Integrated Master in Bioengineering

Siloxanes in seaweed: possible sources, trends and distribution levels

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Resumo

Devido às suas propriedades, os metilsiloxanos voláteis (VMSs) são compostos químicos massivamente produzidos, com uma vasta gama de aplicações industriais e domésticas. O seu largo uso em produtos de cuidado e higiene pessoal tem sido referido como uma das principais fontes da sua libertação continuada no ambiente, atingindo áreas marinhas e costeiras, onde se acredita serem propensos a bioacumular e biomagnificar nas redes tróficas, com possíveis efeitos tóxicos e danos ecológicos. A análise de algas é passível de fornecer informações importantes quanto à sua distribuição nestes ambientes, reforçando o conhecimento sobre as mais prováveis fontes de contaminação, bem como possíveis tendências sazonais e/ou geográficas.

Neste estudo, um método analítico baseado na extração com a tecnologia QuEChERS seguido de análise por cromatografia gasosa de espetrometria de massa (GC/MS) foi desenvolvido e validado para a determinação de três VMSs lineares (L3-L5) e quatro cíclicos (D3-D6) em algas, depois de otimizadas algumas condições experimentais. Um volume de 10 mL de *n*-hexano foi usado para extrair 2.5 g de amostra num banho de ultrassons, seguido de um passo para remoção de água e de um outro de limpeza em fase sólida dispersa, após os quais o extrato foi centrifugado, concentrado e analisado. Obteve-se uma resposta linear numa gama de concentrações entre 1 e 750 $\mu g.L^{-1}$, com valores de R^2 superiores a 0.996 e limites de deteção e quantificação na gama dos $pg.g^{-1}$. Foi obtida uma média global de recuperações de 93 ± 7%, bem como valores satisfatórios de precisão, inferiores a 10 e 15% para testes de repetibilidade e de precisão intermédia, respetivamente.

Em temos de comparação de algas, sete espécies (*Ulva lactua*, Porphyra sp., *Codium tomentosum*, *Fucus vesiculosus*, uma espécie não identificada de kelp, *Palmaria palmata* e *Schizymenia dubyi*) recolhidas em duas praias da Região Norte de Portugal foram analisadas, revelando praticamente nenhumas deteções de VMSs, exceto para a espécie *F. vesiculosus*, onde se quantificaram sobretudo VMSs cíclicos. Outras análises foram realizadas para aferir a distribuição temporal da presença dos contaminantes-alvo em algas de *F. vesiculosus* e *Porphyra* sp. de duas praias da Região Norte e Centro de Portugal, entre os meses de maio e dezembro de 2016, com as concentrações totais de VMSs em cada mês variando entre não detetado (nd) e 37 $ng.g^{-1}_{peso\ seco}$, com os VMSs cíclicos surgindo em concentrações muito superiores aos lineares, e onde os meses de Verão registaram os níveis mais altos de contaminação, sobretudo para os analitos D5 e D6. A análise estendeu-se ainda à determinação de siloxanos numa espécie de erva marinha endémica do Mar Mediterrânico, *Posidonia oceanica,* recolhida em sete locais na Região de Múrcia, no Verão e no Inverno. As concentrações totais de VMSs variaram entre nd e 291 $ng.g^{-1}_{peso\ seco}$, com os cíclicos a serem uma vez mais predominantes, especialmente o D5 e o D6. Amostras dos locais associados a uma maior pressão industrial e urbana registaram, no geral, maiores níveis de contaminação e, em quatro dos sete pontos de amostragem, as concentrações de VMSs foram superiores no Verão, altura em que a população em tais áreas aumenta devido ao afluxo de turistas.

A amostragem de vegetação marinha provou ser uma abordagem adequada para a análise de VMSs em ambientes costeiros, constituindo um ponto de partida relevante para uma apreciação mais alargada sobre estes contaminantes nestes ecossistemas com potencial impacto elevado.

Palavras-chave: Metilsiloxanos voláteis, ambientes costeiros, algas, ervas marinhas, QuEChERS, GC/MS.

Abstract

Due to their unique and valuable combined properties, volatile methylsiloxanes (VMSs) are heavily produced chemicals, with a wide range of industrial and domestic applications. Its usage in personal care products (PCPs) has been referred as one of their main sources of their ubiquitous environmental distribution, reaching marine and coastal areas where they are prone to bioaccumulate and biomagnify in food webs, with possible ecological and toxicological effects. Seaweed analysis can provide important information on the distribution levels of these pollutants in such environments, and enhance our knowledge about possible contamination sources and seasonal or geographical trends.

In this study, an analytical method based on QuEChERS extraction (Quick, Easy, Cheap, Effective, Rugged, and Safe), followed by gas chromatography/mass spectrometry (GC/MS) analysis, was developed and validated for the determination of three linear (L3-L5) and four cyclic (D3-D6) VMSs in seaweed, after enhancement of some of the experimental conditions. Using 10 mL of *n*-hexane, 2.5 g of cut sample were extracted in an ultrasound bath, followed by a water removal step and a dispersive solid-phase clean-up, after which the extract was centrifuged, concentrated and analysed by GC/MS. A good linearity response was obtained, in the range of 1 to 750 $\mu g.L^{-1}$ for each VMS studied, with R^2 values higher than 0.996 and low limits of detection and quantification, on $pg.g^{-1}$ levels. A global mean of recoveries of 93 ± 7% was obtained, and good intraday and interday precision values were achieved (RSD values lower than 10 and 15%, respectively, for most of the target compounds).

In terms of comparison between different seaweed, seven species (*Ulva lactua*, Porphyra sp., *Codium tomentosum*, *Fucus vesiculosus*, an unidentified species of kelp, *Palmaria palmata* and *Schizymenia dubyi*) collected in two beaches in the seashore of the North Region of Portugal were analysed, with almost no VMSs detected, except for *F. vesiculosus* samples, with mostly cVMSs being quantified. Other analyses were conducted in samples of *F. vesiculosus* and *Porphyra* sp. from two urban beaches of the Centre and North of Portugal, over the months of between May and December 2016, to assess the temporal distribution of the target compounds; results revealed that the total concentrations of siloxanes in each month ranged between not detected (nd) and 37 $ng.g^{-1}_{dw}$, with cyclic VMSs appearing in much higher concentrations than linear VMSs, and some of the summer months registering higher values of contamination, mostly for D5 and D6. The validated methodology was also employed in samples of *Posidonia oceanica*, a seagrass endemic to the Mediterranean Sea, collected in seven sites in the Region of Murcia, Spain. Total concentrations of the target compounds ranged between nd and 291 $ng.g^{-1}_{dw}$, with cyclic VMSs being again the most predominant in the majority of cases, especially D5 and D6. Locations associated with higher urban and industrial activities in its surroundings showed, in general, higher levels of contamination, and four out of the seven locations showed higher concentrations in samples collected in summer, a time when population in those areas increased due to a strong afflux of tourists.

Sampling marine vegetation proved to be a suitable approach for the analysis of VMSs in coastal environments, and a starting point for a broader study of these areas under potential impact.

Keywords: volatile methylsiloxanes, coastal environments, seaweed, seagrass, QuEChERS, GC/MS.

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Notation and Glossary

(Sorted alphabetically)

a	Slope
Acet	Acetone
ACN	Acetonitrile
b	Intercept
BDL	Below detection level
C	Concentration
C18	Octadecyl-silica
cVMS	Cyclic volatile methylsiloxane
D3	Hexamethylcyclotrisiloxane
D4	Octamethylcyclotetrasiloxane
D5	Decamethylcyclopentasiloxane
D6	Dodecamethylcyclohexasiloxane
DCM	Dichloromethane
dw	Dry weight
EC	European Community
EC/HC	Environmental Canada and Health Canada
EPA	Environmental Protection Agency
EtAc	Ethyl acetate
GC	Gas chromatography
GC/HRMS	Gas chromatography/high-resolution mass spectrometry
GC/MS	Gas chromatography/mass spectrometry
Hex	*n*-hexane
I.D.	Internal diameter
INCI	International Nomenclature of Cosmetic Ingredients
IS	Internal standard
IUPAC	International Union of Pure and Applied Chemistry
L2	Hexamethylsiloxane
L3	Octamethyltetrasiloxane
L4	Decamethyltetrasiloxane
L5	Dodecamethylpentasiloxane
ld	Lipid weight
LLE	Liquid-liquid extraction
LOD	Limit of detection
Log K_{OW}	Logarithm of the octanol/water partition coefficient
LOQ	Limit of quantification
LRAT	Long range atmospheric transport
lVMS	Linear volatile methylsiloxane
m/z	Mass-to-charge ratio
M4Q	Tetrakis(trimethylsilyloxy)silane
MS	Mass spectrometer
n	Number of repetitions/replications
NA	Not available
nd	Not detected
PCP	Personal care product
PDMS	Polydimethylsiloxane
PSA	Primary and secondary amine
Q1	QuEChERS 1
Q2	QuEChERS 2
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, Safe

r	Pearson correlation coefficient
R^2	Coefficient of determination
Rec	Recovery rate (%)
RF	Response factor (Peak area of the compound/Peak area of the internal standard)
RSD	Relative standard deviation (%)
RT	Retention time
S/N	Signal-to-noise ratio
s_a	Standard deviation of the slope
s_b	Standard deviation of the intercept
SIS	Selected ion storage
SLE	Solid-liquid extraction
SPE	Solid-phase extraction
US EPA	United States Environmental Protection Agency
USE	Ultrasound extraction
VMS	Volatile methylsiloxane
ww	Wet weight
WWTP	Wastewater treatment plant

1 Project Presentation

Silicon (Si) is the second most abundant element in the earth's crust (after oxygen). Elementary Si pure forms are extremely rare in nature, being this chemical element usually found linked with two oxygen atoms forming silica (SiO_2), a major constituent of dust and common sand, or in the form of silicate rock forming minerals, composed of different combinations of silicon with oxygen and several metals (Zulehner et al., 2000). Because of their natural abundance, inorganic silicon matrices have been massively used by mankind in a vast range of applications, from ceramics and glass to electronics. Nevertheless, since the chemical synthesis of the first organosilicon compound in 1863, by chemists Charles Friedel and James Crafts, a new class of organic compounds containing carbon-silicon bonds emerged (Alaee et al., 2013): siloxanes.

Siloxanes are chemically synthetized organic compounds that belong to the organosilicon family. Currently, there is no evidence of natural occurrence of organosiloxanes, whereby the presence of such compounds on the environment are a sign of anthropogenic activity (Rücker et al., 2015). Due to their unique properties, they currently find a role in a wide range of industrial and domestic applications, resulting in high production levels worldwide: in 2017, 6.5 million tons of siloxanes are expected to be produced, with a yearly increase of about 6% until 2022 (Arespacochaga et al., 2015).

Considered innocuous chemicals for a long time, siloxanes were recently classified as emerging pollutants, mostly because of their persistence in the environment and the potential of bioaccumulation and biomagnification in trophic chains with possible toxicological effects (Howard et al., 2010; Mackay et al., 2015). As a result, in the last years, a scientific interest on this subject has emerged and some studies have been conducted to assess the presence of siloxanes in the environment and their effects in trophic chains, as well as potential toxicological hazards (Rücker et al., 2015). Therefore, methodologies of extraction and analysis of these compounds have been developed as well.

Considering that some of the routes of these compounds to the environment are through wastewater treatment plants (WWTPs) discharges and from the common use of siloxanes in personal care products (PCPs) formulations (Mackay et al., 2015), marine and coastal environments are potential hotspots for the presence of siloxanes, mostly because of the usually high urban and industrial development in its surroundings, such as in Portugal. Seaweed analysis can possibly provide important information on the distribution of such pollutants in these environments and improve the state of the art on their sources and behaviour. Therefore, this work aimed to determine the presence of three linear (L3-L5) and four cyclic (D3-D6) volatile methylsiloxanes in macroalgae collected from different sites in the seashore of the North and Centre regions of Portugal and from a marine aquatic plant from the Region of Murcia, Spain, and assess temporal trends on the concentrations of these contaminants in such matrices. To do so, an analytical method using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction followed by gas chromatography-mass spectrometry (GC/MS) analysis was optimised and validated.

2 Introduction

2.1 Siloxanes

2.1.1 Definition

The term "siloxane" is commonly used when referring to compounds consisting of a backbone of alternating silicon (Si) and oxygen (O) atoms, with organic groups attached to the Si atoms, usually alkyl groups (Brook, 2000). Regarding this interpretation, "siloxane" may be perceived as an abbreviation for silicon, oxygen and alkane (Alaee et al., 2013). However, it is important to state that according to the International Union of Pure and Applied Chemistry (IUPAC), the term siloxane refers to "*saturated silicon-oxygen hydrides with unbranched or branched chains of alternating silicon and oxygen atoms (each silicon atom is separated from its nearest silicon neighbours by single oxygen atoms)*" (IUPAC, 1997). When this backbone of alternating silicon and oxygen atoms has organic side chain groups attached to the Si atoms, the resulting molecule is an organic compound generally named organosiloxane (Arespacochaga et al., 2015).

In this work, for short, the term "siloxane" will be used to denote an organosiloxane compound. More specifically, the word "methylsiloxane" will be used when referring to a siloxane in which only methyl groups are present in the organic side chain of the Si and O backbone structure.

2.1.2 Classification

Siloxanes are a large group of organic chemicals with molecular weights ranging from a few hundred to several hundred thousand Daltons, depending on their size and polymerization level. Polymeric forms of siloxanes are usually designated as silicones (Schmitt, 2014). In literature, siloxanes are commonly divided in three major classes: volatile methylsiloxanes (VMSs), polydimethylsiloxanes (PDMSs) and functionalized siloxanes (Wang et al., 2013b). Besides this division, the existence of two basic structural conformations for methylsiloxanes is generally accepted: linear and cyclic. Linear structures are usually expressed as Ln, whereas cyclic ones usually follow the notation Dn, where n represents, in both cases, the number of silicon atoms in the molecule (Rücker et al., 2015).

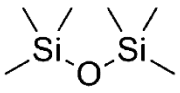
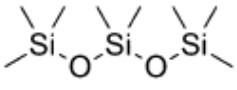
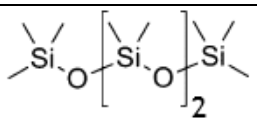
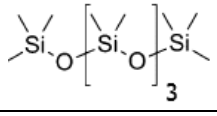
Considering the high use of silicone oils and derivatives in the cosmetic industry, the International Nomenclature of Cosmetic Ingredients (INCI) system also classifies siloxanes in two major groups: dimethicones (linear volatile methylsiloxanes (lVMSs) and PDMSs in general) and cyclomethicones (refers to a mixture of cyclic volatile methylsiloxanes (cVMSs)). Those generic designations are commonly used in the formulations and list of ingredients in cosmetics and toiletries (Lassen et al., 2005).

2.1.3 Physicochemical Properties

Silicon (Si) is the 14th element on the Periodic Table, located on the 3rd period, right under Carbon (C), in the group 14. Therefore, both elements share some physical and chemical properties, showing some similar tendencies on forming chains, rings and polymer networks (Varaprath, 1999). However, the differences between Si and C end up in very distinct properties on the physicochemical behaviour of those atoms with other elements and of the molecules resulting therefrom. For instance, the covalent radius of Si (1.17 Å) is larger than that of C (0.77 Å), which helps to explain that Si-Element bonds are about 20% longer, on average, when compared to C-Element bonds. Besides that, the electronegativity of Si (1.8) is lower than that of C (2.5), resulting in higher polarity of the Si-O bond when compared to the C-O bond (Rücker et al., 2015). These two major differences between Si and C help to understand several macroscopic properties of silicones when compared to carbon-based polymers, such as their high flexibility, low surface tension, very low glass temperature and weak temperature dependence on their viscosity, among others. The high flexibility of the Si-O-Si bond angle (the backbone structure of siloxanes) contributes to a low resistance to linearization and polymerization of the siloxane chain, turning it much more threadlike and deformable. This flexibility, along with the weak interaction between methyl groups in siloxanes' side chains, as well as the length of the molecules, helps to understand the low surface tension and viscosity of these compounds (Carteret et al., 2010; Arespacochaga et al., 2015).

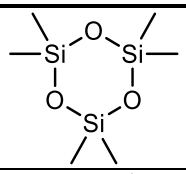
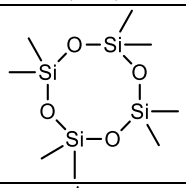
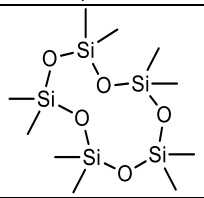
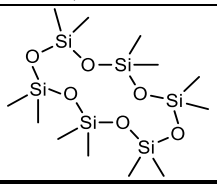
On the present work, eight low molecular weight methylsiloxanes were selected, four lVMSs and four cVMSs. The structure, some physicochemical properties and other related information on the selected lVMSs and cVMSs is listed in Tables 1 and 2, respectively.

Table 1. Chemical structure and some physicochemical properties of lVMSs.

Compound CAS no. Abbreviation Molecular formula	Chemical structure	Molar mass[a] ($g \cdot mol^{-1}$)	Boiling point[b] (°C)	Log K_{ow}[c]	Water solubility[b] ($mg \cdot L^{-1}$, 25 °C)	Vapour pressure[c] (mmHg, 25 °C)
Hexamethylsiloxane 107-46-0 L2 $C_6H_{18}OSi_2$		162.38	107	4.20	9.30×10^{-1}	31.00
Octamethyltrisiloxane 107-51-7 L3 $C_8H_{24}O_2Si_3$		236.53	153	4.80	3.40×10^{-2}	3.90
Decamethyltetrasiloxane 141-62-8 L4 $C_{10}H_{30}O_3Si_4$		310.69	194	5.40	6.74×10^{-3}	0.55
Dodecamethylpentasiloxane 141-63-8 L5 $C_{12}H_{36}O_4Si_5$		384.84	232	6.00	3.09×10^{-4}	0.07

[a]Sanchís et al., (2015), [b]Royal Society of Chemistry, (2014); [c]Kim et al., (2013).

Table 2. Chemical structure and some physicochemical properties of cVMSs.

Compound CAS nr. Abbreviation Molecular formula	Chemical structure	Molar mass[a] ($g \cdot mol^{-1}$)	Boiling point[b] (°C)	Log K_{ow}[c]	Water solubility[b] ($mg \cdot L^{-1}$, 25 °C)	Vapour pressure[c] (mmHg, 25 °C)
Hexamethylcycloclotrisiloxane 541-05-9 D3 $C_6H_{18}O_3Si_3$		222.46	135	4.47	1.56×10^{0}	10.00
Octamethylcyclotetrasiloxane 556-67-2 D4 $C_8H_{24}O_4Si_4$		296.62	176	5.10	5.60×10^{-2}	1.30
Decamethylcyclopentasiloxane 541-02-6 D5 $C_{10}H_{30}O_5Si_5$		370.77	211	5.20	1.70×10^{-2}	0.40
Dodecamethylcyclohexsiloxane 540-97-6 D6 $C_{12}H_{36}O_6Si_6$		444.92	245	6.33	5.50×10^{-3}	0.02

[a]Sanchís et al., (2015), [b]Royal Society of Chemistry, (2014); [c]Kim et al., (2013).

The presented VMSs are oily colourless liquids at ambient temperature, with the exception of D3, which is solid. The eight selected compounds are considered volatile, as their boiling point is lower than 250 ˚C. In all compounds, the boiling point increases with the chain length, with L2 being the most volatile and D6 the least volatile. The vapour pressures of L2, L3 and D3 are considerably high, suggesting that they probably tend to volatilize faster than the other VMSs.

Despite having a polar main chain, methylsiloxanes are water repellent compounds due to the presence of methyl groups attached to Si atoms. Therefore, their water solubility is low, decreasing as the chain length increases. This behaviour is similar to those observed in alkanes: as the size of the molecule increases, the number and area of nonpolar methyl groups also increases, enhancing the hydrophobicity of the molecule. For this same reason, methylsiloxanes do not conduct electricity. All the selected VMSs show a lipophilic nature, with the logarithm of the octanol/water partition coefficient (log K_{ow}) increasing with chain length, ranging from 4.20 (L2) to 6.33 (D6), which makes them soluble in nonpolar organic solvents such as *n*-hexane or toluene. The flexible and polar backbone of siloxanes also appears to be strong and resistant to high temperatures and to chemical reactions such as oxidation, reduction and photodegradation (Kaj et al., 2005; Shi et al., 2015). That makes them usable in applications where carbon based polymers would melt or decompose. However, it also increases the risk of their persistence in the environment and of long-range atmospheric

transport (LRAT), favoured by their volatility and high vapour pressures (McLachlan et al., 2010; Genualdi et al., 2011; Whelan et al., 2013).

Hence, siloxanes are very interesting compounds, not only due to the unique general properties of its backbone structure, but also because of the possible variation of the chemical and physical properties of the polymerized forms. Depending on the types of functional groups selected to the side chains (functionalized siloxanes), as well as the size of the polymers, physicochemical properties of silicones may vary considerably, with an extensive range of uses and applications (Kaj et al., 2005; Buser, 2013; Arespacochaga et al., 2015).

2.2 Industrial Production and Applications

Most of all carbon based synthetic polymers are currently produced using monomers derived from petroleum-based oil. On the other hand, siloxanes are produced from a natural clean source of silica (such as sand or quartz) and methanol, both materials easy to obtain and available for the predictable future with low variance on their cost and availability (Graiver et al., 2003). Consequently, in 2002, the estimated global production of silicones was 2 million tons. A decade later, silicone fluids, elastomers and resins registered a production of 4.7 million tons worldwide, more than doubled in that period. In 2017, 6.5 million tons of these compounds are expected to be produced, with a yearly increase of 6% until 2022. Currently, and since 2009, China is the largest producer and consumer of siloxanes (Arespacochaga et al., 2015; Rücker et al., 2015).

