Agro-Industrial Wastewater Pollution in Greek River Ecosystems

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Abstract In this chapter, the characteristics and environmental impacts of wastewaters from the major agricultural industries on the river ecosystems of Greece are reviewed and discussed, focusing especially on olive mills, orange juice processing factories and cheese processing factories. The high organic load, suspended solids and nutrients of these wastewaters, as well as their toxicity, have deteriorated river water quality and the ecological status of many running waters of Greece. Among the most common effects are eutrophication, the decline of fish and invertebrate populations, species richness loss and the consequent reduction of the river capacity for moderating the effects of polluting substances through internal mechanisms of self-purification. The organic load of the wastewaters, substrate contamination (sewage bacteria) and distance from the wastewater discharge outlet appear to be the most important factors affecting macroinvertebrate assemblages, while typology (i.e. slope, altitude), hydrology (i.e. permanent, intermittent), intensity and volume of the wastewater are the most important determinants of self-purification processes. As these industries are usually located near small-sized streams that are not significantly considered in the Water Framework Directive 2000/60/EC, there is a need for including them in monitoring and assessment schemes as they may considerably contribute to the pollution load of the river basin. Finally, guidelines to manage these wastes through technologies that minimise their environmental impact and lead to a sustainable use of resources are also critical.

Keywords Benthic fauna, Ecological status, Effluents, Olive mills, Toxicity

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Contents

1 Introduction

Throughout the course of human history, the quality and quantity of water were crucial determinants of human health and the health of the Earth's ecosystems. The dramatic yet continuous industrial and agricultural development of the past century has significantly degraded the environment and particularly the soil, lakes, rivers and other aquatic ecosystems. It is estimated that river ecosystems have deteriorated more than any other aquatic ecosystem [1, 2] mainly due to changes in land use, organic and chemical pollution (agrochemicals, solid and liquid industrial and municipal wastewaters), overexploitation of water resources (e.g. water abstraction, overfishing, sand and gravel extraction, etc.), reduction and deforestation of riparian vegetation and unintentional and intentional introduction of exotic and alien species. Pollution episodes are daily, and in many cases, their impact on ecosystems is unpredictable and terrifying.

Rivers play a key role in ecosystems and provide a series of ecosystem functions such as habitat and food source for a wide range of biological species and ecological refuge development. Historically, rivers accommodated communities by providing food and water and a medium for transport, recreation and tourism. Inevitably, many peri-urban and floodplain rivers draining from urban and agricultural areas have been affected significantly during the last decades and remain a sensitive issue in the agenda of river management authorities.

Agricultural industries (referred to as agroindustries hereafter) are major contributors to the worldwide industrial pollution problem. With the tremendous pace of technological development to cover the needs of population overgrowth, the amount and complexity of wastes generated by these industries and their management has been problematic. Now, agroindustries, more than any other industrial sector, require an appropriate approach for successful waste management. There is no wonder that until 2004, more than 1,000 references on the various treatment methods of olive mill wastewaters have been published worldwide [3], and that number has been constantly increasing. Agroindustries such as olive oil mills, fruit processing factories, cheese factories and dairy farms constitute one of the most important pillars of local economy for the Mediterranean countries, including Greece. Agroindustries processing agricultural raw materials such as fruit, vegetables and animal products produce millions of tons of wastewater and large amounts of by-products, which are left untreated or unexploited and end up in the environment. These industrial facilities are usually scattered throughout the countryside, and the raw materials processed are produced at a seasonal rate, thus resulting to wastes varying significantly during the year both in quantity and characteristics.

In this chapter, the environmental impacts of agroindustrial wastewater discharge on river ecosystems of Greece are reviewed and discussed, focusing especially on major industries such as (a) olive mills, (b) orange juice processing factories and (c) cheese processing factories. In addition, impacts from other agroindustries are briefly highlighted.

2 Olive Mill Wastewaters

2.1 Current Production Trends

Worldwide, olive cultivation has increased significantly due to population increase and cultivation intensification using fertilisers, pesticides, irrigation of olive groves and new processing technologies of olives. In Greece, the number of olive trees was estimated to be around 75 million in 1961, while in 2003, their number reached 137 million, an 82.6% increase (Hellenic Ministry of Rural Development and Food). Olive oil production in 1961 was approximately 215 thousand tons, and in 2012, production reached 352 thousand tons, an increase of 64% (Fig. 1). Currently, there are about 14×10^7 olive trees and 450 approved olive mill establishments (Fig. 2), although the real number is estimated to be around 2,800 olive oil mills. Thirty percent (30%) of the olive mills are found in the Peloponnese, 24% in Crete, 9% in Attica, 7% in Western Greece, 7% in Central Greece, 9% in Macedonia and Thrace, 4% in the North Aegean, 4% in the Ionian, 3% in Thessaly and finally, 2% in Epirus and South Aegean, respectively.

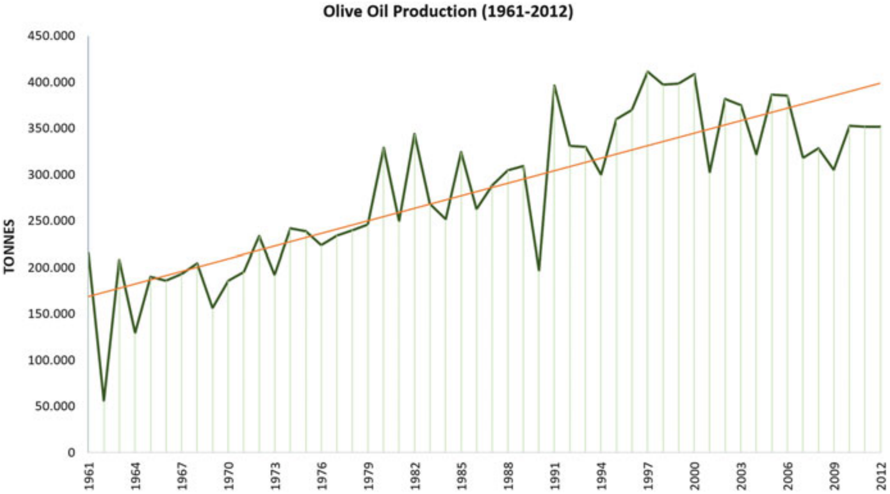

Fig. 1 Olive oil production (tons) in Greece from 1961 to 2012 according to FAO [4]

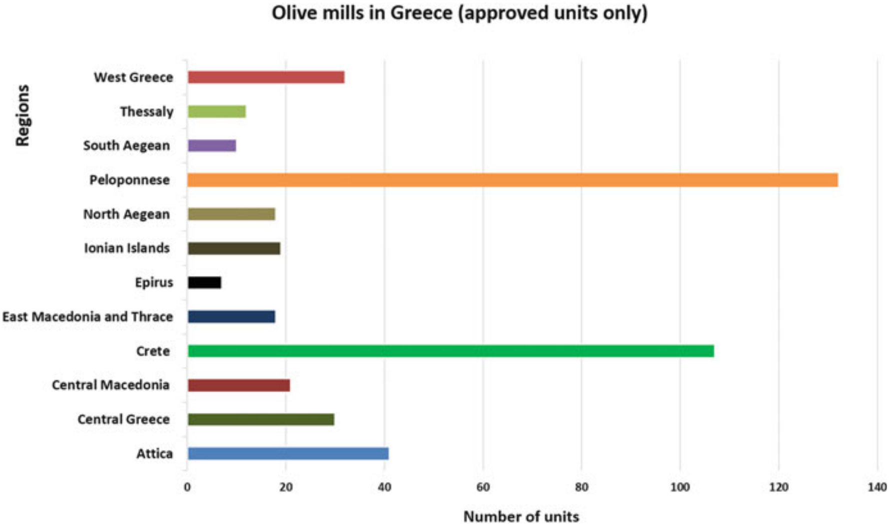

Fig. 2 Approved olive oil mill establishments for 2015 registered at the Greek Ministry of Rural Development and Food

2.2 Polluting Capacity and Characteristics

Olive mill wastewater (OMW) is one of the major and most challenging organic pollutants in olive oil production countries [5, 6]. OMW is the turbid liquid waste generated during the extraction of olive oil, where huge quantities of organic wastes are produced within a short period and usually lasts 3–5 months (November–

March). It is estimated that the volume of OMW produced annually in the Mediterranean region varies between 7×10^6 and 30×10^6 m^3 [7]. Despite the global spread of the olive tree, 95% of the production of olive oil (which yields about 2.5 million tons of olive oil per year) comes from the Mediterranean countries with Spain, Italy and Greece being the largest producers.

The milling process of olives generates about 50% of wastewater, 30% of solid residues and 20% of olive oil. OMW is easily fermentable and its characteristics are variable depending on the method of extraction, type of olive variety, soil and climatic conditions and cultivation methods. Typical OMW composition by weight is 83–94% water, 4–16% organic compounds and 0.4–2.5% mineral salts [8]. The wastewater arising from the milling process amounts to 0.5–1.5 m^3 per 1 ton of olives, depending on the process method [9, 10]. The high pollution ability of OMW is attributed to its remarkably high organic load (BOD: 25–100 g/l; COD: 45–220 g/l) and high content of phenolic compounds [10, 11], its acidity (pH 4–5) as well as the significant concentrations of magnesium, potassium and phosphate salts [12]. In addition, OMW contains many organic compounds such as lipids, sugars, organic acids, tannins, pectins and lignins that contribute to the increase of its organic load [8, 10]. Table 1 presents the major physical and chemical properties of OMW.

