

## Article

# Trace Metals Do Not Accumulate Over Time in The Edible Mediterranean Jellyfish *Rhizostoma pulmo* (Cnidaria, Scyphozoa) from Urban Coastal Waters

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**Abstract:** Jellyfish as food represent a millennial tradition in Asia. Recently, jellyfish have also been proposed as a valuable source of protein in Western countries. To identify health risks associated with the potential human consumption of jellyfish as food, trace element accumulation was assessed in the gonads and umbrella tissues of the Mediterranean *Rhizostoma pulmo* (Macri, 1778), sampled over a period of 16 months along the shallow coastal waters a short distance from the city of Taranto, an area affected by metallurgic and oil refinery sources of pollution. Higher tissue concentrations of trace elements were usually detected in gonads than in umbrella tissue. In particular, significant differences in the toxic metalloid As, and in the metals Mn, Mo, and Zn, were observed among different tissues. The concentrations of vanadium were slightly higher in umbrella tissues than in gonads. No positive correlation was observed between element concentration and jellyfish size, suggesting the lack of bioaccumulation processes. Moreover, toxic element concentrations in *R. pulmo* were found below the threshold levels for human consumption allowed by Australian, USA, and EU Food Regulations. These results corroborate the hypothesis that *R. pulmo* is a safe, potentially novel food source, even when jellyfish are harvested from coastal areas affected by anthropogenic impacts.

**Keywords:** edible jellyfish; novel food; heavy metals; ICP-AES elemental analysis; marine pollution; Gulf of Taranto; Ionian Sea

## 1. Introduction

Heavy metals are usually considered major contaminants in shallow coastal waters worldwide [1,2]. These elements are normal constituents of marine and estuarine environments, often introduced through industrial, agricultural and hydrocarbon-related activities, wastewaters, domestic effluents, and commercial ports [3–5]. Some of these metals are essential trace elements for biological metabolic activities, and their concentrations are finely regulated according to species-specific metabolic requirements in different environmental conditions. At high concentrations, they can be toxic for most organisms. Other elements, such as the metalloid arsenic and metals such as cadmium, lead, and mercury, are classified as non-essential elements, having no identified role in the biochemical pathways of organisms. Metals are highly soluble and may easily enter food webs to accumulate at toxic levels in the biological tissues of wildlife and humans [6–12]. Multiple effects on the

growth and reproduction of marine species have been documented [13–15], while exposure to high levels of non-essential elements (i.e., MeHg) can increase the risk of neurotoxic effects [16–18]. High levels of metals in the food web can significantly affect carnivorous and omnivorous organisms, making them vulnerable to biomagnification of the toxic elements. Jellyfish are key representatives of the gelatinous plankton community and play an important role in biological transfer and carbon cycling in marine food webs [19–21]. They have been identified as one of the most sensitive trophic group for the assessment of Good Environmental Status by the EU Marine Strategy Framework Directive (Directive 2008/56/EC of the European Parliament and of the Council).

In the last few decades, jellyfish outbreaks have been recorded with increasing frequency in coastal areas of sensitive basins worldwide, such as the Mediterranean Sea and the Sea of Japan, negatively affecting human activities such as maritime tourism, aquaculture, and fisheries [22–24]. Their peculiar reproductive and fast growth patterns suggest the use of jellyfish as potential targets for biomonitoring [25,26]. Nonetheless, studies on jellyfish accumulation rates of trace elements are extremely rare, and mostly focused on sedentary jellyfish species, such as *Cassiopea* spp. [26–28]. Hence, understanding heavy metal and metalloid uptake and retention rates of jellyfish in coastal habitats may contribute to clarify their potential as bioindicators for toxic element pollution and to improve the management and protection of marine habitats. Further, jellyfish represent a millennial tradition in Asia [29], supporting fisheries in Eastern countries for jellyfish food businesses. In recent years, jellyfish biomasses have been proposed in Western countries for their potentially sustainable exploitation as novel food for humans or as a source of bioactive compounds for nutraceutical, cosmeceutical, and pharmaceutical purposes [30–37]. In addition, jellyfish have potential as an aquaculture feed ingredient, with gonads rich in yolk proteins and essential fatty acids [21] that may enrich fish feed. In this regard, estimating the levels of heavy metals and metalloids associated with jellyfish is key for the assessment of human health risks related to the potential food exploitation of jellyfish biomasses harvested from coastal waters. The coastal waters of Taranto (northern Ionian Sea, Italy) are influenced by multiple urban and industrial waste discharges, including effluents from steel foundries, oil refineries, and naval shipyards.

The barrel jellyfish *Rhizostoma pulmo* (Macri, 1778) is a large scyphozoan species frequently observed along Mediterranean coasts, estimated to be one of the most abundant coastal jellyfish species in recent years [38]. Subject to seasonal and inter-annual fluctuations, this species recurrently occurs in large numbers in several coastal marine areas with high anthropogenic impact, such as the Mar Menor lagoon (north-western Mediterranean, Spain) [39], Gulf of Trieste (northern Adriatic Sea), and the Gulf of Taranto (northern Ionian Sea) [38]. Due to its rapid growth, large size (up to 50 cm in diameter), high proliferative potential (>48,000 individuals/Km<sup>2</sup>) [32], biochemical composition [32,33,36] and the non-pathogenic species related to its associated microbiome [34,35,40], this species is now regarded as a candidate fishery target as novel food for human consumption or as a feed ingredient for aquaculture.

We investigated trace element accumulation in the different body fractions (i.e., gonads and umbrella) of *R. pulmo* jellyfish collected during a seasonal population bloom in the coastal waters of the Gulf of Taranto (Ionian Sea), which is known to be affected by high anthropic pressures. Jellyfish tissue retention of trace metals has been compared to safety limits allowed by Australian, USA, and EU Food Regulations in order to evaluate the hazard to human health associated with the potential consumption of *R. pulmo* biomass from the Taranto gulf or its use as a feed ingredient.

