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Research Article

Characterization of anthropogenic impacts in Mediterranean intermittent rivers with chemical, ecological and hydrological indicators

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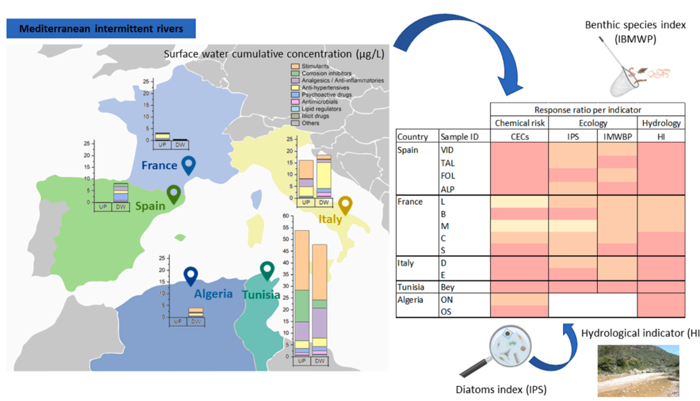
HIGHLIGHTS

- Hotspots of pharmaceutical contamination and environmental risks were identified.
- All indicators declined notably from the upper to the lower parts of the rivers.
- Azithromycin, diclofenac and losartan exceeded PNEC values in all countries.
- Tunisian catchment displayed the most compromised condition across all indicators.

GRAPHICAL ABSTRACT



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ABSTRACT

Water scarcity in the Mediterranean area has increased the number of intermittent rivers, whose flow ceases either occasionally or totally. Key elements to characterize their dynamics are water quality, hydrological, and ecological status, when wastewater effluents dominate flow. Regarding water quality, pharmaceuticals are major pollutants, and serve as indicators of wastewater presence. Intermittent rivers are biodiversity hotspots where their hydrological regime may suffer alterations associated with wastewater effluents, making them harder to characterize than perennial streams. This study aimed to integratively characterize intermittent rivers through chemical, ecological and hydrological status calculating respective indices in twenty Mediterranean intermittent wastewater-impacted rivers located in Spain, France, Italy, Algeria and Tunisia. Pharmaceuticals were used as indicators assessing their frequency of PNEC exceedance and detection; while two ecological indicators and one hydrological indicator were used to evaluate wastewater stress on catchments. All indicators displayed a

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noticeable decline from upper to lower parts of the rivers, proving the effect of anthropogenic stressors on the aquatic environment. The Tunisian catchment displayed the most compromised conditions across all indicators, and the Algerian site even though low concentrations were detected, five compounds exceeded PNEC thresholds. This highlights the need for increased dedication and the adoption of water pollution solutions.

1. Introduction

Water scarcity is becoming more of an issue in the Mediterranean region because of population growth and changes in land use, which are driving up demand. Simultaneously, climate change is causing declining rainfall, further reducing water availability. Consequently, the percentage of intermittent rivers, which are streams that temporarily stop flowing at specific locations, increases [1,2]. The natural water shortage of the intermittent streams makes them more vulnerable to organic pollution and nutrients compared to permanent streams. The aquatic communities suffer extremely from three main stressors organic pollution, nutrients, and water shortage. The continuous emission of effluents from wastewater treatment plants (WWTPs) changes the hydrology of the streams and rivers and the communities to more generalist pollution tolerant communities and drive the specific adapted but highly vulnerable fauna and flora of intermittent rivers to extinction.

In European as well as in Middle East and North Africa (MENA) Mediterranean countries, watershed management is an essential tool to maintain and reach appropriate water quantity and quality in agreement with the European Water Framework Directive (WFD) aims [3]. However, the characterization of wastewater-impacted intermittent waterways in arid and semi-arid regions, have received little attention; the dry season can last for several months and during that time, insufficient dilution of pollutants is a major issue. Point source (PS) and urban WWTP effluents could make up as much as 100% of the intermittent river flow during these periods [4,5]. Water quality, hydrological regime, and ecological status in periods with a high fraction of wastewater effluent contribution to total flow are three fundamental elements to understand the dynamics of intermittent waterways. Until recently, monitoring programs are not specific for temporary rivers. However, sampling protocols and assessment systems developed for perennial water bodies [6,7] are frequently adapted for intermittent water bodies, which suffer from the same stressors, often to an even greater degree. The hydrological status, studying the divergence of actual flow regime from its natural condition, may provide fundamental elements to interpret biological and water quality samplings, especially in temporary rivers where the occurrence of habitats such as pools and riffles influence river ecology and the natural flow regime variability may be confused with anthropogenic impact [8,9]. However, few studies analyzed the hydrological status and flow regime alterations due to WWTPs [10]. Monitoring activities are more challenging than in perennial streams due to the high spatial variability of rainfall events, a variety of environmental factors (such as soil hydraulic properties, land use, and agronomic practices), and the difficulty of establishing reference conditions. The most common method to assess the flow regime alterations is based on hydrological indicators, which are computed by using daily or monthly streamflow recorded over a long period [11–13].

In the context of environmental chemical water monitoring, the WFD includes the surveillance of contaminants of emerging concern (CECs). Nevertheless, despite the efforts made by the daughter directives [14] to extend this scope, the directive's coverage remains restricted to a limited subset of compounds compared to the vast array of compounds found in environmental samples. Pharmaceuticals are one of the major groups present in wastewater effluents and therefore in the dry season they can be the most important group of organic pollutants detected. For several decades, the occurrence of pharmaceuticals in surface waters has been investigated but only a very limited number of studies reported them in intermittent rivers [15]. To the best of our knowledge, this is the first attempt to identify in intermittent rivers key indicators of wastewater pollution with reported resistance to biodegradation.

With the overall goal of generating a map of the chemical, ecological, and hydrological status of Mediterranean intermittent rivers, five study areas comprised of 20 rivers that were impacted by different wastewater effluent discharges or by diffuse PS were studied to (i) analyze the occurrence and spatial distribution of pharmaceuticals; (ii) identify the most critical compounds by evaluating the predicted no-effect concentrations (PNEC) exceedance applying the NORMAN prioritization framework; (iii) evaluate the ecological status by analyzing two biological indicators; (iv) evaluate the impact of anthropogenic pressures and their effect on hydrological alteration; and (v) integrate chemical, ecological, and hydrological indicators to explore differences between intermittent catchments located in countries on the northern and southern shores of the Mediterranean Sea.

