https://dx.doi.org/10.21577/0103-5053.20220106

J. Braz. Chem. Soc., Vol. 34, No. 2, 276-286, 2023

Article

Emerging Contaminants in Sediments as Markers of Anthropogenic Inputs in Santos Estuarine System: Analytical Method and Occurrence Data

Marisa J. S. Chaves,[a] Sergiane C. Barbosa,[a] Ednei G. Primel[,a] and Ítalo B. Castro[b]*

[a]*Laboratório de Análise de Compostos Orgânicos e Metais (LACOM), Escola de Química e Alimentos, Universidade Federal do Rio Grande (FURG), Av Itália, km 8, 96201-900 Rio Grande-RS, Brazil*

[b]*Instituto do Mar, Universidade Federal de São Paulo (IMAR-UNIFESP), Rua Maria Máximo, 168, 11030-100 Santos-SP, Brazil*

The occurrence of emerging contaminants (ECs) in the aquatic systems under influence of urban areas have been considered as an environmental marker of anthropogenic contamination. In this study, 10 ECs were investigated in sediment samples from Santos Bay using a quick, easy, cheap, effective, rugged, and safe method with determination by liquid chromatography tandem mass spectrometry. The method showed acceptable accuracy (51-116%) and precision (relative standard deviation (RSD) ≤ 9), limits of quantification in ng g^{-1} levels and matrix effect lower than 29%. Environmental occurrence of benzophenone-3, caffeine, ketoconazole and triclocarban was detected in two campaigns (2015 and 2019), with concentrations ranging from < 1 to 470 ng g^{-1}. Caffeine and triclocarban were the most abundant target chemicals occurring in up to 71 and 100% of the sample point, respectively. The results indicated that ECs in sediment samples came from diffused sources as the discharge of treated and untreated sewage in the estuary.

Keywords: emerging contaminants, analytical method, sediment, environmental contamination, monitoring

Introduction

A lot of different anthropogenic chemicals and waste materials, including organic and inorganic compounds are related to contamination and/or pollution of aquatic environments. Environmental disturbances caused by such molecules are even more important in coastal and estuarine systems, which are often affected by multiple contamination sources (port activities, industrial and domestic effluents discharges). In addition, estuaries are considered one of the most productive marine ecosystems in the world being highly vulnerable to contamination of water and sediments which generate several impacts on local biota.[1]

Pharmaceuticals, pesticides, industrial chemicals, personal care products and other synthetic or naturally occurring chemicals, whose environmental occurrences are not routinely monitored, but show potential to reach the environment, and cause known or suspected ecological disruption or effects to human health, are known as emerging contaminants (ECs). A wide range of groups, as perfluoroalkyl compounds, plasticizers, microplastics, analgesics, antibiotics, hormones, anti-inflammatory, antidiabetic, and anticonvulsant drugs are included in such group.[2,3] In this regard, ECs have gained attention of environmental agencies and academic researchers due to their presence in aquatic environments.[4,5] Most investigations on ECs residues have been carried out in water samples.[6] However, analysis of sediment samples is interesting since this environmental compartment may accumulate contaminants in levels higher than those observed in the water column, once they provide a wide variety of binding sites, acting as a sink for the deposition of pollutants. Besides, investigation of sediments is important since they are natural repositories of substances present in the water, and sediment layers are a relevant fate of pollutants and may act as sources of contamination for the aquatic food web.[1,7,8]

Although sediments are important indicators of environmental quality, it is considered a complex matrix and ECs are usually detected at low concentrations

*e-mail: eprimelfurg@gmail.com
Editor handled this article: Eduardo Carasek

(ng g^{-1} levels).[8,9] Thus, sample preparation steps are always carried out aiming at decreasing interference and providing cleaner extract for further analysis. Ultrasound assisted extraction followed by solid phase extraction,[10] accelerated solvent extraction followed by solid phase extraction,[11] pressurized liquid extraction[12] and matrix solid phase dispersion[13] have been employed for ECs extraction from sediment samples. In addition to those mentioned, the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method has been also used for the extraction of contaminants from environmental solid samples.[14] Although the method was developed in 2003,[15] the first extraction of ECs from sediment samples was reported in 2014, using citrate QuEChERS[16] and acetate QuEChERS.[17,18] QuEChERS is a useful method that is of fast and easy execution, low consumption of organic solvents and reagents and high efficiency for the simultaneous extraction of many contaminants.[19] Thus, it has been used as a promisor method for analysis of contaminants from several complex environmental samples.[20] It had been reported, among others, for extraction of psychiatric drugs,[21] antibiotics and antidepressants,[9] antivirals,[22] synthetic musks,[23] and multiclass pharmaceuticals[24] from sediment samples.

