Research article

Ecotoxicological and chemical approach to assessing environmental effects from pesticide use in organic and conventional rice paddies

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Abstract: Despite laws and directives for the regulation and restriction of pesticide in farming, the large use of Plant Protection Products (PPPs) in paddy fields is a relevant worldwide cause of contamination of the environment. The aim of this work is to evaluate the environmental impact due to the use of PPPs by using an integrated approach based on chemical analyses and ecotoxicological hazard assessment, supported by statistical tools, in order to overcome the issues related to the traditional tabular evaluation. Samples of soil and water of seven conventional and organic paddies located in Northern Italy have been examined for two years by extensive chemical and ecotoxicological analyses. The results evidenced a direct relationship between the presence of Oxadiazon in water and bioassays responses as the main cause of the toxicity measured. This phenomenon affected both biological and conventional rice fields, due to the free circulation of water through irrigation canals. Therefore, the implementation of organic districts with a water circulation isolated from conventional fields represents a simple and effective countermeasure to safeguard the agricultural practices of organic crops.

Keywords: Environmental Impact Assessment; bioassays; ecotoxicological hazard; pesticides; Oxadiazon.

1. Introduction

Since long, it has been known that the large amount of pesticides applied in the paddy fields, in addition to the common practice of draining the paddy water in irrigation canals that convoy to the freshwater system and eventually into the marine environment, is one of the major cause of pollution worldwide [1].

Plant Protection Products (PPPs), because of their persistence, toxicity, and bioaccumulative properties are of particular concern. They might have adverse ecological effects, causing both short-term (acute) and long-term (chronic), lethal or sub-lethal biological damages; in particular, changes in behavior, metabolism, development, alteration of the food chain or habitat of non-target organisms such as amphibians, and bats, reduction in the populations of natural predators of insect pests [2-17]. In addiction pesticides are known to lower the biodiversity and function of an ecosystem by promoting the dominance of undesired and invasive species [18].

The most common way to assess the biological effects of the use of PPPs derives from toxicological studies with the aim to define the existing relationship between dose of specific compound and toxicity response in laboratory experiments. Relatively few field surveys were conducted with the purpose of correlating the in-situ measurements of pesticide concentration with the bioassays responses, i.e. *Daphnia magna* [19]. Some studies were carried out in controlled microcosms on a range of representative soil organisms

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[20]; while several studies used the Cornell University Environmental Impact Quotient (EIQ) calculator [21] and the ECOTOX Knowledgebase to determine the exposure risk associated with individual pesticides relative to their application rates and aquatic concentrations [22]. Moreover, Sánchez-Bayo and Goka [23] evaluated the ability of four community endpoints (specie richness, abundance, diversity, and similarity indices) to assess impacts of two insecticides (Imidacloprid and Etofenprox). Given that, the ecotoxicity of mixture of PPPs and behavior of pesticide transformation products in the aquatic environment such as paddies are poorly understood.

The European Community established various actions to counter the impact of pesticides on biodiversity through the Directive 2009/128/CE [24], which was implemented in Italy with the Legislative Decree no. 150/2012 [25], that compels a minimization or prohibition of the use of pesticides in the areas designated by the Habitat (92/43/EEC) [26] and Birds (2009/147/EC) [27] Directives and in the protected areas referred to Water Framework Directive (2000/60/EC) [28]. Europe and United States (USEPA) have established Regulation (EC) No. 1107/2009 [29] and the Data Requirements for Conventional Chemicals for pesticides registration, requiring environment fate and ecotoxicity data to be provided [30].

These regulations are referred to a tabular approach, which is limited to the compliance of the chemical thresholds with respect to the toxicity of pure individual pesticides. Moreover, this approach doesn't take into account the simultaneous action of the complex mixtures of contaminants commonly present in aquatic habitats that may result in antagonistic and more often synergistic effects on biota.

One of the most advanced pesticide act is adopted in Japan by the Ministry of Environment [31]. In order to determine the eligibility of the product, acute toxicity tests must be conducted for fish (basically, *Cyprinus carpio*), daphnids (*Daphnia magna*), and algae (*Raphidocelis subcapitata*), and then the minimum value of the 50% effect concentration (EC50 or LC50) is divided by an uncertainty factor that considers the species sensitivity. The Japanese Agricultural Chemicals Regulation Law was revised in 2018, and the method of assessing pesticide registration criteria was also revised [31]. Toxicity tests using aquatic plants such as *Lemna* sp. in addition to algae will be introduced in the setting of criteria for herbicides. The uncertainty factor applied to the algal EC50 was changed from 1 to 10 by default, which is then reduced depending on the number of algal species tested. However, registration criteria for eligibility of pesticides for the new method have not yet been developed until 2021 [32].

A relatively common way to determine hazardous concentration for the protection of ecosystem and to reveal ecological risk is the cumulative distribution function called SSD (Species Sensitivity Distribution) [32, 33]. The 5th percentile of this distribution (called the 5% Hazardous Concentration, HC5) has been used by USEPA [34], the RIVM Institute (The Netherlands) [35], and the European Commission [36] for deriving threshold concentrations that protect most species in a community. Researches based on SSD were conducted for Ecological Risk Assessment (ERA) of several paddy insecticides and herbicides applying the Potentially Affected Fraction (PAF) as index of the magnitude of ecological risk capable of reducing diversity [32, 37-38].

The aim of this work is to investigate the relationship between chemical and ecotoxicological Lines of Evidence (LOEs) in the assessment of environmental impact due to the use of PPPs in Italian paddies, supported by a statistical approach, in a more realistic way than the traditional tabular evaluation. The finding will provide scientifically useful indications on the environmental compatibility of the use of pesticides.

The chemical and ecotoxicological analyzes were carried out in conjunction with the sampling of species and habitats linked to the agroecosystems, in a larger project aimed at testing the measures envisaged by the National Action Plan for the sustainable use of PPPs for Natura 2000 sites and in protected areas, through the comparison of the values emerged in biological and conventional fields. These measures indicate the biological method as the one most compatible with the conservation of biodiversity.

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2. Materials and Methods

2.1. Area of study

The study area is located in the Po Valley, between Piedmont and Lombardy (Italy), a vast agricultural territory in which, for irrigation management needs, rice cultivation is mainly monoculture. The cultivation consists in an uninterrupted succession of rice-field chambers interwoven with a large and articulated irrigation network functional to the distribution of water.

Seven rice paddies, belonging to four organic and three conventional farms, located in the Vercelli plain were investigated from 2018 to 2019 (Figure 1). The zone includes five protected areas and Natura 2000 sites as detailed in Table 1.

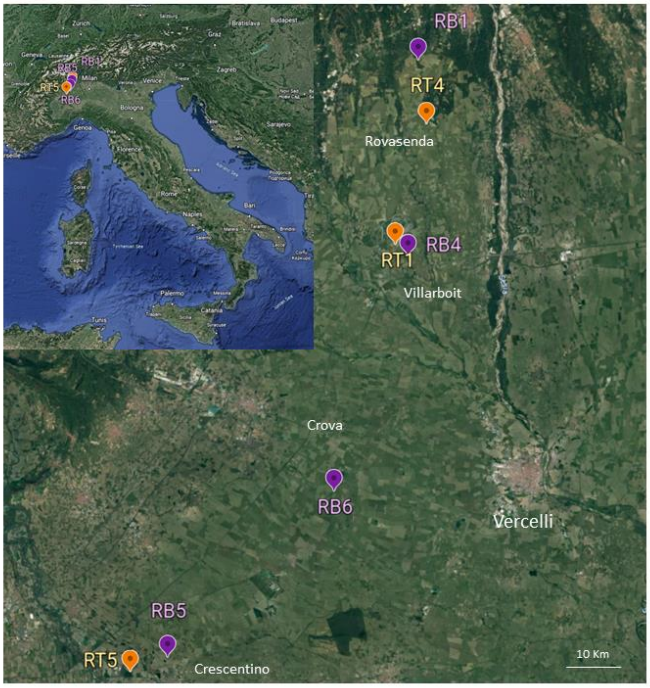

Figure 1. Localization of crops involved in the research project.