2. Materials and methods

2.1. Sampling sites

This study was focused on Mediterranean intermittent streams, for which five main study areas were selected: three located in the northern Mediterranean Sea, including sampling sites in Spain, France and Italy, and two located in the south, in Algeria and Tunisia (Fig. 1). These five areas represent a variety of typical Mediterranean stream conditions, covering a diverse range of climatic, geographic and hydrological characteristics, but with the overall communality that the streams were intermittent. A total of 49 samples were collected, covering twenty intermittent rivers. Sample collection was performed between March 2021 and May 2023 just before the start of the dry season. The duration of the dry season depends on the country but it generally ranges from June to September in most countries, extending a few more months specially in the south of Italy and north Tunisia. Based on the duration of the dry season, these rivers can be considered representative of the Mediterranean streams [16]. The river basins selected in this study are also representative of the respective countries for the anthropogenic pressures (i.e. agriculture, PS and WWTPs) responsible of surface water pollution. Surface water sampling was carried out upstream and downstream of urban WWTP discharges (in Spain and Italy) and along the river network in sites potentially impacted by city pollution and in headwater sites with low impacts (in Algeria, Tunisia and France).

The Spanish river catchment area is located in Catalonia a region in the northeast of Spain (Fig. 1A). Four intermittent streams were selected including the river Ges, Folgueroles Torrent, Riera d'Alpens and Riera de Taradell (VID, FOL, ALP, TAL, respectively in Fig. 1A), each of them being directly impacted by municipal WWTP discharges and high agricultural land uses. A total of 12 samples were collected (three samples for each intermittent stream): an upstream point located 50 m above the WWTP discharge point (DP) and two downstream points, 50 and 250 m downstream of the WWTP DP. The French river catchment, is located in the Montpellier area (Fig. 1B). Five intermittent streams were selected Lirou, Brestalou, Mosson, Cadoule and Salaison (L, B, M, C, S in Fig. 1B), which are surrounded by urban areas and characterized by intensive agricultural land uses. In this case, 2 samples were collected in each studied stream: one upstream of an urban settlement and one downstream, obtaining a total of 10 samples. The selected Italian catchment is located in the Apulia region, south Italy (Fig. 1C) where the Canale D'Aiedda and one of its tributaries were monitored and sampled upstream and downstream of the two most important WWTPs of the basin; Monteiasi and San Giorgio Ionico (D and E, respectively in Fig. 1C) where 3 and 4 samples were collected for each site respectively. A total of 9 samples were collected. The Tunisian basin studied is found in the Grombalia Plain (Fig. 1D), located in northeast Tunisia where the El Bey Wadi was sampled and two of its main tributaries; Tahouna and El Malah Wadis, collecting a total of 8 samples. It flows across an extensively used agricultural region and receives urban and industrial effluents discharged throughout the wadi. Finally, in Algeria, three wadis were selected in the Jijel region (NE Algeria) (Fig. 1E). Kebir Wadi and Mencha Wadi (KW and MW respectively in Fig. 1E) were sampled only downstream to study the effect of urban discharges on the water quality, whereas the Nil Wadi (NW in Fig. 1E), was studied in detail including upstream and downstream points of the main wadi, three of its tributaries (Savoud, Boukraa, and Tasift (SW, BW and TW, respectively in Fig. 1E)) as well as the state of the main wadi after its confluence with its tributaries. A total of 10 samples were collected from the Algerian site. Further information on each river basin can be found in the supplementary material (SM 1). In Tunisia and Algeria, a number of anthropogenic pressures are present from urban and agricultural areas, where discharges flow directly into the wadis; however, these are not well identified. The sources of the target compounds were isolated PSs discharges into the streams in Tunisia and Algeria and urban WWTPs in EU countries.

Once surface water samples were collected, physico-chemical parameters including pH, temperature, conductivity and dissolved oxygen were measured in-situ (Table S1). Additionally, nitrate, ammonia and phosphate content were measured. Abiotic site parameters were evaluated in situ such as lotic/lentic zones relation, artificial flow (coming from WWTPs), shading, eutrophication, between others (further details can be found in Table S2). Samples were transported to the laboratories, filtered at 0.7 μm using fiber glass filters, and stored at −20ºC until processing.

2.2. Target compounds and chemical analysis

The target analytes were comprised of 79 pharmaceuticals (including 21 psychoactive drugs, 21 antimicrobials, 11 antihypertensives, 9 analgesics/anti-inflammatories, 5 illicit drugs, 3 stimulants, 2 lipid regulators, 6 pharmaceuticals classified as others) and 2 corrosion inhibitors, further information on the classification can be found in Table S3 in SM. These compounds were selected due to their widespread consumption and occurrence in surface water [14,17].

The quantitative determination of the analytes was performed following the method described by [18]. Briefly, 500 mL samples were extracted by solid phase extraction (SPE) using custom-built