This tendency of massive production of siloxanes is the response to the worldwide demand of silicones and related products. Due to their hydrophobic properties, these compounds are part of formulations of sealants, adhesives and coatings in water repellent paints used in construction (Arespacochaga et al., 2015), and in paper, leather and textile industries (Graiver et al., 2003) for enhancement of material properties. Siloxanes are also used in the production of rubbers, resins and other matrices with low thermal and electric conductivity, making excellent electric and thermal insulators used in the electronic industry (Rücker et al., 2015). They are also used as antifoaming and wetting agents, reducing the surface tension of liquids. This property makes them a common compound in agrochemical and pharmaceutical formulations and in household and industrial cleaning products (Genualdi et al., 2011). In medicine, siloxanes are used in medical devices and prosthetics, while in the food processing industry, PDMSs are used as anti-foaming agents, as a protective layer on fats and oils against oxygen (additive E900) and in some low-calorie products as an alternative to fats (Andriot et al., 2007). One other major use of siloxanes-derived products is in cosmetics and PCPs. Under the INCI generic designation of dimethicone and cyclomethicone, lVMSs, PDMSs and cVMSs are commonly used to soften, smooth and moisten, making application of creams and lotions easier (Capela et al., 2016a). They can be found on numerous formulations of skin and hair care products,

soaps, perfumes and fragrances, antiperspirants, sunscreens, balms or make-up (Wang et al., 2009; Capela et al., 2016a,b). Due to their high thermal stability, low surface tension, volatility, hydrophobicity (but easily emulsified and hydrolytically stable) and colourless and odourless properties, cVMSs are already considered one of the basic ingredients in PCPs formulations, with D5 and D6 being the predominant cVMSs (Wang et al., 2013b; Dudzina et al., 2014; Capela et al., 2016a). Consequently, PCPs are considered the major source of human exposure to VMSs, as well as one of the most important sources of environmental contamination, mostly due to the evaporation of siloxanes after application of the products, and down-the-drain emissions (Capela et al., 2016a).

2.3 Emergence and Fate of Siloxanes

2.3.1 Release to the Environment

Considering the large (and increasing) amount of siloxanes being produced worldwide, the attention for the presence of these compounds in the environment is recently growing, especially regarding VMSs. After all, these synthetic materials will somehow end up being released into the environment, and their fate in the ecosystems is hard to predict (Rücker et al., 2015). VMSs are mostly released straight away into the atmosphere during industrial manufacturing processes of product formulations containing siloxanes and silicones in general (Badjagbo et al., 2011; Sanchís et al., 2015), but also through emission from landfills disposal, WWTPs and from the normal use of PCPs containing siloxanes (Sanchís et al., 2015; Xu et al., 2014; Mackay et al., 2015). In fact, it is estimated that 90% of the VMSs present in PCPs formulations volatilize during use, while most of the remainder are discharged to wastewater treatment plants (WWTPs), typically the ones present in rinse-off products like soaps, shampoos and conditioners (Allen et al., 1997; Egmond et al., 2013; Mackay et al., 2015; Capela et al., 2016a).

Once in the air, VMSs tend to be mainly present in the gas phase and the reaction with hydroxyl radicals is their main removal mechanism (Sanchís et al., 2015). However, in the atmosphere, cVMSs have average global half-lives of 20.0 days for D3, 10.3 days for D4, 6.7 days for D5 and 5.8 days for D6 (Krogseth et al., 2013), while an estimated half-live for the generality of lVMS is about 9 days (Genualdi et al., 2011). Some studies suggest that with these half-lives VMSs are able to undergo long-range atmospheric transport (LRAT) (Sanchís et al, 2013), reaching areas far away from the emission sources: cVMSs were detected in Arctic air (Genualdi et al., 2011 and Krogseth et al., 2013). Besides that, the presence of VMSs in biota from the Arctic and from Swedish lakes, in areas not receiving known direct inputs of siloxanes, also suggests that wet deposition of these compounds is possible (Warner et al., 2010). When in aqueous environments, due to their low water solubility and high sorption coefficients, siloxanes tend to adsorb to particulate carbon matter (Bletsou et al., 2013). Therefore, and considering

that about 10% of VMSs from PCPs enter the domestic sewage system, most of VMSs will settle down in the WWTPs' sludge line, with a small fraction (1-2%) remaining in the water and being released to the natural aqueous systems (Xu et al., 2014; Mackay et al., 2015). In some WWTPs, siloxanes are even added to the aeration basins as antifoaming agents (Arespacochaga et al., 2015). The resulting sludge streams containing VMSs are then buried in landfills or applied to fertilize soil, promoting mobile transportation of these compounds to new extensions (Xu et al., 2014). Recently, with the use of sludge to produce biogas, the presence of VMSs on such matrices became an important problem due to the deposition of microcrystalline silicon dioxide on the combustion equipment, a product of the oxidization of organosiloxanes present on biogas composition, causing operational problems and reducing the process efficiency (Arespacochaga et al., 2015).

2.3.2 Environmental Concerns and Toxicological Risks

Chemical and physical properties of VMSs favour their persistence in the environment (Sanchís et al., 2015). In the last years, the occurrence of VMSs in the environment has been studied, particularly for cVMSs. Some cVMSs have been reported not only in the air, water and soil/sediment, but also in biota, suggesting potential ecological hazard (Hong et al., 2014). Moreover, some VMSs show high potential for bioaccumulation and biomagnification (Fromme et al., 2015). For instance, cVMSs were identified in the pelagic food webs of two Norwegian lakes and in brown trout in aqueous environments receiving discharges from WWTPs (Borgå et al., 2013), in bottom fish samples in marine environment in Northeast China (Hong et al., 2014), in vegetation, phytoplankton and krill in Antarctic (Sanchís et al., 2015) and in pine needles in Portugal (Ratola et al., 2016).

Investigations conducted to assess the toxic potential of siloxanes focus mostly on cVMSs, particularly D4 and D5. Studies developed in mice suggest that a continuous inhalation exposure to D4 is related to the induction of uterine endometrial adenocarcinomas in female rats (Dekant and Klauning, 2016), as well as the increase of liver weight (hepatic hyperplasia) and the inducing of drug metabolizing liver enzymes (Zhang et al., 2000). D4 exposure in mice was also correlated with hormonal changes on oestrogen levels, resulting in impairment of fertility and reproductive complications (McKim et al., 2001; He et al., 2003; Quinn et al., 2007; Meeks et al., 2007). On the other hand, D5 appears to have no effects in the fertility of mice, but it has been stated to target the liver and the lungs in animal studies, with possible carcinogenic effects related to high chronic exposure (EC/HC, 2008b). In the specific case of marine environments, siloxanes were reported as generally not toxic to organisms at their low values of solubility in the aqueous media (Arespacochaga et al., 2015), but the knowledge on this subject is still scarce.

Several studies are currently being developed by various groups representing governments, industries and academics, in order to support scientific and technological knowledge about the risk

assessment of the presence of these substances in the environment, as well as a better understanding of the toxicological effects to animals and humans. In this sense, environmental risk assessment evaluations on cVMSs have been carried out in Europe and Canada. Environment Canada and Health Canada published in 2008 reports assessing the risk of cVMS to the environment, stating that D4 and D5 were identified as persistent, bioaccumulative, toxic and prone to ecological harm, while D6 was not considered likewise (EC/HC, 2008a,b,c). In 2009, the risk assessment report published by the UK Environment Agency considered D4 and D6 as a threat to the environment, while D5 was considered out of danger (Brooke et al., 2009a,b,c). Recently, the United States Environmental Protection Agency (US EPA) included D4 in a list of chemicals to be reviewed for their further assessment under the Toxic Substance Control Act (Sanchís et al., 2015), and the European Commission classified D4 as an endocrine disruptor, possible of interfering with human endocrine function and eventually impairing human fertility (EC no. 1272/2008). According to a report from the Norwegian Pollution Control Authority in 2007 (Schlabach et al., 2007), Norway has placed D5 on a priority list of substances of which the emissions should be considerably reduced or halted.

2.4 Analytical Methodologies in Siloxanes Detection and Quantification

In order to better understand the behaviour and contamination levels of siloxanes in the environment, it is crucial to have adequate analytical protocols to identify and quantify them in as many matrices as possible.

Analytical determination of siloxanes at trace levels is described in the literature as a demanding task. Some problems derive from organosiloxanes' physical and chemical properties, such as high volatility of lower Ln and Dn methylsiloxanes and the tendency of these compounds to interconversion via hydrolysis or condensation that may mitigate analysis results (Chainet et al., 2011). However, the major problem when analysing the presence of these compounds at trace levels is their ubiquity. Siloxanes, especially VMSs, are present in the air outdoor and indoor, in laboratory equipment and artefacts (caps, vials with O-ring seals, plastic materials, and also in silicon based injection septa, inlet liners with silanized glass wool, or even from bleeding of the fillings of gas chromatography columns), solvents and reagents used during laboratory procedures, and even in clothes and PCPs used by laboratory workers (Wang et al., 2013a; Chainet et al., 2011). Therefore, there is a high potential for the external contamination of samples during collection and handling procedures, mostly by cVMSs.

Potential sources of contamination during sample collection, preparation and analysis must be minimized, and solvents and reagents should be analysed to assess possible residual VMSs concentrations (Wang et al., 2013a). Therefore, to ensure the most accurate results, the usage of PCPs must be avoided and handling of samples should be ideally performed under controlled environments

with air filtration. Appropriate procedural blanks and detection limits should also be applied to avoid erroneous results promoted by external contaminations (Warner et al., 2015).

Different analytical methods have been employed on the detection and quantification of siloxanes at trace levels. Those usually include sample preparation and extraction, a clean-up step and finally, the chromatographic analysis.

2.4.1 Extraction Techniques

Since VMSs are present in different matrices, in order to identify and quantify the target analytes, samples often undergo extraction procedures prior to analysis. Therefore, and depending on the properties of the compounds and the nature of the samples, several techniques are available to isolate and concentrate the compounds under examination, or even to remove contaminants that may influence their detection and quantification. However, considering the concerns associated with the analysis of VMSs at trace levels and the high risk of sample contaminations, the protocols should be optimized to as less steps as possible.

Some of the more common extraction techniques employed in the detection and quantification of siloxanes in biota samples (homogenized or not) are presented in this section. However, it is important to state that in literature, a combination of one or more techniques is usually reported, with the sample pre-treatment varying considerably (Wang et al., 2013b).

2.4.1.1 Liquid-Liquid Extraction (LLE)

LLE is a very commonly used technique for the extraction of the interest analytes from aqueous samples or liquid suspensions, based on mass transfer principles. Typically, an immiscible solvent enters in contact with the liquid sample, towards which the analyte of interest shows high solubility. In order to improve the mass transfer, agitation of the two phases is performed, with recovery of the phase to which the compound of interest has higher solubility (Hong et al., 2014).

2.4.1.2 Solid-Liquid Extraction (SLE)

In the case of SLE, a suitable solvent is used to remove the target analyte from solid material. The choice of the solvent is very important, since the compounds of interest must be soluble in it. If possible, samples are powdered, crushed or milled in order to increase the surface area of the solid particles towards the liquid phase. As for LLE, the two-phase system is agitated to enhance the mass transfer of the analytes to the liquid phase. Recovery of the solvent can be achieved by filtration or centrifugation of the suspension (Warner et al, 2010).

2.4.1.3 Ultrasound Extraction (USE)

Ultrasound extraction is a procedure in which a solvent is added to the liquid or solid matrix and then the sample is subjected to the effect of ultrasound waves. During the sonication process, longitudinal ultrasound waves create regions of alternate compression of the medium, occurring cavitation phenomena that promote the formation of bubbles. Consequently, large amounts of energy are released to the medium, promoting greater solvent penetration in the sample matrix, enhancing the mass transfer of the analytes to the solvent. This technique commonly reduces working times and increases extraction yields. After sonication, the solvent must be collected. A filtration or a centrifugation step is usually applied (Picó, 2013).

2.4.1.4 Solid-Phase Extraction (SPE)

The main principle of solid-phase extraction (SPE) involves the partition of solutes between two different phases, usually a liquid phase (commonly the sample matrix or the solvent with the target analytes) and a solid (sorbent) phase. Based on the affinity of solutes dissolved or suspended in the liquid phase for the sorbents, this technique allows the clean-up of samples and extracts and the concentration of the target analytes prior to instrumental analysis (Zwir-Ferenc and Bziuk, 2006). It is a very common technique, as it is easy to perform and appears to be more efficient and less time-consuming when compared to LLE, with very good recoveries of the analytes.

2.4.1.5 QuEChERS - Quick, Easy, Cheap, Effective, Rugged and Safe

The term QuEChERS is derived from the words Quick, Easy, Cheap, Effective, Rugged and Safe, characteristics of this extraction technique. Developed by Anastassiades et al. (2003), this method was first used for the analysis of residue pesticides in food samples. However, due to the simplicity of the technique and its considerable advantages over other extraction procedures, QuEChERS methodologies have lately been applied to the analysis of many other compounds.

In brief, the sample is put in a tube for a solvent extraction procedure. Mixing and ultrasound extraction are very common steps to promote the contact of the solvent with the sample. Salts and buffers are used to promote the extraction of the analytes to the organic phase and a subsequent clean-up process, through a dispersive solid-phase extraction using selected sorbents, helps to remove undesired components of the extract (Capela et al., 2016b). This is a low solvent consumption, low cost and time saving technique when compared to other extraction procedures, resulting in good recovery rates and reproducible results.

2.4.2 Instrumental Method – Gas Chromatography/Mass Spectrometry

Gas chromatography is an analytic technique that allows the separation and analysis of the different components on a mixture, as long as those can be volatilized without decomposing. After injection and vaporization of the sample, the molecules are transported by an inert carrier gas through a stationary phase. This stationary phase is commonly a microscopic layer of a polymeric fluid atached to an inert solid support, usually a tubular piece of glass or metal called chromatographic column. The constituints of the sample, transported by the carrier gas, will interact differently with the stationary phase, eluting at different times (called retention times).

In order to identify and/or quantify the molecules eluting from the chromatographic column, a variety of detectores are available. However, a mass spectrometer (MS) is the most used for the analysis of organic compounds in complex matrices, providing high sensitivity and specificity, reducing the possibility of false positive results. MS detectors ionize the eluting chemical species and sort the resulting ions based on their mass to charge ratio (*m/z*) (Hübschmann, 2009). Therefore, gas chromatography accoplated to a mass spectrometer detector (GC/MS) allows specific determination of trace amounts of compounds. A brief description of the MS detector is presented in Appendix 1.

A major concern with VMSs analysis at trace levels is the septa used in the GC inlet, usually made of silicone rubber, which can release cVMSs under high temperatures, interfering with the quantitative assessments of the chromatograms. Using a septum-less sampling head is an alternative to reduce these background contaminations (Chainet et al., 2011; Wang et al., 2013b).

Taking into account that the analysis of siloxanes is usually done at trace levels, and considering the properties of VMSs, especially their volatile behaviour and thermal stability, it is easy to understand why in literature almost all instrumental analysis of such compounds are commonly perfomed by GC/MS (Warner et al., 2010, Chainet et al., 2011). A brief review of the available studies on the determination of VMSs in different biota matrices and water from marine environments was accomplished, and the state of the art on this subject is presented below.

3 State of the Art

The thermal stability, resistance to oxidation, reduction and photodegradation of siloxanes seems to favour their presence in the environment (Sanchís et al., 2015). Therefore, considering the massive increasing production of VMSs and their use in PCPs, it is reasonable to assume that they will end up being released to marine environments, with potential to bioaccumulate and biomagnify, despite showing low water solubility (Xu et al., 2014). Because of that, and also taking into account the lack of certainty about the inexistence of ecotoxicological risks and toxicological effects, an interest of the scientific community on this subject has emerged in recent years. As a result, some studies have been conducted on assessing the presence of siloxanes in the ecosystems and its effects in trophic chains.

Nevertheless, to date there is not much information available on the distribution, fate and trends of siloxanes in marine environments. The scenario is even scarcer considering seaweed matrices, with only one study being available in literature, to the author's best knowledge. Therefore, in order to have a better assessment of the level of contamination of marine environments by siloxanes, studies reporting VMSs in sea water and in general biota from marine environments were considered in this section. Table 3 shows an overview of the studies found in literature, conducted in different geographical areas: the European Arctic (Warner et al., 2010) and Nordic countries such as Denmark, Finland, Iceland, Norway and Sweden (Kaj et al., 2005), the Dalian Bay in Northern China (Hong et al. 2014; Jia et al. 2015) and the Antarctic Ocean (Sanchís et al., 2015).

The only work found so far in the literature regarding siloxanes in seaweed was conducted by Jia et al. (2015), in which the trophic transfer of cVMSs in the marine food web of Dalian Bay, in northern China, was studied. The siloxanes found at higher concentrations in sea lettuce (*Ulva pertusa*) samples were D4, D5 and D6, with 6.33 ± 2.31, 5.33 ± 3.39 and 10.25 ± 11.7 $ng.g^{-1}_{ww}$ (wet weight), respectively. Those values were lower when compared to the same compounds concentration in other biota samples with an estimated higher trophic level (average D4, D5 and D6 concentrations detected in 5 different fish species were, respectively, 14.0 ± 8.89, 31.7 ± 29.6 and 19.1 ± 2.2 $ng.g^{-1}_{ww}$). Regarding the concentrations presented in this study, high error values were obtained in most of the quantifications, possibly due to the complexity of the matrices analysed and attesting the difficulties on the determination of siloxanes in naturally contaminated samples at trace levels. Nevertheless, results of this study by Jia et al. (2015) showed a strong biomagnification of cVMS in the marine food web in question. Since the author did not find other studies regarding VMSs contamination in macroalgae, assessing this kind of matrices constitutes an interesting and valuable way to evaluate the presence and behaviour siloxanes in marine environments. Due to their tendency to accumulate pollutants in their tissue and surface area, seaweeds have been used in the biomonitoring of pollutants in aquatic

environments (Roberts et al., 2008). Moreover, as seaweeds occupy low trophic levels in food chains, it is possible that these organisms may be a vector for the accumulation of siloxanes into higher trophic levels (Gutow et al., 2016), thus being important to know if they are prone to retain siloxanes or not.

Determinations of VMSs in other biota samples from marine environments, ranging from phytoplankton and krill (Sanchís et al., 2015), to fish body, mussel and liver (Kaj et al., 2005; Hong et al., 2014), showed, in general, higher concentrations of siloxanes than those present in seaweed and water samples. That may be the result of possible biomagnification in trophic chains, or maybe just a consequence of the tendency identified by Sanchís et al. (2015) of the predominance of siloxanes detected in samples with higher carbon and lipid contents. The study conducted by Warner et al. (2010) in human edible fish from the European Arctic (cod fish and sculpin) reports the presence of only three cVMSs (D4, D5 and D6) in two different locations. Concentrations of D5 are always reported to be higher than those of D6 and D4, with the last being the one found in lower concentrations.

On siloxanes present in marine water samples, only two studies were found. Kaj et al. (2005) assessed the presence of lVMSs and cVMSs in marine water from the Northern Europe (coastal area from Reykjavik, Iceland) and Hong et al. (2014) studied the occurrence of lVMSs, PDMSs and cVMSs in the Dalian Bay, northern China. Both studies concluded that cVMSs showed higher concentrations in marine water than lVMSs, suggesting a predominant usage of cVMSs around the study areas.

Regarding the analytical methods employed, Sanchís et al. (2015) assessed the presence of VMSs in Antarctic matrices using a ultrasound assisted extraction (USE) procedure in order to extract cVMSs and lVMSs from biota matrices (vegetation, phytoplancton and krill). Samples were homogenized and the extraction was carried out with *n*-hexane for 25 min in an ultrasonic bath, followed by centrifugation at 1700 g for 10 min. On another study, a thermal desorption system coupled with GC/MS was adopted for the analysis of VMSs in the Nordic environment by Kaj et al. (2005), with Tenax TA as the trapping agent, and nitrogen. Water and biota samples were successfully analysed with this method. Other adsorbent materials have been referred to allow VMSs extraction, such as XAD® resins, activated carbon, polyurethane foam, tetradecane and the highly retentive nonpolar sorbent ISOLUTE® ENV+ (Wang et al., 2013a). A method for VMSs extraction from biota samples without the need for homogenization was developed by Kierkegaard et al. (2010), thus reducing sample contamination. Biota samples were heated in water and VMSs purged from the tissues and trapped in Isolute ENV+ sorbent cartridges (Kierkegaard et al., 2010; Wang et al., 2013a). Solvent extraction is also a common way to handle water and biota samples, with nonpolar or low polar solvents (alone or combined) usually selected for the extraction of VMSs. For instance, *n*-hexane proved to be an excellent solvent when extracting VMSs, mostly due to its non-polarity and water immiscibility, allowing a one-step sample preparation method consisting on a *n*-hexane extraction with vortexing or mixing, followed by centrifugation, with the supernatant being recovered ready to be injected in GC analyser. Several studies used *n*-hexane extraction with small variations, allowing excellent recovery and repeatability

results (Kaj et al., 2005, Warner et al., 2010; Sanchís et al., 2015), even for small quantities of sample (usually less than 1 g or mL) and low solvent volumes. Other solvents used in extraction of VMSs referred in literature are dichloromethane (DCM), by Hong et al. (2014) for extraction of siloxanes from sea water samples, as well as ethyl acetate (EtAc) and acetonitrile (ACN), individually or in a 1:1 (v/v) mixture with *n*-hexane by Hong et al. (2014), Sparham et al. (2011) and Jia et al. (2015) on biota matrices.

Regarding the extraction and analysis of siloxanes at trace levels, background contamination must be avoided. Therefore, some preventive actions are commonly described in literature, such as limiting or banning PCPs in the lab, use of powder-free nitrile gloves when handling samples, extra care in cleaning the material (often solvent rinsed), usage of controlled air environments and frequent performance of procedural blank analysis (Warner et al., 2010; Chainet et al., 2011; Wang et al., 2013b). When preparing samples for the analysis of VMSs, the solvent evaporation steps (for instance by rotary evaporation or nitrogen blowdown) can result in the volatilization and subsequent loss of the target analytes. Therefore, these procedures should be avoided (Rücker et al, 2015).

Recently, new advantageous and environmentally more conscientious analytical approaches have been employed for the determination of siloxanes in terrestrial vegetation, among other matrices. Ramos et al. (2016) has developed a solvent-saving approach using QuEChERS technology to assess the presence of siloxanes in pine needles. QuEChERS methodology, when compared to more conventional extraction techniques, has been reported to have several advantages, being an interesting time and cost saving procedure, easy to apply and prone to reduce handling of extracts (Homem et al., 2013). This method was successfully employed by Ratola et al. (2016) to evaluate levels of contamination in pine needles in eight different sites in the Portuguese territory, with results showing total concentrations of siloxanes (L2-L5 and D3-D6) between 2 and 118 $ng.g^{-1}_{dw}$. In that study, the predominance of cVMSs in comparison to lVMSs was reported, in line with the previous mentioned works on siloxanes presence in biota samples from marine environments. Soils and passive air samples were also analysed and especially in the latter, there was a significant incidence of the total siloxanes in summer on the two beach sites selected. This again justifies a close attention that should be given to coastal tourist-packing environments in the study of siloxanes behaviour.

Analytical methods using QuEChERS applied in seaweed samples have been reported in literature: it has been used for the determination of bisphenol A and tetrabromobisphenol A, two organic pollutants, in seaweeds (Cunha et al., 2017), while Piovar et al. (2013) employed it for the extraction of purified and stable fucoxanthin from a macroalgae rich in that carotenoid. However, to the author's best knowledge, QuEChERS-based extractions have never been used for the determination of siloxanes in seaweed.

