Chemical and Microbiological Contamination in Limpet (Patella spp.) of the Portuguese Coast

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Abstract

Coastal production areas can be impacted by anthropogenic contamination from urban, agro-industrial and leisure activities. Some contaminants, such as chemical substances might also have a telluric origin. Non filter feeding univalve mollusks, such as limpets, which are collected in rocky shores either for sale or for auto-consumption, are very appreciated in Portugal, but have been excluded from provisions on the classification of production areas, although can present relevant contamination.

Thus, the aim of this study was to assess the microbiological and toxic metal contaminations in limpets (*Patella* spp) of the Portuguese coast, taking into account the production area and seasonal variation, and comparing their contamination levels with those occurring in bivalve mollusk indicator species, mussel (*Mytilus edulis*). The risks associated to the consumption of limpet meals were also assessed. For that, microbial total and fecal levels and cadmium, lead and mercury contents in limpets and mussels samples from three coastal areas over several months were analyzed based on standard methodologies. Contents of mercury and lead in limpets from the three areas studied, were always below the limits of 0.50 mg kg^{-1} and 1.5 mg kg^{-1} allowed by the EU, respectively. Regarding cadmium, levels in limpet were always above the limit of 1.0 mg kg^{-1}, reaching about 3.0 mg kg^{-1} in some samples. These values probably indicate contamination from telluric origin (soil or rocks) in the coastal studied areas. Results indicated that microbiological contamination of fecal origin was low and in general below the detection level. Contamination levels did not show a clear seasonal pattern. The two mollusk species, limpets and mussels, differed statistically in all contaminants analyzed, being cadmium the most of concern, and always higher in limpets than in mussel samples. Thus, the potential risk associated with limpet consumption, taking into account the cadmium tolerable weekly intake (TWI), was investigated, being possible to reach a reliable recommendation of less than a monthly meal of 160 g. As recreational picking of limpets is common in Portugal, official recommendations of maximum periodic human consumption should be published and enforcement increased in forbidden areas.

Keywords: limpets, mussels, mercury, lead, cadmium, E. coli, risk assessment

1. Introduction

Mollusks, together with finfish and crustaceans, are among the most internationally traded food commodities. The first are very appreciated worldwide due to their special organoleptic characteristics and nutritional value. As a consequence of the global demand, in 2017, the world annual production reported for mollusks of 23 355 249 tons accounted for 6.4% and 21.7% of total capture and aquaculture productions, respectively (FAO, 2019).

In Portugal, per capita consumption of seafood is one of the highest of European Union (EU) countries, being estimated in 57.1 kg/(person.year) (FAO, 2019). At national level, 128 438 tons of seafood were landed in Portugal in 2018, with mollusks representing about 15% of the total landings (INE, 2019).

The Portuguese coastline, with more than 900 km long, has plentiful rocky shores (Ferreira et al., 2008), which harbor several bivalve and univalve mollusks, being limpets (*Patella* spp.) one of the most abundant mollusks. Limpets have been exploited by humans since the Paleolithic period and used as food and bait in several parts of the world, including Mexico, United States of America, Australia, South Africa, Chile and Macaronesia (Henriques et al., 2017). In Portugal, the consumption of marine gastropods, represented mostly by univalve mollusks, limpets included, is part of the gastronomic cultural heritage of several coastal regions, appreciated by the local population and tourists, and presents a relevant social and economic importance (Sousa et al., 2019). Living in the intertidal zone, limpets are easy to locate during low tide and, with specific tools, are removed by professional and recreational pickers and used for human consumption. In some Portuguese regions, such as Archipelago of Madeira, its exploitation represents a highly profitable commercial activity (Sousa et al., 2019). Unlike bivalves, which are filter-feeders and concentrate particles in suspension, including contaminants (Anacleto et al., 2013), these univalve mollusks, are grazers and feed, by scraping the rocky substrate with the radula, on microbial biofilms which are primarily composed of cyanobacteria and microalgae, spores, and other propagules of macro algae and invertebrates.

Regardless its nutritional and organoleptic properties, the consumption of univalve and bivalve mollusks is not risk-free and can be implicated in human infections or intoxications. As most coastal waters are exposed to telluric and anthropogenic contamination, and considering that is impossible to determine whether these live animals are fit for human consumption without testing, it is very important to control them at the origin, monitoring their production areas. In EU member states, monitoring programs are in place in order to assess the hygiene and quality of bivalve production areas and classify them. This classification is mainly based on the levels of bacterial indicators related to fecal contamination, such as *Escherichia coli* detected in the flesh of live bivalves, marine gastropods, echinoderms and tunicates (EC, 2004a, EC, 2004b). As, marine gastropods are generally not filter feeder animals, the risk of accumulating microorganisms related to fecal contamination may be considered remote. In addition, no epidemiological information has been reported to link the provisions for classification of production areas with risks for public health associated with marine gastropods which are not filter feeders. As a consequence, such marine gastropods, have been excluded from provisions on the classification of production areas (EU, 2010).