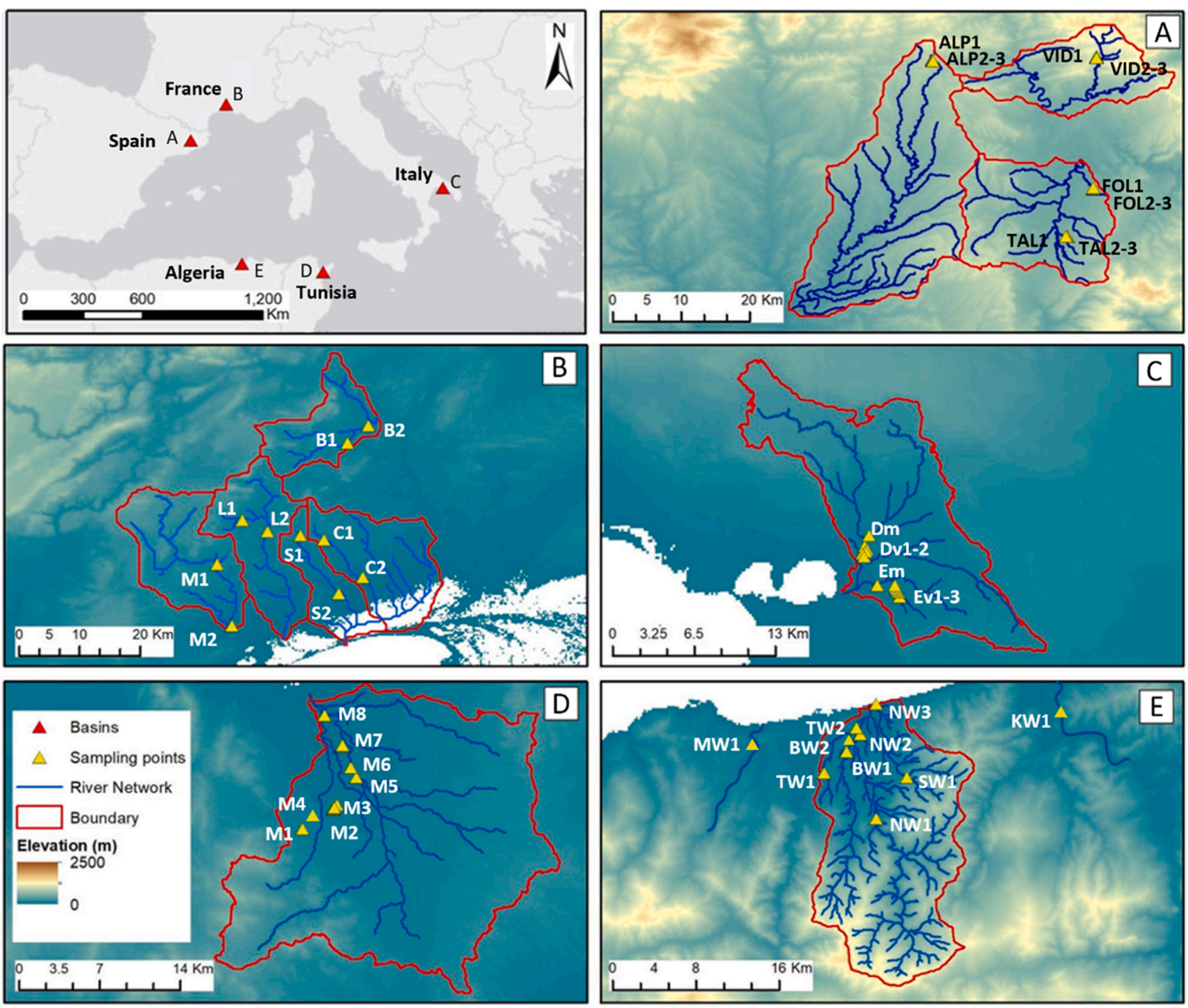

Fig. 1. Studied river basins with sampling locations in Spain (A), France (B), Italy (C), Tunisia (D), and Algeria (E).

multi-sorbent cartridges and were then analyzed by liquid chromatography coupled to a high-resolution Q-Exactive Orbitrap (Thermo Scientific) with electrospray ionization. Chromatographic separation was performed using a Waters Acquity UPLC HSS T3 column (100 ×2.1 mm, 1.8 μm particle size). Positive ionization mode was employed and the mobile phases (0.2 mL min^{-1}) consisted of (A) 100% acetonitrile and (B) 5 mM ammonium formate + 0.1% formic acid in water. Thermo TraceFinder 5.1 software was used for the quantification of the target compounds. A more detailed description of the analytical methodology can be found in [18].

2.3. Prioritization of wastewater pollution chemicals

A prioritization of the studied target compounds was performed where predicted no effect concentrations (PNEC) in freshwater were compared to measured concentrations of the detected compounds. PNEC values were obtained from the NORMAN database [19], those concentrations above the PNEC value would represent ecotoxicological risks. Compounds were then prioritized based on their exceedance to the PNEC threshold value, where a frequency of PNEC exceedance was calculated (FoE), as well as their frequency of detection (FoD). These two frequencies were then used to evaluate chemical wastewater pollution stress on the studied catchments and to prioritize wastewater-borne compounds.

2.4. Diatom and benthic invertebrate analysis

To evaluate the anthropogenic impact from an ecological viewpoint diatoms and benthic invertebrates were sampled. The following are briefly described but further detail on the sample collection can be found in SM3. Diatoms were sampled according to the CEN standard [20], where five fist-sized cobbles were scrubbed using a fresh toothbrush at each sampling site. To the resulting biofilm-scrapings, the hot H_2O_2-HCl method [21] was applied to reach an acceptable diatom cell density and prepare the diatom slides. The finished diatom slides were imaged following the protocol described by [22] for the diatom identification. From the taxalists of each site the ecological quality was calculated using the French Polluosensitivity Index (IPS) [23,24]. This index was developed to characterize different types of pollution including eutrophication, pH and other parameters. It ranges from 0–20, where 0 is the lowest possible value and 20 the highest.

Benthic invertebrates were sampled using a handnet (25 × 25 cm, 500 μm) in a representative 100 m reach of the sites. Substrate composition was assessed in 10% increments, and subsamples proportional to the substrate composition were taken. The subsamples were transferred into white trays and inspected for benthic invertebrates. Each invertebrate specimen was directly identified at least on family level and recorded. A magnification glass was used for identification. The individuals were later on released back to the streams. The final taxa abundances were extrapolated to one square meter.

The ecological quality for the benthic invertebrates was calculated by applying the Spanish Index of Biological Monitoring Working Party (IBMWP) [25]. This index was developed to characterize different types of pollution and has a low score of 0, but does not provide a fixed upper value. We calculated, according to [26], the 95th percentile of the range of all values, as the fixed upper value.

Finally, for a better comparison of the results of both organism groups, both indices were transformed to percentage related to the possible range of values. Here, low values represent good quality and high values represent poor quality.

Samples were collected from Spain, France, Italy and Tunisia. However, the ecological state of the Algerian sites was not studied due to travel restrictions to access the sampling sites.

2.5. Hydrological indicator

River basin hydrology and landscape factors control pollutant transport and instream processes. Hydrological alterations such as water abstractions or water discharge may have a huge influence on river ecology and water quality. Hence, linking hydrological regime to biogeochemical processes is fundamental to understand catchment functioning and water quality. In this study, a hydrological indicator (HI) was defined to improve the understanding of the relationship between flow regime and water quality. In the present paper, since daily streamflow were not available for the investigated basins, a readily accessible HI was used to quantify the overall alteration of the streamflow due to PSs at the sampling time estimated through data and expert judgement. HI may assume five values depending on the ratio R_Q (Eq. 1).

$$R_Q = \frac{PS\ discharge}{Q_d} \quad x \quad 100 \tag{1}$$

Where: *PS discharge* is the contribution to the streamflow from the point sources (localized upstream of the sampling site) recorded the day of the sampling [$m^3\ s^{-1}$]. Q_d is the streamflow recorded in the river section of the monitoring site [$m^3\ s^{-1}$]. Low values represent small hydrological deviations from natural flow regime and high values indicate poor hydrological status (high deviation from its natural flow regime). HI is then classified in five groups; HI = 0 if R_Q ranges from [0–20 %], HI = 25 (R_Q = [21–40 %]), HI = 50 (R_Q = [41–60 %]), HI = 75 (R_Q = [61–80 %]) and HI = 100 when R_Q = [81–100 %]. High values of HI indicate potentially polluted sites. Further information can be found in Table S4 in the SM.