Although disposal of untreated OMW in aquatic systems is not allowed in Greece, it is estimated that approximately 1.5 million tons of OMW are disposed of every year in rivers, streams (Fig. 3 and 4), lakes and even in the sea [5]. The effective treatment of OMW requires expensive and advanced technologies that most olive mills lack. The usual treatment and disposal practice followed in Greece involves neutralisation with lime and disposal in evaporation ponds/lagoons. Disposal of OMW causes significant environmental pollution with unforeseeable effects on the quality of soil, surface and groundwater [17, 18] and poses a serious risk to aquatic and terrestrial biota and subsequently to the health of corresponding ecosystems.

2.3 Toxicity of Olive Mill Wastewaters

OMW and its polyphenolic fraction can be toxic to aquatic organisms [14, 15, 17, 19, 20], to bacteria and yeast [21] and to seed germination [22]. Moreover, it has been shown to affect the physical and chemical properties of the soil and its microbial community [7, 18, 23, 24], while several studies have verified its phytotoxic effects and antimicrobial activity [25]. Finally, OMW can be toxic to anaerobic bacteria which may inhibit conventional secondary and anaerobic treatments in municipal treatment plants [26].

Many related toxicological studies have evaluated the toxicity of the whole OMW effluent with standard toxicity test organisms such as *Daphnia pulex*, *Daphnia magna* and *Thamnocephalus platyurus* [15, 17, 20, 25] or with the luminescent bacteria *Vibrio fischeri* [27]. Paixão et al. [15] have shown that the LC_{50} acute toxicity of OMW can range from 1.08% to 6.83% for *Daphnia magna*,

Table 1 Physicochemical characteristics of OMW

Parameters	Units	Press mill	Three-phase centrifugation system
Density	(g/cm3)	1–1.2	1–1.1
Salinity	mmhos/cm	8–16	8–16
pH		4.2–5.3	4.6–5.2
Conductivity	mmhos/cm	12–18	8–16
Total solids	g/l	70–173	45–103
Total suspended solids	g/l	2–7	2.5–5
BOD_5	g/l	60–100	25–50
COD	g/l	65–190	45–110
Total phenols	g/l	12–19	6–10
Hydroxytyrosol	g/l	0.07–0.9	0.04–0.4
Phenolic acids	g/l	0.5–0.6	0.2–0.3
Tannins-Lignins	g/l	3–12	3–10
Pectins	g/l	2–5	1.5–3
Fats and oils	g/l	1.5–3	0.5–1.64
Total sugars	g/l	17–32	11–21
Glycerol	g/l	0.1	0.062
Organic acids	g/l	2–7	2–4
Polyalcohols	g/l	3–6	2–4
Total N (Kjeldahl)	g/l	1–1.5	0.7–0.9
Organic N	g/l	0.1–1.1	0.1–1
Total proteins	g/l	20–37	11–23
Ash	g/l	7–11	4–8
TOC	g/l	50–70	35–45
Total phosphorus as P_2O_5	g/l	0.5–0.9	0.5–0.6
Nitrate	mg/l	20–23	10–12
Chloride	mg/l	219.48	124
Sulphate	mg/l	75–115	52–75
Iron as FeO	mg/l	35–48	16–32
Potassium as K_2O	g/l	2–3	2–2.5
Sodium as Na_2O	mg/l	300–500	200–300
Calcium CaO	mg/l	350–380	120–270
Magnesium MgO	mg/l	74–200	48–50
Silicate (SiO_2)	mg/l	24–31	16–22
Manganese	mg/l	16–20	11–12
Zinc	mg/l	16–20	11–14
Copper	mg/l	8–10	6–9
Lead	mg/l	0.5–2	0.4–0.7
Cobalt	mg/l	0.2–0.9	0.1–0.5
Nickel	mg/l	0.5–1.5	0.3–1.5

Data assembled from: [8, 10, 13–16]

Fig. 3 Illegal OMW discharge in Skatias stream of the Evrotas river basin (Peloponnese, S. Greece)

Fig. 4 OMW discharge through a pipeline in Skatias stream (Evrotas River, (Peloponnese, S. Greece)

0.73% to 12.54% for *Thamnocephalus platyurus* and 0.16% to 1.24% for the luminescent bacteria *Vibrio fischeri*. Similarly, Rouvalis et al. [20] also showed that the acute toxicity of OMW could vary from 1.7% to 12.4% for *Daphnia pulex* and 3.3% to 8.9% for *Thamnocephalus platyurus*.

More recently, the toxicity of the whole OMW effluent to aquatic macroinvertebrates has also been studied [14, 28]. The 24-h LC_{50} values of OMW range from 2.64% to 3.36% for *Gammarus pulex* and 3.62% to 3.88% for *Hydropsyche peristerica* [19]. Based on a five-class hazard classification system developed by Persoone et al. [29], for wastewaters discharged into the aquatic environment, olive mill wastewaters are classified as highly toxic [19]. OMW concentrations can also be lethal to crustaceans even at lower volumes. The 24-h LC_{50} value for the Palaemonidae species of Pamisos River in South Peloponnesus was 0.7% [33]. Table 2 summarises all known toxicological studies of OMW that have been conducted in Greece.

Table 2 Toxicological studies conducted in Greece evaluating OMW toxicity to plant and animal species

OMW Origin	Taxonomic group	Test organism	Endpoint	Toxicity values	References
Chania	Bacteria	*Vibrio fischeri*	Mortality (EC_{50})	OMW	[27]
				15 min – 0.47% (Conf. range: 0.37–0.6%)	
				30 min – 0.5% (Conf. range: 0.4–0.64%)	
				Electrochemically oxidised (60 min) OMW	
				15 min – 1.36% (Conf. range: 1.17–1.58%)	
				30 min – 1.13% (Conf. range: 1–1.29%)	
				Electrochemically oxidised (120 min) OMW	
				15 min – 0.06% (Conf. range: 0.05–0.07%)	
				30 min – 0.06% (Conf. range: 0.05–0.07%)	
				Electrochemically oxidised (180 min) OMW	
				15 min – 0.22% (Conf. range: 0.13–0.38%)	
				30 min – 0.04% (Conf. range: 0.01–0.14%)	
				(toxicity increased due to the formation of organochlorinated by-products)	
Crete	Bacteria	*Vibrio fischeri*	Mortality (EC_{50})	5 min – Untreated OMW 0.219% (±0.044)	[30]
				Treated OMW (1 cycle of treatment): 1.612 ± 0.687%	
				Treated OMW (2 cycles of treatment): 4.606 ± 1.51%	
				15 min – Untreated OMW: 0.187 ± 0.035%	
				Treated OMW (1 cycle of treatment): 1.361 ± 0.528%	
				Treated OMW (2 cycles of treatment): 4.374 ± 1.54%	

Crete	Crustaceans	*Artemia franciscana*	Mortality (EC_{50}) 24–48 h	Slight toxicity of OMW was observed at high dilutions (1.25 and 2.5% v/v). 100% mortality occurred following a 24 h exposure to OMW at 20% v/v and a 48 h exposure OMW at 10% v/v	[30]
Magnesia	Crustaceans	*Artemia franciscana* (Artoxkit M test)	Mortality (EC_{50}) 24 h	12 samples of seawater: < 10%, with the exception of two sites with toxicity values of 47 and 23% (estuarine areas with low salinity waters and possibly unfavourable conditions for the survival of *A. franciscana*)	[31]
Fthiotida and East Attica	Crustaceans	*Artemia* sp.	Mortality (LC_{50}) 24 h	Untreated OMW: 4.5%	[25]
				Biotreated OMW with *Pleurotus ostreatus*: 12.5%	
Fthiotida and East Attica	Crustaceans	*Daphnia magna*	Mortality (LC_{50})	48 h – Untreated OMW: 2.5% (was not affected by the treatment with *Pleurotus* strains, mortality of the population reached zero at very low OMWs concentration <2%)	[25]
Chania	Crustaceans	*Daphnia magna* (Daphtoxkit F ^{TM} magna)	Immobilisation 24 h	OMW: 5% (Conf. range: 2.5–7.5%)	[27]
				Electrochemically oxidised OMW (60 min): 50%	
				Electrochemically oxidised OMW (120 min): 40%	
				Electrochemically oxidised OMW (180 min): 25%	
				(Increased toxicity after oxidation of OMW)	
Magnesia	Crustaceans	*Daphnia magna* (Daphtoxkit F^{TM} magna)	Mortality (EC_{50}) 24 h	24 h – 12 samples of freshwater: < 5%, with the exception of one site (high amount of agrochemicals draining from the neighbouring farmlands) with toxicity value of 15%	[31]
Achaia (OMW) and Kalamata (OMW)	Crustaceans	*Daphnia pulex* (Daphtoxkit F^{TM} pulex)	Mortality (LC_{50}) 24–48 h	24 h – anaerobically treated OMW: 7.64% ± 4.5, anaerobically treated OMW: 8.83% ± 6.5 (18% of the samples toxic and 82% very toxic)	[32]
				48 h – anaerobically treated OMW: 4.38% ± 2.6, 4.38% ± 2.6, anaerobically treated OMW: 4.48% ± 2.9 (5% of the samples toxic and 95% very toxic)	