In addition, several polluting substances can be accumulated in the edible parts of these marine invertebrates. These substances include persistent organic pollutants (POP), and inorganic chemical contaminants (Viñas et al., 2018; Reguera et al., 2018).The sources of these pollutants in the aquatic environment may be of anthropogenic origin, namely domestic and industrial sewage and those originating from agricultural activities, or of natural origin, such as those resulting from the processes of soil erosion and products expelled in volcanic eruptions. The dangers resulting from the presence of these compounds in the aquatic environment implies not only their persistence and toxicity but also a considerable degree of concentration in the food chain, which constitutes a risk factor for human health. Thus, some elements as mercury (Hg), cadmium (Cd) and lead (Pb), are considered toxic elements that can be assimilated, stored and concentrated by living organisms, through the food chain, causing physiological effects that are sometimes serious. Its concentration in aquatic organisms is influenced by geographical and environmental factors, but also by age, biological cycle, sexual maturation state, migratory behavior, food, among others. For this reason, the level of contaminants in seafood may raise public health concerns, and international organizations such as EFSA (EFSA, 2012a, 2012b, 2012c) established maximum tolerable levels for human consumption. Furthermore, European Union (EU) has also set maximum levels for certain contaminants, in foodstuffs, including seafood (EC, 2006). In particular, high levels of contaminants present in certain foodstuffs may pose specific risks associated to the consumption of meals prepared with contaminated ingredients.

The quantification of these risks is a major issue and innovative mathematical models based on a full probabilistic approach better represent the complexity of the underlying reality. In particular, the application of the Extreme Value Theory (EVT) has been proposed as a solution to tackle this challenge (Tressou et al., 2004; Ventura et al., 2018). Such mathematical approach relies on the modeling of the possible intake distributions taking into account known consumption frequencies or plausible scenarios. The probability of surpassing the corresponding contaminant threshold is used as a way to assess the risk level. Precisely, in the case of Cd, it is possible to use such an approach, being its tolerable weekly intake, TWI, 2.5 µg/(kg b.w. × week), as established by EFSA's Panel on Contaminants in the Food Chain, CONTAM (CONTAM, 2011). This risk quantification provides a more accurate basis to issue advice concerning consumption frequencies.

On the other hand, few authors have studied the chemical and microbial contamination of limpets and addressed the risk of its human consumption. In this context, the aim of this work was to evaluate the microbial total and fecal levels and the contamination by potentially toxic metals detected in limpets (*Patella* spp), from three rocky coastal areas of the Portuguese mainland, and to compared the contamination levels with those detected in the bivalve indicator species, mussel (*Mytillus edulis*). The risks associated to the consumption of limpet meals were also assessed.

2. Material and Methods

2.1 Material

The study was carried out between February 2019 and July 2019, with periodic collection of univalve and bivalve mollusks from representative sampling points located in three Portuguese coastal production areas (PA), namely L1 - North, L5 - Center and L7 – South (Fig 1). A total of 30 samples were collected, comprising 15 samples of limpets and 15 of mussels, each with about 30 individuals. Each sample was divided into two units, one intended to microbiological and another to chemical testing, and stored in tagged food grade plastic bags. All samples were transported to the laboratory in isothermal boxes, between 0°C and 10 °C.

The unit intended to chemical testing was also used to measure total shell length, in order to assure that all individuals were complying with the minimum legal size of 2 cm and 5 cm, established for limpets and mussels, respectively (DGRM, 2019). After this measurement, soft tissues were removed and homogenized in a food blender. Samples were vacuum sealed in individual plastic bags, coded for easy identification and stored at −21ºC until use for chemical analysis.

A representative test sample of 10 live individuals, weighing at least 50 g, was prepared for microbiological analysis (ISO 6887-3, 2017). Mollusks were thoroughly washed using potable running water and a brush, to remove any material adhering to the shells, and dried with absorbent paper. Subsequently, the flesh and intervalvar liquid, when applicable, were aseptically extracted into a sterile container using sterile scalpels and forceps.

2.2 Methods

Total mercury (Hg) was analyzed by atomic absorption spectrometry using an automatic Hg analyzer (LECO apparatus AMA 254, USA) based on the standard EPA methodology (EPA, 2007). Cadmium (Cd) and lead (Pb) were determined by graphite furnace atomic absorption spectrometry using the methodology indicated on NP EN 14084 (2003) on a Spectr AA-240Z Agilent spectrometer. These methodologies were well described in Afonso et al. (2013).

All laboratory ware was cleaned with 200g L^{-1} of HNO_3 during 24 hours and rinsed with ultrapure water (18.2Mµ cm) and chemicals used were of reagent grade. Quantification for all elements were made by the external calibration method using commercial standard solutions (1 g L^{-1}) for Hg, Cd and Pb (Merck, Darmstadt, Germany). Detection limits were calculated from the residual standard deviation of the response and the slope of the calibration curve for each element. The values obtained were 0,004 (Hg), 0,002 (Cd) and 0.02 (Pb) $mgkg^{-1}$ wet weight.

All analyses were performed at least in duplicate and analytical data for elements was reported as mg kg^{-1} of mollusk on a wet weight basis. Certified reference material DORM-4 (fish protein from National Research Council of Canada) was tested under the same conditions as the samples in order to assess the accuracy of the analytical methods. The results obtained in this study were in good agreement with the certified values (Table 1).

Total viable counts (TVC) were assessed in duplicate by surface plating on marine agar (Difco) and incubation at 30˚C for 72 hours. Results were expressed as colony formation unit (CFU) per 100g of mollusk.

