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Tracking the Causes of a Mass Fish Kill at a Mediterranean River within a Protected Area

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Abstract: In this study, an extreme event observed at the intermittent Mediterranean Bogdanas River within the territory of the protected area of the National Park of Lakes Koronia-Volvi and Macedonian Temp that led to a mass fish kill was investigated. We aimed to define the main pressures affecting water quality and biota, specifically fish. No organic poisons, pesticides or heavy metal concentrations were detected in fish tissue, while high values of BOD_5, COD, TN and conductivity were measured in water samples. These results, combined with the prevailing hydroclimatic factors (high temperatures and low water flow), lead to the assumption that mass fish mortality was triggered by high organic loads discharged from an upstream point source of pollution, and in particular an active landfill.

Keywords: Bogdanas River; mass fish kill; landfill; heavy metals; organic pollution

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1. Introduction

A new policy for water resources management was entered into force on 22 December 2000 by the European Union under the Framework of the Directive 2000/60/EC (WFD). This key tool promotes Community actions in the field of water policy between the Member States for protecting the aquatic ecosystems and attaining good status of all water resources. The approach for integrated management is mainly aimed at the geographical scale of the River Basins, while special mention is made for the protected areas within river basins.

The harmonization of Greek legislation with the Community Directive-Framework 2000/60/EC was reached with Law 3199/2003 (Government Gazette 280 A/09.12.2003) and Presidential Decree 51/2007 (Government Gazette 54/A/08.03.2007). In 2010 the National Water Committee designated 14 River Basin Districts [1] in the country and one year later the national monitoring network [2] was established (over 2000 monitoring points) and put in operation, contributing to the first cycle of the River Basin Management Plans (RBMPs), followed by the 1st revision, published by the Special Water Secretariat in 2017 [3].

Despite the great number of the protected areas in Greek river basins and the efforts made for improving their ecological status in the frame of the implementation of WFD, there are still water bodies threatened from insufficient operation of environmental legislation and ad-hoc management practices [4,5].

Indeed, many water bodies suffer heavily by anthropogenic activities and processes that affect their water quality, degrade their ecological role and the ecological services they support and impact the biodiversity that they harbour [6–9], which may include a unique, endemic and extremely range restricted fauna [10]. Unfortunately, extreme episodes such as massive loss of biota, and especially of fish, are not uncommon in Greece, e.g., [9–12], indicating the inadequate protection of the surface water bodies. Actually, the official records of mass fish kills published in the literature are fewer by far than the actual events. The number of the records of such events in the grey literature is more indicative of their higher frequency, as has also been observed worldwide [13].

Here, even though the term "fish kill" is loosely defined in the literature [14], we use it to refer to a high fish-mortality event, which is not part of the natural life cycle of fish, and is neither caused by predation or harvest, during which a minimum of 25 fish specimens are found dead per 1 km^2 in a lentic or per 1 km in a lotic water body, within a 48-h period [13]. Despite their patchiness in time and space, as well as the magnitude of occurrence, mass fish kills may severely impact local fish populations, leading occasionally even to their collapse.

Species-specific mass fish kills (in multi-species ecosystems) are mainly caused by a single factor (i.e., a disease), which, in most cases, can easily be identified [15]. In contrast, identifying the primary causes of multi-species mass fish kill events is rather difficult, as they result from multiple general stressors interacting in complex additive, synergistic or even antagonistic ways [15–17]. The time lag between the beginning of the phenomenon and the time that this becomes perceived and investigated hinders determination [13]. However, understanding these interactions is fundamental for implementing preventative measures to reduce the frequency and magnitude [13] of catastrophic events. To that end, information regarding the evolution of the phenomenon in time, combined with the enforcement of long-term water quality monitoring and the knowledge of the prevailing environmental conditions, is critical. In this context, the establishment of an international protocol for mass fish kill events and the retention of official records, as has already been proposed, [13] is crucial.

