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Environmental exposure and risk assessment of pesticide mixtures in aquatic organisms from the Tagus River Basin

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ABSTRACT

The widespread use of pesticides impacts human, animal and ecosystem health. Pesticide pollution causes significant freshwater biodiversity decline in Europe. In the present work, the occurrence of a large number of currently used pesticides (199) and mixtures was investigated in 90 samples from the fluvial environment, collected from a European emblematic watershed characterized by different land use, livestock farm and population density influences. Multiple pesticides were detected in high concentrations in all matrices (8097 ng/L, 3223 ng/g dry weight, 252 ng/g d.w., max. in water, sediment and fish, respectively). Herbicides exhibited a ubiquitous presence in water and sediment, while insecticides predominated in fish. Glyphosate and its transformation product aminomethylphosphonic acid (AMPA) reached the highest median concentrations in water and sediment, while pyrimethanil M605F002 and p,p`-DDE highlighted in fish. In addition to agricultural inputs, other anthropogenic sources (e.g., urban runoff, wastewater inputs) were found to influence pollution in rivers. The sum of all individual pesticides, metabolites and degradation products surpassed the threshold value (500 ng/L) established in the European Water Framework Directive for surface water in 11 out of 13 locations, and at least one exceedance for individual compounds was observed in all sites studied. Although only 7 % of the single pesticides were designated high risk, pesticide mixture ratios evinced potential ecological risk for aquatic and sediment-dwelling organisms. Furthermore, a total of 27 different hazardous pesticides were identified in the aquatic ecosystems.

1. Introduction

Freshwater ecosystems are among the most diverse natural environments on Earth, providing critical habitats for a wide array of species such as aquatic plants, fish, reptiles, birds and mammals. Pesticides pose a significant threat to the integrity of freshwater ecosystems because they can impact individual aquatic species, entire communities, and whole river ecosystems (Halbach et al., 2021).

Despite the sales of pesticides in the European Union have declined by 19 % since 2011, they still reached about 292000 t in 2023 (Eurostat, 2025a), with Spain being one of the main European agricultural producers and consumers of pesticides (Eurostat, 2025b). Unfortunately, less than 15 % of the applied pesticides reach their intended targets, leading to their undesirable dispersion into the environment (Singh et al., 2023). The primary route of pesticide propagation from agricultural fields and application areas is through runoff into aquatic systems, which results in a significant number of pesticides detected in European waterbodies (Casado et al., 2019; Wolfram et al., 2021; Navarro et al., 2024). In the European Union member states, the chemical quality of surface waters is regulated by the Water Framework Directive (WFD). This EU Directive defines environmental quality standards (EQS; Directive, 2013/39/EU) to safeguard and maintain an optimal ecological status. In October 2022, a proposal for a directive was released to amend previous European water legislation, broadening and updating the EQS for inland surface waters, other surface waters and biota (European Commission, 2022). Because organisms are exposed to a mixture of substances, this proposal establishes an EQS for the mixture of pesticides in surface water.

Although most of these emerging pollutants are detected at low levels, many of them raise relevant ecotoxicological concerns, particularly when they are present as components of complex mixtures. Little is known about their long-term combined effect at low environmental

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concentrations under realistic field conditions. However, there is an intrinsic exposure that strongly jeopardizes non-target organisms and humans and drives biodiversity decline (Ccanccapa et al., 2016a; Peris et al., 2022; Zaller et al., 2022; Silva et al., 2023; Wolfram et al., 2023; Feng et al., 2025; Rodríguez-Seijo et al., 2025). The impact of pesticides can be attributed mainly to their physico-chemical properties, which govern their dynamics and fate in the environment, with persistence, bioconcentration and biomagnification being the major issues involved in direct and through food chain transmission into biotic systems (Singh et al., 2023; Rodríguez-Seijo et al., 2025). Sublethal pesticide levels can negatively affect biodiversity, reproduction, physiology, and behavior (Rasmussen et al., 2013; Wijewardene et al., 2021). Cascading effects on trophic interactions, such as the loss of vegetation and phytoplankton, the decrease of aquatic invertebrate populations or disturbances in aerial insectivorous bird migratory patterns, can be induced and indirectly generate a prey reduction and habitat degradation in the aquatic ecosystem (Cavallaro et al., 2019; Lorenz et al., 2025).

Critical knowledge gaps still remain as regards the environmental fate, exposure and combined effects of pesticide mixtures. Therefore, a biomonitoring approach to characterize complex mixtures of hazardous pesticides into the receiving freshwater environment poses a challenge to establish inherent interactions related to their fate and risks to the aquatic life. Thus, the present research involves the occurrence of a mixture of pesticides (159 active substances and 40 metabolites) in fluvial ecosystems, encompassing the water, sediment and biota compartments. Their distribution, partition behavior and potential risk to non-target organisms, considering both direct exposures and those mediated through the food chain, were examined. Furthermore, a prioritization of hazardous pesticide mixtures in the aquatic ecosystem was also estimated to offer valuable perspectives into the ecological inferences of pesticide behavior. The current study represents a strong contribution to the field and advances beyond previous efforts in terms of compound coverage, multi-compartment integration, temporal scope and environmental risk estimation and prioritization methodology.

2. Material and methods

2.1. Study design

A total of 38 grab samples of surface waters, 28 sediments and 24 fish samples were collected in thirteen locations (S1-S13) along the Tagus River Basin during the period 2020–2022. Details of the sampling design, sample preservation and study area have been previously described (Royano et al., 2023, 2024). The sampling site distribution covered a range of demographic pressures, land use and livestock production typologies in order to ensure its representativeness (Table S1, Figure S1).

2.2. Chemical analysis

The present study involved 199 compounds (159 active substances and 40 metabolites: 69 fungicides, 61 herbicides, 66 insecticides and 1 synergist). Different methodologies for multi-residue, glyphosate and amino-methylphosphonic acid (AMPA) and organochlorinated pesticide (OCP) analysis were optimized and validated in each matrix. Water samples extraction was based on solid-phase extraction (SPE) (Navarro et al., 2024; Royano et al., 2023). Sediments and fish samples were extracted using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) approach for multi-residue/organochlorine pesticides and agitation and derivatization procedure followed by SPE (only in the case of fish matrix) for glyphosate/AMPA (Navarro et al., 2023). HPLC (High Performance Liquid Chromatography) analyses were performed on UHPLC-MS/MS (Ultra-High Performance Liquid Chromatography–tandem Mass Spectrometry; ExionLC Shimadzu-SCIEX Triple Quad 3500; Table S2) and GC (Gas Chromatography) analyses were conducted in a GC–MS/MS (Gas Chromatography–tandem Mass Spectrometry Agilent HRGC 8890-Agilent Triple Quad 7010 C; Table S3). See chemicals, sample preparation and instrumental analysis details at supplementary material (SM).