The enumeration of beta-glucuronidase-positive *E. coli* was carried out in duplicate using the most probable number (MPN) technique in a five-tube, three-dilution format (ISO 16649-3, 2015). Briefly, an initial suspension of 1 in 10 in Maximum Recovery Diluent (MRD; Oxoid) was aseptically prepared, and homogenized during 60 s in a Stomacher 400 (Seward Laboratory System). Subsequent serial decimal dilutions were performed in MRD. The resuscitation step was performed by inoculating minerals modified glutamate broth (MMGB, Oxoid Ltd., Basingstoke, Hampshire, UK) with diluted homogenates and incubation at 37±1˚C for 24±2 hours. The presence of *E. coli* was confirmed by sub-culturing acid producing tubes into agar containing 5-bromo-4-chloro-3-indolyl-glucuronidase activity (TBX, Oxoid), which was incubated at 44±1˚C for 21±3 hours. Presence of blue to blue-green colonies in TBX plates was considered as positive result. The number of positive tubes at each dilution was used to look up the corresponding MPN value and results were expressed as MPN per 100g of mollusk. When appropriate, microbiological results were $\log_{10}$ transformed.

2.3 Statistical Analysis

In this work, the STATISTICA 7 software (Stat-sof, Inc. USA, 2004) was used for data processing. In order to verify the normality and the homogeneity of variance data the Kolmogorov-Smirnov´s test and Levene´s test were used, respectively. In order to determine the difference of the various contaminants during seasons and sampling location, a one-way analysis of variance was performed (one-way ANOVA). The level of significance (p) was set at 0.05 for all analyses.

2.4 Risk assessment

Risk assessment was done using the software @ RISK® —advanced risk analysis for spreadsheets, from Palisade Corporation (Ithaca, NY, USA), version 4.5, 2005. A semi-probabilistic approach was applied. This combines variability in the concentration data with fixed consumption scenarios (ranging from one meal every six months to one weekly meal for limpet samples from the three studied areas of the Portuguese Coast, North, Center, and South). Probability distribution fitting, intake level calculation and risk assessment determination were performed according to Cardoso *et al.* (2010). In particular, best probability distributions were chosen according to χ^2 tests for goodness of fit. The risks were quantified through the probability of exceeding the Cd tolerable weekly intake, TWI (2.5 µg/(kg b.w. × week)). There are two alternatives for estimating the risk: the plug-in (PI) and the tail estimation (TE) based estimators. Both were used, the PI estimator for large probabilities and the TE estimator for the other situations (especially, for very low probabilities). The statistical methodology based on the EVT was applied to the Cd data of the studied limpet groups as described by Ventura et al. (2018). For all the calculations, a meal of 160 g and an average human body weight of 60 kg (FAO/WHO, 2003) were used.

3. Results and Discussion

3.1 Metal and microbiological contamination

Results of Hg, Cd and Pb content for limpets, according to the production area and the season, are shown in table 2.

All samples throughout the study showed Cd mean values above the legal limit (1.0 mg kg^{-1}). Regarding the studied production areas, the area with the highest value range was L5 – Center production area, with values ranging between 1.4 and 2.8 mg kg^{-1}, thus representing the area most contaminated by this metal. The levels obtained in this area for winter and spring seasons were statistically different, both of which did not differ statistically from summer. In L1 - North production area, the range of levels obtained was the lowest, between 1.2 and 1.8 mg kg^{-1}. Nevertheless, the different seasons differed statistically from each other. The same did not occur in L7 - South production area, in which the levels obtained from the different seasons did not differ statistically from each other. The results presented here agree with those described by Cravo et al. (2004) and Cravo & Bebiano (2005) on limpets, *Patella aspera*, collected from a marina and an estuary in the Algarve, Portugal. Those limpets also presented high levels of Cd, ranging between 3.47 mg g^{-1} and 9.19 mg g^{-1} (dw) in the marina area and between 1.03 mg g^{-1} and 2.55 mg g^{-1} (dw) in the estuary area. These Cd high levels can be associated to the coastal rocks since they are predominantly schists, relatively enriched in Cd (Cravo et al., 2004). A study performed by Turkmen et al. (2005) in *P. caeruela* also showed high levels of Cd, between 2.4 and 5 mg kg^{-1} (dw). However, Bergasa et al. (2007) obtained lower levels, than the present study, in Canarian Islands, Spain, demonstrating that these islands area are weakly influenced by anthropogenic activities.

Due to the high levels of Cd contamination, harvesting of limpets has been temporarily forbidden in some of the Portuguese coastal production areas. However, the recreational picking in the areas has not been refrained.

With regard to Hg, some of the results obtained are below the limit of quantification (LQ = 0.011 mg kg^{-1}) (for example in the summer in the three production areas). Mercury quantifiable results were much lower than the legal limit (0.50 mg kg^{-1}) (EU, 2006). These levels are similar to those obtained by other authors (Connan & Tack, 2010; Shefer et al., 2015; Pérez et al., 2019), in different species of limpets captured in North Cotentin (France), Israeli Mediterranean coast and Coruña (Spain).