Mass fish kill events have been reported in all types of marine and freshwater ecosystems [14–16,18,19] and had been attributed to diseases, parasites, changes in water physicochemical parameters caused by natural phenomena, or anthropogenic activities (i.e., extreme temperature values, acidification, eutrophication, pollution, droughts, harmful cyanobacterial blooms) and more commonly to the synergistic effect of two or more causal factors [15–17,19]. However, the mass fish kills triggered by anthropogenic activities outnumber those caused by natural phenomena [13]. Agricultural runoffs, biotoxins and chemical pollutants are among the most common primary causal factors of mass fish kills [13]. The range and frequency of these events, extremely disruptive to the fish fauna, has increased in the last decades [13,14] and are often considered as proxies of ecosystem degradation [13,20]. Moreover, they are rather frustrating for policymakers and the general public [13].

The present study follows a mass fish kill observed at the Mediterranean Bogdanas River. In particular, data concerning the water quality characteristics prior to, during, and after the mortality event were considered to define the major pressures affecting water quality and biota.

2. Materials and Methods

2.1. Study Area

The Bogdanas River is located in Northern Greece and forms part of the Mygdonia basin and the Central Macedonia District (EL10). EL10 is the second largest River Basin District (RBD) of Greece (16.269 km^2 in size area), with 58.09% of its surface waters having a "less than good" ecological status (maintained of physical, chemical and biological conditions) while when only the priority substances are considered, 0.95% of the surface waters have a "less than good" chemical status [21].

The whole Mygdonia basin area is protected and managed by the Management Authority of Koronia-Volvi-Chalkidiki (MAKVC). The Bogdanas River comprises the main water supply of a Ramsar lake (Lake Koronia), which forms part of the National Park of Lakes Koronia-Volvi and Macedonian Temp. Moreover, the river is of great ecological importance, since it hosts a variety of Mediterranean river habitat types and a highly diverse fauna [22]. Furthermore, it comprises a Special Protected Area (SPA) and a Natura 2000 site (Directives 2009/147/EC and 92/43/EEC) and is also included in the national monitoring network under the frame of WFD.

Agriculture constitutes the primary land use within the watershed, along with the existence of several livestock units and small crafts and industries. The river has an intermittent flow and in its upper part (425 m a.s.l.) receives the surplus water amounts of Mavrorachis' landfill. The river outflows into the northwest part of Lake Koronia, after crossing an area of low altitude (73 m a.s.l.). The lake, over the last two decades, has faced numerous environmental issues [23–25]. Although wastewater treatment plants exist, their operation is periodically insufficient, resulting in the outflow of the excess untreated leachates into the Bogdanas River [26].

The Bogdanas River has been included in the Greek national surface water monitoring network and is periodically monitored under the requirements of the implementation of WFD. The ecological status of the river, at its monitored site (downstream of station S7), has been assessed as poor, while its chemical status has been improved from lower-than-good (first cycle RBMP, Central Macedonia District, EL10, 2009–2015) [27] to good (1st update of RBMP, Central Macedonia District, EL10, 2015–2021) [28]. In the ongoing 2nd update of the EL10 District RBMP (2019), the Bogdanas River is included in the Surface Water Systems under high pressure due to intense water extraction in its basin.

A rough picture of the river's water physicochemical features is presented in Table 1. Specifically, the provided mean values represent annual means of the parameters measured by the MAKVC, in several sites along the river seasonally, in the frame of the implementation of its surface water monitoring program in the area and the national monitoring program of the Special Secretariat for water.

Table 1. Water physicochemical features of Bogdanas River for the period 2010–2017. Data from the monitoring program of the Management Authority of Koronia-Volvi-Chalkidiki and the national monitoring program of the Special Secretariat for water.