2.3. Quality assurance

The optimization and validation of the different analytical methodologies followed the SANTE/2020/12830 (SANTE, 2021a) and SANTE/11312/2021 (SANTE, 2021b) requirements, see SM for complete validation results. The limits of quantification (LOQs) were defined as the lowest validated level for each analyte which fulfilled recovery (70–120 %) and precision (RSDr ≤ 20 %) criteria. LOQs ranged between 1 and 114 ng/L in surface water (being 1 ng/L for 75 % of pesticides studied, Table S4), 0.2 and 23 ng/g d.w. (dry weight hereafter) in sediments (83 % ≤ 1 ng/g d.w., Table S5) and 0.2 and 50 ng/g d.w. (dry weight hereafter) in fish (71 % ≤ 1 ng/g d.w., Table S6). The limit of detection (LOD) was calculated as the concentration giving a signal-to-noise ratio (S/N) of 3 at the lowest validated level (LOQ) in matrix. LODs ranged between 4 pg/L (o,p'-DDD) and 2 ng/L (quizalofop-P) in water (Table S4), 4 pg/g d.w. (hexachlorobenzene) and 3 ng/g d.w. (dicamba) in sediment (Table S5) and 6 pg/g d.w. (pymetrozine) and 7 ng/g d.w. (chlorothalonil) in fish (Table S6). Procedural and instrumental blanks were analyzed to check the possibility of cross-contamination.

2.4. Calculations and statistical evaluation

2.4.1. Calculation of mass flow rate

Mass flow rates (F) of individual compounds (kg/y) were calculated to reflect the discharge of pesticides in surface water considering the pesticide concentration detected in surface water (in kg/m^3) and the mean daily flow river (in m^3/y) measured at the time of sampling campaigns in each location (Table S1) (Navarro et al., 2020; see SM for details).

2.4.2. Calculations of partition coefficients and BAFs

Partition coefficient (Log K_d) between water and sediment compartments was estimated as the ratio of median concentrations of sediment (μg/kg d.w.) and water (μg/L) measured at the same sampling points. Bioaccumulation factors (BAFs) were determined as the ratio fish concentration (μg/kg d.w.)/water concentration (μg/L) (Castaño-Ortiz et al., 2024; see further details at SM).

2.4.3. Environmental risk assessment calculations

The environmental risk assessment was performed based on concentration addition (CA) and independent action (IA) concepts (Backhaus and Faust, 2012). The two approaches allowed to provide a more conservative (CA; RQ_{mix}) and a higher-end (IA; RQ_{STU}) estimation of toxicity ecological risk:

i) Risk chronic quotients were calculated to estimate the potential ecological risk of pesticides to water-column organisms (RQ_{water}, e.g., algae, pelagic invertebrates, fish), sediment-dwelling aquatic organisms (RQ_{sed}, e.g., benthic macroinvertebrates) and fish-eating predators (RQ_{oral}, e.g., birds, mammals) at general (RQ_{50}) and worst (RQ_{max}) scenarios (Eq. 1; European Commission, 2003; Navarro et al., 2024; Rodríguez-Seijo et al., 2025).

$$RQ_{50\ or\ max} = \frac{MEC_{50\ or\ max}}{PNEC} \tag{1}$$

MEC was the measured environmental concentration (MEC_{50}, median; MEC_{max}, maximum) of pesticides in each compartment (water, sediment and fish) and PNEC (predicted no effect concentration) was obtained from the available long-term toxicity data (no-observed effect concentration, NOEC; Table S7; PPDB, 2025) divided by an assessment factor (AF).

Risk quotients for mixtures (RQ_{mix}) of pesticides were also calculated in each compartment (Eq. 2).

$$RQ_{mix\_50\ or\ max} = \sum \frac{MEC_{50\ or\ max\_i}}{PNEC_i}$$

ii) Eq. (2)Toxic units (TU) and the sum of toxic units (STU) for each trophic level (algae, invertebrates and fish) are used to estimate the combined acute risk (RQ_{STU}) in the water compartment (Eqs. 3 and 4):

$$TU_i = \frac{MEC_{50\ or\ max\_i}}{EC50(LC50)_i} \tag{3}$$

Where the most sensitive acute toxicity values (median lethal, LC50, and median effective, EC50, concentrations; Table S7; PPDB, 2025) were considered for the organisms groups of algae, invertebrates and fish.

The total toxicity of the pesticide mixture (RQ_{STU}) was based on the sum of toxic units (STU) for the most sensitive trophic level (algae, invertebrates or fish) applying an assessment factor of 1000 (Casillas et al., 2022; Rocha and Rocha, 2023).

$$RQ_{STU} = \max\left(STU_{algae}, STU_{invert}, STU_{fish}\right) \times AF \tag{4}$$

Complete details related to environmental risk assessment are included in SM.

2.4.4. Prioritization of hazardous pesticides

A selection of the top three pesticides of concern for aquatic and benthic organisms and fish-eating predators was established based on a score value calculated according to the methodology outlined in Abrantes et al. (2024). The score was calculated by multiplying the risk quotient ($RQ_{water\ 50}$, $RQ_{sed\ 50}$ and $RQ_{oral\ 50}$) and the detection frequency for each analyte.

2.4.5. Statistical calculations

Statistical analyses were performed with the software SPSS 14.0 and Statgraphics Centurion XVII.I for Windows. Kruskal-Wallis and U-Mann Whitney Tests were conducted to evaluate differences between groups with regard to sampling points, compounds (with detection frequency, Df,>30 %), compartments, land use influence and other relevant factors. Associations between compounds (with Df>30 %) were assessed by Spearman Rho correlations. Principal Component Analysis (PCA) was accomplished to establish relationships between the content of pesticides and their distribution. In this PCA, values < LOD were replaced by the LOD divided by the square root of 2 (De la Torre et al., 2020).

3. Results and discussion

3.1. Occurrence of pesticides in the aquatic ecosystem

A total of 168 out of 199 pesticides (97 approved and 54 non-approved, PPP Status EC1107/2009, and 17 metabolites; Table S7) were detected in 90 aquatic ecosystem samples. The highest number of analytes were identified in water (n = 127) followed by sediment (n = 103) and fish (n = 76) (Table S8). A widespread distribution (4–94 pesticides/sample; min-max) highlighted the omnipresence of these hazardous compounds in the environment studied (Fig. 1). No temporal trends ($p > 0.05$) in all three years of sampling were observed in the different compartments assessed (Figure S2), denoting that sampling period and rainfall or flow conditions were probably similar and did not influence in the pesticide concentrations.