The Santos and São Vicente Estuarine System (SSES) is located on the southeastern coast of Brazil and in the central portion of the Baixada Santista Metropolitan Region. Three large estuarine channels (Santos, São Vicente and Bertioga) form the SSES covering approximately 44,100 m^2.[5] The area presents different ecological features including mangroves, islands and rocky shores sheltering a wide biological diversity. Although its great ecological importance, large amounts of contaminants are released into this estuary, resulting in high sediment contamination and toxicity, as previously reported.[5,25] According to such studies, intense industrial activities associated with legal and illegal inputs of domestic sewage has led SSES to a scenario of environmental degradation. Moreover, the high ship and boat traffic in Santos port also contributed to aggravate pollution rates currently observed in SSES.[26] In fact, the occurrence of different classes and levels of contaminants including polychlorinated biphenyls (PCBs),[27] aliphatic and aromatic hydrocarbons,[25] antifouling biocides,[26] pharmaceuticals,[28] estrogens,[5] microplastics,[29] cocaine and benzoylecgonine[30] have been recently reported in environmental samples from SSES.

Although many studies have been carried out around the world, in developing countries, as Brazil, the lack of information about levels of emerging contaminants in sediments hinder the adoption of mitigation strategies. Thus, this kind of investigation contributes to the worldwide effort to provide more data on environmental concentrations and impacts of ECs in different aquatic environments, helping to establish lists of priority substances and guide future environmental directives. This study aimed to evaluate for the first time the spatial and temporal distribution of 10 ECs, in two sampling campaigns (2015 and 2019), in surface sediments from Santos and São Vicente Estuarine System (southeastern coast of Brazil).

Experimental

Chemicals and reagents

Benzophenone-3 (BP-3), diclofenac (DIC), ketoconazole (KTZ) and propranolol (PPN) were purchased from US Pharmacopeia (USP) (Maryland, USA). Bisphenol A (BP A), caffeine (CAF), carbamazepine (CBZ) and caffeine-D_9 (CAF-d_9) from Sigma-Aldrich (São Paulo, Brazil), ibuprofen (IBU), triclocarban (TCC) and triclosan (TCS) from Dr. Ehrenstofer GmbH (Augsburg, Germany) and ibuprofen-D_3 (IBU-d_3) from Fluka (São Paulo, Brazil). The purity of standards was all higher than 95%. Individual standard solutions were prepared in methanol at a concentration of 1000 mg L^{-1}. A mixture containing all ECs was prepared by appropriate dilution of individual stock solutions in methanol. All the solutions were kept in a freezer at −18 °C. Methanol (MeOH) and acetonitrile (MeCN) high performance liquid chromatography (HPLC) grade were purchased from J. T. Baker (Phillipsburg, USA). Acetic acid and sodium chloride (NaCl) P.A. were acquired from Merck (Darmstadt, Germany), primary secondary amine (PSA) and C18 Bondesil from Varian (Palo Alto, USA) and anhydrous magnesium sulfate ($MgSO_4$) from J.T. Baker (Phillipsburg, USA). Ultrapure water was produced by a Direct-Q UV3® (Millipore, USA).

Sampling

Surface sediment samples (upper 2 cm) were collected using a stainless-steel Ekman grab from 14 sampling sites distributed along SSES. The two sampling campaigns were performed during December. Thus, no seasonal effects are expected as similar weather conditions were observed in 2015 and 2019. The selection of sampling sites were chosen based on previous studies[5] which identified potential EC sources for the region (Figure 1). The sampling campaigns were held at December 2015 and December 2019 and the samples were freeze-dried and frozen (−20 °C) and stored for subsequent analysis.[31] Chemical analyzes were performed within 6 months after each sampling campaign

using the same method. All sediment samples were also analyzed with regard to total organic carbon content (TOC) using TOC-L SSM 5000 A (Shimadzu, Kyoto, Japan) according to Kristensen *et al*.[32]

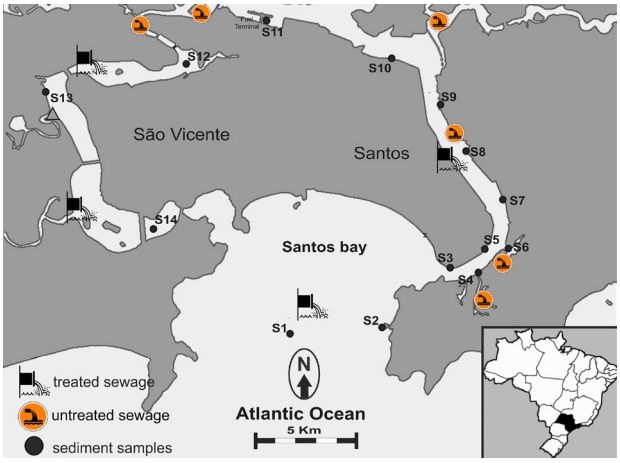

Figure 1. Sampling sites of surface sediments in the Santos-São Vicente Estuarine system.

