Safety Assessment of Brown Seaweed Species and Their Extracts

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INTRODUCTION

The edible algae species *Bifurcaria bifurcata* (BB), *Ascophyllum nodosum* AN, and *Fucus spiralis* FS, found along the European Atlantic coast, contain valuable compounds with significant biological activities, including antioxidant, antimicrobial, anticancer, and neuroprotective properties, making them attractive for various applications in the food, cosmetic, and pharmaceutical industries [1].

Figure 1 Macroalgae collected for this study.

To maximize the extraction of these bioactive chemicals, advanced techniques like microwave-assisted extraction are being explored to improve efficiency over traditional methods [2] .

Besides the biotechnological potential of these algae, ensuring food safety is crucial. A thorough assessment of contaminants, including heavy metals and organic pollutants such as polycyclic aromatic hydrocarbons (PAHs), PCBs, flame retardants, and pesticides, is required to meet strict food safety standards.

METHODOLOGY

1 Algae collection

The algae were washed, sorted, classified, freeze-dried .The extracts were prepared by microwave-assisted extraction [3].

2 Analytical determinations

Microelements (quantified by ICP-OES): Iron (Fe),Manganese (Mn),Copper (Cu),Zinc (Zn). For Mercury (Hg) quantification a cold vapor atomic absorption spectrometry. ICP-MS (Inductively Coupled Plasma Mass Spectrometry) was used in the determination of Iodine (I), Arsenic (As), and Lead (Pb) [4]**.**

The algae and algal extracts were analyzed by gas chromatography with electro-capture detection (GC-ECD) and gas chromatography with flame photometric detector (GC-FPD) for the presence of old and new pollutants such as 22 pesticides, 4 PCBs, and 15 flame retardants. [5].

PAHs were quantified by HPLC-FLD/DAD equipped with a C18 column (CC 150/4 Nucleosil 100-5 C18PAH, 5µm particle size; Macherey-Nagel, Duren, Germany). The eluents were acetonitrile and ultrapure water[6].

