

# ASSESSMENT OF HEAVY METALS AND PAHs IN FISH WASTE VALORISATION FOR NUTRACEUTICAL PURPOSES

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## ABSTRACT

This study presents results on contaminant quantifications for the food safety of initial biomass (fish waste) and its extract product (fish oil) resulting from the activities carried out within the VITADWASTE project. VITADWASTE is a PRIN (Progetti di Rilevante Interesse Nazionale) project funded by Italian Ministry of University Education and Research, which aims to use a multidisciplinary and sustainable approach to transform biological waste into a valuable resource, to develop a safe and effective vitamin D3 nutraceutical product for human use, based on the principles of circular economy and bioeconomy. Fish waste includes by-products such as bones, guts, heads, skin and fins, or species below the minimum conservation size and tissues not suitable for human consumption, which together account for about 20-80% of the total catches and therefore improving the management of such biomass is essential. In the VITADWASTE project, an innovative green extraction method has been developed to recover vitamin D3 from fish waste, to produce a fish oil usable for nutraceutical implementations. Here, the results of Heavy Metals (HMs) and Polycyclic Aromatic Hydrocarbons (PAHs) concentrations are reported in the fish wastes and the fish oil according to the EU regulation. The consistently low concentrations of contaminants, always within regulatory limits, confirm the safety of both the raw material and the extracted product for human use, and support the adoption of SFE as a sustainable and efficient strategy for the valorisation of marine biomass within a circular economy framework.

## 1. INTRODUCTION

Fish plays an important role in human diet due to its high-quality proteins and beneficial effects. In particular, fatty fish, such as bluefish, contain more micronutrients than other meats, including vitamin D in fish oil and minerals like iron, selenium, zinc, iodine, magnesium and calcium, being a good food source for humans. In recent years, fish production, including that from aquaculture, has seen significant growth, reaching 223 million tons (FAO 2024, Umesh et al., 2025). In addition, the amount of fish waste from fishing has increased. Fish waste includes both com-

mercial species below the minimum conservation reference size and tissues not suitable for human consumption (such as bones, guts, heads, skin and tails), which together account for about 19% and 20-80% of the total catches, respectively (Kruijssen et al., 2020). Meanwhile, the fish by-products from industrial processing can result in waste levels of up to 70% (Dawson et al., 2025).

According to EU strategies, which aim for sustainable economic and environmental growth, and in line with Sustainable Development Goals (SDGs) and circular economy objectives, it is necessary to promote new challenges for the valorisation of fish waste. Up to date, fish waste is



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partly used to produce fishmeal, fertilizers (Radziemska et al., 2019; Lopes et al., 2021), and low-profit fish oil, or as raw material for direct feeding in aquaculture (Meena et al., 2024), while the rest is discarded. Indeed, research is growing in interest in such biomass for their bioactive compound's richness (Caruso et al., 2020; Rigueto et al., 2023).

In this context, VITADWASTE project aims to convert and reuse fish waste into a valuable substrate to obtain extract rich in bioactive compounds, particularly vitamin D3 and omega-3 fatty acids, suitable for pharmaceutical and nutraceutical sectors.

For this reason, this work aligns with SDG 12 (Responsible Consumption and Production), specifically with Objective 12.5: "substantially reduce waste generation by 2030 through prevention, reduction, recycling, and reuse," as well as with SDG 3: "ensuring healthy lives and promoting well-being for all at all ages."

Given that fish have the capacity to bioaccumulate a range of environmental contaminants through their diet and habitat, including polycyclic aromatic hydrocarbons (PAHs), heavy metals (HMs), and other contaminants of emerging concern (CECs), any valorisation process must rigorously assess and ensure the safety of these residues. This is particularly important when the final products are intended for human consumption (De Giovanni et al., 2022) or use in the nutraceutical sector.

For food safety, Regulation (EU) 2023/915 sets limits for contaminant levels in fish and certain food supplements, although fish-derived supplements are not as extensively regulated as others. The threshold limits from Regulation 2023/915 relevant to the samples considered in this study are presented below.