(continued)

Table 2 (continued)

OMW Origin	Taxonomic group	Test organism	Endpoint	Toxicity values	References
Achaia	Crustaceans	*Daphnia pulex* (Daphtoxkit F^{TM} pulex)	Mortality (EC_{50}) 48 h	OMW: 1.7–12.4%	[20]
				Toxic Unites (TU) = 8.1–59.2, toxic to very toxic	
Laconia (Evrotas River)	Crustaceans	*Gammarus pulex*	Mortality (LC_{50}) 24 h	OMW: 2.64–3.36%	[19]
				TU = 29.76–37.88	
				Class IV – high acute toxicity	
Fthiotida and East Attica	Crustaceans	*Heterocypris incongruens*	Mortality,% Growth Inhibition	No effect on growth inhibition between untreated and treated OMW. Toxicity slightly decreased after treatment. The concentration of OMW that caused 50% mortality increased from 3.7% (in the untreated OMW) to 5% (in the treated OMW)	[25]
Messinian rivers	Crustaceans	Palaemonidae shrimp	Mortality (LC_{50}) 24 h	OMW: 0.7%	[33, 34]
				TU of OMW: 143 – Class V, Very High Acute Toxicity	
Achaia	Crustaceans	*Thamnocephalus platyurus* larvae (Thamnotoxkit F)	Mortality (LC_{50}) 24 h	Untreated OMW: 0.94 ± 0.66%, TU = 106.4	[26]
				Anaerobically treated OMW: 1.6 ± 0.48%, , TU = 62.5	
				(TU > 100 : extremely toxic, TU = 11–100 : very toxic)	
Achaia (OMW) and Kalamata (OMW)	Crustaceans	*Thamnocephalus platyurus* larvae (Thamnotoxkit F)	Mortality (LC_{50}) 24 h	Anaerobically treated OMW: 1.77% ±0.8	[32]
				Anaerobically treated OMW: 2.32% ± 1	
				(8% of the samples are extremely toxic and 82% very toxic)	
Achaia	Crustaceans	*Thamnocephalus platyurus* larvae (Thamnotoxkit F)	Mortality (LC_{50}) 24 h	OMW: 3.3–8.9%	[20]
				TU = 11.1–30.4, very toxic	

Laconia (Evrotas River)	Trichoptera	*Hydropsyche peristerica*	Mortality (LC_{50}) 24 h	OMW: 3.62 (Conf. Limits: 3.19–4.11%) – 3.88% (Conf. Limits: 3.45–4.37%)	[14]
				TU = 25.77 (Conf. Limits: 22.88–28.99) – 27.62 (Conf. Limits: 24.33–31.35)	
				Class IV – high acute toxicity	
Patras	Mollusks	*Mytilus galloprovincialis*	Stress indices in tissues	Mussels exposed to either 0.1 or 0.01% (v/v) OMW for 5 days. Decreased neutral red retention (NRR) assay time values, inhibition of acetylcholinesterase (AChE) activity. Increase of micronucleus (MN) frequency and DNA damage were detected in haemolymph/haemocytes and gills	[35]
Messenia	Fungi	*Pleurotus* strains	Biomass	OMW toxicity as evaluated by the mycelium growth of *Pleurotus* strains was influenced significantly by the phenolic content of OMW samples obtained during three successive crop years; in contrast, the olives harvest period did not affect *Pleurotus* biomass production	[36]
Achaia	Fish larvae	Zebrafish *Danio rerio* embryo	Mortality (LC_{50}) 24–48 h	24 h – Untreated OMW: 0.43% (Std = 0.19), TU = 231.4, extremely toxic	[26]
				Anaerobically treated OMW: 2.33% Std=0.77), TU = 42.9, very toxic	
				48 h – Untreated OMW**:** 0.33% (Std=0.15), TU = 304.4, extremely toxic	
				Anaerobically treated OMW**:** 1.85% (Std=0.57), TU = 54, very toxic	
				(TU > 100 : extremely toxic, TU = 11–100 : very toxic)	

(continued)

Table 2 (continued)

OMW Origin	Taxonomic group	Test organism	Endpoint	Toxicity values	References
Achaia (OMW) and Kalamata (OMW)	Fish larvae	Zebrafish *Danio rerio* embryo	Mortality (LC_{50}) 24–48 h	24 h – anaerobically treated OMW: 1.99% ±1.1, anaerobically treated OMSW: 2.09% ±0.7	[32]
				48 h – anaerobically treated OMW: 1.52% ±0.8, anaerobically treated OMSW: 1.63% ± 0.8	
				(26% of the samples are extremely toxic and 74% very toxic)	
Crete	Mammals	Wistar rats	Mortality, body weight, signs of toxicity such as tremor, convulsion, salivation, etc.	Oral administration of 2,000 mg OMW per kg body weight. No mortality, no signs of toxicity or abnormal behaviour during the 14-day observation period, normal body weight development	[30]
Fthiotida and East Attica	Plants	*Lepidium sativum*	Seed germination	Untreated OMW – Germination Index (G.I.): 0, Biotreated OMW with *Pleurotus ostreatus* strains LGAM P113 and P115 – increased G.I., toxicity significantly decreased	[25]
Chania	Plants	Lettuce	Seed germination	OMW is strongly phytotoxic (even at 1:4 dilution) and completely hinders plant growth	[37]
Kalamata	Plants	*Lycopersicon esculentum* Mill.	Plant growth	Highly significant reduction of growth observed. Root was more sensitive to OMW than the upper parts of the tomato plant which may be because the root face OMW toxicity directly, while toxicity to other parts is indirect	[38]
Kalamata	Plants	*Spinacia oleracea* L. cv Virofly	Seed germination, plant growth, shoot and root elongation and biomass	1:10 diluted OMW suppressed seed germination by 30%	[39]
				1:20 diluted OMW suppressed seed germination by 22% ($p < 0.01$)	

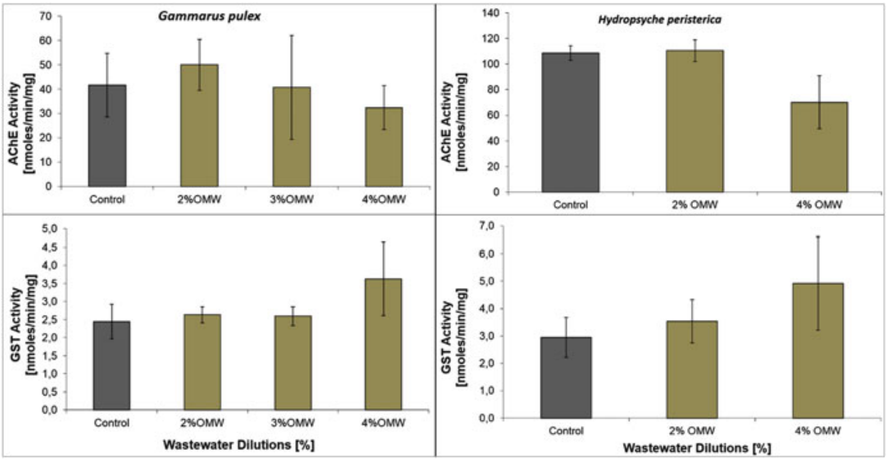

Fig. 5 AChE and GST activities of *G. pulex* (*left*) and *H. peristerica* (*right*) exposed to olive mill wastewaters for 24 h. Results are expressed as the mean ± SD per wastewater concentration samples

Sublethal concentrations of OMW can also cause damage at lower levels of biological organisation [14, 28]. For example, the enzyme activities of the caddisfly *Hydropsyche peristerica* and amphipod *Gammarus pulex* were affected when exposed to OMW [19]. The acetylcholinesterase (AChE) activity of *H. peristerica* and *G. pulex* decreased after 24 h of exposure (Fig. 5). In contrast, the glutathione S-transferase activity of the two species has been shown to increase as OMW concentration increases (Fig. 5). Inhibition of AChE was also observed in the mussel *Mytilus galloprovincialis* when exposed to either 0.1 or 0.01% (v/v) OMW for 5 days [35]. Specifically, decreased neutral red retention (NRR) assay time values, inhibition of acetylcholinesterase (AChE) activity, as well as a significant increase of micronucleus (MN) frequency and DNA damage were detected in haemolymph/haemocytes and gills, compared with values measured in tissues of control mussels [35].

