

Occurrence of trace elements in Mediterranean mussels (*Mytilus galloprovincialis* Lamarck, 1819) from an experimental pilot farm in the Calich Lagoon (Sardinia, Italy)

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Abstract

The present study aimed to determine trace elements in Mediterranean mussels (*Mytilus galloprovincialis*) from an experimental pilot farm of the Calich Lagoon, a typical Sardinian brackish area (Italy). Two sampling sessions were scheduled in February and May 2019 and the occurrence of 24 metals (Hg, Ag, Al, As, Be, Bi, Cd, Co, Cr, Cu, Fe, Ga, In, Mg, Mn, Mo, Ni, Pb, Rb, Se, Sn, Ti, V, Zn) in bivalves was considered. Environmental conditions of water (temperature, salinity, pH, dissolved oxygen, and chlorophyll *a*) were also measured *in situ*. A high significant ($P < 0.001$) difference was reported for temperature, pH, and dissolved oxygen. Our results showed a significant seasonal variation of Mo ($P < 0.001$); Cd, V ($P < 0.01$); Ni, Pb and Co ($P < 0.05$) in examined *M. galloprovincialis* samples; as all values were higher in February than those for May session samples, meanwhile the highest levels were reported for Mg (mean \pm s.d. 1151 \pm 263 mg kg⁻¹ wet weight), Al (mean \pm s.d. 341 \pm 192 mg kg⁻¹ w.w.), and Fe (mean \pm s.d. 212 \pm 75 mg kg⁻¹ w.w.) in February samples. The European Union uppermost values (EC Reg. 1881/2006) for Cd, Hg, and Pb were never overpassed. The results confirmed the role of *M. galloprovincialis* as one of the most

appropriate biological indexes to track the presence of trace elements in brackish environments. It could be concluded that the current ecology of the Calich Lagoon suggests that compatibly with the transitional ecosystem, the classification as a bivalves' production area and the implementation of extensive shellfish farming can improve its production capacities. The knowledge of the lagoon ecology is an essential tool for its sustainable exploitation, preserving biodiversity, and mitigating the effects of anthropogenic activities on public health.

Introduction

Wetlands and coastal lagoons are sensitive areas with peculiar ecological characteristics determined by the interaction between land and sea (Castro *et al.*, 1999; Cataudella *et al.*, 2015) and are subjected to increased economic development and ecological population pressure (Almeida and Soares, 2012). Heavy metals generated from anthropogenic sources such as industrial effluent, urban run-off, mining operations, and atmospheric emissions are transported through rivers or by air and finally accumulate in coastal areas (Olmedo *et al.*, 2013; Azizi *et al.*, 2018). Chemical pollution in coastal shellfish-growing waters is a worldwide problem (Almeida and Soares, 2012) and is recognized as a public health issue (Lozano *et al.*, 2010). Several bivalve species live in estuaries that are subjected to several anthropogenic pressures, thus being exposed to high levels of trace elements (Forstner and Wittmann, 1979). The incorporation rate of chemical contaminants in shellfish depends on biotic factors (*e.g.* species, age, sex, soft-body weight, gametogenesis, and physiological status) and abiotic factors (*e.g.* availability of contaminants in the environment, filtration rate, temperature, salinity, pH, chemical species and interaction with other elements) (Phillips, 1980; Fernandez-Tajes *et al.*, 2011). Shellfish for human consumption could constitute a persistent risk to humans as final consumers at the top of the food chain (Hillwalker *et al.*, 2006; Sunlu, 2006). Since mussels are filter feeders; need to filter a huge volume of water in order to get enough food to eat, consequently they trap trace elements from sediments, suspended particulate materials, water column, and food sources (Livingstone, 1993; Van der Oost *et al.* 2003; Laffon *et al.*, 2006; Jeffs, 2013). Bioindicators were extensively used to monitor the occurrence of trace elements in coastal environments (Rainbow *et al.*, 2004). Shellfish can bioconcentrate chemical contaminants (Langston and Spence, 1995;