Table 1. Identification code, the municipality and type of agronomic management (RT = conventional; RB = organic) of the seven rice paddies considered.

Code	Municipality	Protected Areas of the Natura 2000 Network	Agronomic management	Year
RT1	Villarboit	ZSC/ZPS IT1120014 Druma River marsh	Conventional	2018-2019
RT4	Rovasenda	SIC IT1120026 Station of *Isoetes malinverniana*	Conventional	2018-2019
RT5	Crescentino	-	Conventional	2018-2019
RB1	Rovasenda	ZSC IT1120004 Baraggia of Rovasenda/EUAP0349 Natural Reserve of Baragge	Organic	2018-2019

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RB4	Villarboit	ZSC/ZPS IT1120014 Druma River marsh	Organic	2018
RB5	Crescentino	-	Organic	2018-2019
RB6	Crova	ZPS IT1120021 Paddies of Vercelli	Organic	2019

2.2. Sample collection

The sampling campaigns took place in 2018-2019 and has been strongly influenced by the irrigation level and weather conditions. In 2018, soil sampling was performed on the paddy embankment (*em*) and inside the paddy chamber (*ch*), when the growing season was suitable. In 2019, because of long flooding events, samples were collected only in the paddy embankments and the paddy field RB4 was replaced with RB6 (Table 1).

The sampling strategy for water and soil samples was divided in two phases for each year: one sampling at the beginning of the growing season, before the phytosanitary treatment (t_0), and a second one after the phytosanitary treatments (t_1), for a total of 4 sampling events in two years. For each phase, in presence of water flow, water samples were taken at entry (*in*) and exit (*out*) of the paddy field. In the absence of water flow, two water samples were taken near the channels of entry (*in*) and exit (*out*) from the paddy field. This sampling strategy allowed verifying the possible contributions of contaminants already present in the irrigation water before entering into the paddy field chamber.

Water samples were kept into decontaminated glass bottles and stored in the dark at -20 °C till further analyses.

Four aliquots of soil from each paddy embankment were collected, one on each side of the chamber; the aliquots were then pooled, homogenized, sieved (2 mm) and stored at -20 °C.

2.3. Chemical characterization

2.3.1. Soil samples

The soil samples were dried and homogenized by grinding with an IKA® mill equipped with a beater blade.

Total Carbon (TC) and Total Nitrogen (TN) were determined with a CHNS analyzer Vario Micro Cube Elementar and referred to dry weight.

Inorganic carbon was removed by progressive additions of HCl, then evaporated at ~ 50 °C. The analyzer performed a controlled combustion (~ 900 °C), then a catalytic oxidation (Chromium oxide) and finally a reduction by metallic copper. The CO_2 and N_2 developed were determined by a thermal conductivity detector after their gas chromatographic separation.

Quality control was performed by daily Acetanilide analysis and by repeated measurements of two standard soils (Boden Standard AIVA Analysentechnik and Soil standard 2.1 Elementar).

For Ca, K and Mg analyses, the sample was dried at 35 °C for 48 hours and homogenized in an agate mortar, it was then subjected to a microwave acid digestion, using a 1:3 mixture of nitric and hydrochloric acid [39]. Instrumental determination was performed by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES) [40], at the following wavelengths: 766.492 nm for K; 422.673 nm for Ca; 279.800 nm for Mg, referring concentrations to dry weight. The limit of quantification (LOQ) for these elements was 0.010 %. Quality control was performed analyzing certified reference materials (sediment PACS-2 and soil SRM 2709).

The following PPPs were determined in dried and homogenized soil samples: Chlorpyrifos, Penconazole, Metalaxyl, Metrafenone, Pendimenthalin, Metolachlor, Alpha

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Endosulfan, Beta Endosulfan, Oxadiazon, Boscalid, Deltamethrin, Lambda Cyhalothrin, Oxifluorfen, Tebuconazole, Folpet, Dimethomorph, Kresoxim methyl.

PPPs were extracted by Pressurized Fluid Extraction, dehydrated and concentrated under a nitrogen flow, filtered and analyzed by Gas Chromatography coupled with a triple quadrupole Mass Spectrometer (GC/MS/MS) in MRM mode. Identification was based on the presence of at least two characteristic transitions for each analyte. Quantification was performed using perdeuterated internal injection standards. The LOQ of the method was 0.1 mg Kg^{-1} for each analyte. The method was developed by recovery tests on spiked samples; the quality control involved the analysis, at each sequence, of blanks, replicates, and recovery tests.

2.3.2. Water samples

The determination of Ca, K, Mg and S was performed on whole sample, after acidification with 2 % nitric acid, by Atomic Emission Spectroscopy technique with inductively coupled plasma [40] at the same wavelengths for soil samples. Limits of quantification were 4 mg l^{-1} for Calcium and 1 mg l^{-1} for Mg, K and S.

Copper determination was carried out by AAS technique with a graphite furnace [41] at a wavelength of 327.4 nm, with a quantification limit (LOQ) of 1 μg l^{-1}.

The quality control was performed using different certified reference materials: NIST 1643f for Ca, K, Mg and Cu and SRM2709 for S.

The same PPPs of soil were detected. A solution of deuterated internal standards was added to 200 ml of aqueous sample, and an extraction with dichloromethane was performed three times by separation funnel shaking. The extract was dehydrated with anhydrous sodium sulfate to the final volume by nitrogen blowing and then analyzed by GC/MS in SIM mode. Identification was performed by comparison of retention indices with those of standards and comparison of the relative abundances of the ions.

Quality control was ensured by the analysis, for each batch of samples, of standards, method blanks, recovery tests on spiked samples. The limit of quantification of the method (LOQ) for Folpet and Deltamethrin was 0.20 μg l^{-1}, for all other substances was 0.10 μg l^{-1}.

2.4. Ecotoxicological characterization

Ecotoxicological assays were started simultaneously on all test species, using the same sample aliquot of chemical analyses.

Different batteries of bioassays were set up in order to optimize the ecological representativeness of test-species with respect to the environmental characteristics of the crops under study. The main features of the toxicity tests are detailed in Table 2.

Table 2. Battery of bioassays used for ecotoxicological assessment of soil and water samples of rice paddies.

Sampling point	Species	Common name	Environmental matrix	End-point	Exposition	Method
Embankment and paddy chamber soil	*Lepidium sativum*	Watercress	Soil	Germination and root elongation	72 h	ISO 18763:2016
	Sinapis alba	Mustard				
	Sorghum saccharatum	Sorghum				
	Aliivibrio fischeri	Bacterium	Eluate	Biolumin.	30 min.	ISO 11348-3:2019

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Water	*Raphidocelis subcapitata*	Green algae	Water	Growth rate	72 h	ISO 8692:2012
	Daphnia magna	Water flea	Water	Immobilization	24 h	ISO 6341:2013
	Spirodela polyrhiza	Duckmeat	Water	Leaves growth	72 h	ISO 20227:2017
	Aliivibrio fischeri	Bacterium	Water	Biolumin.	30 min.	ISO 11348-3:2019

All bioassays were performed according to standardized ISO methods in the ISPRA certified laboratories, according to UNI EN ISO 9001:2015.

2.5. Ecotoxicological hazard assessment

ISPRA and Public University of Ancona (Italy) have already developed several synthetic indices for the ecological risk assessment in marine environments [42, 43], transposed in 2016 in the Technical Annex of Ministerial Decree No. 173/2016, which regulates dredging activities in relation to dumping.

