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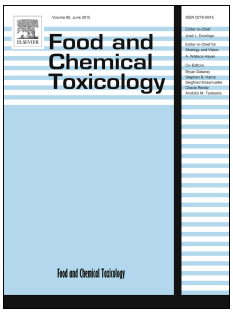


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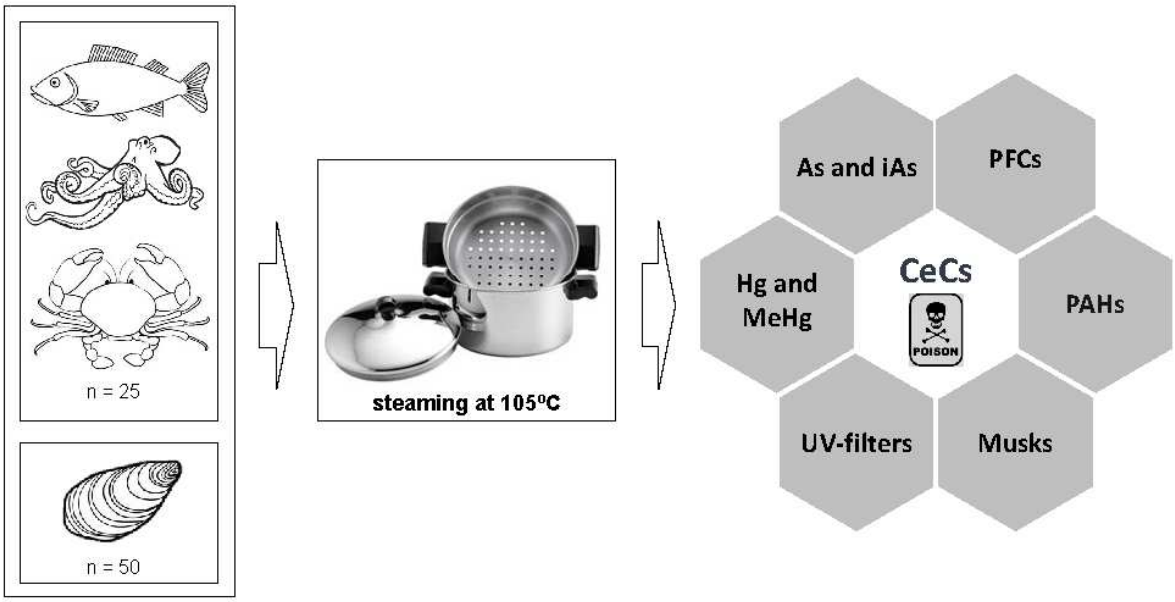

**Effects of steaming on contaminants of emerging concern levels in seafood**

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**Abbreviations:** 4-MBC - 3-(4-Methylbenzylidene)camphor; AHTN - 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene; ANOVA - analysis of variance; AsV – arsenic V; BaP - benzo(a)pyrene;BMDL - benchmark dose lower limit; BP1 - Benzophenone 1; Cd - cadmium; CeCs - contaminants of emerging concern; Cr – chromium; Cu -copper; DBENZO - Hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate; DHMB - 2,2-Dihydroxy-4,4-dimethoxybenzophenone; DHA - docosahexaenoic acid; DORM-4 – dogfish muscle reference material; DPMI - 6,7-dihydro-1,1,2,3,3-pentamethyl-4(5H)-indanone; dSPE - dispersive solid-phase extraction; EC – European Commission; ECHA – European chemicals agency ; EFSA – European Food Safety Authority; EHS - 2-Ethylhexyl salicylate; EPA - eicosapentaenoic aci; ERM-BC211 – rice reference material; GC–IT-MS/MS - gas chromatography-ion trap-tandem mass spectrometr; GC-MS - gas chromatography-mass spectrometry; HBGVs - health-based guidance values; Hg – mercury; HHCB - 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(g)-2-benzopyran; HHCB-lactone - 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-

hexamethylcyclopenta-(g)-2-benzopyran-1-one; HPLC - high performance liquid chromatography; HS - 3,3,5-Trimethylcyclohexylsalicylate; iAs - inorganic arsenic; ICP-MS - inductively coupled plasma mass spectrometer; ISTD – internal standards; Kow - n-octanol/water partition coefficientLC-IT-MS/MS - liquid-chromatography-ion trap tandem mass spectrometry; LOD - limit of detection; LOQ - limit of quantification; MeHg – methyl mercury; MOE – margins of exposure; MS- mass spectrometry; NOAEL – no observed adverse effect level; OC – Octocrylene; PAH2 - sum of benzo(a)pyrene, chrysene; PAH4 - sum of benzo(a)pyrene, chrysene, benz(a)anthracene, benzo(b)fluoranthene; PAH8 - sum of benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene, dibenzo(a,h)anthracene, indeno(123cd)pyrene, benzo(ghi)perylene; PAHs - polycyclic aromatic hydrocarbons; Pb – lead; PCBs - polychlorinated biphenyls; PCPs - personal care products; PFBA – perfluorobutanoate; PFBS - perfluorobutane sulfonate; PFCs - perfluorinated compounds; PFDcA – perfluorodecanoate; PFDoA - perfluorododecanoate; PFDS - perfluorodecane sulfonate; PFHpA – perfluoroheptanoate; PFHpS - perfluoroheptane sulfonate; PFHxA - perfluorohexanoate, PFHxS - perfluorohexane sulfonate;PFNA - perfluorononanoate; PFOA – perfluoroctanoate; PFOS - perfluorooctane sulfonate; PFPeA – perfluoropentanoate, PFTeA - perfluorotetradecanoate, PFTrA - perfluorotridecanoate; PFUnA - perfluorundecanoate; POPs - persistent organic pollutants; QuEChERS - quick, easy, effective, rugged and safe; RSD - relative standard deviation; TAs - total arsenic; TDI - tolerable daily intake; THg - total mercury; TORT-2 - lobster hepatopancreas reference material; TWI - tolerable weekly intake; UF - safety/uncertainty factor; UL - tolerable upper intake level.

**Abstract**

Seafood consumption is a major route for human exposure to environmental contaminants of emerging concern (CeCs). However, toxicological information about the presence of CeCs in seafood is still insufficient, especially considering the effect of cooking procedures on contaminant levels. This study is one among a few who evaluated the effect of steaming on the levels of different CeCs (toxic elements, PFCs, PAHs, musk fragrances and UV-filters) in commercially relevant seafood in Europe, and estimate the potential risks associated with its consumption for consumers. In most cases, an increase in contaminant levels was observed after steaming, though varying according to contaminant and seafood species (e.g. iAs, perfluorobutanoate, dibenzo(ah)anthracene in *Mytilus edulis*, HHCB-Lactone in *Solea* sp., 2-Ethylhexyl salicylate in *Lophius piscatorius*). Furthermore, the increase in some CeCs, like Pb, MeHg, iAs, Cd and carcinogenic PAHs, in seafood after steaming reveals that adverse health effects can never be excluded, regardless contaminants concentration. However, the risk of adverse effects can vary. The drastic changes induced by steaming suggest that the effect of cooking should be integrated in food risk assessment, as well as accounted in CeCs regulations and recommendations issued by food safety authorities, in order to avoid over/underestimation of risks for consumer health.

## 1. Introduction

Seafood is an important food item for a healthy and balanced diet, being its consumption widely recommended to prevent several diseases, such as hypertension, coronary heart disease and cancer (Bayen et al., 2005; Schmidt et al., 2015). Seafood health benefits are mainly associated to its low cholesterol levels and high levels of essential nutrients, such as amino acids (e.g. cysteine, lysine, and methionine), polyunsaturated n-3 fatty acids [e.g. eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA)], vitamins (e.g. vitamin A and vitamin D) and minerals (e.g. selenium, iodine) (Bayen et al., 2005; Bhavsar et al., 2014). Nevertheless, like other types of food, it can accumulate high levels of chemical contaminants, including persistent organic pollutants (POPs; e.g. dichlorodiphenyltrichloroethane, polychlorinated biphenyls, dioxins) and toxic elements [mercury (Hg), cadmium (Cd), lead (Pb) and arsenic (As)], through environmental exposure, representing a risk to human health (Alves et al., 2017; Domingo,

2011; Marques et al., 2011). Since seafood can be one of the major dietary routes of human exposure to environmental contaminants, the interest in assessing levels of contaminants of emerging concern (CeCs) in seafood is growing within the scientific community and regulatory authorities (Aznar-Alemany et al., 2017).

Although most seafood is cooked before consumption, the current risk assessment and limits set by European authorities for the presence of chemical contaminants are mainly based on the analysis of uncooked/raw products (Marques et al., 2011). The diversity of culinary and industrial procedures is enormous for each product, varying according to the region, local traditions and cultural heritages, thus hampering the inclusion of cooking, processing and eating habits in risk assessment and regulations. However, it is known that the nutritional value of seafood products can be strongly affected by cooking procedures (Alves et al. 2017; Maulvault et al., 2012). Similarly, chemical contaminants' concentration can drastically change according to cooking procedures and seafood species (Domingo, 2011). Therefore, human risks associated to seafood consumption may be under- or overestimated (Marques et al., 2011).

Presently, most available studies have assessed the effects of cooking on levels of some chemical contaminants in seafood [e.g. Hg (Alves et al. 2017; Maulvault et al., 2012; Perugini et al., 2013; Schmidt et al., 2015), Cd (Amiard et al., 2008; Ersoy et al., 2006; Houlbrèque et al., 2011), As (Devesa et al., 2001; Ersoy et al., 2006; Maulvault et al., 2012), PFCs (Bhavsar et al., 2014), PBDEs (Aznar-Alemany et al.,2017; Bayen et al., 2005; Hori et al., 2005), PCBs and dioxins (Bayen et al., 2005; Hori et al., 2005)], but as far as CeCs are concerned the information is still limited.

In this context, this study aims to evaluate the effect of steaming on CeCs levels (toxic elements, perfluorinated compounds (PFCs), polycyclic aromatic hydrocarbons (PAHs), musk fragrances and UV-filters) in commercially-relevant seafood species consumed in Europe and to assessing the potential risk associated to seafood consumption.