LC-MS/MS analysis

Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis were carried out with an Alliance Separations Module 2695 Liquid Chromatography (Waters, Milford, USA) equipped with autosampler, quaternary pump, column oven and degassing system, coupled to Micromass® Quattro Micro™ API (Waters, Milford, USA) mass spectrometry detector with electrospray ionization. Multiple reaction monitoring (MRM) was applied for detection. In order to obtain an accurate and sensitive analysis of the selected compounds, data on MRM transitions, spray voltage and collision energy were determined for each analyte by direct infusion of single standard solutions into the ion source. In positive and negative ionization modes, the ECs were separated using a Kinetex C8 analytical column (3.0 × 50 mm × 2.6 µm) purchased from Phenomenex (Torrance, USA). Different mobile phase combinations were tested to achieve the best chromatographic separation, including water, MeOH, and MeCN, in addition to additives, as formic and acetic acids. Final separation was carried out with MeOH (A) and water with 0.1% of acetic acid (B) in gradient elution mode with a flow rate of 0.3 mL min^{-1}. The initial composition was 95% B, which decreased linearly to 70% B in 4 min, and to 5% B in 15 min, maintained at this condition until 17 min and then, returned to the initial composition (95% B) in 1 min, where it remained for 5 min, totalizing an analysis time of 23 min. The injection volume was 10 µL.

Sample preparation

For the extraction of the target chemicals, a modified QuEChERS method was evaluated and extraction conditions were studied.[33] Freeze-dried sediments (10 g) were weighed into a polypropylene centrifuge tube (50 mL). Then, 10 mL ultrapure water and 10 mL of MeCN was added to the tube, which was vortex-stirred for 1 min and followed by addition of 4 g $MgSO_4$ and 1 g NaCl. The extract was centrifuged at 8000 rpm for 5 min and the supernatant (1.5 mL) was collected in a polypropylene centrifuge tube (25 mL) for performing the clean-up. In the clean-up step, 25 mg C18 and 150 mg $MgSO_4$ were used. The tube was vortexed for 1 min and centrifuged at 8000 rpm for 5 min. The clean extract was analyzed by LC-MS/MS.

To ensure the quality of the results, some procedures were adopted during the analysis. Blank experiments of the materials (laboratory glassware) and reagents (salts and solvents) used were prepared in the laboratory daily during the experiments and processed identically to sediment samples. The target chemicals were not detected (below limit of detection) in blanks. In addition, during the extraction, 50 µL of a mix 10 mg L^{-1} containing the surrogate standards (caffeine-d_9 and ibuprofen-d_3) were added to the lyophilized samples to verify the accuracy of the method (final concentration of the surrogate in the sample 50 ng g^{-1}). Thus, quantification errors caused by failures in the extraction process, as well as possible instrumental fluctuations were monitored.

Method validation

The method was validated according to the recommendations of the INMETRO.[34] For the method validation a sediment sample (TOC 1.1%) available at the laboratory was used. Limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy (recovery), precision (intra- and inter-day repeatability) and matrix effects (ME) were evaluated.

LOD and LOQ were calculated using matrix-matched samples spiked in low concentrations, and it was established by the signal-to-noise ratio of 3:1 and 10:1 of individual peaks, respectively, using the Masslynx 4.1 software.

Linearity was studied by the construction of calibration curves by matrix matched calibration with five different concentrations for each analyte, starting from LOQ to 10LOQ. Each solution was analyzed three times in the LC-MS/MS and the linear regression data were obtained with the aid of the software.

The accuracy of the method was evaluated by recovery tests at three levels of concentration (LOQ, 5LOQ and

10LOQ), using equation 1. While for the precision, the relative standard deviation (RSD) was evaluated.

$$R\ (\%) = \frac{A_1 - A_2}{A_3} \times 100 \quad (1)$$

where A_1 and A_2 are chromatographic peaks areas in the fortified and non-fortified samples, respectively, and A_3 is the area corresponding to the concentration added in the samples after treatment.

The evaluation of the matrix effect was carried out by comparing the slope (sensitivity) of the calibration curves prepared in solvent (MeCN) and in matrix extract (matrix matched calibration). Equation 2 was used for the calculation of ME.

$$ME\ (\%) = \frac{C_1 - C_2}{C_2} \times 100 \quad (2)$$

where C_1 and C_2 are the slope of the curves prepared by external calibration in the matrix extract and in the solvent, respectively.

Statistical analysis

Normality and homogeneity of data (analyte concentrations and TOC amounts) were verified using Shapiro-Wilk and Levene tests, respectively. Temporal differences in analyte levels were analyzed by *T*-tests. Alternatively, non-parametric statistics (Mann-Whitney U tests) were used when *T*-test assumptions were not achieved. Spearman nonparametric correlation analysis was used to investigate the relationships between TOC% and analyte concentrations. Analysis of variance (ANOVA) followed by Tukey's post-hoc tests were used to compare recoveries. All statistical analyses were performed using Statistica® (version 13.0, Statsoft, USA)[35] with a significant level of 0.05.

