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Original article

Characterization and Environmental Impact of Olive Mill Wastewater Generated from the Three-Phase Extraction Process

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Abstract

Olive mill wastewater (OMWW) is the main pollutant from the three-phase extraction system of olive oil production. The disposal of OMWW into surface waters represents an important environmental problem in Albania due to huge quantities in short periods (November-February) and high concentrations of organic compounds mainly phenols which cause ecological issues for the ecosystem, such as soil contamination and water pollution.

This study focused on characterization of vegetation water effluents generated from three–phase extraction processes of olive oil production to evaluate their environmental impact.

Samples of OMWW were collected from different three-phase olive mills operating in southern and central parts of Albania. Physicochemical characterization and multivariate analysis were performed. The results of the physicochemical analysis showed that samples of OMWW had an acid pH (4.4-5.3), high levels of organic load expressed in terms of BOD5 (29.8-48.3 g/l), and COD (126-216.8 g/l), higher levels of total nitrogen (423-635mg/L), oils and grease (5.5-8.5 g/L) compared to allowed effluent discharge limits according to Albanian standards. The biodegradability index of OMWW analyzed exceeds the thresh¬old of 3, confirming that our samples are partially or no biodegradable and the polyphe¬nol concentration was 5.5-8.42 g/l.

Keywords: Olive Mill Wastewater, Total Phenolic Content, Environmental Impact, Characterization.

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INTRODUCTION

The consumption of olive oil, well-known for its biological activities, is increasing worldwide due to its health benefits and great nutritional properties (Maazoun et al., 2022; Dermeche et al., 2013). Unfortunately, the olive oil industry is among the agro-industries that generate the most abundant wastes and byproducts with a substantial negative impact on the environment in the Mediterranean area (Benincasa et al., 2022; Neifar et al, 2013).

The production, the type, and the quantity of wastes and byproducts depend on extraction systems (traditional, two- or three-phase mills) used by olive mills (Khwaldia et al., 2022). The three-phase olive oil extraction system requires adding a large amount of water and therefore generates a significant volume of wastewater with a negative impact on the environment compared to the traditional and two-phase systems (Torres and Maestri, 2006; Obied et al., 2005). In this context, olive mill wastewater (OMWW) is one of the largest residues of the olive sector, varying from 1.2 to 1.8 m^3 per ton of olives (Haddad et al., 2017).

The vegetation water has severe environmental impacts including soil contamination, greenhouse gas emissions, the release of unpleasant odors, and growth inhibition of plants and insects (Souilem et al., 2017). Olive mill wastewater (OMWW) has long been thought to be the most polluting effluent and bothersome waste generated by the olive oil industry (Benincasa et al., 2022) since this by-product is characterized by seasonal large volumes (Kalogerakis et al., 2013), high organic load, high quantities of sugars, tannins, the presence of phenolic compounds, polyalcohols, pectins, organohalogenated pollutants, and lipids, which are highly acidic and toxic to plants, bacteria, aquatic organisms and may inhibit the biological degradation of OMWW (Cassano et al, 2013).

The olive oil industry has recently been under greater pressure to address social and environmental challenges in its supply chains throughout product lifecycles (Maazoun et al., 2022). The challenge of treating and disposing of OMWWs is wreaking havoc on the producing countries due to the high organic content (Yangui and Abderraba, 2018). These effluents are characterized by high chemical oxygen demand (COD), strong specific olive oil smell, intensive dark-brown up to black color, high degree of organic pollution, resistance to biodegradation depending on their state of degradation, olives cultivars, and their phytotoxic and antibacterial properties (Niaounakis and Halvadakis, 2006; Capasso et al., 1992). Apart from water (83-92%), the main components of OMWW are phenolic compounds, sugars, and organic acids. It also contains valuable resources such as mineral nutrients, especially potassium, which could be reused as a fertilizer (Aranda et al., 2007; Dermeche et al., 2013).