3 Analytical results

Pollutants: retention time, calibration, and detection limits

Compound	Rt (min)	Calibration curve	R^2	LOD µg/kg
Organochlorine pesticides (ECD)				
α-HCH	21.774	$Y=2.67x10^6X+2.65x10^5$	0.994	1.279
β-HCH	23.390	$Y=8.86x10^5X-1.60x10^5$	0.994	1.289
HCH	26.179	$Y=5.40x10^5X+2.18x10^5$	0.991	1.535
Aldrin	27.805	$Y=1.28x10^6X+6.67x10^5$	0.990	1.613
Endosulfan I	31.636	$Y=2.67x10^6X+2.65x10^5$	0.993	1.365
1.365p-p' DDE	32.542	$Y=2.16x10^5X+2.03x10^5$	0.998	0.709
Dieldrin	32.757	$Y=3.18x10^4X-1.01x10^4$	0.995	1.118
o-p' DDT	34.142	$Y=5.48x10^5X+4.12x10^5$	0.991	1.591
p-p' DDD	34.713	$Y=8.98x10^5X+4.06x10^5$	0.998	0.806
Organophosphorus pesticides (FDP)				
Dimethoate	12.290	$Y=1.95x10^5X-4.5x10^4$	0.995	0.425
Chlorpyrifos-methyl parathion-methyl	15.439	$Y=3.71x10^5X-2.32x10^5$	0.998	0.737
Malathion	16.997	$Y=1.735x10^5X+3.53x10^4$	0.996	1.093
Chlorpyrifos	17.431	$Y=1.64x10^5X+6.44x10^4$	0.998	0.721
Chlorfenvinphos	18.748	$Y=6.69x10^4X+2.93x10^3$	0.9969	1.037
Pyrethroids pesticides (ECD)				
Bifenthrin	37.178	$Y=1.66x10^6X+1.00x10^6$	0.996	1.176
Cyhalothrin	39.022	$Y=3.79x10^4X+4.26x10^5$	0.995	1.734
Cyhalothrin isomers	39,442	$Y=4.45x10^5X+4.87x10^5$	0.997	1.546
Cypermethrin	43.645	$Y=4.00x10^5X+1.59x10^5$	0.996	1.492
Cypermethrin isomers	44.119	$Y=1.17x10^5X+2.81x10^4$	0.997	1.295
Fenvalerate	46.109	$Y=1.74x10^5X+4.77x10^4$	0.998	0.933
Fenvalerate isomers	46.704	$Y=2.41x10^5X+1.32x10^4$	0.998	1.035
Deltamethrin	47.777	$Y=5.71x10^5X+2.50x10^5$	0.996	1.587
Deltamethrin isomers	48.598	$Y=1.66x10^5X+1.33x10^5$	0.997	1.343
Polychlorinated biphenyls (PCBs) (ECD)				
2,4,4'-Trichlorobiphenyl (PCB 28)	26.390	$Y=2.26x10^6X+2.48x10^5$	0.992	1.433
2,3',4,4',5-Pentachlorobiphenyl (PCB 118)	34.214	$Y=4.80x10^5X+5.26x10^5$	0.996	1.282
2,2',4,4',5,5'-Hexachlorobiphenyl (PCB 153)	34.823	$Y=4.13x10^5X+3.38x10^5$	0.996	1.154
2,2',3,4,4',5,5'-Heptachlorobiphenyl (PCB 180)	38.509	$Y=3.26x10^5X+2.27x10^5$	0.996	0.967
Brominated flame retardants (ECD)				
2,4,4'-tribromodiphenyl ether (BDE 28)	34.318	$Y=4.00x10^5X+2.11x10^5$	0.992	1.468
2,2′,4,4′-TetraBDE,2,2′,4,4′-Tetrabromodiphenyl ether (BDE47)	38.918	$Y=1.27x10^5X+6.44x10^4$	0.995	1.251
2,2',4,4',5,6'-Hexabromodiphenyl ether (BDE 100)	42.104	$Y=2.97x10^5X+2.17x10^5$	0.997	1.201
2,2',4,4',5-Pentabromodiphenyl ether (BDE 99)	43.216	$Y=5.22x10^4X+6.73x10^4$	0.998	1.226
2,2',4,4',5,6'-hexabromodiphenyl ether (BDE154)	46.823	$Y=8.11x10^5X-4.82x10^4$	0.996	1.637
2,2',4,4',5,5'-Hexabromodiphenyl ether (BDE 153)	48.901	$Y=2.74x10^5X+8.34x10^4$	0.998	0.934
2,2',3,4,4',5,6-Heptabromodiphenyl ether (BDE 183)	58.109	$Y=5.81x10^5X+1.65x10^5$	0.999	0.781
2,2',3,4,4',5',6-Heptabromodiphenyl ether (BDE 183 Isomers	51.267	$Y=4.52x10^5X+3.39x10^5$	0.998	1.219

Pollutants: retention time, calibration, and detection limits (cont.)

Compound	Rt (min)	Calibration curve	R^2	LOD µg/kg
Phosphorus flame retardants (FDP)				
tripropyl phosphate (TPrP)	6.846	-	-	-
tri-iso-butyl phosphate (TiBP)	8.488	$Y=5.24x10^4X+8.66x10^4$	0.993	1.45
TnBP	10.545	$Y=9.77x10^4X+5.84x10^4$	0.996	0.911
Tris(2-chloroethyl)phosphate (TCEP)	12.688	$Y=5.59x10^4X+1.04x10^2$	0.993	1.191
tris(2-ethylhexyl) phosphate (TEHP)	22.473	$Y=4.28x10^4X+2.56x10^3$	0.9925	1.266
tris(2-butoxyehyl) phosphate (TBEP)	22.086	$Y=4.07x10^4X+1.76x10^4$	0.998	2.776
tri-cresyl phosphate (TCP)	23.957	$Y=4.07x10^4X+1.76x10^4$	0.997	0.833
tri-cresyl phosphate (TCP) isomers		$Y=1.25x10^4X-8.60x10^3$	0.998	0.576
tri-cresyl phosphate (TCP) isomers		$Y=2.16x10^5X-1.44x10^3$	0.999	0.451
tri-cresyl phosphate (TCP) isomers		$Y=1.15 X X-7.35x10^3$	0.998	0.775