The regulation defines maximum allowable levels of heavy metals like lead (Pb), cadmium (Cd), and mercury (Hg) in fish, expressed in terms of wet weight (ww) of the edible muscle tissue, unless the entire fish is intended for consumption. The limit for Pb is set at 0.3 mg/kg. For cadmium, the general threshold is 0.05 mg/kg, though this increases to 0.1 mg/kg for mackerel (*Scomber* spp.) and to 0.25 mg/kg for anchovy (*Engraulis* spp.) and sardine (*Sardina pilchardus*). Mercury has a general limit of 0.5 mg/kg, but this rises to 1 mg/kg in the case of red mullet (*Mullus barbatus barbatus*).

Regarding polycyclic aromatic hydrocarbons (PAHs), the regulation only provides limits for smoked fish. In such cases, the maximum permitted concentration for benzo(a)pyrene is 2 µg/kg, while the sum of benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene, and chrysene must not exceed 10 µg/kg.

At present, there are no specific regulatory limits for PAHs in fish oils. However, for other food supplements governed by Regulation 2023/915, where fish-derived products are excluded, the threshold for benzo(a)pyrene is set at 10 µg/kg. Regarding oils and fats, the regulation states: "oils and fats placed on the market for the final consumer or for use as food ingredients, except cocoa butter and coconut oil. This maximum level applies to vegetable oils used as an ingredient in food supplements", and for these, the limit is set at 2 µg/kg. Therefore, the improvement of fish waste management is essential to address environmental con-

cerns and to fully utilize and valorise this biomass, for high value commercial purposes (Coppola et al., 2021).

The purpose of this paper is to assess if the fish oil derived from fish biomass waste, produced with Supercritical Fluid Extraction (SFE) at pilot scale, is suitable for human consumption and appropriate for nutraceutical applications, by evaluating the concentrations of potential contaminants which could be bioaccumulated by fishes and therefore transferred to the fish oil.

## 2. MATERIALS AND METHODS

### 2.1 Fish sampling and pretreatment

Five fish species from the Adriatic Sea were chosen for their good source of Vitamin D3 (Ostermeyer et al., 2006). Specifically, three pelagic species such as anchovy (*Engraulis encrasicolus*), sardine (*Sardina pilchardus*), and mackerel (*Scomber scombrus*), and two demersal species such as red mullet (*Mullus barbatus barbatus*) and European hake (*Merluccius merluccius*) were chosen for the study.

Fishing activities were conducted in the northern and central Adriatic Sea using commercial fishing vessels. Two types of fishing gear were employed: pelagic nets, used primarily for catching small pelagic species that inhabit the upper layers of the water column, and trawl nets, used for harvesting demersal species typically found near the seabed.

The collected fish waste was refrigerated on board and then transported to the laboratory, where it was separated by species and immediately homogenized using an automatic mill. The homogenized fish waste was then frozen at -20°C and subsequently freeze-dried.

### 2.2 Sardine oil production

Sardine oil was extracted using a Luwar supercritical fluid pilot plant (ENEA Research Centre Casaccia, Rome) with a 564 ml extraction vessel. The extraction was performed in duplicate, and each test treated 100 g of lyophilized sardine waste. The extraction pressure was 310±10 bar, the temperature was 40±0.4°C, and the supercritical fluid density was 916.0±4.9 kg/m<sup>3</sup>. The supercritical CO<sub>2</sub> flow rate was 0.46±0.03 kg/min. The process duration was calculated from the achievement of operating conditions and was 150 minutes for both extractions. The recovery yield was 14.6±0.1%, calculated as the ratio of the amount recovered from the plant to the dry matrix loaded (w/w).

### 2.3 Contamination analysis

Contaminant analysis were performed in both fish waste (HMs, PAHs) and fish oil (Cd, Hg, Pb).

#### 2.3.1 HMs analysis

For HMs analysis, pools for each species were performed, as two aliquots were collected after the homogenization process, and used for the contaminant analysis of those regulated by EU 2023/915 (Hg, Cd, Pb) and others HMs and metalloids non regulated (Al, Ag, As, Fe, Mn, Ni, Co, Cu, Cr, Se, Zn) which will be available in the supplementary material (S1.1. and S1.2.).

