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Solvent-saving approaches for the extraction of siloxanes from pine needles, soils and passive air samplers

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In this study, a solvent-saving analytical strategy was validated to quantify the levels of 8 volatile methyl siloxanes (VMSs) in pine needles, soils and air (measured by sorbent-impregnated polyurethane foam passive samplers, SIPs). Different extraction solvents and sample handling procedures were tested and the protocol that reached the highest recoveries employed QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) and was adapted to pine needles and soils. For SIPs, another method was developed in parallel, as QuEChERS could not be applied to this matrix due to logistic and operative constraints. Thus, extraction was performed using classic Soxhlet extractors and a short clean-up step, limited to the removal of water by a solid-phase extraction (SPE) column containing sodium sulphate. The quantification of the target compounds was performed by gas chromatography/mass spectroscopy (GC/ MS), with identical set-ups for the three matrices. Similar validation protocols were applied and yielded limits of detection (LODs) from 1.8 to 10.8 ng kg^{-1} (dry weight) for pine needles, from 3.4 to 19.8 ng kg^{-1} (dw) for soils and from 4.7 to 10.2 ng SIP^{-1} (dw) for SIPs. The overall mean recoveries were 75 ± 11%, 69 ± 17% and 87 ± 8%, respectively. The application of the methodologies to naturally contaminated samples collected in an urban and a remote site revealed siloxane levels comparable to other studies in the literature and a predominance of the cyclic siloxanes over the linear ones, which were frequently not detected.

Introduction

The analysis of siloxanes in environmental matrices is quite a challenging task, mainly due to their ubiquitous use since they were first produced in the 1940s.[1] The recent attention given by the scientific community to these chemicals (in particular to organosiloxanes) derives from reports mentioning persistence and possible harmful effects to the environment and ecosystems.[2] Siloxanes are organosilicon compounds consisting of alternating Si–O bonds as a backbone with organic side chains which can be classified as either linear or cyclic according to their structure.[3] These anthropogenic compounds have a wide range of use and are incorporated into a variety of household and industrial applications, such as in cosmetics and other

personal care products, textiles, pharmaceuticals, electronics, furniture, food and construction, among others.[4–6]

A special focus has been given to volatile methyl siloxanes (VMSs), which are organosilicons with low molecular weights and high vapour pressures that can be derived from PDMS hydrolysis.[7,8] Their presence was shown in several environmental matrices such as outdoor and indoor air,[9–11] soil[12,13] aquatic media,[14,15] sediments,[16] wastewater and sludge,[17–19] biota[20,21] or vegetation.[22] Regarding the extraction and quantification of VMSs in these complex matrices, also the analytical approaches reflect an increasing development in the last years. Three recent reviews reported a thorough list of options that include extraction by Soxhlet, sonication or pressurized liquid extraction (PLE), clean-up by solid-phase extraction (SPE) with several types of sorbents in glass columns or cartridges and most often employing quantification by gas chromatography with mass spectrometry detection (GC/MS) (ref. 1, 2, and 7 and references therein). However, although the analytes are relatively easy to identify using chromatographic techniques, the aforementioned presence of silicon-based materials virtually everywhere adds a strong complexity due to the possibility of external contaminations of the samples.[23] Not only from the people handling the samples during collection or in the laboratory due to the use of personal care products but also especially from the presence of organosiloxanes in several parts of the chromatographic equipment and consumables, including capillary columns and even solvents.[7] Extra care is needed to minimise or eliminate these sources of contamination, particularly when dealing with samples of matrices that are hard to analyse *per se* and with levels of target chemical close to the limits of detection.[24]

With these assumptions, and to help the enhancement of the still limited knowledge about the fate and behaviour of siloxanes,[1] expedite and reliable analytical methods were developed in this work for the extraction and quantification of VMSs in air, soil and vegetation samples, with a focus toward solvent-saving approaches. This included an original application of the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction method to soil and pine needles. Although having been employed for the biomonitoring of several semi- volatile organic compounds (SVOCs), to our best knowledge this is the first time this vegetation species was tested in the assessment of VMS levels. Different extraction solvents and sample handling procedures were tested and for air samples (measured with the deployment of solvent-impregnated poly- urethane foam disks – SIPs), another method was developed in parallel using classic Soxhlet extractors and a cleanup limited to the removal of water by a SPE column containing sodium sulphate. This environment-friendly strategy yields effective tools to expand the range of monitors for siloxanes and to enlarge the databases available for the understanding of these under-the-spotlight chemicals.

Experimental

Chemicals and materials

All standards of the target siloxanes (L2: hexamethyldisiloxane, L3: octamethyltrisiloxane, L4: decamethyltetrasiloxane, L5: polydimethylsiloxane, D3: hexamethylcyclotrisiloxane, D4: octamethylcyclotetrasiloxane, D5: decamethylcyclopentasilox- ane, and D6: dodecamethylcyclohexasiloxane) and of the internal standard (M4Q: tetrakis(trimethylsiloxy)silane) were purchased individually from Sigma-Aldrich (St. Louis, MI, USA) with a purity of 97 to $99%. Individual (1 g L^{-1}) and mix (5 mg L^{-1}) stock solutions and working standard mixes were prepared from them (in hexane) and stored in the dark in amber glass vials at −20 °C until use. Several solvents were tested (analytical grade Prolabo), provided by VWR (Fontenay-sous-Bois, France): dichloromethane (DCM), hexane (Hex), acetone (Acet) and acetonitrile (ACN). For the preparation of clean-up columns and QuEChERS, alumina (neutral aluminium oxide 90, particle size 0.063–0.200 mm) and sodium sulphate (Na_2SO_4) were supplied by Merck (Darmstadt, Germany), anhydrous magnesium sulphate ($MgSO_4$) and sodium acetate ($NaCH_3COO$) by Sigma- Aldrich and PSA bonded silica and C18 by Supelco (Bellefonte, PA, USA). Na_2SO_4 and $MgSO_4$ were baked at 450 °C overnight before use in a Nabertherm N 120/65 HA furnace (Lilienthal, Germany), as well as all non-graduated glassware. Helium (99.9999%, the GC carrier gas) and nitrogen (99.995%, for solvent drying) were acquired from Air Liquide (Maia, Portugal).