Thereupon, a QuEChERS-GC/MS methodology seems to be a very interesting approach for the analysis of trace levels of VMSs in seaweed, contributing for the better knowledge of the dispersion and behaviour of such contaminants in coastal environments.

Table 3. Overview of the studies on the determination of siloxanes in macroalgae, other marine biota and sea water.

Matrix	Analytes	Extraction method	Instrumental method	%Rec (from spike sample)	LOD ($ng \cdot g^{-1}_{ww}$)	LOQ ($ng \cdot g^{-1}_{ww}$)	C ($ng \cdot g^{-1}_{ww}$) min – max (mean)	References
Macro algae – sea lettuce (Northern China, Dalian Bay)	D4 D5 D6 D7 L4-L17	SLE 30 min (hex/EtAc 1:1 v/v) x 3 Solvent-Exchange to Isooctane	GC/MS	Ranging from 71 ± 11 (L17) to 103 ± 11 (D6)	2.60 2.12 1.68 1.08 ranging from 0.57 (L5) to 1.32 (L4)	5.07 3.86 3.39 1.95 ranging from 0.36 (L6) to 3.96 (L4)	(6.32 ± 2.31) (5.83 ± 3.38) (10.5 ± 11.7) (2.73 ± 1.52) ND	Jia et al., 2015
Muscle from fish – 5 different species (Northern China: Dalian Bay)	D4 D5 D6 D7 L4- L17	SLE 30 min (hex/EtAc 1:1 v/v) x 3 Solvent-Exchange to Isooctane	GC/MS	Ranging from 71 ± 11 (L17) to 103 ± 11 (D6)	2.60 2.12 1.68 1.08 ranging from 0.57 (L5) to 1.32 (L4)	5.07 3.86 3.39 1.95 ranging from 0.36 (L6) to 3.96 (L4)	(14.0 ± 8.98) (31.7 ± 29.6) (19.1 ± 12.2) (3.36 ± 2.29) ND	Jia et al., 2015
Invertebrates – crab, mollusk, clamworm (Northern China: Dalian Bay)	D4 D5 D6 D7 L4- L17	SLE 30 min (hex/EtAc 1:1 v/v) x 3 Solvent-Exchange to Isooctane	GC/MS	Ranging from 71 ± 11 (L17) to 103 ± 11 (D6)	2.60 2.12 1.68 1.08 ranging from 0.57 (L5) to 1.32 (L4)	5.07 3.86 3.39 1.95 ranging from 0.36 (L6) to 3.96 (L4)	(6.51 ± 2.90) (8.92 ± 6.03) (14.0 ± 8.48) (3.14 ± 1.98) ND	Jia et al., 2015
Fish-body (Northeast China, Dalian Bay)	D4 D5 D6 D7 L9 L10 L11 L4, L5, L6, L7, L8, L12, L13, L14, L15, L16, L17	SLE 30 min (hex/EtAc 1:1 v/v) x 3 Solvent-Exchange to Isooctane	GC/MS	98 ± 9 103 ± 11 105 ± 12 96 ± 10 88 ± 11 86 ± 14 91 ± 9 ranging from 71 ± 12 (L17) to 100 ± 8 (L4)	0.99 1.01 0.50 0.20 MDL: 0.69 MDL: 0.67 MDL: 0.72 ranging from 0.84 (L14) To 5.87 (L4)	1.15 1.41 0.64 0.43 NA NA NA NA	0.213 – 0.615 (0.424) 0.451 – 1.150 (0.826) 0.891 – 1.880 (1.310) 0.311 – 0.567 (0.426) BDL – 1.20 (0.656) BDL – 1.32 (0.593) BDL – 1.88 (0.925) ND	Hong et al., 2014

NA – not available; ND – not determined; BDL – below detection limit

Table 3. Overview of the studies on the determination of siloxanes in macroalgae, other marine biota and sea water (cont.).

Matrix	Analytes	Extraction method	Instrumental method	%Rec (from spike sample)	LOD ($ng \cdot g^{-1}$)	LOQ ($ng \cdot g^{-1}$)	C ($ng \cdot g^{-1}$) min - max (mean)	References
					($ng \cdot g^{-1}_{ww}$)	($ng \cdot g^{-1}_{ww}$)	($ng \cdot g^{-1}_{ww}$)	
Biota: marine fish, sea mammals, eggs (Coastal areas of Nordic Environment: Denmark, Faroe Islands, Finland, Iceland, Norway, Sweden)	D3 D4 D5 D6 L2 L3 L4 L5	SLE 5 min (hex)	GC/HRMS	NA	50 5 5 5 0.4 0.3 0.4 0.5	150 15 15 15 NA NA NA NA	BDL - 74 BDL – 70 BDL – 2200 BDL – 74 BDL BDL BDL – 1.1 BDL	Kaj et al., 2005
Fish liver: atlantic cod (European Arctic: Adventfjorden)	D4 D5 D6	SLE 30 min (hex)	GC/HRMS	83 ± 7 (^{13}C-D4) 84 ± 7 (^{13}C-D5) 91 ± 11 (^{13}C-D6)	($ng.g^{-1}_{lw}$) MDL: 10.8 MDL: 6.5 MDL: 2.1	($ng.g^{-1}_{lw}$) NA NA NA	($ng.g^{-1}_{lw}$) BDL 45.5 – 358 (176) 5.3 – 13.8 (10.1)	Warner et al., 2010
Fish liver : atlantic cod (European Arctic: Kongsfjorden)	D4 D5 D6	SLE 30 min (hex)	GC/HRMS	83 ± 7 (^{13}C-D4) 84 ± 7 (^{13}C-D5) 91 ± 11 (^{13}C-D6)	MDL: 2.2 MDL: 1.5 MDL: 0.7	NA NA NA	BDL ng/lipid weight 12.7 – 29.1 (18.3) 10.7 – 52.8 (25.6)	Warner et al., 2010
Fish liver: sculpin (European Arctic: Adventfjorden)	D4 D5 D6	SLE 30 min (hex)	GC/HRMS	83 ± 7 (^{13}C-D4) 84 ± 7 (^{13}C-D5) 91 ± 11 (^{13}C-D6)	MDL: 10.8 MDL: 6.5 MDL: 2.1	NA NA NA	BDL ng/lipid weight 54.3 – 2150 (530.8) BDL – 30.6 (17.7)	Warner et al., 2010
					($ng.g^{-1}_{dw}$)	($ng.g^{-1}_{dw}$)	($ng.g^{-1}_{dw}$)	
Phytoplankton (Antarctic Ocean: Drake Passage, Bransfield Strait, South Scotia, Bellingshausen Weddell Seas)	D3 D4 D5 D6 L3 L4 L5 L6	USE 25 min (hex)	GC/MS/MS	72 – 81 73 – 85 73 – 85 71 – 91 83 – 90 74 – 92 75 – 92 74 - 92	- - - - 0.046 0.12 0.39 0.33	0.49 0.74 0.48 0.31 0.16 0.39 1.3 1.1	BDL - 10.0 (4.22) 0.30 – 3.50 (0.93) 0.30 – 27.0 (3.24) 0.10 – 8.80 (1.17) 6.10 – 88.0 (29.1) BDL - 17.0 (7.41) BDL - 15.0 (11.0) BDL – 120 (9.90)	Sanchís et al., 2015

NA – not available; ND – not determined; BDL – below detection limit

Table 3. Overview of the studies on the determination of siloxanes in macroalgae, other marine biota and sea water (cont.).

Matrix	Analytes	Extraction method	Instrumental method	%Rec (from spike sample)	LOD	LOQ	C min – max (mean)	References
				($ng.g^{-1}_{dw}$)	**($ng.g^{-1}_{dw}$)**	**($ng.g^{-1}_{dw}$)**	**($ng.g^{-1}_{dw}$)**	
Krill (Antarctic Ocean: Drake Passage, Bransfield Strait, South Scotia, Bellingshausen Weddell Seas)	D3	USE 25 min (hex)	GC/MS/MS	72 – 81	-	0.49	4.48 – 154 (36.4)	Sanchís et al., 2015
	D4			73 – 85	-	0.74	12.3 – 117 (48.9)	
	D5			73 – 85	-	0.48	21.3 – 63.1 (36.6)	
	D6			71 – 91	-	0.31	11.5 – 72.7 (35.2)	
	L3			83 – 90	0.046	0.16	BDL – 81.6 (69.6)	
	L4			74 – 92	0.12	0.39	BDL	
	L5			75 – 92	0.39	1.3	BDL	
	L6			74 - 92	0.33	1.1	BDL	
					(ng/V_{sample})	**($ng \cdot L^{-1}$)**	**($ng \cdot L^{-1}$)**	
Sea Water (Coastal area from Reykjavik, Iceland)	D4	Purge & Trap extraction 20 min to 2 h (nitrogen through Tenax TA adsorbent)	GC/MS	NA	4.7	NA	<30L	Kaj et al., 2005
	D5				2.5		<30	
	D6				2.6		<30	
	L2				0.03		<0.5	
	L3				0.04		<0.5	
	L4				0.03		<0.5	
	L5				0.25		<0.3	
				($ng \cdot L^{-1}$)	**($ng \cdot L^{-1}$)**	**($ng \cdot L^{-1}$)**	**($ng \cdot L^{-1}$)**	
Sea Water (Northeast China, Dalian Bay)	D4	LLE 1 h (DCM) x 3	GC/MS	105 ± 9	4.43	7.26	BDL – 55.8 (6.93)	Hong et al., 2014
	D5			110 ± 11	2.75	3.72	0.830 – 40.3 (12.2)	
	D6			104 ± 10	1.99	2.76	0.77 – 56.6 (16.5)	
	D7			101 ± 12	MDL: 0.79	NA	BDL – 21.5 (5.66)	
	L8			97 ± 11	MDL: 1.24	NA	BDL – 1.55 (0.855)	
	L9			90 ± 13	MDL: 1.41	NA	BDL – 4.72 (1.14)	
	L10			91 ± 15	MDL: 0.97	NA	BDL – 4.97 (1.15)	
	L11			93 ± 8	MDL: 1.03	NA	BDL – 2.02 (0.935)	
	L12			89 ± 6	MDL: 1.03	NA	BDL – 1.06 (0.706)	
	L4, L5, L6, L7, L13, L14, L15, L16, L17			ranging from 83 ± 10 (L16) to 105 ± 9 (L4)	MDL: from 1.45 (L14) to 10.76 (L4)	NA	ND	
						NA	ND	
						NA	ND	

NA – not available; ND – not determined; BDL – below detection limit

4 Technical Description

4.1 Chemicals and Materials

Individual standards of the VMSs selected for analysis (hexamethyldisiloxane (L2), octamethyltrisiloxane (L3), decamethyltetrasiloxane (L4), dodecamethylpentasiloxane (L5), hexamethylcyclotrisiloxane (D3), octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexasiloxane (D6)), with purity levels higher than 97%, were purchased from Sigma-Aldrich (St. Louis, MO, USA) and stored at -20 °C, as well as tetrakis(trimethylsilyloxy)silane (M4Q), used as internal standard. Analytical grade *n*-hexane (Hex), dichloromethane (DCM) and acetone (Acet) purchased from VWR (Fontenay-sous-Bois, France), were used as solvents in different processes of the work. Technical grade acetone was also used in syringes and glass material wash. Anhydrous magnesium sulphate ($MgSO_4$) and sodium acetate (CH_3COONa), acquired from Sigma–Aldrich (St. Louis, MO, USA), primary and secondary amine (PSA)-bonded silica and octadecyl-silica (C18), from Supelco (Bellefonte, PA, USA), were used for the QuEChERS preparation. Florisil® (magnesium silicate, particle size 0.150-0.250 mm) and alumina (neutral aluminium oxide 90, particle size 0.063-0.200 mm), purchased from Merck (Darmstadt, Germany), were also used as sorbents in preliminary tests. $MgSO_4$, Florisil® and alumina were baked overnight at 450 °C before use. Nitrogen (99.995%), for sample evaporation, and helium (99.9999%), used as carrier gas in the GC/MS system, were supplied by Air Liquide (Maia, Portugal).

4.2 Standards Preparation

All solutions and proper dilutions were prepared in *n*-hexane and protected from light in amber glass vials or using aluminium foil. When stored until use, preparations were kept in the dark at -20 °C. Individual stock solutions of each standard at approximately 1.0 $g.L^{-1}$ were prepared, from which a mix stock solution containing all the eight siloxanes under study at a concentration of 5.0 $mg.L^{-1}$ and a diluted M4Q individual stock solution at 5.0 $mg.L^{-1}$ were obtained. Using the previous stock solutions and proper dilutions, eight calibration standards, with concentrations of each target analyte ranging from 1 to 750 $\mu g.L^{-1}$, and a final concentration of internal standard of 250 $\mu g.L^{-1}$, were prepared as well. Mix solutions at 3 different concentration levels (25, 100 and 500 $\mu g.L^{-1}$) were used to spike samples in preliminary and method validation tests, and M4Q solutions at 250 $\mu g.L^{-1}$ were prepared to be used in the extraction of the samples as the internal standard.

4.3 QuEChERS Preparation

Two different QuEChERS were employed in the extraction procedure. Even though commercial QuEChERS are available, both QuEChERS 1 and 2 were prepared in the lab, since this procedure was found to be economically favourable and equally good in performance, allowing better control of the mass ratio of the compositions selected (Homem et al., 2013; Ramos et al., 2016). QuEChERS 1 (Q1) contained 6 g of anhydrous $MgSO_4$ and 1.5 g of CH_3COONa and was used to promote phase separation: anhydrous magnesium sulphate is hygroscopic, absorbing the water present in the sample, while sodium acetate ensures the maintenance of the polarity in the aqueous phase, promoting the migration of the analytes to the organic phase. QuEChERS 2 (Q2) consisted of 900 mg of $MgSO_4$, 300 mg of PSA-bonded silica and 150 mg of C18 and was used to remove undesired compounds in a dispersive solid-phase clean-up step: anhydrous $MgSO_4$ ensures water removal, the polarity of PSA bonded silica helps in the adsorption of polar impurities and C18, nonpolar, promotes removal of nonpolar interferences. Both QuEChERS 1 and 2 were prepared in 50 mL polypropylene conical bottom centrifuge tubes using a Mettler Toledo AG 245 analytical balance, and stored in a desiccator until use.

4.4 Optimization of the Extraction Procedure

The development of the extraction procedure was initially based on the protocol validated and employed by Ramos et al. (2016) and Ratola et al. (2016) for the extraction and quantification of siloxanes in pine needles: 2.5 g of sample were spiked with IS at 15 $ng.g^{-1}$ and extracted with 10 mL of DCM/Hex, 1:1, in an ultrasonic bath after 3 min of vortex. After cooling to room temperature, Q1 was added and the mixture was vortexed for 3 min and centrifuged for 10 min at 4000 rpm (2670 *g*). The supernatant was then transferred to another tube containing Q2, again vortexed and centrifuged in the same conditions as before. The supernatant was collected and dried under a nitrogen stream till dryness, and finally dissolved in 150 µL of *n*-hexane and collected for analysis. First of all, aiming to assess the contamination levels of siloxanes from lab related sources, prior to the initiation of preliminary tests for development of the extraction procedure, procedural blanks were performed in duplicate (n=2) in three different circumstances: extractions in the lab bench, in the fume hood with the ventilation system turned on and in the fume hood with the ventilation system turned off. Extracts were then analysed and the response factors (ratio of the compound's peak area per the internal standard's peak area) were compared. After this first test, extractions were performed in the location prone to less VMSs contamination.

Some preliminary tests were performed starting from the described protocol. The solvent evaporation step was enhanced, with two strategies for the solvent evaporation step being tested: one according to the protocols employed by Ramos et al. (2016) and Ratola et al. (2016), and another

avoiding the total dryness of the extracts (instead of drying the extract completely under the nitrogen stream and recover it with 150 µL of *n*-hexane, the solvent was evaporated to a volume less than 150 µL, measured with a microliter syringe, and *n*-hexane was then added, again with a microliter syringe, to complete the final extract volume of 150 µL). Those two different approaches were tested by spiking two samples with the VMSs under analysis at 6 $ng.g^{-1}$. Three different extraction solvents were also tested: pure *n*-hexane (Hex) and two solvent mixtures (Hex/DCM 1:1 and Hex/Acet 1:1). To assess the performance of each solvent, extractions were performed in triplicate (n=3) with samples spiked with the target analytes at 6 $ng.g^{-1}$. Finally, the resultant recoveries obtained for four different compositions of the Q2 were also analysed: the quantities of $MgSO_4$ (900 mg), which acts as a drying agent ensuring the removal of any remaining water, and C18 (150 mg), used to remove nonpolar contaminants, such as lipids and waxes, were kept constant. An amount of 300 mg of a sorbent (or mixture of sorbents) aiming the removal of polar compounds, like sugars, fatty acids and pigments, was the parameter varied in the composition of Q2, with the use of PSA, alumina, Florisil® and a combination of the three (100 mg of each) being tested. The performance of the four different compositions of Q2 was assessed by the extraction, for each one of them, of three samples spiked with a mix solution of siloxanes at 6 $ng.g^{-1}$, as described before, using *n*-hexane as solvent.

4.5 Naturally Contaminated Samples

In this work, six sampling campaigns were performed in low tide conditions in the months of May, June, July, August, September and December 2016, in three different locations in the seashore of the North Region of Portugal, in the surroundings of Porto Metropolitan Area, and in one other further south, close to Aveiro (Figure 1). Those sites were selected considering different anthropogenic pressures in their vicinities: a beach close to the industrial complex of the Matosinhos refinery (Conchinha), a beach with high level or urbanization in its surroundings (Carneiro) and another one with high occupation levels during summer, but with less urban development in its vicinities (Miramar). The site near Aveiro, beach of Barra, was selected because of its high levels of occupation during summer, with a different background when compared to the locations from the North Region of Portugal. Due to the lack of time, it was not possible to analyse all the collected material in those locations in all months. Therefore, only some seaweed samples were selected for analysis with two major goals being selected: identify possible species of seaweed as more suitable for the determination of siloxanes in this kind of matrices, and assess temporal trends on the presence of these compounds in samples from a specific location. Regarding the first goal, two locations in the North Region of Porto were selected to analyse all the species collected in the month of August: beaches of Conchinha (five different species: an unidentified species of kelp, *Ulva lactua*, *Porphyra* sp., *Codium tomentosum* and *Fucus vesiculosus*) and Carneiro (six different species: *U. lactua*, *C. tomentosum*, an unidentified

species of kelp, *Porphyra* sp., *Palmaria palmata* and *Schizymenia dubyi*). Those were the locations (and month) where more different material was available. However, samples were collected in an unusual cloudy and foggy day for the season, with almost no people in the beaches at the time of sampling. On both locations, samples were collected according to their availability in the dry zone of the beach, except for *F. vesiculosus* in the beach of Conchinha, which was collected from the rocks. The second approach consisted of analysing the same species of macroalgae in a specific location, in different months. For this, *Porphyra* sp. from the beach of Miramar and *F. vesiculosus* from Aveiro region (beach of Barra), collected monthly from May to December (except in October and November), were analysed. Both samples were collected in different sites of those beaches, from rocks in the dry zone during low tide conditions. This aimed to investigate possible seasonal trends on the siloxanes presence in seaweed in these spots.

On a third level it was also possible to apply the analytical protocol validated to a different kind of marine plant from a region with different socio-geographic and climatic patterns (the Mediterranean Sea in the Region of Murcia, southeast of Spain). Therefore, *Posidonia oceanica*, a seagrass species with ribbon-like leaves endemic to the Mediterranean Sea, was also collected in seven different sites in the Region of Murcia, Spain, in two campaigns (July 2015 and February 2016). The seven locations (Figure 1) were again chosen to cover different backgrounds: a remote site in Cala Blanca, a beach in the small town of La Azohía, an urban beach close to the entrance of the Port of Cartagena and in the vicinities of the refinery of Cartagena (Cala Cortina), and the remaining four in La Manga del Mar Menor (a spit of 21 km long and about 100 m wide, separating the Mediterranean Sea from the Mar Menor), with two locations selected in Mar Menor and two sites in the Mediterranean Sea, all of them in beaches with high occupancy levels, especially during summer months. Mar Menor is a salt water lagoon with high levels of pollution reported, mostly due to the impacts of mining and agricultural activities, as well as a major urban development in its surroundings during the last decades (Conesa et al., 2007), thus being an interesting spot to analyse and compare with samples from the Mediterranean Sea. Samples were collected from similar sites in both summer and winter sampling campaigns, in low tide conditions in the tide zone of the selected site, inside water.

Overall, collection of samples was performed using gloves, with approximately 50 to 100 g of sample being stored in plastic bags after being shaken and drained to remove sand and water from the macroalgae surface. Finally, samples were kept refrigerated and protected from light until proper storage in the lab at -20 °C until analysis. In Figure 2, pictures of the different species of macroalgae and the marine plant analysed in this work are presented, where major macroscopical differences between the different species can be observed (*U. lactua* and *Porphyra* sp. are thin, flat surfaced algaes, while *P. palmata* and *S. dubyi* are fleshy leather textured brownish-red fronds; kelps are a group of large brown seaweeds, with its thallus consisting of flat or leaf-like structures known as blade*; C. tomentosum* is a branched structure green algae with thin branches with a circular cross section, and

F. vesiculosus is a brown algae also with a branched structure but with longer and wider fronds with a more irregular surface, where spherical air filled bladders are present). All samples were extracted in duplicate (n=2), and procedural blanks were performed for each batch of samples analysed.