LoD (Limit of detection 3*($S_{y/x}$) /slope)($S_{y/x}$) standard deviation of the calibration curve slope FDP- Flame photometric detector; ECD Electro-capture detector

PAHs: retention time, calibration, and detection limits

Compound	Rt (min)	Calibration curve	R^2	LOD µg/L
Naphthalene	10.3	$Y=2.31x10^4X-6.48x10^3$	1.000	1.5
Acenaphthylene	11.7	$Y=5.03x10^2X-3.01x10^4$	0.996	36.4
Acenaphthene	13.3	$Y=3.41x10^4X-1.54x10^5$	0.999	5.2
Fluorene	14.0	$Y=6.42x10^5X-6.77x10^4$	0.999	0.5
Phenanthrene	15.3	$Y=1.37x10^5X-2.42x10^4$	0.999	0.6
Anthracene	16.5	$Y=8.03x10^4X-5.92x10^3$	0.999	0.4
Fluoranthene	17.5	$Y=4.51x10^4X-3.13x10^4$	0.999	1.1
Pyrene	18.4	$Y=5.24x10^4X-2.68x10^4$	0.999	0.7
Benz(a)anthracene	21.3	$Y=1.16x10^5X-2.41x10^4$	0.999	0.6
Chrysene;	22.1	$Y=3.87x10^4X-2.69x10^3$	0.999	0.6
Benzo(b)fluoranthene	24.1	$Y=6.42x10^5X-4.82x10^4$	1.000	1.9
Benzo(k)fluoranthene	25.2	$Y=2.35x10^5X-1.30x10^4$	0.999	0.6
Benzo(a)pyrene	26.2	$Y=4.90x10^5X-3.11x10^4$	0.999	0.7
Bibenz(a,h)anthracene	26.7	$Y=7.16x10^4X-5.19x10^4$	0.999	2.4
Benzo(g,h,i)perylene	28.1	$Y=4.48x10^4X-1.18x10^4$	0.999	0.3
Indeno(1,2,3-cd)pyrene	29.0	$Y=2.00x10^5X-1.76x10^4$	0.998	1.1
Dibenzo(a,l)pyrene	30.5	$Y=5.04x10^3X-8.50x10^3$	0.996	2.2

LOD (Limit of detection 3x ($S_{y/x}$) /slope)($S_{y/x}$) standard deviation of the calibration curve slope, Bold names correspond to priority pAHs (EURO LEX, 2023)

Metals and metalloids composition mg/kg dw

	BB	AN	FS	BB(ext)	AN(ext)	FS(ext)	LOQ	RDI (mg/day)
Cu	45.791^A	1.570^{BC}	3.867^C	1.281^a	1.112^a	<loq	0.60	1.6*
Fe	2612^A	113^{BC}	431^C	$11,87^a$	18.46^{ac}	44.9^b	1.00	11
Zn	12.734^A	18.381^B	15.326^{AB}	6.856^A	12.314^{bc}	8.819^a	0.20	7.5-16.3
I	196.76^A	463.05^{BC}	224.79^A	304.88^a	1145.85^{bc}	303.41^a	5.00	0.15*
As	69.67^A	33.12^B	46.07^B	128.33^a	70.01^b	61.39^b	2.50	-
Pb	<LOQ	<LOQ	<LOQ	<LOQ	<lOQ	<lOQ	1.25	-
Hg	<LOQ	<LOQ	<LOQ	0,06	<lOQ	<lOQ	0,040	-

Results are the average of at least n=3 with an SD<10% LOQ- limit of quantification RDI-recommended dose intake *adequate intake, different letter represent different results $p < 0.05$ [29]

No pesticides detected
No flame retardants detected
No Priority PAHs detected.