For a long time, OMWW has been regarded as a hazardous waste with a negative environmental impact and an economic burden on the olive oil industry (Obied et al., 2008). However, if properly managed, it is an inexpensive and convenient source of natural antioxidants, mainly due to their high polyphenolic content that can be extracted and applied as natural antioxidants for the food and pharmaceutical industries (Niaounakis and Halvadakis, 2006).

Olive oil production is one of the major agricultural sectors in Albania, with 15.500 tons produced in the 2022 crop year according to the International Olive Council (IOC). In Albania, approximately 80% of olive mills still use continuous three-phase extraction systems that have not yet been replaced by two-phase systems, which significantly reduce the volumes of OMWW discharges (Pikuli and Devolli, 2024; Topi et al., 2014). The disposal of OMWW into surface waters represents an important environmental problem due to huge quantities in short periods (November-February) and high concentrations of organic compounds mainly phenols which cause ecological issues for the ecosystem, such as soil contamination and water pollution.

Albanian environmental regulations restrict the discharge of OMWW effluents into the environment if they do not meet the wastewater discharge limits to water bodies according to national standards (VKM177, 2005). However, there is still no emergency plan to avoid the illegal discharge of OMWW.

This study focused on the characterization of OMWW effluents generated from the three-phase extraction system of olive mills operating in southern and central parts of Albania to assess their environmental impact.

MATERIAL and METHOD

Sampling of OMWW

Olive oil production is one of the major agricultural sectors in Albania, with 15.500 tons produced in the 2022 crop year according to the International Olive Council (IOC). The highest efficiency in olive oil production is reached mainly in the southern and central parts of Albania, which are characterized by a Mediterranean climate with hot, dry summers and mild rainy winters (M.A.F.C.P, 2009; Topi et al., 2014).

Samples of OMWW were collected from seven three-phase olive mills operating in the southern (Vlora region) and central parts (Elbasan and Tirana regions) of the country during harvest seasons in November 2022-February 2023. This study selected 3 olive mills operating in the Vlora, 2 olive mills operating in Elbasan, and 2 olive mills operating in the Tirana regions (Fig 1). Samples of vegetation waters for chemical analyses were collected in duplicates immediately after the extraction of the olive fruits and transported directly to the laboratory. Fresh OMWW samples were characterized by deep dark brown up to black color, with a strong specific olive oil smell. Locations, average water consumption in olive oil production (liter/ton of processed olive), and extraction type used in studied olive mills are presented in the Table below.

Table 1. Olive mill location, type of phase extraction, and water consumption

Olive mill location	Nr. mills	Extraction type	Water consumption (L/t)
Vlora	3	3-phase	960
Elbasan	2	3-phase	1200
Tirane	2	3-phase	980

Figure 1. Location of olive mills wastewater sampling stations

Physicochemical parameters

The characterization of OMWW effluents was based on the study of various physicochemical parameters such as pH, electrical conductivity (EC), total suspended solids (TSS), biological oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP), calcium, potassium, sodium, oil and grace, and total phenolic content (TPC).

Collected samples of OMWW were stored in a plastic container at 4^0C, in a dark place, and left to settle for two weeks before analyses. All analyses were performed in triplicate according to the standard analytical methods for the examination of water and wastewater (APHA, 2017).

The pH and electrical conductivity (EC) were measured in situ using a multi-parameter water analyser WTW 3620 IDS. The TSS was determined gravimetrically by drying well homogenized samples respectively at 103°C - 105°C for 24 h according to the standard method by the filtration process. BOD content was determined by the Winkler method, while the COD concentration was determined also according to standard method 5220D with an accuracy of 0.1 mg COD per litre which employs potassium dichromate as an oxidizing agent (APHA, 2017).

Total nitrogen (TN), expressed as the sum of nitrite nitrogen, nitrate nitrogen and Kjeldahl nitrogen was determined according to the method described by Bremner and Mulvaney, 2015.