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Andral *et al.*, 2011; Saavedra *et al.*, 2004; Mertens *et al.*, 2005; Farrington *et al.*, 2016) and among them, *Mytilus galloprovincialis* has been widely used in biomonitoring of Mediterranean coastal ecosystems to evaluate the status of chemical contamination (Cirillo *et al.*, 2010; Stanković *et al.* 2011; Rouane-Hacene *et al.*, 2015; Belivermiş *et al.*, 2016; Azizi *et al.*, 2017). It is widely considered as one of the most suitable biological indicators of chemical pollution, due to its sedentary filter feeders' nature, wide geographical distribution, ease of sampling, and remarkably tolerance to chemical pollution (Emmanouil *et al.*, 2008; Gupta and Singh, 2011; Waykar and Deshmukh, 2012; Zuykov *et al.*, 2013). The development of a more competitive and environmentally friendly shellfish farming is a priority objective of the EU. The future development of European shellfish farming is based on three key elements: *a*) competitiveness through strong research support; *b*) better land use along coastal areas and in river basins; *c*) sustainability through environmentally friendly production methods and high standards in terms of animal/human health and consumer protection. The Calich

Lagoon is a typical Mediterranean lagoon of the northwest of Sardinia (Italy) located in an area of strong touristic impact (Baralla *et al.*, 2017). In the late years, different authors have studied its ecology (Ielmini *et al.*, 2014; Pulina *et al.*, 2017, 2018; Bazzoni *et al.*, 2018; Satta *et al.*, 2020), proposed sustainable exploitation strategies (Chessa *et al.*, 2005; 2007; Pais *et al.*, 2006; 2007; Cannas *et al.*, 2011) and highlighted the occurrence of bacteria, viruses, biotoxins and trace elements in native shellfish (Sedda *et al.*, 2016; Baralla *et al.*, 2017; Esposito *et al.*, 2018; Bazzoni *et al.*, 2019; Esposito *et al.*, 2021). The main aims of the present study were: *a*) to evaluate the occurrence of 24 trace elements in *M. galloprovincialis* from an experimental pilot farm of the Calich Lagoon realized in the frame of the EU Interreg program RETRALAGS (TRAnsfronter network of LAGunes, lakes, and ponds) 2014-2019 (Pilot Action T2.1.2 “Development of an experimental bivalve mollusc pilot farm in the Calich Lagoon”); *b*) to verify the compliance with the European Regulations for cadmium (Cd), mercury (Hg), and lead (Pb) in shellfish; *c*) to assess the abiotic factors (temperature, salinity, pH, dissolved oxygen and chlorophyll *a*) of the lagoon water.

Materials and methods

Study site

The Calich Lagoon is situated in the Porto Conte Regional Natural Park, along the north-western coast (40°35' 47.5" N; 8°17' 59.9" E) of Sardinia (Italy) (Figure 1). The lagoon extends for 92 ha with a depth varying between about 0.5 and 1.5 m. It is connected with the Alghero Gulf through a natural channel (60-m wide and 2-m deep) hosting a medium-size shipyard and a tourist harbor. The lagoon receives freshwater from natural fluvial tributaries and an artificial channel (Pulina *et al.*, 2017; Esposito *et al.*, 2021). The Calich Lagoon suffers from high eutrophication due to urban, agricultural, and industrial activities (Fenza *et al.*, 2014; Baralla *et al.*, 2017; Bazzoni *et al.*, 2018): its catchment area extends for about 42,000 ha, receiving water from several surrounding municipalities. Water temperature in the Calich Lagoon follows a seasonal trend with the highest values between July and August (Baralla *et al.*, 2017). Natural beds of highly valuable shellfish species (*e.g.* grooved carpet shell (*Ruditapes decussatus*), olive green cockle (*Cerastoderma glaucum*), and Mediterranean mussel (*M. galloprovincialis*) are present in the lagoon, but despite their abundance, the Calich Lagoon has not yet

been classified as a shellfish production area (Esposito *et al.*, 2018; Bazzoni *et al.*, 2019; Esposito *et al.*, 2021).

Experimental mussel pilot farm

The experimental mussel pilot farming was carried out in suspension, on a small “Trieste” type farm consisting of 2 single ropes supported by wooden poles fixed deeply in the muddy sediment of the lagoon and emerging from the water for about 1 meter (Figure 1). The mussels were inserted in n.60 droppers seeded with plastic stockings (80 cm length and 38 mm mesh) filled with tiny mussels (≈ 3.5 cm length and 1.9 cm width). The experiment began in December 2018 and continued until the achievement of the minimum commercial size in May 2019 (5 cm in length).

Mussel sampling

Specimens of *M. galloprovincialis* were collected in February and May 2019 from the droppers from one sampling point of the experimental pilot farm (Figure 1). A total of 30 adult mussels per sampling were collected (*i.e.*, 60 in total). After sampling, mussels were washed with clean lagoon water, measured, weighed, and immediately transported at +4°C in a cold box to the laboratory of the Dipartimento di Medicina Veterinaria in Sassari. The samples were cleaned and eviscerated: the soft part of each bivalve sample was removed separately from the shell, washed with distilled water, and sent frozen to the laboratories of the Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d’Aosta in Turin to be analyzed.