Concerning Pb (Table 2), all obtained levels were below the legal limit (1.5 mg kg^{-1}) (EU, 2006) for these species. Limpets from L5 - Center production area were the ones with significantly lower levels, whereas limpets from L1 - North production area showed significantly higher levels. These differences may have occurred due to the capture area, since L1 – North production area corresponds to the most populous and industrialized area, being the most polluted of the three areas studied. Lead levels detected in all production areas did not differ statistically during the three seasons. Lead contents obtained by Cravo & Bebiano (2005) for limpets of the species *Patella aspera*, were less than 0.1 mg kg^{-1}, being therefore lower than those obtained in this work. However, Pérez-López et al. (2003), showed Pb concentrations between 0.95 and 3 mg kg^{-1} (dw), in limpets captured in Galicia, Connan & Tack (2010) obtained levels between 0.10 and 0.44 mg kg^{-1} (ww) in France and, Idrissi-Azzouzi et al. (2017) contents between 0.8 and 1.3 mg kg^{-1} (dw) in Moroccan Atlantic coast, which can be considered similar to those found in the present study. Other authors obtained higher levels of Pb than those obtained in this work, due to the impact of untreated industrial, agricultural and sewage contamination (El Serafy et al., 2003) or mining activities (Kelepertzis, 2013). Although, there are some papers that refer significant differences for the metal content in different seasons (El Sefary et al., 2003; Idrissi-Azzouzi et al., 2017), in the present study a seasonal pattern was not registered. The same was also observed by Connan & Tack (2010) in which a seasonal cycle of metal levels was not clearly detected.

TVC data displayed in table 3, shows that limpets collected from L7 - South production area presented higher contamination compared to limpets collected from other production zones, with values ranging between 6.51 and 7.21 log UFC / g. L5 - Center production area was the least contaminated, as TVC values varied between 5.64 and 5.97 log UFC / g. Limpet samples collected during summer, from L1 - North and L7 – South production areas were statistically different from those collected during winter and during winter and spring from L1 - North and L7 – South production areas, respectively. Nevertheless, samples from L5 – Center production area did not differ statistically with season. Regarding the three production areas studied, the levels of total viable microorganisms recorded in summer did not differ statistically among the three production areas. However, the three production areas showed levels of contamination statistically different from each other in winter. In spring, the contamination levels of samples from L7 – South production area were statistically different from those recorded in L1 and L5. This variability in limpets TVC levels may be associated with different macro algae contents among production areas and seasons, as algae present specific associated microbiota (Martin et al., 2015).

All *E. coli* results observed in limpet samples from L1 - North and L5 - Center production areas were below the detection limit (< 18 MPN/100g) (Table 3). Some limpets collected from L7 - South production area, during winter and spring seasons, revealed some contamination of fecal origin, in spite of being below the legal limit of 230 MPN/100g (EC, 2004). Although limpets are not filter-feeder, studies have shown that they can present relevant *E. coli* levels if impacted by low quality waters (Silva et al., 2018). Nevertheless, no evidences were found that L7 - South production area presented lower quality waters.

So, in general, microbial contamination levels present in limpets were higher in L7 - South production area, but no clear seasonal pattern was observed.

3.2 Comparison between limpet and mussel contamination

A comparison between the main study subject, limpet, and a reference indicator mollusk species also of the Portuguese coast, mussel (*Mytillus edulis*), is warranted for a proper assessment of the relative propensity of limpet to assimilate and accumulate contaminants.

The two mollusk species differed statistically in all contaminants analyzed (Table 4). With regard to chemical contaminants, limpet showed higher levels of Cd and Pb than mussel samples (Table 4). The contaminant of most concern was Cd, since its value in limpets was above the legal limit, as mentioned above. One explanation for these results is that the limpet feeds on the rocky substrate where it lives, being able to concentrate the metals that make up the rock. Cubbada et al. (2001) referred that limpets are influenced by metals accumulated in the algae on which they graze and generally can accumulate higher levels of Cd than other marine gastropod species. A study carried out by Pérez et al (2019), for the chemical contaminants present in limpet and mussels captured in "O Portiño" in Coruña, Spain, revealed levels of these contaminants higher than those obtained in this work. This is due to the fact that Coruña is a place of great fishing activity, with some risk of pollution. In this study, it was also found that limpets had a higher Cd content than mussel samples.

Concerning microbiological contaminants, limpets presented higher levels of total microorganisms than mussels; the opposite trend was observed for *E. coli* levels, with mussels presenting the highest levels of fecal contamination (Table 4). As mussel are filter feeders, they concentrate the microorganisms present in the water column, such as those of fecal origin. On the opposite, limpets are grazers, so the higher levels of total microorganisms may be associated with the algae used for feeding, which can present higher levels of bacteria (mainly algal polysaccharide-degrading bacteria) in comparison to the water column (Martin et al., 2015).

3.3 Cadmium risk assessment

The specific Cd risk associated to the consumption of limpet meals, expressed as a probability of exceeding the Cd TWI, is displayed in Table 5.

The lowest consumption frequency scenario, a meal every six months, generated very low probabilities of surpassing the Cd TWI, < 9.5×10^{-3} %, that is, almost no risk was observed. On the other hand, a non-negligible risk was already observed for a limpet meal every month. However, probabilities ranged from 0.057 % in the case of North Coast limpets to 1.9 % for Centre Coast samples. Finally, a weekly meal of limpet brought about very high probabilities of exceeding the Cd TWI, above 70 %.

The results attained by the risk assessment point to an advisable limitation of limpet consumption regardless of the geographical origin: less than a monthly meal. This is a consequential outcome of very high Cd levels in limpets from the Portuguese Coast. This same contaminant has been found in several groups of mollusks, such as bivalves and cephalopods (Bustamante et al., 1998; Ventura et al., 2018). Since the current study is the first semi-probabilistic (a distribution was fitted to Cd contents, but not to consumption frequencies) analysis of Cd risk in limpets, similar studies are only found for other shellfish groups (Ju et al., 2012; Ventura et al., 2018). These studies advise against excessive consumption frequency of several species of shellfish. Accordingly, the situation of limpet in the studied areas of the Portuguese Coast agrees with the previously mentioned studies.