## 2. Materials and Methods

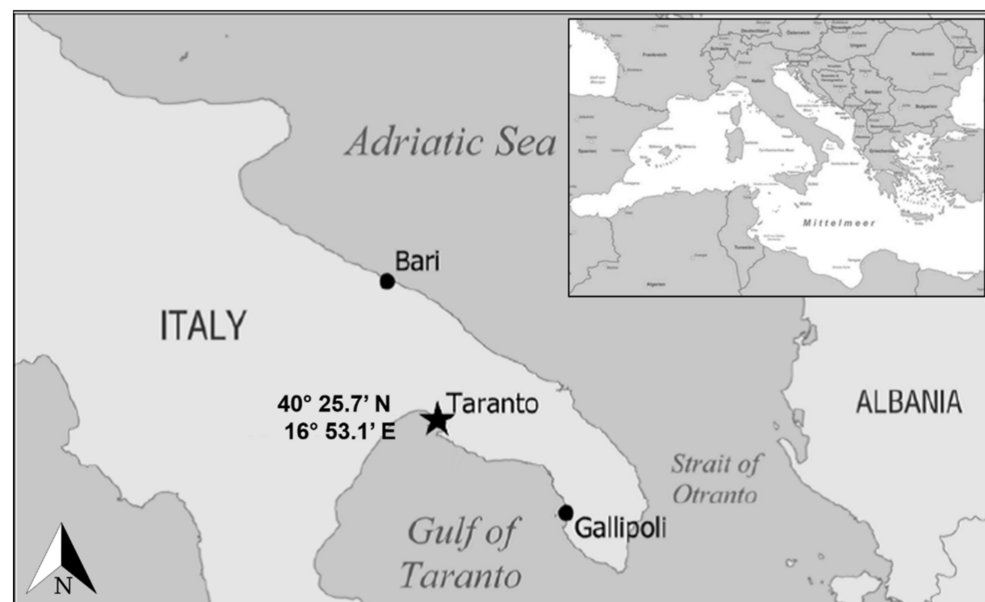
### 2.1. Sampling Area

The Gulf of Taranto (north-western Ionian Sea, Southern Italy) is a deep marine area delimited from east to north to west by the coasts of the Apulia, Basilicata, and Calabria regions. The Gulf of Taranto has been significantly affected by different human pressures

such as industrial and agricultural installation, commercial fishing, and aquaculture [40]. Being largely exposed to industrial pollution, the city has been identified as an “Area of High Environmental Risk” (Italian Law n.349/1986, Institution of the Ministry of Environment, and regulations regarding the environmental damage). The intensive maritime traffic and the particulate matter carried through rivers of the Basilicata region flowing into the Ionian Sea (Bradano, Basento, Cavone, Agri, and Sinni) concur to influence coastal water contamination levels. Sampling sites were located a few kilometers southwest of the Taranto industrial area, which is known as a Hg-contaminated site [41–43].

## 2.2. Sample Collection

A total of 21 healthy, adult medusae of *Rhizostoma pulmo* (Macri 1778) were collected at shallow depths (1–3 m depths) along the coastal waters of Ginosà Marina (Gulf of Taranto, Ionian Sea; Figure 1) from summer 2016 to winter 2017 (T1 = August 2016; T2 = September 2016; T3 = October 2016; T4 = November 2016; T5 = March 2017; T6 = June 2017; T7 = December 2017).



**Figure 1.** Map of the sampling location indicated with a star (Ginosà Marina, Gulf of Taranto, Ionian Sea) in the Mediterranean Sea.

At each sampling, three jellyfish were individually collected underwater within sterile plastic bags and transferred on board a small boat equipped for first sample processing. Namely, jellyfish were: a) rapidly rinsed with 0.2 µm-filtered seawater (FSW) to remove all visible material adhering on the jellyfish body and tentacles, b) individually sealed within sterile plastic bags, and c) immediately transported to the laboratory within a refrigerated box at 5 °C. In the laboratory, jellyfish were further washed in sterile FSW, checked at the stereomicroscope for sex determination and gonadal maturity, and measured (inter-rhopalial umbrella diameter). Gonads were carefully dissected from the umbrella on a clean ceramic chopping board using a ceramic knife. For chemical analyses, two fractions (gonads and umbrella) were isolated from each jellyfish, washed with double-distilled water, frozen in liquid nitrogen, and stored at −80 °C before the lyophilization process (48 h).

## 2.3. Elemental Analysis

Concentrations of 21 elements (Ag, Al, As, B, Ba, Bi, Cd, Co, Cr, Cu, Fe, Hg, In, Li, Mn, Mo, Ni, Pb, Te, V, and Zn) in jellyfish samples were measured using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES, Thermo Fisher Scientific, Waltham, MA, USA). Freeze-dried samples were weighted and mixed with 4 mL of H<sub>2</sub>O<sub>2</sub> and 6 mL of

super pure HNO<sub>3</sub> 69% (Carlo Erba, Milano, Italy), then treated at 180 °C for 10 min using a microwave digestion system (START D, Milestone, Bergamo, Italy). The samples were then cooled, diluted with ultrapure water to a final volume of 20 mL, filtered through syringe filters (pore size: 0.45 µm), and then measured for element content using an ICP-AES (iCap 6000 Series, Thermo Scientific, Waltham, MA, USA) spectrometer. The spectrometer was previously calibrated for quantitative analysis with five standard solutions containing known elemental concentrations of 0.001, 0.01, 0.1, 0.5, and 1.0 mg/L. A multi-standard solution was used for all elements except for Hg, which was measured separately using a single element standard solution. The calibration lines showed correlation coefficients (*r*) greater than 0.99 for all measured elements. The ICP Multi-Element Standard Solution VIII (Merck, Darmstadt, Germany), consisting of 24 elements in dilute nitric acid, was used to construct the calibration lines. The Hg standard solution, used to construct the calibration line for this element, was the CPAchem (Stara Zagora, Bulgaria) Hg Mono-Element Calibration Standard in 10% nitric acid. The detection limit of the various elements corresponds to the lowest concentration of the calibration lines. Results were expressed as the average ( $\pm$ standard deviation) of three different measurements, with elemental concentrations expressed as ppm (mg/kg of dry weight). The Certified Reference Material used for quality control and quality assurance was the CPAchem (Stara Zagora, Bulgaria) Multi-Element Standard Solution.

#### 2.4. Statistical Analysis

To assess differences in elemental concentration in jellyfish tissues (gonads and umbrella) across the different samplings, multivariate and univariate analyses (PERMANOVA) [44,45] were carried out, based on Euclidean distances of previously normalized data, using 9999 random permutations of the appropriate units [46], on two factors: Fraction (Fr, as a fixed factor with 2 levels) and Sampling Time (Ti, as a random factor with 7 levels), with  $n = 3$  for each combination of factors. To examine the generality of patterns in elemental concentrations, MDS plots [45] were drawn and Pearson's correlation coefficient was used to determine the correlation values between metals and jellyfish tissues. When significant differences were encountered ( $p < 0.05$ ), post-hoc pairwise tests for the fixed factor were carried out to ascertain the consistency of the differences. Because of the limited number of samples and the number of unique permutations in the pairwise tests, *p*-values were obtained from Monte Carlo samplings. The analyses were performed using the software PRIMER, version 6 [46]. Linear regressions between each elements' concentration and jellyfish size were performed, separately for the umbrella and gonads, with the function `lm` of the library `stats` of the R Environment for Statistical Computing [47]. Pearson's correlation matrices and *p*-values were calculated using the function `rcorr` of the R package `Hmisc`, version 4.1.

### 3. Results

Trace element concentrations are reported in Table 1. Ag, Bi, and In concentrations were measured for both umbrella tissue and gonads, but the values were below the detection limit for all samples. All results are reported in dry weight. The measured water contents of *R. pulmo* umbrella and gonadal tissue fractions were  $95.50 \pm 0.61\%$  and  $93.7 \pm 1.9\%$ , respectively. This affords a conversion factor (CF) of 0.045 for *R. pulmo* umbrella and 0.063 for gonadal tissue fractions respectively, allowing us to convert ppm/dry weight to ppm/wet weight, with  $m_M(\text{mg/kg wet weight}) = m_M(\text{mg/kg dry weight}) \times \text{CF}$ .