2.6. Response ratio of indicators

To evaluate the state of Mediterranean catchments and integrate chemical, ecological and hydrological indicators, a response ratio was calculated. The contribution of each indicator was calculated as a response ratio (R), i.e. the ratio of the different studied indicators at the impacted sites (downstream) compared to the condition at the upstream sites, where: R= 1 indicated no change or no effect, R< 1 a decrease and R> 1 an increase on water stress [27].

$$R = \frac{Indicator\ of\ Impacted(downstream)}{Indicator\ of\ upstream} \tag{2}$$

2.7. Statistical analyses

Statistical analyses were performed using OriginPro, Version 2023b (OriginLab Corporation, Northampton, USA). One-way analysis of variance (ANOVA) was conducted to assess differences in concentration levels among the different studied countries and to study the influence of human stressors on the intermittent streams in each country. Upon finding significant ANOVA results ($p < 0.05$), Tukey's HSD post-hoc test was applied to identify specific pairwise differences. To assess the correlation between the different response ratios of each indicator, a Spearman correlation test was conducted at a significance level of $p < 0.05$.

3. Results and discussion

3.1. Occurrence of CECs in Mediterranean basins

Of the 81 target contaminants, 72 were detected in at least one of the studied samples. Among these, 8 wastewater-borne compounds (caffeine and its metabolite 1,7 dimethylxanthine, benzotriazole, acetaminophen, carbamazepine, cotinine, lidocaine, and tramadol) were detected in all 49 studied samples, in both upstream and downstream points.

In Fig. 1, the cumulative average concentrations of the nine categories, at the upstream and downstream sampling points are shown for each of the studied countries. Samples from Tunisia displayed the highest concentrations in both upstream and downstream points with cumulative average concentrations of 50 µg/L being similar to those published in a worldwide review [28]. The cumulative average concentration in the samples collected from Italian sites ranked second. In both cases, the upstream points were highly impacted by pharmaceutical concentration, mainly contaminated by the stimulants class. In Italy, it was mainly due to a specific stream (Em, Fig. 2C), which was heavily contaminated, probably illegal PSs were present upstream of the site or septic tanks of isolated houses that partially treated domestic wastewater. The same happened with the French upstream points, however in this case higher drug concentrations were found in the upstream than downstream points. This was attributed to two main streams (Lirou and Mosson) where significantly high concentrations of all classes were observed, which then further decreased downstream, proving attenuation along the streams [29]. The upstream points of Lirou and Mosson are situated near the downtown of two cities, explaining the presence of several compounds. In the case of Algeria and Spain, the clear impact of anthropogenic sources on the studied streams is observed, and enables us to determine the contamination PS, in Spain it clearly corresponded to the WWTP discharge while in Algeria it is linked to urban waste, which is discharged into the wadi without any previous primary treatment.

The distribution, between countries, of contaminants class was evaluated (Fig. S7) where the total median water concentrations, for all countries, followed the order Σstimulants (19 µg/L) > Σantihypertensives (12 µg/L) > Σanalgesics / anti-inflammatories (7 µg/L) > Σcorrosion inhibitors (4 µg/L) > Σpsychoactive drugs 4 (µg/L) > Σantimicrobials (2 µg/L) > Σothers (1 µg/L) > Σillicit drugs (0.1 µg/L) > Σlipid regulators (0.1 µg/L). At a country individual level the Spanish sites, did not follow this trend, and the major contaminant class were the psychoactive drugs, while the stimulants class were extremely low (1 µg/L and 0.009 µg/L respectively). However, the stimulant class predominated in the majority of the Mediterranean basins except for Spanish study area. Caffeine is a commonly found compound at high concentrations in surface waters; its high ubiquity is due to the high consumption of caffeine-containing products. Caffeine concentration ranged from 4.6 to 61,000 ng/L, its highest was detected in the Tunisian study site, (site M5 Fig. 2B). Such high caffeine concentrations are an indicator of untreated domestic sewage discharges, also observed in previous studies [30,31]. Caffeine concentration levels reported in surface water worldwide also detected similar concentrations [28,32,33]. Other than stimulants, Tunisian samples were also highly contaminated by corrosion inhibitors and analgesics/anti-inflammatories classes, and significant differences ($p < 0.05$) were found between Tunisia and the rest of the countries for the stimulants, analgesics / anti-inflammatories lipid regulators class. The Tunisian upstream points studied were highly impacted, due to the presence of different PS, especially the location of factories near the upstream points, which clearly have a high impact. Contrastingly, in the Algerian case study, very low concentrations were detected, demonstrating the existence of low-concentration discharges compared to other sites. In the Spanish case study, psychoactive drugs were the predominant class with concentration up to 3700 ng/L, much higher than the other northern Mediterranean sites. Based on data from the OECD [34], Spain is in the top countries with higher consumption of psychoactive drugs with a daily dose twice as high as in France and Italy. In our study we were able to observe this difference in consumption and to possibly explain the difference observed in cumulative concentrations of this category. As for Algeria and Tunisia, no data on consumption is available. In our study the highest psychoactive drug concentrations corresponded to carbamazepine with maximum concentration of 190 and 3100 ng/L respectively and are similar to those reported previously by [35]. Lamotrigine was also detected at high levels in both sites (Algeria and Tunisia) with maximum concentrations of 160 and 210 ng/L, respectively, much lower than in the northern Mediterranean studied sites from Spain and Italy (660 and