3.1.1. Surface water

A large number of compounds (127 out of 199) with 70 substances per sample (median) were detected in surface water. The total amount of pesticides (∑Pesticides: sum of 42 fungicides, 48 herbicides, 36 insecticides and 1 synergist) reached relatively high levels (6.95 ng/L and 8097 ng/L, min-max; 2986 ng/L, median; Table S8). Herbicides (2677 ng/L, median total concentration) showed a high predominance

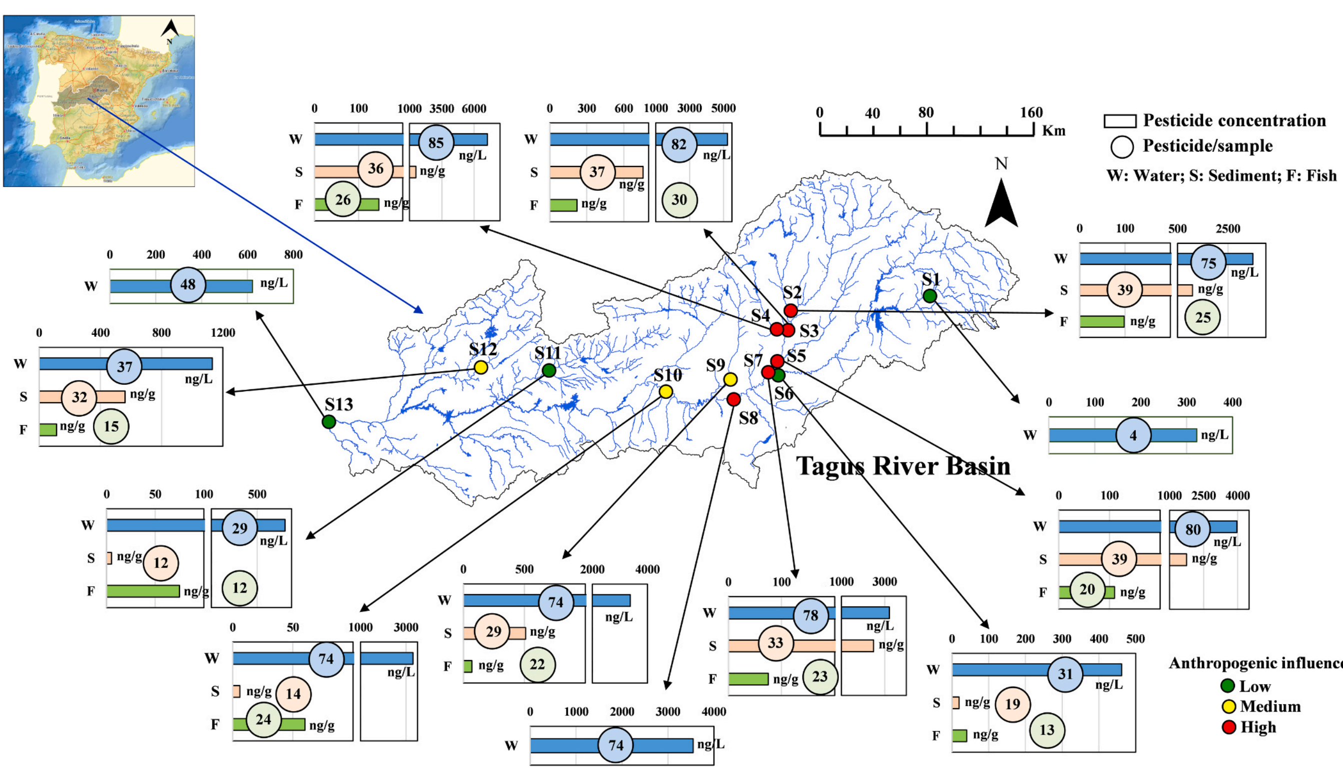

Fig. 1. Distribution of pesticides in the aquatic environment. Details of median concentration in water (ng/L), sediment (ng/g d.w.) and fish (ng/g d.w.) and median number of pesticides per sample.

($p < 0.01$) compared to insecticides (119 ng/L, median) and fungicides (92.3 ng/L, median) (Figure S2, Table S8), tendency also observed in other European river surface waters (Casado et al., 2019; Navarro et al., 2024). As shown in Figure S2, this result was consistent across all three years of sampling. Herbicides are the most widely used pesticides, accounting for 81 % of the total amount applied to Spanish crops, including olives, wheat and barley (González García et al., 2022).

There were notable statistical differences between the individual compounds (Table S9). The transformation product of glyphosate, AMPA, reached the highest concentration (2136, 53.8–6648 ng/L; median, min-max) followed by glyphosate (108, 7.65–332 ng/L), metolachlor ESA (105, 2.03–464 ng/L), and to a lesser extent imidacloprid (48.2, 0.19–226 ng/L), mecoprop-P, (31.0, 1.63–294 ng/L) and azoxystrobin o desmethyl (29.6, 1.90–484 ng/L). Although most of pesticides presented low detection frequency (Df; 59 % with Df>30 %), AMPA and the insecticide imidacloprid were the most ubiquitous compounds (97 %, Df) followed closely by 2,4-D, acetamiprid, azoxystrobin, carbendazim, fluopyram and metolachlor-S (92 %) (Table 1). Notably, imidacloprid was found in nearly all water samples despite its unauthorized use as plant protection product (PPP) in the EU since 2020 (European Commission, 2020; MAPA, 2025; PPDB, 2025; Table S7). However, imidacloprid is currently authorized as biocide and veterinary medicinal formulations (CIMAVET, 2025; ECHA, 2025; MISAN, 2025; Table S7), which might explain its high occurrence and underline these uses as the main source of contamination to the aquatic environment (De la Casa-Resino et al., 2022). Interestingly, its metabolites imidacloprid 5 hydroxy (3.96, 1.40–11.4 ng/L, 21 %; median, min-max, Df) and imidacloprid desnitro (4.54, 0.46–47.9 ng/L, 82 %) were also detected at lower concentrations. At this point, it is remarkable that metabolites and degradation products such as AMPA, metolachlor ESA and azoxystrobin o desmethyl, correlated with their respective parent compounds (glyphosate, metolachlor, azoxystrobin; r_s>0.6; $p < 0.01$, Table S10) and exhibited higher median concentrations than those (Table S8). The high predominance and concentrations of these metabolites have been previously observed in waterbodies (Feng et al., 2025; Konečná et al., 2023; Slaby et al., 2023). Glyphosate and 2,4-D are the most widely used herbicides in Spain and commonly applied on olives, grapes, sunflower, wheat and barley (González García et al., 2022); some of these crops are cultivated in the surroundings of the watershed studied (Royano et al., 2023). Glyphosate is also used in forestry and for rail and road infrastructure maintenance, therefore these sources cannot be ruled out (MITERD, 2023).