**Table 1.** Trace element concentrations for Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Hg, Li, Mn, Mo, Ni, Pb, Te, V, and Zn, (mean  $\pm$  SE) measured in umbrella and gonads of *R. pulmo* at each sampling time. All concentrations are expressed as mg/kg dry weight. Individuals were sampled by scuba diving in Ginos Marina (Ionian Sea 40°25.7' N, 16°53.1' E; Taranto, Italy) in seven sampling times over 16 months (T1 = August 2016; T2 = September 2016; T3 = October 2016; T4 = November 2016; T5 = March 2017; T6 = June 2017; T7 = December 2017, n = 3).

		T1		T2		T3		T4		T5		T6		T7		Total Average	
Al	Umbrella	54.54	$\pm$ 12.38	46.96	$\pm$ 28.45	36.55	$\pm$ 10.07	30.96	$\pm$ 12.50	7.76	$\pm$ 1.26	1.69	$\pm$ 1.05	0.64	$\pm$ 0.05	42.78 $\pm$ 17.32	
	Gonads	3.91	$\pm$ 1.91	3.71	$\pm$ 1.17	1.99	$\pm$ 0.33	3.27	$\pm$ 0.12	0.89	$\pm$ 0.29	2.04	$\pm$ 0.62	3.35	$\pm$ 0.92	2.74 $\pm$ 0.38	
As	Umbrella	1.42	$\pm$ 0.17	2.02	$\pm$ 0.10	1.93	$\pm$ 0.21	2.42	$\pm$ 0.29	2.90	$\pm$ 0.60	1.45	$\pm$ 0.46	0.87	$\pm$ 0.19	1.81 $\pm$ 0.18	
	Gonads	13.16	$\pm$ 1.73	18.05	$\pm$ 4.73	16.65	$\pm$ 4.67	22.44	$\pm$ 9.48	23.24	$\pm$ 8.02	24.78	$\pm$ 3.01	25.98	$\pm$ 2.97	20.61 $\pm$ 2.02	
B	Umbrella	49.93	$\pm$ 0.69	49.88	$\pm$ 5.14	53.84	$\pm$ 2.76	49.99	$\pm$ 0.89	61.67	$\pm$ 0.40	39.96	$\pm$ 7.10	55.08	$\pm$ 1.01	50.58 $\pm$ 1.86	
	Gonads	32.23	$\pm$ 6.04	29.38	$\pm$ 6.11	38.40	$\pm$ 4.75	38.07	$\pm$ 0.94	24.54	$\pm$ 9.18	50.50	$\pm$ 13.64	30.67	$\pm$ 1.66	34.83 $\pm$ 2.89	
Ba	Umbrella	0.23	$\pm$ 0.03	0.22	$\pm$ 0.06	0.13	$\pm$ 0.00	0.26	$\pm$ 0.08	0.16	$\pm$ 0.00	0.18	$\pm$ 0.04	0.17	$\pm$ 0.01	0.19 $\pm$ 0.02	
	Gonads	0.71	$\pm$ 0.18	0.42	$\pm$ 0.12	0.21	$\pm$ 0.06	0.49	$\pm$ 0.07	0.11	$\pm$ 0.04	0.31	$\pm$ 0.08	0.23	$\pm$ 0.02	0.35 $\pm$ 0.05	
Cd	Umbrella	0.23	$\pm$ 0.06	0.26	$\pm$ 0.06	0.24	$\pm$ 0.05	0.23	$\pm$ 0.03	0.15	$\pm$ 0.05	0.04	$\pm$ 0.01	0.15	$\pm$ 0.07	0.18 $\pm$ 0.02	
	Gonads	1.10	$\pm$ 0.09	0.63	$\pm$ 0.15	0.70	$\pm$ 0.16	0.77	$\pm$ 0.12	0.84	$\pm$ 0.33	0.78	$\pm$ 0.29	2.76	$\pm$ 0.26	1.08 $\pm$ 0.17	
Co	Umbrella	0.04	$\pm$ 0.01	0.05	$\pm$ 0.01	0.04	$\pm$ 0.00	0.03	$\pm$ 0.00	0.02	$\pm$ 0.00	0.03	$\pm$ 0.00	DL	$\pm$ DL	0.03 $\pm$ 0	
	Gonads	0.22	$\pm$ 0.01	0.09	$\pm$ 0.00	0.12	$\pm$ 0.02	0.10	$\pm$ 0.01	0.05	$\pm$ 0.01	0.20	$\pm$ 0.05	0.18	$\pm$ 0.02	0.14 $\pm$ 0.02	
Cr	Umbrella	0.10	$\pm$ 0.02	0.49	$\pm$ 0.37	0.08	$\pm$ 0.03	0.06	$\pm$ 0.03	0.08	$\pm$ 0.01	0.15	$\pm$ 0.08	DL	$\pm$ DL	0.13 $\pm$ 0.06	
	Gonads	0.13	$\pm$ 0.05	0.07	$\pm$ 0.01	0.09	$\pm$ 0.01	0.12	$\pm$ 0.02	0.24	$\pm$ 0.14	0.22	$\pm$ 0.03	0.12	$\pm$ 0.05	0.14 $\pm$ 0.02	
Cu	Umbrella	1.56	$\pm$ 0.21	1.54	$\pm$ 0.11	1.09	$\pm$ 0.18	1.17	$\pm$ 0.07	1.00	$\pm$ 0.06	0.91	$\pm$ 0.11	0.80	$\pm$ 0.16	1.12 $\pm$ 0.08	
	Gonads	5.81	$\pm$ 1.28	3.24	$\pm$ 0.30	3.45	$\pm$ 0.57	5.11	$\pm$ 0.33	2.88	$\pm$ 0.87	8.72	$\pm$ 2.92	6.46	$\pm$ 0.75	5.1 $\pm$ 0.6	