Samples

Pine needles and soil samples for the method validation were collected in the premises of the University of Porto, in order to analyse them immediately after collection. Needles from *Pinus pinea* L. species were collected from the bottom branches of the same tree and soil was collected with a stainless steel tool from the 0–10 cm layer. Pine needles were analysed and cut into 1 cm pieces and soil was sieved to remove litter such as roots, stones and leafs and the <2 mm particle fraction was chosen for assays. Complementary sample conditioning was tested and will be described below. These samples and others from a rural site (Midões) were also used for the application of the developed methodology to a field-based environment. Naturally contaminated pine needles from Midões were wrapped in aluminium foil and sealed in plastic bags whereas soils were placed in solvent-rinsed amber jars. All samples were frozen until analysis and defrosted at ambient temperature immediately before it. The moisture content of pine needles and soils was determined to allow the final expression of results on a dry-weight basis. For this, triplicate 2.5 g samples were dried in an oven at 80 °C until constant weight. The material tested for the air samples is used for passive sampling: SIPs. To make them, pre-cleaned poly- urethane foam (PUF) disks (14 cm diameter; 1.35 cm thick; surface area, 365 cm^2; mass, 4.40 g; volume, 207 cm^3; density, 0.0213 g cm^{-3}) from Tisch Environment (Cleves, OH, USA) were impregnated with finely ground XAD-4 supplied by Supelco (Bellafonte, PA, USA) in a hexane slurry (11 g of XAD-4 in 1.7 L of hexane) and dried under vacuum in a Buchi R-200 rotary

evaporator (Flawil, Switzerland) and stored in solvent-rinsed airtight containers until analysis. XAD-4 is a polymeric material that enhances the field performance of passive air sampling dedicated to assess siloxanes.[25] For the field-based tests, SIPs were deployed in the same sites as where pine needles and soils were collected, for a period of three months.

Validation protocol

The developed analytical protocol was validated regarding the linearity of response of the detector using seven calibration standards (1, 10, 50, 100, 200, 250 and 500 mg L^{-1}), the limits of detection (LOD) using the signal-to-noise ratio of 3, recovery assays performed with triplicate samples spiked with 250 mg L^{-1} of the siloxane mix (L2–L5 and D3–D6) and 125 mg L^{-1} of M4Q as internal standard plus one blank, repeatability also with triplicate samples and interday precision with five replicates analysed in different days, using the same spiking levels. For pine needles and soils, 2.5 g of samples were used and for SIPs, pre-cleaned disks, to follow the final protocol.

Extraction of pine needles and soil

A preliminary test was conducted for pine needles, based on the experience acquired during the development of a multi- component protocol for the extraction of PAHs, musks, BFRs, PCBs and HCB from this matrix.[26] Thus, 2.5 g of *Pinus pinea* needles were cut into approximately 1 cm strips, spiked with 30 ng g^{-1} of internal standard M4Q and 60 ng g^{-1} of a siloxane mix (L2, L3, L4, L5, D3, D4, D5, and D6) and extracted with 100 mL of DCM/Hex (1 : 1) or Acet/Hex (1 : 1) – the tested solvent mixes – for 15 min in a 720W Selecta ultrasonic bath (J.P. Selecta, Barcelona, Spain). After cooling down to room temperature, the solvent was separated from the needles and volume reduced to approximately to 1 mL by rotary evaporation. Solid-phase extraction columns were prepared by packing 5 g of activated alumina into glass column, topped with a small amount of anhydride sodium sulfate. The spiked extract was applied to the column and eluted with 50 mL DCM/Hex (1 : 1). After volume reduction to approx. 1 mL by rotary evaporation, the extract underwent a subsequent clean-up in a gel permeation chromatography (GPC) column prepared using 6 g of S-X3 Bio- Beads® from Bio-Rad (Hercules, CA, USA). Elution was carried out with 40 mL of DCM/Hex (1 : 1) of which the first 15 mL were rejected. The remaining eluate was reduced by rotary evaporation and then by nitrogen blow down to near dryness. Finally, the extract was redissolved in 150 µL of *n*-hexane before injection in the GC/MS.

The definitive option relied on a recent solvent-saving technique based on QuEChERS, a kind of dispersive SPE. This technique had already been attempted by our group for the analysis of pesticides in pine needles[27] and employed with success for the assessment of musks in personal care products.[28] A wide choice of pre-prepared QuEChERS mixtures is commercially available, either in polypropylene tubes or sachets. However, having proven previously that QuEChERS prepared in the lab show equal performance as the commercial ones, QuEChERS were prepared in-house for the sake of economy, following the

protocol of Homem *et al.*[28] For each pine needles and soil samples, two QuEChERS were prepared and used sequentially: QuEChERS 1 (Q1) was used in the partitioning step and contained 6 g of $MgSO_4$ and 1.5 g of CH_3COONa. QuEChERS 2 (Q2) was used for the cleanup of the extract and contained 900 mg of $MgSO_4$, 300 mg of PSA and 150 mg of C18. In the end, the analytical protocol validated was: 2.5 g of pine needles (3–5 mm pieces) or 2.5 g of fresh soils (sieved to the <2 mm fraction) spiked with 15 ng g^{-1} of internal standard M4Q were placed in a 50 mL polypropylene conical bottom centrifuge tubes and extracted for 15 min in an ultra- sonic bath with 10 mL of DCM/Hex (1 : 1). At this point, after cooling down to room temperature, extracts were filtered with PTFE 0.2 mm from Supelco, but only for soil samples. Then, to both pine needles and soils, Q1 containing 6 g $MgSO_4$ + 1.5 g CH_3COONa was added and mixed in a vortex for 3 min. Then, the phases were separated in a Rotofix 32A centrifuge from Hettich (Kirchlengern, Germany) for 10 min at 4000 rpm and the supernatant was transferred to a clean 50 mL centrifuge tube containing Q2 (900 mg $MgSO_4$ + 300 mg PSA + 150 mg of C18). The mix was again vortexed for 3 min and centrifuged for 10 min at 4000 rpm and the supernatant was transferred to an amber GC/MS vial, evaporated to near dryness with N_2 and finally topped with 150 μL of Hex before chromatographic analysis.

Extraction of air samples (SIPs)

Soxhlet extraction with different solvents was tested followed by only one additional step where the obtained extract was filtered and desiccated using a SPE column containing sodium sulfate. Consequently, SIP disks were put into a 200 mL Soxhlet extractor, spiked with 150 ng of a siloxane mix and 75 ng of internal standard (M4Q) and extracted overnight with the solvents to be tested: Hex, Hex/Acet 1 : 1 or DCM/Hex 1 : 1.

Then, the extracts were passed through a glass column containing anhydride Na_2SO_4. The eluate was then reduced to approximately 1 mL by rotary evaporation, transferred to glass vials, evaporated to near-dryness by N_2 and redissolved in 300 mL Hex before injection in the GC/MS.