Fish waste mineralization was performed using a MARS-6 Microwave Assisted Acid Digestion, by adding 3 ml of ultrapure nitric acid and 3 ml of hydrogen peroxide to about 0.5 g of homogenized and freeze-dried fish and then the solution was diluted to 20 ml using ultrapure water (18.2 MΩ cm at 25°C).

Sardine fish oil digestion was performed by microwave-assisted acid digestion using a MARS6 microwave and the CEM method "Microwave Digestion of Fish Oil (No Capsule)", by adding to 0.2 g of fish oil 10 ml of ultrapure HNO<sub>3</sub>. After the digestion, the solution was diluted with 10 mL of Milli-Q ultrapure water (18.2 MΩ cm at 25°C), yielding a 20 ml clear and colorless solution, which was then analyzed by GF-AAS for Pb and Cd.

Inductively coupled plasma optical emission spectroscopy (ICP-OES) was used for all the HMs determinations in the previously mineralized fish waste, except for mercury. Microwave digestion program and ICP-OES operative parameters are extensively reported in Girolametti et al., (2024).

Hg determinations for both fish waste and oil were carried out using Thermal Desorption Atomic Absorption Spectrometry (TD-AAS) with a Direct Mercury Analyzer (DMA-1). Fish waste Hg quantification were performed in the freeze-dried sample, while fish oil was directly measured without further preparations. Three replicates were analyzed for each sample, by directly measuring 0.1 g of dry weight (dw) for fish waste and 0.02 g of fish oil. The TD-AAS program consisted in three different temperature steps: 1 minute at 200°C, followed by 2 minutes at 650°C, and a final 1-minute hold at 650°C.

The contaminant concentrations in the fish waste are expressed as mg/kg of wet weight (ww) by normalizing their dw concentrations with the water content removed from the sample through the freeze-drying procedure, determined by differential weighing before and after the drying process.

Pb quantification in the fish oil was performed using the standard additions method, while Cd was determined by calibration curve. For both elements, a palladium-based matrix modifier was used to enhance GF-AAS measurement accuracy.

The Limits of Detection (LODs) were evaluated using the calibration curve method and are reported in Table 1 for the regulated heavy metals (HMs), and in Table S1 for the non-regulated ones.

### 2.3.2 PAH analysis

PAHs extraction from fish waste was performed using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and

Safe) technique, which involves a first extraction phase using small amounts of solvent (acetonitrile), separated from the aqueous matrix with magnesium sulfate and sodium chloride, followed by a further purification phase through a 0.2 µm filtration, with a slight modification of the method described by Frapiccini et al., (2018).

16 PAHs were investigated using for calibrations a standard PAH solution (EPA 610 PAHMIX, Supelco, Bellefonte, PA, USA) with dichloromethane:methanol (1:1 v/v) containing naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene, indeno(1,2,3cdi)pyrene.

The quantification of PAHs was performed using UH-PLC equipped a FLD Dionex UltiMate 3000 (Termofisher, Italy). Acenaphthylene was not considered because of it does not have fluorescent properties, therefore could not be measured through UHPLC-FLD.

The chromatographic separation of PAHs was performed over a 40-minute run using a reversed-phase gradient elution of acetonitrile (ACN) and water (v/v%). The gradient started at 10% ACN and linearly increased to 100% ACN, which was maintained for the final 5 minutes of the run to ensure effective column cleaning prior to subsequent injections. A fixed injection volume of 1 µL was employed for each analysis. Detailed conditions of UHPLC-FLD analysis are reported in Table 2.

Limit of detection (LOD) of PAHs were evaluated directly from the calibration curve with Chromeleon software using Hubaux-vos at 95% confidence interval.

## 3. RESULTS AND DISCUSSION

### 3.1 Heavy Metal(loid)s and PAHs concentrations

#### 3.1.1 In the Fish waste

The contaminants investigated in the five species of the fish waste are HMs and PAHs. Particular attention was given to the three heavy metals regulated by EU 2023/915: Hg, Cd and Pb, which were measured in both the sample typologies. The concentrations of HMs are expressed in mg/kg<sub>ww</sub>. The results of the non-regulated HMs will be available in the supplementary material (Figure S1 and Figure S2).