Fe	Umbrella	6.68	$\pm$ 1.67	5.64	$\pm$ 1.72	3.77	$\pm$ 0.36	3.27	$\pm$ 0.49	1.82	$\pm$ 0.05	5.10	$\pm$ 1.66	1.51	$\pm$ 0.50	4.01 $\pm$ 0.54	
	Gonads	20.89	$\pm$ 3.26	11.03	$\pm$ 0.05	13.01	$\pm$ 3.55	8.93	$\pm$ 1.64	12.07	$\pm$ 4.09	44.90	$\pm$ 11.31	35.66	$\pm$ 13.52	20.93 $\pm$ 3.67	
Hg	Umbrella	0.17	$\pm$ 0.05	0.02	$\pm$ 0.02	DL	$\pm$ DL	DL	$\pm$ DL	0.01	$\pm$ 0.01	DL	$\pm$ DL	0.02	$\pm$ 0.02	0.03 $\pm$ 0.07	
	Gonads	0.03	$\pm$ 0.02	0.07	$\pm$ 0.02	DL	$\pm$ DL	DL	$\pm$ DL	0.06	$\pm$ 0.03	DL	$\pm$ DL	0.04	$\pm$ 0.00	0.03 $\pm$ 0.04	
Li	Umbrella	4.40	$\pm$ 0.04	4.07	$\pm$ 0.18	4.16	$\pm$ 0.10	4.05	$\pm$ 0.06	4.32	$\pm$ 0.08	3.47	$\pm$ 0.59	4.56	$\pm$ 0.08	4.08 $\pm$ 0.12	
	Gonads	2.49	$\pm$ 0.56	3.16	$\pm$ 0.25	3.38	$\pm$ 0.50	3.09	$\pm$ 0.18	1.89	$\pm$ 0.72	3.56	$\pm$ 1.07	2.15	$\pm$ 0.15	2.82 $\pm$ 0.23	
Mn	Umbrella	0.32	$\pm$ 0.26	0.05	$\pm$ 0.20	0.21	$\pm$ 0.04	0.34	$\pm$ 0.11	0.08	$\pm$ 0.00	0.27	$\pm$ 0.09	0.10	$\pm$ 0.01	0.26 $\pm$ 0.04	
	Gonads	2.39	$\pm$ 0.21	1.09	$\pm$ 0.26	1.93	$\pm$ 0.85	1.51	$\pm$ 0.18	1.00	$\pm$ 0.33	3.22	$\pm$ 1.76	4.84	$\pm$ 1.70	2.28 $\pm$ 0.42	
Mo	Umbrella	0.20	$\pm$ 0.03	0.15	$\pm$ 0.01	0.17	$\pm$ 0.03	0.15	$\pm$ 0.01	0.14	$\pm$ 0.01	0.13	$\pm$ 0.03	0.17	$\pm$ 0.00	0.16 $\pm$ 0.01	
	Gonads	0.43	$\pm$ 0.05	0.23	$\pm$ 0.02	0.29	$\pm$ 0.07	0.20	$\pm$ 0.01	0.15	$\pm$ 0.05	0.37	$\pm$ 0.11	0.30	$\pm$ 0.01	0.28 $\pm$ 0.03	
Ni	Umbrella	0.19	$\pm$ 0.01	0.29	$\pm$ 0.16	0.10	$\pm$ 0.01	0.07	$\pm$ 0.01	0.09	$\pm$ 0.01	0.10	$\pm$ 0.02	0.08	$\pm$ 0.02	0.13 $\pm$ 0.03	
	Gonads	0.15	$\pm$ 0.03	0.10	$\pm$ 0.01	0.19	$\pm$ 0.02	0.15	$\pm$ 0.02	0.09	$\pm$ 0.04	0.17	$\pm$ 0.05	0.11	$\pm$ 0.02	0.14 $\pm$ 0.01	
Pb	Umbrella	0.71	$\pm$ 0.41	0.04	$\pm$ 0.03	0.01	$\pm$ 0.01	0.59	$\pm$ 0.50	DL	$\pm$ DL	0.02	$\pm$ 0.01	0.05	$\pm$ 0.03	0.2 $\pm$ 0.1	
	Gonads	0.37	$\pm$ 0.03	0.07	$\pm$ 0.04	0.19	$\pm$ 0.18	1.43	$\pm$ 0.54	0.05	$\pm$ 0.01	0.12	$\pm$ 0.07	0.30	$\pm$ 0.13	0.36 $\pm$ 0.12	
Te	Umbrella	0.09	$\pm$ 0.09	0.02	$\pm$ 0.01	0.03	$\pm$ 0.01	0.02	$\pm$ 0.01	0.06	$\pm$ 0.01	0.04	$\pm$ 0.02	0.52	$\pm$ 0.02	0.13 $\pm$ 0.04	
	Gonads	0.01	$\pm$ 0.01	0.00	$\pm$ 0.00	0.02	$\pm$ 0.01	0.01	$\pm$ 0.01	0.04	$\pm$ 0.03	0.14	$\pm$ 0.05	0.67	$\pm$ 0.02	0.13 $\pm$ 0.05	
V	Umbrella	31.48	$\pm$ 0.32	29.54	$\pm$ 1.44	31.72	$\pm$ 0.46	29.55	$\pm$ 0.26	31.79	$\pm$ 1.01	27.05	$\pm$ 4.85	34.80	$\pm$ 0.79	30.77 $\pm$ 0.81	
	Gonads	17.95	$\pm$ 4.03	22.65	$\pm$ 2.53	24.40	$\pm$ 3.63	22.06	$\pm$ 0.88	13.72	$\pm$ 5.28	23.94	$\pm$ 10.89	15.84	$\pm$ 1.20	20.08 $\pm$ 1.84	
Zn	Umbrella	18.40	$\pm$ 6.33	22.99	$\pm$ 2.36	16.32	$\pm$ 3.05	16.44	$\pm$ 3.08	8.78	$\pm$ 1.36	8.93	$\pm$ 2.76	7.35	$\pm$ 1.73	13.71 $\pm$ 1.6	
	Gonads	93.6	$\pm$ 10.43	66.98	$\pm$ 6.49	90.29	$\pm$ 10.39	80.68	$\pm$ 11.14	67.15	$\pm$ 29.16	129.87	$\pm$ 52.77	123.89	$\pm$ 20.94	93.21 $\pm$ 9.54	