## 2. Material and Methods

### *2.1. Sampling species and culinary treatment*

Thirteen seafood species were selected based on the following assumptions: i) being frequently consumed in EU countries; and ii) previously reported as containing high levels of specific CeCs

(Cunha et al., 2018; Jacobs et al., 2015; Vandermeersch et al., 2015; Vilavert et al., 2017). The selected seafood species of commercial size consumed in Europe collected in different markets, are summarized in Table 1. The selected species include, sole (*Solea* sp.), mackerel (*Scomber scombrus*), farmed seabream (*Sparus aurata*), mussels (*Mytilus galloprovincialis* and *Mytilus edulis*), plaice (*Pleuronectes platessa*), brown crab (*Cancer pagurus*), octopus (*Octopus vulgaris*), farmed salmon (*Salmo salar*), monkfish (*Lophius piscatorius*), cod (*Gadus morhua*), tuna (*Katsuwonus pelamis*) and hake (*Merluccius australis* and *Merluccius capensis*). For fish, muscle tissue (fillets) were collected without skin, while for cephalopods and crustaceans the mantle and abdominal muscle tissues were sampled ($n$ = 25). For bivalves, the edible part with the intervalvar liquid was collected ($n$ = 50). Each sample was divided in two portions, one for culinary treatment (steaming) and another for raw seafood assessment. Steaming was performed as follows: seafood samples were wrapped up in aluminum foil, steamed in an oven (Combi-Master CM 6, Rational GroßkÜcken Technik GmbH, Germany) at 105 ºC during 15 min for fish, crustaceans and cephalopods, and at the same temperature during 5 min for bivalves. Raw and steamed samples were homogenized with a grinder (Retasch Grindomix GM200, Germany) using polypropylene cups and stainless steel knives at 10,000 *g* until complete visual disruption of the tissue, frozen at -80 ºC, freeze-dried for 48 h at -50 °C at low pressure (approximately$10^{-1}$ atm), re-homogenized and kept at -20ºC until further analysis.

### *2.2. Contaminant analysis*

#### *2.2.1. Targeted contaminants*

The target contaminants were from five different chemical groups:

i) Toxic elements: Total mercury (THg), methyl-mercury (MeHg), total arsenic (TAs), inorganic arsenic (iAs), cadmium (Cd), chromium (Cr), copper (Cu) and lead (Pb);

ii) Perfluorinated compounds (PFCs): perfluorobutanoate (PFBA), perfluoropentanoate (PFPeA), perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), perfluoroctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDcA), perfluorundecanoate (PFUnA), perfluorododecanoate (PFDoA), perfluorotridecanoate (PFTrA), perfluorotetradecanoate (PFTeA), perfluorobutane sulfonate (PFBS), perfluorohexane

sulfonate (PFHxS); perfluoroheptane sulfonate (PFHpS), perfluorooctane sulfonate (PFOS) and perfluorodecane sulfonate (PFDS);

iii) Polycyclic aromatic hydrocarbons (PAHs): acenapthylene, acenapthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(j)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, indeno(123cd)pyrene, dibenzo(ah)anthracene and benzo(ghi)perylene;

iv) Musk fragrances [6,7-dihydro-1,1,2,3,3-pentamethyl-4(5H)-indanone (DPMI), 4-acetyl-1,1-dimethyl-6-tert-butylindane (ADBI), 6-acetyl-1,1,2,3,3,5-hexamethylindane (AHMI), 5-acetyl-1,1,2,6-tetramethyl-3-isopropylindane (ATII), 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(g)-2-benzopyran) (HHCB), 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene (AHTN), 2,4,6-trinitro-1,3-dimethyl-5-tert-butylbenzene (MX), 1,1,3,3,5-pentamethyl-4,6-dinitroindane (MM) and 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(g)-2-benzopyran-1-one (HHCB-lactone);

v) UV-filters: 2-Ethylhexyl salicylate (EHS), 3,3,5-Trimethylcyclohexylsalicylate (HS); Isoamyl-4 methoxycinnamate (IMC), 3-(4-Methylbenzylidene)camphor (4-MBC), 2-Ethylhexyl 4-(dimethylamino)benzoate (EPABA); 2-Ethylhexyl 4-methoxycinnamate (EHMC), Octocrylene (OC), benzophenone 3 (BP3), benzophenone 1 (BP1), 2,2-Dihydroxy-4,4-dimethoxybenzophenone (DHMB) and Hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate DBENZO).

### *2.2.2. Toxic elements*

#### *2.2.2.1. Total and organic Mercury (THg and MeHg)*

Mercury concentrations (total and MeHg) were quantified by atomic absorption spectrometry, using an automatic Hg analyser (AMA 254, LECO, USA), according to Maulvault et al. (2015). For total Hg determination, 10-20 mg of solid sample was placed on a sample boat of the automatic analyser. After drying and combustion, samples enter in a decomposition tube, where they undergo amalgamation at 700 °C, and the dissolved elemental mercury (Hg) is pre-concentrated, released and detected at a wavelength of 254 nm. For the quantification of MeHg, 150 mg of freeze-dried samples were hydrolyzed in hydrobromic acid (10 mL, 47% w/w, Merck), followed by MeHg extraction with toluene (35 mL, 99.8% w/w, Merck) and removed from toluene using an aqueous solution of cysteine (1% L-cysteinium chloride in 12.5% anhydrous sodium

sulfate and 0.775% sodium acetate, SIGMA). Then 100 µL of liquid sample (cysteine extracts containing MeHg) is analysed in the automatic Hg analyser. THg and MeHg accuracy was evaluated with Lobster hepatopancreas reference material (TORT-2) from the National Research Council of Canada (Ontario, Canada). The obtained values for Hg (0.332 ± 0.004 mg $kg^{-1}$) and MeHg (0.140 ± 0.009 mg kg-1) were in agreement with the certified values (0.27 ± 0.06mg $kg^{-1}$ and 0.152 ± 0.013 mg $kg^{-1}$, respectively). Detection limits for this analysis can be found in Table 2.

*2.2.2.2. Inorganic Arsenic (iAs)*

Inorganic arsenic was quantified by anion exchange HPLC (High Performance Liquid Chromatography) (1260 HPLC Agilent Technologies, Waldbronn, Germany) coupled on-line to an ICP-MS, according to Rasmussen et al. (2012). Freeze-dried samples were weighed (0.2 - 0.5 g) into 15 mL polypropylene plastic tubes and 10 mL of extraction solution was added (0.06 M nitric acid, SCP Science, Courtaboeuf, France, in 3% hydrogen peroxide, Merck). Tubes were placed in a water bath (90 ± 3 °C) for 60 ± 3 min. After cooling at room temperature, tubes were centrifuged for 10 min and an aliquot of the supernatant was removed for arsenic speciation analysis. The supernatants were then filtered through 0.45 µm polytetrafluoroethylene filters in Mini-UniPrep HPLC vials (Whatman International, Maidstone, Kent, UK) prior to analysis. Aliquots of the extract (5 µL) were injected onto the HPLC–ICP-MS system. The determination of iAs followed the standard procedure (EN 16802:2016) issued by the European Committee for Standardization (CEN, 2016). Separation of AsV from other As species was obtained following Sloth et al. (2005) protocol. The iAs accuracy was evaluated by DORM-4 (Dogfish muscle) from the National Research Council of Canada (Ontario, Canada) and ERM-BC211 (rice) from the Institute of Reference Materials and Measurements, (Geel, Belgium). ERM-BC211 is certified for iAs (0.124 ± 0.011 mg $kg^{-1}$), whereas DORM-4 is only certified for total As, and not for inorganic arsenic, but a target value for iAs has recently been established in a collaborative trial at 0.270 ± 0.040 mg $kg^{-1}$ (Sloth, 2015) and the value obtained in this study (0.277 mg $kg^{-1}$) was in agreement with the collaborative trial results. Detection limits for this analysis can be found in Table 2.

*2.2.2.3. Total Arsenic (TAs), Cadmium (Cd), chromium (Cr), copper (Cu) and lead (Pb)*

Five elements were determined by inductively coupled plasma mass spectrometer (ICP-MS) (Agilent 8800 ICP-QQQ-MS, Santa Clara, USA). Subsamples of homogenized freeze-dried seafood (0.2 - 0.5 g) were digested in closed vessels in a microwave oven (Multiwave 3000, Anton Paar, Graz, Austria) with 4 mL nitric acid (68% w/w) and 2 mL MilliQ water. The digests were diluted to a volume of 20 mL and sample aliquots were further diluted 10 times with acids to obtain ~2% $HNO_3$ and 1% HCl (c/v) aqueous solutions. ICP-MS equipped with a micromist concentric quartz nebulizer and a Scott type double-pass water-cooled spray chamber was run in no gas ($^{111}$Cd, $^{202}$Hg, $^{206}$Pb), helium ($^{55}$Mn, $^{59}$Co, $^{65}$Cu, $^{66}$Zn) and oxygen ($^{56->72}$Fe, $^{52->68}$Cr, $^{75->91}$As, $^{78->94}$Se) modes, respectively, with 0.2 s integration time per mass. Typical plasma conditions were 1550 W RF power, 15 L min$^{-1}$ plasma gas, 1.05 L min$^{-1}$ carrier gas and 0 L min$^{-1}$ makeup gas. Cell gas flows were 5 mL min$^{-1}$ for helium and 30% oxygen with stabilization times of 30 s, 10 s and 30 s for helium, no gas, and oxygen modes, respectively. Instrument parameters were optimized by autotune in the MassHunter software (Agilent, Santa Clara, USA). Internal standards (ISTD; $^{115}$In and $^{209}$Bi) were added online (5 µg L$^{-1}$) via a t-piece using the peristaltic pump. Blank samples were analysed in the same conditions as samples and were subtracted to all results. Analytical accuracy was assessed through the analysis of the CRM Dogfish muscle (DORM-4). The values obtained in this study for As (6.9 mg kg$^{-1}$), Cd (0.310 mg kg$^{-1}$), Cr (2.10 mg kg$^{-1}$), Cu (16.4 mg kg$^{-1}$) and Pb (0.328 mg kg$^{-1}$) were in agreement with certified values (6.8 ± 0.64 mg kg$^{-1}$, 0.306 ± 0.015 mg kg$^{-1}$, 1.87 ± 0.16 mg kg$^{-1}$, 15.9 ± 0.9 mg kg$^{-1}$ and 0.416 ± 0.053 mg kg$^{-1}$, respectively). The detection limits for these analyses can be found in Table 2.

