fate.pdf

[e]: http://www.pesticideinfo.org/Detail_Chemical.jsp?Rec_Id=PC34303

378   **Table 2** Mean recoveries (%) with their relative standard deviation (RSD, %), limit of detection (LOD, ng

379   g^{-1}) and limit of quantification (LOQ, ng g^{-1}) of the studied insecticides

| | Fortification levels (ng g^{-1})[a] | | | | | |
| | 10 | | 2 | | | |
Compounds	Mean	RSD	Mean	RSD	LOD[b]	LOQ[b]
RESME	104	3	75	1	0.4	1.2
BIFE	103	2	100	8	0.1	0.3
FEN	107	3	107	3	0.2	0.7
λ-CYHA	95	9	96	9	0.1	0.4
PERME	94	5	98	11	0.1	0.5
CYFLU	97	4	102	4	0.3	1.1
α-CYPER	96	7	97	10	0.2	0.8
τ-FLUV	92	8	107	4	0.3	1.0
ESFEN	101	8	106	7	0.3	1.0
DELTA	99	7	75	2	0.3	0.9

[a]: (n=8); [b]: (n=10)

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382 **Table 3** Soil characteristics

	0-40 cm			40-60 cm		
	Min.	Max.	Mean ± RSD	Min.	Max.	Mean ± RSD
EC 1/5 (dS m^{-1})	0.4	2.0	0.6 ± 0.4	0.4	2.8	0.7 ± 0.5
pH	7.2	8.1	7.6 ± 0.2	0.5	8.4	7.6 ± 1.3
Carbonate (g Kg^{-1})	278	502	358 ± 38	260	530	357 ± 55
OC g Kg^{-1}	18.6	104.9	31.2 ± 18.5	4.3	60.1	23.4 ± 11.3
SOC g Kg^{-1}	0.0	1.1	0.4 ± 0.3	0.0	1.2	0.4 ± 0.3

383 EC: electrical conductivity (dS m^{-1}); OC: organic carbon; SOC: soluble organic carbon

384 **Table 4** Levels (ng g^{-1}) and detection rate (% det.) of PYs during plow and rice production period from
385 33 soil sampling points at different depths.

Plow period									
	0-40 cm					40-60 cm			
	Min.	Max.	Mean	% det.		Min.	Max.	Mean	% det.
RESM	0.0	52.0	19.5	97.0	RESM	1.6	53.4	23.9	100.0
BIFE	nd	nd	nd	nd	BIFE	nd	nd	nd	nd
FENP	nd	44.9	6.2	24.2	FENP	13.7	29.8	8.1	42.4
CYHA	nd	nd	nd	nd	CYHA	nd	1.5	0.0	3.0
PERM	nd	nd	nd	nd	PERM	nd	nd	nd	nd
CYFL	nd	54.2	20.3	90.9	CYFL	nd	27.3	11.0	81.8
CYPE	nd	17.9	5.0	69.7	CYPE	nd	11.1	1.7	42.4
FLUV	nd	nd	nd	nd	FLUV	nd	nd	nd	nd
ESFE	nd	57.0	19.4	84.8	ESFE	nd	46.3	19.7	90.9
DELT	nd	nd	nd	nd	DELT	nd	nd	nd	nd
Rice production period									
	0-40 cm					40-60 cm			
	Min.	Max.	Mean	% det.		Min.	Max.	Mean	% det.
RESM	2.0	62.3	23.2	100.0	RESM	4.5	57.9	28.2	100.0
BIFE	nq	32.2	4.2	100.0	BIFE	nq	13.5	3.0	100.0
FENP	nd	47.5	13.9	97.0	FENP	nq	40.2	13.3	100.0
CYHA	nd	20.7	3.0	93.9	CYHA	nd	41.1	7.7	97.0
PERM	nd	nd	nd	nd	PERM	nd	nd	nd	nd
CYFL	nq	39.0	15.7	100.0	CYFL	nq	54.9	22.0	100.0
CYPE	nd	26.2	3.9	84.8	CYPE	nd	31.9	4.1	97.0
FLUV	nd	nd	nd	nd	FLUV	nd	nd	nd	nd
ESFE	nd	57.1	23.4	87.9	ESFE	nd	48.8	20.3	97.0
DELT	nd	nd	nd	nd	DELT	nd	nd	nd	nd

386 nd: not detected; nq: not quantified

387

388 **Table 5** Statistical/Non-parametric outcomes (Mann-Whitney and Spearman tests).