DL = below the detection limit.

These concentrations were generally higher in jellyfish gonads than in umbrella tissue (Table 1) and only chrome (Cr) and nickel (Ni) concentrations exhibited no differences among the factors and interactions investigated (Table 2). Thallium and bismuth were not detected in any tissue, whereas indium and silver were found only in two samples of gonads and at low concentrations, so they were not further considered in the analyses. The concentration of lead (Pb) ranged from 0 to 1.59 ppm. Across sampling times, the concentration of Pb was always higher in gonads than in the umbrella fraction; nevertheless, differences were not significant (Table 2). However, significant differences between sampling times were observed (Table 2). Mean concentrations of arsenic (As), lithium (Li), manganese (Mn), molybdenum (Mo), and zinc (Zn) found in gonads were significantly higher than umbrella in all cases.

**Table 2.** Results of PERMANOVA testing for the differences between jellyfish fraction (umbrella and gonads) and sampling time on elemental composition and each elemental concentration analyzed (Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Te, V, and Zn). Ti = time, Fr = fraction, df = degrees of freedom; MS = mean squares; Pseudo-F = F critic; \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$ ; ns = not significant.

	df	Pseudo-F	<i>p</i>	Pseudo-F	<i>p</i>	Pseudo-F	<i>p</i>	Pseudo-F	<i>p</i>	Pseudo-F	<i>p</i>	Pseudo-F	<i>p</i>
		<b>Al</b>		<b>As</b>		<b>B</b>		<b>Ba</b>		<b>Cd</b>		<b>Co</b>	
Ti	6	2.97		0.72		0.31		5.18		10.95		6.30	
Fr	1	20.82		78.32	***	29.72		16.51		112.34		124.78	
Ti × Fr	6	2.65	*	0.73		3.21	**	2.59	*	12.00	***	6.66	***
		<b>Cr</b>		<b>Cu</b>		<b>Fe</b>		<b>Li</b>		<b>Mn</b>		<b>Mo</b>	
Ti	6	0.93	ns	2.36		3.87		0.45		1.80		3.42	*
Fr	1	0.01	ns	62.81		38.86		31.42	***	29.46	***	30.28	***
Ti × Fr	6	1.50	ns	2.87	*	3.83	**	2.26		1.96		2.14	
		<b>Ni</b>		<b>Pb</b>		<b>Te</b>		<b>V</b>		<b>Zn</b>		<b>All</b>	
Ti	6	1.12	ns	4.83	**	88.53		0.33		0.77		2.98	
Fr	1	0.05	ns	1.58		0.93		26.75	***	67.52	***	28.40	
Ti × Fr	6	1.96	ns	1.12		3.23	*	1.23		1.31		2.31	***
		<b>Hg</b>											
Ti	3	3.76											
Fr	1	0.10											
Ti × Fr	3	6.82	**										

Regardless of the different sampling times, total mean concentrations of As and Mn were about 10-fold greater in gonads than in umbrella (As =  $20.61 \pm 5.35$  and  $1.86 \pm 0.46$  ppm, Mn =  $2.28 \pm 1.12$  and  $0.23 \pm 0.08$  ppm, respectively) reaching 30- and 50-fold higher at T7, respectively (Table 1). Similarly, Zn showed a trend of higher concentration in gonadal tissue, with values 16 times higher at T7 when compared to the data obtained from the umbrella fraction (Table 1). Only for Li and V, concentrations were higher in umbrella tissue for all sampling times. The essential trace element Zn was most concentrated in the gonads (range: 15.04–235.20 ppm). No significant differences in mercury (Hg) or barium (Ba) concentration were detected between *R. pulmo* tissues, except for Hg at T1 and for Ba at T7 (Tables 2 and 3). The concentrations of the remaining elements (aluminium (Al), boron (B), cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), lithium (Li), and tellurium (Te)) were significantly different between tissues only in specific sampling times. Gonadal tissue values of Co and Cu were significantly higher than those of umbrella tissues, except at T5 for Co, and T5 and T6 for Cu (Table 3). Cd and Fe values were generally higher in gonadal than umbrella tissues for all sampling times, however, analysis of variance showed significant differences only at T1 and T4 for both elements, at T7 for Cd, and at T2 and T6 for Fe (Table 3). The concentration of Al, Li, and the metalloid B were consistently high in umbrella tissue, with significant differences observed exclusively at specific sampling times (Table 3).

**Table 3.** Results of the pairwise tests contrasting element concentration composition and single elements between the umbrella (B) and gonads (G) of jellyfish within each sampling time. T = T value, P(MC) = probability level after Monte Carlo simulations. \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$ ; ns = not significant.

	t	P(MC)	t	P(MC)	t	P(MC)	t	P(MC)	t	P(MC)
	B vs. G		B vs. G		B vs. G		B vs. G		B vs. G	
	ALL		Al		B		Ba		Cd	
T1	3.25	**	4.04	*	2.91	*	2.63	ns	8.16	**
T2	1.49	ns	1.52	ns	2.57	ns	1.41	ns	2.21	ns
T3	2.60	*	3.43	*	2.81	*	1.28	ns	2.67	ns
T4	2.21	*	2.22	ns	9.24	***	2.21	ns	4.49	*
T5	2.63	*	5.33	**	4.04	*	1.24	ns	2.05	ns
T6	1.91	ns	0.28	ns	0.69	ns	1.48	ns	2.57	ns
T7	4.79	**	2.95	*	12.58	***	2.86	*	9.82	***
	Co		Cu		Fe		Li		Te	
T1	9.61	***	3.28	*	3.88	*	3.39	*	0.89	*
T2	5.20	**	5.26	**	3.13	*	2.95	*	1.99	ns
T3	4.12	*	3.94	*	2.59	ns	1.53	ns	1.04	ns
T4	9.59	***	11.67	***	3.32	*	5.05	**	0.55	ns
T5	2.58	ns	2.16	ns	2.51	ns	3.36	*	0.47	ns
T6	3.00	*	2.68	ns	3.48	*	0.07	ns	1.71	ns
T7	7.84	**	7.40	**	2.52	ns	14.05	***	5.83	**

Pearson's correlation matrices for concentrations of trace elements in jellyfish tissues are reported in Table 4 for umbrella and gonadal tissue, respectively.

A highly significant positive correlation was observed between Cr and Ni in umbrella tissue ( $r = 0.942$ ,  $p < 0.0001$ ), which was not significant in the gonads. The correlations were significant in both umbrella and gonads, but slightly higher in the gonads, between: B and Li ( $r = 0.838$  umbrella,  $r = 0.912$  gonads,  $p < 0.0001$  for both), Li and V ( $r = 0.912$  umbrella,  $r = 0.974$  gonads,  $p < 0.0001$  for both), Cu and Zn ( $r = 0.842$  umbrella,  $r = 0.887$  gonads,  $p < 0.0001$  for both). The opposite situation was observed for B and V ( $r = 0.802$  umbrella,  $r = 0.887$  gonads,  $p < 0.0001$  for both), Zn and Cd ( $r = 0.725$  umbrella,  $r = 0.573$  gonads,  $p < 0.001$  in both cases), Cu and Cd ( $r = 0.737$  umbrella,  $p < 0.001$  and  $r = 0.434$  gonads,  $p < 0.01$ ), and Fe and Co ( $r = 0.728$  umbrella,  $r = 0.745$  gonads,  $p < 0.001$ ). Several significant negative correlations were observed, all concentrated in the umbrella tissue: Co and Te ( $r = -0.797$  umbrella,  $p < 0.0001$ ), As and Te ( $r = -0.560$ ,  $p < 0.001$ ), and Pb and Zn ( $r = -0.450$ ,  $p < 0.01$ ). Groups of elements showing positive correlations with values between 0.5–0.7 in umbrella, but between 0.4–0.5 in gonads, were found for many pairs of elements, including Fe (Fe and Cr, Fe and Ni, Fe and Cu) and Mo and V (Table 4). The correlation between Fe and Zn was higher in gonads than in umbrella tissue ( $r = 0.807$ ,  $p < 0.001$  and  $r = 0.647$ ,  $p < 0.01$  respectively), and slightly less so for Fe and Mn ( $r = 0.755$  gonads, and  $r = 0.684$  umbrella,  $p < 0.001$ ). The correlation between Pb and Ba ( $r = 0.667$ ,  $p < 0.0001$ ) was observed only in umbrella tissue, while the only significant correlation for aluminum in the gonads was Al and Ba ( $r = 0.588$ ,  $p < 0.01$ ).