2.4 Effects on Water Quality, Aquatic Organisms and Ecological Status

Olive mill wastewaters are being discharged, untreated or partially treated in hundreds of torrents and streams throughout the country. The most visible effect of OMW pollution is the discolouring of surface waters, which is attributed to the oxidation and subsequent polymerisation of tannins that give dark-coloured polyphenols [13]. The main cause of the problem is the very high organic content, which is not easily biodegradable, while high concentrations of polyphenolic compounds result in toxicity and environmental degradation. Most mills are family businesses of small capacity which cannot afford the cost of installing treatment systems, ending up in disposing the wastewater in adjacent water bodies. In fact, the main recipients of wastewaters in Greece are streams and torrents (58.3%), soil (19.8%), rivers (6%), water (5.3%) and lakes (0.038%) [40]. There have been numerous studies on the effects of pollution in Greece's running waters, resulting from the synergistic effect of multiple stressors, such as pesticides, fertilisers and hydromorphological degradation; however, the pure effects of OMW on the aquatic biota and ecological status of stream ecosystems have been poorly investigated (Table 3).

The first one dates back to 1993, where Voreadou [44] within the context of her doctorate thesis studied the impacts of OMW in several streams of Crete. The results of her study showed a dramatic decline of the benthic macroinvertebrate community during wastewater discharge, while the intensity of the effects was proportional to the volume and duration of water in the stream bed. In streams receiving OMW with high water velocity that retained water for 7–8 months, species richness declined by 41%, while in streams with less water supply and flow duration, species richness loss reached approximately 71% [45]. Voreadou [44], apart from the phenol toxicity capacity of OMW, also attributed the reduction of biodiversity to the formation of a greasy layer on the water surface from the lipid content of the wastewater, thus preventing the entry of light and oxygen and the accumulation of solid components in the stream bottom that may enter the body of aquatic organisms.

Recently, the effects of OMW on the stream macroinvertebrates, water quality and river ecological status were thoroughly and systematically studied in the Evrotas river basin in South Peloponnese [14, 19, 28, 42]. Benthic macroinvertebrates and environmental parameters were monitored for two years, thus following the biennial cycle of olive growth and production and hydrological variation (dry – wet years) in order to assess spatial and temporal responses of stream fauna to high and low OMW yield years. Furthermore, two different hydrologic years (wet and dry year) were covered during the two-year monitoring period, thus allowing evaluation of hydrologic regime variation to OMW pollution intensity and effects.

The results of these studies revealed the spatial and temporal structural deterioration of the aquatic community due to OMW discharge with consequent reduction of the river capacity for reducing the effects of polluting substances through internal mechanisms of self-purification. OMW, even highly diluted, had significant impacts on the aquatic fauna and the ecological status of the Evrotas River. The vast majority of macroinvertebrate taxa were eliminated, and only a few tolerant Diptera species (i.e. Chironomidae, Simuliidae, Syrphidae) survived with very limited abundances (1–4 individuals/1.25 m^2). Macroinvertebrate assemblages downstream the OMW outlets were dominated by Diptera species, whereas Ephemeroptera, Plecoptera and Trichoptera (EPT) were almost depleted during and after the OMW discharge period.

Overall, the effects of OMW on water chemistry were more pronounced on the second year of the sampling campaign due to the higher olive fruit production that

Table 3 Monitoring and assessment studies conducted in Greece evaluating OMW effects to running waters

River basin	Stream name	Investigated topic	Endpoint (State/Effect)	References
Pamisos, Nedon, Aris, Belikas and Epis	Pamisos, Nedon, Aris, Belikas and Epis and their estuaries	Effects of OMW on water quality	Water quality (Downgraded especially in November and December. Elevated levels of phenols, high concentrations of ammonium and inorganic phosphorus)	[34]
Epis	Epis and its estuary	Effects of OMW on water quality	Physicochemical quality (Increased values of Mn, Cu, Ni, phenols, ammonium, nitrates)	[41]
Evrotas	Kotitsanis, Vordoniatis, Yerakaris and Skatias tributaries	Effects of OMW on benthic macroinvertebrates and ecological status	Water pollution (increased COD, BOD_5, TSS, chloride, phenols, sewage bacteria levels, decreased O_2 concentrations)	[42]
			Macroinvertebrate assemblages (decreased number and abundance of taxa, degraded biocommunity structure)	
			Biological quality and ecological status (downgraded from good and high to moderate and bad)	
Evrotas	Kotitsanis, Vordoniatis, Yerakaris and Skatias tributaries	Effects of OMW on small streams	Physicochemical quality (good to moderate)	[42]
			Biological quality and ecological status (moderate to bad)	
			Water pollution (high levels of BOD_5, COD, TSS and phenols, low DO)	
Evrotas	Evrotas River	Effects of OMW on water and effects of disposing OMW	Water physicochemical quality (low levels of COD, phenols and nutrient levels in Evrotas river. Increased phenols in Skoura and Vrontamas station due to the olive mills)	[43]
			Attenuation capacity of river sediments (High	

(continued)

Table 3 (continued)

River basin	Stream name	Investigated topic	Endpoint (State/Effect)	References
			attenuation capacity. Phenols were reduced from 2.0 to 1.0 mg/l and COD from 30.3 to 6.3 mg/l in 68 days)	
			Soil physicochemical quality after irrigation with treated OMW (increased conductivity, pH, nitrogen, nitrate-N and organic matter, lower ammonia-N)	
			Groundwater quality (increased phenolic compounds, ammonia, TOC and COD. Leaching of loads from the surface to the groundwater)	
Aposelemis	Prinopotamos	Effects of OMW on water quality and benthic fauna	Benthic macroinvertebrate community loss during wastewater discharge. Intensity of the effects was proportional to the volume and duration of water in the stream bed. Streams receiving OMW with high water velocity that retained water for 7–8 months, species richness declined to 41%, while in streams with less water supply and flow duration, species richness loss reached approximately 71%	[44]

yielded a greater quantity of wastewater. During the OMW discharge period, BOD_5, COD and TSS were extremely high, causing a significant decrease in dissolved oxygen concentrations and creating anoxic conditions in many cases. A significant increase in chloride and total phenols concentration was also observed in the downstream sites during the wastewater discharge period as well as a marked increase in nutrients [42]. Mean concentrations of COD, BOD_5, total phenols, total suspended solids (TSS) and chloride were higher in the sites receiving OMW, while sewage bacteria flourished as a result of OMW residue on the stream substratum during the wastewater discharge period [42]. Dissolved oxygen concentration showed no marked variation among periods in the upstream sites in contrast to

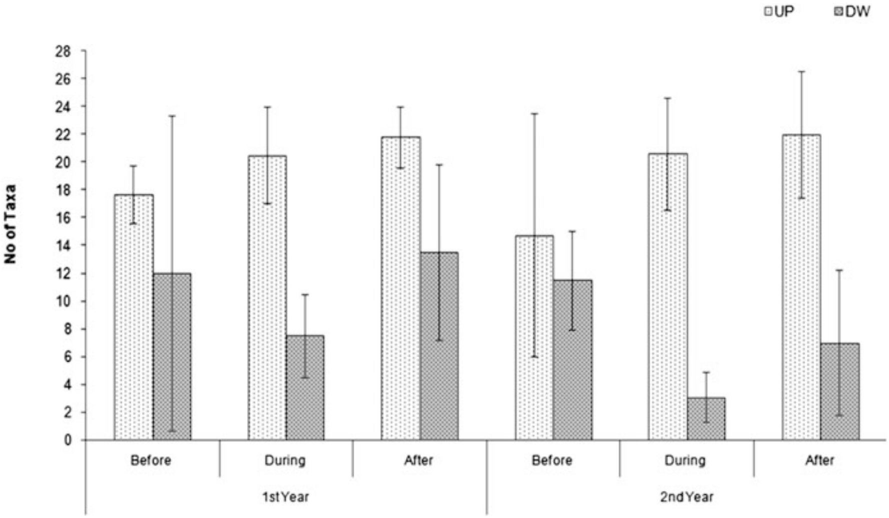

Fig. 6 Mean (± SD) number of taxa before, during and after the OMW discharge period for the two-year sampling campaign. *UP* upstream sites; *DW* downstream sites ([42], with permission)

the downstream sites, where oxygen concentration decreased during and after the wastewater discharge period, especially in the second year of sampling. Total phenols were detected only during the wastewater discharge period and were significantly higher in the second sampling year compared to the previous one.

Species richness downstream the OMW outlets was markedly lower than upstream of the olive mills (Figs. 6, 7, and 8). During the wastewater discharge period, the number and abundance of taxa were significantly decreased; the effects during year two being more pronounced due to the prolonged drought in the years 2006 and 2007 (Fig. 6). Upstream sites that were used as control presented good and high ecological status, whereas the ecological status of the sites affected from OMW pollution ranged from moderate to bad. Effects were more pronounced at lowland intermittent streams (Fig. 8), thus showing that intermittency and prolonged drought in combination with wastewater discharge significantly affect stream fauna and ecological status. Stream typology (i.e. slope, altitude) and hydrology of the stream site (i.e. perennial or intermittent) and the intensity and volume of the wastewater were the most important determinants of self-purification processes [42].