Chromatography

The analysis of siloxanes is challenging as siloxane-containing GC/MS components, namely injector, septum and column, are prone to bleeding and cause background levels that may interfere with quantification. Therefore, some modifications to the Varian 4000 GC/MS system (Lake Forest, CA, USA) working in electron impact mode (70 eV) were made in order to reduce this kind of interference. The conventional CP-1177 split/splitless injector was adapted with a Merlin Microseal System from Supelco (Bellefonte, PA, USA) that was referred to help on the problem of siloxane bleeding.[29] Another important source of bleeding is the polydimethylsiloxane film contained in most chromatographic columns. For our purpose, a special DB-5 ms ultra-inert column (30 m × 0.25 mm I.D., 0.25 μm film thick-ness) from Agilent (Santa Clara, CA, USA) was chosen due to its very low bleeding behavior.

Therefore, chromatographic separation was performed using the above-mentioned column with helium at 1 mL min^{-1} as the carrier gas and a GC oven temperature program as follows: starting 35 °C (hold for 5 min), then increased to 160 °C at 10 °C min^{-1}. Several injector temperatures (150 °C, 200 °C and 250 °C) as well as injection liners with and without glass wool were tested. The best response was achieved using an injection liner without glass wool and an injection temperature of 200 °C. The injection volume was 1 μL in splitless mode. Helium (at 1.0 mL min^{-1}) was the carrier gas and the temperatures of the transfer line and ion source were 250 °C and 200 °C, respectively. Detection of the target compounds was performed using the ion-trap mass spectrometer operating in electron ionization mode (70 eV) and time-scheduled selected ion storage (SIS) acquisition according to Table 1. M4Q was used as the internal standard, after showing better performances than TKS in the preliminary tests, and the quantification of the target chemicals was performed using the Mass Spectrometry Workstation 6.6 software from Varian.

Quality assurance/quality control

As mentioned previously, when studying VMSs there are some sources of potential external contamination. Laboratory personnel was instructed to refrain or limit to a minimum the use of personal care products, as some of the target chemicals may be included in their formulations. Special care was taken with the chromatographic equipment. Besides the use of a Merlin microseal adapted to the injector and a low-bleed capillary column, septa were changed and the liner was cleaned by sonication frequently, as these are two of the main sources of silicon contamination into analysed samples.[7] Given the level of volatility of the target VMSs, the complete dryness of the extracts in the analytical protocol was avoided to reduce losses by evaporation. Every new bottle of solvent was analysed to check if they were clear of VMSs. All glassware or related material involved in the preparation and analysis of samples was baked overnight at 450 °C and rinsed with acetone and hexane before use. These efforts reduced considerably the external contamination by VMSs, but were not enough to eradicate them completely, especially in some cases for D5 and D6. For these reasons, laboratory blanks were performed recurrently and whenever needed, the results were corrected accordingly (subtracting the blank concentrations). Nevertheless, it was possible to obtain low LODs in this first attempt of using QuEChERS for the analysis of VMSs in pine needles and soils and for the Soxhlet approach for SIPs. Results were not corrected for recovery, which was checked using the internal standard M4Q.

Results and discussion

Preliminary tests

As a first attempt, the adaptation of a previously developed protocol[26] based on ultrasonic assisted extraction (USE) followed by cleanup with solid phase extraction (SPE) and gel permeation chromatography (GPC) was tested for pine needles, as this was one of the three matrices in study for which, to our knowledge,

there were no reports of VMS analysis and quantification. To set-up the appropriate amount of elution solvent for the SPE cleanup, elution profile assays were established following the SPE protocol described above, extracting pine needles with a total of 100 mL of two solvent mixes that were tested separately (DCM/Hex (1 : 1) and Acet : Hex (1 : 1)), and collecting 10 mL fractions of the eluate. The fractioning behavior of the SPE column was clearly visible. While the first fraction is clear, as this is a sample taken before extract application onto the column, the following fractions show different tones. First yellowish, probably due to the carotenes which elute first as they are mostly apolar, then showing high amounts of a viscous greenish mass (probably chlorophylls), and finally the fractions appear with increasing transparency. A 50 mL volume was considered enough for the elution and the overall recoveries, although better for DCM/Hex, were still low, as can be seen in Fig. 1. Increasing recoveries with the boiling point of the siloxanes can be noticed, but not exceeding 26%. The siloxanes studied in this work are volatile chemicals, and the recoveries are naturally influenced by the number of solvent-reduction steps in the extraction protocols. Using a classic SPE extraction protocol, there are a few of these steps and despite all the efforts taken in the process, it is inevitable that the "lighter" siloxanes (smaller molecules, more prone to be lost by evaporation) do not display not as good recoveries as larger molecules such as the cyclic siloxanes D5 or D6, for instance. Given the poor results of this approach, it was decided to test alternative approaches, relying on "green" solvent-saving techniques.

QuEChERS extraction of pine needles

In this case a rather recent methodology based on QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) was tested. This technique offers several advantages over traditional methods: (a) fast and easy to perform and therefore less susceptible to error; (b) uses disposable containers and devices, minimising the risk of contamination; (c) low consumption of resources (solvents, sorbents, working time) and easy handling. A typical QuEChERS protocol involves extraction, partitioning and cleanup steps.[30] In brief, the extraction of the target compounds from the matrix is most often carried out using sonication, to improve the mass transfer of the analytes. In the next step, buffer salts such as sodium acetate or sodium citrate are added to control the pH level in order to stabilise the analytes and allow a better performance of the dispersive SPE sorbents in the cleanup step. Additionally, a drying agent (usually magnesium sulfate) is added to remove any remaining water and sometimes also to promote the migration of less polar organic analytes to the solvent phase.[28] A good mixing prior to centrifugation enhances the subsequent phase separation. Finally, a cleanup step by dispersive SPE can be performed to achieve an extract that allows good chromatographic performance, minimising matrix effects. The most commonly employed sorbents are primary secondary amine (PSA), octadecylsilica (C18) and graphitized carbon black[31] and a wide choice of pre-prepared QuEChERS mixtures is commercially available, either in polypropylene tubes or sachets. In our case, it was decided to employ the composition previously used in our group for the determination of synthetic musk fragrances in personal care

products[28] and proceed to the optimisation of the extraction conditions, again starting with pine needles.