The model, by means of a modular structure corresponding to several LOEs, combines in a weighted way the chemical, biological and ecotoxicological characteristics of sediments. Regarding the ecotoxicological LOE, the Hazard Quotient (HQ_{eco}) integrates the results of the bioassays based not only on the biological measured effects, but also on the severity of the end-point (growth, bioluminescence, survival, embryonic development, etc.), the ecological relevance of the tested environmental matrix (pore water, elutriate, whole sediment, etc.) as well as the type of exposure (chronic or acute) [42]. In particular, weighted criteria to elaborate results from standardized ecotoxicological bioassays are based on specific thresholds and weights assigned to each bioassay depending on the biological endpoint, tested matrix, time of exposure, and the possibility of hormetic responses. The cumulative Hazard Quotient referred to battery bioassays ($HQ_{Battery}$) is obtained by the summation (Σ) of the weighted effects (E_w), i.e., the variations measured for each test compared to specific thresholds, corrected for the statistical significance of the difference (w), the biological importance of the endpoint and exposure conditions (w_2) [42]:

$$HQ_{Battery} = \sum_{K=1}^{N} Effect_w(k) \cdot w_2$$

The $HQ_{Battery}$ is normalized to a scale ranging from 0 to 10, where 1 is the battery threshold when all the bioassays exhibit an effect equal to the threshold, while 10 indicates that all the assays exhibit 100 % of effect. The $HQ_{Battery}$ is then assigned to one of five classes of hazard, from Absent to Severe [43].

The flexible structure of HQ index and weighted criteria can be easily adapted to other applications, simply by varying the test species and weights of the different variables considered environmentally relevant.

In the case of rice fields, the toxicity thresholds and weights assigned to variables considered in the integrated index for estimating ecotoxicological Hazard Quotient (HQ_{eco}) are detailed in Table 3.

Table 3. Weights assigned to the variables considered in the integrated index for estimating ecotoxicological Hazard Quotient (HQ_{eco}).

Species	End-point (weight)	Matrix (weight)	Exposure (weight)	Toxicity threshold (%)
Aliivibrio fischeri	Biolumiscence (1.8)	Water (0.9)	Acute (1.0)	15

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		Soil eluate (1.0) Soil (*em*) (0.7) Soil (*ch*) (0.8)		25
Daphnia magna	Survival (2.2)	Water (0.9) Eluate (1.0)	Acute (1.0)	10
Eisenia sp.	Survival (2.2)	Soil (em) (0.7) Soil (*ch*) (0.8)	Acute (1.0)	10
	Growth (1.3)	Soil (*em*) (0.7) Soil (*ch*) (0.8)		20
Lepidum sativum	Germination (2.0)	Soil (*em*) (0.7) Soil (*ch*) (0.8)	Acute (1.0)	30
	Radical elongation (1.1)	Soil (*em*) (0.7) Soil (*ch*) (0.8)		
Raphidocelis subcapitata	Algal growth (1.6)	Water (0.9) Soil Eluate (1.0)	Chronic (0.8)	10
Sinapis alba	Germination (2.0)	Soil (*em*) (0.7) Soil (*ch*) (0.8)	Acute (1.0)	30
	Radical elongation (1.1)	Soil (*em*) (0.7) Soil (*ch*) (0.8)		

The weight of the different types of end-point varies according to the severity of the biological effect, with a maximum value for mortality (2.2) and a minimum value for root elongation (1.1). According to the same principle, acute toxicity has greater weight (1.0) than chronic toxicity (0.8).

2.6. Statistical analyses

In order to compare the ecotoxicological results obtained between organic and conventional paddies, a specific t-test was applied for inhomogeneous variance at confidence level of 95 % (p = 0.05). Finally, with the aim to assess possible relationships between the measured ecotoxicological responses and the chemical characteristics detected in the various matrices, a multivariate analysis (PCA) was carried out on the raw data. Statistical elaborations were carried out using R (version 4.2.1) and RStudio (version 2022.07.1) with the installed packages lme4, vegan, factoextra, ggplot2.

3. Results and discussion

3.1. Chemical characteristics of soil

Table 4 shows the raw data referred to organic content (TOC, TC), macro-elements and inorganic pesticides (Cu and S) sought for characterization of samples.

Table 4. Chemical characterization of soil samples from paddies (RB = organic cultures; RT = conventional cultures). In 2019 RB4 was substitute with RB6. Only values > LOQ are reported (em = paddy embankment soil; ch_ins = inside paddy field chamber soil; ch_in = paddy field chamber entrance soil; ch_out = paddy field chamber exit soil).

2018

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Sample				TOC (%)	TC (%)	TN (%)	Ca (%)	K (%)	Mg (%)	Cu (mg kg^{-1})	S (mg kg^{-1})
RB1	em		t_0	1.465	1.325	0.113	0.62	0.64	0.50	15.20	215.38
			t_1	2.193	2.004	0.173	0.61	0.67	0.53	10.34	285.99
	ch	ins	t_0	1.244	1.250	0.102	0.59	0.58	0.49	9.45	244.88
		in	t_0	1.398	1.431	0.118	0.52	0.51	0.47	15.53	213.17
			t_1	1.684	1.721	0.167	0.40	0.35	0.41	12.73	194.69
		out	t_0	1.214	1.369	0.127	0.49	0.49	0.47	15.53	213.17
			t_1	1.482	1.164	0.132	0.50	0.57	0.47	12.73	194.69
RB4	em		t_0	t_0	1.696	0.109	0.68	0.51	0.42	6.163	221.43
			t_1	t_1	1.552	0.123	0.62	0.41	0.38	5.567	283.86
	ch	in	t_0	1.98	1.985	0.167	0.56	0.46	0.37	15.664	297.50
			t_1	1.334	1.32	0.089	0.8	0.28	0.29	10.257	170.38
		out	t_0	1.597	1.842	0.139	0.55	0.39	0.35	10.287	256.31
			t_1	1.25	1.259	0.097	0.47	0.38	0.33	9.752	288.28
RB5	em		t_0	1.195	1.960	0.200	1.28	0.41	1.14	16.21	325.53
			t_1	1.807	2.005	0.178	1.64	0.49	1.10	10.98	380.43
	ch	ins	t_0	2.053	2.285	0.182	1.50	0.45	1.03	13.12	468.17
		in	t_0	1.304	1.411	0.131	1.57	0.40	1.13	15.53	317.78
		out	t_1	0.987	0.862	0.070	1.49	0.37	1.08	15.53	317.78
RT1	em		t_0	1.564	1.584	0.141	0.50	0.34	0.33	15.20	307.51
	ch	ins	t_0	1.539	2.006	0.412	0.62	0.46	0.38	10.152	249.4
			t_1	1.614	1.764	0.14	0.53	0.41	0.36	9.921	274.8
		in	t_0	1.134	1.266	0.113	0.48	0.37	0.33	17.358	226.81
			t_1	1.227	1.198	0.125	0.55	0.33	0.30	15.002	263.42
		out	t_0	1.718	1.799	0.159	0.46	0.27	0.30	14.931	320.52
			t_1	1.523	2.251	0.25	0.57	0.59	0.50	13.983	275.59
RT4	em		t_0	1.303	1.399	0.116	0.55	0.52	0.53	14.00	313.68
	ch	ins	t_0	1.822	1.946	0.128	0.55	0.55	0.51	15.21	385.54
			t_1	1.651	2.836	0.151	0.57	0.59	0.50	13.23	301.94
		out	t_1	1.674	1.667	0.175	0.55	0.65	0.47	17.90	349.90
RT5	em		t_0	2.055	2.106	0.199	1.69	0.41	0.98	13.17	329.78
	ch	ins	t_0	2.300	2.313	0.207	1.46	0.46	1.12	15.31	670.39
			t_1	2.142	2.246	0.201	1.59	0.58	1.21	18.51	615.68
		in	t_0	2.623	2.650	0.273	1.57	0.42	1.03	17.57	574.08
			t_1	2.068	2.118	0.215	1.58	0.53	1.24	22.36	630.69
		out	t_0	2.275	2.445	0.235	1.52	0.42	1.07	18.02	457.29
			t_1	1.891	1.873	0.182	1.51	0.40	1.05	15.97	416.75
2019											
RB1	em		t_0	1.856	1.874	0.167	0.59	0.69	0.50	16.00	259.72
			t_1	2.072	2.330	0.216	0.53	0.55	0.50	21.79	301.81
	ch	ins	t_0	1.595	1.702	0.142	0.42	0.38	0.45	15.58	247.54