Factor	Subfactor	Average rank (Mann-Whitney)						
		RESM	CYFL	CYPE	ESFE	BIFE	FENP	CYHA
	WWTPs	70.3a[1]	79.6a	76.9a	85.2a	31.9a	29.2a	39.2a
Water origin	Rivers	64.6a	59.9b	61.3b	57.1b	34.3a	35.7a	30.6a
	0-40	58.5a	69.4a	77.0a	67.2a	33.7a	33.2a	30.9a
Soil depth	40-60	74.5b	63.6a	56.0b	65.8a	33.3a	33.8a	36.1a
		Spearman coefficients						
		RESM	CYFL	CYPE	ESFE	BIFE	FENP	CYHA
Distance to WWTP		0.216*	0.418**	0.254**	0.288**	0.046	0.006	0.3*

389 [1] Different letter means $p < 0.005$ (inside the same factor); * ($p<0.05$); ** ($p<0.001$)

390 **Table 6** Ecotoxicology

Compound	Kd (L Kg^{-1})	Maximum equilibrium concentration in water (ng L^{-1}) in the studied area	EC$_{50}$ (ng L^{-1})	NOEC (ng L^{-1})	
			Algae	Invertebrates *Daphnia magna*	Fish *Pimephales promelas*
BIFE	7787	4	-	1.3[a]	40[a]
CYHA	9803	2	> 1000000[c]	3.8[a]	31[a]
CYFL	7787	7	> 991000[c]	20[a]	140[a]
CYPE	9803	3	> 1300000[c]	20[b]	77[a]

391 [a]: Fojut TL et al, 2012
392 [b]: Hill IR, 1985
393 [c]: Maund SJ et al, 2012
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395

396   **Figure captions**

397

398   **Fig 1** Map of the sites sampled in the rice fields at the Natural Park in Valencia, Spain

399   **Fig 2.** Hydrological cycle of rice production and the two sampling periods

400   **Fig 3.** Spatial representation of CYFL and ESFE A) First sampling of top soil, B) First sampling of deep
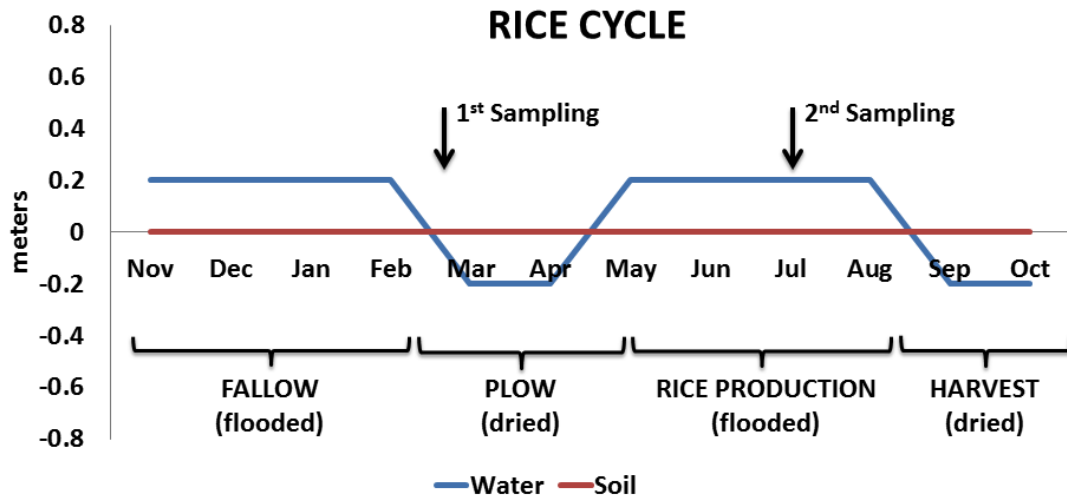401   soil