Choice of the extraction solvent. Two pure solvents, *n*-hexane (Hex) and acetonitrile (ACN), and two solvent mixtures, DCM/ Hex 1 : 1 and Acet/Hex 1 : 1, were tested. This choice of the pure solvents was made in order to cover a wide range of polarities and miscibility, as the capacity of the solvent to wet the matrix plays an important role in the recovery of the analytes. The mixtures were chosen to obtain a combination of properties from each solvent. In case of the Acet/Hex mixture, the non- polarity of Hex, which matches that of the siloxanes, is combined with the capacity of the acetone to wet the sample. On the other hand, the mixture DCM/Hex covers a large spectrum of polarities but at the same time avoiding the extraction of water, which may interfere with the process.

Following the determination of the respective recoveries (Fig. 2), DCM/Hex seems to be the most appropriate extraction solvent. However, as sample preparation may also affect the extraction efficiency, also Acet/Hex was considered for further testing. Statistics confirm this choice, as a dependent *t*-test for paired samples with a 95% confidence interval reveals that there is no significant difference between the recoveries using Hex/DCM and Acet/Hex, whereas the pure solvents have significantly lower recoveries. It is interesting to notice that, as in the preliminary tests with the SPE method, recoveries follow an inverse correlation with the volatility of the individual siloxanes. This reinforces the extreme care one must take in the solvent-reducing steps and to reduce them to a minimum, due to the possible losses by evaporation explained previously. This may also be the reason why the standard deviations of the recoveries are much higher for the more volatile siloxanes (L2, D3, L3, D4). The instability of these compounds due to their higher volatilities makes the consistency of the recoveries more difficult to obtain. In any case, they are now much higher than in the classic SPE approach, reaching over 80% for all siloxanes except L2, D3 and L3 for DCM/Hex. Using QuEChERS the solvent evaporation steps are down to one and this can be one of the reasons for this better performance.

Freeze-drying of pine needles. As mentioned previously, effective removal of water is important and is usually carried out by employing drying agents such as $MgSO_4$. Another possibility of reducing water levels is by acting directly on the sample using, for instance, freeze-drying (also known as lyophilisation)

In this process, a previously frozen sample is subject to vacuum at ambient temperature. Due to the vacuum the ice will not melt into water, but rather sublimate into vapor, preserving the more volatile analytes.[32] For these tests, pine needles were cut into 3–5 mm pieces and frozen for at least 48 h. Before lyophilisation, 2.5 g samples were placed into 50 mL polypropylene centrifuge tubes, covered with perforated aluminum foil and freeze-dried for 72 h. After drying, the needles were again weighted to determine the moisture level and spiked with 30 ng g^{-1} of a siloxane mix containing 15 ng g^{-1} of M4Q and extracted with either DCM/Hex or Hex/Acet using the same procedure as in the choice of solvent. The moisture of the pine needles (*P. pinea*) was on average 38 ± 3%, lower than the 59% reported by Ratola *et al.*,[33] which

employed oven-drying at 80 °C until constant weight instead. Fig. 3 compares fresh and freeze-dried needles, extracted either with DCM/Hex or Hex/Acet. Highest mean recoveries were achieved for DCM/Hex and the dependent *t*-test shows a significant difference between DCM/Hex and Hex/ Acet, but no significant difference was found between fresh or freeze-dried needles, mean recoveries being 72 ± 8% in both cases. This is not surprising as this solvent mixture is hydro- phobic and therefore no water co-extraction would be expected. In the assays above, the spiking of the matrix was carried out after freeze-drying. This was performed deliberately in order to evaluate only the extraction efficiency of the dried samples and not the freeze-drying process itself. However, in naturally spiked samples, analytes are present already before freeze-drying and therefore, the losses during lyophilisation must also be evaluated. For this reason, pine needles were spiked before drying and compared to the ones spiked after 72 h of lyophilisation. As DCM/Hex showed the best results, extraction was performed only with this solvent mixture. Recoveries show that overall there are some losses of compounds during lyophilisation. In fact, the freeze-drying process reduced the mean recovery from 72% to 64%, while standard deviation increased from 7% to 12%. Other authors also report losses when dealing with the extraction of SVOCs from environmental samples.[34] Taking this into account and the time length of the whole process, freeze-drying was discarded in the remaining analysis.

The differences between cut and ground needles (with a pestle and mortar) were also tested, and for DCM/Hex the mean recoveries of the individual siloxanes were similar (67 ± 7% for cut and 63 ± 15% for ground needles). In this case, given the slightly worse reproducibility (higher SD) and the tendency to form a paste that adhered to the surface of the mortar of ground needles, it was decided to continue with the cut version.

QuEChERS extraction of soils

After the good results obtained for pine needles with the QuEChERS methodology, an adaptation of this method for soils was attempted. Again, the extraction solvent and freeze-drying of the matrix were tested.

Choice of the extraction solvent. Based on the previous experience, one pure solvent (acetone) and two solvent mixtures (DCM/Hex (1 : 1) and Hex/Acet (1 : 1)) were chosen. For recovery determination, fresh soil was sieved to remove litter such as roots, stones and leafs and the <2 mm particle fraction was collected for the assays. Then, 2.5 g of soil was weighted into 50 mL polypropylene conical bottom centrifuge tubes and spiked with 30 ng g^{-1} of a siloxane mix and 15 ng g^{-1} of M4Q as the internal standard. After resting for about 20 min for impreg- nation, 10 mL of solvent were added and extracted for 15 min in an ultrasonic bath. The following procedure is the same as the one described for pine needles with one exception. Having found that some soil particles were interfering with the dispersive extraction, it was decided to test if filtration of the extract after the sonication had an impact on the recovery of the target VMSs. Two types of filters were employed: PTFE 0.2 μm and glass fibre. No appreciable retention of the analytes and the internal standard was found for both types, but PTFE 0.2 μm were chosen in the end due to their generally better

performance (Table 2).

Regarding the solvents tested in the extraction, Fig. 4 shows that pure acetone delivered the worst recoveries, ranging from zero (all the linear siloxanes) to 180% (D6). Hex/Acet obtained slightly better mean recoveries than DCM/Hex (66% and 56%, respectively). However, the standard deviation is much worse (mean of 52% for Hex/Acet and of 13% for DCM/Hex).

Freeze-drying of soils. As water may again affect recovery, freeze-drying of the soil was tested. Sieved soil was freeze-dried as a bulk for 5 days until constant weight was achieved. The mean moisture level of the soil was 15.4% (as opposed to the 25% obtained with oven drying until constant weight). The procedure to test recoveries was identical to the pine needles and again there was no improvement compared to the fresh soil. Consequently, freeze-drying was also discarded in this case.