A study conducted in the Epis River in Messenia (Peloponnese, S. Greece) by Anastasopoulou et al. [41] revealed high concentrations of phenols (36.1–178 mg/l), ammonium (7.3–9.5 mmol/l), phosphate (6.1–7.5 mmo/l), COD (53.4 g/l) and certain heavy metals especially during December when the olive oil production reaches a peak in the area. Increased values of Mn, Cu and Ni were recorded in the river water, while the calculation of the sediment enrichment factor confirmed the ecosystem's deterioration due to these trace metals. Increased phenol concentrations were also detected in the Messenian Gulf during the olive-harvesting period

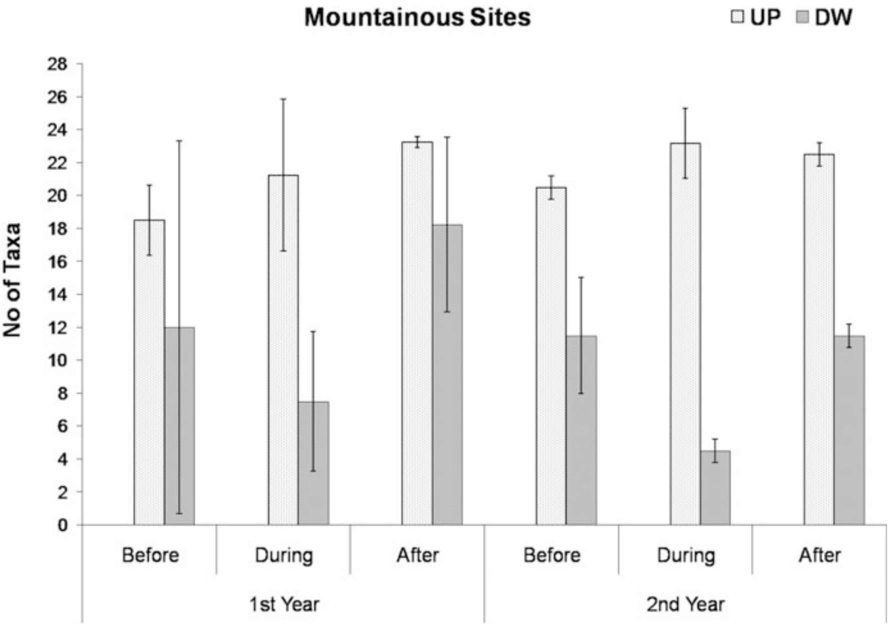

Fig. 7 Mean (± SD) number of taxa upstream and downstream the OMW outlet in mountainous (permanent) sites ([42], with permission)

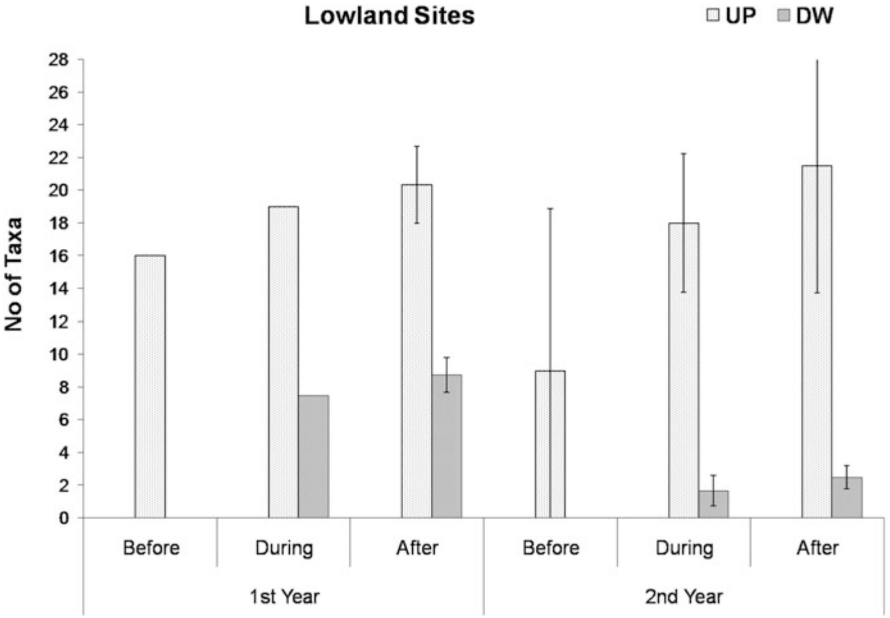

Fig. 8 Mean (±SD) number of taxa upstream and downstream the OMW outlet in lowland (intermittent) sites ([42], with permission)

(96–207 ppb). Concerning the concentration of heavy metals, high values of Fe (515 ppb) and Mn (486 ppb) were detected in the water body of Epis before its estuaries [41]. Before the production period, the concentration of these trace metals were found at much lower levels (48.9 and 118 ppb, respectively). Both zinc and lead's concentrations did not appear to differ greatly before and during the production period. According to the Greek Nutrient Classification System [46], the quality of all the sites assessed before the olive oil production period of 2008–2009 was classified as high. During the production period, the physicochemical quality of the sites downstream the olive mills varied from moderate to poor. Based on benthic macroinvertebrate fauna, the biological quality of the Epis River ranged from bad to moderate [47].

Another study conducted in the region of Messenia from 2008–2011 [34], in which several rivers and streams that discharge into the coastal zone of the Messenian gulf were included, showed that OMW have deteriorated the water quality of the gulf. The studied rivers were classified as good or moderate, and in some cases, poor, whereas the sites at the coastal zone of the Messenian Gulf were characterised as good or moderate [34]. Five months after the oil-productive period (May 2011), water quality has not been recovered due to OMW. This is also supported by the biological results (macroinvertebrates) in the studied rivers influenced by OMW, which were obtained in the framework of a monitoring programme that was carried out at the Prefecture of Messenia during 2011–2014 [48]. According to the STAR_ICMi [49] and BMWP [50] biological indices, the ecological status at most of the sampling sites of Pamisos River, Epis River, Belikas River and Aris River was classified as good or moderate during the summer period, with no influence of OMW, and as poor or bad during the wet (olive oil production) period [48].

Increased nutrient and metal concentrations are also reported from experiments carried out in evaporation lagoons in order to test for changes in the chemical properties of the soil [51]. Disposal of untreated OMW at evaporation lagoons without using protective materials (e.g. impermeable membranes) resulted in significant changes in soil chemical properties. Soil samples collected one month after the completion of waste disposal were characterised by enhanced content in nitrogen, organic matter, exchangeable K, Mg, cation exchange capacity, available Mn and Fe as well as increased electrical conductivity and decreased $CaCO_3$ [51]. Changes in soil properties depended on depth and distance from the disposal lagoon.

Although not carried out in running waters, a study performed by the Institute of Infectious and Parasitic Diseases of the Centre of Veterinary Institutions of Thessaloniki [98] on the effects of OMW in fish farms in the Gulf of Amvrakikos surfaced conclusions already known. Specifically, fish deaths occurred in aquaculture due to (a) non-water-soluble OMW components superimposed on the gills of the fish thus blocking respiration, (b) pH decrease of seawater at values well below 8 (which is the normal value in the region), (c) high levels of BOD and COD leading to anoxic conditions and (d) weakening of the organism, making them susceptible to microbial infections.

2.5 Current Legislation

The disposal of OMW, on both freshwater bodies and soils, may also affect groundwater quality [52], especially in calcareous rocks, which have high permeability. In Greece, according to the law Y2/2600/2001, the limit for the content of phenolic compounds in drinking water is 0.5 μg/l. The concentration of phenols in OMW usually ranges from 0.1 to 0.5 mg/l, which certifies that the direct disposal of wastewater into water bodies is hazardous. The acceptable limits designated for European countries for the disposal of OMW in various recipients are presented in Table 4.

Until today, there are no specific rules for the treatment, management and disposal of OMWs in surface water bodies. The Ministry of Development, Competitiveness, Infrastructure, Transport and Networks and the Ministry of the Environment, Energy and Climate Change (YPEKA) have published a guideline on the management of OMW that is included in Category B of the Ministerial Decision (MD) 1958/2012 (Government Gazette B21/13-01-2012). The guidelines set for the application of the term E3 of the Common Ministerial Decision (CMD) Φ15/4187/266/2012 (Government Gazette B'1275/11-04-2012) on standard environmental commitments for certain industrial activities refers to pretreatment methods so as to avoid the direct discharge of wastewaters to water recipients. With the 191645/03-12-2013 Circular, the Secretariat for Water of the Ministry of Environment, Energy and Climate Change states that within the implementation of the measures of the Basin Management Plans of the water districts in the country, they will proceed to the modernisation of waste management legislation with the issue of a Common Ministerial Decision (CMD). The new CMD will replace the articles of the Sanitary Provision EIB/221/1965 on the disposal of liquid waste into surface water bodies and will essentially abolish it. Until the adoption of this new CMD, the decisions of the regional units should be followed.