*2.2.3. Perfluorinated compounds (PFCs)*

PFCs were analysed according to the method described by Kwadijk et al. (2010). As internal standard, 50 ng $^{13}C_4$-PFOS and $^{13}C_4$-PFOA in 350 µL acetonitrile were added to 2 g of sample in a 15 mL polypropylene tube. Eight mL of acetonitrile (HPLC grade, Promochem) were added to the sample, shaken for 30 min. and subsequently centrifuged for 10 min. at 3,220 *g*. Supernatants were transferred to 50 mL polypropylene tubes and the extraction was repeated twice. Extracts were dried using sodium sulphate and concentrated to 10 mL using a TurboVap. Afterwards, 10 mL of hexane (picograde, Promochem) was added and samples were vigorously shaken for 5 min., centrifuged for 5 min. at 3,220 *g*, and the hexane layer was

removed. This procedure was repeated twice and extracts were concentrated to 700 µL. Samples were transferred to polypropylene eppendorfs, where 50 mg of ENVIcarb (Supelco) were added. Samples were vortexed for 1 min., and centrifuged for 5 min. at 7,270 *g*. Extracts were then transferred to a vial and stored at 4 °C until analysis by liquid-chromatography-ion trap tandem mass spectrometry (LC-IT-MS/MS Thermo Finnigan, Waltham, United States). The accuracy of the method was confirmed by an internal reference sample (pike perch, Wageningen Marine Research) in each series of samples. Results for the internal reference sample were all satisfactory (< 2s). Calibration curves ranged from 0.5 – 500 ng $mL^{-1}$, with an $R^2 \geq 0.995$ for all compounds. The methods intra-day and inter-day repeatability, expressed as relative standard deviation (RSD%), was typically <20% for all analytes. The detection limits for this analysis can be found in Table 2.

*2.2.4. Polycyclic aromatic hydrocarbons (PAHs)*

Sample preparation for PAH analysis followed the methodology described by De Witte (2014). Samples were extracted by accelerated solvent extraction (Dionex, ASE350). Cells of 22 mL were filled with dried sample, 2.5 g of florisil (Merck, 0.150–0.250 mm) and diatomaceous earth (Sigma Aldrich, Celite 545) and a mixture containing acenaphthene $d_{10}$, anthracene $d_{10}$, pyrene $d_{10}$, benzo(a)anthracene $d_{12}$, benzo(a)pyrene $d_{12}$ and indeno(123cd)pyrene $d_{12}$ in iso-octane was added as recovery standards. Cells were then extracted at 100 °C with a mixture of hexane (Merck, Suprasolv, P98.0%) and acetone (Biosolve, Pesti-S,P99.9%) (3:1). For the extraction, 3 cycles of 5 min static time each were programmed. The extract was evaporated to 1 mL by a Turbovap II evaporator (Zymark) and eluted with 15 mL hexane on a glass column filled with 2 g of aluminum oxide (Merck, Aluminium oxide 90 active basic), deactivated with 10% of type 1 water. A second evaporation step to 1 mL was performed, followed by the extract elution with 10 mL of hexane on a glass column filled with 1 g of silicon oxide (Merck, Silica gel 60). After evaporation and reconstitution to 0.5 mL of iso-octane (Merck, Lichrosolv, P99.0%), samples were transferred to vials for analysis by gas chromatography-mass spectrometry (Agilent 7890A GC with an Agilent 5975C MS-detector) with chrysene $d_{12}$ in toluene added to the vial as injection standard. The detection limits for this analysis can be found in Table 2.

*2.2.5. Musk fragrances*

The analytical method used is described in detail by Trabalón et al. (2015), and was based on QuEChERS (Quick, Easy, Effective, Rugged and Safe) extraction, followed by gas chromatography-ion trap-tandem mass spectrometry determination (GC–IT-MS/MS, Varian, Walnut Creek, CA, USA), equipped with a 3800 gas chromatograph, a 4000 ion trap mass detector, a 1079 programmable vaporising temperature injector and a CombiPal autosampler (CTCAnalytics, Zwigen, Switzerland). Homogenized freeze-dried samples were weighted (0.5 g) and mixed in 10 mL of ultrapure water and 10 mL of acetonitrile. Then according to the Standard Method EN15662, an extraction salt packet (Scharlab) was added and centrifuged. The acetonitrile layer (supernatant) was removed and transferred to a 15 mL centrifuge tube containing 2 g of florisil (Sigma-Aldrich) for the dSPE (dispersive solid-phase extraction) clean-up. Tubes containing each sample were centrifuged and the supernatant was evaporated under a gentle stream of nitrogen to a final volume of approximately 1 mL. The internal standard (d15-MX) was added and the extract was reconstituted to 2 mL with ethylacetate (GC grade purity >99.9%, Prolabo). Extracts were filtered with a 0.22 mm PTFE syringe filter and analysed by GC–IT-MS/MS. Matrix matched calibration curves were performed for the quantification by spiking hake, salmon and mussel samples at different levels, and good linearity was achieved ($R^2$ > 0.98). The detection limits were calculated as three times the signal-to-noise ratio (Table 2). Intra-day and inter-day repeatability were expressed as relative standard deviation (RSD%) ($n$ = 5, 50 ng $g^{-1}$), being lower than 21% for all analytes.

*2.2.6. UV-filters*

Individual standard solutions of UV-filters were prepared in methanol (HPLC grade from Sigma-Aldrich) at concentrations of 2 mg $mL^{-1}$, accordingly to Cunha et al. (2017). Briefly, 2 g of freeze-dried sample were added to 100 µL of BPd10 (IS, 2 mg $L^{-1}$) into a 40 mL amber glass vial tube. Then, 7 mL of deionized water and 10 mL of MeCN were added, vortexed, and placed on a wrist action shaker for 10 min. Four g of anhydrous $MgSO_4$ and 1 g of NaCl were added, shake vigorously by hand for 5 min. and centrifuged at 4,736 *g* for 3 min. MeCN extract were transferred (3 mL) to a 20 mL vial tube, diluted with 7 mL of deionized water and then 4 mL of hexane:tertbutylmethylether (3:1 v/v) was added. Afterwards, it was gently shaken by hand for 30 s and centrifuged at 4,736 *g* for 1 min. to remove the organic phase, and then 4 mL of hexane:benzene (3:1 v/v) was added. For fish samples, the organic phases were combined and

evaporated to dryness using a gentle nitrogen stream at room temperature. In contrast for mussel samples the organic phases were combined with 200 mg of Z-Sep+, vortexed during 1 min., centrifuged at 4,736 *g* for 3 min., and the top layer was evaporated to dryness using a gentle nitrogen stream at room temperature. Finally, analytes were silylated, 50 µL of BSTFA were added, derivatized during 5 min. in a household microwave (600 W) and injected (1 µL of the extract) in the GC-MS system. The GC-MS/MS equipment consisted of an Agilent 7890B chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with 7693 autosampler (Agilent Tecnologies) and coupled to a triple quadrupole mass spectrometer Agilent 7000C MS (Agilent Technologies). GC separation was performed in a DB-5MS capillary column (30 m x 0.25 mm I.D., 0.25 µm film thickness; J & W, USA), following Cunha et al. (2017).Mass Hunter Quantitative Analysis software (v. B.02.03) (Agilent Technologies) was used for data processing. Matrix matched calibration curves were performed for quantification by spiking blank extracted mackerel sample at different levels, and good linearity was achieved ($R^2$ > 0.996). The detection limits were calculated as three times the signal-to-noise ratio (Table 2). Intra-day and inter-day repeatability were expressed as relative standard deviation (RSD %) (*n* = 6, 25 ng $g^{-1}$), being lower than 20% for all analytes.

2.4 *Consumers health risk assessment*

Consumers' risks associated with the ingestion of 150 g of cooked seafood were evaluated based on: i) Tolerable weekly intake (TWI) (THg and MeHg, EFSA, 2012; Cd, EFSA, 2011; PFOS, EFSA, 2008b), ii) Tolerable daily intake (TDI) (Cr, EFSA 2014a), iii) Tolerable Upper Intake Level (UL) (Cu, EFSA, 2015), iv) Benchmark Dose Lower Limit ($BMDL_{10}$) for BaP (benzo(a)pyrene), PAH2 (sum of benzo(a)pyrene, chrysene), PAH4 (sum of benzo(a)pyrene, chrysene, benz(a)anthracene, benzo(b)fluoranthene) and PAH8 (sum of benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene, dibenzo(a,h)anthracene, indeno(123cd)pyrene, benzo(ghi)perylene), EFSA, 2008a]; and v) Benchmark Dose Lower Limit ($BMDL_{01}$) for iAs (EFSA, 2014b) and Pb (EFSA, 2010). Margins of exposure (MOE) were calculated for $BMDL_{10}$ by dividing this value with the estimates of dietary exposure. A MOE of 10,000 or higher is typically considered of low concern for genotoxic carcinogenic compounds like PAHs (EFSA, 2005). Based on the available NOAEL (No

Observed Adverse Effect Level) values for (PFDoA, Kato et al., 2015), AHTN (ECHA, 2008a), HHCB (ECHA, 2018b), and EHS (ECHA, 2017a), TDI and TWI, were calculated by dividing NOAEL values by a safety/uncertainty factor (UF) of 100 (accounting for species differences and human variability) (Renwick, 2002).

### 2.5. *Statistical analysis*

Data were analysed for normality and variance homoscedasticity using Kolmogorov–Smirnov and Levene's tests, respectively. The t-test student for dependent samples was performed to test significant differences between contaminants levels in raw and steamed seafood, for each compound and seafood species. Whenever data (or transformed data) did not met the normality and variance homoscedasticity assumptions, non−parametric Mann–Whitney U test was used. Furthermore, differences between species were also analysed by One-way ANOVA, followed by Tukey's post-hoc test for pair wise multiple comparisons. When ANOVA assumptions were not met, Kruskal–Wallis test was performed, followed by non-parametric multiple comparison test. Statistical analysis was performed at a significance level of 0.05, using the STATISTICA™ software (Version 7.0, StatSoft Inc., Tulsa, Oklahoma, USA).