Determination of physicochemical parameters of water

The monthly temperature, pH, salinity, dissolved oxygen, and chlorophyll *a* were

recorded in the pilot farm water of Calich Lagoon from December 2018 to May 2019. The physicochemical parameters of water were recorded *in situ* on three bathymetries (30, 60, and 100 cm) using an Ocean Seven 316 Plus CTD (Idronaut, Brughiero, Italy). One water sample from each bathymetry was examined, for a total of 18 water samples. Data were averaged.

Detection of trace elements in Mediterranean mussels

A Direct Mercury Analyser (DMA80, Milestone, Shelton, CT, USA), based on thermal decomposition, amalgamation, and atomic absorption, was utilized for Mercury (Hg) detection according to Esposito *et al.* (2018). The limit of quantification of the method (LOQ) was 0.010 mg kg⁻¹. The following 23 elements Silver (Ag) Aluminum (Al), Arsenic (As), Beryllium (Be), Bismuth (Bi), Cadmium (Cd), Cobalt (Co), Chrome (Cr), Copper (Cu), Iron (Fe), Gallium (Ga), Indium (In), Magnesium (Mg), Manganese (Mn), Molybdenum (Mo), Nickel (Ni), Lead (Pb), Rubidium (Rb), Selenium (Se), Tin (Sn), Titanium (Ti), Vanadium (V), Zinc (Zn) were quantified using an inductively coupled plasma mass spectrometer (ICP-MS Xseries II, Thermo Scientific, Bremen, Germany) according to Squadrone *et al.* (2016). Samples (1.0 g of each) were first homogenized then subjected to microwave acid (HNO₃ and H₂O₂) mineralization process (oven ETHOS 1 from Milestone, Shelton, CT, USA). Recovery was checked by using the CRM-SRM 1566b from NIST and was in the range of 82–104%. The LOQ was 0.010 mg kg⁻¹. Three distinct measures were made, and results were averaged.

Statistical analysis

The differences between the



Figure 1. Map of Italy showing the location of Calich lagoon. Inset is the location of Italy on the world map. The study area is indicated with the red circle “○” and the sampling location with the red dot “●”.

physicochemical parameters of the Calich Lagoon water in relation to the sampling month and the concentrations of trace elements in mussels in relation to the sampling session were compared and analyzed with a one-way ANOVA (<https://statpages.info/anova1sm.html>). Moreover, a multiple pairwise comparison between the means of groups through a Tukey HSD (honestly significant differences) *post-hoc* test was carried out (<https://statpages.info/anova1sm.html>). The results were considered statistically significant when $P < 0.05$.

Results and discussion

Physicochemical parameters of water

Table 1 and Figure 2 show the values of temperature, salinity, pH, dissolved oxygen, and the concentration of chlorophyll *a*, of the

water, recorded using a multiparametric probe at the experimental pilot farm. The results confirmed how the values of salinity, dissolved oxygen, pH and chlorophyll *a* were in the desirable ranges for lagoon environments with livestock purposes (Specchiulli *et al.*, 2008; Serra *et al.*, 2011). A high significant ($P < 0.001$) difference was reported for temperature, pH, and dissolved oxygen (Table 1). Regarding the abiotic factors, the above-mentioned parameters temperature and salinity appeared almost always within the survival limits of *M. galloprovincialis* (5°C and 15 ‰ respectively) and only for short periods significant variations that could be related to a reduction in growth rates and an increase in mortality rates were reported (Table 1). The values of chlorophyll *a*, a fundamental parameter for evaluating the bearing capacity of the breeding environment, were between 0.5 and 1.9 µg/L (Table 1). These values were in accordance with previous studies (Serra *et al.*, 2011; Fenza *et al.*, 2014;

Baralla *et al.*, 2017; Bazzoni *et al.*, 2018) and confirmed the Calich Lagoon as a highly eutrophic ecosystem. However, it has been shown that increased chlorophyll *a* enhancing the food source for bivalve molluscs, may favour the entry of chemical pollutants through food (Wang and Fisher, 1996; Chong and Wang, 2001). The differences among the physicochemical water parameters and between the sampling months (ANOVA and Tukey test) are reported in Table 2.