Physicochemical Parameter	Mean	SE	Min	Max
TEMP (°C)	14.6	0.93	11.2	19.5
TURB (NTU)	29.8	18.53	5.5	140.0
pH	8.2	0.19	7.3	8.9
Cond (μS/cm)	1183	445	464	6230
DO (mg/L)	8.1	1.29	1.6	15.5
BOD_5(mg/L)	5.7	1.82	2.6	12.8
NO_2-N (mg/L)	0.099	0.0537	0.001	0.87
NO_3-N (mg/L)	2.451	0.4108	0.165	10.4
NH_4-N (mg/L)	1.174	0.9558	0.037	7.982
PO_4-P (mg/L)	1.021	0.8239	0.060	4.637
TN (mg/L)	3.497	1.4723	1.835	7.772

2.2. Sampling

In June of 2015, a mass fish kill was observed in the upper and middle part of the Bogdanas River during a field survey conducted by the MAKVC. In order to identify the extent and causes of this catastrophic disaster, a thorough inspection along the river was conducted by the agency staff. To this framework, in situ measurements (AP-2000 Aquaread multi sensor) of water temperature (T, °C), conductivity (Cond, μS/cm), dissolved oxygen (DO, mg/L) and pH were conducted by multi sensor probes in sites S2 (which receives the wastes of the landfill), S3 (upstream the landfill, reference site) and S4 (downstream the landfill, after merging with the main river channel) (Figure 1). Moreover, water samples were collected and sent at Qlab Analytical Laboratory to measure biological oxygen demand (BOD_5, mg/L), chemical oxygen demand (COD, mg/L), total suspended solids (TSS, mg/L), nitrate nitrogen (NO_3-N, mg/L), nitrite nitrogen (NO_2-N, mg/L) and ammonium nitrogen (NH_4-N, mg/L), phosphate-phosphorus (PO_4-P, mg/L), total nitrogen (TN, mg/L) and fats and oils. In addition, water samples were sent to the Laboratory of Toxicology of the Veterinary Center (Athens) to determine the presence of toxic substances.

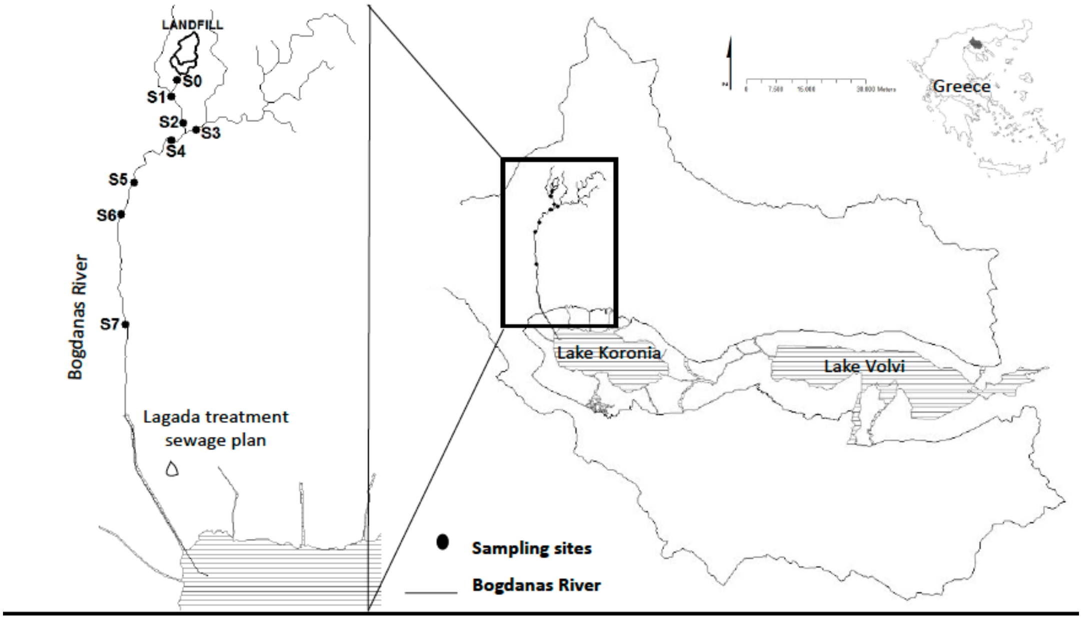

Figure 1. Bogdanas River (Mygdonia basin). Mavrorachis Landfill and sampling sites (S0-S7) are indicated.