It should also be referred that the performed risk assessment was built on assumptions. Namely, there is the assumption that the used threshold (Cd TWI) is a binary outcome (hazard *vs* no hazard) parameter for the health effect. This may not be the case and this kind of threshold values contain some uncertainty (Ventura et al., 2018). In particular, it is difficult to establish a reference for the whole population, which has a high variability of health situations and genetic characteristics. Whenever a reference is set, safety factors are used, which lower the tolerable intake level for Cd TWI. Another problem is connected to the paucity of consumption surveys, leading to arbitrary and uninformed consumption frequency scenarios. For instance, a weekly consumption of a limpet meal may be unrealistic. Moreover, the assumed average body weight of 60 kg (FAO/WHO, 2003) and the used meal size of 160 g are also assumptions. Finally, there are conjectures underlying the used mathematical models, such as the application of the EVT (Tressou et al., 2004).

4 Conclusions

Univalve and bivalve mollusks are very appreciated worldwide due to their special nutritional and organoleptic characteristics. In Portugal, univalve mollusks, such as limpets, are captured in coastal areas by professional or recreational pickers and destined for sale or auto-consumption. Nevertheless, its consumption is not risk-free and can cause human infections or intoxications.

The levels of Hg and Pb detected in limpets, from the three areas studied, were always below the limits of 0.50 mg kg^{-1} and 1.5 mg kg^{-1} allowed by the EU, respectively. Concerning Cd, the levels registered in limpets were always above the limit of 1.0 mg kg^{-1}, reaching about 3.0 mg kg^{-1} in some samples. These values probably indicate contamination from telluric origin (soil or rocks) in the coastal areas studied. Due to the high levels of Cd contamination, harvesting of limpets has been temporarily forbidden in some of the Portuguese coastal production areas. However, the recreational picking in the areas has not been refrained. Microbiological contamination of fecal origin in limpets was low and in general below the detection level, supporting EU legislation which excludes marine gastropods from production areas classification provisions. In general, contamination levels in limpets did not show a clear seasonal pattern.

The two mollusk species studied, limpets and mussels, differed statistically in all contaminants analyzed, being Cd the most of concern and always higher in limpets than in mussels.

This study also enabled to quantify the Cd intake risk associated to different limpet consumption scenarios, thereby pointing to an advisable limpet consumption of less than a monthly meal of 160 g.

As recreational picking of limpets is common in Portugal, official recommendations of maximum periodic human consumption should be published and enforcement increased in forbidden areas.

Credit authorship contribution statement

S. Pedro: Conceptualization, Methodology, Validation, Formal Analysis, Investigation; Writing – original draft, Funding Acquisition**. J. Caetano:** Methodology, Investigation, Writing – original draft. **C. Cardoso:** Methodology and Validation of Risk assessment, Manuscript Revision**. M. Mourato:**

Conceptualization, Methodology, Supervision. **H. M. Lourenço:** Conceptualization, Methodology, Validation, Investigation; Writing – original draft, Funding Acquisition**.**

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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References

Afonso, C., Lourenço, H. M., Cardoso, C., Bandarra, N. M., Carvalho, M. L., Castro, M. & Nunes, M. L. (2013). From fish chemical characterization to the benefit-risk assessment – Part A. *Food Chemistry*, *137*, 99-107. http://dx.doi.org/10.1016/j.foodchem.2012.10.014

Anacleto, P., Pedro, S., Nunes, M. L., Rosa, R. & Marques A. (2013). Microbiological composition of native and exotic clams from Tagus estuary: Effect of season and environmental parameters. *Marine Pollution Bulletin*, *74*, 116–124. https://doi.org/10.1016/j.marpolbul.2013.07.019

Bergasa O., Ramírez R., Collado C., Hernández-Brito J. J., Gelado-Cabarello M. D., Rodríguez-Somozas M., & Haroun R. J. (2007). Study of metals concentration levels in Patella piperata throughout the Canary Islands, Spain. *Environmental Monitoring and Assessment*, *127*, 127-133. https://doi.org/ 10.1007/s10661-006-9266-x

Bustamante, P., Caurant, F., Fowler, S.W., & Miramand, P. (1998). Cephalopods as a vector for the transfer of cadmium to top marine predators in the north-east Atlantic Ocean. *Science of the Total Environment*, *220*, 71-80. https://doi.org/10.1016/S0048-9697(98)00250-2

Cardoso, C., Bandarra, N., Lourenço, H., Afonso, C., & Nunes, M.L. (2010). Methylmercury risks and EPA+DHA benefits associated with seafood consumption in Europe. *Risk Analysis*, *30*, 827-840. https://doi.org/10.1111/j.1539-6924.2010.01409.x

Connan, O. & Tack, K. (2010). Metals in marine environment (mollusc *Patella* sp., fish *Labrus bergylta*, crustacean *Cancer pagurus*, beach sand) in a nuclear area, the North Cotentin (France). *Environmental Monitoring and Assessment*, *165*, 67–86. https://doi.org/ 10.1007/s10661-009-0927-4

CONTAM, EFSA Panel on Contaminants in the Food Chain (2011). Statement on tolerable weekly intake for cadmium. EFSA Journal, 9(2). https://doi.org/10.2903/j.efsa.2011.1975