Sorbent-impregnated polyurethane foam disks (SIPs) extraction

SIPs differ from pine needles and soils, and therefore the extraction method takes this into account. Due to the shape and material, QuEChERS extraction is not a viable option, and a classical approach had to be employed. As SIPs are only exposed to air during deployment, interfering compounds are minimal and exempt from intensive cleanup methods. For this reason, Soxhlet extraction with different solvents (Hex and the mixtures DCM/Hex (1 : 1) and Acet/Hex (1 : 1)) was tested followed by only one simple clean-up step with sodium sulfate. Some differences could be noticed among the solvents tested already when adding them to the Soxhlet. The mixture Hex/Acet caused the release of a significant amount of XAD-4 resin from the SIP disks that eventually led to the clogging of the sodium sulfate column. With Hex and DCM/Hex this effect was only residual, so no problems were seen during extraction and cleanup. The best mean recoveries and respective standard deviations in this test were also found for DCM/Hex (84 ± 5%), followed by Acet/Hex (79 ± 45%). Thus, DCM/Hex was chosen as the extraction solvent also for SIPs.

Method validation

All these optimization assays allowed the establishment of an analytical protocol for the extraction and cleanup of siloxanes from all matrices in an expedite and solvent-saving manner. The protocols for the three matrices then underwent the appropriate validation procedure to establish parameters of linearity, limits of detection and reproducibility, using samples spiked with 250 μg L^{-1} of a mix containing all target siloxanes and 125 μg L^{-1} of M4Q as the internal standard. New recovery assays were also performed with the same spiking levels. The results can be seen in Tables 3–5 for pine needles, soils and SIPs, respectively.

A good linear behaviour was obtained for all compounds and matrices at concentrations, with coefficients of determination (R^2) ranging between 0.9946 and 0.9997 overall. Despite all the problems with possible external contaminations in the lab and during the GC/MS operation, low LODs were obtained: from 1.8 to 10.8 ng kg^{-1} (dry weight) for pine needles, from 3.4 to 19.8 ng kg^{-1} (dw) for soils and from 4.7 to

10.2 ngSIP^{-1} (dw) for SIPs. These values are in line with other studies in the literature. Although there is no possible comparison for pine needles, Sânchez-Brunete *et al.*[12] found LODs of 0.5–1.1 ng kg^{-1} for cyclic VMSs D4, D5 and D6 in soils, only slightly lower than the current work. The values for pine needles and soils can be considered similar, although somewhat higher for the latter matrix. For SIP disks, the reported levels vary between 7 and 40 ngSIP^{-1} using similar air sampling media and analyzing linear and cyclic VMSs[10,35] and 0.011 to 25 ngSIP^{-1} analysing only the cyclic ones.[9,25] Our LODs fall within this range, but not surpassing 10.2 ngSIP^{-1} (D3). In terms of repeatability and interday precision, acceptable values were also found for this kind of complex matrix. The mean RSD for the repeatability was 11%, 17% and 8% for pine needles, soils and SIPs, respectively, whereas for the interday precision the values were slightly higher (mean RSD of 21%, 37% and 20%, respectively). These values are influenced by the lower precisions found for the more volatile compounds (mainly L2 and D3). This reinforces the care that must be taken with the solvent reduction steps, as they may be responsible for these higher deviations. Considering the type of matrices and the low levels dealt with, these values reflect a good performance of the proposed methods.

Regarding the recovery assays, Fig. 5 shows a comparison between the three matrices. In general, and in line with the results of the previous test, the recovery performances tend to increase as the volatility of the siloxane compounds decreases. Nevertheless, the values for SIPs are not so affected by this tendency, except in the case of L2, the most volatile siloxanes in study, which cannot reach 20% recovery in any of the matrices. The mean recovery for SIPs was 87% (min 17% for L2, max 116% for L4), whereas pine needles and soils reached 75% (min 18% for L2, max 115% for L5) and 69% (min 15% for L2, max 111% for L5), respectively. These values can be considered as good under these conditions and again in line with the studies in the literature regarding soils[12] and SIPs.[9,10,25,35] Pieri *et al.*[10] reported a higher range of recoveries for linear and cyclic VMS in SIPs (82–95%). There is also a consistency of results and trends between matrices, particularly soils and pine needles, also favoured by the fact that the analytical protocol is very similar. The performances obtained for the VMSs that occur more frequently in the commercial formulations and for which there is more information available (D4, D5 and D6)[36,37] are very good (over 80% recovery except for D4 in soils), but there is of course a need to try to improve these indicators for the least volatile siloxanes. One of the possible measures is to use deuterated siloxanes compounds as surrogates for the assays, but these are still very expensive compared to the target chemicals. In any case, M4Q has been often used in similar siloxane assessments

Naturally contaminated samples. The field application of the optimised protocols was carried out with samples collected in an urban (Porto) and a remote (Midões) site and the results are shown in Table 6. For all matrices, the cyclic siloxanes are more detected and appear in higher concentrations, which are the linear ones. In fact, L2 and L4 are not detected at all. This is a common pattern in similar

studies in the literature, most likely reflecting the more extensive use of the cyclic VMSs. It is also not a surprise that the levels for the urban site (Porto) are consistently higher than those of the remote area (10.2 *versus* 4.5 ng g^{-1} for pine needles; 14.6 *versus* 2.8 ng g^{-1} for soils; 1880.6 *versus* 129.9 ngSIP^{-1} for SIPs). Being anthropogenic chemicals, urban pressure is associated with the majority of siloxanes sources, and Porto is the second biggest conurbation in Portugal, with a relevant industrial implantation as well. On the contrary, only the most used VMSs (D4, D5 and D6) are detected in Midões, where the absence of strong sources is clear. Information is scarce for soils and non-existent for pine needles, but since VMSs are considered "fliers"[38] it is not surprising that the atmosphere is an important route for their transport and deposition into those two matrices. D5, arguably the most studied of the target compounds (particularly regarding its atmospheric behaviour, including modelling approaches[38,39]), is the one with an overall stronger incidence in all matrices, again suggesting a direct correlation with its use in the product formulations.

The performance of the solvent-saving methodologies proposed is encouraging, but there are still some aspects to improve. In fact, despite all our efforts, it was impossible to eliminate completely the external contamination in some cases, which obviously affects the LODs and the detection of the target chemicals in some cases. Even if the total replacement of the siloxanes-containing materials of the laboratory equipment is almost impossible (and very costly), there should be an investment in searching for alternatives for at least some of the potentially more important sources (injectors, capillary columns, solvents, *etc.*). In any case, in comparison to the classic methodologies that are also used by our work team, the savings in terms of operation time, cost of analysis and solvent use can go up to 42%, 85% and 95%, respectively, if an extra clean-up step with GPC columns is used in the classic approach, these numbers can reach 61%, 88% and 97%, respectively, which constitutes an extremely relevant enhancement of the analytical conditions.