Results and Discussion

LC-MS/MS analysis

Conditions established in the mass spectrometer (monitored transitions, collision energy, cone voltage and ionization mode) are shown in Table 1. Two most intense product ions and the optimal collision energy (CE) were chosen to define the quantifier and qualifier transitions. The qualifier transition was used by means of confirmation of the identity of the compound, avoiding the quantification of false positives. The relative ratios of the chosen transitions obtained with standards measured in the same sequence and under the same conditions as the samples were used as reference values. It is indicated that ion ratios should not deviate more than 30% (relative) from the reference value.[36] Ibuprofen showed only a stable transition.

Ion source temperature, desolvation temperature, desolvation gas flow rate and the cone gas flow rate were 100 ºC, 500 ºC, 500 L h^{-1} and 50 L h^{-1}, respectively.

Once defined the conditions in the mass spectrometer, parameters that influence the chromatographic separation were evaluated. Solvents such as MeOH and MeCN were tested, and for detectability enhancement, the effect of the acidity was investigated by testing neutral phases and phases with the addition of 0.1% (v/v) of formic or

Table 1. MS/MS optimized parameters for quantitative and qualitative analysis of emerging contaminants

Analyte	Transitions (*m/z*)		CE / eV		Cone / V	ESI mode	Retention time / min
Benzophenone-3	229 > 150.7	229 > 104.6	17	19	27	+	14.4
Bisphenol A	227 > 135.9	227 > 211.8	19	19	39	–	13.4
Caffeine	195 > 137.7	195 > 109.6	19	21	33	+	7.7
Carbamazepine	237 > 193.7	237 > 178.6	19	39	27	+	11.4
Diclofenac	294 > 249.7	294 > 213.8	11	21	19	–	14.8
Ibuprofen	205 > 160.7	–	7	–	9	–	14.9
Ketoconazole	531 > 81.6	531 > 134.8	41	39	49	+	11.3
Propranolol	260 > 182.7	260 > 115.7	19	19	29	+	9.3
Triclocarban	313 > 159.6	313 > 125.6	15	25	35	–	15.5
Triclosan	289 > 35	287 > 35	9	7	18	–	15.7
Caffeine-d_9	204 > 144	–	16	–	34	+	7.7
Ibuprofen-d_3	208 > 164	–	9	–	19	–	14.9

CE: collision energy; ESI: electrospray.

acetic acids. The best results were achieved by employing a mobile phase composed of MeOH (A) and water with 0.1% of acetic acid (B) in gradient elution mode. The initial composition was 5% A, which increased linearly to 30% A in 4 min, and to 95% A in 15 min, maintained at this condition until 17 min and then, returned to the initial composition (5% A) in 1 min, where it remained for 5 min, totalizing an analysis time of 23 min. Flow rate of mobile phase was 0.3 mL min^{-1} and the injection volume was 10 µL. Figure 2 shows the multiple-reaction monitoring chromatograms for the most intense transitions obtained using the conditions described above. It is important to highlight that the use of methanol as an elution solvent has the advantage of lower cost and less toxicity, when compared to acetonitrile. In addition, it is important to note that, even working with a tandem mass spectrometer, which allows the quantification of compounds with the same retention time, a better chromatographic resolution improves the limit of detection and reduces the matrix effect, thus increasing the sensitivity of the method.[37]

QuEChERS method evaluation

Initially, the effect of the acidification of the MeCN (extraction solvent) was evaluated by adding 0.1% acetic acid, since pH is an important parameter in the stability of base or acid-sensitive compounds.[38] The recovery results and the matrix effect using these conditions are shown in Figure 3. The use of MeCN without acidifying generated better recovery for most compounds and low matrix effect (< 20%). Only ketoconazole recovered below 70% in both conditions. The acidification of the extraction solvent led to an increase of the extraction of interfering compounds from the matrix, which generated a higher matrix effect (enrichment of the chromatographic signal). Thus, once the aim of extraction is not only extracting the target analytes from the matrix to the extraction solvent but also guarantee low co-extractives of the matrix components as far as possible, MeCN without acidifying was chosen, aiming the better integrity of analysis and the equipment.