The total phosphorus (TP) measurement was performed by using a WTW Spectrophotometer 7100 VIS according to standard methods for examination of water and wastewater (APHA, 2017) while Na, Ca, and K were determined using Flame Atomic Absorption Spectrometer (Analytik Jena GmbH - novAA 400 P).

Oil and grease in vegetation water was determined by liquid-liquid extraction method as described by Rodier et al., (1996).

Extraction of phenolic compounds from OMWW and evaluation of TPC

Extraction of phenolic compound was performed according to the analytical methodology described by De Marco et al., (2007) and Leouifoudi et al., (2014) with some modifications. To remove lipid fraction, samples of vegetation waters were acidified with HCl to pH 2 and washed with hexane: a volume of 100 ml of acidified OMWW sample were well mixed for 5 minutes with hexane (1:1 v/v) and centrifuged for 20 minutes at 3500 rpm. The phases were separated and the washing process was repeated four times.

Liquid-liquid extraction was performed by using ethyl acetate as an extraction solvent. A volume of 50 ml of prewashed OMWW samples were well mixed for 10 min with 100 ml of ethyl acetate and centrifuged for 20 min at 6000 rpm. The phase separation and extraction process was repeated four times. Ethyl acetate was evaporated under vacuum, dry matter was dissolved in methanol and used for total phenolic content determination. Ethyl acetate and hexane were recovered through a rotary evaporator under vacuum at room temperature.

The total phenolic content (TPC) was evaluated by spectrophotometry using the Folin-Ciocalteu method according to Spinelli et al., (2018), with some modifications described by Pikuli and Devolli, (2024) calibrated against gallic acid (GA) as the reference standard and results were expressed as GA equivalent (GAE).

Statistical analysis

All collected data were subject to statistical analysis (ANOVA and PCA) using the SPSS software ver.29. Descriptive statistics, mean, standard deviation (SD) and confidence interval (CI) were used to analyze related OMWW properties. Pearson correlation was performed to evaluate the correlation between measured OMWW properties in the three studied regions.

Principal component analysis (PCA) was conducted to find the combination of the most influencing factors of OMWW physicochemical properties.

RESULTS and DISCUSSION

This research aims to characterize the physicochemical properties of OMWW samples collected from different three-phase olive mills operating in southern and central parts of Albania. Descriptive statistics of the physicochemical properties of OMWW in three studied regions are presented in the table below.

The study is focused on the environmental impact of OMWW generated from the three-phase extraction process. The results obtained from physicochemical analyses were evaluated according to discharge limits allowed by the Albanian authorities. As can be seen from the results presented in Table 2, all OMWW samples are considered acidic (pH 4.4-5.3) compared to the allowed limits (6-9) for wastewater discharge (VKM 177, 2005).

Table 2. Statistical results of physicochemical parameters of OMWW samples collected in the studied regions.

Parameter	**Unit**	***Vlora region***			***Elbasan region***			***Tirana region***		
		Mean	SD	CI	Mean	SD	CI	Mean	SD	CI
pH	-	4.83	0.26	0.30	4.83	0.37	0.42	4.83	0.37	0.42
EC	mS/cm	9.68	2.12	2.40	9.41	1.45	1.64	9.41	1.45	1.64
TSS	g/L	23.97	4.54	5.14	30.93	6.21	7.02	30.93	6.21	7.02
TN	mg/L	621.67	13.60	15.39	460.33	32.40	36.66	460.33	32.40	36.66
TP	mg/L	263.00	77.16	87.32	195.33	39.84	45.08	195.33	39.84	45.08
COD	g/L	201.70	4.58	5.18	172.47	34.00	38.47	172.47	34.00	38.47
BOD	g/L	45.23	6.81	7.71	34.27	3.56	4.02	34.27	3.56	4.02
Oil & grease	g/L	6.11	0.49	0.56	7.50	0.73	0.82	7.50	0.73	0.82
TPC	g/L	8.11	0.23	0.26	6.20	0.57	0.64	6.20	0.57	0.64
Na	g/L	0.77	0.03	0.04	1.03	0.05	0.06	1.03	0.05	0.06
K	g/L	6.27	0.26	0.30	3.75	0.41	0.47	3.75	0.41	0.47
Ca	g/L	0.72	0.12	0.14	0.95	0.06	0.07	0.95	0.06	0.07