Table 4 Acceptable levels on the disposal of OMW in water bodies in different European countries

Parameters [mg/l]	Disposal in surface waters			Disposal at sea		Disposal at sewage systems		
	Greece	Italy	Croatia	Greece	Croatia	Greece	Italy	Croatia
pH	6–9	5.5–9.5	6.5–8	6–9	6.5–8	6–9	5.5–9.5	5–9.5
BOD	40	$\leq$40	25	40	25	500	$\leq$250	250
COD	120	$\leq$160	125	120	125	1,000	$\leq$500	700
Total suspended solids	40	$\leq$80	35	50	35	500	$\leq$200	80
Lipids and oils	5	–	25	5	25	40	100	–
Total phenols	0.5	$\leq$0.5	0.1	0.5	0.1	5	$\leq$1	10

Source: IMPEL Olive Oil Project 2003

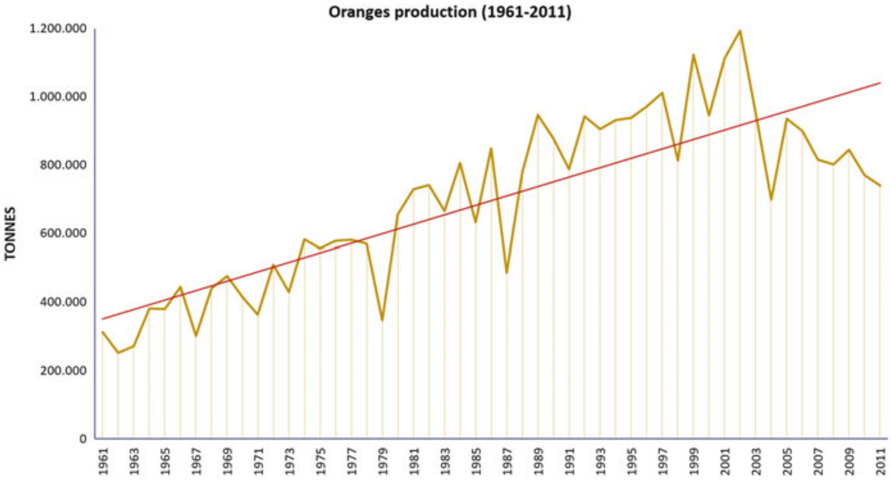

Fig. 9 Orange production (tons) in Greece from 1961 to 2011 according to the FAO (World Food and Agriculture Organization)

3 Orange Juice Processing Wastewaters (OJPW)

3.1 Current Production Trends

According to the Hellenic Ministry of Rural Development and Food, the total production of citrus fruit in Greece is about 1 million tons per year, of which only about a third is destined for juicing. The citrus fruit-cultivated area in Greece is estimated at approximately 53,000 hectares. Of these, 40,000 hectares are oranges [4]. The cultivated land and production of oranges have increased significantly in recent decades. In 1961, there were 17,700 hectares of orange trees that yielded 321,000 tons of oranges, and today, the cultivated area is about 40,000 hectares producing about 900,000 tons of oranges (Fig. 9). From this quantity, 34% of the fruit is produced in the prefecture of Argolida, 23% in Laconia, 17.5% in Arta, 9% in Chania and Crete, 5% in Ilia, and 3% in Etoloakarnania and Corinthia, respectively. The Greek citrus processing industries are mainly located in the regions of Argolida, Arta, Laconia and Crete.

3.2 Polluting Capacity and Characteristics

The processing of orange fruit gives about 45% fresh juice and 50% solids that consist of the pulp and peel of the fruit. The remaining 5% consists of a collection of cells, essential oils and limonene. A fraction of the juice and pulp are various compounds of flavonoids, such as hesperidin, neohesperidin, rutin, narirutin, naringin and nobiletin [53]. Orange juice production gives about 70% waste, of which 75–80% is solid and 20–25% liquid. Solid waste is composed of the peel and pulp, while the effluent comes from the washing of fruits and the production of various by-products. It is estimated that one ton of oranges produces 1.5 million litres of wastewater. In most citrus juice plants, the citrus wastewater undergoes biological treatment. The solid waste is usually transported to a landfill. However, disposal of waste in illegal dumps and steep cliffs, as well as the uncontrolled disposal of solid waste (pulp and shredded stems) in streams and rivers after being mixed with the wastewater is frequent (Figs. 10 and 11).

The composition and physicochemical characteristics of OJPW vary greatly depending on the variety, the maturity of the orange fruit and the production

Fig. 10 Illegal OJPW discharge in Tyflo stream of the Evrotas river basin, Peloponnese, S. Greece

Fig. 11 OJPW discharge in the Tyflo stream (Evrotas River, Peloponnese, S. Greece)

conditions. Wastewaters generated from orange juice production have high organic load (BOD: 20–1,400 mg/l; COD: 100–2,000 mg/l) and can be toxic due to the high concentration of organics, including terpene-containing oils and flavonoids [54]. The complex and insoluble carbohydrates, proteins, fibres, high nitrogen and sodium levels [55, 56] increase the organic load of OJPW and, in addition to the limonene levels (90–95% in citrus peel oil; 0.3%–0.8% in wastewater), decrease the effective treatment and disposal of the effluent [56]. Table 5 summarises the chemical and physicochemical composition of OJPW.

Table 5 Composition and physicochemical characteristics of OJPW

Parameters	Units	OJPW
pH		4–6.5
Acidity (citric acid)	g/l	0.1–0.2
Electrical conductivity	μS/cm	500–3,700
BOD_5	mg/l	409–4,000
COD	mg/l	435–13,650
Total solids	mg/l	640–840
Total suspended solids	mg/l	300–2,800
Total dissolved solids	mg/l	540
Alkalinity (CaCO3)	mg/l	800–815
Ash	mg/l	424
Total sugars	g/l	6–30
Total phenols	mg/l	1.5–8
Limonene	mg/l	50–200
D-Limonene	%	0.02–0.5%
Organics	%	94.7
Hesperidin	mg/l	1,000–3,000
Pectin	mg/l	1,200–9,000
Fats and oils	mg/l	2,045
Dry mass (DM)	g/kg	110
Proteins	g/kg	53.8
Fibres	g/kg	164
Nitrite (NO_2)	mg/l	1–3
Organic nitrogen [ON]	g/l	7.28
Potassium [K]	mg/l	1,578
Manganese [Mn]	mg/l	0.3–0.7
Iron [Fe]	mg/l	0.33–3.9
Total phosphorus [TP]	mg/l	188
Phosphorus [P]	mg/l	0.4–2.4
Calcium [Ca]	mg/l	30–60
Chloride [Cl]	mg/l	80–160
Sodium [Na]	mg/l	135–205

Sources: [55–60]

3.3 Toxicity, Effects on Water Quality, Aquatic Organisms and Ecological Status

Up to date, there is only one study available that documents the toxicity of OJPW on aquatic organisms [14, 19]. In that study, two test organisms were used for testing the acute toxicity of the wastewater: *Gammarus pulex* and *Hydropsyche peristerica*. Mortality for 50% of the amphipod *G. pulex* population occurred at 25.26% wastewater dilution concentration and 17.16% for *H. peristerica*. The latter showed to be more sensitive to OJPW toxicity than *G. pulex*. Based on the five-class hazard classification system used for wastewaters discharged into the aquatic environment [29], OJPW belongs to class III (acute toxicity).

The effects of OJPW were also evaluated at the molecular level of the two-test species by assessing changes in their AChE and GST enzyme activities [14, 28]. OJPW caused the decrease of AChE of *G. pulex* after 24 h of exposure (Fig. 12). Unlike the activity of AChE, the GST activity of *G. pulex* increased at higher concentrations of the effluent (Fig. 13). The same changes were also observed in the enzymatic activities of *H. peristerica* after 24 h of exposure. AChE concentration decreased at increasing concentrations of OJPW (Fig. 12), while activity of GST increased in conjunction with higher concentrations of the wastewater (Fig. 13).

As with olive mills, wastewaters of the citrus juice processing industry can have profound effects on freshwater ecosystems. Two streams of the Evrotas river basin

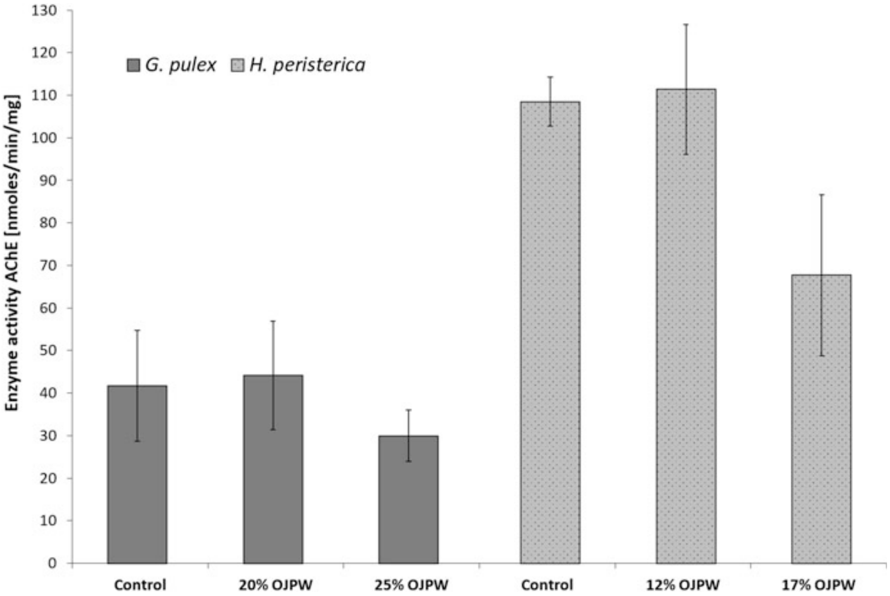

Fig. 12 AChE activities of *G. pulex* (*left*) and *H. peristerica* (*right*) exposed to orange juice processing wastewaters for 24 h. Results are expressed as the mean ± SD per wastewater concentration samples