402   **Fig 4.** Representation of the area where BIFE levels (> 10 ng g^{-1}) may present negative effects to aquatic
403   invertebrates

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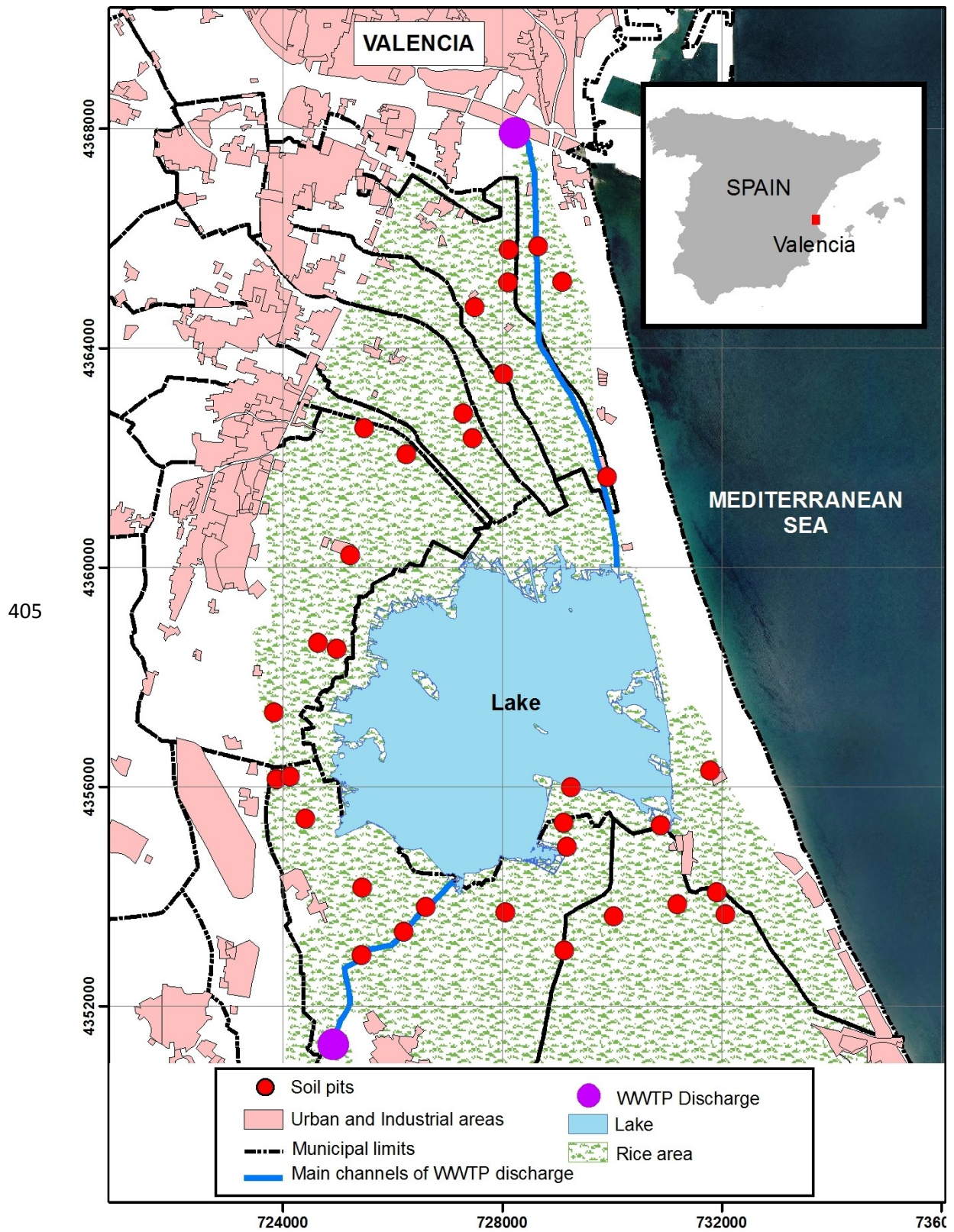
406   **Fig. 1** Map of the sites sampled in the rice fields at the Natural Park in Valencia, Spain
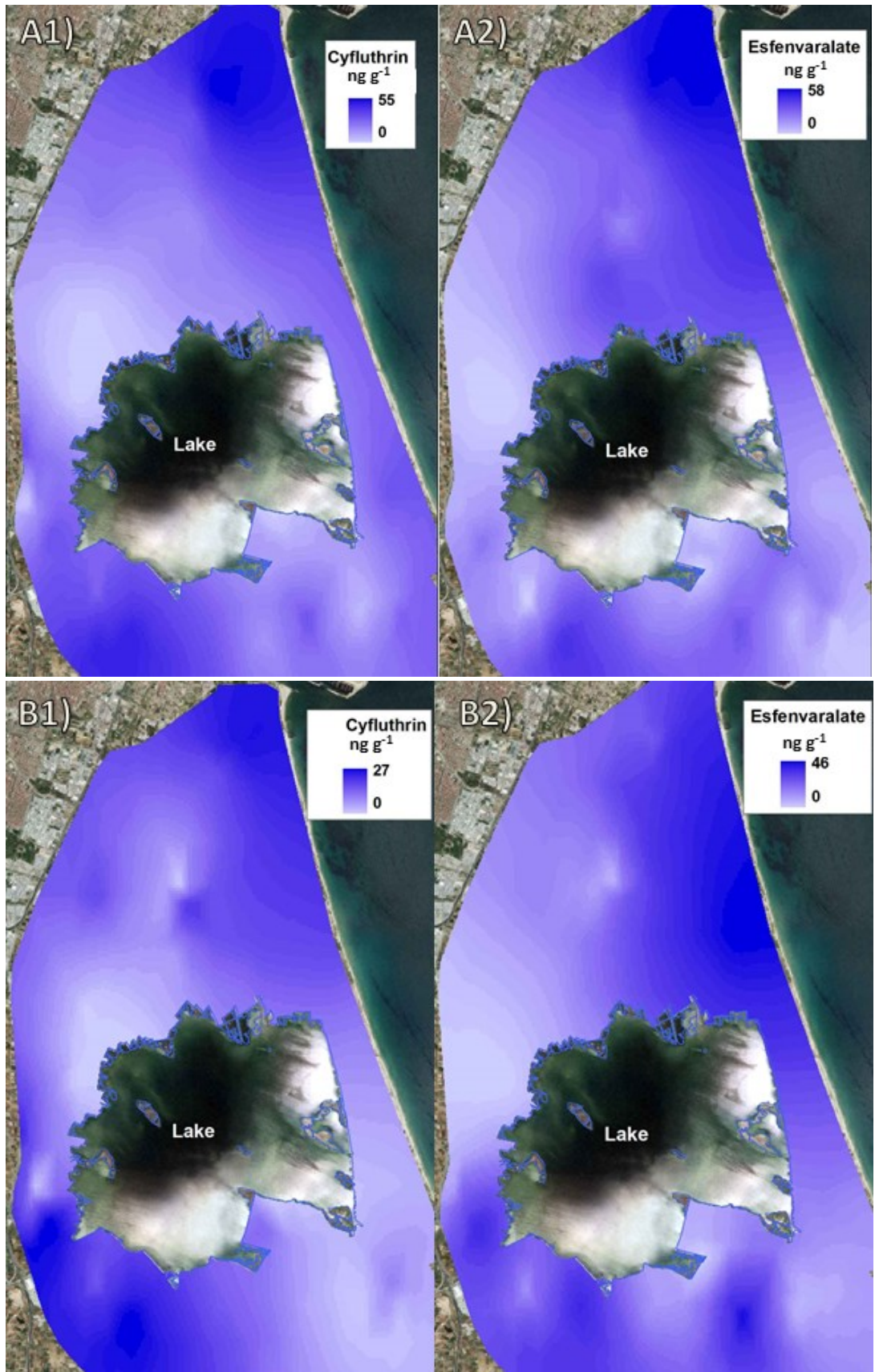
407