## 3. Results

#### *3.2.1. Toxic elements*

From the nine species analysed for THg and MeHg, significantly higher levels ($p$ <0.05) were found in steamed samples for *Solea* sp.*, O. vulgaris, S. scombrus, L. piscatorius, P. platessa* and *K. pelamis* (Fig. 1). Yet, in *M. capensis*, despite THg levels significantly increased (23%) after steaming, MeHg levels significantly decreased (18%). The highest THg and MeHg increase (%) in steamed samples were observed in *O. vulgaris* (47% and 38%, respectively), followed by *L. piscatorius* (30% and 32%, respectively). Significant differences in THg levels were also found between species in steamed samples ($p$ < 0.05) according to the following order: *Solea sp. < P. platessa = S.aurata < S. scombrus < K. pelamis < L. piscatorius = M. capensis = M. austalis < O. vulgaris.* On the other hand, MeHg levels were significantly different ($p$ < 0.05) between species after steaming according to the following order: *Solea* sp. < *S.aurata*

= *S. scombrus* = *P. platessa* < *M. capensis* = *K. pelamis* < *L. piscatorius* = *M. austalis* < *O. vulgaris* (Fig. 1).

Concerning other elements, significant differences ($p$ < 0.05) between raw and steamed samples were found in *M. galloprovincialis* (TAs, iAs, Cu, Cd, Cr and Pb), *M. edulis* (TAs, iAs, Cu, Cr and Pb) and *C. pagurus* (Cd) (Fig. 1). On the one hand, steaming resulted in a strong increase (%) of the following elements: iAs (88% in *M. edulis* and 50% in *M. galloprovincialis*), Cr (69% in *M. galloprovincialis*) and Pb (60% in *M. galloprovincialis*). On the other hand, Cr levels decreased (28%) in steamed samples of *M. edulis*. Significant differences ($p$ <0.05) in TAs, iAs, Cu, Cd, Cr and Pb levels were observed between species in steamed samples accordingly to the following order: *M. edulis* < *M. galloprovincialis* < *C. pagurus*, in TAs and Cd; *M. galloprovincialis* < *M. edulis* < *C. pagurus*, in iAs and Cu; *M. galloprovincialis* < *M. edulis* in Cr; and *M. edulis* < *M. galloprovincialis* in Pb (Fig. 1).

*3.2.2. Perfluorinated compounds (PFCs)*

Out of all analysed PFCs, only 5 compounds were detected in raw and steamed samples of *K. pelamis* and *P. platessa*, i.e. PFUnA, PFDoA, PFTrA, PFTeA and PFOS (Fig. 2). On the other hand, PFBA and PFDcA, which were not detected (< LOD) in raw samples, were detected in steamed samples of *M. edulis* and *K. pelamis*, respectively (Fig. 2). Furthermore, PFDcA, which was detected in raw samples of *M. edulis*, was not detected after steaming (< LOD) (Fig. 2). Steaming resulted in significant increase ($p$ <0.05) of PFTrA, PFBA and PFDcA levels, as well as a significant decrease ($p$ <0.05) of PFUnA, PFDoA, PFOS and PFDcA levels (Fig. 2). The highest decrease (%)was observed for PFDcA (>100% in *M. edulis*) followed by PFUnA (68%) and PFOS (53%). On the contrary, highest decreases (%)were observed for PFBA and PFDcA (>100%; *M. edulis* and *K. pelamis*, respectively), followed by PFTrA (50%). PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFBS, PFHxS, PFHpS, PFDS were not detected (< LOD) in the analysed species (i.e. *P. platessa, M. australis, M. capensis, K. pelamis* and *M. edulis*). Significant differences ($p$ <0.05) in PFOS levels were observed between species (i.e. *P. platessa* < *K. pelamis*), as well as in PFDcA (i.e. *M. edulis* < *K. pelamis*), after steaming (Fig. 2).

*3.2.3. Polycyclic aromatic hydrocarbons (PAHs)*

Out of all analysed PAHs, 14 compounds were detected in raw and steamed *M. galloprovincialis*, *M. edulis* and *C. pagurus* (Fig. 3). Acenapthylene (*M. galloprovincialis* and *M. edulis*) and fluoranthene (*C. pagurus*), which were detected in raw samples, were not detected (< LOD) after steaming (Fig. 3). Conversely, benzo(a)pyrene and dibenzo(ah)anthracene were not detected in raw *M. edulis*, but steamed samples revealed quantifiable levels of these compounds (Fig. 3). Steaming resulted in significant increase ($p$ <0.05) of chrysene, fluoranthene, benzo(a)pyrene, benzo(e)pyrene, benzo(a)anthracene, benzo(ghi)perylene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, dibenzo(ah)anthracene and indeno(123cd)pyrene levels and in statistical decrease ($p$ <0.05) of fluorine levels (Fig. 3). Steaming also resulted in significant increased or decreased ($p$ <0.05) levels of phenanthrene and pyrene according to species (Fig. 3). The highest increase (%) after steaming was observed for benzo(a)pyrene (> 100%; *M. edulis*) and dibenzo(ah)anthracene (>100% and 77%; *M. edulis* and *M. galloprovincialis*, respectively), followed by benzo(e)pyrene, benzo(a)anthracene and benzo(j)fluoranthene (75%, 74% and 73%, respectively in *M. edulis*) (Fig. 3). On the other hand, the highest decrease of ratio levels was observed in acenapthylene (>100%; *M. edulis* and *M. galloprovincialis*) and fluoranthene (>100%; *C. pagurus*), followed by fluorene (52%; *M. galloprovincialis*) and pyrene (32%; *M. edulis*). Furthermore, fluorene, phenanthrene, chrysene, fluoranthene, pyrene, benzo(a)pyrene, benzo(e)pyrene, benzo(a)anthracene, benzo(ghi)perylene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, dibenzo(ah)anthracene and indeno(123cd)pyrene levels in steamed samples were significantly different ($p$ <0.05) between species accordingly to the following order: *M. galloprovincialis* < *M. edulis* (fluorene); *C. pagurus* < *M. edulis* < *M. galloprovincialis* (phenanthrene, chrysene, fluoranthene, benzo(a)fluoranthene, benzo(j)fluoranthene); and *M. edulis* < *M. galloprovincialis* (pyrene, benzo(a)pyrene, benzo(e)pyrene, benzo(a)anthracene, benzo(ghi)perylene, benzo(k)fluoranthene, dibenzo(ah)anthracene, indeno(123cd)pyrene) (Fig. 3).

*3.2.4. Musk fragrances*

Among musk fragrances, only 3 compounds revealed detectable levels (> LOD) in raw and steamed samples of *Solea* sp., *P. platessa*, *C. pagurus*, *S. scombrus* and *M. galloprovincialis*,

i.e. HHCB, HHCB-Lactone and AHTN (Fig. 4). Moreover, AHTN, DPMI and HHCB-Lactone levels, which were not detected (< LOD) in raw samples of *M. galloprovincialis* and *Solea* sp., were quantified after steaming (Fig. 4). Conversely, DPMI levels were detected in raw samples of *Solea sp*. and *M. edulis*, but not detected (< LOD) after steaming (Fig. 4). Steaming resulted in significantly increased ($p$ <0.05) levels of HHCB (*Solea* sp., *C. pagurus* and *M. galloprovincialis*), HHCB-Lactone (*S. scombrus*) and AHTN (*Solea* sp., *P. platessa* and *S. scombrus*), but significantly decreased ($p$ <0.05) HHCB (*S. scombrus*) and AHTN (*C. pagurus*) levels (Fig. 4). Yet, the highest increase (%) was observed for DPMI (>100%; *M. galloprovincialis*), HHCB-lactone (>100%; *Solea* sp), AHTN (>100% and 75%; *M. galloprovincialis* and *Solea* sp., respectively) and HHCB (87% and 60%; *M. galloprovincialis* and *Solea* sp., respectively) after steaming. On the other hand, the highest decrease 8%) was registered for DPMI (>100%) in steamed samples of *Solea* sp. and *M. edulis,* followed by HHCB and AHTN in steamed samples of *S. scombrus* (37%) and *C. pagurus* (21%), respectively (Fig. 4). Musk fragrances levels in steamed samples were significantly different ($p$ <0.05) between species (i.e. HHCB: *P. platessa* < *M. galloprovincialis* < *Solea* sp. < *S. scombrus* < *C. pagurus*; HHCB-lactone: *Solea* sp. < *S. scombrus*; DPMI *Solea* sp. = *M. edulis* < *M. galloprovincialis*; AHTN: *M. galloprovincialis* < *P. platessa* < *S. scombrus* = *Solea* sp. < *C. pagurus*) (Fig. 4).

*3.2.5. UV-filters*

Within UV-filters, only EHS, HS and DHMB presented detectable levels in raw and steamed samples of *S. scombrus, M. galloprovincialis* and *L. piscatorius*, respectively (Fig. 5). Yet, EHS (i.e. *S. aurata, S. salar* and *G. morhua*), HS (i.e. *S. aurata* and *S. salar*), DHMB (i.e. *S. aurata*), OC (i.e. *S. aurata, G. morhua* and *L. piscatorius*) and BP1 (i.e. *S. aurata* and *M. galloprovincialis*) were quantified in raw samples, but not detected after steaming (< LOD) (Fig. 5). The opposite trend occurred for EHS (*L. piscatorius*), HS (*S. scombrus and L. piscatorius*), 4-MBC (*M. edulis*) and DBENZO (*S. scombrus*) (Fig. 5). Steaming resulted in significantly increased ($p$ <0.05) levels of EHS (>100% and 55%) and HS (>100%) in *L. piscatorius* and *S. scombrus*, as well as 4-MBC (>100%) in *M. edulis* and DBENZO (>100%) in *S. scombrus*. Significantly decreased ($p$ <0.05) levels were detected for EHS (>100%; *S. aurata, S. salar* and *G. morhua*), HS (>100%; *S. aurata, S. salar* and 62%; *M. galloprovincialis*), DHMB (>100%; *S.*

*aurata* and 36%; *L. piscatorius*), OC (>100%; *S. aurata; G. morhua* and *L. piscatorius*) and BP1 (>100%; *S. aurata* and *M. galloprovincialis*) (Fig. 5). Also, EHS, HS and DHMB levels in steamed samples were significant different ($p$ <0.05) between species by the following order: *S. aurata = S. salar = G. morhua < S. scombrus < L. piscatorius* (EHS); *S. aurata = S. salar < L. piscatorius = M. galloprovincialis < S. scombrus* (HS) and *S. aurata < L. piscatorius* (DHMB) (Fig. 5).