OMWW effluents in the three studied regions contain considerably higher quantities of organic load expressed by COD and BOD, higher levels of TSS, TN, and TP than the permitted level according to Albanian discharge standards. COD and BOD levels varied from 126 g/L to 216.4 g/L and 29.8g/L to 48.3 g/L, respectively. This indicates the high oxygen demand for the complete oxidation of the organic matter contained in these effluents, which reflects their very high polluting powers to the environment (Tabet et al., 2006). A high electrical conductivity value was observed (EC 6.5-12.65 mS/cm), reflecting the high salt content in these effluent wastewater streams.

The values of K, Na, and Ca in the current study were higher than those reported from different studies regarding the presence of K, Na, and Ca in OMWW effluents (Alburquerque et al., 2004; Abu Khayer et al., 2013; Topi et al., 2014). Based on this, we can conclude that the high K, Na, and TN levels could be attributed to fertilizer and organic matter use in the study areas (Tekaya et al., 2013; Gharaibeh et al., 2022).

Total phenolic content (TPC) of OMWW effluents ranged from 5.5 g/L to 8.42 g/L, which is significantly higher than the results reported by other studies (Benamar et al., 2020; Bargougui et al., 2019). This high concentration that gives them antimicrobial power could limit any natural biodegradation and consequently cause a deep disturbance in the ecosystem (Elayeb et al., 2021).

The biodegradability index (Ib) expressed by the COD/BOD5 ratio indicates the importance of pollutants with little or no biodegradability. The calculated values of OWWW's biodegradability index (Ib) for each studied region are displayed in Table 3.

Table 3. The biodegradability index (Ib), COD/BOD ratio

Ib index	*OMWW-Vlora*	*OMWW-Elbasan*	*OMWW-Tirane*
COD/BOD	5.13	5.03	5.30

Obtained results show that OMWW is rich in organic matter expressed as BOD and COD with an Ib index higher than 5, which indicates that OMWW effluents are not suitable for biological treatment and therefore must be considered a physico-chemical treatment before discharge to the environment or sewer system (Vlyssides et al., 1998).

The value of the Ib index exceeds the limit threshold of biodegradability (Ib=3) therefore OMWW effluents present a high, polluting power with toxic effects and are considered among the "strongest" industrial effluents. The uncontrolled discharge of untreated Olive Mill Wastewaters on the soil and water has the disadvantage of spreading materials that are foul-smelling and probably pathogenic in the environment (Khdair et al., 2019).