Cravo, A., Bebianno, M. J., & Foster, P. (2004). Partitioning of trace metals between soft tissues and shells of *Patella aspera. Environment International*, *30*, 87–98. https://doi.org/10.1016/S0160-4120(03)00154-5

Cravo, A., & Bebianno, M. J. (2005). Bioaccumulation of metals in the soft tissue of *Patella aspera*: Application of metal/shell weight indices. *Estuarine, Coastal and Shelf Science*, *65,* 571-586. https://doi.org/10.1016/j.ecss.2005.06.026

Cubadda, F., Conti, M. E., & Campanella, L. (2001). Size-dependent concentrations of trace metals in four Mediterranean gastropods. *Chemosphere*, *45*, 561– 569 https://doi.org/10.1016/S0045-6535(01)00013-3

DGRM. (2019). https://www.dgrm.mm.gov.pt/documents/20143/121104/ Tabela+de+Tamanhos+M%C3%ADnimos+de+Captura+10-11-17.pdf/3be9f518-f518-4d37-c64c-126ac40f40b6/ Accessed 30 September 2019.

EC, 2004a. Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. *Official Journal of European Union*, 139, 55–205.

EC, 2004b. Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organization of official controls on products of animal origin intended for human consumption. *Official Journal of European Union*, *139*, 206–320.

EFSA. (2012a). Cadmium dietary exposure in the European population. *EFSA Journal*, *10*(1), 2551. https://doi.org/10.2903/j.efsa.2012.2551

EFSA. (2012b). Lead dietary exposure in the European population. *EFSA Journal*, *10*(7), 2831. https://doi.org/10.2903/j.efsa.2012.2831.

EFSA. (2012c). Scientific opinion on the risk for public health related to the pressure of mercury and methylmercury in food. *EFSA Journal*, 10(12):2985. https://doi.org/10.2903/j.efsa.2012.2985.

El-Serafy, S. S., El-Gamal, M. M., & El-Sayed, D. S. (2003). Seasonal variations of trace metals levels in water and the limpet *Patella caerulea* of Alexandria coast, Egypt. *Egyptian Journal of Aquatic Biology and Fisheries*, *7*, 283 – 312.

EPA (2007). Test method 7473: Mercury in solids and solutions by thermal decomposition, amalgamation and atomic absorption spectrometry. SW-846, USA, Environment Protection Agency, 17p.

EU (2006). Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs, *Official Journal of European Union*, *364*, 5-24.

EU (2010). Commission Regulation (EU) No 558/2010 of 24 June 2010 amending Annex III to Regulation (EC) No 853/2004 of the European Parliament and of the Council laying down specific hygiene rules for food of animal origin, Official Journal of the European Union. 159, 18-21.

FAO/WHO (2003). Evaluation of certain food additives and contaminants. Rome, Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO Technical Report Series, 922*, 188p.

FAO/WHO (2011). Safety evaluation of certain food additives and contaminants: prepared by the Seventy-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). World Health Organization). *WHO Food Additives Series*, *64*, 551p.

FAO. (2019). FAO yearbook. Fishery and Aquaculture Statistics 2017. Rome, 108p.

Idrissi Azzouzi, L. M., Aakameb, R. B., El Abidib, A., & Naciric, M. (2017). Contamination levels of metals (Cu, Cr, Cd and Pb) in *Patella rustica* from the Moroccan Atlantic coast. *International Journal of Environmental & Science Education*, *12*, 2347-2361.

Ferreira, O., Dias, J. A. & Taborda, R. (2008). Implications of Sea-Level Rise for Continental Portugal. *Journal of Coastal Research*, 24 (2), 317-324.https://doi.org/10.2112/07A-0006.1

Henriques P., Delgado J. & Sousa R. (2017). Patellid limpets: An overview of the biology and conservation of keystone species of the rocky shores. In: Ray S, editor. Organismal and Molecular Malacology. *IntechOpen*, pp. 71-95.

ISO 16649-3 (2015). Microbiology of the food chain - Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* - Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide, 10p.

ISO 6887-3 (2017). Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -- Part 3: Specific rules for the preparation of fish and fishery products, 16p.

Ju, Y.-R., Chen, W.-Y., & Liao, C.-M. (2012). Assessing human exposure risk to cadmium through inhalation and seafood consumption. *Journal of Hazardous Materials*, *227-228*, 353-361. https://doi.org/10.1016/j.jhazmat.2012.05.060

Kelepertzis, E. (2013). Heavy metals baseline concentrations in soft tissues of *Patella* sp. from the Stratoni coastal environment, ne Greece. *Ecological Chemistry and Engineering*, *20*, 141-149. https://doi.org/10.2478/eces-2013-0011

Martin, M., Barbeyron, T., Martin, R., Portetelle, D., Michel, G. & Vandenbol, M. (2015) The Cultivable Surface Microbiota of the Brown Alga *Ascophyllum nodosum* is Enriched in Macroalgal-Polysaccharide-Degrading Bacteria. *Frontier for Microbiology*, *6*, 1-14. https://doi.org/10.3389/fmicb.2015.01487

NP EN 14084 (2003). Foodstuffs – Determination of trace elements – Determination of lead, cadmium, zinc, copper and iron by atomic absorption spectrometry (AAS) after microwave digestion, CEN, Brussels, 16p.