3.1.2. Sediment

A total of 103 pesticides and up to 44 substances per sample (34 pesticides/sample, median) were found in sediments. Similar to water, herbicides (611 ng/g d.w., dry weight hereafter; median) dominated over insecticides (47.0 ng/g d.w.) and fungicides (7.84 ng/g d.w.) (Table S8, Figure S2), as other studies also reported (Peris et al., 2022). High concentrations were reached (3223 ng/g d.w., max; 753 ng/g d.w., median) due to the significant ($p < 0.05$) contribution of AMPA (779 ng/g d.w., median), glyphosate (59.6 ng/g d.w.), permethrin (36.0 ng/g d.w.) and o,p'-DDT (21.8 ng/g d.w.) (Table S8). The prevalence of glyphosate and AMPA have been previously identified in sediments (Fantón et al., 2021; Konečná et al., 2023; Lajmanovich et al., 2023). In spite of the very low levels of imidacloprid, (0.26, 0.07–1.14 ng/g d.w.; median, min-max), it was the most commonly encountered (100 %, Df), as happened in water, followed by p,p'-DDE, permethrin, terbutryn (86 %) and pendimethalin, piperonyl butoxide and terbuthylazine (82 %). Important differences ($p < 0.05$; Df > 30 %) were observed for acetamiprid (0.15 ng/g d.w., 39 %; median, Df), carbendazim (0.15 ng/g d.w., 64 %), fipronil sulfone (0.14 ng/g d.w., 79 %), hexachlorobenzene (0.10 ng/g d.w., 79 %), imidacloprid (0.26 ng/g d.w., 100 %), prometryn (0.31 ng/g d.w., 75 %), pyriproxyfen (0.14 ng/g d.w., 32 %) and terbuthylazine (0.24 ng/g d.w., 82 %) whose concentrations were lower than the rest of compounds (Table S11). In general, results were in accordance with other studies that frequently quantified DDTs and their metabolites, neonicotinoids and pyrethroids in sediments (Peris et al., 2022). Sediments are the major sink of organic pollutants in aquatic systems, enabling the identification of historical sources and exposure pathways. However, retained contaminants can be resuspended in the water column and transferred through the aquatic food chain (Pizzini et al., 2021; Peris et al., 2022). Interesting correlations were observed between chemical families, such as organochlorines (r_s>538, $p < 0.05$), pyrethroids (r_s>552, $p < 0.05$), triazines (r_s=951, $p < 0.01$) and triazoles (r_s>507, $p < 0.05$), metabolites (glyphosate/AMPA, imidacloprid/imidacloprid desnitro or DDT/DDE/DDD; r_s>566, $p < 0.05$) and application synergies (piperonyl butoxide/pyrethroids r_s=841, $p < 0.01$) (Table S12).

3.1.3. Fish

Lower number of compounds (22 pesticides/sample, median) and concentrations (85.2 ng/g d.w., 23.4–252 ng/g d.w.; dry weight hereafter, median, min-max) were detected in fish samples (Table S8). Although no significant differences were observed among types of pesticide, insecticide total concentration was slightly higher (32.1 ng/g d.w., median) compared to herbicides (20.6 ng/g d.w.) and fungicides (9.94 ng/g d.w.).

Interestingly, several metabolites presented high concentrations such as pyrimethanil M605F002 (27.1 ng/g d.w., 8 %; median, Df), p,p'-DDE (18.8 ng/g d.w., 79 %) and metalaxyl CGA 62826 (14.6 ng/g d.w., 25 %) as well as the pyrethroids lambda-cyhalothrin (23.4 ng/g d.w., 4 %) and esfenvalerate (13.3 ng/g d.w., 8 %), and oxyfluorfen (11.0 ng/g d.w., 50 %), pendimethalin (10.4 ng/g d.w., 88 %) and tetraconazole (9.95 ng/g d.w., 4 %). Concretely, p,p'-DDE, and fenhexamid (6.40 ng/g d.w., 79 % Df) stood out for their high significant ($p < 0.05$) values (Table S13). However, others frequently occurred were found in very low amounts such as 2,4-D (100 %, 1.31 ng/g d.w.; Df, median), trifloxystrobin CGA 321113 (92 %, 1.67 ng/g d.w.) and prosulfocarb (83 %, 2.53 ng/g d.w.). Neonicotinoids, such as acetamiprid and imidacloprid that correlated with each other (Table S14), and fipronil and fludioxonil were also quantified in statistically ($p < 0.05$) low levels (<0.30 ng/g d.w.; Df<79 %) (Table S8 and S13). Pyrethroid concentrations were aligned with previous values obtained in muscle and viscera of fish (Lajmanovich et al., 2023). A high dominance of p,p'-DDE (3.20–215 ng/g w.w., wet weight, 68 %; min-max, Df) has been also reported in fish (Eqani et al., 2013). Although DDT use is no longer approved, the notable presence of its metabolites in fish and sediment samples, where the contribution of o,p'-DDT was also significant, demonstrates its persistence and bioaccumulation in the environment (Table S7).

3.1.4. Relationship of pesticides among aquatic compartments

The pesticide composition differed among the three aquatic compartments studied due to their specific physico-chemical properties (Figure 2 and S3), however, a total of 38 compounds (19 % of pesticides analyzed) were simultaneously detected in all matrices. Imidacloprid, fludioxonil, terbutryn and piperonyl butoxide were among the first 20 analytes with the highest contribution and Df > 30 % in the three matrices (Fig. 2). Astonishingly, several pesticides simultaneously detected correlated significantly (Table S15), highlighting their possible common origin and dynamic transfer among the different aquatic compartments. This is the case of DDT metabolites (r_s>0.667, $p < 0.05$), difenoconazole (r_s>0.900, $p < 0.01$), piperonyl butoxide (r_s=-0.561, $p < 0.05$), azoxystrobin (r_s>-0.574, $p < 0.05$), pendimethalin (r_s>-0.591, $p < 0.05$), diflufenican (r_s=-0.629. $p < 0.05$), fipronil (r_s>-0.691, $p < 0.05$), fenhexamid (r_s>-0.900, $p < 0.01$) and propoxur (r_s>-0.900, $p < 0.01$).

In general, highly soluble compounds with low organic carbon-water partition coefficients (Log K_{oc}) and low potential particle bound transport indexes (PPBT) (Table S7) such as neonicotinoids, some carbamates (propamocarb and propoxur), dimethoate, MCPA, mecoprop-P,

Table 1
Detection frequency (%) of pesticides in the aquatic environment at the Tagus basin.

FUNGICIDES	W	S	F
Ametoctradin	-	7	-
Azoxystrobin	92	68	13
Azoxystrobin O desmethyl	87	-	-
Boscalid	87	54	13
Carbendazim	92	64	-
Chlorothalonil 4 hydroxy	32	-	-
Cymoxanil	11	-	-
Cyproconazole	45	-	-
Cyprodinil	-	4	4
Difenoconazole	42	36	4
Dimethomorph	61	7	-
Dimoxystrobin	-	4	-
Epoxiconazole	8	4	-
Fenbuconazole	-	4	-
Fenhexamid	16	11	79
Fenpropidin	21	54	38
Fenpropimorph	-	-	4
Fludioxonil	74	71	79
Fluopicolide	24	-	8
Fluopyram	92	21	-
Fluopyram benzamide	71	-	-
Fluoxastrobin	-	4	-
Flutolanil	21	11	-
Fluxapyroxad	66	4	4
Hexachlorobenzene	-	79	58
Imazalil	18	50	-
Kresoxim methyl	3	4	-
Mandipropamid	5	21	-
Meptyldinocap phenol	11	-	-
Metalaxyl-M	76	11	-
Metalaxyl CGA 62826	76	-	25
Metconazole	-	-	4
Metrafenone	8	4	8
Myclobutanil	45	25	-
Penconazole	21	11	4
Pencycuron	-	7	4
Prochloraz	55	54	-
Prochloraz BTS 44595	68	-	-
Prochloraz BTS 44596	18	-	8
Propamocarb	66	-	-
Propiconazole	74	71	8
Prothioconazole desthio	13	11	8
Pyraclostrobin	8	7	13
Pyrimethanil	63	4	-
Pyrimethanil M605F002	18	-	8
Pyriofenone	-	-	4
Quinoxyfen	-	7	-
Spiroxamine	-	-	4
Tebuconazole	89	79	29
Tetraconazole	39	11	4
Tolylfluanid DMST	58	-	-
Tricyclazole	58	7	-
Trifloxystrobin	3	11	13
Trifloxystrobin CGA 321113	74	29	92
Zoxamide	-	-	17