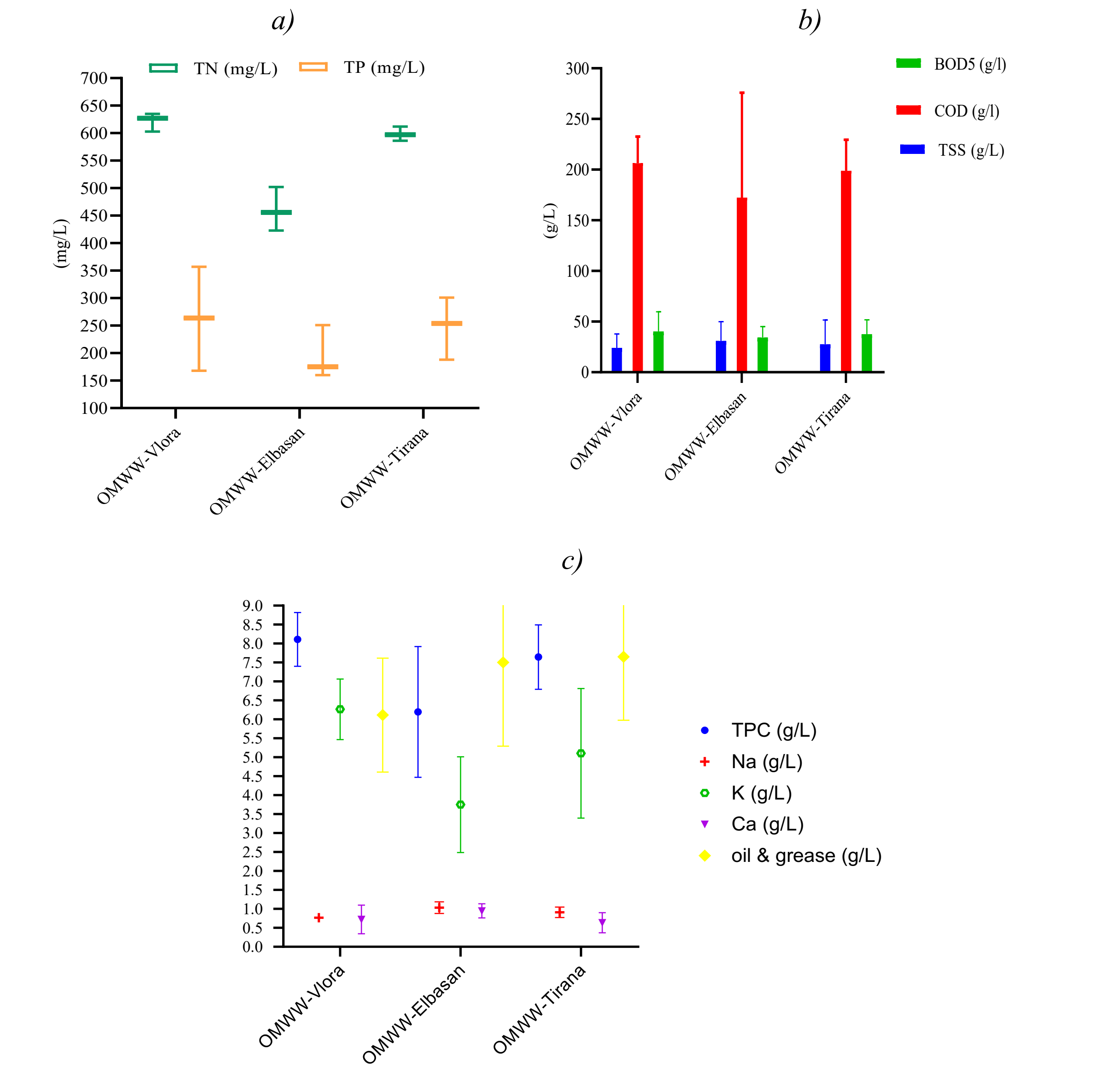

Figure 2. Spatial changes of physicochemical parameters in three studied regions: *a) Vlora region; b) Elbasan region and c) Tirana region.*

A multivariate analysis was performed to assess the variability of the physicochemical properties of OMWW collected from olive mills operating in three different areas. The data of spatial changes of physicochemical parameters in three studied regions are displayed in Fig. 2. Compared to other studied areas, OMWW samples collected in the Vlora region, contain a higher level of TN, TP, organic load, TPC, and K. The composition and characteristics of OMWW effluents usually vary depending on many factors, including the geographic location and climate, type, degree of maturity of olive fruits, processing procedures, and the oil extraction method (Dermeche et al., 2013).

Pearson correlation was performed to evaluate the relationship between the physico-chemical properties of OMWWs in the three studied regions. The tables below (tables 4, 5, and 6) present the Pearson correlation matrix between the physicochemical parameters of OMWW in three different studied areas.