Pérez, S. Sánchez-Marín, P., Bellas, J., Viñas, L., Besada, V. & Fernández, N. (2019). Limpets (*Patella* spp. Mollusca, Gastropoda) as model organisms for biomonitoring environmental quality. *Ecological Indicators*, *101*, 150-162. https://doi.org/10.1016/j.ecolind.2019.01.016

Pérez-López, M., Alonso, J., Nóvoa-Valiñas, C., & Melgas, M. J. (2003). Assessment of heavy metal contamination of seawater and marine limpet, *Patella vulgata* L., from Nothrwest Spain. *Journal of Environmental Science and Health Part A: Environmental Science Engineering*, *38*, 2845-2856. https://doi.org 10.1081/ESE-120025835

Reguera, P., Couceiro, L., & Fernández, N., (2018). A review of the empirical literature on the use of limpets *Patella* spp. (Mollusca: Gastropoda) as bioindicators of environmental quality. *Ecotoxicology and Environmental Safety, 148,* 593-600. https://doi.org/10.1016/j.ecoenv.2017.11.

Shefer, E., Silverman, J., & Herut, B. (2015). Trace metal bioaccumulation in Israeli Mediterranean coastal marine mollusks. *Quaternary International*, *390*, 44-55 https://doi.org/10.1016/j.quaint.2015.10.030

Silva, C. S. E., Novais, S. C., Simões, T., Caramalho, M., Gravato, C., Rodrigues, M. J., Maranhão, P. & Lemos, M. F. L. (2018). Using biomarkers to address the impacts of pollution on limpets (*Patella depressa*) and their mechanisms to cope with stress. *Ecological Indicators*, *95*, 1077–1086. https://doi.org/10.1016/j.ecolind.2017.09.046

Sousa R., Riera, R., Vasconcelos, J., Gouveia, L., Pinto, A. R., Delgado, J., Alves, A., González, J. A., Freitas, M. & Henriques, P. (2019). Artisanal harvest of shellfish in the Northeastern Atlantic: The example of limpet and topshell fisheries in the Archipelago of Madeira. In: Ray S, editor. Invertebrates - Ecophysiology and Management. *IntechOpen*, pp. 1-18.

Tressou, J., Crépet, A., Bertail, P., Feinberg, M.H. & Leblanc, J.C. (2004). Probabilistic exposure assessment to food chemicals based on extreme value theory. Application to heavy metals from fish and sea products. *Food and Chemical Toxicology*, *42*, 1349–1358. https://doi.org/10.1016/j.fct.2004.03.016

Türkmen M., Türkmen A., Akyurt I., & Tepe Y. (2005). Limpet, *Patella caerulea* Linnaeus, 1758 and Barnacle, *Balanus* sp., as biomonitors of trace metal availabilities in İskenderun Bay, Northern East Mediterranean Sea. *Bulletin of Environmental Contamination and Toxicology*, *74*, 301-307. https://doi.org/10.1007/s00128-004-0584-9

Ventura, M., Cardoso, C., Bandarra, N.M., Delgado, I., Coelho, I., Gueifão, S., Ribeiro, M., Costa, M.H., & Castanheira, I. (2018). Bromine, arsenic, cadmium, and lead in several key food groups: an assessment of relative risk. *International Journal of Environmental Analytical Chemistry*, *98*, 1398-1412. https://doi.org/10.1080/03067319.2018.1559307

Viñas, L., Pérez-Fernández, B., Soriano, J. A., López, M. & Bargiela, J. (2018). Limpet (*Patella sp*) as a biomonitor for organic pollutants. A proxy for mussel? *Marine Pollution Bulletin, 133*, 271-280. https://doi.org/10.1016/j.marpolbul.2018.05.046

Tables 1 to 5

Table 1 – Quality assurance of elements analyzed using the certified reference material Dorm4 (average ± standard deviation) (n ≥ 4).

Elements	**Certified value** (mg kg^{-1})	**Present work** (mg kg^{-1})
Cd	0.299 ± 0.018	0.299 ± 0.024
Hg	0.412 ± 0.036	0.396 ± 0.011
Pb	0.404 ± 0.062	0.389 ± 0.025

Dorm-4, fish protein certified reference material for trace metals (National Research Council of Canada, Canada).

Table 2 – Cadmium, mercury and lead contents (mean ± standard deviation) expressed in mg kg^{-1}, observed in limpets, according to the production area and the season.

	Season	**Production area**		
		L1 - North	**L5 - Center**	**L7 - South**
Cd	Winter	1.2 ± 0.1^{a,1}	2.8 ± 0.5^{b,2}	2.3 ± 1.0^{a,1,2}
	Spring	1.8 ± 0.1^{c,1}	1.4 ± 0.2^{a,1}	1.4 ± 0.9^{a,1}
	Summer	1.5 ± 0.0^{b,1}	2.3 ± 0.0^{a,b,2}	1.7 ± 0.1^{a,1}
Hg	Winter	0.011 ± 0.001^{a,1}	0.015 ± 0.003^{2}	< LQ
	Spring	0.012 ± 0.001^{b,1}	< LQ	0.014 ± 0.000^{2}
	Summer	< LQ	< LQ	<LQ
Pb	Winter	0.43 ± 0.04^{a,3}	0.12 ± 0.02^{a,1}	0.34 ± 0.05^{a,2}
	Spring	0.37 ± 0.05^{a,2}	0.14 ± 0.01^{a,1}	0.32 ± 0.01^{a,2}
	Summer	0.46 ± 0.01^{a,3}	0.14 ± 0.00^{a,1}	0.32 ± 0.01^{a,2}

For each element: Means with different lowercase letters indicate that there are statistical differences between the variables (p <0.05). Means with different numbers within a row indicate that there are statistical differences between the variables (p <0.05); LQ = 0.011 mg kg^{-1}.