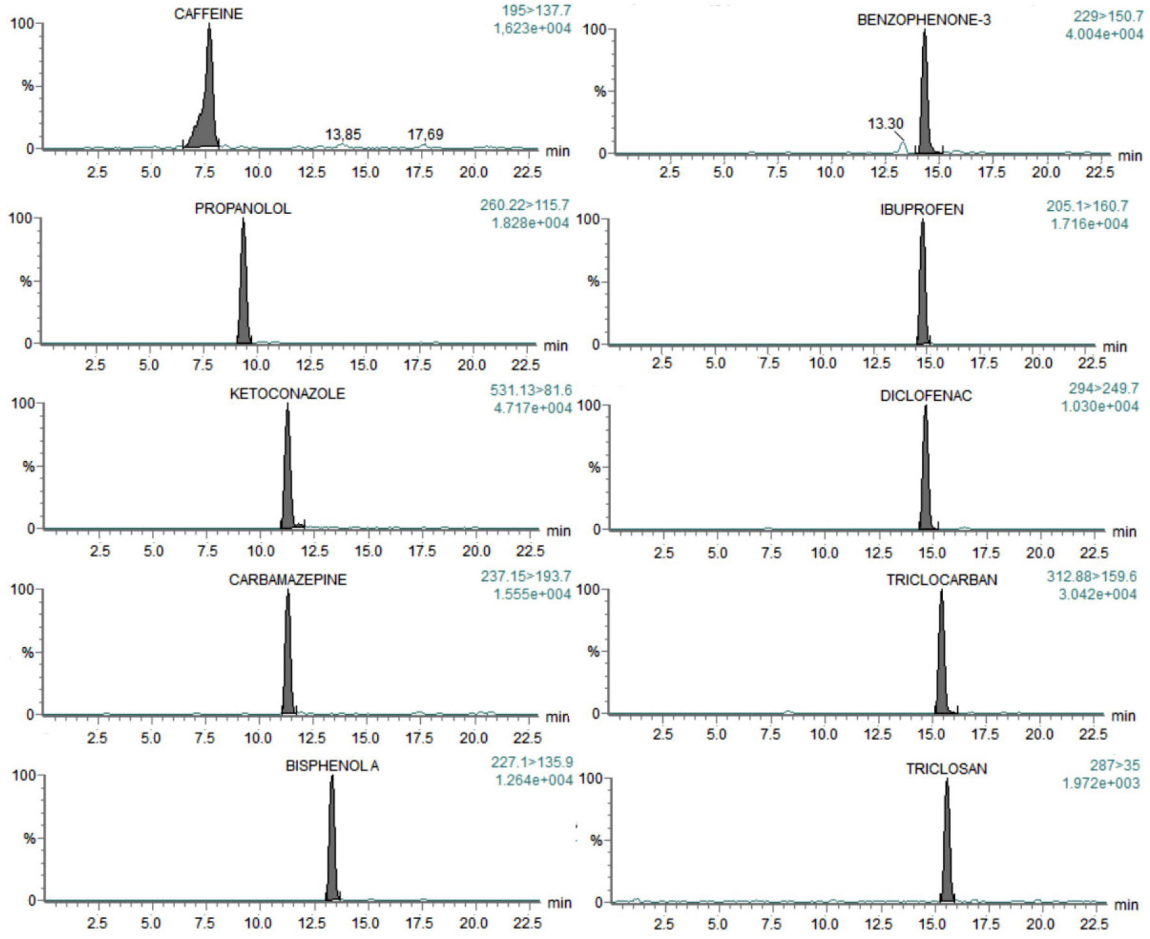

Figure 2. Multiple-reaction monitoring chromatograms for the analytes under study in the best chromatographic conditions (standard solution diluted in a blank sediment extract at a concentration equivalent to 10LOQ).

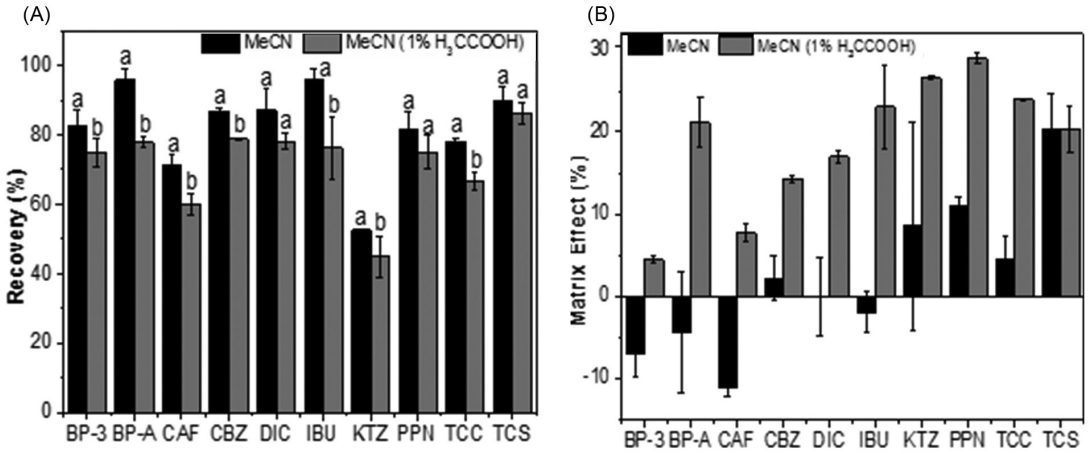

Figure 3. Recovery of emerging contaminants from sediment (A) and matrix effect (B) obtained with the use of different extraction solvents (a and b represent statistically significant differences using the *T* test) (MeCN-acetonitrile; MECN 1% H_3CCOOH-acetonitrile with 1% acetic acid).