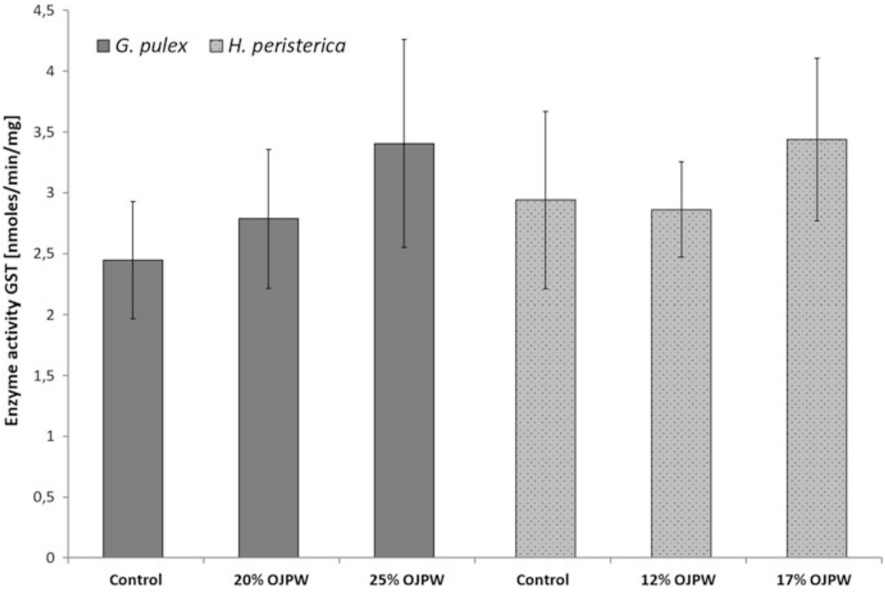

Fig. 13 GST activities of *G. pulex* (*left*) and *H. peristerica* (*right*) exposed to orange juice processing wastewaters for 24 h. Results are expressed as the mean ± SD per wastewater concentration samples

have been receiving untreated or partially treated wastewaters from two orange juice processing plants for many decades. Ecological quality monitoring and assessment carried out in these two streams revealed significant loss of the benthic fauna, since in almost all months of monitoring, only a few individuals of the Dipteran families of Chironomidae and Simuliidae were found [28]. The Tyflo stream flowing through the Riviotissa settlement on the suburbs of Sparta was represented solely by *Chironomus plumosus*-gr with very limited abundance (usually 1–3 individuals/1.25 m^2). Even months after the end of the wastewater discharge period, no recovery was observed in benthic fauna composition while the ecological status of the stream remained poor throughout the monitoring period. At the Mylopotamos stream in the Aghia Kyriaki settlement (3 km south of Sparta), the situation was the same as with the Tyflo stream, apart from a burst of *Chironomus plumosus*-gr. and Tubificidae worm abundances after the end of the wastewater discharge period [19, 28].

Apart from Evrotas, there are many more freshwater systems that receive wastewaters from fruit and vegetable juice processing units (oranges, peaches, apples, apricots, carrots, pomegranates, grapes, etc.) throughout Greece, such as Aliakmonas, Axios, Pinios, Louros, etc. Studies carried out in these systems involve their ecological status assessment and include a variety of stressors such as pesticides, nutrient pollution from fertilisers, organic pollution (olive mills, wastewater treatment plants, slaughterhouses), hydromorphological modifications, etc. [61–68].

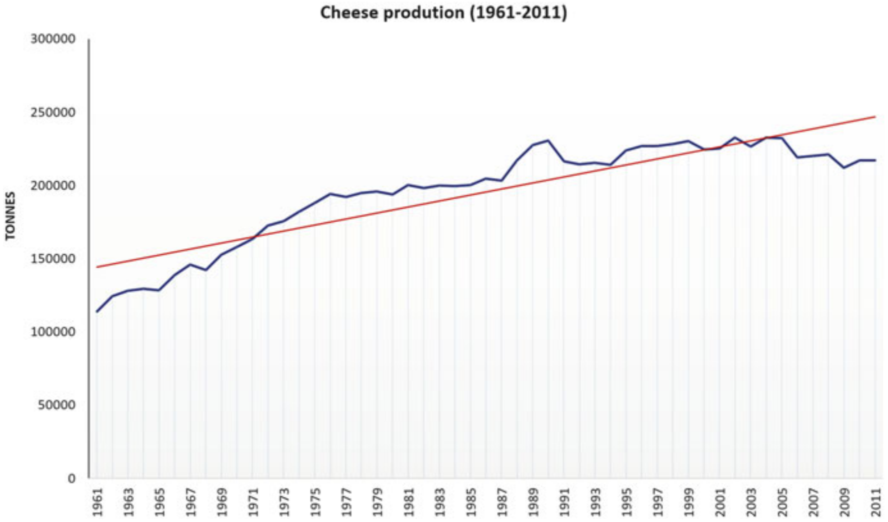

Fig. 14 Cheese production (tons) in Greece from 1961 to 2011 according to FAO (World Food and Agriculture Organization). Data include goat, sheep and cow cheese

4 Cheese Whey Wastewaters (CWW)

4.1 Current Production Trends

Cheese production in Greece is a traditional area of activity, as it has been reported by several historical sources as one of the main trade activities in ancient and more recent times. Over the years and with the assistance of financial institutions through granting investment incentives from the state (under development laws and EU regulations), the industry has made significant developments (Fig. 14). A key feature of the industry is the large number of industries, mainly primary production farms. The majority of these industries include, mainly, small size and capacity units at local level characterised by high dispersion and usually a lack of required modern mechanical equipment. Similar to olive mills, the exact number of cheese production units that currently operate in Greece is unknown. For example, in 2011, from the 96 registered production units of Crete, only 60 had operational permission, while the true number is speculated to be around 400 [99].

4.2 Polluting Capacity and Characteristics

The dairy industry is one of the main sources of industrial wastewater generation in Europe [69]. It is based on the processing and manufacturing of raw milk into products such as yogurt, butter, cheese and various types of desserts by means of several different processes, such as pasteurisation, coagulation, filtration, centrifugation, etc. Dairy factory wastewaters commonly contain milk, by-products of processing operations, cleaning products and various additives that may be used during the production [70]. The water requirement of a dairy plant for washing and cleaning operations corresponds to 2–5 l of water per litre of processed milk. The characteristics of dairy effluents may vary significantly, depending on the final products, system type and operation methods used in the manufacturing plant [71].

The cheese manufacturing industry generates three main types of effluents; cheese whey (resulting from cheese production), second cheese whey (resulting from cottage cheese production) and the washing water of pipelines, storage and tanks that generates a wastewater called cheese whey wastewater (CWW). The latter, also contains cheese whey and second cheese whey and is a strong organic and saline effluent whose characterisation and treatment have not been sufficiently addressed. CWW generation is roughly four times the volume of processed milk [71].

Cheese whey wastewater is white in colour and usually slightly alkaline in nature and becomes acidic quite rapidly due to the fermentation of milk sugar to lactic acid. It is characterised by an unpleasant odour of butyric acid, high organic content (COD up to 70 g/l, BOD up to 16 g/l) and relatively high levels of total suspended solids (up to 5 g/l) [72, 73]. Due to salt addition during the cheese production process, sodium and chloride levels are extremely high (2.1–2.8 g/l). The values reported in total nitrogen (0.5–10.8 mg/l) and phosphorus (6–280 mg/l) indicate a serious risk of receiving water eutrophication [71]. It also contains lactose, proteins and fats (45, 34 and 6 g/l, respectively) and has a high biodegradability index (BOD/COD $\approx$ 0.46–0.80) (Table 6) that suggests the suitability of biological process application [71].

Table 6 Composition and physicochemical characteristics of CWW

Parameters	Units	CWW
pH		4–8.7
Electrical conductivity [EC]	mS/cm	11–13
BOD_5	g/l	0.9–15
COD	g/l	0.8–77
Total solids [TS]	g/l	1–63.5
Total susp. solids [TSS]	g/l	0.25–5
Turbidity	NTU	1,300–2,000
Total organic carbon [TOC]	g/l	0.55–35
Total Kjeldahl nitrogen [TKN]	g/l	0.11–0.83
Total phosphorus [TP]	mg/l	6–280
Total nitrogen [TN]	mg/l	0.5–11
Fats and Oils	g/l	0.1–5.7
Proteins	g/l	1.88–9
Lactose	g/l	0.1–44
Chloride	g/l	2–2.5
N-NH4	mg/l	8–161

Sources: [71, 74, 75]

Fig. 15 CWW discharge in Voulgaris stream, Lesbos Island

If discharged untreated into the waterways (e.g. Fig. 15), CWW can cause serious environmental problems. Although cheese whey contains valuable fertiliser components such as nitrogen, phosphorus and potassium, application on land compromises the physical and chemical structure of the soil resulting to crop yield decline [76, 77] and reduces aquatic life by depleting the dissolved oxygen of the water [78, 79] and may eventually pollute the groundwater.