### *3.3. Consumers health risk assessment*

Based on the available health-based guidance values (HBGVs), the exposure to contaminants through the consumption of 150 g seafood day$^{-1}$ varied according to species and compound (Table 3). In general, human exposure to CeCs increased with the consumption of 150 g of seafood after steaming. Consumption of *O. vulgaris*, especially after steaming, increased human exposure to MeHg, representing 60% of the tolerable weekly intake (TWI) for adults and exceeding TWI for children (i.e. 8 years old). In case of children, higher exposure to MeHg increased with the consumption of steamed *L. piscatorius* and *M. australis* (66% TWI), as well as *M. capensis* and *K. pelamis* (51% TWI). Also, the consumption of 150 g of steamed *C. pagurus* brown meat, provided remarkably higher intakes of Cu (62% UL) for both adults and children, and Cd exposure increased with the consumption of steamed *C. pagurus* brown meat, reaching intakes of 66% in adults TWI and exceeding children Cd TWI. The consumption of *M. galloprovincilis* after steaming, increased consumer exposure to Pb, exceeding Pb $BMDL_{01}$ in both adults and children. In contrast, intake of *M. edulis* exceeded $BMDL_{01}$ values of Pb (in raw and steamed samples) and iAs (in steamed samples) only for children. Regarding PAHs, the consumption of steamed *M. galloprovincialis* enabled higher exposure to carcinogenic PAHs, where MOE were exceeded for all PAHs in children and in PAH4 and PAH8 for adults. Concerning, the other CeCs (PFCs, Musk fragrances and UV-filters), exposure through the consumption of 150 g of seafood did not increase with the culinary treatment (steaming), with intakes being below 1% of health-based guidance values (HBGVs).

## 4. Discussion

In recent years, there has been a growing research interest to assess the effects of cooking procedures on seafood contamination levels. Yet, still limited information is available in what concerns CeCs. This study reveals that the concentration of most CeCs generally increases after steaming. However, data also point out that the changes induced by cooking practices depend on the type of compound and on the seafood species. Increased levels of toxic elements after cooking were previously associated with the loss of water, volatilization and degradation of lipids, carbohydrates and proteins, resulting in weight loss and consequently in increased concentration of contaminants (Ganbi, 2010; Maulvault et al., 2012). Another potential explanation for such trend is the higher affinity of some toxic elements to tissue proteins, forming stable complexes that do not easily leach out by simple cooking processes, such as steaming and boiling (Schmidt et al., 2015). In line with the present study, increases in total Hg concentrations were also observed for a diversity of cooking processes in several species (Ganbi, 2010; Kalogeropoulos et al., 2012; Maulvault et al., 2012; Perugini et al., 2013; Torres-Escribano et al., 2011). For instance, increases in Hg levels were observed in boiled fillets of *Epinephelus areolatus* (Ganbi, 2010), grilled *Xiphias gladius*, *Galeorhinus galeus*, *Sarda* sp. and *Thunnus* sp. (Torres-Escribano et al., 2011), grilled and fried *Aphanopus carbo* (Maulvault et al., 2012), pan-fried and grilled *Sardina pilchardus* and *M. merluccius* (Kalogeropoulos et al., 2012), and boiled *Nephrops norvegicus* (Perugini et al., 2013). The iAs increase in cooked samples may be explained by the conversion of organic As species into iAs during the cooking process (Devesa et al., 2001). Increases in As and iAs levels were also reported in bivalves after steaming (Devesa et al., 2001), in sardine, hake and tuna after frying, grilling, roasting and boiling (Perelló et al., 2008) and in *A. carbo* after grilling and frying (Maulvault et al., 2012). Concerning Pb, increased levels were also reported in fried sardine, hake and tuna, as well as in grilled, roasted and boiled hake (Perelló et al., 2008) and in grilled and pan-fried *S. pilchardus* (Kalogeropoulos et al., 2012). Increases in Cu levels were registered in boiled *E. areolatus* (Ganbi, 2010), in pan-fried *S. pilchardus* and *M. galloprovincialis* and in grilled and pan-fried *M. merluccius* (Kalogeropoulos et al., 2012). Increases in Cd levels were observed in boiled *Mytilus chilensis* (Houlbrèque et al., 2011), in pan-fried *M. merluccius*, *S. pilchardus* and *M. galloprovincialis* and in grilled *S. pilchardus* (Kalogeropoulos et al., 2012). At last, increases in Cr levels were recorded in pan-fried *M.*

*merluccius*, *S. pilchardus* and *M. galloprovincialis*, and in grilled *M. merluccius* (Kalogeropoulos et al., 2012).

On the other hand, decreases in element content were also reported in our study (e.g. MeHg in steamed *M. capensis* and Cr in steamed *M. edulis*), and can possibly be associated with solubilisation or volatilization, drip loss and degradation of the complex Hg-proteins by protein denaturation and/or hydrolysis (Devesa et al., 2001; Ganbi, 2010; Houlbrèque et al., 2011). Decreases in Hg and MeHg were previously reported by Perreló et al. (2008) in grilled sardine and fried and roasted hake, as well as by Schmidt et al. (2015) in roasted and fried *Thunnus albacares*, *Arapaima gigas* and *Brotula barbata*. Higher losses of MeHg can occur with changes in Hg-cysteine complexes, once MeHg predominantly binds to proteins (Schmidt et al., 2015). Moreover, decreases in Cr levels were also reported in fried, boiled and roasted *E. areolatus* (Ganbi, 2010) and in grilled *M. merluccius* (Kalogeropoulos et al., 2012). Contrastingly, decreases in As levels were previously reported in fried *Dicentrarchus labrax* (Ersoy et al., 2006), in Pb levels of baked *D. labrax* (Ersoy et al., 2006), in Cd and Pb levels of fried and grilled tuna (Perelló et al., 2008), in Pb, Cu and Cd levels of fried, boiled and roasted *E. areolatus* (Ganbi, 2010) and in Cd levels of grilled *M. merluccius* (Kalogeropoulos et al., 2012). Although, carnivorous fish species usually have higher MeHg levels, in this study *O. vulgaris* revealed the highest MeHg levels, likely reflecting the feeding habits of this species that are based on small pelagic fish species, thus being subjected to the bioaccumulation and biomagnification patterns of mercury along the food chain (Maulvault et al., 2015). Within the other toxic elements, iAs predominates in seawater and sediments, where filter-feeding organisms', such as mussels, accumulate higher iAs levels (Vandermeersch et al., 2015). On the other hand, brown crab meat (particularly the hepatopancreas) presents high levels of Cd and Cu, reflecting the Cd detoxifying role of this organ in crustaceans (Barrento et al. 2009). The high levels of Cu in brown crab hepatopancreas are likely associated with the accumulation of this element required for the respiratory pigment haemocyanin of crustacean's haemolymph (Barrento et al. 2009).

As for the other CeCs, limited studies assessed the effect of cooking on contamination levels in seafood. The decreased PFCs levels registered in the current study (i.e. PFUnA, PFDoA, PFDcA and PFOS) were in line with previous studies undertaken in other seafood species and

with other culinary treatments. Indeed, Del Gobbo et al. (2008) observed decreases in PFOA, PFNA, PFDA, PFUA, PFDoA, PFTeA and PFOS levels in several seafood species (cuttlefish, sea squirt, grouper, red snapper, catfish, monkfish, yellow croaker, grey mullet, whitting, skate and octopus) after baking, boiling or frying. Also, PFUnA, PFDoA, PFTrA, PFHxS and PFOS levels decreased in common carp after boiling and frying (Bhavsar et al., 2014). Like toxic elements, PFCs have higher affinity for tissue proteins and, therefore, losses are likely due to leaching into the cooking media caused by the disruption of PFCs aggregation to proteins (Del Gobbo et al., 2008). Nonetheless, increases in PFCs (i.e. PFDA, PFUnA, PFDoA, PFBS and PFOS) levels were also reported in several fried and grilled seafood species (*M. galloprovincialis*, *Parapenaeus longirostris*, *Loligo vulgaris*, *Spicara smaris*, *Atherina boyeri*, *S. pilchardus*, *Engraulis encrasicolus* and *Boops boops*; Vassiliadou et al. 2015), as well as PFOS levels in baked, boiled and fried chinook salmon, lake trout and walleye (Bhavsar et al., 2014). Such increase of PFCs levels in cooked seafood could be related with mass loss through evaporation during the cooking procedures (Vassíliadou et al., 2015). As far as PAHs are concerned, it is important to highlight the general increase in levels of eight PAH compounds considered as carcinogenic for humans (benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, dibenzo[a,h]anthracene, indeno[1,2,3-c,d]pyrene and benzo[ghi]perylene) in the current study. It is known, that PAHs occur as a result of the incomplete combustion or pyrolysis of organic materials and their presence in seafood are mainly associated with atmospheric contamination, industrial food processing and even with home cooking practices, especially grilling/barbecuing, roasting and smoking (EFSA, 2008). Moreover, PAHs are lipophilic, have low aqueous solubility, and are mainly accumulated in lipid tissues, thus higher levels are generally found in seafood with higher fat content (Storelli et al., 2003). Increases of PAHs (i.e. fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene and phenanthrene), levels after cooking have also been reported for fried sardine, fried, grilled, boiled and roasted hake and fried and grilled tuna (Perelló et al., 2009). In contrast, decreases in PAHs (i.e. phenanthrene, fluoranthene, benzo(a)anthracene, crysene and pyrene) were previously reported in grilled and fried sardine, grilled, fried and boiled hake, and in grilled tuna (Perelló et al., 2009). Like most neutral organic contaminants, decreases may be due to moisture loss or evaporation during the cooking procedure (Domingo, 2011). It is also