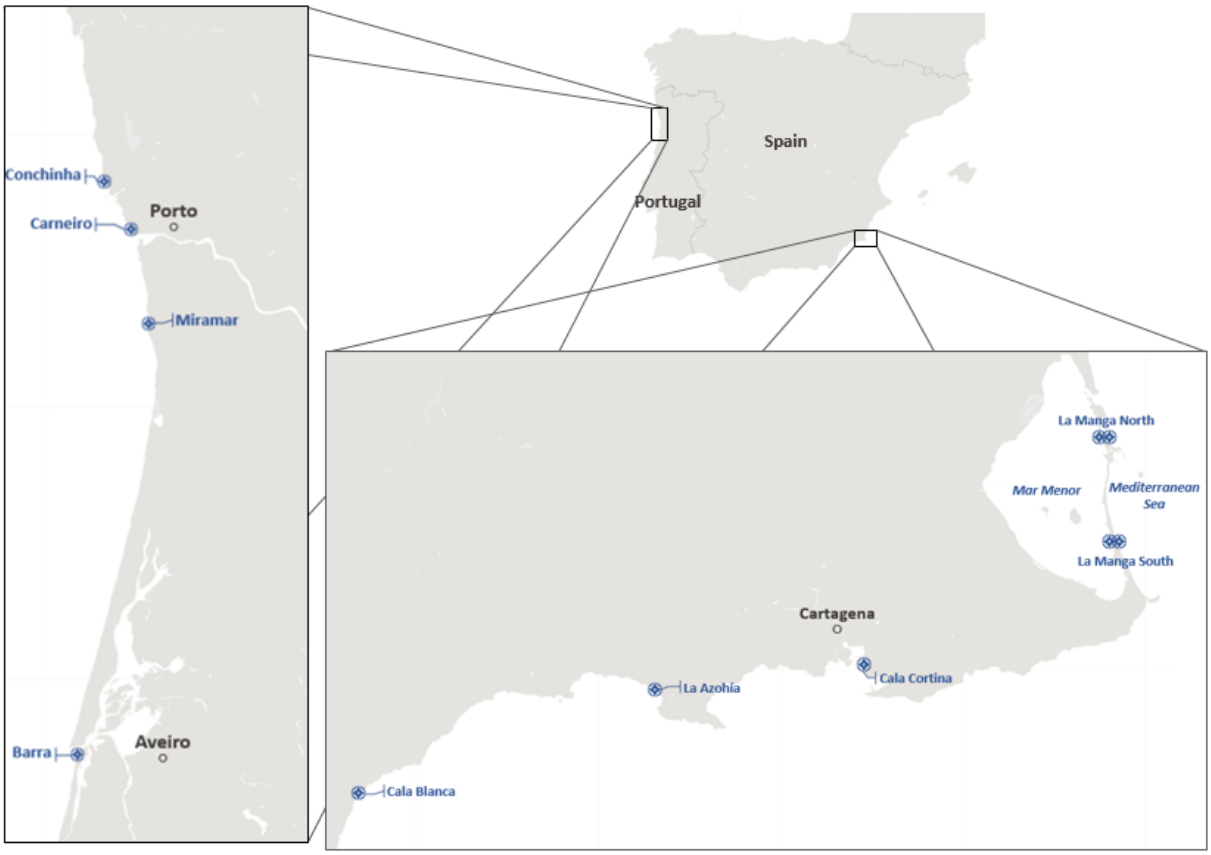


Figure 1. Geographical locations of the different sampling sites in Portugal and Murcia (Spain).

Figure 2. Different species of marine vegetation collected in the different sampling campaigns.

4.6 Extraction Procedure

To begin with the extraction procedure, the samples were defrosted at room temperature immediately before use and cut into small pieces. Approximately 2.5 g of sample was then weighted directly into a 50 mL polypropylene conical bottom centrifuge tube followed by addition of 10 mL of solvent (*n*-hexane) and spiked with M4Q, the internal standard, at 15 $ng.g^{-1}$. The mixture was agitated in the vortex (IKA VORTEX 3) for 3 min, after which it was sonicated in a JP Selecta ultrasonic bath (Barcelona, Spain) with a nominal power of 420 Watts for 15 min, and then left to cool to room temperature to avoid the loss of analytes. The QuEChERS 1 was then added and the mixture was vortexed for 3 min and centrifuged for 10 min at 4000 rpm (2670 *g*). The supernatant was transferred to another tube, already containing the QuEChERS 2, and the mixture was vortexed and centrifuged as before, with the resulting supernatant being collected in a 12 mL amber glass vial. The extract volume was reduced under a gentle nitrogen stream till approximately 1 mL and transferred to a 1.5 mL glass vial and again exposed to a nitrogen stream till less than 150 µL of volume. Finally, the volume of extract was measured with a microliter syringe and transferred to a glass microvolume insert inside a 1.5 mL amber glass vial, with *n*-hexane being added to set a final volume of extract of 150 µL. Extracts were stored at -20 °C prior to their analysis by GC/MS.

4.7 Instrumental Analysis

Instrumental analysis was performed using a Varian Ion Trap GC-MS system (Walnut Creek, CA, USA), equipped with a 4000-GC gas chromatograph, a 240-MS ion trap mass spectrometer, a CP-1177 split/splitless injector adapted with a Merlin microseal system (a microvalve alternative to the conventional silicone rubber septa) and an auto-sampler model CP-8410. To prevent bleeding from common chromatographic columns and minimize background contaminations of siloxanes, compound separation was obtained in a low-bleed Agilent DB-5ms ultra-inert column (30 m × 0.25 mm I.D., 0.25 µm film thickness) at a constant flow of helium (1.0 $mL.min^{-1}$), in a total time of analysis of 30 min. The temperature programme was the following: 35 °C hold for 5 min, raised at 10 $°C.min^{-1}$ to 95 °C, then 5 $°C.min^{-1}$ to 140 °C and finally 35 $°C.min^{-1}$ to 300 °C (hold for 5.43 min). Injection (1 µL) was in split mode, with the split ratio of 100, with a liner without glass wool. Temperatures of manifold, ion trap, transfer line and injector were maintained at 50, 200, 250 and 200 °C, respectively. The filament emission current was 50 µA. The mass spectrometer was operated in the electron ionization (EI) mode (70 eV) and for quantitative analysis of target compounds, time-scheduled selected ion storage (SIS) mode was applied. The main parameters are presented in Table 4.

Table 4. SIS mode parameters for the detection and quantification of the different target compounds by GC/MS.

Segment Description		Identification and Quantification Parameters		
Time Range (min)	**Mass Ranges (m/z)**	**Target Compound**	**Retention Time (min)**	**Qualifier and Quantifier[a] Ions (m/z)**
0.00 - 4.50	Ionization Off	-	-	-
4.50 - 6.50	130-132, 146-150	L2[b]	-	131, **147**
6.50 - 8.00	132-134, 190-192, 206-210	D3	7.26	133, 191, **207**
8.00 - 10.00	72-74, 130-134, 220-224	L3	8.75	73, 133, **221**
10.00 - 11.70	191-194, 264-268, 280-284	D4	11.06	193, 265, **281**
11.70 - 13.50	190-194, 206-210, 294-279	L4	12.51	191, **207**, 295
13.50 - 17.75	248-251, 266-270, 354-358	D5	14.29	251, **267**, 355
14.75 - 17.00	146-150, 280-284, 368-371	M4Q	15.00	147, **281**, 369
		L5	16.64	147, **281**, 369
17.00 - 19.00	324-328, 340-344, 427-431	D6	18.28	325, **341**, 430
19.00 - 30.00	Ionization Off			

[a]Quantifier ions in **bold**; [b]Due to several factors explained in section 5.1, L2 ended up being excluded from the instrumental analysis.

4.8 Data Treatment

Quantification of the target compounds was performed using the VARIAN, INC. Mass Spectrometry Workstation software, version 6.9.3. Univariate statistic tests (*F*-test for analysis of variance and independent two-sample *t*-test for equal sample sizes and equal or unequal variance (depending on the case) for a 95% confidence interval were applied to the results in order to clarify their interpretation and discussion, according to Miller et al., (1989).

4.9 Quality Assurance and Experimental Control

The analysis of siloxanes can be challenging due to their ubiquitous presence, including in the components of the gas chromatographers, for instance. Strict measures and tests had to be performed in order to minimize external contaminations. So, all glass material was rinsed with distilled water and acetone and the non-calibrated pieces were subjected to heating at 400 °C for at least 1 h before usage. Analysts avoided the use of lotions, perfumes, hand creams and other PCPs, and gloves were constantly changed during the manipulation of samples. Instrumental analysis was also enhanced to eradicate possible contamination sources of siloxanes: the injector was adapted with a Merlin microseal system as an alternative to the conventional rubber septa, silanized glass wool was removed from the inlet liner and a low bleed ultra-inert column was used to minimize the bleeding phenomena during chromatographic analysis. At the end of each chromatographic run, a temperature of 300 °C was kept for about 5.5 min as a clean-up step to remove impurities possibly remaining in the column. Chromatographic blanks were also frequently employed by injection of pure *n*-hexane to avoid possible memory effects and guarantee the column rinsing. Procedural blanks (extractions performed

with no sample) were also performed with every extraction batch and whenever needed, the final results were blank corrected.

4.10 Waste Management

The wastes generated from the experimental work consisted mainly of organic solvents (mostly *n*-hexane) and solid materials resulting from the extraction procedure with trace amounts of siloxanes. Those were collected in proper labelled containers and stored protected from light and ignition sources for further treatment by the Environmental Management System of FEUP – EcoFEUP. Acetone used in material rinsing was collected and recovered by distillation for other possible utilizations.

5 Results and Discussion

5.1 Development and Validation of the Analytical Method

A new analytical method for the determination of VMSs in seaweed was developed based on a previous approach for the extraction and quantification of the same compounds in pine needles (Ramos et al., 2016; Ratola et al., 2016). Optimizations of the employed protocol and the tests for method validation with analysis of real samples were performed with a single species of macroalgae, *Fucus vesiculosus*, which was the most commonly found on the sampling campaigns. Instrumental analysis was carried as described in the section 4.6 of this work, an approach based on the aforementioned publications with slight modifications in the temperatures programme and in the parameters of the time-scheduled SIS mode. However, of the eight VMSs initially considered for this work, L2 ended up being excluded from the analysis due to problems on its detection and quantification. Its properties, mostly its low boiling point when compared to the other analytes, caused the compound to overlap with the solvent elution, thus frustrating its proper identification and quantification. An example of the separation achieved for the target compounds is presented in Figure 3, where a chromatogram of a solution in *n*-hexane containing all siloxanes under analysis at 100 µg.L^{-1} and the IS at 250 µg.L^{-1} is shown (only in the time interval of between min 4.5 and 19, since during the other time periods ionization mode was turned off and, therefore, the signal intensity was zero).

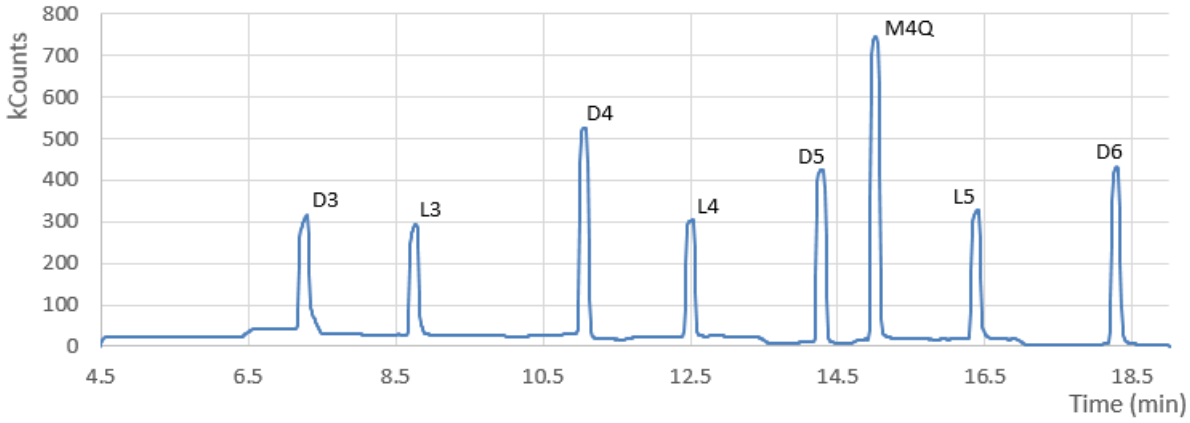


Figure 3. Chromatogram in SIS mode of a mix solution of siloxanes in *n*-hexane at 100 µg.L^{-1} and IS at 250 µg.L^{-1}.

A series of preliminary tests were conducted to improve and adapt the previously reported extraction protocol used with pine needles to the seaweed matrices. However, due to the concerns about siloxanes presence in lab equipment and indoor air, possible external contamination sources were first studied and procedural blanks were performed in different conditions to determine the best approach to minimize contamination of samples.

5.1.1 Procedural Blanks Analysis and Assessment of Contamination Sources

Ideally, the manipulation of samples and extracts should be carried in a controlled air room or facility (usually called “clean room”), due to the ubiquitous presence of siloxanes in the indoor air. Since this was not possible, the closest to a controlled air facility available was the fume hood. Therefore, procedural blanks were performed in duplicate (n=2) in the lab bench, in the fume hood with the ventilation system turned on, and in the fume hood with the ventilation system turned off, in order to study possible differences on the contamination levels of VMSs in those different circumstances. The results are presented in Figure 4.

The aim of this first assay was to assess if the contamination levels of siloxanes from laboratory sources (especially from the indoor air) could be reduced somehow. In all the circumstances tested, results showed the presence of only cVMSs, the ones commonly referred to be used in PCPs. Nevertheless, procedural blanks performed in the fume hood with the ventilation system turned off presented lower levels of D4, D5 and D6 when compared to the other two conditions. Similar levels of contamination between the extractions performed in the lab bench and in the fume hood with the ventilation turned on may be explained by the fact that when the exhaust fan is working, the air from the lab gets inside the fume hood and, therefore, no considerable differences were observed.

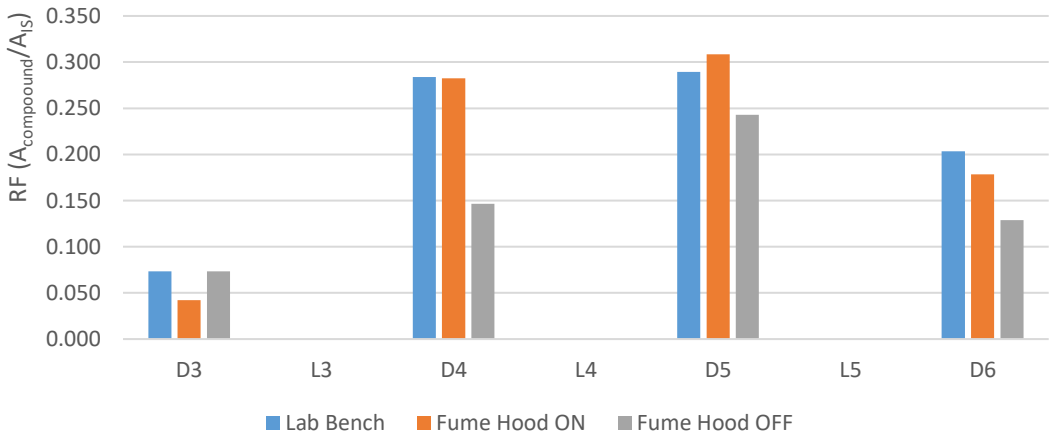


Figure 4. Contamination level of siloxanes in procedural blanks. Bars represent the average value of two determinations.

Considering the results in this first test, the manipulation of samples and extracts during the extraction procedure was from then on done in the fume hood with no ventilation.

To assess possible contaminations from the solvent used and bleeding phenomena from the GC/MS equipment, chromatographic blanks were also performed by direct injections of *n*-hexane, but siloxanes were not detected.

5.1.2 Preliminary Tests – Development of the Extraction Procedure

As stated in section 4.4, the development of the extraction procedure was initially based on the protocol validated and employed by Ramos et al. (2016) and Ratola et al. (2016) for the extraction and quantification of siloxanes in pine needles. To determine differences in the recovery rates of the target compounds in the various tested variables, samples were spiked with VMSs at 6 $ng.g^{-1}$ and IS at 15 $ng.g^{-1}$. An example, the chromatogram of a sample of *F. vesiculosus,* with and without the VMSs spike addition, is presented in Appendix 2.

A vortex time of 5 min and a mass of sample of 5 g were also tested, with no major differences found in terms of recoveries of the compounds. However, increasing the mass of sample and the vortex time also led to an increase of the temperature of the samples during vortex agitations and ultrasound extraction, resulting in longer waiting times until the extracts cooled to room temperature, also increasing the possibility of volatilization of the target compounds. Therefore, the mass of sample and the vortex agitations were kept the same.

5.1.2.1 Enhancement of the Solvent Evaporation Step

Since the analytes under study are volatile, the major losses are expected during the solvent evaporation step. In fact, results reported by Ramos et al. (2016) indicate that recovery rates increase as the volatility of the siloxanes decreases. Therefore, the evaporation step was rearranged to avoid the total dryness of the extracts. Recovery rates of the target compounds were determined in two different solvent evaporation strategies, as described in section 4.4, and the results are presented in Figure 5.

The no-dryness step tested showed the best performance, with recoveries ranging from 63% (D3) to 105% (D6), with an average of 85% for all siloxanes, while the prior solution of total dryness resulted in recoveries ranging from less than 1% (D3) to 106% (L5), with an average for all compounds of 57%. Results are a clear improvement, especially for the most volatile compounds (D3, L3, D4 and L4), and it is interesting to note that the highest recoveries are still associated to the least volatile compounds, with results for D5, L5 and D6 being similar between the two procedures.

In view of the results obtained, the following preliminary tests were performed not completely drying the extract in the solvent evaporation step.

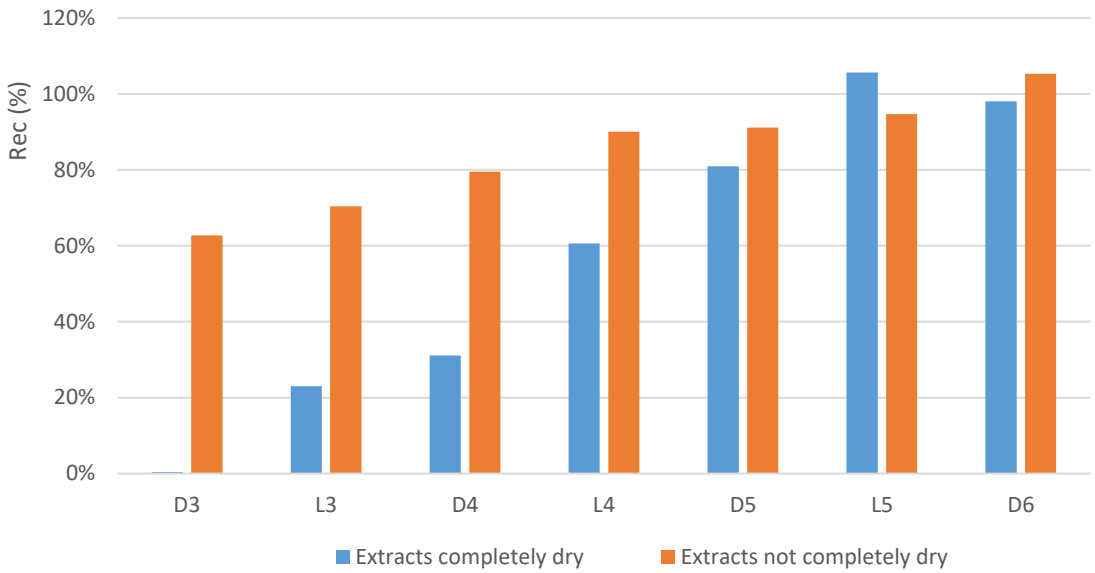


Figure 5. Recovery rates of the target compounds in the two different protocols tested for the solvent evaporation step. Bars represent the average value of two determinations.

5.1.2.2 Choice of the Extraction Solvent

Three different extraction solvents were tested: pure *n*-hexane (Hex) and two solvent mixtures (Hex/DCM 1:1 and Hex/Acet 1:1). *n*-hexane is a nonpolar solvent, which appears in literature as a common extraction solvent for VMSs, having been successfully applied to different matrices, including sediment and fish liver (Warner et al, 2010), phytoplankton and krill (Sanchís et al., 2015), and other biota samples (Kaj et al., 2005). Siloxanes are nonpolar compounds, but with a polar backbone, therefore the two solvent mixtures were chosen to increase the range of polarities of the extraction solvent at two different levels (dichloromethane is less polar than acetone), and to allow a better capacity to wet the sample and hopefully increase the extraction of the interest analytes. To assess the performance of each solvent, extractions were performed in triplicate (n=3) with samples spiked with the target analytes at 6 $ng.g^{-1}$.

Three factors were considered for the choice of the extraction solvent. The main focus was given to the recovery rates of the target compounds (Figure 6), but also important were the levels of siloxanes in procedural blanks performed in triplicate for each solvent used, as well as the appearance of extracts and the subsequent peak resolution in the chromatograms. For GC/MS analysis, the cleanliness of the extracts is very important, since there is the risk to have interferents attached to the chromatographic column, eventually compromising its performance, or influencing the separation quality.

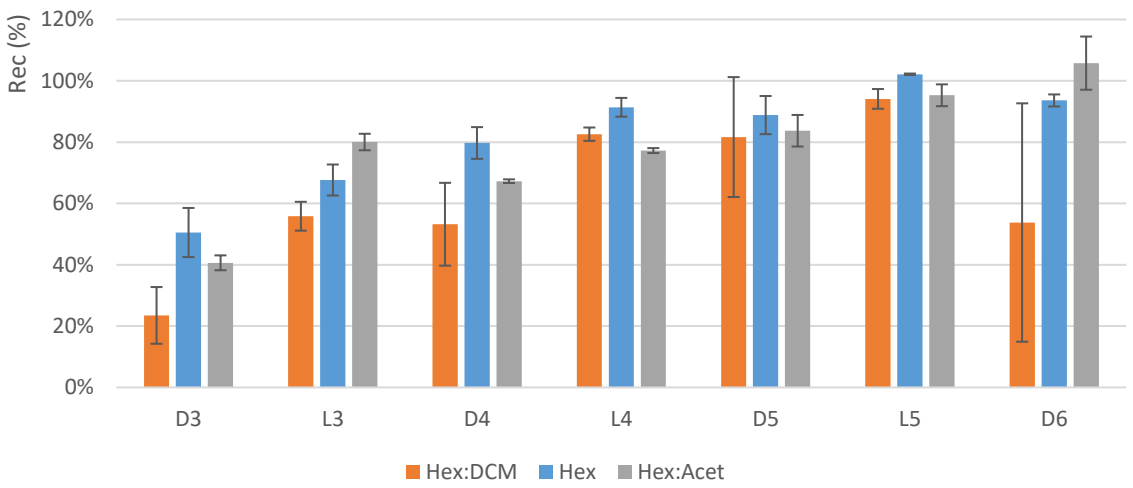


Figure 6. Recovery rates of the target compounds for each of the extraction solvents tested, (n=3), *(error bars represent the standard deviation)*.