Other significant correlations in umbrella tissue (Pearson's coefficients ranging from 0.4–0.5) were found for Cu (Cu and Pb, Cu and Mo, Cu and Ni), Ni (Ni and Ba, Ni and Zn, Ni and Co), Cr (Cr and Ba, Cr and Fe, Cr and Zn, Cr and Co) and Pb (Pb and Cu, Pb and Fe, Pb and Mn). Of these, only Cu and Mo ( $r = 0.871$ ,  $p < 0.0001$ ), Cu and Ni ( $r = 0.434$ ,  $p < 0.05$ ), Ni and Zn ( $r = 0.675$ ,  $p < 0.001$ ), Ni and Co ( $r = 0.505$ ,  $p < 0.05$ ), and Cr and Fe ( $r = 0.437$ ,  $p < 0.05$ ) had a corresponding correlation in gonadal tissue. Pb showed no significant correlation with any other element in the gonads. On the other hand, the association of Fe and Ni was significantly positive ( $r = 0.586$ ,  $p < 0.001$  umbrella tissue and  $r = 0.443$ ,  $p < 0.05$  gonads).

**Table 4.** Pearson's correlation matrix for trace elements in umbrella tissue of *R. pulmo*. Number of samples = 21.  $p < 0.0001$  '\*\*\*\*';  $p < 0.001$  '\*\*\*',  $p < 0.01$  '\*\*',  $p < 0.05$  '\*'.

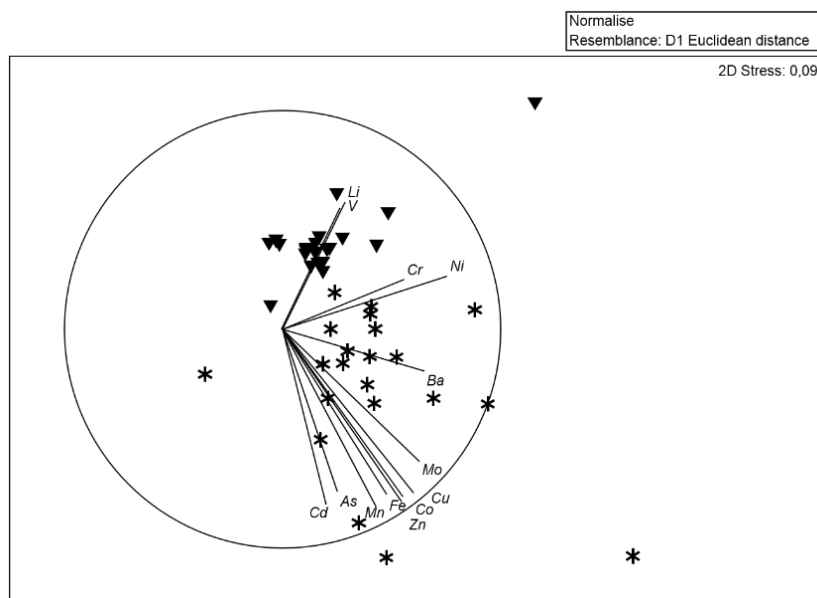
	Al	As	B	Ba	Cd	Co	Cr	Cu	Fe	Li	Mn	Mo	Ni	Pb	Te	V
As	0.082															
B	−0.253	0.357														
Ba	0.047	0.092	0.076													
Cd	0.728 ***	0.399	0.065	0.045												
Co	0.429	0.283	−0.134	0.253	0.326											
Cr	0.062	0.054	0.024	0.490 *	−0.028	0.461 *										
Cu	0.736 ***	0.288	−0.121	0.372	0.737 ***	0.674 ***	0.334									
Fe	0.497 *	−0.015	−0.206	0.550 **	0.264	0.728 ***	0.544 *	0.693 ***								
Li	−0.037	0.086	0.838 ****	0.22	0.186	−0.12	−0.064	0.061	0.045							
Mn	0.456 *	0.141	−0.311	0.685 ***	0.322	0.492 *	0.224	0.607 **	0.684 ***	−0.059						
Mo	0.299	0.028	0.305	0.362	0.394	0.162	0.035	0.508 *	0.484 *	0.600 **	0.41					
Ni	0.151	−0.061	0.07	0.479 *	0.05	0.486 *	0.942 ****	0.434 *	0.586 **	0.083	0.188	0.146				
Pb	0.241	0.035	−0.021	0.667 ***	0.259	0.335	0.002	0.459 *	0.440 *	0.141	0.642 **	0.418	0.058			
Te	−0.328	−0.560 **	0.172	−0.062	−0.222	−0.797 ****	−0.267	−0.429	−0.396	0.365	−0.386	0.196	−0.191	−0.137		
V	−0.169	0.075	0.802 ****	0.138	0.127	−0.2	−0.042	−0.063	0.003	0.912 ****	−0.084	0.587 **	0.037	0.019	0.411	
Zn	0.720 ***	0.292	−0.049	0.245	0.725 ***	0.719 ***	0.448 *	0.842 ****	0.647 **	0.051	0.475 *	0.385	0.470 *	0.296	−0.450 *	−0.012

	Al	As	B	Ba	Cd	Co	Cr	Cu	Fe	Li	Mn	Mo	Ni	Pb	Te	V
As	0.06															
B	0.24	0.254														
Ba	0.588 **	−0.202	0.353													
Cd	0.256	0.4	0.071	−0.038												
Co	0.257	0.128	0.463 *	0.385	0.477 *											
Cr	−0.034	0.474 *	0.413	0	0.059	0.09										
Cu	0.325	0.391	0.762 ****	0.361	0.434 *	0.773 ****	0.33									
Fe	0.17	0.438 *	0.523 *	0.066	0.505 *	0.745 ***	0.437 *	0.781 ****								
Li	0.338	0.13	0.912 ****	0.37	−0.088	0.261	0.286	0.549 **	0.289							
Mn	0.121	0.352	0.416	0.079	0.730 **	0.752 ****	0.153	0.755 ****	0.714 ***	0.24						
Mo	0.415	0.145	0.605 **	0.546 *	0.317	0.871 ****	0.195	0.775 ****	0.644 **	0.472 *	0.638 **					
Ni	0.316	0.061	0.794 ****	0.357	0.046	0.505 *	0.26	0.553 **	0.443 *	0.747 ****	0.399	0.642 **				
Pb	0.204	−0.055	0.149	0.388	0.014	0.033	−0.034	0.093	−0.11	0.132	0.024	−0.041	0.261			
Te	0.069	0.233	−0.141	−0.28	0.829 ****	0.265	−0.019	0.22	0.434 *	−0.323	0.504 *	0.021	−0.236	−0.105		
V	0.334	0.151	0.887 ****	0.36	−0.021	0.281	0.278	0.529 *	0.283	0.974 ****	0.282	0.477 *	0.743 ***	0.129	−0.297	
Zn	0.244	0.493 *	0.700 ***	0.161	0.573 **	0.759 ****	0.29	0.870 ****	0.807 ****	0.531 *	0.844 ****	0.754 ****	0.675 ***	0.053	0.248	0.556 **



The MDS plot showed the clustering of different trace elements among the investigated fractions across several sampling times (Figure 2). Based on Pearson’s correlation coefficients >0.6, As, Cd, Co, Cu, Fe, Mn, Mo, and Zn were preferentially associated to gonadal tissue fractions, whereas Li and V were mostly associated to umbrella fractions (Figure 2).