Killed fish species (Figure 2a) were identified and individuals were collected from various sites along the river. The dead fish were inspected for external wounds (apart from natural decomposition) or signs indicating disease (i.e., a virus/bacterium infection). Individuals in relatively good condition were frozen and used for further toxicological and heavy metal analyses conducted also by the Laboratory of Toxicology of the Veterinary Center.

In October 2015, a second pollution event was observed at the Bogdanas River, followed by the presence of foams at site S7 (Figure 2b), while black colored waters were observed at S2 (Figure 2c,d). Water conductivity and pH were immediately measured at stations S2 and S3, and water samples were collected to estimate COD and heavy metal concentrations (Zn, Cu, Cd, Cr), in order to identify the status of pollutants in the system. Additional samplings were conducted one month later (in November 2015) for the assessment of water quality, based on measurements of several physicochemical parameters (pH, T, Cond, DO) and sediment quality, based on the determination of heavy metal (As, Cd, Cr, Cu, Hg, Ni and Pb) concentrations.

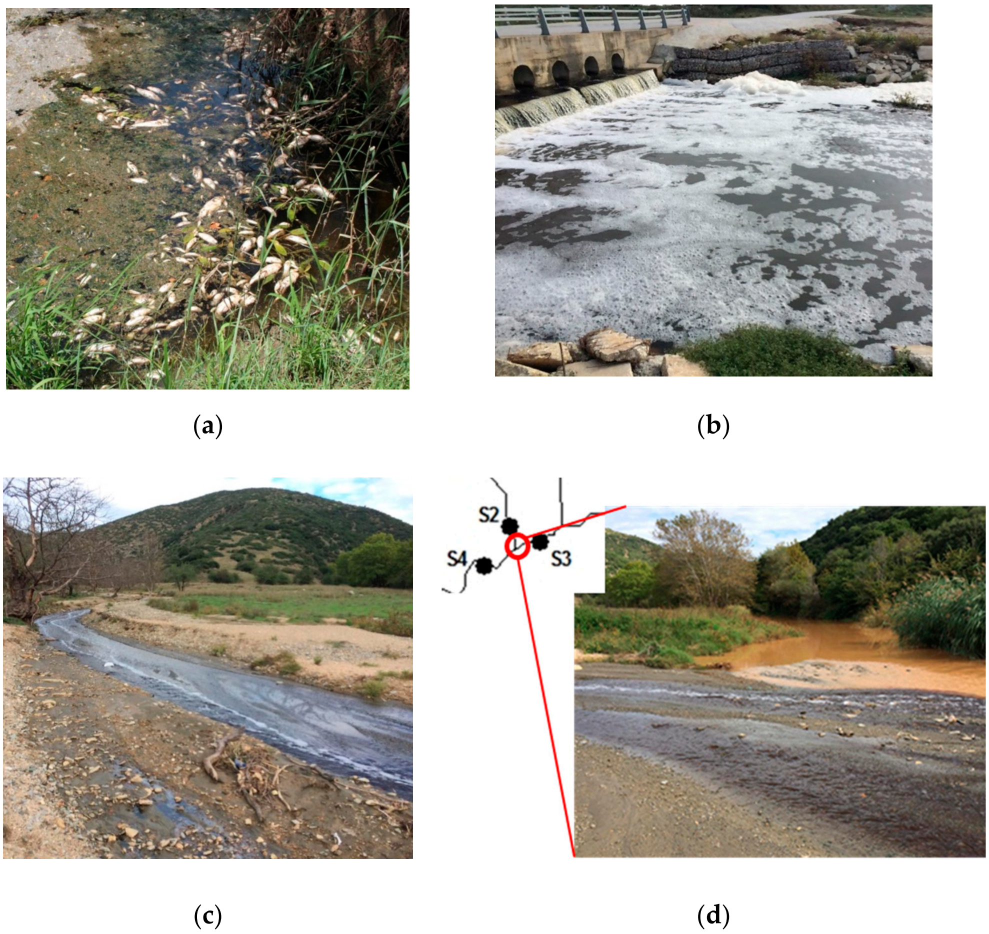

Figure 2. (**a**) Dead fish observed, June 2015, (**b**) foam in S7, (**c**) black colored water at site S2, (**d**) the confluence of stream branches above S4, October 2015, Bogdanas River.

2.3. Analyses

Nutrient analyses in water samples conducted at Qlab Analytical Laboratory were based on appropriate methodologies [29]. The Hg concentrations in the dead fish tissues were estimated using atomic absorption spectrophotometry with hybrid production in continuous flow while the concentrations of Cd and Pb were determined by atomic absorption spectrophotometry with a graphite furnace. The presence of toxic substances, i.e., organic poisons and pesticides, was investigated by conducting a toxicological analysis on fish tissue based on Gas chromatography–mass spectrometry. The detection of toxic substances (organic poisons and pesticides) in water and fish tissue samples and fats and oils in water samples was conducted followed certified methods (Gas chromatography–mass spectrometry). The estimations of the heavy metal (Zn, Cu, Cd, Cr) concentrations in the water samples were conducted using Merck's test kits and photometric methods. In addition, sediment samples pretreatment and analyses were in accordance with the standard methodology (ISO 11464:2006, Soil quality—Pretreatment of samples for physicochemical analysis, ISO 16729:2013—Digestion of nitric acid soluble fractions of elements, ISO/TS 16965:2013, Soil quality—Determination of trace elements using inductively coupled plasma mass spectrometry—ICP-MS).

3. Results

The water's physicochemical parameters measured during the mass fish kill in June 2015 are provided in Table 2. The dead fish observed along the upper and middle part of the Bogdanas River, and particularly downstream of S4 belonged to three families and eight species (Table 3), four of which are endemic to the system and the Balkan Peninsula and two are non-indigenous [30,31]. No injuries or signs of diseases, such as popped eyes, hemorrhaging and lesions [15], were observed in dead fish. However, the majority of fish specimens were in advanced decomposition stage. Therefore, heavy metal concentrations were estimated in a limited number of samples ($n = 5$) of *Cyprinus carpio* that were found in better condition. The measured fish tissue heavy metal concentrations were within the proposed limits [32] (Table 4) and were far below the levels that could cause a mass fish kill, e.g., [33,34]. The toxicological analyses conducted on fish tissue and water samples did not reveal the presence of organic poisons and/or pesticides, indicating that fish deaths could not be attributed to toxic micro pollutants.

Table 2. Physicochemical parameters measured in water samples of Bogdanas River. With bold the values that exceed limits. Government Gazette (GGE) 1079: [35], Directive 2006/44/EC: [36].