The average recovery for all the siloxanes using Hex/DCM 1:1 as solvent was 64%, lower when compared to the averages of 82% and 79% obtained for *n*-hexane and Hex/Acet 1:1, respectively. Hex as solvent showed statistically significant difference recoveries for D3, L3, D4, L4 and L5 when compared to Hex/DCM 1:1, while when comparing results for Hex with Hex/Acet 1:1, only L4 registered a higher statistically significant difference. Despite *n*-hexane and Hex/Acet 1:1 showed very similar results in terms of recovery rates, procedural blanks performed with *n*-hexane showed the least values of contamination when compared to those of Hex/DCM 1:1 and Hex/Acet 1:1 (Figure 7). In fact, Hex/Acet 1:1 revealed statistically significant higher values of contamination for D4, D5 and D6 when compared to those obtained with Hex as solvent.

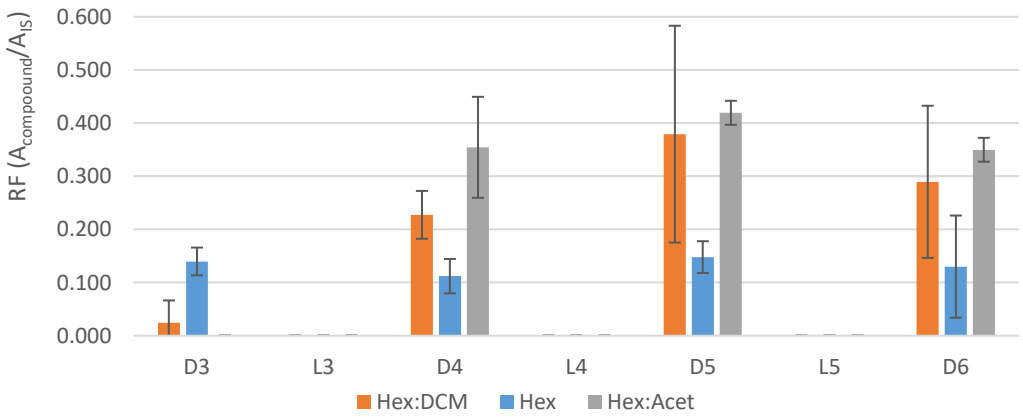


Figure 7. Contamination levels in procedural blanks performed with each one of the solvents tested, (n=3), *(error bars represent the standard deviation)*.

Besides that, while extracts performed with *n*-hexane appeared to be transparent to the naked eye, the same did not occur for the solvent mixtures. Increasing the polarity range of the extraction solvent resulted in a higher capacity to remove other compounds from the samples, which could be

easily verified by inspecting the colour of the extracts. The Q2, the clean-up step on the extraction procedure, played an important role on removing part of these substances, as can be seen in Figure 8. However, the final extracts obtained with the solvent mixtures after solvent recovery still showed a dark green colour, especially the ones obtained with Hex/Acet 1:1.

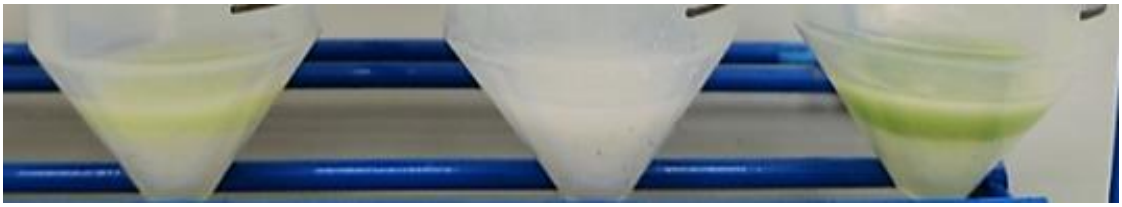


Figure 8. Appearance of the Q2 wastes after the clean-up step with each one of the extracts performed with the different solvents tested, (from left to right, Hex/DCM, *n*-hexane and Hex/Acet).

Considering all these results, *n*-hexane was selected as the extraction solvent, for showing the best compromise between high recovery rates, low contamination levels on procedural blanks and cleanliness of final extracts.

5.1.2.3 Choice of QuEChERS 2

QuEChERS 2 is responsible for the clean-up step in the extraction procedure, where common dispersive solid-phase sorbents are used. Its composition was tested in four different arrangements, as stated in section 4.4. The recovery results for each VMSs obtained on each of the four arrangements of Q2 tested are presented in Figure 9. Procedural blanks were also performed in triplicate for each one of the conditions, with no major differences observed between the different Q2 tested.

Q2 containing Florisil® was the one with lowest global average recovery values considering all the siloxanes under analysis (66%), with poor results particularly for D3 and D6 (lower than 40%); recoveries for D5 and D6 using Q2 containing Florisil® were also statistically significant lower than the recoveries obtained with other compositions for Q2 tested. PSA, alumina and the mixture containing all the three sorbents showed very similar results for most of the analytes, with statistical analysis showing no significant differences for most of the compounds. However, major significant differences were observed for D3, with average recoveries of only 1% using the Q2 containing alumina and the Q2 containing the three sorbents showing average recoveries of only 34%, while the Q2 containing PSA registered the higher value of recovery, 51%.

Considering these results, no changes were made to the composition of Q2, with PSA being selected as the sorbent to be used (300 mg), with 900 mg of $MgSO_4$ and 150 mg C18.

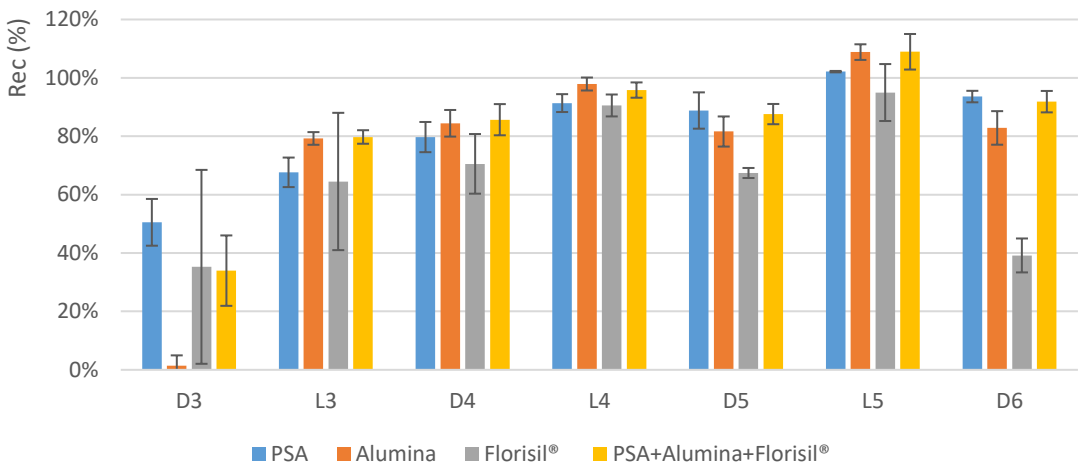


Figure 9. Recovery levels of the target compounds obtained with each one of the different Q2 compositions, (n=3), *(error bars represent the standard deviation)*.

5.1.3 Method Validation

From the results obtained in the optimisation tests, a method to detect and quantify three linear (L3-L5) and four cyclic (D3-D6) VMSs from seaweed was established, with the performance of the selected procedural conditions being tested. The validation of the analytical method was achieved by determining quantification parameters such as the linearity response of the GC/MS instrumental approach, its associated statistical criteria and limits of detection and quantification, and by assessing the precision and accuracy, as described below.

5.1.3.1 Quantification Parameters

The linearity response was assessed by direct injection in the GC/MS of eight calibration standards with concentrations of all siloxanes under analysis ranging from 1 to 750 µg.L^{-1}, and a concentration of M4Q as the internal standard of 250 µg.L^{-1}. Calibration curves were obtained by correlating the concentrations with the resultant response factors (RF), and evaluated according to the criteria proposed by Harris (2003). Linear responses were good, with correlation coefficients (*r*) higher than 0.995 for all the siloxanes, ranging from 0.998 (D4) to 0.999 (D3, L3, L4 and D5), and relative standard deviations of the slope lower than 5%, ranging from 2.6% (D4) to 0.6% (D3, L3, L4 and L5). The only validation criteria that was not fulfilled for all the compounds under analysis was the intercept containing the origin ($b-s_b < 0 < b+s_b$), a parameter that ensures that for a null concentration an insignificant response of the detector is obtained, not occurring for D3 and D6. In Appendix 3, RF values obtained for each compound at different concentrations are presented, as well as the equations of the resulting calibration curves and its statistical validation parameters and graphical representations. Coefficients of determination (R^2) were also determined (Table 5), ranging between 0.9971 and 0.9998.

The limits of detection (LOD) and the limits of quantification (LOQ) for each siloxane were determined based on the signal-to-noise ratios (S/N) of the analytes. A S/N of 3 was applied to calculate LOD, while a S/N of 10 was used to determine the LOQ. In order to better compare the results obtained with other studies, values are presented in Table 5 in μg of compound per L of extract ($\mu g.L^{-1}$) and also in pg of compound per g of wet and dried sample ($pg.g^{-1}_{ww}$ and $pg.g^{-1}_{dw}$), to better compare them to the results obtained in other studies regarding siloxanes in marine environment matrices. LOD and LOQ values obtained were very satisfactory, being lower than those reported by Jia et al. (2015) for sea lettuce and fish, and for those stated by Hong et al. (2014) for the analysis of siloxanes in marine fish. LOQs for the compounds under study were also lower or in the same range of those reported by Sanchís et al. (2015) in the determination of siloxanes in Antarctic vegetation, phytoplankton, and krill.

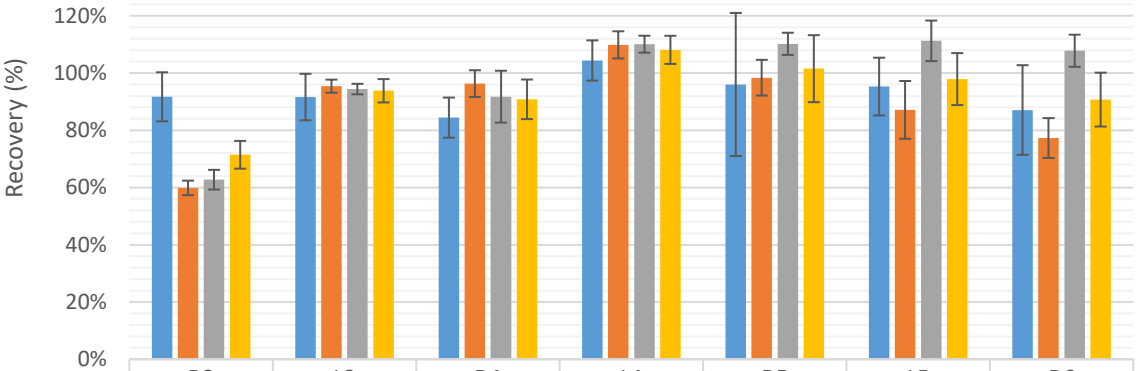
Table 5. Linearity range, coefficents of determination and limits of detection and quantificatin for each siloxane studied.

	Linearity Range ($\mu g.L^{-1}$)	R^2	LOD			LOQ		
			$\mu g.L^{-1}$	$pg.g^{-1}_{ww}$	$pg.g^{-1}_{dw}$	$\mu g.L^{-1}$	$pg.g^{-1}_{ww}$	$pg.g^{-1}_{dw}$
D3	1 - 750	0.9998	0.128	7.69	32.3	0.427	25.6	108
L3		0.9998	0.250	15.0	62.9	0.833	50.0	210
D4		0.9959	0.142	8.49	35.6	0.472	28.3	119
L4		0.9998	0.227	13.6	57.2	0.758	45.5	191
D5		0.9992	0.047	2.80	11.7	0.155	9.32	39.1
L5		0.9998	0.183	11.0	46.0	0.610	36.6	153
D6		0.9971	0.097	5.81	24.3	0.323	19.4	81.2

5.1.3.2 Accuracy and Precision

Accuracy measures the degree of proximity between the obtained and the expected results. It was tested by using the standard addition method, assessing the recovery rates of five spiked samples (n=5) at three different concentration ranges of the target compounds (1.5, 6 and 30 $ng.g^{-1}$). Results are presented in Figure 10.

Average recoveries for each VMS ranged from 71 ± 5% (D3) to 108 ± 5% (L4), while a global mean recovery rate of 93 ± 7% was achieved, if all the analytes and the three spiked levels are considered. For this kind of analysis, recovery results are considered good if close to 100%, with variations between 80 and 120% being commonly accepted. This was achieved for all siloxanes at all concentrations tested, except for D3 in the samples spiked at 6 and 30 $ng.g^{-1}$ (probably from losses due to volatilization of the analyte, since its boiling point is the lowest of the target siloxanes, and its vapour pressure is the highest). Therefore, the accuracy results obtained were good, with high recovery rates and satisfactory low standard deviations overall.



	D3	L3	D4	L4	D5	L5	D6
1.5 ng/g	92%	92%	84%	104%	96%	95%	87%
6 ng/g	60%	95%	96%	110%	98%	87%	77%
30 ng/g	63%	94%	92%	110%	110%	111%	108%
Average	71%	94%	91%	108%	102%	98%	91%

Figure 10. Recoveries of the different siloxanes at different spike levels, (n=5), (error bars represent the standard deviation).

Precision measures the degree of proximity between the results obtained for the same sample. Repeatability (intraday precision), was evaluated by the relative standard deviation (RSD) of five repeated extractions of samples spiked at three different concentrations (1.5, 6 and 30 $ng.g^{-1}$). Interday precision was also measured, assessed by the RSD of nine replicate extractions in different days, again at the three selected spike levels. Results are shown in Table 6.

Table 6. Intraday and interday precision for each siloxane studied, at different spike levels.

	Intraday Precision, n=5 (RSD)			Interday Precision, n=9 (RSD)		
	1.5 $ng.g^{-1}$	6 $ng.g^{-1}$	30 $ng.g^{-1}$	1.5 $ng.g^{-1}$	6 $ng.g^{-1}$	30 $ng.g^{-1}$
D3	9%	4%	5%	24%	6%	10%
L3	9%	2%	2%	11%	15%	2%
D4	8%	5%	10%	8%	9%	8%
L4	7%	4%	3%	7%	11%	6%
D5	26%	6%	4%	28%	9%	6%
L5	11%	12%	6%	14%	11%	5%
D6	18%	9%	5%	19%	11%	6%
Average	13%	6%	5%	16%	10%	6%

Good intraday precisions were obtained, especially for samples spiked at higher concentration levels, with RSD below 10% for the majority of siloxanes. The spike level of 1.5 $ng.g^{-1}$ showed the highest RSD, with D6 (18%) and D5 (26%) being the lowest values. Since the amount of siloxanes in those spiked samples was smaller when compared to those of the other spike levels, possible losses during the experimental procedure and greater vulnerability to the effects of possible contamination in the procedural blanks are reasonable justifications for the obtained results.

Interday precision values were acceptable, with most of siloxanes at different spiked levels showing RSD below 15%. Again, as expected, the spike level with lower concentration showed higher values of RSD, ranging from 7% (L4) to 28% (D5).

Considering the complexity of the selected sample matrix (due to natural variability of biological materials, such as seaweed, and to the difficulty on granting their homogeneity for analysis), and the challenges associated with the analyses of these volatile compounds at trace levels, the intra and interday precision results obtained were good.

5.2 Analysis of Naturally Contaminated Samples

The seaweed samples collected were analysed with two major goals: identify possible species of seaweed as more suitable for the determination of siloxanes in this kind of matrices, and assess temporal trends on the presence of these compounds in samples from a specific location. On a third level it was also possible to apply the analytical protocol validated to a different kind of marine plant from a region with different socio-geographic and climatic patterns (the Mediterranean Sea in the Region of Murcia, southeast of Spain).

All samples were extracted in duplicate (n=2), and procedural blanks were performed for each batch of samples analysed. Figure 11 shows the levels of siloxanes reported in the procedural blanks performed for each group of samples analysed.

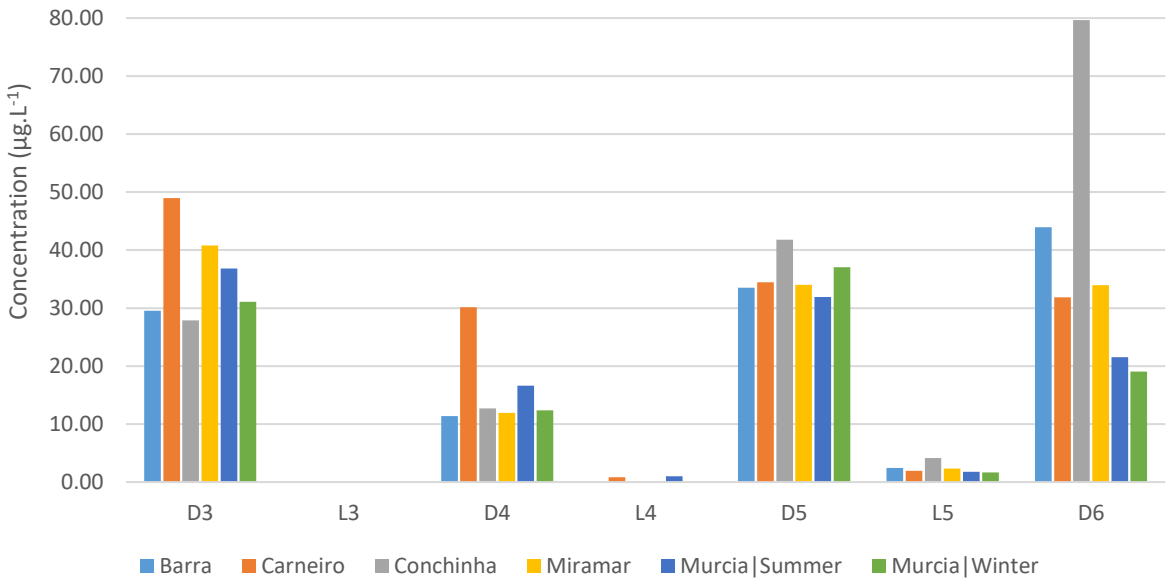


Figure 11. Overview of the contamination levels of siloxanes in procedural blanks, for each group of samples analysed.

Despite all efforts on trying to reduce contaminations from lab-related and other sources, siloxanes were consistently detected in the procedural blanks in all the extraction batches performed. Low levels were detected for lVMSs, with L4 appearing in two batches with concentrations lower than 1 $\mu g.L^{-1}$, and L5 being present in all of them ranging from 1.69 to 4.15 $\mu g.L^{-1}$. However, cVMSs appear in higher concentrations in all procedural blanks, mostly for D3, D5 and D6, in concentrations between 11.36 and up to 79.67 $\mu g.L^{-1}$. Since the latter are the most commonly used in PCPs, those values suggest that the efforts taken by the laboratory staff in reducing the use of these products and in changing some parts of the gas chromatograph may not have been enough. To overcome these difficulties, the use of a cleaned air room or facility would be recommended in the future.

Since some contamination was found in the procedural blanks, the concentrations in the extracted samples were corrected by subtracting the VMSs concentrations in the blanks to the concentrations found in the samples.

Finally, the concentrations of siloxanes in each sample were presented in terms of mass of analyte per mass of dried sample. Therefore, the dry weight (dw) of each sample was determined according to a procedure proposed by Littler et al. (1985) for the determination of the water content in macroalgae: approximately 2.5 g of each species used was left to dry at 105 °C (n=2) for about 8 hours, after which their mass was weighted and dried again at the same temperature for repetitive periods of one hour till constant weight was achieved. The results are presented in Appendix 4, Table 4.2.

5.2.1 Assessing the Presence of Siloxanes in Different Seaweed Species

The levels of detected siloxanes in samples from Conchinha beach (Table 7) showed different results between most species, with L3 and L4 being not detected in any sample and D4 being found below LOQ in only one (*F. vesiculosus*). The compound present in more samples was D3, only not being detected in *Porphyra* sp., with concentrations between below LOQ (kelp) and 2.7 $ng.g^{-1}_{dw}$ (*F. vesiculosus*), followed by D5, found in three out of the five samples, with concentrations between 4 and 27 $ng.g^{-1}_{dw}$. The species that showed higher presence of siloxanes was *F. vesiculosus*, with only cVMSs quantified in a total concentration of 123 $ng.g^{-1}_{dw}$. However, in some samples, it was registered a considerable difference between the concentrations of siloxanes in the two determinations performed. This can be the result of the difficulty on granting a homogenised sample for analysis and also possible external contaminations, increasing the variability of possible differences between the duplicated determinations for each sample.

Table 7. Average values of the concentration of siloxanes in the samples of different species of seaweed, (n=2), collected in Conchinha beach in August 2016.

	Concentration ($ng.g^{-1}_{dw}$)				
	Sample1 Kelp	**Sample *2*** *Ulva lactua*	**Sample3** *Porphyra* sp.	**Sample 4** *Codium tomentosum*	**Sample 5** *Fucus vesiculosus*
D3	<LOQ	0.70	nd	2	2.7
L3	nd	nd	nd	nd	nd
D4	nd	nd	nd	nd	<LOQ
L4	nd	nd	nd	nd	nd
D5	nd	7	4	nd	27
L5	nd	0.24	nd	nd	<LOQ
D6	nd	nd	nd	nd	93
∑cVMS	-	8	4	2	123
∑lVMS	-	0.24	-	-	-
TOTAL	-	8	4	2	123

Considering the results obtained for the samples in Carneiro beach (Table 8), it is interesting to note that L5 was the only siloxane being detected in all samples, with concentrations ranging between below the limit of quantification (*Palmaria palmata* and *Codium tomentosum*) to 4.71 $ng.g^{-1}_{dw}$ (*Porphyra* sp.), while almost all other analytes were not perceived in the analysis, with only D5 and D6 being detected in two samples (Kelp and *Porphyra* sp.), but below LOQ in most cases. It is obvious that almost no siloxanes were quantified in the species under analysis. However this can also be the result of the contaminations observed in the procedural blanks.

Table 8. Average values of the concentration of siloxanes in the samples of different species of seaweed, (n=2), collected in Carneiro beach in August 2016

	Concentration ($ng.g^{-1}_{dw}$)					
	Smple 1 *Ulva lactua*	**Sample 2** *Codium tomentosum*	**Sample 3** Kelp	**Sample 4** *Porphyra* sp.	**Sample5** *Palmaria palmata*	**Sample6** *Schizymenia dubyi*
D3	nd	nd	nd	nd	nd	nd
L3	nd	nd	nd	nd	nd	nd
D4	nd	nd	nd	nd	nd	nd
L4	nd	nd	nd	nd	nd	nd
D5	nd	nd	<LOQ	<LOQ	nd	nd
L5	0.29	<LOQ	0.25	4.71	<LOQ	0.41
D6	nd	nd	1.4	<LOQ	nd	nd
∑cVMS	-	-	1.4	-	-	-
∑lVMS	0.29	-	0.25	4.71	-	0.41
TOTAL	0.29	-	1.7	4.71	-	0.41

Summarizing the results of these two sites in August 2016 (beaches of Conchinha and Carneiro), almost all compounds were not detected in the different species, except for L5, which was noticed but in very low or non-quantifiable concentrations. *F. vesiculosus* appeared to be an exception,

with high concentrations of mostly cVMSs reported. Sanchís et al. (2015) stated that the contamination of siloxanes in biota samples tends to increase with higher contents of lipids and organic carbon. *F. vesiculosus* was the algae with higher dry mass percentage from the species analysed, which can be a possible explanation for the results obtained. This species of macroalgae also has a floating structure known as pneumatocyst that contains gas which provides buoyancy to the algae, increasing the surface area of this organism. This can also be an explanation for the results obtained, but further studies would be necessary.