Conclusions

Expedite and reliable methodologies for the extraction and quantification of siloxanes in air (SIPs), pine needles and soils were developed in this work. And more importantly, after a considerable number of tests that had to be performed in search of the best solution, the option for solvent-saving techniques was a successful one and allowed us to aim for similar strategies in future efforts. For now it was possible to overcome the challenges posed in the extraction of the chosen matrices and present a protocol based on short clean-ups and recent extraction technologies (QuEChERS), which reaches low limits of detection and good recoveries, which increase with the number of siloxane groups (Si-O-Si) and are between 67% and 115% for the most used siloxanes (D4, D5 and D6) and have mean values of 69% to 87% for all compounds in all matrices. Care must be taken to avoid cross-contamination of the samples, as siloxanes are ubiquitous not only in the environment but also in the equipment commonly used in a laboratory. The modifications made to the usual procedures both in sample handling and GC/MS

operation were helpful but did not allow the complete eradication of the external contamination, being this an important aspect to improve in the future. Siloxanes are chemicals of emerging concern and this study intends to back the setting-up of field-based sampling campaigns, providing a valid option to quantify them in several environmental matrices.

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Notes and references

1 D. Ru¨cker and K. Ku¨mmerer, Chem. Rev., 2015, 115, 466.

2 D. G. Wang, W. Norwood, M. Alaee, J. D. Byer and S. Brimble, Chemosphere, 2013, 93, 711.

3 N. J. Fendinger, R. G. Lehmann and E. M. Mihaich, in Organosilicon Materials; The Handbook of Environmental Chemistry, ed. G. Chandra, Springer, New York, 1997, vol. 1, pp. 1–25.

4 D. Graiver, K. W. Farminer and R. Narayan, J. Polym. Environ., 2003, 11, 129.

5 R. Dewil, L. Appels and J. Baeyens, Energy Convers. Manage., 2006, 47, 1711.

6 T. Dudzina, N. von Goetz, C. Bogdal, J. W. H. Biesterbos and K. Hungerbu¨hler, Environ. Int., 2014, 62, 86.

7 F. Chainet, C.-P. Lienemann, M. Courtiade, J. Ponthus and O. F. X. Donard, J. Anal. At. Spectrom., 2011, 26, 30.

8 N. de Arespacochaga, C. Valderrama, J. Raich-Montiu, M. Crest, S. Mehta and J. L. Cortina, Renewable Sustainable Energy Rev., 2015, 52, 366.

9 S. Genualdi, T. Harner, Y. Cheng, M. MacLeod, K. M. Hansen, R. van Egmond, M. Shoeib and S. C. Lee, Environ. Sci. Technol., 2011, 45, 3349.

10 F. Pieri, A. Katsoyannis, T. Martellini, D. Hughes, K. C. Jones and A. Cincinelli, Environ. Int., 2013, 59, 363.

11 L. Ahrens, T. Harner and M. Shoeib, Environ. Sci. Technol., 2014, 48, 9379.

12 C. S´anchez-Brunete, E. Miguel, B. Albero and J. L. Tadeo, J. Chromatogr. A, 2010, 1217, 7024.

13 L. Xu, Y. Shi, T. Wang, Z. Dong, W. Su and Y. Cai, Environ. Sci. Technol., 2012, 46, 11718.

14 E. Y. Companioni-Damas, F. J. Santos and M. T. Galceran, Talanta, 2012, 89, 63.

15 W.-J. Hong, H. Jia, C. Liu, Z. Zhang, Y. Sun and Y.-F. Li, Environ. Pollut., 2014, 191,

175.
16 M. J. Whelan, Chemosphere, 2013, 91, 1566.
17 J. Sanch´ıs, E. Mart´ınez, A. Ginebreda, M. Farr´e and D. Barcel´o, Sci. Total Environ., 2013, 443, 530.
18 C. Cortada, L. Costa dos Reis, L. Vidal, J. Llorca and Canals, Talanta, 2014, 120, 191.
19 D. G. Wang, M. Aggarwal, T. Tait, S. Brimble, G. Pacepavicius, L. Kinsman, M. Theocharides, S. A. Smyth and M. Alaee, Water Res., 2015, 72, 209.
20 N. A. Warner, A. Evenset, G. Christensen, G. W. Gabrielsen, K. Borg˚a and H. Leknes, Environ. Sci. Technol., 2010, 44, 7705.
21 A. Kierkegaard, A. Bignert and M. S. McLachlan, Chemosphere, 2013, 93, 774.
22 J. Sanch´ıs, A. Cabrerizo, C. Galb´an-Malag´on, D. Barcel´o, M. Farr´e and J. Dachs, Environ. Sci. Technol., 2015, 49, 4415. 23
23 S. Varaprath, D. H. Stutts and G. E. Kozerski, Silicon Chem., 2006, 3, 79.
24 N. A. Warner, G. Kozerski, J. Durham, M. Koerner, R. Gerhards, R. Campbell and D. A. McNett, Chemosphere, 2013, 93, 749.
25 I. S. Krogseth, X. Zhang, Y. D. Lei, F. Wania and K. Breivik, Environ. Sci. Technol., 2013, 47, 4463.
26 J. A. Silva, N. Ratola, S. Ramos, V. Homem, L. Santos and Alves, Anal. Chim. Acta, 2015, 858, 24.
27 R. Arau´jo, N. Ratola, J. L. Moreira, L. Santos and A. Alves, Environ. Technol., 2012, 33, 2359.
28 V. Homem, J. A. Silva, C. Cunha, A. Alves and L. Santos, J. Sep. Sci., 2013, 36, 2176.
29 J. de Zeeuw, Am. Lab., 2005, 37, 18.
30 O. D. Prestes, M. B. Adaime and R. Zanella, Scientia Chromatographica, 2011, 3, 51, in Portuguese.
31 UCT – United Chemical Technologies, Inc., QuEChERS Informational Booklet, Bristol, PA, USA, 2014, p. 24, Available at https://www.unitedchem.com/sites/default/files/docs/product-displays/QuEChERS_Booklet_2014.pdf.
32 N. Fidalgo-Used, E. Blanco-Gonz´alez and A. Sanz-Medel, Anal. Chim. Acta, 2007, 590, 1.
33 N. Ratola, J. M. Amigo, M. S. N. Oliveira, R. Arau´jo, J. A. Silva and A. Alves, Environ. Exp. Bot., 2011, 72, 339.
34 Y. Zhang, N. Lin, S. Su, G. Shen, Y. Chen, C. Yang, W. Li, H. Shen, Y. Huang, H. Chen, X. Wang, W. Liu and S. Tao, Environ. Pollut., 2014, 191, 250.
35 Y. Cheng, M. Shoeib, L. Ahrens, T. Harner and J. Ma, Environ. Pollut., 2011, 159, 2380.
36 SCCS – Scientific Committee on Consumer Safety, Opinion on cyclomethicone D4/D5, 22 June 2010.
37 D. N. Brooke, M. J. Crookes, D. Gray and S. Robertson, Environmental Risk Assessment Report, Dodecamethylcyclohexasiloxane, Environment Agency of England and Wales, Bristol, U.K, 2009.
38 S. Xu and F. Wania, Chemosphere, 2013, 93, 8358.
39 M. S. McLachlan, A. Kierkegaard, K. M. Hansen, R. van Egmond, J. H. Christensen and C. A. Skjoth, Environ. Sci. Technol., 2010, 44, 5365.