Concentration of trace elements in Mediterranean mussels

Mussels were sampled in February (≈4.5 cm length and 2.3 cm width) and at the achievement of the minimum commercial size in May 2019 (5 cm length and 2.6 cm width). Metal concentrations are shown in Table 3. There was a clear seasonal variation of trace element bioaccumulation in examined *M. galloprovincialis* samples; as our results showed that nearly all parameters

Table 1. Temporal variation of physicochemical water parameters in the experimental farm of Calich Lagoon from December 2018 to May 2019 (average values ± s.d. of single sample/ bathymetry = 3 samples/ month = 18 total samples).

Sampling month	Parameters				
	Temperature (°C)	Salinity (mg/L)	Dissolved Oxygen (mg/L)	Chlorophyll a (g/L)	pH
Dec (2018)					
A*	13.5	44	8.2	1.29	7.7
B*	15.4	31.9	6.4	1.34	7.4
C*	16.5	34.5	5.6	0.64	7.6
Averaged values ± s.d.**	15.13±1.51	23.6±16.67	6.73±1.33	1.09±0.39	7.56±0.15
Jan (2019)					
A	10.9	16.0	4.3	0.39	8.2
B	13.2	32.5	3.8	0.64	8.1
C	13.8	35.6	3.7	0.48	8.1
Averaged values ± s.d.	12.63±1.53	28.03±10.53	3.93±0.32	0.50±0.12	8.13±0.05
Feb (2019)					
A	10.7	1.8	4.4	1.31	7.9
B	10.7	1.8	4.4	1.30	7.9
C	12.2	26.1	3.5	1.30	7.6
Averaged values ± s.d.	11.20±0.86	9.9±14.82	4.1±0.51	1.30±0.00	7.8±0.17
Mar (2019)					
A	14.8	17.7	6.9	1.67	8.2
B	14.8	18.3	7.1	1.95	8.2
C	14.5	33.5	6.5	2.3	8.2
Averaged values ± s.d.	14.7±0.17	23.16±8.95	6.83±0.30	1.97±0.31	8.2±0.00
Apr (2019)					
A	19.8	18.6	5.2	1.37	8.5
B	17.0	32.3	6.3	3.41	8.6
C	16.5	34.0	6.9	1.15	8.5
Averaged values ± s.d.	17.76±1.77	28.3±8.44	6.13±0.86	1.97±1.24	8.53±0.05
May (2019)					
A	14.0	23.4	7.2	0.71	9.0
B	14.0	23.5	7.0	0.71	9.0
C	13.9	23.7	6.6	0.98	8.9
Averaged values ± s.d.	13.96±0.05	23.53±0.15	6.93±0.30	0.8±0.15	8.96±0.05
Differences from ANOVA and Tukey test	p<0.001	n.s.***	p<0.001	n.s.	p<0.001
Desirable range in Lagoon environment	8-26****	15-40****	5.8-10*****	-	-
Survival limits of <i>M. galloprovincialis</i>	5****	<15****	5*****	-	-

*A, B and C corresponding to the bathymetries 30, 60, and 100 cm, respectively; **s.d.= standard deviation; ***n.s.= not significant; ****Adapted from Keskin and Ekici, 2021; *****Adapted from Gosling, 2003.

were higher in February than May session samples. ANOVA and Tukey test showed a significant sessional variation of Mo ($P < 0.001$); Cd, V ($P < 0.01$); Ni, Pb, Co ($P < 0.05$). The highest levels were found for Mg (mean \pm s.d. 1151 \pm 263 mg kg⁻¹ wet weight), Al (mean \pm s.d. 341 \pm 192 mg kg⁻¹ w.w.), and Fe (mean \pm s.d. 212 \pm 75 mg kg⁻¹ w.w.) in February samples. It is well known that the levels of trace elements in mussels register fluctuations depending on the period of the year in which they are collected (Claisse, 1992). The highest metals concentrations are usually recorded in winter and spring and the lowest in summer and autumn (Piras *et al.*,