5.2.2 Determination of Siloxanes in Seaweed from the Beaches of Barra and Miramar – Assessing Sources and Temporal Trends

Average concentrations of siloxanes reported in the analysis of *F. vesiculosus* collected in the beach of Barra, in Aveiro, in six different months between May and December 2016, and *Porphyra* sp. from the beach of Miramar collected in different days but in the same periods, are presented in Tables 9 and 10, respectively. In both cases, in every month, cVMSs were predominant, when compared to the total amounts of lVMSs. This is consistent with results on the determination of siloxanes in biota samples in marine environments by Kaj et al. (2005), Hong et al. (2014) and Warner et al. (2010).

In Barra beach, total concentrations of lVMSs ranged between below LOQ (in December and August) to 0.396 $ng.g^{-1}_{dw}$ (in July), with only L5 being detected. In the same location, total concentrations of cVMSs ranged between not detected to 36 $ng.g^{-1}_{dw}$ (in July), with D5 and D6 being the most commonly detected and at higher concentration levels, especially in the months of June and July. However, as observed in the results before, considerable variations between the duplicate determinations were obtained, mostly in samples with higher values of concentrations found.

Table 9. Average values of the concentration of siloxanes in the samples of *Fucus vesiculosus*, (n=2), collected in Barra beach, Aveiro, between May and December 2016.

Concentration ($ng.g^{-1}_{dw}$) in samples of *Fucus vesiculosus*, beach of Barra						
	May	**June**	**July**	**August**	**September**	**December**
D3	1.5	1.19	2.0	0.36	nd	nd
L3	nd	nd	nd	nd	nd	nd
D4	0.6	0.4	1.0	nd	nd	nd
L4	nd	nd	nd	nd	nd	nd
D5	4	2.3	7	2.8	2.3	<LOQ
L5	0.16	0.23	0.396	<LOQ	0.25	<LOQ
D6	<LOQ	19	26	nd	1.1	<LOQ
∑cVMS	6	23	36	3.2	3	-
∑lVMS	0.16	0.23	0.396	-	0.25	-
TOTAL	6	23	37	3.2	4	-

Table 10. Average values of the concentration of siloxanes in the samples of *Porphyra* sp., (n=2), collected in Miramar beach between May and December 2016.

Concentration ($ng.g^{-1}_{dw}$) in samples of *Porphyra* sp., beach of Miramar						
	May	**June**	**July**	**August**	**September**	**December**
D3	nd	nd	nd	nd	nd	<LOQ
L3	nd	nd	nd	nd	nd	nd
D4	3	<LOQ	1.1	0.7	1.1	1.1
L4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
D5	12	7	8	5	10	8
L5	<LOQ	<LOQ	0.27	0.6	<LOQ	<LOQ
D6	nd	<LOQ	3	16.9	<LOQ	<LOQ
∑cVMS	15	7	12	22	11	9
∑lVMS	-	-	0.27	0.6	-	-
TOTAL	15	7	12	23	11	9

In samples of *Porphyra* sp. from Miramar, lVMSs were only above the LOQ in the months of July (0.27 $ng.g^{-1}_{dw}$) and August (0.6 $ng.g^{-1}_{dw}$), despite L4 and L5 being found above LOD in every months. On the other hand, cVMSs were present in every samples, between 7 $ng.g^{-1}_{dw}$ (June) and 23 $ng.g^{-1}_{dw}$ (August), with D4 and D5 being the most commonly detected, D5 always at higher concentrations; however, and despite D6 was only quantified in June and August, it is the compound found at a higher concentration, registering a concentration in August of 16.9 $ng.g^{-1}_{dw}$. Again, as observed in samples from Barra, cVMSs are found in much higher concentrations than lVMSs.

Besides this difference in the quantified values of cVMSs over lVMSs being consistent with the literature studies on the determination of siloxanes in marine biota, these results are also in line with the prevalence of cyclomethicones use in wash-off PCPs and the resulting higher mass loadings of these compounds to WWTPs when compared to lVMSs (Egmond et al., 2013; Xu et al., 2013, Capela et al., 2016a). In fact, WWTPs are a possible route of these pollutants into marine environments. Another direct source is probably the release of siloxanes due to its presence in PCPs used by the population attending the beaches during the bathing season.

To better analyse the variation on the concentrations of total VMSs in these two different locations, total concentrations of VMSs are presented, per month, in Figure 12.

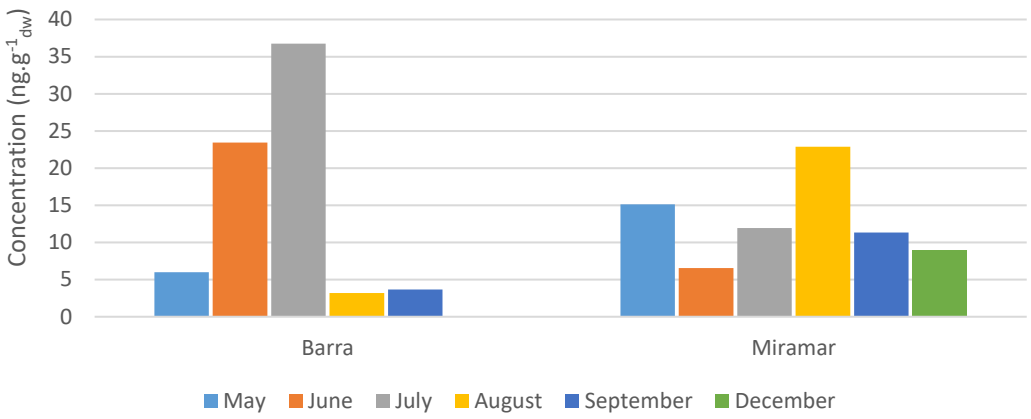


Figure 12. Total concentration of siloxanes in *Fucus vesiculosus*, from Barra beach, and *Porphyra* sp., from Miramar beach, determined in the different sampling months.

In Barra, June and July were the months when the total concentration of siloxanes was higher, up to 37 $ng.g^{-1}_{dw}$ (July), with no detected values in December. This can possibly be explained by the direct input of the siloxanes present in PCPs used by the beach goers during summer. However, following that explanation, similar levels would be expected in August, but total concentrations in that month were considerably lower. Unusual bad weather conditions in the time of sample collection in August may be an explanation to such a result, since the number of people in the beach decreased considerably when compared to a regular summer day.

In Miramar, August is the month in which highest concentrations are observed (23 $ng.g^{-1}_{dw}$), followed by May (15 $ng.g^{-1}_{dw}$). In spite of this (mostly due to the presence of D5 and D6, again possibly related to the use of PCPs by the population attending the beach), in this site, the total concentration of siloxanes detected is more constant during the other months. In fact, the total VMSs in the other months ranged between 7 and 12 $ng.g^{-1}_{dw}$, in June and July, respectively, with D5 being the major contributor. There is a small stream, Ribeira do Espírito Santo, with a total length of 12 km, which flows through small urbanized areas until entering the ocean in this spot. It would be interesting to assess the incidence of siloxanes in that stream to evaluate if it has any contribution on the background concentrations of VMSs observed, despite of their typically more hydrophobic nature.

Overall, in samples from Barra, the total concentration of siloxanes is higher in the first months of summer, with no levels recorded in December. In samples collected in July, the concentrations found were significantly higher than those recorded for the other months, with statistical analysis ensuring those results. Concentrations reported in samples collected in June, were also significantly higher when compared to the months of August, September and December, but no statistically significant difference was found when compared to the results obtained in samples collected in May. In the case of the beach of Miramar, there is a background level of contamination in samples during all the sampling months, with August and May showing higher concentrations, followed by July and

September. However, no significant statistically difference between the results obtained for samples collected in the different months was found, considering a 95% confidence interval.

The results of the naturally contaminated samples suggest a possible seasonal trend, with siloxanes being less present in colder months and, especially in the beach of Barra, with higher contamination levels during the first months of Summer time. However, further analysis should be carried out, possibly with a higher sampling frequency. Nevertheless, in both cases, cVMSs are undoubtedly the more common siloxanes contaminants, a tendency observed in every month on both locations, even in samples from Barra in December (despite below LOQ, two cVMSs, D5 and D6, were detected, while only one lVMS, L5, was above the LOD in the analysis).

5.2.3 Determination of Siloxanes in *Posidonia oceanica* from the Region of Murcia – Assessing Sources and Temporal Trends

P. oceanica, a seagrass species endemic to the Mediterranean Sea, was collected in seven different sites in the Region of Murcia (Spain), in July 2015 and in February 2016 (summer and winter sampling campaigns, respectively). Despite not being an algae, the analytical method developed was also successfully employed in this marine plant.

The concentrations determined in the extracts of those samples are registered in Table 11 and, in Figure 13, the total concentrations of the target compounds are compared between the two seasons for each one of the sampling sites. Since those seven locations were chosen to cover different anthropogenic pressures, results will be analysed individually for each spot.

The sample from Cala Blanca was collected in an inhospitable beach in a remote area, with no urban development in its surroundings. No siloxanes were quantified in the summer campaign, but in the winter all cVMSs were detected, although in very low levels (D3, D4 and D6 ranging from 1.8 $ng.g^{-1}_{dw}$ (D3) to 4 $ng.g^{-1}_{dw}$ (D6)). This was the only spot where the target compounds were not found in the summer, which is consistent with its remote location. However, the winter results are odd, since this level of contamination would not be expected. In fact, this was the location that registered the second highest total concentration of siloxanes in winter. Having no local sources of siloxanes, the levels found may be more subject to the common incidence of VMSs in the atmosphere, which was reported as higher in winter by several authors, due to stronger transformation phenomena occurring in summer such as the reaction of VMSs with OH radicals in the air (McLachlan et al., 2010).

Table 11. Average values of the concentration of siloxanes in the samples of *Posidonia oceanica,* (n=2), collected in seven different sites in the Region of Murcia, surroundings of Cartagena, in both summer (July 2015) and winter (February 2016) sampling campaigns.

		Concentration ($ng.g^{-1}_{dw}$)						
	Season	Cala Blanca (Remote site)	La Azohía (Urban Beach)	Cala Cortina (Urban Beach, entrance of The Port of Cartagena)	La Manga, North Mediterranean Sea (Small Beach)	La Manga, North Mar Menor (Small Beach)	La Manga, South Mediterranean Sea (Urban Beach)	La Manga, South Mar Menor (Urban Beach)
D3	Summer	nd	nd	nd	nd	nd	nd	nd
	Winter	1.8	<LOQ	nd	<LOQ	0.9	1.3	2.0
L3	Summer	nd	nd	nd	nd	nd	nd	nd
	Winter	nd	nd	nd	nd	nd	nd	nd
D4	Summer	nd	nd	2.0	<LOQ	nd	nd	nd
	Winter	2.3	1.4	nd	0.5	0.62	2.1	nd
L4	Summer	<LOQ	nd	<LOQ	<LOQ	nd	<LOQ	<LOQ
	Winter	<LOQ	0.53	0.58	nd	<LOQ	<LOQ	<LOQ
D5	Summer	nd	12.3	156	22	6.4	4.7	21
	Winter	<LOQ	nd	<LOQ	nd	nd	<LOQ	20
L5	Summer	nd	<LOQ	2.3	<LOQ	<LOQ	nd	0.41
	Winter	<LOQ	<LOQ	<LOQ	nd	nd	nd	<LOQ
D6	Summer	nd	3	132	<LOQ	nd	nd	2.6
	Winter	4	1.4	2.8	nd	nd	3	74
∑cVMS	Summer	-	15	290	22	6.4	4.7	23
	Winter	8	2.9	2.8	0.5	1.5	6	96
∑lVMS	Summer	-	-	2.3	-	-	-	0.41
	Winter	-	0.53	0.58	-	-	-	-
TOTAL	Summer	-	15	291	22	6.4	4.7	24
	Winter	8	3.4	3.4	0.5	1.5	6	96

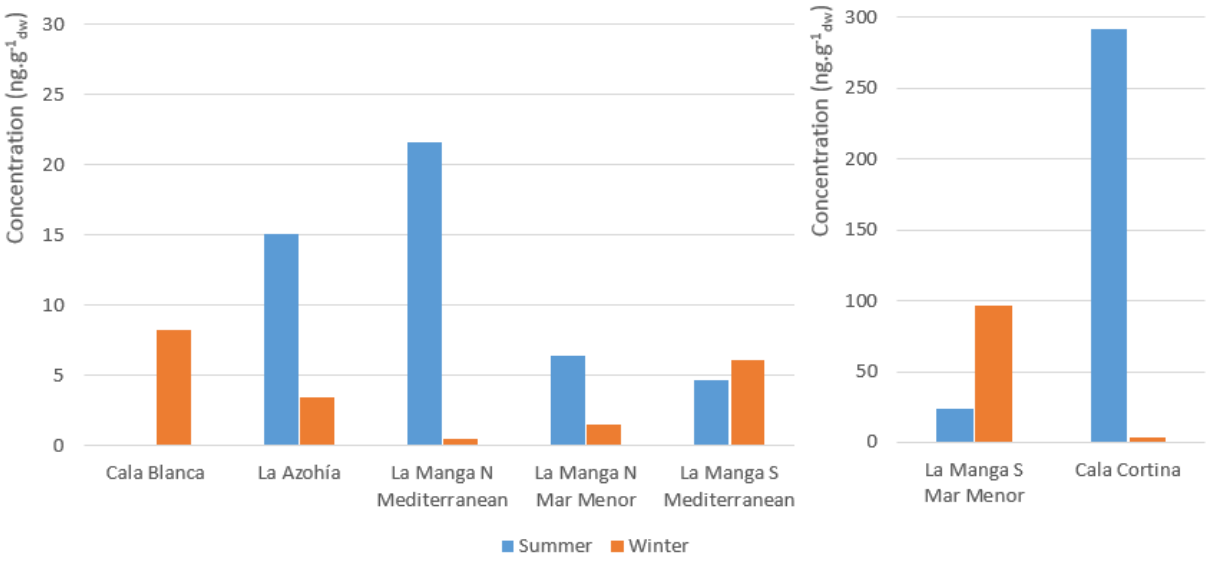


Figure 13. Total concentration of siloxanes in samples of *Posidonia oceanica* from the different sampling sites, distributed by season. Note the different scales in the concentration axes.

The total concentration of siloxanes in the samples from the small town of La Azohía show a strong difference between seasons. Despite being a small urbanized area, in summer the population substantially increases due to the afflux to its beaches in the Mediterranean Sea. The total concentration of siloxanes quantified in the warm season (15 $ng.g^{-1}_{dw}$) is more than four times higher to that of the cold one (3.4 $ng.g^{-1}_{dw}$), with the prevalence of D5 (81%), followed by D6 (19%), with no lVMSs quantified. In winter, only D4, D6 and L4 were quantified, with concentrations between 0.53 $ng.g^{-1}_{dw}$ (L4) and about 1.4 $ng.g^{-1}_{dw}$ (for both D4 and D6). These results suggest that the presence of siloxanes is related to the increase of the population during the summer, hence increasing the use of PCPs in these areas, such as sunscreens and other body lotions. Similar seasonal trends were observed in three other sampling locations, where total concentration of VMS in summer surpasses that in winter: in both samples from La Manga North (in both beaches from Mediterranean Sea and Mar Menor) and in the samples collected in the site located in Cala Cortina.

In La Manga North, the difference between the total VMSs concentration between seasons was considerably higher in the Mediterranean Sea side, where in summer it was almost 50 times higher than in winter. Despite the different values between the two determinations of the same sample in summer, D5 was the only quantified siloxane (22 $ng.g^{-1}_{dw}$), while D4 was the only one present in winter in quantifiable amounts (0.5 $ng.g^{-1}_{dw}$). In the side of Mar Menor, cVMSs were also the only ones quantified in both samples from summer and winter. In the warm season, only D5 was detected above LOQ, in a concentration of 6.4 $ng.g^{-1}_{dw}$, much lower to that of the sample collected in the Mediterranean Sea location. In winter, at this side of the La Manga seaside spot, only D3 and D4 were quantified, with only 0.9 and 0.62 $ng.g^{-1}_{dw}$, respectively.

P. oceanica samples collected in the urban beach in Cala Cortina were the ones that showed the highest concentration levels of siloxanes. In summer, the total concentration of VMSs determined was 291 $ng.g^{-1}_{dw}$, of which less than 1% of lVMSs. The cVMSs that most contributed to this value were D5 (53%) and D6 (45%), with D4 being quantified at a much lower concentration (less than 1% of the total). This was the sampling site with higher anthropogenic pressure: an urban beach, close to the city of Cartagena, at the entrance of the Port of Cartagena and in the vicinities of a big refinery and a power plant. Therefore, this location is prone to the effects of PCPs usage by the population during the bathing season, but other possible sources can be hypothesised. For instance, silicone based polymers are commonly used as coatings in ship hulls, producing a surface which discourages the settling and accumulation of marine fouling, mostly in cruise and fast container ships (Bohlarder, 1999). However, total concentrations in winter are much lower to those of summer, with L4 and D6 showing concentrations of only 0.58 and 2.8 $ng.g^{-1}_{dw}$, respectively, and L5 and D5 being detected below LOQ. This suggests the importance of the afflux of sunbathers in the summer.

Finally, in La Manga South, both sites in Mediterranean Sea and Mar Menor showed higher total concentrations of VMSs in the samples collected in winter, with the difference between seasons being higher in the Mar Menor side. This result was not consistent with the seasonal trend verified for the latter four sites analysed, which suggested that another unidentified considerable source of VMSs in these locations was present in winter. In the side of Mar Menor, total concentration of siloxanes in winter was of 96 $ng.g^{-1}_{dw}$, of which 76% were due to the presence of D6, 21% to D5 and 2% to D3 (no lVMSs were quantified), while the summer samples registered a total VMSs concentration of with 23 $ng.g^{-1}_{dw}$, mostly due to D5 (87%) and D6 (11%), with L5 also being quantified (2% of the total). Contamination levels of siloxanes in the sample from Mar Menor were much higher than those observed in the side of the Mediterranean Sea, especially in winter. In the Mediterranean spot, the sample collected in this season showed a total concentration of siloxanes of 6 $ng.g^{-1}_{dw}$, with D3, D4 and D6 having 21, 34 and 45% incidence, respectively, while the summer sample only revealed the quantifiable presence of D5, at 4.7 $ng.g^{-1}_{dw}$. It is interesting to notice that in both cases, while D6 prevails in samples from in Winter, D5 is the most present siloxane in the summer samples, suggesting that two different contamination sources in both seasons may exist and, due to the proximity between the two locations, only separated by the La Manga spit, the contamination source may be the same.

Overall, considering the results obtained from the samples of *P. oceanica* in these seven different sites, the prevalence of cVMSs over lVMSs was clear in both seasons, just as in the samples from the beaches of Barra and Miramar from Centro and North Region of Portugal, in line with the results found in the literature regarding contamination levels of siloxanes in marine biota. It was also possible to identify higher levels of contamination during summer time. In fact, statistical analysis confirmed the prevalence of the studied VMSs in samples collected in the summer in four different spots associated to urban areas, with tourism industry increasing the population during the warm

season, also increasing the anthropogenic pressure over the selected environments. On the other hand, two of the three locations where the concentration of total siloxanes was higher in winter are geographically near (La Manga South, in the Mediterranean Sea and in Mar Menor), although with substantial differences in the magnitude of the contamination values, but still showing some similarities in the profile of siloxanes amount quantified. Samples from the remote location of Cala Blanca also showed high values of siloxanes in winter, when compared to the generality of the scenario in the other urbanized sites. This result is intriguing, but also calls attention for the ubiquity of these compounds and how their behaviour, transport and distribution in the environment is a challenging task to take on.

6 Conclusions

An analytical method based on QuEChERS extraction followed by GC/MS was validated for the analysis of three linear (L3-L5) and four cyclic (D3-D6) VMSs in seaweed, after improvement of some of the experimental parameters, with *n*-hexane being selected as the extraction solvent, the composition of QuEChERS 2 being developed and the solvent evaporation test enhanced. Linearity responses were good, ranging from 1 to 750 $\mu g.L^{-1}$ for each siloxane studied, with R^2 values between 0.9959 (D4) and 0.9998 (D3, L3, L4 and L5). The LODs and LOQs obtained were low, and suitable for the detection of these contaminants at trace levels, ranging from 11.7 to 62.9 $pg.g^{-1}_{dw}$ (LODs) and 39.1 to 210 $pg.g^{-1}_{dw}$ (LOQs). The recovery rates were good, with a global average considering all the siloxanes under study at three different spike levels (1.5, 6 and 30 $ng.g^{-1}$) of 93 ± 7%. Intraday and interday precision tests, based on five and nine determinations, respectively, at the same spike levels, yielded very good results, with RSD values lower than 15% for the majority of the target compounds.

From the analysis of several species of seaweed, collected in two beaches in the North Region of Portugal in August 2016, almost all the target compounds were not detected. An exception was *Fucus vesiculosus*, which showed higher values of cVMSs in one of the locations, also being the species with higher dry mass percentage in its composition.

On the determination of the siloxanes concentration in two different species of seaweed from two different locations over the months of between May and December 2016, total results varied between below LOQ to 37 $ng.g^{-1}_{dw}$, with cVMSs appearing in much higher concentrations than lVMSs, mostly D5 and D6 in most of the determinations. Higher total concentrations of the target compounds were observed in some of the summer months, however, seasonal trends were not clear in both locations, with contamination sources not being clearly identified.

Regarding the application of the validated method to a seagrass collected in the Region of Murcia, in seven different sites in two different seasons (summer and winter), total concentrations of siloxanes in the different sampling sites varied between below LOQ and 291 $ng.g^{-1}_{dw}$ in summer and 0.5 to 96 $ng.g^{-1}_{dw}$ in winter, with cVMSs being the most predominant in the majority of cases, especially D5 and D6. Locations associated with higher urban and industrial activities in its surroundings showed, in general, higher levels of contamination and four out of the seven locations showed higher concentrations in samples collected in summer, a time when population in those areas increases due to beach-related tourism.