SYNERGIST	W	S	F
Piperonyl butoxide	82	82	96

HERBICIDES	W	S	F
2,4-D	92	-	100
AMPA	97	68	-
Atrazine	61	7	-
Bentazone	74	7	-
Bromoxynil	32	-	8
Carfentrazone	21	-	-
Carfentrazone ethyl	-	4	-
Chloridazon	18	-	4
Chlorpropham	16	-	-
Chlorotoluron	79	39	-
Clomazone	32	7	-
Diflufenican	63	68	71
Diflufenican AE-B107137	63	4	-
Dimethenamid-P	11	-	-
Diuron	84	61	38
Ethofumesate	61	18	-
Flazasulfuron	8	-	-
Florasulam	21	-	-
Fluazifop-P	11	4	-
Flufenacet	16	-	4
Flumioxazine	-	4	-
Fluroxypyr	34	-	-
Foramsulfuron	13	4	-
Glyphosate	89	64	-
Haloxyfop-P	55	-	-
Isoproturon	74	25	-
Isoxaben	-	4	-
Lenacil	26	-	-
Linuron	26	-	8
MCPA	79	-	-
Mecoprop-P	68	-	-
Metamitron	29	-	4
Metamitron desamino	61	4	13
Metazachlor	58	-	-
Methabenzthiazuron	5	-	-
Metobromuron	11	7	-
Metolachlor-S	92	7	17
Metolachlor ESA	61	-	-
Metolachlor OA	87	-	-
Metribuzin	39	-	8
Metsulfuron methyl	16	-	-
Napropamide-M	8	-	4
Nicosulfuron	68	-	-
Oryzalin	-	4	-
Oxyfluorfen	-	-	50
Pendimethalin	5	82	88
Prometryn	-	75	63
Propaquizafop	-	-	4
Propyzamide	29	4	-
Prosulfocarb	68	71	83
Pyraflufen ethyl	-	18	4
Pyroxsulam	-	4	-
Quizalofop-P	16	21	21
Rimsulfuron	-	4	-
Terbuthylazine	87	82	-
Terbuthylazine desethyl	87	4	8
Terbutryn	82	86	79
Thiencarbazone methyl	18	-	4

INSECTICIDES	W	S	F
Acetamiprid	92	39	46
Acetamiprid N desmethyl	79	11	4
Bifenthrin	-	32	4
Chlorantraniliprole	71	4	-
Chlorpyrifos	32	79	54
Chlorpyrifos methyl	-	-	4
Chlorpyrifos methyl TCPy	37	-	21
Clothianidin	29	4	-
Cypermethrin	-	61	25
o,p'-DDD	5	71	54
p,p'-DDD	5	71	67
o,p'-DDE	-	4	50
p,p'-DDE	3	86	79
o,p'-DDT	-	32	-
p,p'-DDT	-	36	-
Deltamethrin	-	57	-
Dieldrin	5	7	4
Dimethoate	68	-	-
Dinotefuran	68	-	-
Emamectin	-	4	-
Esfenvalerate	8	-	8
Fipronil	71	25	63
Fipronil sulfone	61	79	54
Flonicamid	42	-	-
Flupyradifurone	76	4	-
Imidacloprid	97	100	71
Imidacloprid 5 hydroxy	21	-	-
Imidacloprid desnitro	82	61	-
Indoxacarb	8	-	-
Lambda-Cyhalothrin	-	21	4
Lindane (gamma-HCH)	18	4	-
Methiocarb sulfon	-	-	25
Methoxyfenozide	26	-	-
Permethrin	11	86	17
Phosmet	-	7	-
Phoxim	-	4	13
Pirimicarb	74	-	-
Pirimicarb desmethyl	24	-	-
Pirimiphos methyl	-	29	17
Pirimiphos methyl DEAMPY	61	-	-
Pirimiphos methyl desmethyl	55	-	-
Propoxur	63	14	8
Pymetrozine	5	-	-
Pyrethrin I	3	-	-
Pyriproxyfen	-	32	4
Spinetoram	-	11	-
Spinosyn A	21	11	-
Spinosyn D	-	11	-
Spirotetramat enol	74	-	-
Spirotetramat keto hydroxy	39	4	-
Spirotetramat mono hydroxy	-	-	4
Tau-Fluvalinate	-	4	-
Thiacloprid	61	-	4
Thiamethoxam	84	-	-

	W	S	F
∑PESTICIDES	63	51	38

W: water; S: sediment; F: fish. "-": Not detected. Mean detection frequency (%) was shown.

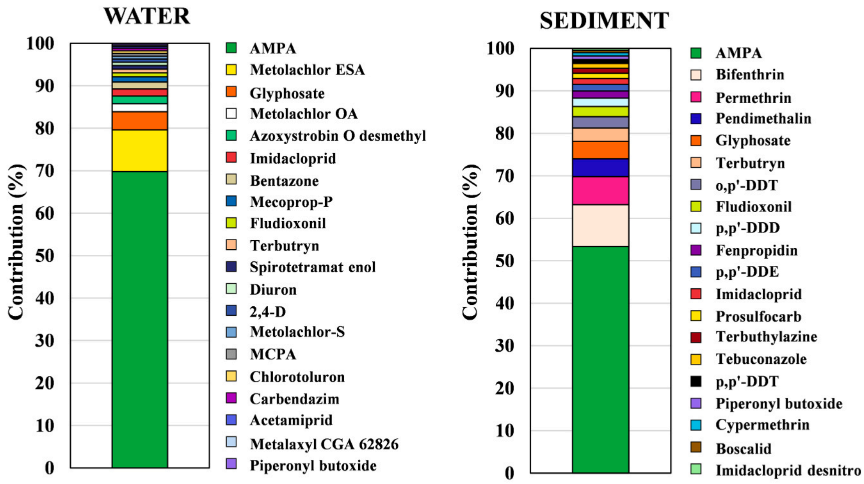

Fig. 2. Contribution (%) of each pesticide to the total in water, sediment and fish. Only the first 20 pesticides with higher contribution and Df > 30 % in each matrix are shown.

metalaxyl-M and metolachlor metabolites (ESA and OA) remained preferably in the aqueous phase (Table S8). In contrast, pyrethroids and organochlorines with higher hydrophobicity, sorption affinity and bioaccumulation (Log $K_{oc} \geq 4$, Log $K_{ow} \geq 5$; Table S7) were more prominent in sediments and biota. Up to 50 compounds were detected in these two matrices, highlighting that 25 % of the target pesticides may accumulate in sediments and fish and cause risks to the aquatic life.