worth mentioning that low molecular weight (LMW, 2–3 aromatic rings) PAHs levels, such as acenaphtylene and fluorine, readily evaporate, thus likely explaining the decreased levels after steaming (Albers, 2003). Additionally, PAHs levels in aquatic organisms depend on the ability of these organisms to metabolize them. While fish have a well-developed detoxification system, bivalve mollusks, like mussels, are not able to efficiently metabolize PAHs and, therefore, can accumulate high molecular weight (HMW) PAHs (Zelinkova and Wenzl, 2015). Within personal care products (PCPs), there is a raising concern about the potential toxicological effects of musk fragrances and UV-filters for consumers. In the current study, steaming increased most musk fragrances concentration (e.g. HHCB-lactone), whereas the opposite trend was observed for UV-filters (e.g. DHMB, OC, BP1). Despite the presence of UV-filters and musk fragrances has been previously reported in seafood (Cunha et al., 2015; Trabalón et al., 2015), limited information concerning the effect of cooking is currently available. Like other lipophilic compounds (e.g. PAHs and PCBs), changes in musk fragrances and UV-filters contents after cooking could be due to chemical changes promoted by heat exposure during steaming (Alves et al., 2017). Within compounds, differences may be explained by their physico-chemical properties (e.g. water solubility, vapor pressure and polarity). Also, isomerization of UV-filters can occur and both isomers and enantiomers (optical isomers) may differ in biological behavior during the cooking procedure (Gago-Ferrero et al., 2010). Increases and decreases in musk fragrances and UV-filters, may also be the result of reconversion after thermal treatment to parent compounds (McEneff et al., 2013) or into metabolites, e.g. degradation of HHCB into HHCB-Lactone (Cunha et al., 2015).

Organic contaminants with higher log $K_{ow}$ (n-octanol/water partition coefficient), such as PAHs ($K_{ow}$ = 3.94 – 6.68; ECHA, 2009), UV-filters ($K_{ow}$ = 3.93 – 6.16; Kotnik et al., 2014; Rodil et al. 2009) and musk fragrances ($K_{ow}$ = 4.0 – 5.9; ECHA, 2008a, 2008b) are hydrophobic and lipophilic, thus being associated with fatty tissues. In this context, cooking processes promoting the reduction of fat are likely to decrease the levels of these contaminants (Domingo, 2011). Conversely, toxic elements and PFCs are generally associated with protein tissues, therefore, being less affected by mild cooking procedures, such as steaming (Bhavsar et al., 2014). Yet, in our study, the results for both toxic elements and PFCs, as well as for organic contaminants, do not seem to follow this trend. This could be due to the distinct characteristics of the analysed

seafood species and contaminants (Bhavsar et al., 2014). Another reason for the differences observed from previous studies may be due to the different cooking procedures assessed. Also, chemicals with very high log $K_{ow}$ values (i.e. > 4.5) may potentially bioconcentrate in living organisms, thus explaining the differences in contaminants concentration among species (ECHA, 2017b). Previous studies demonstrated that steaming not only reduce the moisture content, but also increase the relative ratio of protein and polar lipid fractions (Bhavsar et al., 2014; Castro-González et al., 2014), which can explain the increase of most CeCs after steaming.

In terms of risk assessment of consumers' exposure to CeCs in steamed seafood, the current results reveal that steaming generally increased contaminants levels, resulting in a higher risk of contaminant exposure to seafood consumers, especially when the observed levels were close to toxicity levels or toxicological safety thresholds. Currently, TWI, TDI, UL and $BMDL_{01}$ are established for most toxic elements. Despite the general increase observed in toxic elements levels during cooking procedures, in the present study they were overall below the toxicological safety thresholds established by EFSA. Yet, increased exposure to MeHg was registered through the consumption of steamed *O. vulgaris*, as well as to iAs levels in steamed *M. edulis*, and Cu and Cd levels in *C. pagurus* brown meat, which may represent a risk for European consumers, particularly children. Moreover, potential adverse effects of Pb, including developmental neurotoxicity in children and nephrotoxicity in adults (EFSA, 2010), through the consumption of steamed mussels cannot be excluded, once the estimated dietary intakes exceeds the $BMDL_{01}$ intake values for both adults (*M. galloprovincialis*) and children (*M. galloprovincialis* and *M. edulis*). So far, EFSA (2008a) has also set maximum levels for one carcinogenic PAH individually (BaP) and for the combination of carcinogenic PAHs (PAH2, PAH4 and PAH8). The general increase in PAHs levels in steamed *M. galloprovincialis*, resulted in MOEs below 10,000 for both adults (i.e. PAH4 and PAH8) and children (i.e. BaP, PAH2, PAH4, PAH8), which indicates the possibility that a carcinogenetic effect cannot be excluded on some consumers (EFSA, 2008a). It should be emphasized that despite in general, cooking procedures tend to increase contaminants concentration in seafood, contaminants' bioaccessibility generally decreases contaminant levels likely absorbed, thus reducing risks to consumers (Alves et al., 2017; Amiard et al., 2008). To sum up, the general increase of CeCs

levels in seafood after steaming may exacerbate health risks for adults and children. Indeed, the consumption of steamed octopus, brown crab and mussels lead to a higher human exposure to toxic elements (i.e. MeHg, iAs, Cu, Cd and Pb) and carcinogenic PAHs (i.e. BaP, PAH2, PAH4, PAH8), for which reference values are available.

## 5. Conclusions

The present study provides new insights into the effect of steaming on seafood CeCs levels, highlighting the importance to undertake further research on human exposure to these contaminants through seafood consumption, including the effect of cooking processes. To the authors' knowledge, the effect of cooking is assessed for the first time integrating a range of CeCs and the potential risks associated with seafood consumption. Results clearly indicate that steaming can indeed affect the levels of most CeCs in seafood products, though strongly varying according to the chemical properties of each contaminant, seafood species and cooking procedure. Steaming resulted in significant increases of most toxic elements, PAHs and musk fragrances, as well as significant decreases in most PFCs and UV-filters. Considering the scarcity of data related with the cooking effect on CeCs levels, these results also evidence the overall increased levels of musk fragrances and decreased levels of UV-filters after steaming. Based on the currently available recommendations set for some toxic elements and PAHs, the increase of contaminant levels in seafood after steaming indicates that adverse health effects can never be excluded, regardless of contaminant. Given the fact that seafood is mainly consumed after cooking, it is strongly recommended to include a heating step (or heating factor) in monitoring and risk assessment studies. Moreover, to enhance seafood consumers' confidence, further studies should be undertaken covering a diversity of CeCs from distinct chemical groups, integrating the most consumed seafood species and the different regional culinary habits (e.g. frying, grilling, roasting and boiling), as well as contaminants bioaccessibility and bioavailability. Such information will allow to have more realistic data concerning CeCs levels in seafood for consumers exposure assessment, enabling food safety authorities to adjust the HBGVs of contaminants in seafood products, and to provide more reliable recommendations (taking into account both risks and benefits) associated with seafood consumption.

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**Table 1.** Seafood species used to assess the effect of culinary processing in contaminants of emerging concern (CeCs) levels.

| Species | Origin | Market country | N | Total length (mm) | Weight (g) | Moisture (%) | | Contaminants analysed (raw vs cooked) |
|---|---|---|---|---|---|---|---|---|
| | | | | | | raw | cooked | |
| *Gadus morhua* | North Sea | Denmark | 25 | 780 - 870 | 4500 - 6000 | 81.0 | 75.7 | UV-filters |
| *Katsuwonus pelamis* | Azores | Portugal | 25 | n.a. | 235 - 139[a] | 67.6 | 56.2 | Hg, MeHg; PFCs |
| *Lophius piscatorius* | Atlantic Ocean | Portugal | 25 | 570 - 590 | 3365 - 3448 | 82.4 | 77.2 | Hg, MeHg; UV-filters |
| *Merluccius australis* | South America | Portugal | 25 | n.a. | 2500 - 3500 | 74.7 | 67.1 | Hg, MeHg; PFCs |
| *Merluccius capensis* | South Africa | Portugal | 25 | n.a. | 2400 - 3000 | 78.9 | 75.0 | Hg, MeHg; PFCs |
| *Pleuronectes platessa* | Channel | Belgium | 25 | 330 - 370 | 332 - 555 | 78.2 | 71.4 | Hg, MeHg; Musk fragrances; PFCs |
| *Salmo salar* | Farmed (DanSalmon) | Denmark | 25 | 520 - 560 | 1480 - 1678 | 59.3 | 63.1 | UV-filters |
| *Sparus aurata* | Farmed | Italy | 25 | 260 - 310 | 381 - 526 | 72.4 | 70.1 | Hg, MeHg; UV-filters |
| *Scomber scombrus* | Atlantic Ocean | Spain | 25 | 250 – 320 | | 70.2 | 65.0 | Hg, MeHg; UV-filters |
| | Goro | Italy | 25 | 189 – 285 | 48 – 269 | 75.2 | 72.5 | UV-filter (EHS); Musk fragrances |
| *Solea sp.* | Goro | Italy | 25 | 215 - 250 | 97 - 159 | 77.8 | 72.4 | Hg, MeHg; Musk fragrances |
| *Octopus vulgaris* | Mediterranean | Spain | 25 | 350 - 440 | | 80.1 | 72.7 | Hg, MeHg |
| *Cancer pagurus* | North Sea | The Netherlands | 25 | 153 - 205 | 546 - 1440 | 60.5 | 59.2 | toxic elements; UV-filters; Musk fragrances; PAHs |
| *Mytilus edulis* | North Sea | The Netherlands | 50 | 44 - 68 | 5.9 - 18.5[b] | 79.2 | 77.0 | iAs, As; Musk fragrances |
| | France | France | 50 | 31 - 50 | 2.6 - 9.9[b] | 75.3 | 70.2 | Hg, MeHg, Cd, Cu, Cr, Pb; UV-filters; Musk fragrances; PAHs; PFCs |
| *Mytilus galloprovincialis* | Goro | Italy | 50 | 42 - 62 | 6.0 - 19.9 | 82.1 | 76.6 | Musk fragrances |
| | Farmed (Atlantic Ocean) | Spain | 50 | 49 - 74 | 2 - 11[b] | 85.3 | 80.7 | As, iAs, Cd, Cu, Cr, Pb; UV-filters; PAHs |

total length (mm) and total weight (g), range minimum and maximum; moisture, average values; N, number of specimens; n.a, data not available; [a] slice weight; [b] flesh weight; PFCs, perfluorinated compounds; PAHs, polycyclic aromatic hydrocarbons