The methodology employed was successful for the analysis of siloxanes in the selected matrices. Therefore, seaweed and other marine vegetation proved to be suitable samples for the determination of the contamination and distribution levels of VMSs in coastal environments, with potential to be used as bioindicators of such contaminants in these ecosystems.

7 Limitations and Future Works

The analysis of siloxanes at trace levels is, by itself, a demanding task. Biological matrices, such as seaweed, turn these efforts even more complicated, mostly due to the difficulty on granting its homogeneity for analysis and to the natural variability of the samples (even within the same species, due to its natural intraspecific variations, different ages and growth stages, among others). An alternative to be studied for the sample processing carried out in this validated method (cut the sample in small pieces) could be freeze the sample with liquid nitrogen and ground it to obtain a more uniform and homogeneous material, and also to increase the area of contact to the solvent.

Despite the interesting results obtained in this work, there are some other improvements on the developed method to possibly be considered in the future, not tested so far due to unavailability of materials or technical conditions. For instance, to reduce potential contamination of samples from indoor air, samples and extracts should be manipulated in a clean air room or facility. The internal standard, M4Q, could also be changed, since it presented some irregular behaviour in some assays; ideally, stable isotope standards (deuterated siloxanes, or ^{13}C-labelled siloxanes) should be used instead, to grant more reproducible and reliable results.

During the development of this project, other problems and limitations occurred. Due to technical failures in the GC/MS equipment, some experimental work had to be rescheduled or changed, which led to a delay on the final work presentation.

Finally, and regarding the results obtained in this work, it would be interesting to analyse other samples already collected during the sampling campaigns performed in Portugal in different locations around the selected areas, which were not all processed due to the lack of time. This could be a first step to monitor the presence of siloxanes in marine and coastal environments, assessing possible geographical and temporal trends, in order to better disclose the main sources of these compounds in the selected sites and to develop and increase our knowledge on the distribution and behaviour of these contaminants in such environments.

8 References

Alaee, M., Wang, D.-G., Gouin, T., Cyclic volatile methyl siloxanes in the environment, *Chemosphere* 2013, 93, 709-710.

Allen, R.B., Kochs, P., Chandra, G., "Industrial organic materials, their environmental entry and predicted fate", *in Organosilicon Materials* Vol.3, Part H, *Anthropogenic Compounds* by Chandra, G., *Ed. Springer-Verlag* 1997, Berlin.

Anastassiades, M., Lehotay, S.J., Stajnbaher, D., Schenck, F.J., Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce, *Journal of AOAC International* 2003, 86, 412-431.

Andriot, M., Chao, S.H., Colas, A., Cray, S., Buyl, F., DeGroot, J.V., Dupont, A., Easton, T., Garaud, J.L., Gerlach, E., Gubbels, F., Jungk, M., Leadley, S., Lecomte, J.P., Lenoble, B., Meeks, R., Mountney, A., Shearer, G., Stassen, S., Stevens, C., Thomas, X., Wolf, A.T, "Silicones in Industrial Applications", *in Inorganic Polymers by De Jaeger*, R., Gleria, M., *Nova Science: New York*, 2007.

Arespacochaga, N., Valderrama, C., Raich-Montiu, J., Crest, M., Mehta, S., Cortina, J.L., Understanding the effects of the origin, occurrence, monitoring, control, fate and removal of siloxanes on the energetic valorization of sewage biogas – A review, *Renewable and Sustainable Energy Reviews* 2015, 52, 366-381.

Badjagbo, K., Furtos, A., Alaee, M., Moore, S., Sauvé, S., Direct analysis of volatile methylsiloxanes in gaseous matrixes using atmospheric pressure chemical ionization-tandem mass spectrometry, *Analytical Chemistry* 2009, 81, 7288-7293.

Bletsou, A.A., Asimakopoulos, A.G., Stasinakis, A.S., Thomaidis, N.S., Kannan, K., Mass loading and fate of linear and cyclic siloxanes in a wastewater treatment plant in Greece*, Environmental Science & Technology* 2013, 47, 1824-1832.

Bohlander, J., Review of options for in-water cleaning of ships, *MAF Biosecurity New Zealand,* Technical Paper No: 2009/42, 2009.

Borgå, K., Fjeld, E., Kierkegaard, A., McLachlan, M.S., Consistency in trophic magnification factors of cyclic methyl siloxanes in pelagic freshwater food webs leading to brown trout, *Environmental Science & Technology* 2013, 14394-14402.

Brook, M.A., Silicon in organic, organometallic, and polymer chemistry, *Wiley*: New York, 2000.

Brooke, D.N., Crookes, M.J., Gray, D., Robertson, S., Environmental Risk Assessment Report: Octamethylcyclotetrasiloxane, 2009a, Environment Agency of England and Wales, Bristol.

Brooke, D.N., Crookes, M.J., Gray, D., Robertson, S., Environmental Risk Assessment Report: Dodecamethylcyclohexasiloxane, 2009c, Environment Agency of England and Wales, Bristol, U.K.

Brooke, D.N., Crookes, M.J., Gray, D., Robertson, S., Environmental Risk Assessment Report: Decamethylcyclopentasiloxane, 2009b, Environment Agency of England and Wales, Bristol.

Buser, A., Kierkegaard, A., Bogdal, C., MacLeod, M., Scheringe, M., Hungerbuhler, K., Concentrations in ambient air and emissions of cyclic volatile methylsiloxanes in Zurich, *Environmental Science & Technology* 2013, 47, 7045-7051.

Capela, D., Alves, A., Homem, V., Santos, L., From the shop to the drain – volatile methylsiloxanes in cosmetics and personal care products, *Environmental International* 2016a, 92-93, 50-62.

Capela, D., Homem, V., Alves, A., Santos, L., Volatile methylsiloxanes in personal care products – using QuEChERS as a "green" analytical approach, *Talanta* 2016b, 155, 94-100.

Carteret, C., Labrosse, A., Vibrational properties of polysiloxanes: from dimer to oligomers and polymers. 1. Structural and vibrational properties of hexamethyldisiloxane $(CH_3)_3SiOSi(CH_3)_3$, *Journal of Raman Spectroscopy* 2010, 41, 996-1004.

Chainet, F., Lienemann, C.-P., Courtiade, M., Ponthus, J., Donard, O.F.X., Silicon speciation by hyphenated techniques for environmental, biological and industrial issues: a review, *Journal of Analytical Atomic Spectrometry* 2011, 26, 30-51.

Conesa, H.M., Jiménez-Cárceles, F.J., The Mar Menor lagoon (SE Spain): a singular natural ecosystem threatened by human activities, *Marine Pollution Bulletin* 2007, 54, 839-849.

Cunha, S., Oliveira, C., Fernandes, J.O., Development of QuEChERS-based extraction and liquid chromatography-tandem mass spectrometry method for simultaneous quantification of bisphenol A and tetrabromobisphenol A in seafood: fish, bivalves, and seaweeds, *Analytical and Bioanalytical Chemistry* 2017, 409, 151-160.

Dekant, W., Klauning, J.E., Toxicology of decamethylcyclopentasiloxane (D5), *Regulatory Toxicology and Pharmacology* 2016, 74, 66-76.

Dudzina, T., Goetz, N., Bogdal, C., Biesterbos, J., Hungerbuhler, K., Concentrations of cyclic volatile methylsiloxanes in European cosmetics and personal care products: prerequisite for human and environmental exposure assessment, *Environmental International* 2014, 62, 86-94.

EC/HC (Environment Canada and Health Canada), Draft Screening Assessment for the Challenge Octamethylcyclotetrasiloxane (D4), 2008a, Ottawa, Canada.

EC/HC (Environment Canada and Health Canada), Draft Screening Assessment for the Challenge Decamethylcyclopentasiloxane (D5), 2008b, Ottawa, Canada.

EC/HC (Environment Canada and Health Canada), Draft Screening Assessment for the Challenge Dodecamethylcyclohexasiloxane (D6), 2008c, Ottawa, Canada.

Egmond, R., Sparham, C., Hastie, C., Gore, D., Chowdhury, N., Monitoring and modelling of siloxanes in a sewage treatment plant in the UK, *Chemosphere* 2013, 93, 757-775.

European Parliament and the Council of the European Union Regulation (EC) no. 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) no. 1907/2006. *Official Journal of the European Union* 2008, 353, 1−1354.

Fromme, H., Cequier, E., Kim, J.-T., Hanssen, L., Hilger, B., Thomsen, C., Chang, Y.-S., Völkel, W., Persistent and emerging pollutants in the blood of German adults: occurrence of dechloranes, polychlorinated naphthalenes, and siloxanes, *Environment International* 2015, 85, 292-298.

Genualdi, S., Harner, T., Cheng, Y., MacLeod, M., Hansen, K.M., Egmond, R., Shoeib, M., Lee, S.C., Global distribution of linear and cyclic volatile methyl siloxanes in air, *Environmental Science & Technology* 2011, 45, 3349-3354.

Graiver, D., Farminer, K.W., Narayan, R., A review of the fate and effects of silicones in the environment, *Journal of Polymers and the Environment* 2003, 11, 129-136.

Gutow, L., Eckerlebe, A., Giménez, L., Saborowski, R., Experimental evaluation of seaweeds as a vector for microplastics into marine food web, *Environmental Science & Technology* 2016, 50, 915-923.

Harris, D., Quantitative Chemical Analysis, 6th ed., *W.H Freeman & Company*, 2003, New York.

He, B., Rhodes-Brower, S., Miller, M.R., Octamethylcyclotetrasiloxane exhibits estrogenic activity in mice via ER alpha, *Toxicology and Applied Pharmacology* 2003, 192, 254-261.

Homem, V., Silva, J.A., Cunha, C., Alves, A., Santos, L., New analytical method for the determination of musks in personal care products by quick, easy, cheap, effective, rugged, and safe extraction followed by GC-MS, *Journal of Separation Science* 2013, 36, 2176-2184.

Hong, W.-J., Jia, H., Liu, C., Zhang, Z., Sun, Y., Li, Y.-F., Distribution, source, fate and bioaccumulation of methyl siloxanes in marine environment, *Environmental Pollution* 2014, 191, 175-181.

Howard, P.H., Muir, D.C.G., Identifying new persistent and bioaccumulative organics among chemicals in commerce, *Environmental Science & Technology* 2010, 44, 2277-2285.

Hübschmann, H.-J., Handbook of GC/MS - fundamentals and applications, second edition, *WILEY VCH Verlag GmbH & Co. KGaA* 2009.

IUPAC. Compendium of Chemical Terminology, (the "Gold Book"), 2nd ed. Compiled by A. D. McNaught and A. Wilkinson. *Blackwell Scientific Publications*, 1997, Oxford.

Jia, H., Zhang, Z., Wang, C., Hong, W., Sun, Y., Li, Y., Trophic transfer of methyl siloxanes in the marine food web from coastal area of northern China, *Environmental Science & Technology* 2015, 49, 2833-2840.

Kaj, L., Schlabach, M., Andersson, J., Palm Cousins, A., Schmidbauer, N., Brorström-Lunden, E., Siloxanes in the Nordic Environment, *TemaNord* 2005:593.

Kierkegaard, A., Adolfsson-Eric, M., McLachlan, M.S., Determination of cyclic methylsiloxanes in biota with a purge and trap method, *Analytical Chemistry* 2010, 82, 9573-9578.

Kim, N., Chun, S., Cha, D., Kim, C., Determination of siloxanes in biogas by solid-phase adsorption on active carbon, *Korean Chemistry Society* 2013, 34, 2353-2357.

Krogseth, I.S., Kierkegaard, A., McLachlan, M.S., Breivik, K., Hansen, K.M., Schlabach, M., Occurrence and seasonality of cyclic volatile methyl siloxanes in Arctic air*, Environmental Science & Technology* 2013, 47, 502-509.

Lassen, C., Hansen, C., Mikkelsen, S., Maag, J., Siloxanes-consumption, toxicity and alternatives (1st ed., Vol No 1031), *COWI A/S*, 2005, Denmark.

Lebedev, A.T., Comprehensive environmental mass spectrometry, ILM Publications 2012.

Littler, M.M., Littler, S.S., Handbook of phycological methods: volume 4: ecological field methods: macroalgae, Cambridge University Press, 1985, Cambridge, New York.

Mackay, D., Powell, D.E., Woodburn, K.B., Bioconcentration and aquatic toxicity of superhydrophobic chemicals: a modelling case study of cyclic volatile methyl siloxanes, *Environmental Science & Technology* 2015, 49, 11913-11922.

March, R.E., An introduction to quadrupole ion trap mass spectrometry, Journal of Mass Spectrometry 1997, 32, 351-369.

McKim Jr., J.M., Wilga, P.C., Breslin, W.J., Plotzke, K.P., Gallavan, R.H., Meeks, R.G., Potential estrogenic and antiestrogenic activity of the cyclic siloxane octamethylcyclotetrasiloxane (D4) and the linear siloxane hexamethyldisiloxane (HMDS) in immature rats using the uterotrophic assay, *Toxicological Sciences* 2001, 63, 37-46.

McLachlan, M.S., Kierkegaar, A., Hansen, K.M., van Egmond, R., Christensen, J.H., Skøth, C.A., Concentrations and fate of decamethylcyclopentasiloxane (D5) in the atmosphere, *Environmental Science & Technology* 2010, 44, 5365-5370.

Meeks, R.G., Stump, D.G., Siddiqui, W.H., Holson, J.F., Plotzke, K.P., Reynolds, V.L., An inhalation reproductive toxicity study of octamethylcyclotetrasiloxane (D4) in female rats using multiple and single day exposure regiments, *Reproductive Toxicology* 2007, 23, 192-201.

Miller, J.C., Miller, J.N., Statistics for analytical chemistry, second edition, *Ellis Horwood Ltd.,* 1989.

Picó, Y., Ultrasound-assisted extraction for food and environmental samples, *Trends in Analytical Chemistry*, 2013, 43, 84-99.

Piovar, A., Seraglia, R., Bresin, B., Caniato, R., Filippini, R., Fucoxanthin from *Undaria pinnatifida*: photostability and coextractive effects, *Molecules* 2013, 16, 6298-6310.

Quinn, A.L., Regan, J.M., Tobin, J.M., Marinik, B.J., McMahon, J.M., McNett, D.S., Sushynski, C.M. Crofoot, S.D., Jean, P.A., Plotzke, K.P., *In vitro* and *in vivo* evaluation of the estrogenic, androgenic, and progestagenic potential of two cyclic siloxanes, *Toxicological Sciences* 2007, 96, 145-153.

Ramos, S., Silva, J.A., Homem, V., Cincinelli, A., Santos, L., Alves, A., Ratola, N., Solvent-saving approaches for the extraction of siloxanes from pine needles, soils and passive air samples, *Analytical Methods* 2016, 8, 5378-5387.

Ratola, N., Ramos, S., Homem, V., Silva, J.A., Jiménez-Guerrero, P., Amigo, J.M., Santos, L., Alves, A., Using air, soil and vegetation to assess the environmental behaviour of siloxanes, *Environmental Science and Pollution Research International* 2016, 23, 3273-3284.

Roberts, D.A., Johnston, E.L., Poore, A.G.B., Contamination of marine biogenic habitats and effects upon associated epifauna, *Marine Pollution Bulletin* 2008, 56, 1057-1065.

Rücker, C., Kümmerer, K., Environmental Chemistry of Organosiloxanes, *Chemical Reviews* 2015, 115, 466-524.

Sanchís, J., Martínez, E., Ginebreda, A., Farré, M., Barceló, D., Occurrence of linear and cyclic volatile methylsiloxanes in wastewater, surface water and sediments from Catalonia, *Science of the Total Environment* 2013, 443, 530-538.

Sanchís, J., Cabrerizo, A., Galbán-Malagón, C., Barceló, D., Farré, M., Dachs, J., Unexpected occurrence of volatile dimethylsiloxanes in Antarctic soils, vegetation, phytoplankton, and krill, *Environmental Science & Technology* 2015, 49, 4415-4424.

Schlabach, M., Andersen, M.S., Green, N., Schøyen, M., Kaj, L., Siloxanes in the Environment of the Inner Oslofjord - TA-2269/2007, Norwegian Pollution Control Authority, 2007.

Schmitt, M., Analysis of silanes and of siloxanes formation by Raman spectroscopy, *Royal Society of Chemistry Advances* 2014, 4, 1907-1917.

Shi, Y., Xu, S., Xu, L., Cai, Y., Distribution, elimination, and rearrangement of cyclic volatile methylsiloxanes in oil-contaminated soil of the Shengli oilfield, China, *Environmental Science & Technology* 2015, 49, 11527-11535.

Snyder, D.T., Peng, W.-P., Cooks, R.G., Resonance methods in quadrupole ion traps, Chemical Physics Letters 2017, 668, 69-89.

Sparham, C., Egmond, R., Hastie, C., O'Connor, S., Gore, D., Chowdhury, N., Determination of decamethylcyclopentasiloxane in river and estuarine sediments in the UK, *Journal of Chromatography A* 2011, 1218, 817-823.

Sparkman, D., Penton, Z., Kitson, F., Gas chromatography and mass spectrometry: a practical guide (2nd edition), Elsevier Science and Technology 2011, Oxford.

Varaprath, S., Synthesis of 14C-labeled cyclic and linear siloxanes, *Journal of Organometallic Chemistry* 1999, 572, 37-47.

Wang, D.-G., Alaee, M., Steer, H., Tait, T., Williams, Z., Brimble, S., Svoboda, L., Barresi, E., DeJong, M., Schachtschneider, J., Kaminski, E., Norwood, W., Sverko, E., Determination of cyclic volatile methylsiloxanes in water, sediment, soil, biota, and biosolid using large-volume injection-gas chromatography-mass spectrometry, *Chemosphere* 2013a, 93, 741-748.

Wang, D.-G., Norwood, W., Alaee, M., Byer, J.D., Brimble, S., Review of recent advances in research on the toxicity, detection, occurrence and fate of cyclic volatile methyl siloxanes in the environment, *Chemosphere* 2013b, 93, 711-725.

Wang, R., Moody, R.P., Koniecki, D., Zhu, J., Low molecular weight cyclic volatile methylsiloxanes in cosmetic products sold in Canada: implication for dermal exposure, *Environmental International* 2009, 35, 900-904.

Warner, N. A., Krogseth, I. S., Whelan, M. J., Comment on "Unexpected occurrence of volatile dimethylsiloxanes in Antarctic soils, vegetation, phytoplankton, and krill", *Environmental Science & Technology* 2015, 49, 7504-7506.

Warner, N., Evenst, A., Christensen, G., Gabrielsen, G., Borga, K., Leknes, H., Volatile siloxanes in European Arctic: assessment of sources and spatial distribution, Environmental Science & Technology 2010, 44, 7705-7710.

Whelan, M.J., Breivik, K., Dynamic modelling of aquatic exposure and pelagic food chain transfer of cyclic volatile methyl siloxanes in the Inner Oslofjord, *Chemosphere* 2013, 93, 794-804.

Xu, L., Shi, Y., Cai, Y., Occurrence and fate of volatile siloxanes in a municipal wastewater treatment plant of Beijing, China, *Water Research* 2013, 47(2), 715-724.

Xu, S., Kozerski, G., Mackay, D., Critical review and interpretation of environmental data for volatile methylsiloxanes: partition properties, *Environmental Science & Technology* 2014, 48, 11748-11759.

Zhang, J., Falany, J.L., Xie, X., Falany, C.N., Induction of rat hepatic drug metabolizing enzymes by dimethylcyclosiloxanes, *Chemico-Biological Interactions* 2010, 124, 133-147.

Zulehner, W., Neuer, B., Rau, G., 2000. Silicon, Ullmann's Encyclopedia of Industrial Chemistry. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

Zwir-Ferenc, A., Biziuk, M., Solid phase extraction technique – trends, opportunities and applications, *Journal of Environmental Studies* 2006, 15, 677-690.

Appendix 1 GC/MS Detector Description

Mass spectrometry is an analytical tool that deals with ionised chemical species, whereby a crucial step involves the ionisation of the molecules. There are several ionization techniques available, commonly divided into two major groups: “soft” and “hard” (Lebedev, 2012). In the “soft” ionization methods, the fragmentation of the compounds is not pronounced, usually resulting in a molecular ion, providing important information about the molecular mass and elemental composition. This type of ionization is usually achieved by chemical ionization, where the molecules under analysis react at high pressure with ionized species of a reagent gas, such as methane, ammonia or benzene, previously ionized, resulting in the neutral molecules of the analyte reacting with the reagent gas ions. On the other hand, “hard” ionization results in a higher level of fragmentation of the target compounds, allowing a better structural elucidation based on the masses of the fragment ions. Electron ionization (EI) mode is an example of a “hard” ionization method, in which the target molecules are vaporized into the ion source in conditions of high vacuum, where free electrons emitted by a filament interact with them, eventually knocking off another electron from the neutral molecules, yielding a positively charged ion. These recently-formed radical cations may be ionized multiple times, resulting in a relative abundance of different fragments, depending on the nature of the sample molecules and of the energy of the electrons. The standard energy of the electrons applied is 70 eV, but it can be increased or reduced in order to promote or reduce fragmentation, influencing the number of ions formed. Therefore, EI mode is considered a universal method, suitable for the study of many classes of chemical compounds (Lebedev, 2012).

The resulting ionised fragments represent the most stable pieces of the broken molecule, and their separation is performed in a mass analyser. In a modern GC/MS equipment, the most common mass analysers are quadrupoles and ion traps (Hübschmann, 2009). These mass analysers contain four long electrodes with appropriate hyperbolic cross sections, arranged at the corners of a square (Figure 1.1), on which direct current and radio frequency voltages are applied to create a quadrupole field (Snyder et al., 2017). In ion traps, those electrodes are wrapped into a circle, and rapid changes in the electric field in time result in the ions being trapped in the centre before being mass-selectively activated and ejected through the end-cap electrodes so that the mass analysis can occur. Ion fragments of the selected masses are usually detected in an electron multiplier, a high vacuum tubular structure ending in an anode that amplifies the response of the detected ions by a process called secondary emission: when a charged particle hits the electron multiplier surface, secondary electrons are released, with their number depending on the characteristics of the electron multiplier material surface, the nature of the incident primary particle, its angle and energy (March, 1997; Snyder et al., 2017).