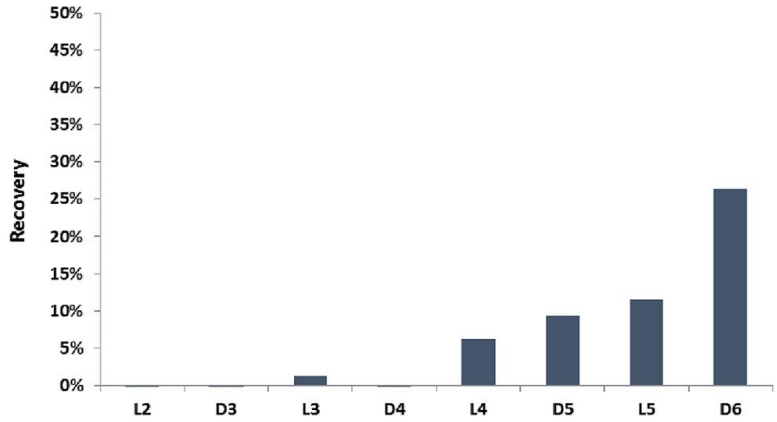

Fig. 1 Recovery of siloxanes using USE extraction and cleanup by SPE (alumina) and GPC columns.

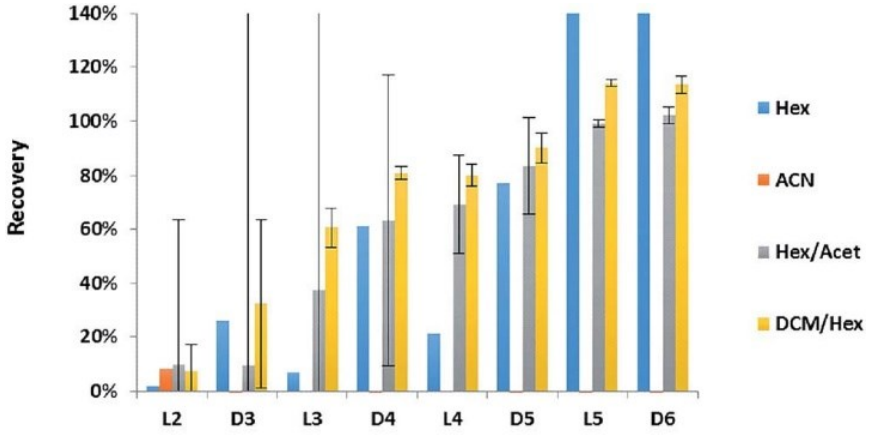

Fig. 2 Comparison of the recoveries of several extraction solvents of spiked pine needles using QuEChERS (error bars depict standard deviation).

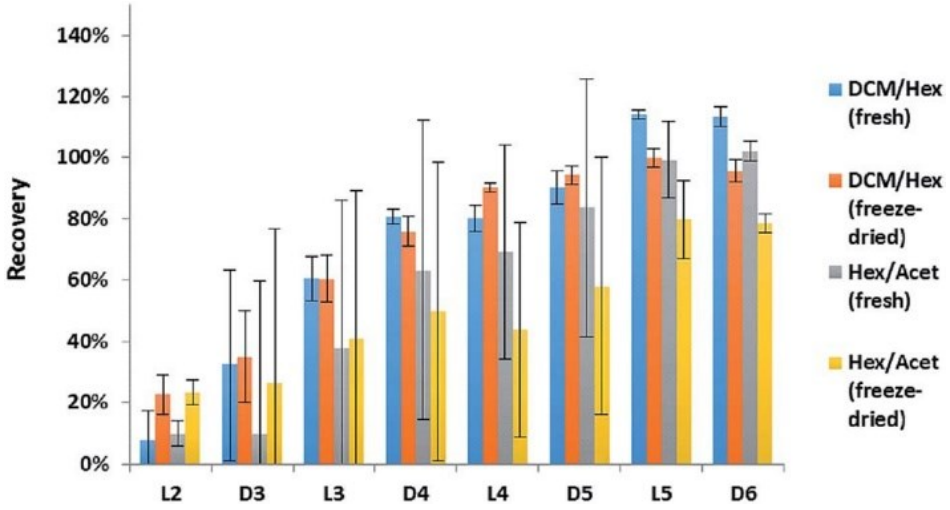

Fig. 3 Comparison of the extraction recoveries of siloxanes between fresh and freeze-dried pine needles.

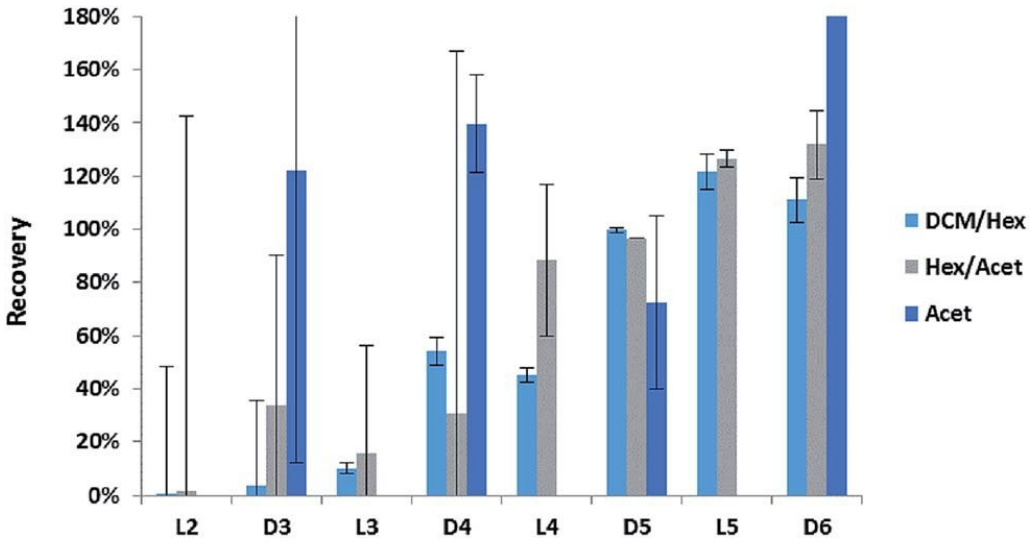

Fig. 4 Comparison of recoveries of spiked fresh soil using different extraction solvents.