Sediment-water partition coefficients (Log K_d) were calculated for 57 compounds (Table S16). The lower the value of the coefficient, the higher the concentration of the pesticide in water. Then, the sediment-water exchange of pesticides can be classified as low (Log $K_d < 2$) and high (Log $K_d > 2$) (FAO, 2000; Castaño-Ortiz et al., 2024). The highest values (Log $K_d > 3.6$) obtained for pp'-DDE, p,p'-DDD, o,p'-DDD, permethrin, fenpropidin, difenoconazole corroborated their more readily adsorbable behavior. As expected, compounds with high solubility such as bentazone, carbendazim, acetamiprid, acetamiprid N desmethyl and imidacloprid presented the lowest values (Log $K_d < 0.9$). Results were close to values obtained in previous studies in the Tagus river (Log $K_d \approx 2.5–4.4$, calculated for suspended particle matter, Cruzeiro et al., 2016). Similarly, bioaccumulation factors were estimated for 37 pesticides (Table S17). The values obtained for p,p,'-DDE (BAF > 80000 L/kg), o,p′-DDD (> 9000 L/kg) and pendimethalin (> 15000 L/kg) higher than 5000 demonstrated their bioaccumulative behavior (Arnot and Gobas, 2006).

3.1.5. Spatial distribution in the study area

The geographic distribution of pesticides was firstly explored considering possible sources such as land use, livestock farms, wastewater treatment plant (WWTP), and population density influence (Table S18). Interestingly, sites located near WWTP facilities and more densely populated revealed statistically ($p < 0.05$) higher concentrations. Contamination of the freshwater ecosystem by pesticides in urban areas cannot be neglected. In addition, these areas exhibited higher impact on levels than agricultural ones, denoting the urban applications as one of the main sources of pesticides in the river course studied (Table S18). Generally, runoff water from agricultural areas used to be the major origin of pesticides in the aquatic systems. However, these results suggest that wastewaters related to urban areas may also be one of the main pathways of pesticide contamination in the environment. The presence of these compounds in urban WWTPs is primarily associated to non-agricultural applications, such as the management of industrial vegetation and grass (in parks, gardens, on roadways, pipelines, etc.), public health and safety measures (in areas where mosquitoes or rodents are controlled), or commercial forestry and horticulture (Köck-Schulmeyer et al., 2013). Previous studies concluded that biocide and pesticide input from urban sources was as high as agricultural one (Wittmer et al., 2010, 2011). Apparently, livestock farms closeness did not affect the pesticide distribution.

Spatially, the maximum concentrations were observed at site S4 (7035 ng/L, ∑Pesticides, median) for water, S7 for sediment (2486 ng/g d.w.) and S3 for fish (221 ng/g d.w.) (Figure S4, Table S19), all of them with a notable industrial and urban influence. Areas characterized by an important demographic weight showed statistically higher levels. On the contrary, more remote locations and lowly impacted appeared less polluted by pesticides. The lowest levels were obtained at S1 for water (322 ng/L), S10 for sediment (5.91 ng/g d.w.) and S6 for fish (39.8 ng/g d.w.).

Mass flow rates of pesticides in the Tagus watershed ranged between 0.44 and 5981 kg/y (Table S20). Similarly to the tendency related to concentrations, urban locations (i.e. S8 > S3 > S7 > S10 > S5) presented higher pesticide discharge. The annual pesticide loads from the Tagus river were estimated to be 816 kg (median; 816 kg in 2020, 1407 kg in 2021 and 1152 kg in 2022). These values are in accordance with pesticide loads reported in other Mediterranean rivers (Ccanccapa et al., 2016a). This estimate corresponds to the autumn period, when pesticide release is usually lower than in spring or application period (Ccanccapa et al., 2016b, Szöcs et al., 2017; Vormeier et al., 2023). However, the indirect and long-term effects of pesticide constant presence in the aquatic ecosystem can affect the habitat quality and biodiversity (Lorenz et al., 2025). The contribution of the individual pesticides to the three matrices revealed a similar profile at almost all locations (Figure S5). In general, AMPA was the predominant compound in water although metolachlor ESA and chlorpyrifos increased in importance at S11 and S1, respectively, denoting a possible different agricultural input. The high prevalence of some metabolites such as AMPA, metolachlor ESA and azoxystrobin o desmethyl exhibited metabolite/parent ratios higher than 1 (16.8, 19.4 and 5.95, median, respectively). Remarkably, lower values were found in rural and agricultural locations for AMPA/glyphosate and azoxystrobin o desmethyl/azoxystrobin ratios compared to urban areas, suggesting fresh pesticide input due to possible runoff from the fields. However, the imidacloprid desnitro/imidacloprid ratios (0.03–0.66, min-max) were lower in urban sites, denoting the major biocide use of imidacloprid in these areas. On the other hand, chlorpyrifos has been recently listed under Stockholm Convention (POP, 2025) and although its PPP use was banned since 2020 in Spain and the EU (Table S7; MAPA, 2025), low levels can still be detected in the sediment (79 %, Df) and fish (54 %) samples (Table S8 and Figure S3). The AMPA prevalence was also observed in sediment excluding four locations with low urban impact (S6, S9, S10 and S11), where pyrethroids highly contributed. Although slight variations were noted in the fish matrix, p,p'-DDE stood out as the main compound in almost all samples.

Relationships between the content of pesticides and their distribution in the aquatic ecosystem were also explored by principal component analysis (PCA) (Fig. 3; Table S21). Two principal components (PC) depicted 81 % of the variance. The first component (PC1) explained 60 % of the variance and was determined by the total concentration of pesticides, followed by herbicides, sum of glyphosate and AMPA, insecticides and fungicides. The second component (PC2, 21 %) was mainly defined by organochlorines and pyrethroids (pesticides with low solubility in water). As shown in the score plots (Figs. 3a and 3b), the distribution of the different aquatic samples revealed higher pollutant levels with influence in PC1 in the water compartment compared to sediment and fish that were scattered in the negative side of this component. On the other hand, water samples were distributed in the negative side of PC2, indicating a lower content of these two families. Possible source associations were also examined (Figure 3d, 3e and S6, Table S18). The anthropogenic influence was mainly observed at sites with low pressure, showing significantly lower ($p < 0.05$) levels (PC1 and 2; Fig. 3d, Table S18). Similarly, land use related to agriculture (Figure S3e) reflected statistically ($p < 0.05$) lower pollutant values than urban use (Table S18). The score plot distribution related to each sampling site revealed that samples from low anthropogenic areas (S1, S6, S11 and S13) presented lower levels for total concentration of pesticides, herbicides, glyphosate, AMPA, insecticides and fungicides compared to those with medium or high demographic pressure (S8 and S10) (Fig. 3c). Significant differences ($p < 0.05$) observed in some cases corroborated these results (Table S22). Likewise, the score plot also reflected lower levels for organochlorines and pyrethroids at low urban impacted areas (S1, S6, S11 and S13) than high ones (S2 and S7).