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		in	t_0	1.342	1.395	0.129	0.59	0.74	0.55	20.66	261.70
			t_1	1.206	1.360	0.125	0.55	0.72	0.55	21.13	277.80
		out	t_0	0.608	0.967	0.079	0.52	0.63	0.59	22.03	182.40
			t_1	1.210	1.160	0.105	0.42	0.46	0.48	24.59	224.55
RB5	em		t_0	1.464	1.813	0.169	1.72	0.40	1.04	3.19	296.80
			t_1	1.808	1.921	0.196	1.54	0.36	1.13	4.32	295.49
	ch	in	t_0	1.029	1.313	0.118	1.64	0.37	1.00	6.34	250.78
			t_1	1.262	1.292	0.118	1.40	0.25	0.99	8.60	373.48
		out	t_0	2.155	2.176	0.198	1.59	0.50	1.05	3.40	531.56
			t_1	1.954	2.677	0.239	1.45	0.40	1.02	5.67	550.07
RB6	em		t_0	2.551	2.531	0.24	1.32	0.47	1.38	24.410	352.79
			t_1	1.946	2.216	0.203	1.02	0.33	1.04	20.760	290.34
	ch	in	t_0	1.741	2.037	0.177	1.38	0.45	1.14	32.690	269.46
			t_1	2.27	3.235	0.291	1.33	0.61	1.14	26.030	432.05
		out	t_0	1.536	1.748	0.155	1.3	0.59	1.16	24.240	325.40
			t_1	2.442	2.512	0.218	1.24	0.49	1.59	26.480	399.43
RT1	em		t_0	2.087	2.107	0.165	0.46	0.42	0.42	16.400	275.21
			t_1	1.526	1.655	0.178	0.57	0.53	0.35	14.700	244.93
	ch	in	t_0	1.392	1.464	0.152	0.58	0.51	0.38	19.230	315.53
			t_1	0.831	1.538	0.141	0.63	0.54	0.40	22.400	302.42
		out	t_0	1.216	1.435	0.122	0.29	0.17	0.28	15.700	305.08
			t_1	1.168	1.327	0.105	0.59	0.36	0.31	15.470	312.22
RT4	em		t_0	2.365	3.403	0.287	0.33	0.25	0.36	19.82	339.13
			t_1	1.668	1.669	0.169	1.54	0.62	1.34	31.01	592.29
	ch	in	t_0	1.917	1.926	0.182	0.46	0.40	0.40	20.62	432.04
			t_1	1.845	1.987	0.175	0.45	0.36	0.40	18.99	345.50
		out	t_0	1.810	1.922	0.182	0.52	0.32	0.36	16.39	218.78
			t_1	1.906	2.178	0.193	0.56	0.63	0.49	22.67	479.67
RT5	em		t_1	2,324	2,458	0,251	1,33	0,38	1,05	18,02	476.87
	ch	in	t_1	1.034	1.752	0.19	1.78	0.48	1.10	17,38	548.90
		out	t_0	1.087	1.894	0.182	1.61	0.41	1.07	19.68	483.60

The percentages of Total Nitrogen (TN) reflect a type of soil "well endowed", according to the classification of Giardini [45], while the C/N ratios are generally >11. The high percentage of organic component leads to nitrogen stabilization, making it less bioavailable. The ratios did not change substantially between organic and conventional paddies, with median values of 11.22 and 10.58, respectively. The amount of organic matter did not show significant differences between the organic and conventional fields ($p = 0.335$) throughout the study period, whereas conventional paddies resulted with significant higher content of Cu and S in 2018 ($p = 0.005$ and 0.004 respectively). For both metals, the highest values were always found in the conventionally treated rice fields, especially in RT5. The relatively high values measured could be associated with the large use of copper sulphate, as classic verdigris in the entire area.

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Table 5 reports the results for the pesticides for which at least one sample showed quantifiable concentrations (> LOQ). In particular, the PPPs concentrations were < LOQ in all samples from organic crops, while few cases of quantifiable concentrations of Oxadiazon, Oxyfluorfen and Pendimethalin were found in samples from conventional paddies.

The low number of quantifiable results does not allow evaluating differences between the various types of samples from rice fields (chamber, embankment, soil inlet or outlet) or even just between before (t_0) and after treatment (t_1).

Table 5. Concentration of PPPs measured in soil samples of paddies (μg Kg^{-1}). Only data from fields for which at least one value has been quantified are reported (Em = paddy embankment; ch_ins = inside paddy field chamber; ch_in = paddy field chamber entrance; ch_out = paddy field chamber exit).

Sample				Pendimethalin	Oxyfluorfen	Folpet	Oxadiazon
2018							
RT1	em		t_0	< 0.1	0.1	< 0.1	< 0.1
	ch	ins	t_0	< 0.1	0.3	< 0.1	< 0.1
			t_1	< 0.1	0.2	< 0.1	< 0.1
		in	t_0	< 0.1	< 0.1	< 0.1	< 0.1
			t_1	< 0.1	< 0.1	< 0.1	< 0.1
		out	t_0	< 0.1	0.1	< 0.1	< 0.1
			t_1	< 0.1	< 0.1	< 0.1	< 0.1
RT4	em		t_0	< 0.1	< 0.1	< 0.1	< 0.1
	ch	ins	t_0	< 0.1	< 0.1	< 0.1	< 0.1
			t_1	< 0.1	< 0.1	< 0.1	< 0.1
		out	t_1	< 0.1	< 0.1	< 0.1	0.2
RT5	em		t_0	< 0.1	< 0.1	< 0.1	< 0.1
	ch	ins	t_0	< 0.1	< 0.1	< 0.1	< 0.1
			t_1	0.3	< 0.1	< 0.1	< 0.1
		in	t_0	0.2	< 0.1	< 0.1	< 0.1
			t_1	0.9	< 0.1	< 0.1	< 0.1
		out	t_0	< 0.1	< 0.1	< 0.1	< 0.1
			t_1	< 0.1	< 0.1	< 0.1	0.4
2019							
RT1	em		t_0	< 0.1	0.2	0.1	< 0.1
			t_1	< 0.1	< 0.1	< 0.1	< 0.1
	ch	in	t_0	< 0.1	< 0.1	< 0.1	< 0.1
			t_1	< 0.1	< 0.1	< 0.1	< 0.1
		out	t_0	< 0.1	< 0.1	< 0.1	< 0.1
			t_1	< 0.1	< 0.1	< 0.1	< 0.1
RT4	em		t_0	< 0.1	< 0.1	< 0.1	< 0.1
			t_1	< 0.1	< 0.1	< 0.1	< 0.1
	ch	in	t_0	< 0.1	< 0.1	< 0.1	0.5
			t_1	< 0.1	< 0.1	< 0.1	< 0.1
		out	t_0	< 0.1	< 0.1	< 0.1	0.3

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			t_1	< 0.1	< 0.1	< 0.1	0.3
RT5	em		t_1	0.2	0.4	<0.1	0.6
	ch	in	t_1	<0.1	0.3	<0.1	0.3
		out	t_0	< 0.1	< 0.1	< 0.1	0.9

Despite the many active ingredients sought, most measures were below the LOQ. The finding demonstrates that, despite frequent treatments with PPPs over the years, the irrigation cycles in rice fields favour soil leaching, which therefore retains little residue of contaminants in the chamber, promoting their transfer to the water.

3.2. Chemical characteristics of water

Table 6 shows the concentrations of macro elements (Ca, K, Mg), inorganic and organic pesticides found in the water samples for which at least one value has been quantified, except for sample RT4_in_t_0 because it was not in sufficient quantity to carry out the analyses.