Parameter	GGE 1079	2006/44/EC	June 2015			October 2015		November 2015						
			S2	S3	S4	S2	S3	S1	S2	S3	S4	S5	S6	S7
DO (mg/L)		4	7.9	8.5	7.8			9.2	6.1	9.8	10.9	6.7	6.6	10.2
T(°C)	<30		25.2	26.9	**31.3**			13.4	15.7	11.1	11.7	16.1	14.1	12.5
Cond (μS/cm)	1000		**5610**	663	**1010**	**3860**	475							
pH	6–8.5	6–9	8.26	8.15	7.84	8.24	7.99	8.44	8.38	8.38	**8.55**	7.10	8.07	8.23
BOD_5 (mg/L)	25	6	**1810**	<10	13									
COD (mg/L)	125		**6405**	15	37	**1382**		**9710**	**10580**	659	**1322**	**1247**	**1527**	**1027**
TSS (mg/L)	35		**192**											
NO_3-N (mg/L)			9.8	<1	14.71									
NH_4-N (mg/L)		0.156	**229.3**	**0.3**	**0.52**									
PO_4-P (mg/L)	1	0.131	**0.58**	**0.81**	**0.44**									
TN (mg/L)	10		**235**	0.4	3.6									
Fats/Oils (mg/L)	7		<5	-	-									
Cl (mg/L)	0.5		**1035.1**	**46.1**	**99.3**									
SO_4 (mg/L)	1000		52.76	29.28	<5									
Zn (mg/L)	5					0.85								
Cu (mg/L)	0.5					0.134								
Cd (mg/L)	0.4					0.072								
Cr (mg/L)	0.5					0.12								

Table 3. Fish species suffered deaths at Bogdanas River, in June 2015.

Family/Species	Common Name	Origin
Cyprinidae		
Cyprinus carpio Linnaeus, 1758	European carp	N
Squalius orpheus Kottelat and Economidis, 2006	Maritza chub	E
Barbus strumicae Karaman, 1955	Strumica barbel	E
Pachychilon macedonicum (Steindachner, 1892)	Macedonian moranec	E
Rhodeus amarus (Bloch, 1782)	European bitterling	N
Carassius gibelio (Bloch, 1782)	Prussian carp	I
Cobitidae		
Cobitis strumicae Karaman, 1955	Struma spined loach	E
Centrarchidae		
Lepomis gibbosus (Linnaeus, 1758)	Pumpkinseed	I

N: native, E: endemic in Balkans, I: introduced.

Table 4. Concentrations of heavy metals (mg/kg wet weight), in *Cyprinus carpio* flesh from Bogdanas River.

Heavy Metal	Concentration (mg/kg)	Maximum Allowed Values Based on Regulation 1881/2006/EC (mg/kg)
Hg	0.11 ± 0.02	0.5
Cd	0.0055 ± 0.0008	0.05
Pb	<0.050 *	0.3

* detection limit.

On the contrary, the values of water physicochemical parameters, nutrients and other elements measured at that period, both in situ by the MAKVC and in external analytical laboratories, were much higher than the maximum permissible limits (Table 2) and the mean values recorded the previous years in the system (Table 1). Conductivity values at site S2, which receives directly the wastewaters of Mavrorachis' landfill, were 5 times higher than the highest permissible value adopted by the Government Gazette 1079 B/5-7-2010 for the determination of surface water uses and special conditions for the disposal of sewage and industrial waste to each recipient of the Prefecture of Thessaloniki [35] and almost ten times higher than the reference site (S3). The same pattern was obvious for BOD_5 and COD values, which were 70 and 50 times respectively higher at site S2 than the highest limits for sewage and liquid waste disposal to surface water bodies. In addition, BOD_5 values were extremely higher than the optimum values proposed for Cyprinidae species in Directive 2006/44/EC on the quality of fresh waters needing protection or improvement in order to support fish life [36]. Moreover, the TN concentration was 23 times higher than the upper limit, while at sites S3 and S4 the measured values were within the suggested limits. Furthermore, ammonium concentrations exceeded the proposed limit [34] at all sites. On the contrary, the concentrations of fats and oils were very low, below the detection limits of the applied methods.

In October 2015, in the highly pungent and black colored waters at site S2, a high conductivity value of 3860 µS/cm was estimated (Table 2), exceeding by almost 4 times the maximum permissible limit [35] and thus indicating a high organic load.

In addition, very high conductivity values, almost ten times the limit, were recorded at sites S1 and S2 in November 2015 (Table 2). Hg concentrations in the sediment samples (Table 5) were below the method detection limits in all sampling sites as Cd concentrations at sites S1, S2 and S5.