3.2. Compliance with EQS

Only 16 out of 128 pesticides (13 %) detected in water in the present study have an environmental quality standard (EQS) established in the Water Framework Directive (WFD) (Table S23). In the case of sediments and biota, there are currently no EQS for pesticides. The compliance with the threshold values established for inland water (AA-EQS: Annual Average Environmental Quality Standard; MAC-EQS: Maximum Allowable Concentration Environmental Quality Standard) has been explored for each location studied (Table S24). Surprisingly, total concentration (sum of 128 pesticides; 2778; 6.95–8097 ng/L; mean, min-max) surpassed 5-fold the AA-EQS (500 ng/L) for the sum of all individual pesticides, metabolites and degradation products (European Commission, 2022), revealing a potential negative ecological impact and risk for the aquatic organisms. Only two sampling sites (S1 and S6) remained below this reference value 243 ng/L and 495 ng/L, respectively, mean). However, all sites presented at least one exceedance of the EQS for individual compounds, with acetamiprid, chlorpyrifos, diuron, esfenvalerate, glyphosate, imidacloprid, nicosulfuron, permethrin and terbutryn exceeding the thresholds most frequently (Figure S7). Three of them (chlorpyrifos, diuron and terbutryn) were already banned for all uses when the sampling was carried out, and other two (imidacloprid and permethrin) were only approved as biocide or veterinary medicinal products (Table S7). In 2020, imidacloprid showed the highest absolute number of exceedances of effect thresholds in Europe's surface waters (EEA, 2023). Previous studies have also reported levels exceeding reference values for neonicotinoids, pyrethroids, chlorpyrifos, nicosulfuron and terbutryn (Cruzeiro et al., 2017; Vormeier et al., 2023; Navarro et al., 2024; Lorenz, et al., 2025). It is remarkable that AMPA, glyphosate and imidacloprid also exceeded national threshold values established for surface waters in Spain (Table S23; MITERD, 2021).

3.3. Environmental risk assessment in the aquatic ecosystem

Environmental risk assessment was estimated covering direct exposure of organisms and exposure via the food chain for predators to provide an overview of the potential pesticide chronic risk (Tables S25-27). Ratios based on water concentrations for individual pesticides (RQ_{water}) indicated low risk (RQs < 0.1) in almost all cases (Table S25). However, ratios for mixtures (RQ_{mix}) denoted medium ($0.1 < RQ < 1$) or high ($RQ > 1$) risk for aquatic organisms both at general and worst scenarios in most sites studied (Table S25). In general, locations with low anthropogenic influence (S1, S6, S11 and S13) remained at low risk. Although EQS exceedance was observed for several individual pesticides (Table S24), only esfenvalerate and terbutryn revealed high risk. Esfenvalerate has been identified as highly toxic to fish inducing physiological disturbances and behavioral changes as hypoactivity (Wang, et al., 2020; Navruz et al., 2023). In the case of terbutryn, embryonic development toxicity, including mortality, reduced body length and eye size and yolk sac edema, has been reported in early life stages of fishes (Min et al., 2023). Interestingly, potential risk was also estimated for carbendazim, fipronil sulfone and fludioxonil whose concentrations were below the ecological thresholds and considered safe for aquatic organisms. These findings arouse concern due to the bioaccumulation capacity of these five pesticides in fish at low levels (0.15–13.3 ng/g d. w., 8–79 %, median, Df; Table S8). Furthermore, several studies have demonstrated that carbendazim and fipronil sulfone exposure induces developmental and biochemical disturbance in fish (Andrade et al., 2016; Kim and Lee, 2023) while fludioxonil affects the growth, morphology and metabolism of microalgae (Liu et al., 2022). Significant risk for pyrethroids, fipronil sulfone and terbutryn has been previously pointed out in small water bodies related to agricultural fields on European scale (Navarro et al., 2024). Ecological risk for benthic organisms (RQ_{sed}) was mainly dominated by insecticides (Table S26). As can be expected, carbendazim, fipronil sulfone and terbutryn also exhibited risk for sediment-dwelling organisms. Some studies reveal the toxicity effect of these pesticides on sediment-dwelling macroinvertebrates community (Cuppen, et al., 2000; Brust et al., 2001; Maul et al., 2008). Higher number of individual pesticides presented medium or high risk in sediment compartment (36 %) compared to the aqueous phase (10 %). Sediments act as a pollution source due to the fact that pesticides can be deposited and retained in them and bioaccumulate in sediment-dwelling organisms, originating possible negative ecological impacts and risk among the food chain (Peris et al., 2022). These results evidence the need to include this compartment in the monitoring schemes. Negligible risk ($RQ_{oral}<0.01$) for fish-eating predators (birds or mammals) for single compounds or mixtures was observed (Table S27), which reveals a higher risk for organisms with a direct exposure to pesticides.

Ecotoxicological assessment was also performed separately for three taxonomic group receptors (algae, invertebrates, and fish) to consider the combined acute effects from water compartment (Table S28). This approach revealed high acute risk ($RQ_{STU}>1$) for aquatic organisms in almost all locations studied. As can be expected, herbicides showed higher influence on algae taxonomic level while insecticides impacted mainly invertebrate and fish groups, tendency in line with previous studies (Vormeier et al., 2023; Wolfram et al., 2023). Little is known about the multiple effects of pesticide cocktail, but results underlined greater potential acute (RQ_{STU}) and chronic (RQ_{mix}) risks compared to the single compounds. While RQ_{mix} values represent a more conservative and protective approach based on the most sensitive taxonomic group, RQ_{STU} values reflect biological interpretation focused on the separate taxons that are used as receptors.

The main factor of limitation and uncertainty of the estimation is the lack of ecotoxicological information related to some pesticides and their metabolites or degradation products (Table S7). Nevertheless, the two concepts applied integrate a pragmatic risk assessment to provide valuable information on the widespread pesticide cocktail in the aquatic ecosystem.