In the analysed fish waste samples, the concentrations of Hg, Cd and Pb never exceeded the limits set by EU 2023/915 (Table 3). To facilitate the comparison between measured values and regulatory limits, the ratio between the Regulatory Limit (R.L.) and the Measured Value (M.V.) is also reported in Tables 3 and 5.

**TABLE 1:** Limits of detection (LOD) for the regulated HMs (Cd, Pb and Hg). Concentrations on mg/kg are evaluated considering the digestion volume and dilution utilized for the quantifications, where necessary.

Analytical Instrument	Matrix	Cd		Pb		Hg
		µg/L	mg/kg	µg/L	mg/kg	mg/kg
ICP-OES	fish waste	0.05	0.002	3.7	0.15	
GF-AAS	fish oil	0.13	0.014	2.1	0.201	
DMA	fish waste and oil					0.003

**TABLE 2:** Chromatographic and analytical parameters for PAH determination by UHPLC-FLD.

PAH	Retention time (min)	$\lambda_{ex}$ / $\lambda_{em}$	Calibration range (ng/ml)	Calibration algorithm	R <sup>2</sup>	LOD (ng/ml)
naphthalene	8.305	265ex/360em	10-495	Linear	0.99997	6.0
acenaphthene	11.439	265ex/360em	10-543	Linear	0.99999	3.6
fluorene	12.005	265ex/360em	2.5- 97.8	Linear	0.99990	2.0
phenanthrene	13.289	265ex/360em	1.3-50	Linear	0.99973	1.6
anthracene	14.398	265ex/410em	4.5-50	Linear	0.99975	1.6
fluoranthene	15.777	290ex/480em	9.7-96.3	Linear	0.99992	2.3
pyrene	16.617	270ex/390em	1.4-51	Linear	0.99995	3.0
benzo(a)anthracene	19.914	265ex/410em	1.0-49	Linear	0.999997	0.1
chrysene	20.481	265ex/410em	0.9-48.2	Linear	0.99993	1.1
benzo(b)fluoranthene	23.148	265ex/410em	1.5-94.2	Linear	0.999996	1.3
benzo(k)fluoranthene	24.281	265ex/410em	0.9-48.7	Linear	0.99998	0.4
benzo(a)pyrene	25.331	265ex/410em	1.4-51	Linear	0.99990	1.0
dibenz(a,h)anthracene	27.331	265ex/410em	2.5-96.2	Linear	0.99986	1.9
benzo(g,h,i)perylene	28.131	290ex/480em	1.8-97.2	Linear	0.99991	2.4
indeno(1,2,3cdi)pyrene	28.827	265ex/410em	3.9-47	Linear	0.99999	1.0

**TABLE 3:** Comparison of HM concentrations measured in the fish waste samples and the regulatory limits of the EU 2023/915. R.L./M.V. expresses the factor by which the Measured Values (M.V.) falls below the Regulatory Limit (R.L).

Fish Waste	Cd			Hg			Pb	
	Measured Value (mg/kg <sub>ww</sub> )	Regulatory Limit (mg/kg <sub>ww</sub> )	R.L./M.V.	Measured Value (mg/kg <sub>ww</sub> )	Regulatory Limit (mg/kg <sub>ww</sub> )	R.L./M.V.	Measured Value (mg/kg <sub>ww</sub> )	Regulatory Limit (mg/kg <sub>ww</sub> )
Red Mullet	0.0035 ± 0.0002	0.05	14.3	0.045 ± 0.005	1	22.2	< 0.037	0.3
Sardine	0.0138 ± 0.0008	0.25	18.2	0.027 ± 0.001	0.5	18.8	< 0.037	0.3
European Hake	0.0064 ± 0.0024	0.05	7.9	0.048 ± 0.001	0.5	10.4	< 0.037	0.3
Mackerel	0.0034 ± 0.0008	0.1	29.4	0.062 ± 0.001	0.5	8.1	< 0.037	0.3
Anchovy	0.017 ± 0.001	0.25	14.4	0.038 ± 0.002	0.5	13.1	< 0.037	0.3

Pb concentrations were undeterminable due to values below the detection limit, attesting to be below 0.037 mg/kg<sub>ww</sub> in all the samples (fish waste concentration as mg/kg<sub>ww</sub> was evaluated by assuming LOD as minimum concentration and using the average digestion mass, volume, dilution and % dry weights for the calculations), with concentrations at least 8.1 times lower than the regulatory limit.