**Table 2.** Contaminant limit of detection (LOD, µg $kg^{-1}$ w.w.) and limit of quantification (LOQ, µg $kg^{-1}$ w.w.) of the CeCs analysed for steamed samples

| | LOD (µg $kg^{-1}$ w.w.) | LOQ (µg $kg^{-1}$ w.w.) |
|---|---|---|
| **Elements** | | |
| Hg & MeHg | 0.5 – 2 | 1 – 4 |
| As & iAs | <0.002 | <0.006 |
| Cd | 0.03 | 0.10 |
| Cu | 0.04 | 0.12 |
| Cr | 0.07 | 0.21 |
| Pb | 0.04 | 0.12 |
| **PFCs** | <0.01 | <0.04 |
| **PAHs** | 0.01 – 0.23 | 0.15 – 0.47 |
| **UV-filters** | 0.30 – 1.52 | 1 – 5 |
| **Musks*** | 0.30 – 3.00 (0.40 – 4.00) | 2.00 – 11.00 (2.00 – 12.00) |

*Musk fragrances values for fish matrix and in parentheses for mussels' matrix

**Table 3.** Percentage of the health-based guidance values (HBGVs) established for CeCs, considering the consumption of a portion size of 150 g of seafood.

| | *Solea sp.* | | *Sparus aurata* | | *Octopus vulgaris* | | *Scomber scombrus* | | *Lophius piscatorius* | | *Pleuronectes platessa* | | *Merluccius australis* | | *Merluccius capensis* | | *Katsuwonus pelamis* | | *Cancer pagurus* | | *Mytilus galloprovincialis* | | *Mytilus edulis* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | raw | cooked | raw | cooked | raw | cooked | raw | cooked | raw | cooked | raw | cooked | raw | cooked | raw | cooked | raw | cooked | raw | cooked | raw | cooked | raw | cooked |
| **Toxic elements** | | | | | | | | | | | | | | | | | | | | | | | | |
| Hg | 2 (5) | 3 (6) | 7 (13) | 7 (13) | 20 (40) | 30 (59) | 8 (16) | 10 (20) | 11 (22) | 15 (29) | 5 (10) | 7 (13) | 15 (31) | 15 (31) | 13 (25) | 16 (31) | 10 (20) | 12 (24) | - | - | - | - | - | - |
| MeHg | 4 (9) | 5 (10) | 14 (28) | 14 (28) | 44 (88) | 60 (>TWI) | 13 (26) | 16 (33) | 25 (50) | 33 (66) | 11 (22) | 14 (28) | 36 (71) | 33 (66) | 31 (62) | 25 (51) | 21 (41) | 26 (51) | - | - | - | - | - | - |
| iAs | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 20 (39) | 20 (41) | 15 (29) | 22 (44) | 40 (80) | 75 (>$BMDL_{01}$) |
| Cu | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 67 | 62 | 3 | 4 | 4 | 5 |
| Cd | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 61 (>TWI) | 66 (>TWI) | 14 (28) | 17 (33) | 7 (13) | 7 (15) |
| Cr | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.1 (0.2) | 0.2 (0.4) | 0.5 (1.1) | 0.4 (0.8) |
| Pb | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 65 (>$BMDL_{01}$) | >$BMDL_{01}$ | 60 (>$BMDL_{01}$) | 67 (>$BMDL_{01}$) |
| **PFCs** | | | | | | | | | | | | | | | | | | | | | | | | |
| PFDoA | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.1 (0.2) | 0.1 (0.2) | - | - | - | - | - | - |
| PFOS | - | - | - | - | - | - | - | - | - | - | 0.1 (0.1) | 0.1 (0.1) | - | - | - | - | 0.1 (0.3) | 0.1 (0.1) | - | - | - | - | - | - |
| **PAHs** | | | | | | | | | | | | | | | | | | | | | | | | |
| BaP | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 37 (73) | 58 (>MOE) | - | - |
| PAH2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 2 (5) | 3 (5) | 71 (>MOE) | 99 (>MOE) | 6 (12) | 8 (16) |
| PAH4 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 2 (3) | 2 (4) | 81 (>MOE) | >MOE | 8 (16) | 13 (26) |
| PAH8 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 79 (>MOE) | >MOE | 9 (18) | 13 (27) |
| **Musks** | | | | | | | | | | | | | | | | | | | | | | | | |
| HHCB | 0.0 (0.0) | 0.0 (0.0) | - | - | - | - | 0.0 (0.0) | 0.0 (0.0) | - | - | 0.0 (0.0) | 0.0 (0.0) | - | - | - | - | - | - | 0.0 (0.0) | 0.0 (0.0) | - | - | - | - |
| AHTN | 0.0 (0.0) | 0.0 (0.0) | - | - | - | - | 0.0 (0.0) | 0.0 (0.0) | - | - | 0.0 (0.0) | 0.0 (0.0) | - | - | - | - | - | - | 0.0 (0.0) | 0.0 (0.0) | - | 0.0 (0.0) | - | - |
| **UV-filters** | | | | | | | | | | | | | | | | | | | | | | | | |
| EHS | - | - | 0.0 (0.0) | - | - | - | 0.0 (0.0) | 0.0 (0.0) | - | 0.0 (0.0) | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

Percentages were calculated according to the HBGVs set and considering an adult average body weight (bw) of 75 kg and in parenthesis an 8 years old children of 35 Kg. Tolerable weekly intake (TWI), Benchmark Lower Limit (BMDL), Tolerable Upper Intake Level (UL), Tolerable Daily Intake (TDI). Toxic elements: Hg (TWI) = 4 $\mu g.kg^{-1}$ of individual bw, MeHg (TWI) = 1.3 $\mu g.kg^{-1}$ of individual bw, iAs ($BMDL_{01}$) = 0.3 $\mu g.kg^{-1}$ of individual bw, Cu (UL) = 5$mg.day^{-1}$, Cd (TWI) = 2.5 $\mu g.kg^{-1}$ of individual bw, Cr (TDI) = 300 $\mu g.kg^{-1}$ of individual bw and Pb ($BMDL_{10}$) = 0.63 $\mu g.kg^{-1}$ of individual bw for adults (chronic kidney disease) and Pb ($BMDL_{01}$) = 0.5 $\mu g.kg^{-1}$ of individual bw for children (developmental neurotoxicity). PFCs: PFDoA (TWI) = 7 $\mu g.kg^{-1}$ bw, PFOS (TWI) = 1.05 $\mu g.kg^{-1}$ bw. PAHs: BaP ($BMDL_{10}$) = 0.07 $mg.kg^{-1}$ bw, PAH2 ($BMDL_{10}$) = 0.17 $mg.kg^{-1}$ bw, PAH4 ($BMDL_{10}$) = 0.34 $mg.kg^{-1}$ bw, PAH8 ($BMDL_{10}$) = 0.49 $mg.kg^{-1}$ bw. Musks: HHCB (TWI) = 3500 $\mu g.kg^{-1}$ bw, AHTN (TWI) = 350 $\mu g.kg^{-1}$ bw. UV-filters: EHS (TWI) = 1750 $\mu g.kg^{-1}$ bw. BaP: benzo[a]pyrene; PAH2: benzo[a]pyrene, chrysene; PAH4: benzo[a]pyrene, chrysene, benz[a]anthracene, benzo[b]fluoranthene; PAH8: benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, dibenzo[a,h]anthracene, indeno[1,2,3-c,d]pyrene, benzo[ghi]perylene. MOE (margin of exposure) was calculated by dividing the $BMDL_{10}$ by the mean estimated dietary intake levels. >MOE indicates that the calculated MOE means increased human exposure to contaminants

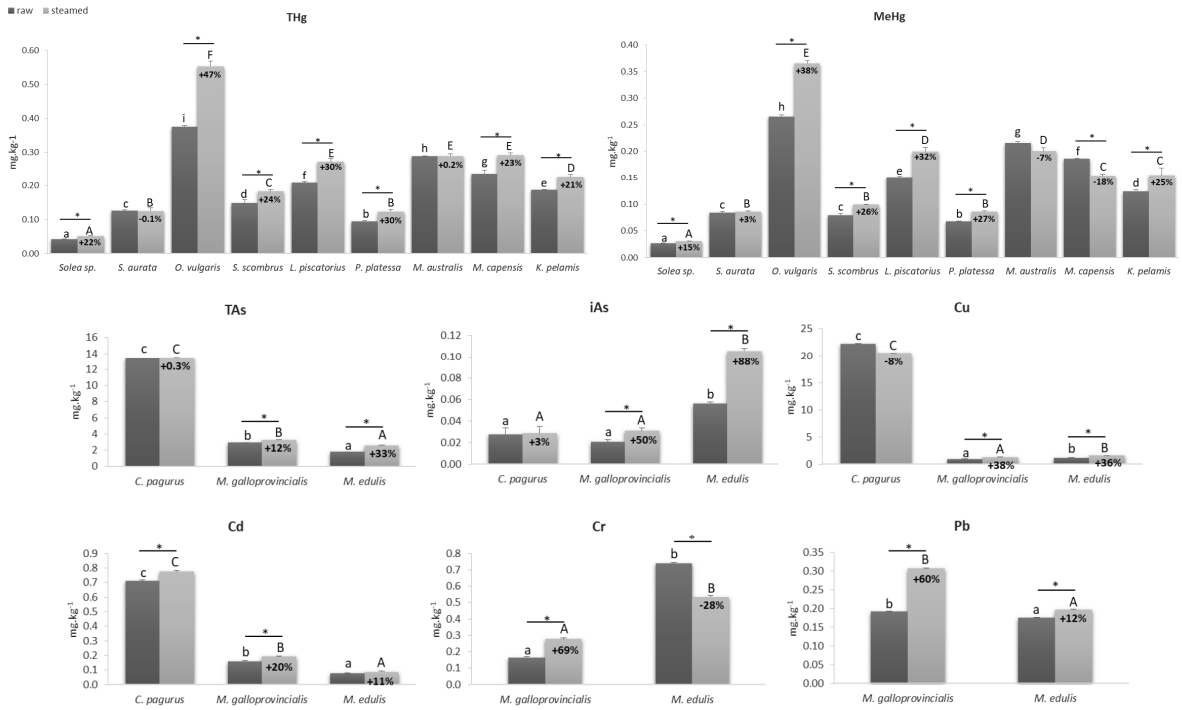
Fig. 1. Toxic elements content content (mg $kg^{-1}$ wet weight) obtained in raw and steamed seafood samples. THg (Total mercury); MeHg (Methyl mercury); TAs (Total arsenic); iAs (Inorganic arsenic); Cu (Copper); Cd (Cadmium); Cr (Chromium); Pb (Lead), and percentages of element content increase (+) and decrease (-) upon steaming. Results are expressed as mean ± standard deviation. Asterisk indicates significant differences ($p < 0.05$) between raw and steamed samples. Different letters (capital letters for steamed; small letters for raw) represent significant differences of element contents between species ($p < 0.05$).