After the definition of the extraction solvent, the effect of different adsorbents in the clean-up step was investigated. The results obtained for the use of PSA, C18, PSA + C18 and without the clean-up step are shown in Figure 4. Significant statistical differences ($p < 0.05$) were observed. Using C18, better recoveries were obtained for most analytes. The main difference was observed in the recovery of acidic pharmaceuticals (diclofenac and ibuprofen) when using PSA. The low recovery of these compounds using PSA can be explained by its alkaline character that interacts with the acid groups of the drugs, retaining them in the adsorbent.[20]

Regarding the matrix effect, most of the compounds presented ME < 10% (except caffeine) when using C18. Therefore, in order to achieve a compromise between recovery and ME, C18 was selected. Figure 5 shows the final experimental procedure for the extraction of 10 emerging contaminants from sediment samples using the QuEChERS method.

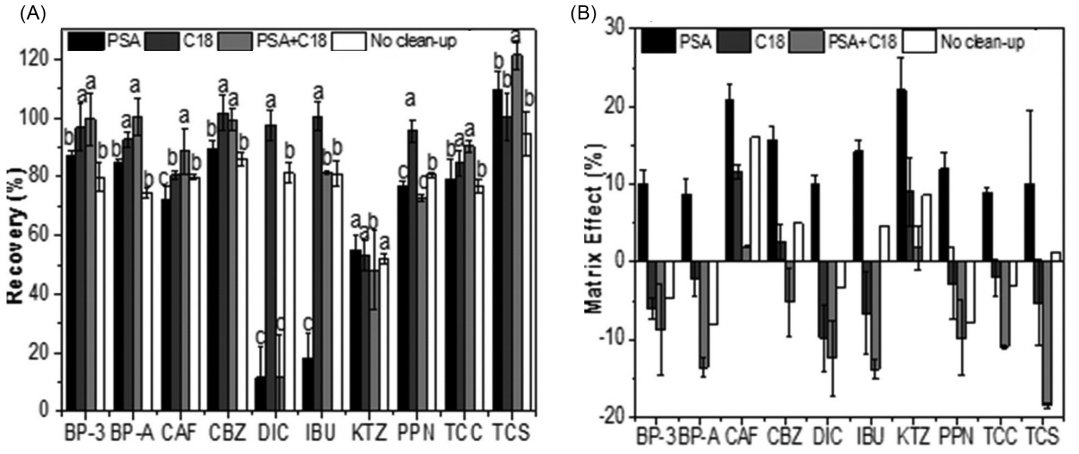

Figure 4. Recovery of emerging contaminants from sediments (A) and matrix effect (B) obtained with the use of different adsorbents in clean-up step (a, b and c represent significant statistical differences using Tukey's test) (PSA-primary secondary amine; C18-octadecyl).

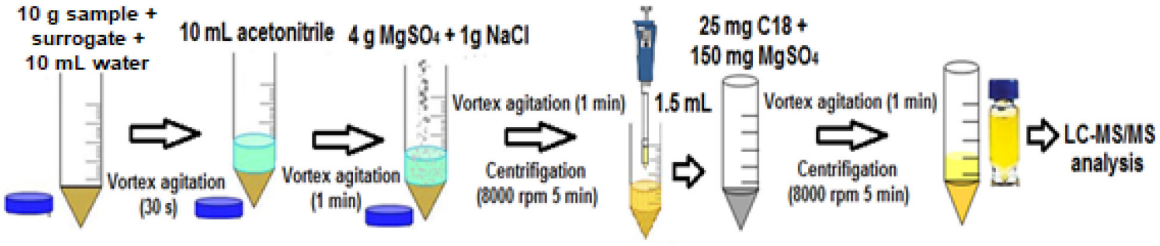

Figure 5. Conditions established for the QuEChERS method for extraction of emerging contaminants from sediment.

Method validation

Calibration parameters evaluated in the method validation are summarized in Table 2. LOD and LOQ ranged from 0.3 to 16.7 ng g^{-1} for LOD and from 1 to 50 ng g^{-1} for LOQ, considering dry weight of sediment samples. Although there is no legislation for these ECs in sediment samples, LOQs results were in agreement with concentrations that have been previously found in sediment samples around the world,[39-42] and it is similar to what have been found in previously published methods employing other extraction techniques as ultrasound assisted extraction (UAE)[43] and pressurized liquid extraction (PLE).[44]

Linearity was assessed in a range from the LOQ to 10LOQ. A linear correlation coefficient (r) > 0.99 was achieved for almost all compounds for both the solvent-based and matrix-matched calibration curves, except triclosan (r > 0.98). Intra-day and inter-day precision, estimated as relative standard deviation (RSD, %), were from 1 to 7% and 1 to 9%, respectively. Recoveries from 51 to 116% were obtained for all analytes.