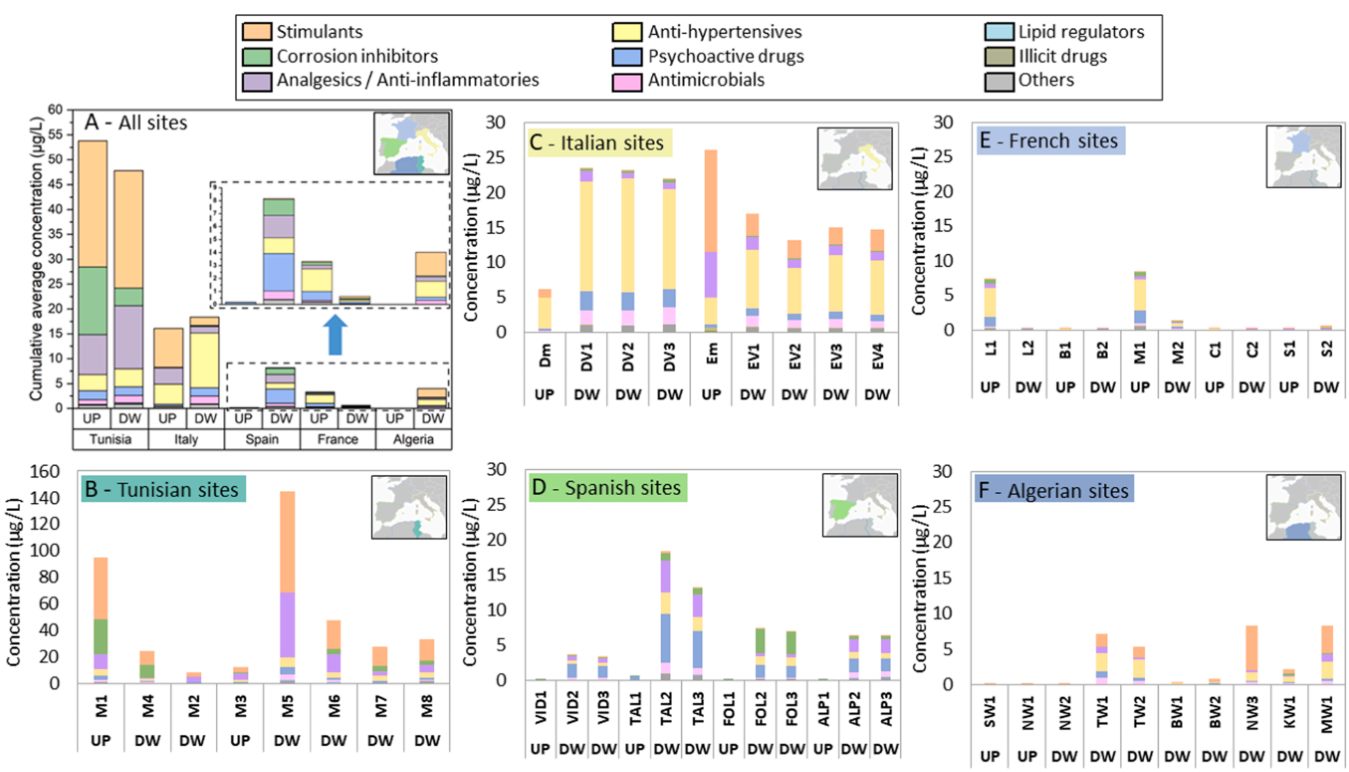

Fig. 2. Cumulative average concentrations (A) across study areas and sampling sites, upstream (UP) and downstream (DW) in descending order of concentrations, classified into 9 main categories. Cumulative average concentration reported here were calculated as the average of the sum concentration of all quantifiable target compounds at each sampling site within the respective UP and DW of the studied river catchment for each country. (B-F) Category concentrations for each country study site.

460 ng/L respectively). In Fig. 2B-F the individual concentration for each country site is shown. In general, the upstream points have lower concentrations of each class, except for some specific cases as described above.

3.2. Prioritized compounds

The measured concentrations of the detected compounds were compared to their lowest PNEC in freshwater organisms. In Table 1, the list of compounds that exceeded their PNEC values is summarized, and hence further attention and regulations are required concerning the reported compounds. The French site had the smallest number of compounds (3) at concentrations above the PNEC values, while the Tunisian sites presented the highest (9). Azithromycin, diclofenac and losartan exceeded the PNEC in all five countries and have been reported to exceed in previous studies [36,37]. Azithromycin presented the highest FoEs, reaching 100% in the Italian and Tunisian catchment. In the Tunisian sites, the PNEC was exceeded in both downstream and upstream points. In general, upstream points did not exceed the PNEC values, except for French sites, where the upstream sites presented the highest concentrations, reaching a maximum of 40% FoE and none of the downstream sites exceeded PNEC values. In the Spanish and Algerian sites, FoE of upstream sites was 0 in all cases, evidencing that no risk is posed until the presence of anthropogenic impacts such as WWTP effluents. The Italian catchment was quite similar, except for 2 compounds, caffeine and losartan, where higher concentrations than PNEC were detected at the upstream sampling site Em. Diclofenac and losartan presented FoEs of 100 % in both Spanish and Italian sites. The findings in this study demonstrate that the presence of azithromycin, diclofenac and losartan pose significant hazards to the ecosystem regardless the location warranting attention on these.

3.3. Ecological status

The relation between physico-chemical parameters and abiotic variables collected from each site were subjected to principal components analysis (Fig. S8) which revealed two main gradients when all sites were analyzed together. Firstly, a large gradient in pH values and dissolved oxygen accompanied by the percentage of lentic flow situations. Secondly, artificial flow induced by effluents of wastewater treatment plants, which accompanied by an increase in conductivity and general chemical pollution. Thus, there is a gradient in organic pollution and additionally in chemical pollution mainly nutrients together with hydrological alteration.

The analysis of indicator species (diatoms and benthic invertebrates) revealed common pollution-tolerant species to be indicative for the impacted sites. *Physa acuta*, an invasive snail native to North America, which proliferated across Europe in recent decades can survive in environments with very low oxygen content and poor water quality, hence it is often found below the effluents of wastewater treatment plants. Furthermore, leeches and chironomids were also identified as indicators of impacted sites. Leeches, which are predators and feed on snails or chironomids, are common in streams with poor water quality. They tolerate low oxygen levels and proliferate when food is abundant. Chironomids comprise many species that are very tolerant to poor water quality because they possess high amounts of red blood cells, enabling efficient oxygen utilization in low-oxygen conditions and are found in large amounts in slow flowing polluted streams. Few significant indicator taxa were identified for unimpacted sites, i.e. some caddisflies and

Table 1
Compounds with PNEC exceedance in Mediterranean intermittent rivers, ordered by country and compound, divided in downstream and upstream sites. Number of studied sites (n), minimum (min.) maximum (max.) and median concentrations, all expressed in ng/L. Frequency of detection (FoD) and frequency of PNEC exceedance (FoE), expressed in %.