Several value-added products can be produced from cheese whey by using various fermentation processes in order to minimise the problems associated with its disposal and improve the economics of the dairy and food processing industry. Usually, the small and medium cheese factories are isolated from centralised wastewater treatment facilities and, in some cases, located next to ecologically sensitive areas, which may cause environmental risks. Land application is often the only practical option for wastewater disposal.

4.3 Toxicity, Effects on Water Quality, Aquatic Organisms and Ecological Status

Despite the fact that several methods for the treatment or utilisation of cheese whey wastewater have been proposed during the last 60 years, more than 50% of wastewater is discharged untreated to waterways [74]. According to statistical data of 2007, 7.5 million tons of CWW are produced every year in Greece [74]. The vast majority of these quantities are discharged untreated or partially treated into the environment, including soil and freshwaters.

Even though CWW discharge is among the main sources of organic pollution in Greek river ecosystems, its effects on aquatic ecosystems have overall been neglected. Many running waters throughout the country receive CWW, but up to date, effects only on the Vouraikos River in Peloponnese have been assessed

[80]. In that study, Karadima et al. [80] found that the ecological quality of the sites close to the cheese production factory ranged from moderate to bad and that there was a significant ecological risk for almost 15 km downstream of the point pollution source. Pollution-tolerant macroinvertebrate taxa such as Chironomidae, Tubificidae, Valvatidae and Lumbricullidae were abundant in the low-quality sites (close to the factory), which also presented low biodiversity values and low numbers of families (between 6 and 7). In contrast, samples from 10 km downstream the cheese production factory presented higher biodiversity, many pollution-sensitive taxa such as Athericidae, Perlidae, Perlodidae and Sericostomatidae and a number of families between 26 and 27 [80].

Cheese whey wastewater has also shown to be toxic to aquatic organisms. Toxicological results of the zebrafish *Danio rerio* embryo bioassay with a mean 7-day LC_{50} was 0.655%, while bioassays on *Daphnia magna* and *Thamnocephalus platyurus* presented a higher LC_{50} value of 3.032% and 1.56%, respectively [80]. Even after treatment with an anaerobic fermentation system for hydrogen production, the CWW samples varied from "very" to "extremely toxic" [81]. Average toxicity values for the zebrafish *Danio rerio* embryo bioassay were 1.55% (24 h) and 0.75% (48 h), for *Thamnocephalus platyurus* 0.69% (24 h) and for *Daphnia magna* 2.51% (24 h) and 1.82% (48 h). Toxicity of CWW was attributed to the chemical compounds PO_4^{-3}, SO_4^{-2}, $N\text{-}NH_3$ and NO_3^- [81].

Similar to olive mills, cheese producing plants are small capacity units that are scattered throughout Greece and cause very serious environmental problems due to their large volume and organic load of wastewaters. To date, an integrated treatment solution at national level has not been implemented, despite the existence of various small-scale treatment technologies. For example, a new, integrated technology for the treatment and utilisation of cheese-dairy wastewater has been developed by the laboratory of Organic Chemical Technology of the National Technical University of Athens and has successfully been tested in a cheese-making factory in Viotia [74]. The proposed technology reduced fat and oil content by 76% and COD by 90%, while biogas was produced (4 m^3/h). The university laboratory concluded that the final effluent could be disposed in water bodies after an aerobic biological refining, however the final effluent should be tested for toxicity as the effluent can still be toxic after treatment as has been shown in some studies (e.g. [81]).

5 Other Agroindustrial Industries

Greece's running waters are also recipients of effluents from other agricultural industries, including animal factory farms, dairy farms, slaughterhouses, food, fruit and meat processing plants, tannery (leather processing plants), wineries and paper and weaving (cotton and textile) industries. The common characteristic of all these industries is the high organic content (BOD and COD) and total solids [82] of their wastewaters that result in oxygen depletion when discharged in receiving waterways [83, 84]. The acceptable lower limit for oxygen concentrations in rivers is usually about 6 mg/l, which is the level in which sensitive fish species (usually trout and salmon) are able to survive [85]. The discharge of organic wastewaters to rivers results in the development of bacterial or fungal-dominated epilithon (stone-attached communities), commonly referred to as sewage fungus or sewage bacteria [86]. These growths degrade river aesthetics, make the riverbed unsuitable for fish and many invertebrate species [19, 28, 44] and gradually decrease the water pH, accompanied by a release of strong odours due to decomposition of organic compounds. The receiving water becomes a breeding place for pollution-tolerant species, which are usually Dipteran species, such as flies and mosquitoes, and may often be carriers of dangerous diseases.

These effects have been observed in many river systems throughout Greece, but no prevention and control measures are implemented. Usually, pollution incidents become obvious when mass fish deaths are witnessed by the local inhabitants. A recent example comes from the Spercheios River in Central Greece, where hundreds of fish died due to oxygen depletion. Fish mortality is attributed to untreated wastewater discharge from the paper mill into a tributary of Spercheios (Asopos stream). The mill operated all year round, but effects are more pronounced during the dry period where flow is at a minimum. Ecological quality of the stream near the paper mill was classified as poor and bad, and only *Chironomus plumosus*-gr and species of the Simuliidae family were detected at very high abundances (Karaouzas et al., unpublished results). The effects of paper mill wastewaters into river ecosystems are documented elsewhere [87, 88].

Although the livestock industry is one of the major industries in Greece, its effects on the environment have been largely overlooked and ignored. The United Nations has declared concentrated animal feeding operations to be “one of the top two or three most significant contributors to the most serious environmental problems, at every scale from local to global” [100]. Wastewater discharge from slaughterhouses causes deoxygenation of rivers [84] and contamination of groundwater [89]. Blood, one of the major dissolved pollutants in slaughterhouse wastewater, has a chemical oxygen demand (COD) of 375 g/l and contains high concentrations of slowly biodegradable suspended solids, including pieces of fat, grease, hair, feathers, flesh, manure, grit, and undigested feed [90]. Furthermore, livestock produce significant amounts of manure, which may overflow due to heavy rainfalls or ruptures leach through the soil into groundwater [91, 92]. Manure is rich in compounds of nitrogen, phosphorus and ammonia. When excessive amounts of these compounds enter into freshwaters, they can lead to lethal algal blooms, causing eutrophication [92].

Elevated nutrient levels due to livestock and food processing industries have been recorded in several rivers of Greece. A general increasing trend of the annual mean values of nitrogen and phosphorous compounds at the Louros River, at its conjunction with the small tributary of Vossa which receives wastes from animal farms, has been observed [93]. Increased values of organic matter (8%) have been found at a site of Asmaki canal (Larissa, Thessaly), where a textile-dyeing plant is operating [94]. Two other sampling sites, in the same canal area where extensive farming and an alcohol producing factory occur, displayed high Cu values due to increased organic matter which strongly retains Cu [94].

High nutrient levels (NH_3-N, NO_2-N and NO_3-N) have also been recorded in Canal 66 that flows through Veria city and discharges into the Aliakmon River [66]. Canal 66 receives agroindustrial wastewaters mainly from canneries, and concentrations are higher during the low flow season than in the high flow season due to the lower discharge [66]. This canal is considered by many the most polluted freshwater body of Northern Greece, and in the press it is often cited as “The Canal of Death” due to the frequent sighting of mass fish deaths.

Concluding, it must be noted that all major rivers in Greece have significant pollution problems, particularly at their downstream parts due to wastewater discharge. These rivers receive pollutants from many other point and non-point pollution sources, thus further deteriorating their ecological quality.

6 Conclusions

Agro-industrial wastewater management is today one of the main concerns for ensuring a sustainable environment. Management of wastewaters, as covered in this chapter, is crucial in view of the high organic matter and high nutrient levels that they contain. Most of these wastewaters can be effectively treated either with aerobic or anaerobic digestion processes [74, 82, 95, 96]. Furthermore, all these wastewaters contain nutrients, salts, organics and oils that can be recycled or utilised for other purposes and with effective treatment can be used to irrigate pasture, thereby conserving potable water. Occasionally, pretreatment strategies (i.e. wetlands, artificial lagoons) are required in order to improve the efficiency of the treatment methodology. In the agricultural sector, methane recovery and use as a clean energy source can be a highly sustainable solution, contributing to a number of environmental objectives, as well as providing social and economic benefits for rural communities.

Finally, and most importantly, new regulations must be implemented for the treatment and management of these agroindustrial wastewaters. These wastewaters are usually discharged in small stream catchments (<10 km^2) which are not considered in the Water Framework Directive 2000/60/EC. Therefore, there is a need for including small streams into monitoring and assessment schemes as small streams contribute to the pollution load of the river basin. Furthermore, guidelines to manage these wastes through technologies that minimise their environmental impact and lead to a sustainable use of resources are critical.

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