The concentrations of the macroelements indicate that Ca is the dominant element (4.27 - 44.25 mg l^{-1}), followed by Mg (up to 15.01 mg l^{-1}) and K (up to 33.93 mg l^{-1}) in similar quantities. No significant statistical differences were found between organic and conventional fields about the content of both macro-elements and inorganic pesticides, in the monitored years. However, a general trend is noticeable towards a lower Ca, Mg and K content in organic rice fields, especially in 2019 ($p = 0.076$ for Ca, $p = 0.097$ for K and $P = 0.099$ for Mg).

No differences related to either the sampling point (*in* or *out*) or the two sampling campaigns following treatment are noticed. The concentrations of Cu e S measured in water are in very low ranges, especially for copper, as are the values found in soil samples from the same fields. Likely, this is due to the limitations on the use of copper sulphate in rice fields from 2012, according to Regulation 1107/2009/EC [29].

Table 6. Chemical characterization of water sample from rice paddies (RB = organic cultures; RT = conventional cultures). In 2019 RB4 was substitute with RB6 (in = paddy field chamber entrance; out = paddy field chamber exit).

Sample			**Ca** (mg l^{-1})	**K** (mg l^{-1})	**Mg** (mg l^{-1})	**Cu** (µg l^{-1})	**S** (mg l^{-1})	**Λ-Cyalothrin** (µg l^{-1})	**Metolachlor** (µg l^{-1})	**Oxadiazon** (µg l^{-1})	**Oxyfluorfen** (µg l^{-1})
2018											
RB1	in	t_0	4.81	1.07	1.12	3.60	1.79	< 0.1	< 0.1	0.15	< 0.1
		t_1	8.86	2.78	2.02	2.85	2.31	< 0.1	< 0.1	0.14	< 0.1
	out	t_0	9.44	19.79	3.08	8.07	2.44	< 0.1	< 0.1	0.13	< 0.1
		t_1	12.76	< 1.00	3.57	< 1.00	< 1.00	< 0.1	< 0.1	0.10	< 0.1
RB4	in	t_0	9.22	5.57	2.25	1.85	2.7	< 0.1	< 0.1	1.68	< 0.1
		t_1	11.00	4.00	3.1	1.94	1.17	< 0.1	< 0.1	0.67	< 0.1
	out	t_0	13.85	3.81	3.31	6.86	3.13	< 0.1	< 0.1	0.21	< 0.1
		t_1	9.89	1.90	2.81	5.67	< 1.00	< 0.1	< 0.1	0.52	< 0.1
RB5	in	t_0	27.39	6.92	8.00	4.92	10.08	< 0.1	< 0.1	1.72	< 0.1
		t_1	34.05	< 1.00	3.57	2.10	5.06	< 0.1	< 0.1	1.09	< 0.1

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	out	t_0	36.59	8.24	8.22	2.73	7.01	< 0.1	< 0.1	0.52	< 0.1
		t_1	36.59	1.53	9.99	3.03	19.58	< 0.1	< 0.1	0.23	< 0.1
RT1	in	t_0	6.29	2.09	1.34	2.67	2.27	< 0.1	< 0.1	1.75	0.23
		t_1	10.9	2.79	2.56	5.58	2.44	< 0.1	< 0.1	0.61	< 0.1
	out	t_0	5.63	2.40	1.09	5.56	3.12	< 0.1	< 0.1	1.24	0.12
		t_1	9.52	33.93	3.05	6.69	1.50	< 0.1	< 0.1	0.35	< 0.1
RT4	in	t_0	n.d.	n.d.	n.d.	n.d.	n.d.	< 0.1	< 0.1	0.13	< 0.1
		t_1	5.60	< 1.00	1.22	6.73	1.63	< 0.1	< 0.1	0.17	< 0.1
	out	t_0	4.27	1.96	< 1.00	3.82	2.43	< 0.1	< 0.1	0.15	< 0.1
		t_1	6.14	1.31	1.85	7.22	< 1.00	< 0.1	< 0.1	0.42	< 0.1
RT5	in	t_0	42.66	3.58	8.72	2.12	10.42	< 0.1	< 0.1	0.82	< 0.1
		t_1	30.73	3.85	15.01	< 1.00	9.25	< 0.1	0.13	0.22	< 0.1
	out	t_0	25.13	16.9	6.68	4.56	9.31	< 0.1	< 0.1	0.26	< 0.1
		t_1	30.16	3.13	6.89	2.59	6.92	< 0.1	0.47	0.35	< 0.1
						2019					
RB1	in	t_0	8.02	6.4	1.74	1.03	1.88	< 0.1	< 0.1	1.56	< 0.1
		t_1	9.58	2.03	1.93	1.16	1.61	< 0.1	< 0.1	0.11	< 0.1
	out	t_0	12.19	13.35	3.19	2.75	2.81	< 0.1	< 0.1	< 0.1	< 0.1
		t_1	8.95	2.00	1.80	1.29	1.31	< 0.1	< 0.1	0.17	< 0.1
RB5	in	t_0	39.14	4.57	9.54	1.92	16.36	< 0.1	< 0.1	0.26	< 0.1
		t_1	44.25	5.99	11.3	2.34	10.08	< 0.1	< 0.1	0.18	< 0.1
	out	t_0	42.17	16.74	8.11	2.23	7.41	< 0.1	< 0.1	< 0.1	< 0.1
		t_1	31.53	6.28	9.95	< 1.00	4.32	< 0.1	< 0.1	0.23	< 0.1
RB6	in	t_0	15.26	1.71	3.45	1.06	5.78	0.17	< 0.1	0.20	< 0.1
		t_1	17.70	1.08	4.73	< 1.00	4.85	< 0.1	< 0.1	< 0.1	< 0.1
	out	t_0	13.88	1.98	3.11	< 1.00	6.61	< 0.1	< 0.1	0.43	< 0.1
		t_1	17.46	< 1.00	3.98	< 1.00	3.81	< 0.1	< 0.1	0.46	< 0.1
RT1	in	t_0	6.05	1.89	1.35	< 1.00	2.34	< 0.1	< 0.1	0.13	< 0.1
		t_1	6.58	2.65	1.37	1.05	< 1.00	< 0.1	< 0.1	0.37	< 0.1
	out	t_0	5.43	3.32	1.11	6.86	3.2	< 0.1	< 0.1	< 0.1	< 0.1
		t_1	12.15	< 1.00	2.34	1.14	< 1.00	< 0.1	< 0.1	0.40	< 0.1

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RT4	in	t_0	6.47	< 1.00	1.04	< 1.00	1.90	< 0.1	< 0.1	0.17	< 0.1
		t_1	6.37	1.40	1.41	1.23	2.06	< 0.1	< 0.1	< 0.1	< 0.1
	out	t_0	5.11	1.57	1.06	2.63	3.01	< 0.1	< 0.1	< 0.1	< 0.1
		t_1	6.11	1.11	1.11	1.44	2.11	< 0.1	< 0.1	0.14	< 0.1
RT5	in	t_0	29.79	3.47	8.47	1.30	15.28	< 0.1	< 0.1	0.29	< 0.1
		t_1	16.98	2.99	4.67	< 1.00	6.17	< 0.1	< 0.1	0.29	< 0.1
	out	t_0	27.45	4.55	4.73	2.24	12.97	< 0.1	< 0.1	47.6	< 0.1
		t_1	24.07	5.61	7.69	2.47	7.64	< 0.1	< 0.1	0.71	< 0.1

Oxadiazon was detected in almost all samples analyzed in concentrations up to 47.6 µg l^{-1} (RT5_out_t_0), showing widespread contamination, although the organic paddies showed concentrations generally lower (0.45 ± 0.52 µg l^{-1}) than conventional ones (2.36 ± 9.44 µg l^{-1}), but still present even before the first treatment of the culture cycle (t_0). The overall variability among the rice fields is such that no statistically significant difference has been identified ($p = 0.343$), considering both the individual years of investigation and the overall comparison among organic and conventional crops.