The results indicated a moderate to strong correlation between the physicochemical parameters of OMWW. In the Vlora region, a high positive correlation was observed between pH with EC, oil & grease and Ca, COD, and TPC, and TN with K, while TPC was negatively correlated with Na, K, and Ca, pH with COD, TN and TPC, and TP with K. In the Elbasan region, a strong positive correlation was observed between pH with TSS and TPC, EC with BOD, TN with COD and oil & grease, and TP with K, while COD was negatively correlated with TP, EC with TSS and Na, TPC with Ca. A significant positive correlation between pH, TPC, and BOD, and a negative correlation between EC and TP, COD with TP, and TN with Ca were noticed in the Tirana region.

A significant positive correlation (r ranged from 0.71 to 0.98) between TPC and COD of OMWW effluents in the three studied areas was noticed. A similar correlation between these parameters was reported by other studies (Gharaibeh et al., 2022). A strong significant positive correlation between TPC and COD of OMWW effluents was reported also by Amaral et al., (2008) who postulated that factors affecting both properties (organic load) could be the same.

Table 4. Pearson correlation matrix of OMWW parameters in Vlora region.

Parameter	*pH*	*EC*	*TSS*	*TN*	*TP*	*COD*	*BOD*	*oil & grease*	*TPC*	*Na*	*K*	*Ca*
pH	1											
EC	**0.96**	1.00										
TSS	0.61	**0.81**	1.00									
TN	-0.92	**-1.00**	**-0.87**	1.00								
TP	0.16	-0.13	-0.69	0.23	1.00							
COD	**-0.94**	**-0.80**	-0.31	0.74	-0.48	1.00						
BOD	-0.75	**-0.91**	**-0.98**	**0.95**	0.53	0.48	1.00					
oil &grease	**0.90**	**0.99**	**0.89**	**-1.00**	-0.29	-0.70	-0.96	1.00				
TPC	**-0.85**	-0.66	**-0.10**	0.58	-0.66	**0.98**	0.29	-0.53	1.00			
Na	0.80	0.58	0.00	-0.50	0.73	**-0.95**	-0.19	0.45	**-1.00**	1.00		
K	0.50	0.22	-0.39	-0.12	**0.94**	-0.76	0.20	0.07	**-0.88**	**0.92**	1.00	
Ca	**0.87**	0.68	0.13	-0.61	0.63	**-0.98**	-0.32	0.56	**-1.00**	**0.99**	**0.86**	1

Table 5. Pearson correlation matrix of OMWW parameters in Elbasan region.

Parameter	***pH***	***EC***	***TSS***	***TN***	***TP***	***COD***	***BOD***	**oil & grease**	***TPC***	***Na***	***K***	***Ca***
pH	1											
EC	-0.65	1.00										
TSS	**0.87**	**-0.94**	1.00									
TN	0.36	0.48	-0.14	1.00								
TP	-0.74	-0.04	-0.32	**-0.89**	1.00							
COD	0.66	0.14	0.22	**0.94**	**-0.99**	1.00						
BOD	-0.59	**1.00**	-0.91	0.54	-0.11	0.21	1.00					
Oil&grease	0.16	0.65	-0.34	**0.98**	-0.79	**0.85**	0.70	1.00				
TPC	**1.00**	-0.60	**0.84**	0.42	-0.78	0.71	-0.54	0.23	1.00			
Na	0.70	**-1.00**	**0.96**	-0.42	-0.04	-0.07	**-0.99**	-0.59	0.65	1.00		
K	-0.28	-0.54	0.22	**-1.00**	**0.86**	**-0.91**	-0.60	**-0.99**	-0.35	0.48	1.00	
Ca	**-1.00**	0.63	**-0.86**	-0.38	0.75	-0.68	0.57	-0.19	**-1.00**	-0.68	0.31	1.00

Table 6. Pearson correlation matrix of OMWW parameters in Tirana region.