Country	Compound	PNEC (ng/L)	Downstream sites						Upstream sites					
			n	Concentration (ng/L)			FoD (%)	FoE (%)	n	Concentration (ng/L)			FoD (%)	FoE (%)
				Min.	Max.	Median				Min.	Max.	Median		
Algeria	Azithromycin	19	8	< 0.17	28	1.5	50	13	2	< 0.17	2.1	1.2	50	0
	Caffeine	1200	8	5.7	5700	880	100	50	2	< 0.23	< 0.23	< 0.23	100	0
	Climbazole	110	8	1.7	290	35	100	25	2	< 0.10	0.37	0.24	100	0
	Diclofenac	50	8	< 0.76	740	81	63	50	2	< 0.76	< 0.76	< 0.76	0	0
	Losartan	12	8	< 0.12	88	4.0	50	25	2	< 0.12	< 0.12	< 0.12	0	0
France	Azithromycin	19	5	< 0.17	2.6	0.57	60	0	5	< 0.17	70	< 0.17	40	40
	Diclofenac	50	5	< 0.76	2.5	< 0.76	20	0	5	< 0.76	480	< 0.76	40	40
	Losartan	12	5	< 0.12	4.7	0.12	20	0	5	< 0.12	81	< 0.12	40	40
Italy	4-Hydroxydiclofenac	220	7	< 3.2	260	150	100	29	2	< 0.49	17	8.6	50	0
	Azithromycin	19	7	150	1100	640	100	100	2	< 0.17	12	6.1	50	0
	Caffeine	1200	7	< 0.23	2490	1930	100	57	2	590	11,000	5800	100	50
	Clarithromycin	120	7	32	160	49	100	43	2	< 0.08	< 0.08	< 0.08	0	0
	Diclofenac	50	7	180	1130	380	100	100	2	< 0.76	43	22	50	0
	Losartan	12	7	40	56	51	100	100	2	1.1	30	16	100	50
Spain	4-Hydroxydiclofenac	220	8	< 3.2	510	40	100	25	4	< 0.49	< 0.49	< 0.49	0	0
	Azithromycin	19	8	2.4	370	74	100	63	4	< 0.17	2.6	1.1	50	0
	Clarithromycin	120	8	< 0.08	380	2.6	75	25	4	< 0.08	0.70	< 0.08	25	0
	Diclofenac	50	8	290	3000	1100	100	100	4	< 0.76	< 0.76	< 0.76	0	0
	Lorazepam	96	8	10	260	58	100	25	4	< 0.09	0.64	0.20	50	0
	Losartan	12	8	42	630	83	100	100	4	< 0.12	1.9	0.78	75	0
	O-desmethylvenlafaxine	880	8	620	3700	860	100	38	4	< 0.32	6.6	3.0	100	0
	Sertraline	9.4	8	1.4	52	3.8	100	25	4	< 0.20	2.4	< 0.20	25	0
	Venlafaxine	880	8	92	970	260	100	13	4	< 0.03	16	2.9	100	0
Tunisia	1 H-Benzotriazole	19,000	5	75	8300	2000	100	0	3	160	23,000	1900	100	67
	Acetaminophen	46,000	5	780	47,100	5000	100	20	3	0.49	10,100	5000	100	0
	Azithromycin	19	5	90	470	320	100	100	3	40	620	49	100	100
	Caffeine	1200	5	8700	60,500	13,000	100	100	3	1050	37,800	1900	100	67
	Carbamazepine	2000	5	360	3110	910	100	20	3	190	1460	360	100	0
	Clarithromycin	120	5	< 0.25	1220	120	100	60	3	28	390	31	100	33
	Climbazole	110	5	190	450	210	100	100	3	32	240	42	100	33
	Diclofenac	50	5	< 0.76	90	< 0.76	20	20	3	< 0.76	23	< 0.76	33	0
	Losartan	12	5	< 0.12	530	39	80	60	3	< 0.12	60	< 0.12	33	33

mayflies, known for their sensitivity to pollution.

Beside benthic invertebrates, the diatom index used (IPS), displayed, particularly in French sites and Spanish upstream sites, very low pollution (Fig. 3A). The downstream sites in Tunisia and Italy had on average the worst values. For the benthic invertebrates where the IBMWP was studied, best scores on average were obtained in the Spanish, French and Tunisian upstream sites and the Spanish downstream sites, while the latter was still lower than the respective upstream sites. The Italian sites showed in comparison to the other countries very low quality values.

The results of the biotic indices clearly indicate that anthropogenic influence impacts both organism groups. While the diatoms mainly react to the water quality parameter, benthic invertebrates also depend on morphological parameters like natural river bottom substrate. Both indices indicated a tendency of the degradation from the upstream sites to the downstream sites, however significant differences ($p < 0.05$) were only observed in the IBMWP index between the UP and DW samples. In comparisons of countries, both indices were significantly different ($p < 0.05$) from each other, where the French sites had the lowest impacts. The Italian sites were most degraded from a morphological point of view as most parts of the drainage system is pure concrete with no natural channel substrates. Benthic invertebrates reacted to this degradation, while benthic diatoms mainly reacted to water quality degradation. The degradation was related to water quality of the wastewater effluent i.e. more efficient wastewater treatment translates to reduce impact in communities. However, in several of our sampling sites the diatom and the benthic invertebrate communities indicated poor water quality, i.e. strong degradation. Thus, our results urgently call for the construction of wastewater treatment plants and the improvement of existing plants.

3.4. Hydrological status

Anthropogenic activities and climate change may alter the natural flow regime with severe impacts for river ecosystems and water quality [38,39]. PS discharges in temporary rivers may induce severe alterations of the flow regime (i.e. timing, magnitude and duration of the low flow) with a shift towards perennial conditions and a near-constant value of the streamflow in dry season [10].

The hydrological status, evaluated for the monitoring sites using the HI, showed that the PS discharges highly altered the natural flow regime with a shift from intermittent to perennial regime as shown previously [10]. For the upstream sampling sites, the HI is zero at all sites located in Spain, the majority of sites located in Algeria, with the exception of TW1, and in three sites in France; this result is indicative of very low or absent hydrological pressures. HI ranges from 25 to 50 in Italy due to the presence of hydrological pressures in the upstream drainage area (i.e. WWTP and domestic effluents from house sewerage plants). Finally, HI is 100 in all upstream sites of Tunisia due to the presence of PS discharges from factories (Fig. 4).

For the downstream sites, HI is 100 in Italy and Tunisia at all sites and in most of the sites located in Algeria since the volume of wastewater discharged from PSs makes up the majority of the streamflow. In French sites, HI ranges from 25 to 75 (Fig. 4). In the case of the Spanish sites, HI varies from 0 to 75, even though downstream sites are clearly affected by WWTP effluent, in some sites the effluents do not constitute a high hydrological pressure. This is probably because the contribution of the WWTPs is quite low compared to the natural streamflow, especially in spring when heavy rainfall occurred the day before the sampling

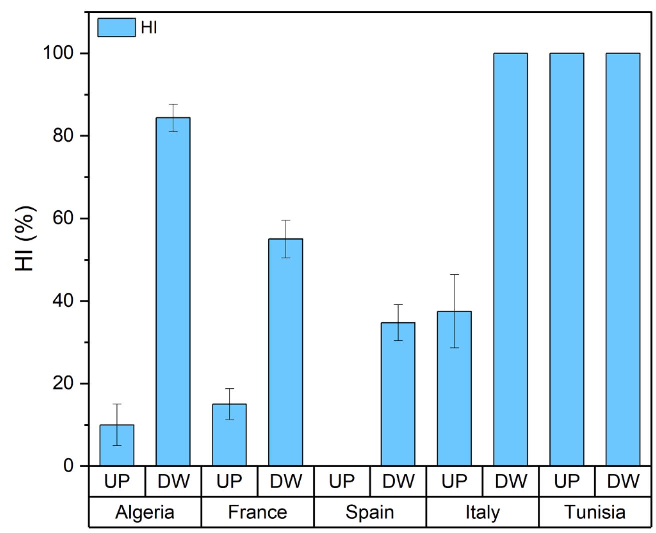

Fig. 4. Average values of the Hydrological Indicator (HI) of flow regime alteration. Error bars show the standard deviation.