2013; Bajc and Kirbiš, 2019). Al showed very high values between the nonessential elements and higher than those described by Squadrone *et al.* (2016) in the Gulf of La Spezia and more important, by Esposito *et al.* (2018; 2021) in native *R. decussatus* and *M. galloprovincialis* collected in the Calich Lagoon. The tolerable weekly intake (TWI) recommended by the European Food Safety Authority (EFSA) for Al is 1 mg Al kg⁻¹ body weight (b.w.) which corresponds to 70 mg of Al in a week in an adult weighing 70 kg. (EFSA, 2008). According to our results, this limit would be exceeded if consuming 315 g of mussels per week. These results

highlight the necessity of routinely monitoring Al levels in mussels of Calich Lagoon (Esposito *et al.*, 2021). No significant sessional variation of Al bioaccumulation was reported. The maximum levels set by European Regulations (EC Reg. 1881/ 2006) for Cd, Hg, and Pb (1.0, 0.5 and 1.5 mg kg⁻¹, respectively) were never exceeded (Bajc and Kirbiš, 2019). Cd concentrations (Table 2) were in accordance with those recorded in the south-western coast of Sardinia (Piras *et al.*, 2013), in the Tyrrhenian coast (Papetti and Rossi 2009), and the Gulf of La Spezia (Squadrone *et al.*, 2016). A provisional

Table 2. Differences among the physicochemical water parameters in the experimental farm of Calich Lagoon from ANOVA and the Tukey test (December 2018-May 2019).

Sampling month	Dec (2018)	Jan (2019)	Feb (2019)	Mar (2019)	Apr (2019)	May (2019)
Dec (2018)	#	DO** - pH***	T* - DO**	pH***	pH***	pH***
Jan (2019)		#	pH*	DO**	T** - pH**	DO** - pH***
Feb (2019)			#	T* - pH**	T*** - pH***	DO** - pH***
Mar (2019)				#	pH*	pH***
Apr (2019)					#	T* - pH**
May (2019)						#

T = Temperature; DO= Dissolved Oxygen; ***p<0.001; **p<0.01; *p<0.05; Salinity and Chlorophyll *a* = differences not significant.

Table 3. Trace elements in mussels from an experimental pilot farm of the Calich Lagoon (mg kg⁻¹ w.w., 30 mussels/month).

Trace element	February 2019				May 2019				Differences from ANOVA and Tukey test	EU reference limits
	Mean*	SD**	Min***	Max****	Mean	SD	Min	Max		
Ag	BQL*****	BQL	BQL	BQL	BQL	BQL	BQL	BQL	-	-
Al	341.342	192.228	143.880	527.869	96.009	23.177	69.787	113.755	n.s.*****	-
As	1.414	0.250	1.216	1.695	1.050	0.230	0.836	1.293	n.s.	-
Be	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	-	-
Bi	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	-	-
Cd	0.076	0.006	0.069	0.081	0.037	0.011	0.027	0.049	p<0.01	1.0
Co	0.084	0.011	0.072	0.091	0.038	0.015	0.026	0.054	p<0.05	-
Cr	0.262	0.123	0.119	0.333	0.115	0.021	0.092	0.130	n.s.	-
Cu	2.320	0.965	1.754	3.434	3.084	2.934	1.127	6.458	n.s.	-
Fe	212.034	75.650	132.741	283.420	96.565	13.423	81.294	106.494	n.s.	-
Ga	0.062	0.034	0.027	0.095	0.015	0.004	0.011	0.019	n.s.	-
Hg	0.013	0.002	0.011	0.014	BQL	BQL	BQL	BQL	-	0.5
In	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	-	-
Mg	1150.789	263.475	848.060	1328.336	1038.138	95.767	952.257	1141.408	n.s.	-
Mn	3.476	0.656	2.757	4.041	2.312	0.802	1.529	3.133	n.s.	-
Mo	0.275	0.029	0.245	0.304	0.073	0.025	0.057	0.101	p<0.001	-
Ni	0.317	0.077	0.227	0.363	0.114	0.031	0.087	0.148	p<0.05	-
Pb	0.138	0.046	0.085	0.169	0.046	0.015	0.032	0.061	p<0.05	1.5
Rb	0.786	0.210	0.560	0.976	0.988	0.558	0.655	1.632	n.s.	-
Se	16.149	0.538	15.786	16.767	8.444	12.367	0.982	22.719	n.s.	-
Sn	0.134	0.061	0.069	0.190	0.059	0.003	0.056	0.063	n.s.	-
Tl	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	-	-
V	0.573	0.095	0.480	0.670	0.151	0.012	0.137	0.161	p<0.01	-
Zn	24.207	3.563	22.130	28.321	21.687	7.298	17.437	30.113	n.s.	-

*Mean of three distinct measurements; **SD= Standard deviation; ***Min= Minimum value; ****Max=Maximum value; *****BQL = below method quantitation limit (LOQ) of 0.010 mg kg⁻¹; *****n.s.= not significant