Parameter	***pH***	***EC***	***TSS***	***TN***	***TP***	***COD***	***BOD***	***oil& grease***	***TPC***	***Na***	***K***	***Ca***
pH	1.00											
EC	-0.25	1.00										
TSS	-0.35	**-0.82**	1.00									
TN	-0.41	**0.99**	-0.71	1.00								
TP	0.42	**-0.98**	0.70	-1.00	1.00							
COD	0.81	0.36	**-0.83**	0.20	-0.19	1.00						
BOD	-0.51	**0.96**	-0.63	**0.99**	**-1.00**	0.09	1.00					
oil&grease	-0.07	**0.98**	**-0.91**	**0.94**	**-0.94**	0.52	**0.90**	1.00				
TPC	**1.00**	-0.23	-0.37	-0.39	0.40	**0.83**	-0.49	-0.05	1.00			
Na	-0.47	**0.97**	-0.66	**1.00**	**-1.00**	0.13	**1.00**	**0.91**	-0.45	1.00		
K	-0.13	**0.99**	**-0.88**	**0.96**	**-0.96**	0.47	**0.92**	**1.00**	-0.11	**0.94**	1.00	
Ca	0.14	**-0.99**	**0.87**	**-0.96**	**0.96**	-0.46	**-0.93**	**-1.00**	0.12	**-0.94**	**-1.00**	1.00

Table 7. Principal component analysis of physicochemical parameters of OMWW

Principal Component	***Extraction Sums of Squared Loadings***		
	Total	**% Variance**	**% Cumulative**
1	5.392	44.933	44.933
2	2.121	17.677	62.61
3	1.77	14.753	77.364
4	1.145	9.542	86.906
5	0.675	5.622	92.528

Principal component analysis (PCA) was conducted to find loadings between physicochemical parameters of OMWWs in studied areas. The number of principal components, the amount of variance explained by each principal component, and OMWW parameter loadings are presented in the tables below (Tables 7 and 8).

Principal components with the eigenvalues > 1 there are four. PC1 explains 44.93% of the total variance with an eigenvalue of 5.39 and the most influenced parameters in loadings are TSS, TN, COD, BOD, TPC, Na, K, and Ca. PC2 explains 17.68% of the total variance dominated by pH while PC3 explains 14.75% of the total variance loading influenced by TP, and oil & grease.

Table 8. Component matrix of all measured parameters.

	Component			
Parameter	**1**	**2**	**3**	**4**
pH	-0.064	**0.827**	-0.214	0.354
EC	-0.105	-0.06	0.615	**0.76**
TSS	**-0.577**	0.438	-0.572	-0.031
TN	**0.955**	0.186	0.044	0.032
TP	0.399	-0.442	**-0.538**	0.187
COD	**0.679**	0.572	0.217	-0.072
BOD	**0.646**	-0.419	0.444	-0.296
Oil&Grease	-0.478	0.45	**0.628**	-0.087
TPC	**0.896**	0.38	-0.206	0.03
Na	**-0.867**	0.12	0.149	-0.206
K	**0.87**	-0.13	-0.041	0.314
Ca	**-0.746**	-0.363	-0.147	0.403

Conclusions

The characterization of OMWW effluents in three different regions and their environmental impact assessment was based on the results obtained from physicochemical analysis compared to allow effluent discharge limits of olive mills wastewater to water bodies according to Albanian standards.

The results indicated that Olive Mill Wastewater effluents in all studied areas exceeded the national standards for wastewater discharged to surface waters and municipal sewage treatment plants.

OMWW effluents in the Vlora region were characterized by higher levels of total phenolic compounds, organic load expressed in terms of COD and BOD, and higher levels of TN, TP, and TSS compared to other studied regions.

Principal Component Analysis (PCA) has served as a useful tool for analyzing and correlating physicochemical parameters of OMWW collected from three different studied areas.

The biodegradability index of OMWW effluents exceeded the limit threshold of biodegradability (Ib >5) due to the high organic load rate (BOD, COD, and total phenolic compounds). These results indicated that such effluents are non-biodegradable and not suitable for biological treatment and therefore must be considered a physicochemical treatment before discharge to the environment or sewer.

Application of cleaner production options and proper environmental waste management systems in olive oil industries are needed to reduce their environmental impact. This may include the adoption of the two-phase mills which significantly reduce water consumption.

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