Figure 6 shows the results of the matrix effect, which were estimated by the relationship between the slope coefficients of solvent-based and matrix-matched calibration curves. Moderated matrix effect (ME > ± 20%) was obtained for caffeine (–26%), in which signal suppression was observed and triclocarban (29%), which had signal enrichment. All others had a low ME (ME < ± 20%).

In summary, the proposed method presents extraction efficiency, precision, matrix effect and limit of quantification similar to other previously published methods. However, some advantages should be emphasized such as the low volume of solvent and short time of extraction. Besides, most of the other sample preparation extraction techniques usually require apparatus, such as a microwave[45] or an ultrasonic bath,[43] and most of them requires a clean-up step usually employing SPE[44,45] or chromatographic columns.[43] In the proposed method, no apparatus is required, except a vortex and a centrifuge. It is important to highlight that these reductions generate many advantages that include decrease in generated waste, consumption of energy, and cost. In summary, the proposed procedure afforded good sensitivity and efficiency being an interesting alternative to extract ECs from sediment samples.

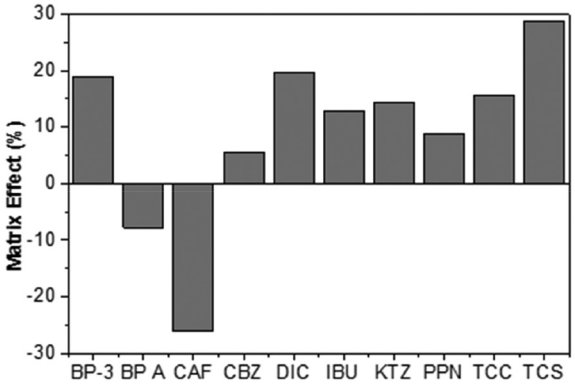

Figure 6. Matrix effect for the selected emerging contaminants.

Determination of anthropic contamination in real samples

The sediments sampled in Santos-São Vicente Estuarine System presented TOC amounts (%) ranging between 0.8 (S1) and 3.5% (S12) in 2015. Similarly, during the campaign performed in 2019 such values varied

Table 2. Limit of detection (LOD), limit of quantification (LOQ), analytical curve, linear correlation coefficient (r), intra- and inter-day precision (RSD) and accuracy (R%)

Analyte	LOD / (ng g^{-1})	LOQ / (ng g^{-1})	Analytical curve	r	Intra-day			Inter-day		
					LOQ	5LOQ	10LOQ	LOQ	5LOQ	10LOQ
					R ± RSD / %	R ± RSD / %	R ± RSD / %	R ± RSD / %	R ± RSD / %	R ± RSD / %
BP-3	1.0	3	$y = 128689x + 431$	0.993	81 ± 6	98 ± 2	88 ± 1	84 ± 7	105 ± 2	77 ± 8
BPA	8.5	25	$y = 7562x + 26$	0.993	102 ± 7	95 ± 4	96 ± 5	104 ± 3	95 ± 1	98 ± 1
CAF	0.3	1	$y = 171170x + 168$	0.997	85 ± 4	89 ± 5	76 ± 1	94 ± 2	88 ± 3	90 ± 1
CBZ	1.0	3	$y = 93199x + 118$	0.996	111 ± 4	97 ± 5	90 ± 4	108 ± 4	111 ± 9	116 ± 4
DIC	8.5	25	$y = 8923x - 13$	0.995	109 ± 4	91 ± 1	97 ± 4	106 ± 7	99 ± 1	100 ± 3
IBU	8.5	25	$y = 6185x - 16$	0.999	100 ± 7	92 ± 2	93 ± 3	99 ± 2	95 ± 4	96 ± 3
KTZ	1.7	5	$y = 29699x + 90$	0.999	52 ± 7	64 ± 5	86 ± 4	58 ± 3	70 ± 5	83 ± 5
PPN	0.3	1	$y = 63371x + 76$	0.996	106 ± 7	98 ± 2	108 ± 2	103 ± 2	96 ± 5	103 ± 3
TCC	0.3	1	$y = 82231x + 44$	0.997	101 ± 1	107 ± 2	107 ± 4	108 ± 4	107 ± 5	110 ± 2
TCS	16.7	50	$y = 1090x - 28$	0.981	85 ± 1	102 ± 2	89 ± 4	83 ± 9	97 ± 4	87 ± 2
IBU-d_3	16.7	50	$y = 42919x - 1306$	0.991	99 ± 2	93 ± 3	90 ± 3	98 ± 6	87 ± 6	72 ± 2
CAF-d_9	4.5	13	$y = 3571030x - 15911$	0.991	81 ± 7	56 ± 3	57 ± 2	83 ± 6	54 ± 3	51 ± 2