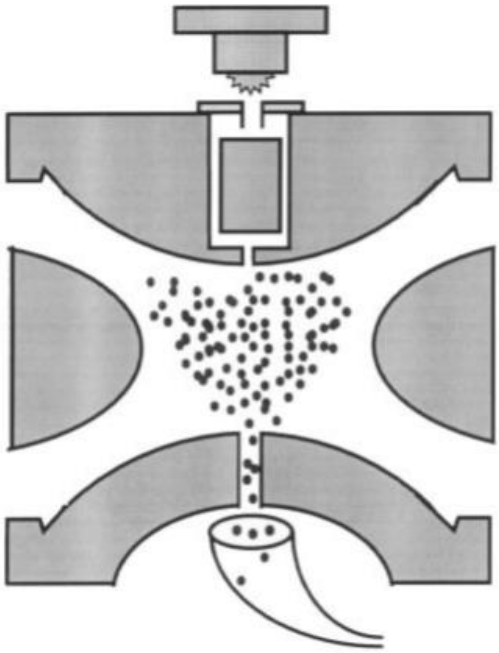


Figure 1.1. Scheme of a quadrupole ion trap mass analyser (adapted from March, 1997).

The recorded intensity of each group of fragments are plotted with their mass-to-charge ratio (*m/z*), resulting in a mass spectrum, usually normalized to 100% for the most abundant ion formed (Lebedev, 2012). The mass spectrum is a unique feature of the targeted compound, like a fingerprint. The representation of the signal intensity *versus* the GC retention time results in the chromatogram. Data acquisition can be performed in full-scan mode (all intensities of every mass fragments at a given time are recorded and displayed) or selected ion storage (SIS) mode (only the certain *m/z* ranges, defined by the operator, are selected for detection during analysis, which usually results in enhancement of selectivity and sensitivity). Modern GC/MS systems are usually controlled by a computer, responsible for the regulation of all physical parameters of the system and for data recording and processing (Sparkman et al., 2011).

Appendix 2 GC/MS Chromatograms

The chromatograms, in SIS mode, obtained after the injection of *Fucus vesiculosus* extracts spiked with IS at 15 ng.g^{-1} and VMSs at 6 ng.g^{-1}, and the same sample spiked with only IS at 15 ng.g^{-1} are presented in Figures 2.1 and 2.2 respectively. In both cases, time axis represents only the period during which the ionization was turned on.

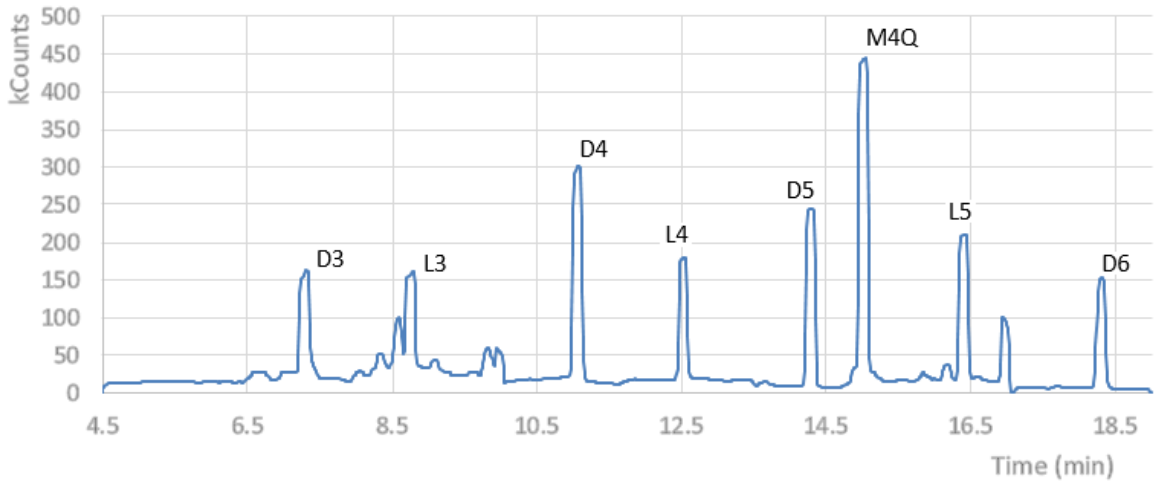


Figure 2.1. Chromatogram of an extract of *Fucus vesiculosus* spiked with VMSs at 6 ng.g^{-1} and IS at 15 ng.g^{-1}.

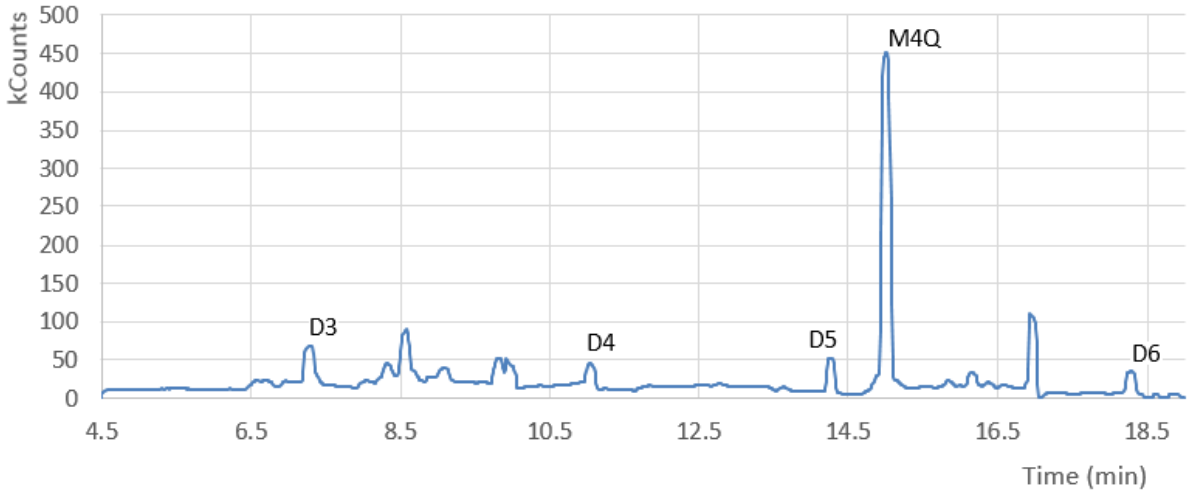


Figure 2.2. Chromatogram of an extract of *Fucus vesiculosus* spiked with IS at 15 ng.g^{-1}.

Appendix 3 Method Validation – Support Information

The response factors (RF) obtained for each VMS under analysis at the different calibration standard concentrations are presented in Table 3.1. Those values were used to create the calibration curves for the different siloxanes and also to calculate their validation parameters (Table 3.2). In Figures 3.1 to 3.7, graphic representations of the resulting calibration curves are presented.

Table 3.1. Response factors (RF) obtained for each compound at different concentration levels, used for the construction of the calibration curves.

	Response Factor (RF)						
Concentration (µg.L^{-1})	**D3**	**L3**	**D4**	**L4**	**D5**	**L5**	**D6**
1.00	0.022	0.004	0.029	0.004	0.019	0.009	0.013
5.00	0.038	0.021	0.068	0.020	0.038	0.026	0.026
25.00	0.098	0.103	0.191	0.086	0.106	0.118	0.039
50.00	0.194	0.211	0.394	0.183	0.237	0.226	0.080
100.00	0.375	0.410	0.721	0.338	0.445	0.455	0.187
250.00	0.983	1.073	1.452	0.908	0.996	1.166	0.317
500.00	1.875	2.050	3.563	1.740	1.999	2.412	0.649
750.00	2.815	3.098	5.404	2.620	2.869	3.551	0.944

Table 3.2. Calibration curves equations and their respective validation parameters.

Compound	**Calibration curve equation** $y = (a \pm s_a).x + (b \pm s_b)$	***r*** **(> 0.9950)**	s_a/a **(< 5%)**	$b-s_b$ / $b+s_b$	$(b-s_b < 0 \wedge b+s_b > 0)$
D3	RF = (0.0037 ± 0.0000).C + (0.0146 ± 0.0074)	0.9999 ✓	0.6% ✓	0.007 / 0.022	×
L3	RF = (0.0041 ± 0.0000).C + (0.0044 ± 0.0078)	0.9999 ✓	0.6% ✓	-0.003 / 0.012	✓
D4	RF = (0.0071 ± 0.0002).C - (0.0190 ± 0.0623)	0.9979 ✓	2.6% ✓	-0.081 / 0.043	✓
L4	RF = (0.0035 ± 0.0000).C + (0.0037 ± 0.0069)	0.9999 ✓	0.6% ✓	-0.003 / 0.011	✓
D5	RF = (0.0038 ± 0.0000).C + (0.0335 ± 0.0144)	0.9996 ✓	1.1% ✓	0.019 / 0.048	✓
L5	RF = (0.0048 ± 0.0000).C - (0.0052 ± 0.0091)	0.9999 ✓	0.6% ✓	-0.014 / 0.004	✓
D6	RF = (0.0012 ± 0.0000).C + (0.0217 ± 0.0090)	0.9986 ✓	2.2% ✓	0.013 / 0.031	×

RF – response factor; C – concentration of compound (µg.L^{-1}); *r* – Pearson correlation coefficient; a – slope; s_a - standard deviation of the slope; s_a/a – relative standard deviation of the slope; b – intercept; s_b - standard deviation of the intercept.

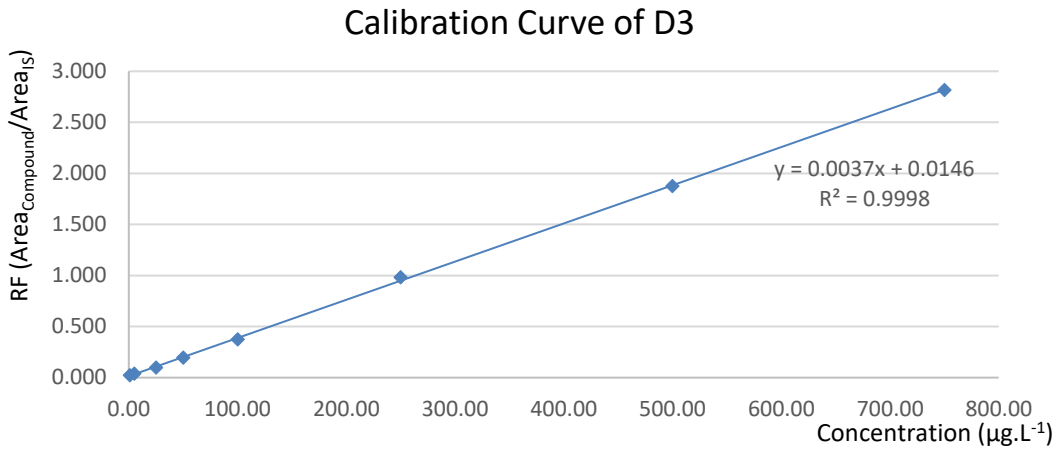


Figure 3.1. Graphic representation of the calibration curve of D3.

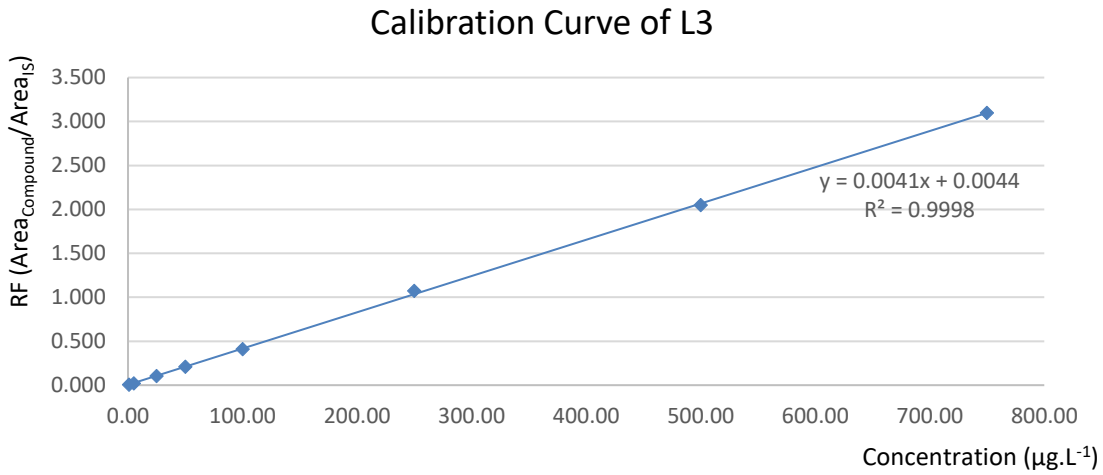


Figure 3.2. Graphic representation of the calibration curve of L3.

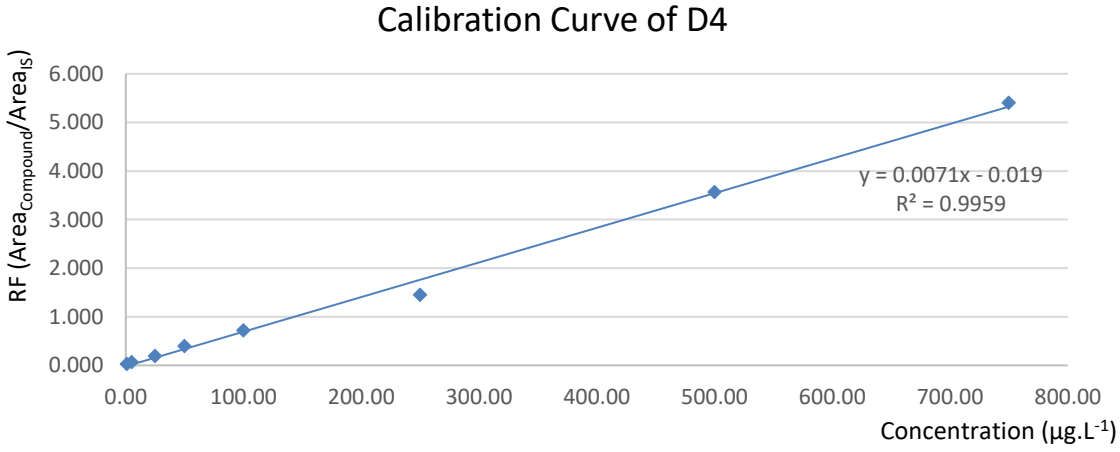


Figure 3.3. Graphic representation of the calibration curve of D4.

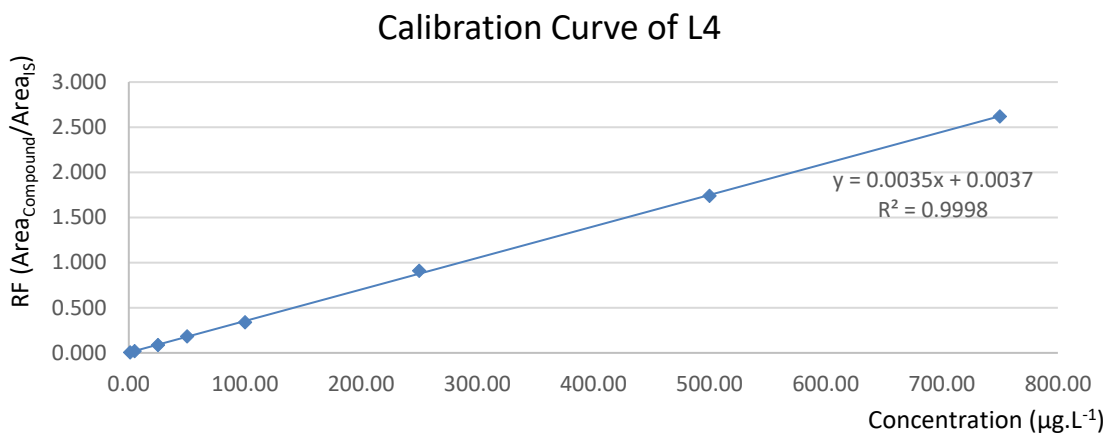


Figure 3.4. Graphic representation of the calibration curve of L4.

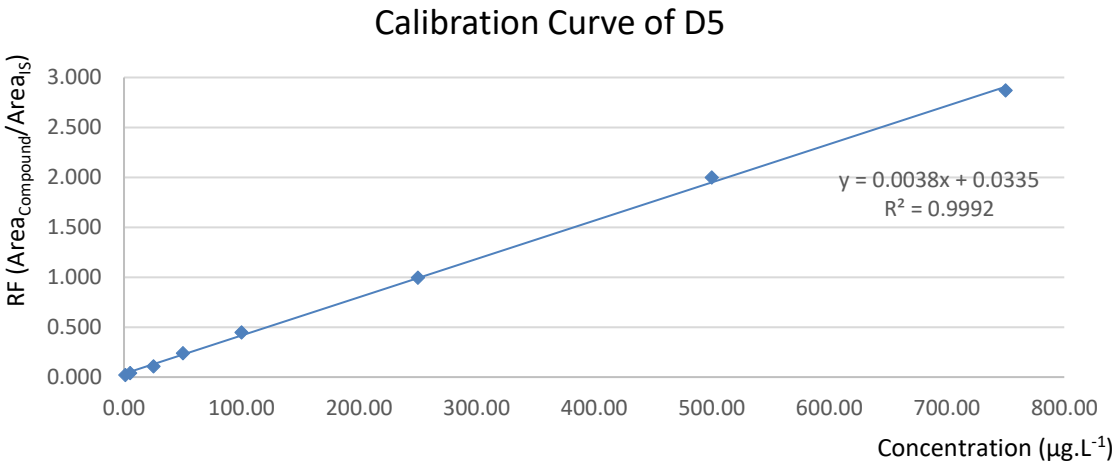


Figure 3.5. Graphic representation of the calibration curve of D5.

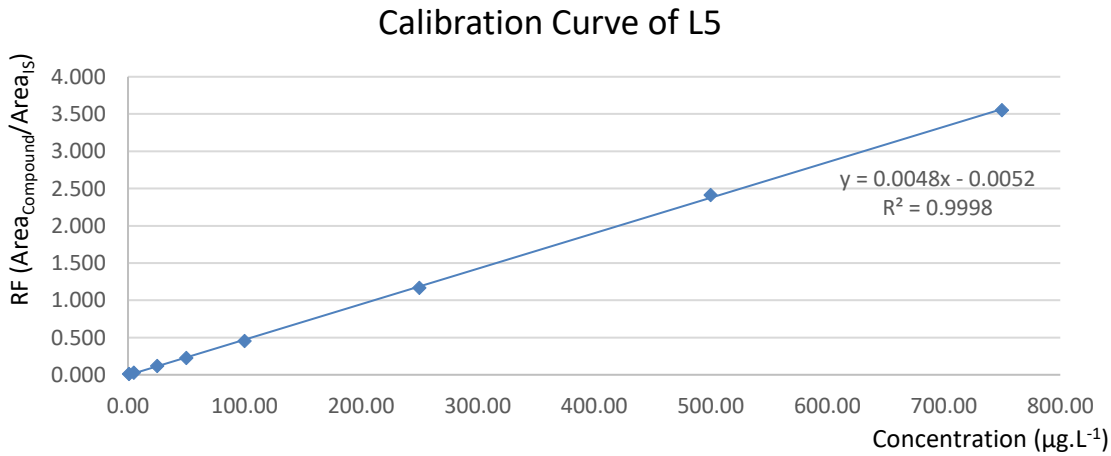


Figure 3.6. Graphic representation of the calibration curve of L5.

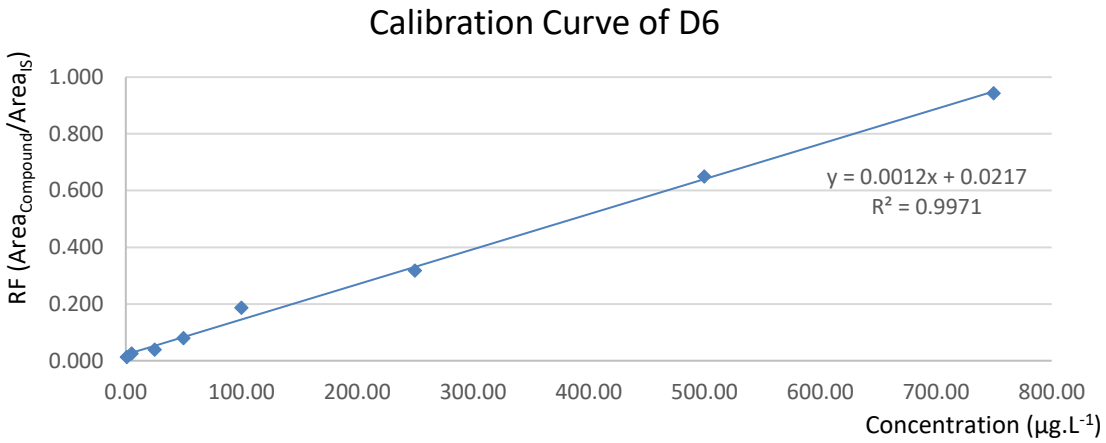


Figure 3.7. Graphic representation of the calibration curve of D6.

Appendix 4 Real Samples Analysis – Support Information

Average values of siloxanes contamination detected in procedural blanks performed with every extraction batch in each group of samples analysed are presented in Table 4.1. In Table 4.2, the average weight determinations of samples before and after the drying process are shown, as well as the water and dry mass percentage obtained for each species analysed.

Table 4.1. Overview of the average values of siloxanes contamination in procedural blanks, performed for each group of samples analysed. Concentrations in µg of compound per L of extract.

	Concentration ($\mu g.L^{-1}$)							
	Barra	**Carneiro**	**Conchinha**	**Miramar**	**Murcia (Summer)**	**Murcia (Winter)**	**Global Average**	**SD**
D3	29.56	48.99	27.9	40.81	36.81	31.06	35.86	8.09
L3	nd	nd	nd	nd	nd	nd	-	-
D4	11.36	30.16	12.73	11.93	16.62	12.37	15.86	7.25
L4	nd	0.86	nd	<LOQ	0.99	<LOQ	0.46	0.54
D5	33.53	34.47	41.78	34	31.93	37.04	35.46	3.51
L5	2.45	1.94	4.15	2.34	1.76	1.69	2.39	0.92
D6	43.96	31.88	79.67	33.97	21.51	19.07	38.34	22.16

Table 4.2. Determinations of the dry weight, water percentage and the percentage of dry mass for each one of the species collected, based on two determinations for each sample.

Sample	Wet Weight (g)	Dry Weight (g)	% Water	% Dry Mass
Codium tomentosum	2.543	0.189	92.56%	7.44%
Fucus vesiculosus	2.532	0.837	66.95%	33.05%
Palmaria palmata	2.574	0.462	82.05%	17.95%
Porphyra sp.	2.575	0.440	82.93%	16.94%
Posidonia oceanica	2.522	0.401	84.09%	16.24%
Kelp	2.546	0.659	74.10%	25.90%
Schizymenia dubyi	2.579	0.535	79.25%	20.75%
Ulva lactua	2.540	0.602	76.31%	23.69%