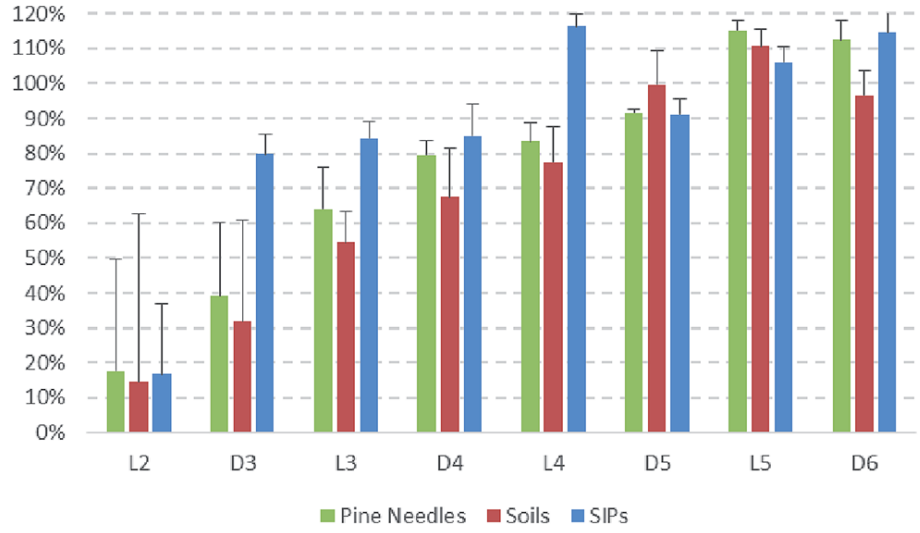

Fig. 5 Comparison of the mean recoveries between the three matrices studied and their respective standard deviation (only positive error bars are shown).

Table 1 GC-MS method parameters for siloxanes (internal standard in *italics*)

Time segment (min)	Retention time (min)	Compound	Ions (*m*/*z*)
2.50–5.00	3.48	L2	72, 73, 147–149
5.00–8.00	7.12	D3	208–211
8.00–10.75	8.62	L3	72, 73, 220–221
10.75–11.00	10.96	D4	265, 281, 282
11.50–13.00	12.26	L4	208–211, 294, 295
13.00–13.80	13.61	D5	267–269, 354–356
13.80–14.25	14.00	*M4Q*	148, 149, 281, 282
14.25–15.30	14.98	L5	148, 149, 281, 282
15.30–20.00	16.10	D6	325, 341, 342, 428–430

Table 2 Retention test for PTFE 0.2 μm and glass fibre filters for siloxanes

	PTFE 0.2 μm	Glass fiber
	Recovery (%)	Recovery (%)
M4Q	97	90
L2	97	93
D3	97	92
L3	103	100
D4	102	102
L4	103	94
D5	109	103
L5	99	96
D6	108	104

Table 3 Results of the method validation parameters for pine needles

	Linearity (μg L^{-1})	R^2	LOD (ng kg^{-1} dw)	Repeatability ($n = 3$, RSD)	Interday precision ($n = 5$, RSD)
L2	1–500	0.9997	1.845	32%	60%
D3	1–500	0.9993	2.223	21%	21%
L3	1–500	0.9988	10.853	12%	15%
D4	1–500	0.9994	2.838	4%	17%
L4	1–500	0.9994	2.795	5%	21%
D5	1–500	0.9988	3.417	1%	15%
L5	1–500	0.9991	3.181	3%	10%
D6	1–500	0.9984	2.278	6%	7%

Table 4 Results of the method validation parameters for soils

	Linearity (μg L^{-1})	R^2	LOD (ng kg^{-1} dw)	Repeatability ($n = 3$, RSD)	Interday precision ($n = 5$, RSD)
L2	1–500	0.9970	3.375	48%	93%
D3	1–500	0.9960	4.066	29%	64%
L3	1–500	0.9980	19.853	9%	54%
D4	1–500	0.9990	5.192	14%	21%
L4	1–500	0.9980	5.114	10%	18%
D5	1–500	0.9990	6.250	10%	11%
L5	1–500	0.9990	5.819	5%	10%
D6	1–500	0.9980	4.167	7%	28%

Table 5 Results of the method validation parameters for SIPs

	Linearity (μg L^{-1})	R^2	LOD (ng_{SIP}^{-1})	Repeatability ($n = 3$, RSD)	Interday precision ($n = 5$, RSD)
L2	1–500	0.9975	6.739	20%	46%
D3	1–500	0.9946	10.216	6%	40%
L3	1–500	0.9989	4.689	5%	9%
D4	1–500	0.9975	7.634	9%	15%
L4	1–500	0.9983	5.640	3%	6%
D5	1–500	0.9981	6.436	5%	18%
L5	1–500	0.9981	5.905	4%	7%
D6	1–500	0.9992	4.917	14%	22%

Table 6 Concentrations of individual and total siloxanes for naturally contaminated samples (n.d. – not detected)

	Porto			Midões		
	Pine needles (ng g^{-1} dw)	Soil (ng g^{-1} dw)	Air (ng_{SIP}^{-1})	Pine needles (ng g^{-1} dw)	Soil (ng g^{-1} dw)	Air (ng_{SIP}^{-1})
L2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D3	0.3	2.7	127.5	n.d.	n.d.	n.d.
L3	0.5	n.d.	34.0	n.d.	n.d.	n.d.
D4	1.3	4.4	586.3	0.4	0.1	35.4
L4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D5	4.3	2.0	740.2	0.6	1.1	70.6
L5	n.d.	0.2	13.8	n.d.	n.d.	n.d.
D6	3.8	5.3	378.8	3.5	1.6	23.9
Total	10.2	14.6	1880.6	4.5	2.8	129.9