Hg and Cd, however, showed quantifiable but still non-harmful concentrations, as they always resulted below the threshold limit in all the studied species (Table 3).

Cd concentrations ranged between a minimum of 0.0034±0.0008 mg/kg in mackerel and red mullet to a maximum of 0.0174±0.0008 mg/kg in the anchovy, with intermediate values for European hake, 0.0064±0.0024 mg/kg, and sardine, 0.0138±0.0008 mg/kg, with values below the regulatory limit from 7.9 to 29.4 times, depending on the

species. The higher Cd concentrations observed in pelagic species such as anchovy and sardine, compared to demersal species, mirror the higher maximum limits established in Regulation EU 2023/915. This is due to the tendency of Cd to accumulate in the phytoplankton, and thus in the upper photic zone of the water column (Mart & Nürnberg, 1986), which leads to an increase in the bioavailability of Cd to these fishes, because of their diet that is typically plankton-phytoplankton based.

Hg concentrations ranged from minimum values of 0.0265±0.0012 mg/kg in sardine to a maximum value of 0.0618±0.0008 mg/kg in mackerel, with intermediate values for anchovy, red mullet, and European hake. The comparison with regulatory limits (R.L./M.V.) shows concentrations from 8.1 to 22.2 times below the threshold (Table 3).

PAH concentrations were low or absent in the fish waste, in particular for the most relevant PAHs (benzo(a)



**TABLE 4:** PAH concentrations in the fish waste (r1 and r2 are referred to different fish waste replicate). < LOD, indicates that the analyte concentration is below the detection limit.

PAH compounds	European hake r1	European hake r2	Mackerel r1	Mackerel r2	Sardine r1	Sardine r2	Anchovy r1	Anchovy r2	Red mullet r1	Red mullet r2
naphtalene	< LOD	< LOD	0.18	0.43	0.46	< LOD	0.58	0.56	1.20	1.05
acenaphthene	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	2.46	2.36
fluorene	< LOD	< LOD	0.60	0.55	< LOD	< LOD	< LOD	< LOD	1.66	1.93
phenanthrene	< LOD	< LOD	< LOD	0.36	0.28	< LOD	1.26	1.23	< LOD	0.32
anthracene	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
fluoranthene	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
pyrene	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.17	0.16
benzo(a)anthracene	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.04	0.04	0.07	< LOD
chrysene	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
benzo(b)fluoranthene	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
benzo(k)fluoranthene	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
benzo(a)pyrene	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
dibenz(a,h)anthracene	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
benzo(g,h,i)perylene	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
indeno(1,2,3cdi)pyrene	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD

pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene) regulated in the EU 2023/915 (Table 4).

Benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene were not identified in nearly all samples, with quantifiable concentrations only for benzo(a)anthracene in the anchovy (0.04 µg/kg) and in only one replicate (0.07 µg/kg) of the red mullet, indicating safety for the consumption.

The highest PAH concentrations for the fish wastes were found for the lowest molecular weight compounds (LMW-PAHs) such as naphthalene, acenaphthene and fluorene; this was particularly evident for the red mullet, where its demersal behavior and preferred muddy habitat plays a key role for the bioaccumulation of such compounds mainly through the diet, even if this does not represent a matter of concern regarding this contamination for humans. Also, LMW-PAHs, due to their lower log K<sub>ow</sub>, are more water-soluble and tend to associate with suspended particles that settle in sediments. This makes them more bioavailable to demersal species that live and feed near the sediment-water interface (Frapiccini et al., 2020).