5 Hazard evaluation

$$EDI = \frac{Ct \times Cr \times EF \times ED}{BW \times AT}$$

EDI= Estimated Daily Intake (mg/kg-day); Ct=toxic concentration mg/kg dw; Cr= Amount of algae consumed in kg dw; EF =exposure frequency (days/years) [7] ED =exposure duration (years) [8] ; BW= body weight [9], AT=averaging time exposure period (ED*365 days).

$$THQ = \frac{EDI}{RfD}$$

RfD=oral reference dosage (mg/kg/day); THQ Target hazard quotient [9-12]

$$HI = \sum_{i=1}^{n} THQ_n \quad i=1,2,3...n$$

HI-Hazard index

$$CR = EDI \times C_{SF}$$

CR= cancer risk, C_{SF}=oral cancer slope factor in (mg/kg/day) [9-12]

$$TCR = \sum_{i=1}^{n} CR_n \quad i=1,2,3... n$$

TCR =Total Cancerinogenic Risk

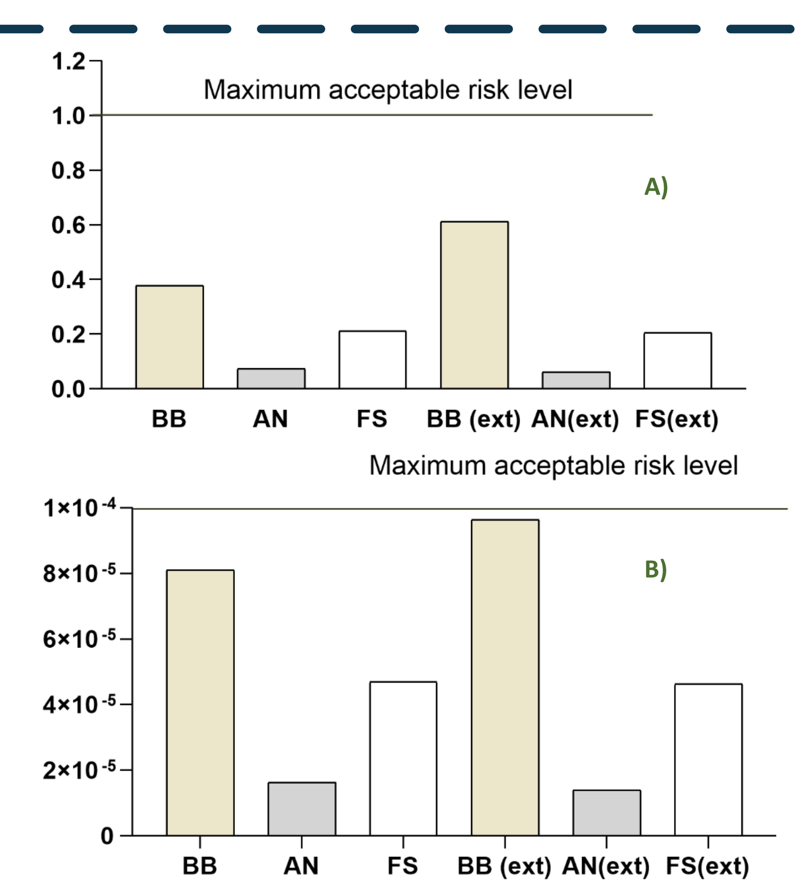

Figure 2 A) Hi leves , B) TCR levels .

6 Conclusions

In this study, a serving size equivalent to 100% of the recommended daily intake (RDI) for I (0.15 mg/day) for each seaweed per week was used for the risk assessment. Based on the I concentration, the amount of seaweed in fresh weight that provides the RDI per day is 2.8 g BB, 1.2 g An, and 2.5 g FS. As for the extracts, 130 mg of AN(ext) and 490 mg of the other macroalgae provide the RDI for Iodine

Considering this intake values,, and based on the results for metals and metalloids since all other pollutants were absent the macroalgae and extracts are safe to consume.

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