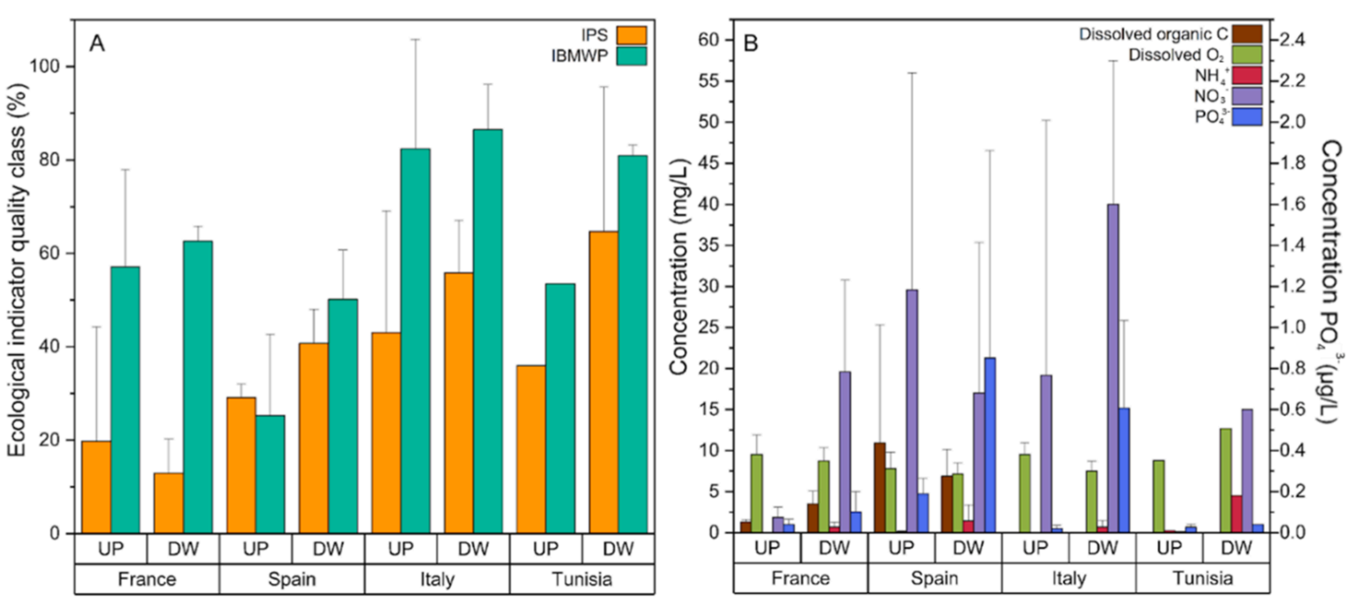

Fig. 3. Average ecological indicator quality class for each studied country, including both IPS and IBMWP (A) and average concentrations of nutrients (nitrogen and phosphate), dissolved oxygen, and dissolved organic carbon (B). Specific site concentrations can be found in Table S2.

campaign. Significant differences were observed between the HI at the upstream and downstream points in France, Italy and Algeria, proving the anthropogenic impact. However, no significant differences were observed in Spain between the UP and DW. This is because the WWTP contribution to the Spanish streams was not as high and no significant hydrological impact was observed for the Spanish sites.

3.5. Response ratio of chemical, ecological, and hydrological indicators on water quality status

To understand better the water quality state of the Mediterranean basins, response ratios were calculated and are resumed in Fig. 5, as a heat map. In general, French sites showed the best water quality values from a chemical, ecological and hydrological point of view; hence we can say that the French catchment was the one with the lowest anthropogenic impact. French sites showed that when HI has a low value, a decrease in chemical stress may occur due to a stronger dilution effect. On the other hand, the Tunisian catchment showed the highest-worrisome values in all indicators. In the case of the Algerian sites, despite the low concentrations detected, similar to those detected in France, response ratios resulted in an increase in water stress when comparing upstream and downstream sites reflecting anthropogenic impacts. As for Spanish and Italian sites, the anthropogenic impact is clearly observed for the chemical and hydrological indicators however, for the ecological indicator no major anthropogenic effect is observed.

The indicators identified are useful for summarizing the anthropogenic impacts; however, they show some limitations. From a hydrological point of view, the HI cannot describe river morphological elements that may greatly influence water quality and river ecology, as the Italian case study showed. On the other hand, the HI did not describe the spatial and temporal variability of the flow regime. Indeed, in the case studies, the river networks show a longitudinal continuum of perennial and intermittent reaches[10,39]. During the dry season, streamflow gradually decreases presenting a sequence of pools, connected by an extremely low streamflow or subsurface flow, disconnected pools, and dry conditions. This peculiarity, which is common in most of the intermittent rivers in the Mediterranean Region [16,40], brings a shift between lotic and lentic conditions that may greatly affect the water quality and river ecology (i.e. invertebrate assemblage) [41]. In addition, floods may have important implications on water quality for remobilizing sediment and pollutants deposited on the river bed during the low flow [42]. Despite its limitation, the HI summarizes and quantifies the impact of PSs contributing to interpreting water quality data and identifying sources of pollutants. These are fundamental steps for river management, especially in data-limited regions.

The ecological indicators show clear degradation from the upstream to the downstream sections, indicating an effect of the anthropogenic stressors on the aquatic communities. This impact is country specific, and at a significant difference as mentioned previously, with stronger impacts in the Tunisian and Italian sites than e.g. in the French [6]. These ecological results mirror the chemical results too as it can be observed in Fig. 5. Nonetheless, we found unequal “starting points” i.e. already degraded communities in several upstream sites independent of the country. Both organism groups proved their ability to detect anthropogenic stress. While sensitivity to oxygen depletion was dominating in benthic invertebrates, the chemical and hydrological stressors served as an additional stress to further deterioration of the communities. As most of the sites suffered from multiple stress factors, diatoms indicated more nutrient enrichment than oxygen depletion or hydrological stress. Although the applied indices were developed for perennial systems [43], they proved sensitivity for intermittent systems after some adjustments. Nonetheless, the applicability in intermittent systems is allegeable as these systems suffer from the same stressors as perennial systems with even more extreme stress during the drying phase [44,45]. At a statistical level, a significant positive correlation was observed between the chemical risk response ratio and the IBMWP with a Spearman correlation coefficient (r) of 0.6. This result indicates a moderate positive relationship between the two variables, suggesting that as the concentration of CECs increase, a corresponding increase in IBMWP is also observed. This implies that chemical stressors may be linked to biological responses in intermittent streams as measured by the IBMWP. In contrast, the correlation values for the other indicators were