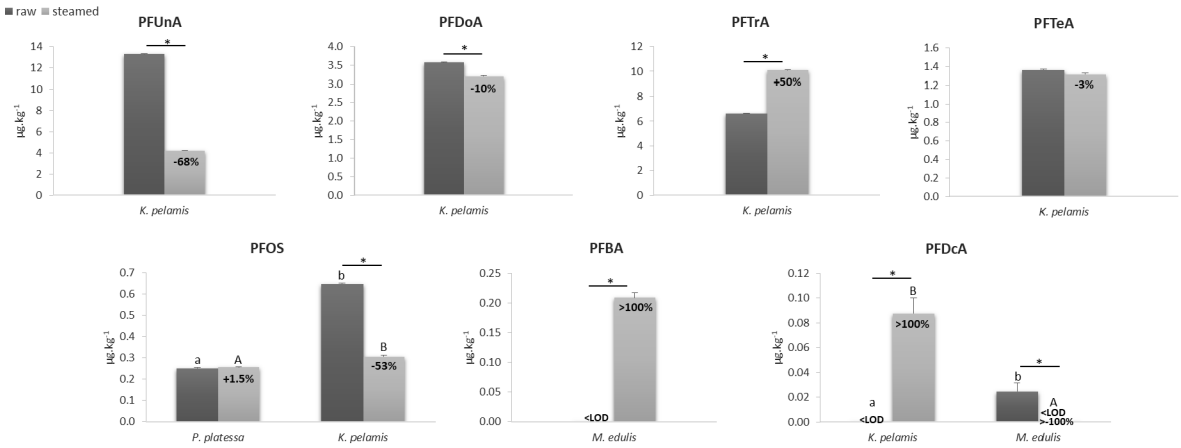
Fig. 2. Perfluorinated compounds (PFCs) content (µg/kg wet weight) obtained in raw and steamed seafood samples. PFUnA (Perfluorundecanoate); PFDoA (Perfluorododecanoate); PFTrA (Perfluorotridecanoate); PFTeA (Perfluorotetradecanoate), PFOS (Perfluorooctane sulfonate) PFBA (Perfluorobutanoate); PFDcA (Perfluorodecanoate), and percentages of PFCs content increase (+) and decrease (-) upon steaming. Results are expressed as mean ± standard deviation. Asterisk indicates significant differences ($p < 0.05$) between raw and steamed samples. Different letters (capital letters for steamed; small letters for raw) represent significant differences of PFCs contents between species ($p < 0.05$).

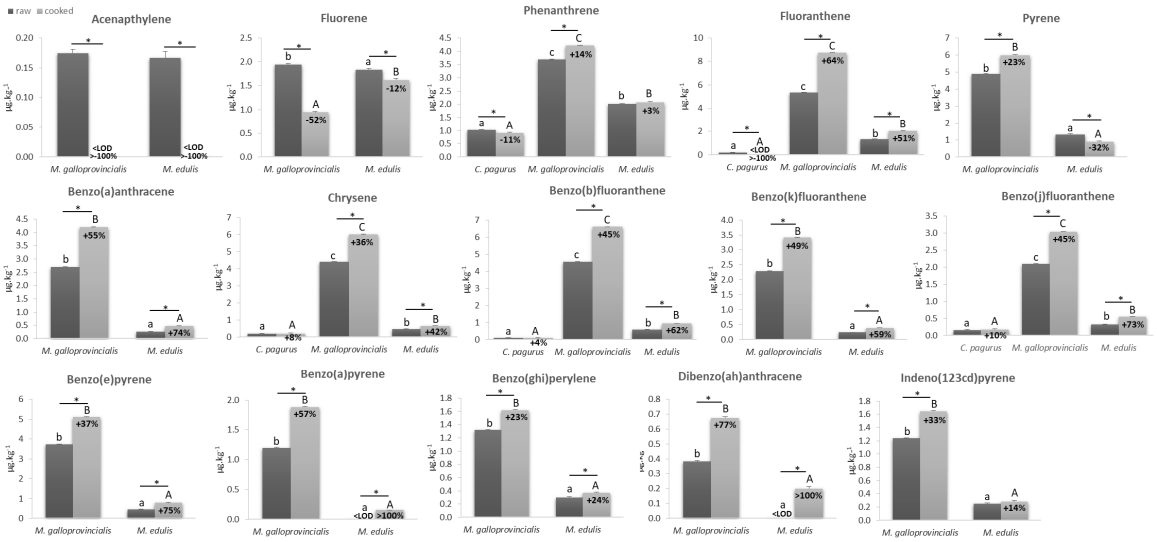
Fig. 3. Polycyclic aromatic hydrocarbons (PAH) content (µg/kg wet weight) obtained in raw and steamed seafood samples and percentages of PAHs content increase (+) and decrease (-) upon steaming. Results are expressed as mean ± standard deviation. Asterisk indicates significant differences ($p < 0.05$) between raw and steamed samples. Different letters (capital letters for steamed; small letters for raw) represent significant differences of PAHs contents between species ($p < 0.05$).

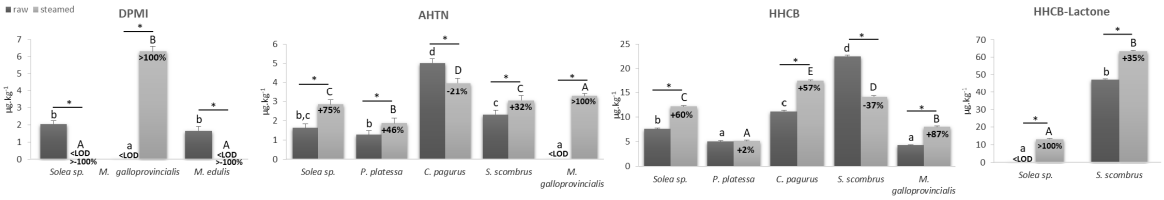
Fig. 4. Musk fragrances content (µg/kg wet weight) obtained in raw and steamed seafood samples. DPMI (6,7-dihydro-1,1,2,3,3-pentamethyl-4(5H)-indanone); AHTN (7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene); HCCB (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(g)-2-benzopyran); HHCB-lactone (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(g)-2-benzopyran-1-one), and percentages of musk fragrances content increase (+) and decrease (-) upon steaming. Results are expressed as mean ± standard deviation. Asterisk indicates significant differences ($p < 0.05$) between raw and steamed samples. Different letters (capital letters for steamed; small letters for raw) represent significant differences of musk fragrances contents between species ($p < 0.05$).

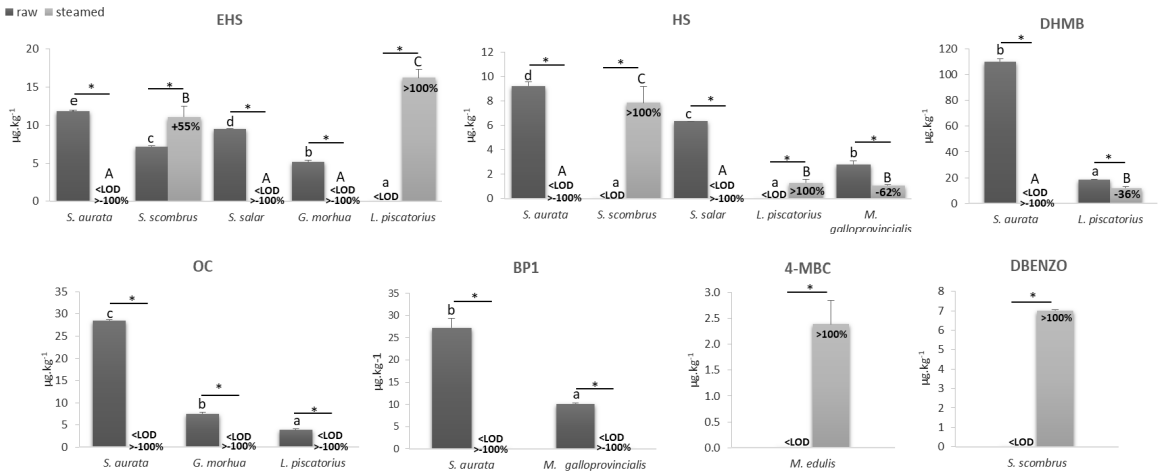
Fig. 5. UV-filters content (µg/kg wet weight) obtained in raw and steamed seafood samples. EHS (2-Ethylhexyl salicylate); HS (3,3,5-Trimethylcyclohexylsalicylate); DHMB (2,2-Dihydroxy-4,4-dimethoxybenzophenone); OC (Octocrylene); BP1 (Benzophenone 1); 4-MBC (3-(4-Methylbenzylidene)camphor); DBENZO (Hexyl 2-[4-(diethylamino)-2-hydroxybenzoyl]benzoate), and percentages of UV-filters content increase (+) and decrease (-) upon steaming. Results are expressed as mean ± standard deviation. Asterisk indicates significant differences ($p < 0.05$) between raw and steamed samples. Different letters (capital letters for steamed; small letters for raw) represent significant differences of UV-filters contents between species ($p < 0.05$).











**Highlights:**

- CeCs levels strongly varied according to the contaminant and seafood species
- Most toxic elements, PAHs and musk fragrances levels increased after steaming
- Most PFCs and UV-filters levels decreased after steaming
- Adverse health effects can never be excluded, regardless of contaminants concentration