Table 5. Heavy metal concentrations (mg/kg) in the sediments of Bogdanas River in November 2015.

Site	As	Cd	Cr	Cu	Pb	Hg	Ni
S1	14.0	<0.13	28	35	12.0	<0.5	20
S2	20.0	<0.13	20	21	6.2	<0.5	17
S3	14.0	0.19	33	122	19.0	<0.5	27
S4	19.0	0.23	51	53	26.0	<0.5	37
S5	8.5	<0.13	13	10	6.4	<0.5	11
S6	27.0	0.27	66	56	33.0	<0.5	50
S7	13.0	0.15	44	27	20.0	<0.5	39

4. Discussion

The subject of the present study was to investigate the causes of the mass fish kill event which took place during June of 2015 in Bogdanas River, impacting almost all known present fish species [22,37–39].

Since no injuries or signs of diseases such as popped eyes, hemorrhaging and lesions [15], were observed in dead fish, it could be hypothesized that their death was primarily caused by environmental factors rather than pathogens. The fact that eight fish species suffered deaths also supports this hypothesis, considering that a contamination effect would probably be a species-specific stressor [15].

The low heavy metal concentrations detected in the tested fish tissues also excluded the heavy metal contamination from the causals of the observed mass fish kill event in the Bogdanas River. It is noted that under certain conditions, heavy metals may accumulate to a high toxic level in living organisms, causing various disturbances in their health and wellbeing [40–42].

Consequently, the observed mass fish kill was due to other external factors, possibly the high organic pollution load flowing out of the Mavrorachis' landfill as has already been documented [43]. This is supported by the very high BOD_5, COD, TN and conductivity values measured at station S2, close to the effluent of the landfill. The high organic load combined with the high temperatures occurring at that period and the relatively low flow and limited river water level, stressed the fish and eventually caused their death. This is also supported by the fact that dead fish of all species and regardless of the specimens' size were observed.

The above are a convincing indication that Mavrorachis' landfill comprises a permanent [26] source of organic pollution in the area, raising concerns for both Lake Koronia, which is in a rehabilitation program, but also for the Bogdanas basin aquifer. In addition, the fact that heavy metal concentrations were in general higher in sediment samples from the downstream of S1 and S2 sites, reveals that additional heavy metal pollution sources exist in the Bogdanas basin. Relevant past reports also exist [26], framing, in specific, dyeing industries existing in the region, as well as the various agricultural activities. In particular, the high Cr concentrations were attributed to dyeing industries, the presence of Pb, Cd and Zn to the uncontrolled landfill runoff, while As concentrations were considered to be agrochemical products [26].

Mass fish kill events tend to re-appear after a first occurrence [44]. In this context, the systematic recording of mass fish kill events in the area should aim to enhance the understanding of their causative factors, thereby providing an insight into the water body functions and ecological conditions. Therefore, establishing, supporting and enhancing monitoring programs designed to collect baseline data on inland surface waters is crucial. The availability of background environmental data would benefit any future action targeting environmental protection, the identification of contamination sources and the insurance of legal compliance. Moreover, they would allow the detection of trends along time, thereby assisting decision makers.

Preventing such pollution phenomena mandates stricter governmental measures to ensure the proper operation of the landfill and avoiding accidental leakage events. Relocation of the landfill could also be considered, after a thorough environmental impact assessment, in order to minimize undesirable effects to the water bodies of the broader area and, consequently, to the fauna that they support. Besides the above regarding a comprehensive program considering the environmental protection, the legislated "polluter pays" principle [45] ought to be applied more strictly and compellingly. Toward that direction, the next cycle of RBMPs conducted in the District EL10 (Central Macedonia) should include stricter regulations on land use and anthropogenic activities followed by an accurate program of measures concerning the area of the National Park of Lakes Koronia-Volvi and Macedonian Temps, thus contributing to the fulfillment of the objectives of the WFD.

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