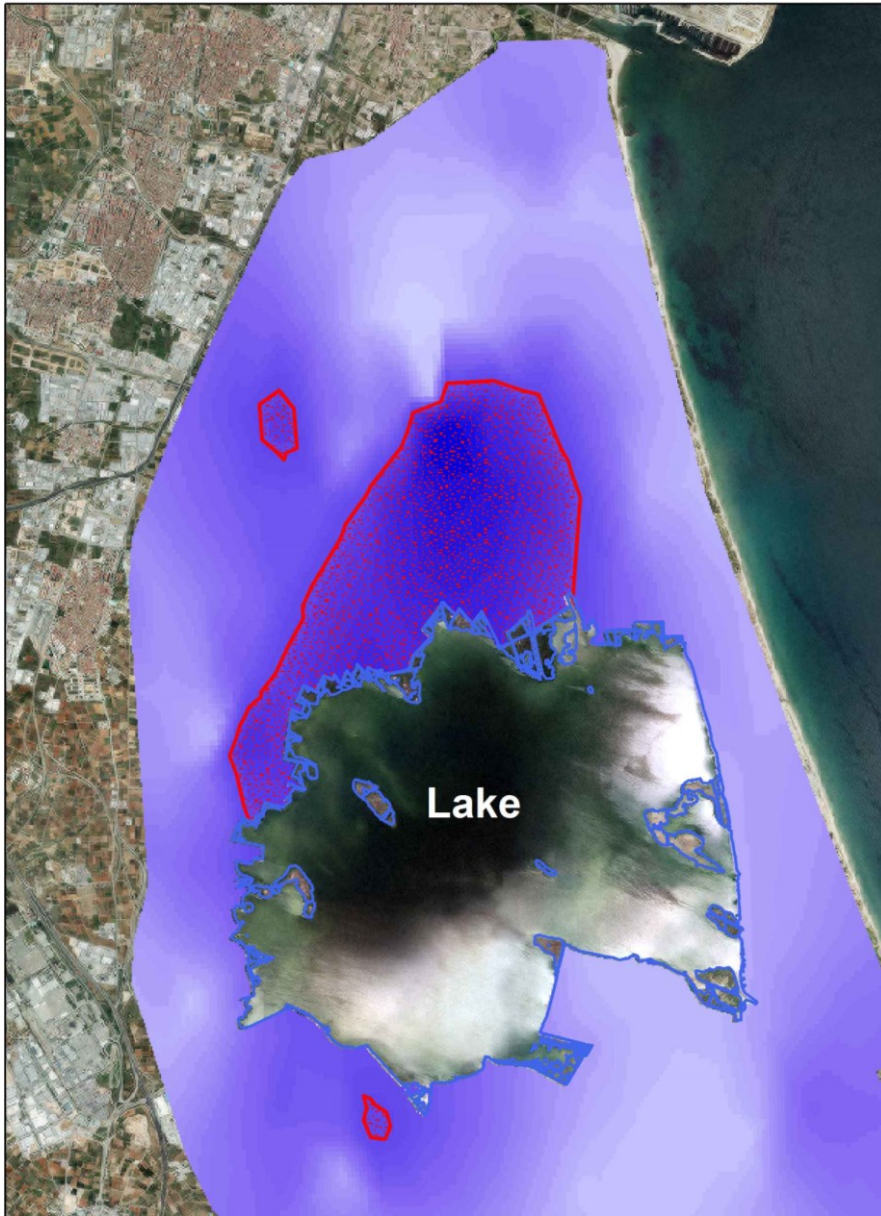Fig. 2 Hydrological cycle of rice production and the two sampling periods

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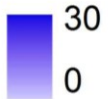**Fig. 3** Spatial representation of CYFL and ESFE A) First sampling of top soil, B) First sampling of deep soil

Lake

[Bifenthrin] (ng g^{-1})
30
0

[Bifenthrin] > 10.1 ng g^{-1}

415

Fig. 4 Representation of the area, marked in red, where BIFE levels (> 10.1 ng g^{-1}) may present a negative effect to aquatic invertebrates, together with their spatial representation.