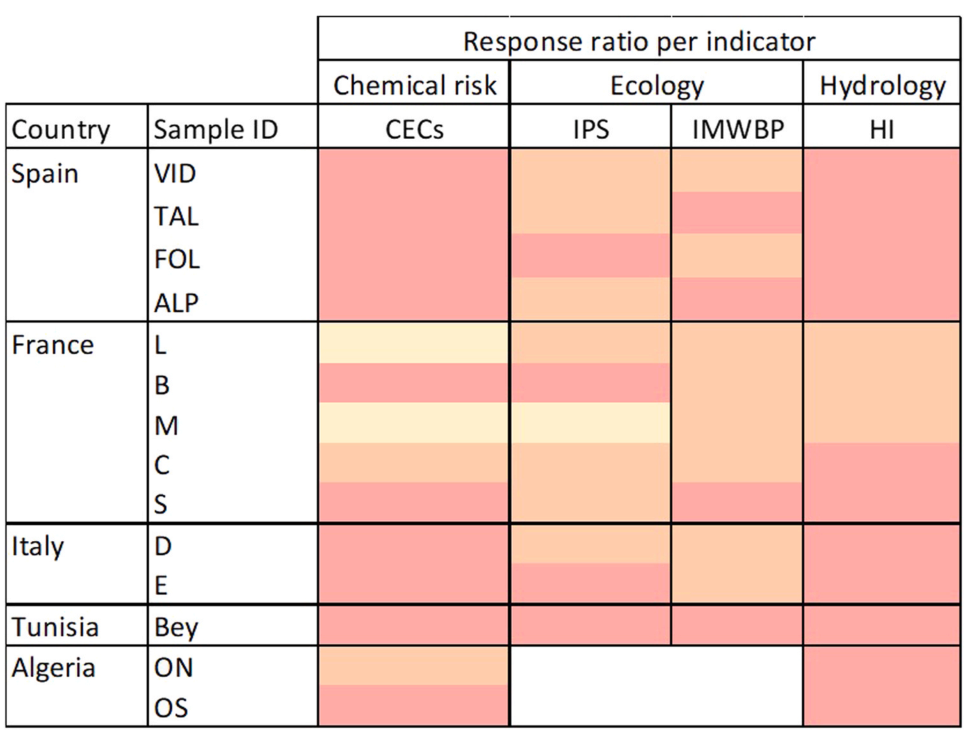

Fig. 5. Heat map of the analysis of response ratios per indicator; red indicating R> 1 increase in stress; orange R= 1 no effect or change; yellow R< 1 decrease in stress.

lower, ranging from 0.05 to 0.2. These values suggest weak correlations that are not statistically significant, indicating that the current data lacks sufficient evidence to draw further conclusions about these indicators relations. To further explore potential correlations between these indicators a higher number of samples are required to enhance statistical correlations. Nonetheless, at a descriptive level, this study represents a first attempt to assess and relate multiple indicators to evaluate human-induced stressors on intermittent streams.

4. Conclusions

This is the first study to compile and compare data from north and south Mediterranean countries, assessing a variety of water indicators including, pharmaceutical pollution, benthic and diatom species indices, and hydrological alteration. The Tunisian catchment presented the most compromised state across all indicators, exhibiting the highest frequency of PNEC exceedance, corroborating the need for intensified efforts to be devoted and the implementation of water pollution mitigations. Conversely, while Algeria presented relatively low CECs concentrations in this study, several compounds still surpass PNEC threshold, proving the need for more monitoring. This highlights the necessity of increased attention to water quality across the Mediterranean, particularly in regions with less monitoring frameworks.

This study also presents a preliminary attempt to assess and relate multiple indicators to evaluate human-induced stressors on intermittent streams. While a significant correlation between CECs concentrations and IBMWP provides valuable insights, further research is necessary to refine the use of these indicators and deepen our understanding of anthropogenic impacts on the Mediterranean aquatic ecosystems.

Furthermore, the relatively poor water quality results obtained for the different indicators showed that maximum efforts should be conducted in the Mediterranean regions to connect urban areas to WWTPs and improve the treatment of the wastewater in the WWTPs. Many new techniques and standards are already at hand and could improve the water quality greatly in the intermittent streams and would additionally aid ecosystem services like irrigational use.

Moreover, the disparity in research attention between north and south Mediterranean countries i.e. European and Northern Africa becomes evident. The lack of or less previous monitoring in the south Mediterranean regions underscores the necessity of increased investigation efforts there rather than in areas where lower risks to the ecosystem and human health are likely, such as the case of the French catchment. Finally, this study emphasizes that a comprehensive assessment of all water indicators is indispensable for a better understanding of water catchment conditions. By combining these integrated approaches for intermittent river characterization with river management and mitigation actions strategies, such as upgrading existing treatment plants and enforcing stringent effluent quality standards as well as the restoration of riparian zones with the establishment of woody riparian vegetation, their health status can be improved. Implementing this strategy, Mediterranean countries can make significant progress in protecting the integrity and functionality of their intermittent streams. Safeguarding these unique aquatic habitats is not only vital for preserving biodiversity but also crucial for maintaining the overall health and sustainability of the Mediterranean basin.

Environmental Implication

Contaminants of emerging concern (CECs) are poorly removed during wastewater treatment and end up in the aquatic environment. This fact is extremely concerning in intermittent rivers in which wastewater effluents could make up as much as 100% of the flow during dry seasons. CECs are well-known "hazardous materials" due to their prevalence and ubiquity in the environment. This work contributes to the understanding of the fate of pharmaceuticals in Mediterranean rivers. Chemical, ecological and hydrological indicators were evaluated to characterize the effect of anthropogenic impacts on intermittent rivers. Our outcomes increase the knowledge of environmental gaps linking different disciplines.

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CRediT authorship contribution statement

Francesco Gentile: Writing – review & editing, Conceptualization. **Samia Khadhar:** Validation, Methodology. **Taha Hocine Debieche:** Writing – original draft, Methodology, Data curation, Conceptualization. **Olga Gómez-Navarro:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Anna Maria De Girolamo:** Writing – original draft, Validation, Methodology, Conceptualization. **Armin W. Lorenz:** Writing – original draft, Methodology, Conceptualization. **Serge Chiron:** Writing – original draft, Conceptualization. **Sandra Perez:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, 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.

Data availability

Data will be made available on request.

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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.jhazmat.2024.135951.

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