**Figure 2.** MDS plot of the elemental composition in two fractions of *R. pulmo*. Plot showing the discrimination among the umbrella (▼) and gonads (\*) based on the elemental composition. Vectors are proportional to the Pearson correlation of the element concentration variables ( $r > 0.6$ ).

Non-essential elements such as As, Cd, Cr, Co, Pb, and Hg were present at higher concentrations in gonads with respect to the values found in umbrella tissues in most specimens. Only vanadium concentrations were significantly higher in umbrella than in the gonads.

To detect the potential bioaccumulation of heavy metals or other elements, we performed a univariate linear regression for each of the investigated elements with non-zero values against the volume of the umbrella, which was used as a proxy for the specimen age. The sampled specimens ranged from 16.9 cm to 42.44 cm in bell diameter, with a mean value of  $27.27 \pm 7.45$  cm. Tukey’s honest significant difference (HSD) method, used to assess significantly different means, indicated a significant ( $p = 0.0132$ ) difference in size only between the sampling times T5 and T6, corresponding to the months of March and June of the same year. The regression was not performed for Hg due to the limited number of samples. The correlations showing a  $p < 0.05$  are reported in Table 5.

**Table 5.** Values of  $R^2$  for significant correlations ( $p < 0.05$ ) for linear regressions of the element concentration vs. specimen size. Number of samples for each regression = 21.

Element	$R^2$	Adjusted $R^2$	$p$ -Value	Correlation Sign	Tissue
Mn	0.1927	0.1502	0.0465	negative	umbrella
B	0.2672	0.2286	0.0164	negative	gonads
Ba	0.2219	0.1810	0.0311	negative	gonads
Li	0.2786	0.2406	0.0139	negative	gonads
V	0.1979	0.1557	0.0433	negative	gonads

A single element correlating with size in the umbrella (Mn, a biologically essential transition metal), and four in the gonads (B, Ba, Li, and V) (Table 5) were found. All observed correlations were negative, and in all cases, all  $R^2$  coefficients were  $<0.28$ .

#### 4. Discussion

In previous studies, a clearly different biochemical composition was observed between somatic tissue and gonads of the semeanostome jellyfish *Pelagia noctiluca* [21,48]. Here, we investigated trace element concentrations in the gonads and umbrella of *R. pulmo* in order to explore its potential application as a novel food or as a source of nutraceuticals [32]. The measured water contents of *R. pulmo* umbrella and gonadal tissue fractions were  $95.50 \pm 0.61\%$  and  $93.7 \pm 1.9\%$ , respectively, which are in good agreement with the 93.2–95.9% range previously reported in [32] for the whole organism. Mean concentrations of Cr and Ni measured in both tissues and across different samplings were lower by at least one order of magnitude when compared to *R. pulmo* umbrella tissue collected in Mar Menor [26] and other scyphozoans [27,49,50].

The concentration range detected for lead in the *R. pulmo* umbrella (0–1.59 ppm) fell under similar intervals found in previous studies on the jellyfish *R. pulmo*, *Cotylorhiza tuberculata* (0.28–1.58 and 0.3–1.47 ppm, respectively; [50,51]) and *Rhopilema nomadica* (0.14–7000 ppm; [52]). On the other hand, significantly positive associations between Fe and Ni, reported in the present study, were not significant in Muñoz-Vera et al. [51]. Regardless of the different sampling times, total mean concentrations of As and Mn were about 10-fold greater in gonadal tissue than in umbrella tissue, in line with previous investigations, whereas concentrations found in spotted jellyfish *Mastigias papua* were seven times higher in gonads than in other tissues [51].

Only Li and V concentrations were higher in umbrella tissue consistently over the sampling times. Vanadium concentrations were previously detected in the ctenophore *Beroe cucumis* (8 mg/kg ash weight (AW); [53]), in the scyphozoan *Cyanea capillata* (5 mg/kg AW; [54]), and in the hydrozoan *Velella lata* ( $>70$  mg/kg AW; [55]). The presence of the element V was constant in all samples and at all collection times (range: 17.43–35.97 ppm). Vanadium is also an important indicator of oil pollution in surrounding environments [56]; therefore, the high vanadium concentration found in the sampled *R. pulmo* individuals could be a consequence of both the massive industrial activities (such as the production of steel and petrochemical refining) and the constant vessel traffic that characterizes the Gulf of Taranto. Vanadium, along with As, Cd, Cu, Cr, Ni, and Pb, has already been reported in marine species collected from Mediterranean areas at potentially high contamination levels [57]. Trace element concentrations in the aquatic biota may vary according to both the environmental level of metal bioavailability and body compartment composition.

The essential trace element Zn was mostly abundant in the gonads when compared with umbrella tissue. The mean concentrations of Zn and As detected in both the umbrella tissues and gonads of *R. pulmo* from Taranto coastal waters were comparable to those recorded elsewhere by other authors [26,27,50]. In particular, the high Zn concentrations recorded in gonadal tissues were in line with those found in jellyfish umbrella and oral arms of *Pelagia noctiluca*, *Cassiopea* sp., *Rhopilema nomadica*, and *Catostylus tagi* [27,49,52,58]. As an enzymatic cofactor, Zn is known to influence the gonadal development of aquatic organisms [59–61].

The range of the concentration of Cu (0.58–1.98 ppm) observed in umbrella tissues in the present study were in agreement with reported literature values for other jellyfish umbrella fractions [27,50], and with the values detected by Muñoz-Vera et al. [51] in *R. pulmo* from Mar Menor; whereas Cu values in gonadal tissues reflected those reported in the umbrella of the jellyfish *Catostylus tagi* [49].

All observed correlations were negative, suggesting, instead of bioaccumulation, a trend of dilution of the micronutrients' concentrations with increasing jellyfish size and age. Conversely to data reported by Muñoz-Vera et al. [51], we observed a few significant negative correlations (Co and Te, As and Te, and Pb and Zn) in the umbrella tissue. Some

of the correlations reported by Muñoz-Vera et al. [51] in *R. pulmo* from Mar Menor, both in umbrella and oral arms, such as Al and Fe (herein not observed in gonads), Al and Pb, Mn and Cr, Mn and Pb (herein not observed in gonads), and Zn and As (herein not observed in the umbrella), were not observed in this study, while the correlation between Mn and Fe was similarly significant.

Overall, gonads showed higher concentrations of trace metals than umbrella fractions, along with the presence of As, Cd, and Cu, probably due to background contamination levels [42,43,62]. Several studies showed that the tissue-specific ability to load metals (including heavy metals) can be influenced by reproductive cycle phases and sex [63,64]. Nonetheless, in agreement with previous studies [57], metal concentrations still remained below the cautionary level required for the potential exploitation of jellyfish biomasses for nutritional, nutraceutical, cosmeceutical, and pharmaceutical applications.