Table 3 – Total viable counts (TVC) and *E. coli* levels (mean ± standard deviation) expressed in Log CFU/g and Log MPN/100g, respectively, observed in limpets, according to the production area and the season.

	Season	**Production area**		
		L1 - North	**L5 - Center**	**L7 - South**
TVC	Winter	5.63 ± 0.22^{a,1}	5.97 ± 0.12^{a,2}	7.21 ± 0.03^{b,3}
	Spring	5.90 ± 0.25^{a,b,1}	5.72 ± 0.28^{a,1}	7.08 ± 0.23^{b,2}
	Summer	6.20 ± 0.00$^{b, 1}$	5,64 ± 0.49$^{a, 1}$	6.51 ± 0.04^{a,1}
E. coli	Winter	n.d	n.d	1.04 ± 0.17
	Spring	n.d	n.d	1.13 ± 0.20
	Summer	n.d	n.d	n.d

For each parameter: Means with different lowercase letters within a column indicate that there are statistical differences between the variables (p <0.05). Means with different numbers within a row indicate that there are statistical differences between the variables (p <0.05); n.d. – not detected (below 18 MPN/100g).

Table 4 – Contaminant levels (mean ± standard deviation) in both studied species.

Contaminants	Mollusk species	
	Limpet	Mussel
Total Viable Counts (log CFU/g)	6.22 ± 0.64^{b}	5.33 ± 0.86^{a}
E. coli (log MPN/100g)	0.99 ± 0.11^{a}	1.25 ± 0.43^{b}
Cd (mg kg^{-1})	1.8 ± 0.7^{b}	0.3 ± 0.1^{a}
Hg (mg kg^{-1})	0.012 ± 0.002^{a}	0.017 ± 0.007^{b}
Pb (mg kg^{-1})	0.29 ± 0.12^{b}	0.15 ± 0.05^{a}

Means with different lowercase letters indicate that there are statistical differences between the variables ($p < 0.05$).

Table 5 – Risk associated to Cd intake through consumption of limpet from different geographical areas of the Portuguese Coast (calculation of the probability in % of exceeding the respective Tolerable Weekly Intake*).

Consumption Scenario	L1 - North	L5 - Center	L7 - South
1 meal every 6 months	1.5 × 10-6	9.5 × 10-3	1.3 × 10-4
1 monthly meal	0.057	1.9	0.52
1 weekly meal	71	71	95

*Tolerable Weekly Intake of Cd: 2.5 µg/(kg bw × week). Values in bold correspond to probabilities calculated through the "plug-in" (PI) estimator.

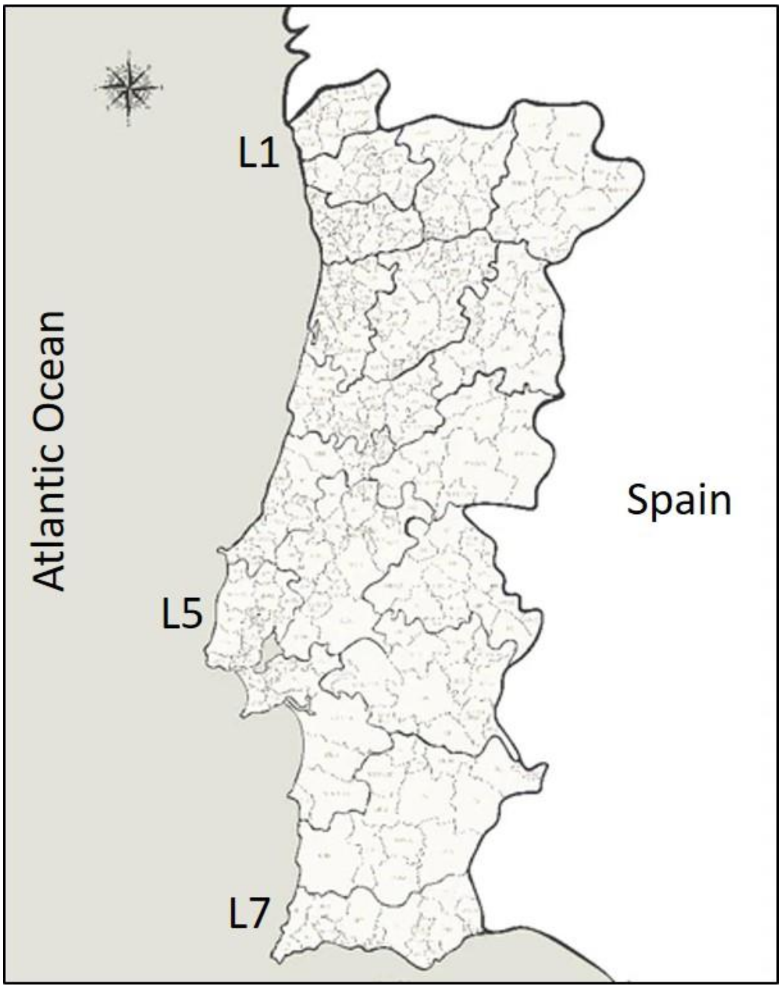

Fig. 1 – Location of sampling sites: L1 – North (41.74283; -8.87833); L5 – Center (38.00468; -9.42503); L7 – South (37.29571; -8.87083).