Other pesticides such as Metolachlor and Oxyfluorfen were detected in very low concentration in sporadic cases (Table 6), whereas the other active ingredients analyzed, they were all below the LOQ of specific method, except for lambda-cyhalothrin in RB6_in_t_0 (0.17 µg L^{-1}).

3.3. Ecotoxicological effects of soil

The soil samples of conventional paddies showed significant toxicity for root elongation (Table 7) and for the bioluminescence inhibition assays on eluate (Table 8), in particular for RT5 and RT4 rice fields, making the difference between organic and conventional crops statistically significant for *L. sativum* ($p = 0.047$) and *S. saccharatum* ($p = 0.001$).

Table 7. Results of phytotoxicity tests with *Lepidium sativum* (Ls), *Sinapis alba* (Sa), *Sorghum saccharatum* (Ss) on samples of soil collected during the campaigns t_0 and t_1 (in bold the significative effects above the threshold). Negative sign indicates biostimulation of root growth in comparison to control (Ch = rise chamber; em = embankment).

2018			Ls		Sa		Ss		2018			Ls		Sa		Ss	
Conventional			(%)	sd	(%)	sd	%	sd	Organic			(%)	sd	(%)	sd	(%)	sd
RT1	ch	t_0	17.81	7.61	1.84	2.75	**-48.62**	7.88	t_0	em	RB4	**30.48**	12.24	21.80	9.86	19.47	7.04
		t_1	**38.20**	4.88	24.53	7.83	4.66	12.09	t_1			26.94	17.37	15.51	14.62	21.04	15.51
RT4	em	t_0	25.84	13.36	**36.27**	14.63	**40.81**	3.88	t_0	em	RB1	18.11	14.57	-6.79	10.25	-12.43	22.14
		t_1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	t_1			23.67	2.34	-1.50	11.38	-11.97	13.09
	ch	t_0	**30.91**	15.18	29.94	10.42	**35.84**	15.39	t_0	ch		-4.05	8.69	3.13	15.74	-20.45	24.46
RT5	em	t_1	**94.34**	0.58	**82.90**	3.25	**32.62**	12.63	t_0	em	RB5	24.41	10.99	7.69	8.27	23.60	14.84
	ch	t_0	25.15	6.45	8.28	6.38	15.96	1.02									
		t_1	**59.71**	9.35	**53.18**	6.48	24.62	7.46	t_1			**57.66**	14.97	23.59	6.33	19.57	23.58
2019			**Ls**		**Sa**		**Ss**		**2019**			**Ls**		**Sa**		**Ss**	
RT1	em	t_0	24.18	5.92	**41.66**	5.33	17.52	12.06	t_0	em	RB6	20.26	3.96	20.71	16.14	14.02	2.83
		t_1	49.06	3.37	**59.85**	6.02	24.37	5.53	t_1			6.07	8.59	9.04	11.63	-10.85	5.62
RT4	em	t_0	22.40	5.52	21.14	2.96	1.60	14.33	t_0	em	RB1	28.56	2.60	-8.25	10.64	5.54	3.60
		t_1	18.19	2.79	**34.99**	10.24	8.75	8.11	t_1			**32.26**	2.70	6.97	20.02	5.90	5.22
RT5	em	t_0	**30.55**	10.42	23.79	0.13	19.44	2.39	t_0	em	RB5	21.11	16.30	10.62	13.76	14.08	11.87
		t_1	**72.78**	4.83	**73.08**	2.18	**58.79**	5.15	t_1			15.85	9.83	11.19	10.04	13.47	8.86

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Table 8. Results of bioassays with *Aliivibrio fischeri* on samples of soil eluate collected during the campaigns t_0 and t_1 campaigns (in bold the effects above the threshold). Negative sign indicates biostimulation in comparison to control (Ch = rise chamber; em = embankment).

2018									
Conventional crops			**%**	**sd (%)**	**Organic crops**			**(%)**	**sd (%)**
RT1	ch	t_0	**-15.60**	7.92	RB4	em	t_0	**21.50**	9.50
		t_1	**31.67**	20.65			t_1	-11.32	18.49
RT4	em	t_0	-3.51	25.78	RB1	em	t_0	-0.24	15.68
							t_1	-28.70	36.55
	ch	t_0	-11.04	21.68		ch	t_0	**25.32**	14.53
RT5	em	t_0	**-18.65**	19.62		ch	t_0	10.88	7.90
	ch	t_0	**25.26**	14.42	RB5	em	t_0	**-24.77**	39.92
		t_1	1.16	21.12			t_1	2.69	4.60
2019									
RT1	em	t_0	10.00	18.21	RB6	em	t_0	**-90.59**	107.65
		t_1	-11.28	32.56			t_1	-11.84	13.97
RT4	em	t_0	**30.57**	33.73	RB1	em	t_0	**-26.33**	58.08
		t_1	10.56	14.81			t_1	-0.33	11.21
RT5	em	t_0	**-24.17**	33.99	RB5	em	t_0	-3.23	8.73
		t_1	**-55.48**	78.87			t_1	**-18.89**	11.82

These results are in agreement with the chemical data measured in conventional paddies regarding traces of Oxadiazon (RT1, RT4 and RT5), and Oxyfluorfen (RT1 and RT5), two herbicide pesticides the first of which could be used in Italy until 30 June 2020, whereas Oxyfluorfen is authorized until 2024 [46].

3.4. Ecotoxicological effects of water

Table 9 reports the results of bioassays expressed as bioluminescence inhibition for *A. fischeri*, average immobilization for *D. magna*, growth rate inhibition for *R. subcapitata* and leaf growth inhibition for *S. polyrhiza* (only for 2018), respectively.

Table 9. Bioassays results expressed as percent effect (%) with standard deviation (ds) on paddy water samples collected during campaigns t_0 and t_1 before and after PPPs treatment; in and out labels refer to samples taken from streams entering and exiting the paddy water chamber; boldface indicates inhibition effects above the toxicity thresholds; negative sign indicates biostimulation in comparison to the control.

Aliivibrio fischeri												
Conventional			**2018**		**2019**		**Organic**			**2018**		**2019**	
			(%)	**ds**	**(%)**	**ds**				**(%)**	**ds**	**(%)**	**ds**
RT1	in	t_0	-11.1	8.4	-4.00	10.00	t_0	in	RB4 (2018) RB6 (2019)	-6.5	6.8	**96.13**	10.24
	out		-8.9	7.4	**95.99**	10.60		out		14.4	13.3	-7.36	7.97
	in	t_1	-5.4	12.8	-4.28	14.08	t_1	in		-3.1	8.5	-19.61	13.75
	out		-5.9	5.4	-12.05	9.74		out		-7.4	5.5	-26.92	17.46
RT4	in	t_0	-11.2	20.8	3.11	17.97	t_0	in	RB1	-8.2	11.9	**94.04**	11.48
	out		-1.5	8.3	**94.95**	11.51		out		**21.2**	26.9	**90.22**	17.59
	in	t_1	-7.1	10.1	-5.35	6.57	t_1	in		-10.0	11.0	**81.88**	18.99
	out		-0.8	10.1	**97.16**	7.52		out		**60.1**	24.7	**46.99**	10.76
RT5	in	t_0	1.3	11.4	**69.53**	13.28	t_0	in	RB5	4.7	9.1	**32.38**	10.02
	out		7.7	4.3	**77.95**	19.08		out		4.9	14.7	**39.99**	21.93

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	in	t_1	-6.4	10.4	**36.77**	10.96	t_1	in		-17.1	17.7	**38.67**	7.59
	out		-15.0	14.9	**42.00**	5.00		out		7.9	10.1	**37.85**	9.30