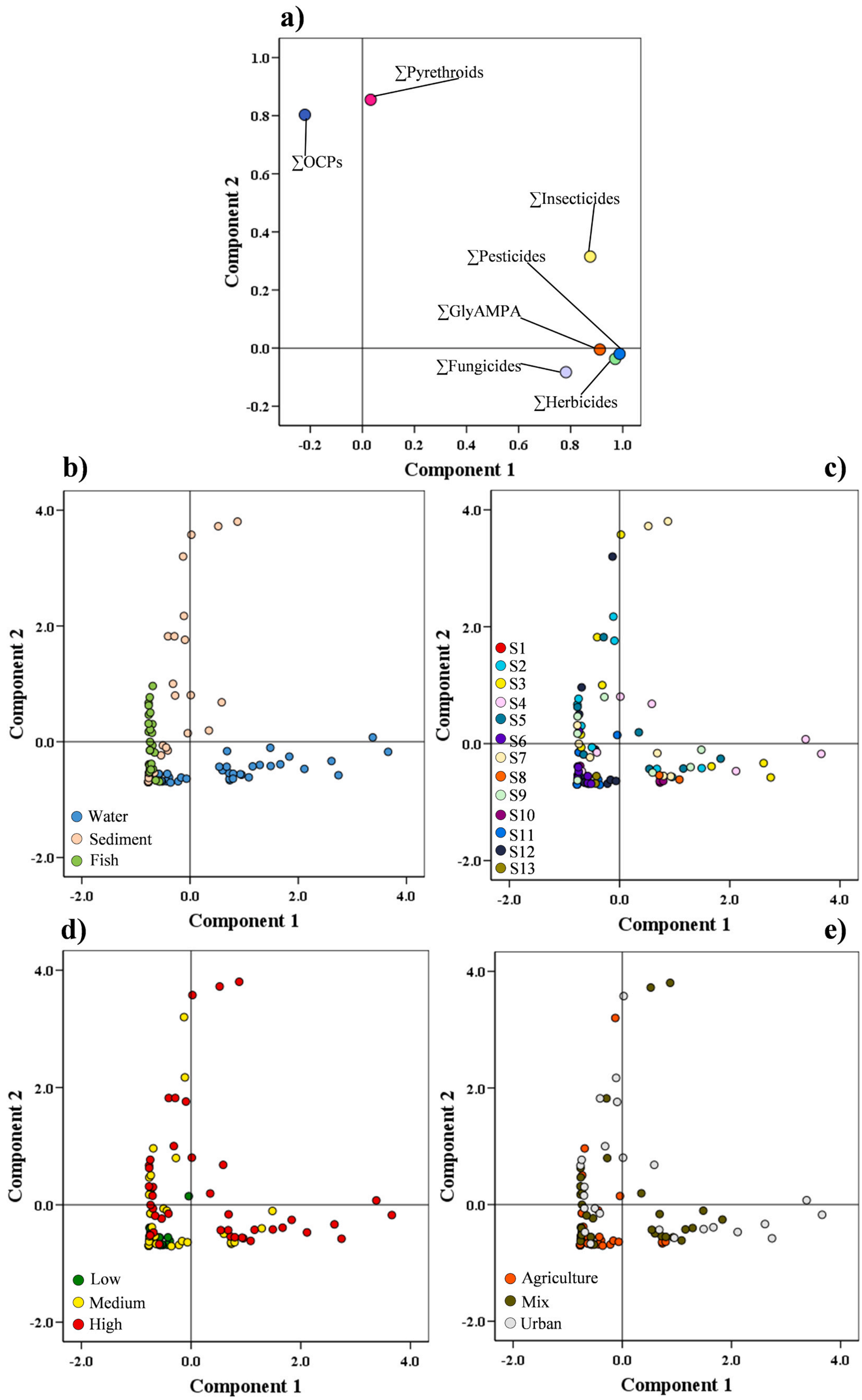

Fig. 3. Diagrams of dispersion related to the two components resulting from a principal components analysis (PCA) derived from the content and distribution of pesticides in the aquatic ecosystem (Component 1 and 2). Loading plots (a) contribution of each variable to each component. Score plots markers set by type of matrix (b), location (c), anthropogenic influence (d) and land use (e), of all samples on each component.

3.4. Prioritization of hazardous pesticides in aquatic ecosystem

It is noteworthy that the ecological risk based on concentrations and the detection frequency of the pesticides studied are essential to assess the concern caused by the different pesticides found in the aquatic ecosystem. A total of 27 different pesticides of concern were identified in the environment studied, concretely, 10 for aquatic organisms, 9 for benthic organisms and 14 for fish-eating predators (Table S29). Fipronil sulfone, terbutryn and carbendazim were the pesticides most representative (observed in the 62 % of locations) in water compartment and the highest concern for aquatic organisms due to their high detection frequency. Remarkably, those sampling points with low anthropogenic influence and land use related to agriculture presented a different and variable profile. In the case of sediment compartment, the predominant mixture (found in 60 % of sites) was comprised by chlorpyrifos, cypermethrin and permethrin, highlighting insecticides as the main class of concern for benthic organisms. The profile observed in fish denoted different mixtures and bioaccumulation behavior in each sampling site, being diflufenican the most recurrent compound in this exposure medium. The cocktail defined by chlorpyrifos, pendimethalin and diflufenican was the most emblematic for fish-eating predators.

Several compounds detected in the aquatic ecosystem studied (36 %) have been included in the PAN International List of Highly Hazardous Pesticides (HHPs; PAN, 2024; Table S7), showing high incidence of severe or irreversible adverse effects on human health (70 %) or the environment (53 %). Interestingly, some of the pesticides scored as a great concern in this study (carbendazim, chlorpyrifos, cypermethrin, fipronil and pendimethalin) are classified as highly hazardous pesticides.

4. Conclusions

Through a multi-compartment approach involving water, sediment, and fish matrices, this study provides a relevant evaluation of the environmental occurrence, partitioning behavior, bioaccumulation potential, and associated ecological risks of a broad survey of pesticides. Mixture-based risk exceeded single-compound risk and predominant pesticide mixtures were identified in the different compartments. Overall, herbicides and insecticides contributed most to the mixture risk for aquatic and benthic organisms. Remarkably, several pesticides of particular concern were identified in the aquatic ecosystem, concretely, fipronil sulfone, terbutryn and carbendazim for aquatic organisms; chlorpyrifos, cypermethrin and permethrin for benthic organisms; and chlorpyrifos, pendimethalin and diflufenican for fish-eating predators. This widespread pollution evidences exposure to mixtures of multiple pesticides across different trophic levels in the aquatic ecosystems whose long-term effects are unknown. Further research is required to understand the potential risks and effects of complex pesticide mixtures on aquatic organisms. Regarding the implications related to water management and regulatory monitoring, it is noteworthy that pesticide total concentration in water surpassed 5-fold the AA-EQS established in the WFD for the sum of individual pesticides. In addition, medium or high risk was observed for a significant number of pesticides in benthic organisms, highlighting the need to establish EQS values for the sediment compartment. These findings underscore the urgency of improving water quality policies, biomonitoring and assessing exposure measures to achieve a quality ecological status. The establishment of integrative monitoring schemes and proper benchmarks for mixtures proves essential to protect the integrity and biodiversity of ecosystems and ultimately the health of humans within the One Health perspective.

CRediT authorship contribution statement

María Ángeles Martínez: Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Adrián de la Torre:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Covadonga Alonso:** Writing – review & editing, Methodology. **Silvia Royano:** Writing – review & editing, Validation, Methodology, Investigation. **Irene Navarro:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2025.119221.

Data availability

Data will be made available on request.

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