Pyrene also showed measurable concentrations of 0.17 and 0.16 µg/kg only in red mullet, while in the rest of

the investigated species was not detectable. Also, traces of Phenanthrene of 1.26 and 1.23 µg/kg were found in the anchovy, even if this does not represent a matter of concern.

### 3.1.2 In the extract product (fish oil)

The fish oil production operations were achieved using the green-innovative Supercritical Fluid Extraction (SFE). This technique was chosen because of SFE uses CO<sub>2</sub> as extracting solvent instead of organic – and generally harmful – solvent of the traditional extracting techniques, which gives a first insight for the possibility to the usage for nutraceutical. Also, the investigated species Vitamin D-3 extraction yield of SFE was compared with traditional methods like Ultrasound Assisted Extraction (UAE) and Solid-Liquid Extraction (SLE), resulting in comparable yields among the different techniques, confirming its suitability. A comparison of Vitamin D-3 concentrations obtained under different SFE extraction conditions are described in detail in a separate study (Alessandroni et al., under review).

Therefore, according to the purpose of the VITAD-WASTE project, the different species of fish waste were tested, and as sardine waste resulted to provide a suitable oil for the nutraceutical usage, considering both the high oil extraction yield and Vitamin D-3 concentration, the contamination analysis for the regulated HMs were then conducted.

The concentrations of the contaminants, expressed as average ± standard deviations, are compared with the maximum allowed concentrations established for the food supplements in the EU 2023/915, which establishes limits of 3, 1 and 0.1 mg/kg respectively for Pb, Cd, and Hg (Table 5).

Hg concentrations in the fish oil were found to be 3.11±0.16 µg/kg, which is approximately 32 times lower than the regulatory limit. Similarly, Pb and Cd concentra-

**TABLE 5:** HMs comparison between the concentrations found in the sardine fish oil and the EU regulation 2023/915 threshold levels. R.L./M.V. expresses the factor by which the Measured Values (M.V.) falls below the Regulatory Limit (R.L.).

HMs	Measured Value µg/kg	Regulatory Limits, EU 2023/915 µg/kg	R.L. / M.V.
Hg	3.11 ± 0.16	100	32
Pb	48.7 ± 6.9	3000	62
Cd	23.4 ± 1.2	1000	43

tions were  $48.7 \pm 6.1 \mu\text{g/kg}$  and  $23.40 \pm 1.18 \mu\text{g/kg}$ , respectively. These values are about 62 times lower than the regulatory threshold for Pb and 43 times lower for Cd, as reported in Table 5. Therefore, based on these results, no health risk was identified for consumers in relation to these contaminants.

## 4. CONCLUSIONS

In this study, the food safety of both the initial product (fish waste) and the extract product (fish oil) derived from the VITADWASTE project was assessed with respect to contamination by heavy metals (HMs) and polycyclic aromatic hydrocarbons (PAHs), in accordance with Regulation (EU) 2023/915.

Previous investigations within the project tested supercritical fluid extraction (SFE) as a sustainable method for recovering bioactive lipophilic compounds from fish waste. After optimizing the extraction process and comparing different species, sardine waste was identified as the most suitable raw material for producing nutraceutical-grade fish oil, which was subsequently analyzed for contaminants.

From a food safety perspective, no critical levels of contamination were observed. Concentrations of both inorganic (Hg, Cd, Pb) and organic (PAHs) contaminants in the raw biomass and extracted oil were well below the thresholds established by EU legislation. HMs in the fish waste were found to be approximately 8 to 29 times lower than regulatory limits, and no heavy PAHs were detected except for minimal traces of pyrene and benzo(a)fluoranthene, without posing any health risks due to their low concentrations. In the extracted oil, HM concentrations ranged from 32 to 62 times below legal thresholds.

These findings confirm the safety of both the raw material and the extracted product for human use and high-light SFE as a viable and sustainable strategy for marine biomass recovery in line with circular economy principles.

Although this represents an initial validation for the food safety, future work will focus on scaling up the process to obtain larger quantities of oil enriched in vitamin D and polyunsaturated fatty acids, aiming to formulate nutraceutical products for evaluation in clinical studies.

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