Daphnia magna

Conventional			2018		2019		Organic			2018		2019	
			(%)	ds	(%)	ds				(%)	ds	(%)	ds
RT1	in	t_0	**37.0**	51.8	0.0	0.0	t_0	in	RB4 (2018) RB6 (2019)	**25.0**	27.8	0.0	0.0
	out		**97.0**	7.1	0.0	0.0		out		7.5	10.4	0.0	0.0
	in	t_1	0.0	0.0	0.0	0.0	t_1	in		0.0	0.0	0.0	0.0
	out		7.5	10.4	0.0	0.0		out		2.5	7.1	0.0	0.0
RT4	in	t_0	**62.5**	51.8	5.0	0.0	t_0	in	RB1	0.0	0.0	5.0	10.0
	out		15.0	23.3	0.0	0.0		out		**97.5**	7.1	**55.0**	19.1
	in	t_1	0.0	0.0	0.0	0.0	t_1	in		2.5	7.1	**45.0**	19.1
	out		**50.0**	53.5	0.0	0.0		out		15.0	9.3	**75.0**	19.1
RT5	in	t_0	**25.0**	20.7	15.0	10.0	t_0	in	RB5	0.0	0.0	15.0	19.1
	out		5.0	9.3	**45.0**	34.2		out		0.0	0.0	**40.0**	23.1
	in	t_1	0.0	0.0	**25.0**	19.1	t_1	in		0.0	0.0	20.0	16.3
	out		0.0	0.0	**25.0**	10.0		out		2.5	7.1	0.0	0.0

Raphidocelis subcapitata

Conventional			2018		2019		Organic			2018		2019	
			(%)	ds	(%)	ds				(%)	ds	(%)	ds
RT1	in	t_0	**92.0**	2.1	**96.8**	1.7	t_0	in	RB4 (2018) RB6 (2019)	**100.0**	11.0	**95.0**	1.2
	out		**91.1**	2.9	**97.2**	1.9		out		**100.0**	4.3	**95.3**	3.1
	in	t_1	**87.0**	0.6	**98.3**	1.7	t_1	in		**89.0**	0.6	**99.4**	2.8
	out		**100.0**	3.8	**97.6**	4.4		out		**86.0**	2.1	**99.4**	2.0
RT4	in	t_0	**100.0**	7.6	**89.1**	4.5	t_0	in	RB1	**100.0**	10.3	**88.5**	3.9
	out		**93.0**	2.3	**97.8**	3.5		out		**84.5**	5.1	**70.3**	7.3
	in	t_1	**87.0**	3.3	**94.5**	1.7	t_1	in		**100.0**	7.2	**100.0**	4.6
	out		**87.3**	2.4	**80.5**	3.9		out		**90.0**	11.9	**77.1**	8.3
RT5	in	t_0	**97.5**	2.9	**55.1**	8.3	t_0	in	RB5	**84.1**	2.2	**100.0**	3.7
	out		**96.3**	3.8	**36.6**	4.5		out		**93.0**	2.3	**78.4**	3.7
	in	t_1	**95.5**	1.7	**99.0**	5.4	t_1	in		**94.3**	1.0	**51.4**	5.9
	out		**94.8**	1.4	**75.3**	5.7		out		**98.7**	2.2	**76.0**	2.6

Spirodela polyrhiza

Conventional			2018		Organic			2018	
			(%)	sd (%)				(%)	sd (%)
RT1	in	t_0	**-37.9**	33.8	t_0	in	RB4/RB6	-16.3	47.2
	out		-0.6	23.8		out		-5.1	35.6
	in	t_1	-10.4	34.4	t_1	in		-19.5	26.8
	out		-8.0	34.5		out		-11.8	37.2
RT4	in	t_0	-15.6	28.0	t_0	in	RB1	-44.6	51.0
	out		-2.6	30.5		out		**-35.9**	32.9
	in	t_1	12.7	32.7	t_1	in		-19.5	45.4
	out		**-34.4**	27.2		out		**-30.2**	42.6
RT5	in	t_0	**25.6**	20.4	t_0	in	RB5	**81.9**	6.8
	out		10.0	27.7		out		**51.5**	10.2
	in	t_1	-0.2	26.2	t_1	in		-1.7	27.8
	out		**23.7**	23.0		out		-3.3	31.9

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In the 2018 campaign, with regard to *A. fischeri* bioassay, toxicity effects were found only in RB1, with particular reference to water leaving organic rice chambers; regarding the 2019 campaign most of the samples showed significant inhibition of bioluminescence, with no statistical differences between conventional and organic crops ($p > 0.05$).

Despite being, the least sensitive among the organisms of the selected battery, *D. magna* showed some important toxicity effects on both conventional and organic paddies for both monitoring campaigns. It should be pointed out that the toxicity data on crustacean are particularly relevant, since, from an ecotoxicological point of view, this is an organism characterized by relatively low sensitivity, but at the same time by high ecological value for the environment in question, as it is a species normally found in the aquatic environments of rice paddies that adopt organic cultivation methods [47].

The green alga *R. subcapitata* was the most sensitive species in the battery of bioassays used: for all monitoring campaigns, water samples showed high toxicity, but without particular differences between inlet and outlet water samples, between conventional and organic rice fields. These ecotoxicological effects could be associated with the presence of at least three herbicides: Metolachlor, Oxadiazon and Oxifluorfen. In particular, Oxadiazon is found in all organic and treated fields (Table 6), although these substances have not been officially used in organic rice fields. Oxadiazon is known to have important toxic effects on *R. subcapitata* [48]. The high toxicity could therefore be linked to the simultaneous presence of these herbicides in the water, considering other possible synergistic effects with other contaminants not researched in this study but found in the past in paddy field waters in Piedmont (https://www.snpambiente.it/2017/07/11/monitoraggio-dei-fitofarmaci-delle-acque-piemontesi-nelle-risaie/).

Except for sample taken during t_0 in paddy RB5, for *S. polyrrhiza* no inhibition values were revealed, but a general tendency towards biostimulation, probably due to the presence of nutrients in the water.

3.5. Ecotoxicological Hazard Index and statistical analysis

The results of bioassay applied to embankment and chamber soil, and water samples were elaborated by the ecotoxicological Hazard Index, producing the hazard levels showed in tables 10.

Table 10. Ecotoxicological hazard (HQ_{eco}) applied to paddy field soil samples. The index is referred to a bioassay battery with 7 test-species. Colors refers to the level of hazard as followed: green = absent; orange = moderate; red = major; black = severe.

2018							2019						
	soil			water				soil			water		
ID	Sampling point and campaign		HQ_{eco}	Sampling point and campaign		HQ_{eco}	ID	Sampling point and campaign		HQ_{eco}	Sampling point and campaign		HQ_{eco}
RB1	em	t_0	0.16	in	t_0	2.37	RB1	em	t_0	0.10	in	t_0	5.13
				out		8.37					out		7.32
	ch	t_0	0.14	in	t_1	2.41			t_1	0.17	in	t_1	6.29
	em	t_1	0.09	out		4.64					out		7.42
RB4	em	t_0	0.59	in	t_0	3.6	RB5	em	t_0	0.10	in	t_0	3.63
				out		3.22					out		5.96
		t_1	0.13	in	t_1	2.37			t_1	0.05	in	t_1	3.94
				out		2.41					out		3.69

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RB5	em	t_0	0.07	in	t_0	2.42	**RB6**	em	t_0	0.38	in	t_0	5.06
				out		3.01					out		2.37
				in		2.37					in		2.37
		t_1	0.30	out	t_1	2.59			t_1	0.01	out	t_1	2.37
RT1	ch	t_0	0.01	in	t_0	3.14	**RT1**	em	t_0	0.35	in	t_0	2.37
				out		7.16					out		5.06
		t_1	0.68	in	t_1	2.37			t_1	0.45	in	t_1	2.37
				out		2.55					out		2.37
RT4	em	t_0	0.29	in	t_0	5.48	**RT4**	em	t_0	0.66	in	t_0	5.06
				out		2.66					in		2.46
	ch	t_1	0.25	in	t_1	2.37			t_1	0.21	in	t_1	2.37
				out		4.84					out		5.06
RT5	em	t_0	0.71	in	t_0	3.61	**RT5**	em	t_0	0.26	in	t_0	4.62
	ch	t_0	0.57	out		2.69					out		3.49
		t_1	0.50	in	t_1	2.37			t_1		in	t_1	4.15
				out		2.37				0.98	out		4.95

With regard to the soil samples, no ecotoxicological hazard was detected (HQ < 1), due to a general absence of toxicity, according with the low presence of pesticides (table 5). Therefore, neither differences between organic fields and their corresponding conventional fields, nor differences between the two survey campaigns, can be highlighted. This is in perfect agreement with the general absence of pesticide residues in soil and sediment samples.