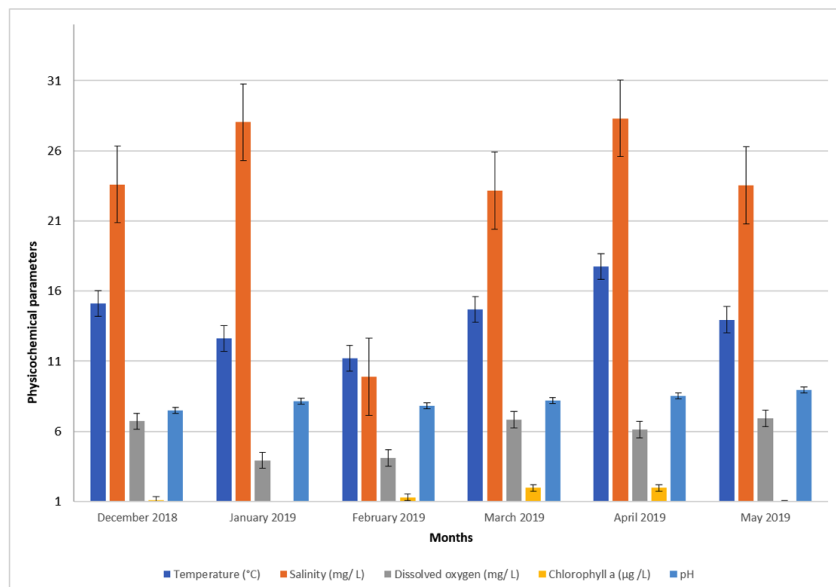


Figure 2. Diagram of physicochemical parameters averages of the Calich Lagoon's water.

tolerable weekly intake (PTWI) of $7 \mu\text{g kg}^{-1}$ b.w. was set for Cd (EFSA, 2009), corresponding to 0.49 mg of Cd in one week for an adult weighing 70 kg. Consequently, an adult would have to have an intake of at least ≈ 1 kg of mussels per day to exceed this PTWI. Lower concentrations were reported in other Italian studies (Cubadda *et al.*, 2006; Lafabrie *et al.*, 2007; Desideri *et al.*, 2010; Bille *et al.*, 2015). On the contrary, high levels were reported by Bat, Üstün and Baki (2012) in mussels caught from the Black Sea in Turkey and Bezuidenhout *et al.* (2015) in the South African coasts. Hg levels (0.011 – 0.014 mg kg^{-1} w. w.) were in the range or even lower of the concentrations previously reported in Italy (Cubadda *et al.*, 2006; Lafabrie *et al.*, 2007; Desideri *et al.*, 2010; Brambilla *et al.*, 2013; Piras *et al.*, 2013; Bille *et al.*, 2015; Squadrone *et al.*, 2016). At the same time, the concentrations of Pb (range 0.032 – 0.17 mg kg^{-1} w. w.) were in accordance with previous Italian studies (Cubadda *et al.*, 2006). According to previous studies reporting very low concentrations for Ag, Be, Bi, In and Tl in *Mytilus* spp. (Rodríguez-Hernández *et al.*, 2019; Esposito *et al.*, 2021), these trace elements scarcely or not investigated in mussels, were always below the method quantitation limit (LOQ) of 0.010 mg kg^{-1} in all the samples (Table 3).

Conclusions

The present study provided useful

information on the occurrence of 24 trace elements in Mediterranean mussels from an experimental pilot farm of the Calich Lagoon. Due to the presence of strong demand for high economic value seafood products on the regional and national market and to the vocation of the Calich Lagoon for shellfish farming, these results could constitute a baseline for the future classification of this lagoon as a shellfish production area. The environmental parameters of water (temperature, salinity, pH, dissolved oxygen, and chlorophyll *a*) reported in Table 1, Table 2 and Figure 2 were in the desirable ranges for lagoon environments with livestock purposes and confirmed the Calich Lagoon as a highly eutrophic ecosystem. The concentrations of Cd, Hg, and Pb in mussels complied with the European Regulations and did not pose a concern for human consumption. On the contrary, Al showed very high values: its incorporation rate in mussels has a strong relationship with the presence of urban, agricultural, and industrial activities that may lead to increase Al concentration in brackish environments. Since food is the major source of human exposure to Al (EFSA, 2008), the concentrations in mussels should be routinely monitored. An integrated approach to the study of the Calich Lagoon ecology is essential for its sustainable exploitation, preserving biodiversity, and mitigating the effects of anthropogenic activities on public health.

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