Finally, to assess suitability for human consumption, metal levels recorded in *R. pulmo* jellyfish from Taranto coastal waters were compared with the maximum permissible level for human consumption established in different countries and with best-known metal concentrations detected by previous studies in whole body, umbrella, and gonads in several jellyfish species collected in different areas (Table 6). As reported by Stabili et al. [33], the water content of *R. pulmo* ovaries was more than 90% and the dry weight of *R. pulmo* ovaries is estimated to be 6%. The range estimated for the whole jellyfish is similar (4.1–6.8%, [32]).

**Table 6.** Mean content of heavy metals and metalloids in jellyfish (mg/kg dry weight, ppm) reported in other studies. Bottom of the table, bold: maximum allowed metal content in marine food according to USA<sup>#</sup> (dry weight, ppm), EU\* (wet weight, ppm), and Australia\*\* (wet weight, ppm) legislations. <sup>#</sup>CFR: Code of Federal Regulations Title 21 Food and Drugs, [https://www.ecfr.gov/cgi-bin/text-idx?SID=d00d5bf8686e0d306a7e63512c39baf6&mc=true&tpl=/ecfrbrowse/Title21/21tab\\_02.tpl](https://www.ecfr.gov/cgi-bin/text-idx?SID=d00d5bf8686e0d306a7e63512c39baf6&mc=true&tpl=/ecfrbrowse/Title21/21tab_02.tpl) (accessed on 11 May 2021); \*Commission Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs and further amendments, <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02006R1881-20201014> (accessed on 11 May 2021); \*\*Australia and New Zealand Food Standards Code: Standard 1.4.1 Contaminants and Natural Toxicants, <https://www.legislation.gov.au/Details/F2015C00052> (accessed on 11 May 2021).

Species	Tissue	As	Cd	Cr	Cu	Pb	Zn	Hg	Ref	
<i>Rhizostoma pulmo</i>	Umbrella	13.6	0	2.26	1.12	0.55	24.08		[51]	
	Umbrella	1.86	0.19	0.14	1.16	0.20	14.17	0.6	Current study	
	Gonads	20.61	1.08	0.14	5.10	0.36	93.21	0.05	Current study	
<i>Cotilioriza tuberculata</i>	Umbrella	6.16	0.02	4.96	0.7	0.86	12.73		[50]	
	Aurelia sp.	Whole animal	0.04		16.1		19.9		[65]	
<i>Aurelia aurita</i>	Whole animal	6.30	$\times 10^{-2}$	0.658	$\times 10^{-2}$	1.3	$\times 10^{-2}$	9.37	$\times 10^{-2}$	[26]
	Whole animal	0.013							[66]	
<i>Carybdea rastoni</i>	Whole animal	0.135							[66]	
<i>Aequorea coerulescens</i>	Whole animal	0.026							[66]	
<i>Spiriodon saltator</i>	Whole animal	0.017							[66]	
<i>Chrysaora melanaster</i>	Umbrella	0.074							[66]	
<i>Pelagia noctiluca</i>	Whole animal	0.197			2	0.6	46		[66]	
<i>Pelagia noctiluca</i>	Whole animal		0.4						[67]	
<i>Mastigias papua</i>	Umbrella	0.039							[68]	
	Gonads	0.578							[68]	
<i>Mastigias</i> sp.	Whole animal	0.177	0.012	0.017	50.8	0.0186	0.586		[26]	
<i>Stromolophus nomurai</i>	Umbrella	0.183							[68]	
<i>Bolinopsis mikado</i>	Whole animal	0.011							[68]	
<i>Beroe cucumis</i>	Whole animal	0.145							[68]	
<i>Cassiopea</i> sp.	Umbrella	2.163	0.161	2.3	1.41		40.2		[27]	
	Whole animal	0.186	0.033	0.045	0.086	9.49	$\times 10^{-3}$	0.572	[26]	
<i>Chironex fleckeri</i>	Whole animal	0.236	0.52	$\times 10^{-3}$	3.21	$\times 10^{-3}$	0.065	$\times 10^{-3}$	0.553	
<i>Copula sivickisi</i>	Whole animal	4.137	0.41	0.09	0.475	0.115	$\times 10^{-3}$	6.028	[26]	
<i>Cyanea</i> sp.	Whole animal	0.473	0.038	0.011	0.063	2.41	$\times 10^{-3}$	0.537	[26]	
<i>Natrestoma</i>	Whole animal	0.104	0.012	7.94	$\times 10^{-3}$	0.032	3.83	$\times 10^{-3}$	0.295	
<i>Rhopilema nomadica</i>	Umbrella		0.6	1.36	2.24	1	5.6		[52]	
<i>Rhopilema esculentum</i>	Umbrella		0.02	0.04	0.03	0.08	0.61		[69]	
<i>Catostylus tagi</i>	Umbrella	n.d.	0.36	$\times 10^{-3}$	1.08	$\times 10^{-3}$	5.94	$\times 10^{-3}$	0.11	[49]
			<b>0.05–1</b>			<b>0.3–1.5</b>		<b>0.5–1</b>	EU (mg/kg fresh)	
		<b>2</b>	<b>2</b>			<b>0.5</b>		<b>0.5–1</b>	Australia (mg/kg fresh)	
			<b>1.5</b>		<b>60</b>	<b>3</b>	<b>200</b>		USA (mg/kg dry)	

Our data showed that heavy metal concentrations in *R. pulmo* collected in Taranto coastal waters were below the recommended threshold levels for human consumption according to the EU Regulation 466/200t/EC, as well as to current safety regulations in Australia and the USA. Other studies, focused on filter-feeder organisms such as mussels,

also showed that metals in mussel seafood can remain within the range of concentrations allowed for consumption and do not pose a hazard to human health [41], although some contaminants above allowed limits for human consumption were reported in some cases [70].

## 5. Conclusions

In the present research, we investigated the concentrations of 21 trace elements in *R. pulmo* jellyfish over a period of 16 months using ICP-AES. The potentially toxic heavy metals Cd, Cr, Co, Pb, and Hg, and the metalloid As, were generally found at higher concentration in gonads than in umbrella tissues, indicating the higher potential of gonads to accumulate these elements. Measurable concentrations of V were observed to be higher in the umbrella fraction than in the gonads. Low levels of Hg were also measured in both the umbrella and gonads. However, no correlation between the size of the specimens and the toxic element concentrations was observed, suggesting the absence of species-specific bioaccumulation patterns. The pair-element correlations found in this study were different from previous results, suggesting an important influence of environmental conditions. In particular, mean concentrations of Cr and Ni were always lower by at least one order of magnitude compared to *R. pulmo* umbrella from Mar Menor and other scyphozoan jellyfish species. In our study, heavy metal concentrations from *R. pulmo* jellyfish were always below the maximum level allowed by European, Australian, and USA regulations. However, further studies on the microelement contamination of marine species will consolidate knowledge on the suitability of Mediterranean jellyfish as a food source, clarifying the relationship between environmental exposure and physiological variability in jellyfish populations.

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