The situation is quite different for water samples. In general, important toxic effects were found on several organisms and for several end-points, such as to determine an ecotoxicological hazard in all samples without exception, sometimes even "major" or "severe" (table 10). The ecotoxicological hazard affects both conventional and organic paddy waters, with the highest hazard measured in the RB1 field, especially in 2019.

This ecotoxicological picture is also in agreement with the findings of the chemical analysis, with particular reference to the ubiquitous presence of Oxadiazon and Oxyfluorfen.

Figure 2 shows the plot of a Principal Component Analysis (PCA) applied to raw data that explains 67,7 % of total variance. Almost all samples appear to be distributed along a gradient oriented with respect to the first component (PCA1), whose main variable contributing to this distribution is the parameter Ca (with the higher factor loading of – 0.924) and secondly Mg and S in the same way (factor loading of - 0.241 and – 0.251, respectively), in accordance with the significantly higher content of these macroelements in organic crops. With respect to the second component (PCA2), the sample RT5t0out2019 is

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clearly distinguished in the factorial space due to the highest Oxadiazon concentration, associated to the highest factor loading (- 0.940).

This herbicide is therefore the substance that most influences the characteristics of paddy water; its use is regularly reported by conventional paddies, while it has not been used by organic ones.

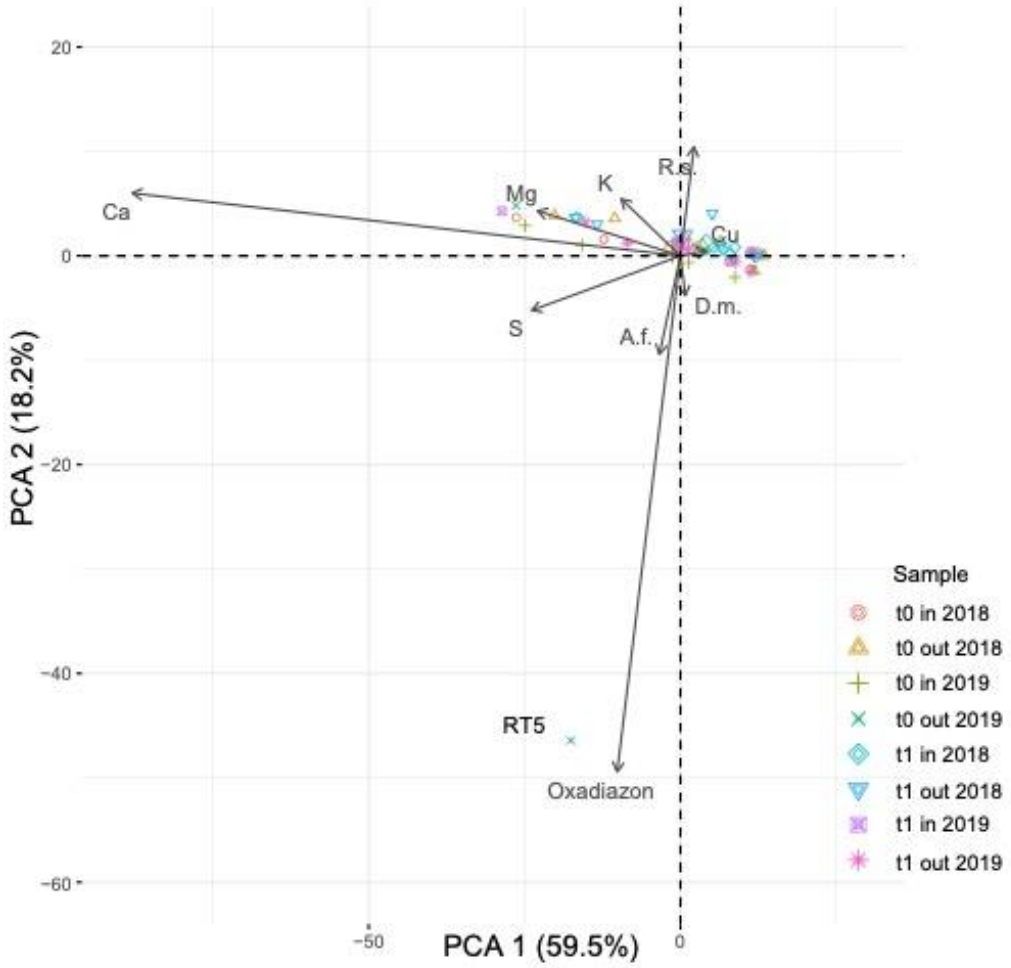

Figure 2 – Plot of PCA applied to chemical and ecotoxicological characteristics of all water samples from rice paddies (t0 and t1 labels refer to the campaigns before and after PPPs treatment; in and out to samples taken from streams entering and exiting the paddy water chamber in 2018 and 2019. Only parameters (arrows) that contribute more than 15% on the variability of the samples are shown.

5. Conclusions

Herbicides are the main PPPs used in rice fields to control herbaceous and aquatic plants competing with rice, fungicides are used to control Blast Disease (i.e. *Pyricularia grisea*) and insecticides to control weevils.

In the chemical analysis of paddy water, the ubiquitous presence of Oxadiazon in all biological and conventional fields was registered, quantitatively higher in the latter, where also other PPPs such as Metolachlor. The widespread and also relatively

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homogeneous concentration of Oxadiazon, which may explain the important and general ecotoxic responses detected in the waters, is probably due to the cascade circulation pattern of irrigation water among the different rice fields. However, it is reasonable to assume that these concentrations will decrease rapidly over time in the runoff water, accounting for volatilization, degradation, leaching to groundwater, and sorption to soil [49].

The presence of residues of other PPPs also in soil samples (Oxyfluorfene and Pendimethalin) could explain the higher frequency of positive, sometimes important, ecotoxicological responses, found in conventional rice fields.

Therefore, this study highlighted the importance to assess the impact of pesticides directly in paddy fields using an integrated approach, because the chemical or ecotoxicological approach alone is unable to consider numerous environmental factors that can occur as the photodecomposition responsible of rapid degradation [1], the hydrolysis influenced by the flooded conditions and pH of the paddy water [50, 51], the volatilization favored by high temperatures [52, 53], and the microbial degradation [54].

Nowadays it is widely recognized that the impact of chemical pollution should be evaluated by giving increasing importance to the assessment of biological effects due to the contaminants, and using an integrated approach with chemical data. An important advantage of these approaches is the added interpretative value derived from the integration of multiple typologies of studies, thus improving the ability to describe and interpret variations of environmental conditions [55]. The Weight Of Evidence Approach has already been validated in several case studies for environmental risk assessment associated with polluted sediments, harbor areas, or complex natural and anthropic impacts on the marine environment [42-44; 55-59].

The application of a multidisciplinary approach has allowed the identification of the main cause of contamination in rice fields in relation to ecotoxicological effects, i.e. Oxadiazon freely circulating in waters. Indeed, it is quite likely that the Oxadiazon applied to conventional rice paddies move to organic from ones through the circulation of water, determining an ecotoxicological response also in organic fields, thus reducing the positive effects of organic farming practices. The realization of organic districts with a water circulation isolated from conventional fields appears to be a valid and effective solution to limit the transfer of the PPPs.

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