BP-3: benzophenone-3; BPA: bisphenol A; CAF: caffeine; CBZ: carbamazepine; DIC: diclofenac; IBU: ibuprofen; KTZ: ketoconazole; PPN: propranolol; TCC: triclocarban; TCS: triclosan.

between 0.3 (S1) and 6.3% (S13) (Table 3). Temporal variability in TOC levels, based on two or more sampling campaigns, are often observed in field sampling, and attributed to variations in oceanographic factors.[46] In addition, physico-chemical parameters may also influence deposition and retention capacity of organic matter in surface sedimentary layers, over time.[47] Moreover, it is important to highlight that any significant changes in wastewater treatment facilities were adopted during the studied period. In its turn, TOC concentrations tend to interfere in the sorption equilibrium among sediments and hydrophobic organic contaminants.[6] Residues of benzophenone-3, caffeine, triclocarban and ketoconazole were found in sediments from SSES during the two sampling campaigns. On the other hand, bisphenol-A, carbamazepine, diclofenac, ibuprofen, propranolol and triclosan were not detected in any analyzed sample (Table 3). Despite to moderate to high K_{ow} and/or K_{oc} values of benzophenone-3 (K_{ow} = 3.79, K_{oc} = 3.10), caffeine (K_{ow} = –0.07, K_{oc} = 2.87), triclocarban (K_{ow} = 4.90, K_{oc} = 3.70) and ketoconazole (K_{ow} = 4.34, K_{oc} = 3.34) no statistically significant correlations ($p > 0.05$) were seen between TOC% and their measured concentrations, considering the two sampling campaigns. This lack of correlations have been reported by several studies assessing nonpolar contaminants in coastal sediments, and frequently attributed to sediment complexity, including kind of organic matter, relative absorbability with inorganic particles, and differential biological activity.[48]

Considering detection frequencies, in 2015, triclocarban was seen in all 14 (100%) analyzed samples followed by caffeine, benzophenone-3 and ketoconazole, which were detected respectively 8 (57%), 5 (36%) and 5 (36%) times. Such frequencies were slightly higher in 2019 when triclocarban, caffeine, benzophenone-3 and ketoconazole were found in 9 (64%), 9 (64%), 14 (100%) and 6 (43%) samples, respectively. In most cases, the environmental occurrence of the studied emerging contaminants was seen in areas close to sewage effluents (see Table 3 and Figure 1), indicating diffused contamination sources related to domestic outfalls as pointed out by Pusceddu *et al*.[5] The absence or limited sewage treatment performed in some countries has been previously identified as a major factor contributing to the occurrence of pharmaceuticals in surface waters.[49] In this regard, it is important to highlight that in the margins of SSES there are several urban areas not covered by sanitation services, which discharge untreated sewage directly into the estuary. Moreover, even when collected by sewage systems, after human consumption and excretion, these compounds are continuously released into SSES since local wastewater treatment plants only remove solids, without primary or secondary treatment prior disposal.[30] After release in the aquatic environment, estuarine discharges, tides and other oceanographic and physicochemical factors promote the spatial distribution of these molecules throughout the environmental compartments along the estuary.[6] Indeed, the high detection frequency and levels found nearby sources of treated (S1,

Table 3. Emerging contaminants detected in sediments from Santos-São Vicente Estuarine system

Sample	Sampling 2015					Sampling 2019				
	TOC / %	BP-3 / (ng g^{-1})	CAF / (ng g^{-1})	TCC / (ng g^{-1})	KTZ / (ng g^{-1})	TOC / %	BP-3 / (ng g^{-1})	CAF / (ng g^{-1})	TCC / (ng g^{-1})	KTZ / (ng g^{-1})
S1	0.8	102.7	n. d.	104.6	16.9	0.3	< 3	n.d.	n. d.	< 5
S2	1.5	n. d.	n. d.	5.8	n. d.	0.5	< 3	n. d.	n. d.	< 5
S3	0.9	n. d.	28.3	47	n. d.	1.9	< 3	1.2	< 1	< 5
S4	1.8	16.8	5.3	333	10.9	2.8	< 3	1.1	< 1	< 5
S5	1.3	n. d.	15.4	470.2	n. d.	1.8	< 3	1.1	< 1	n. d.
S6	1.6	26.5	12.4	21.4	n. d.	1	< 3	< 1	< 1	n. d.
S7	3	n. d.	10.1	46.4	n. d.	0.4	< 3	< 1	< 1	n. d.
S8	2.7	12.3	7.4	274.5	< 5	0.6	< 3	1.4	2.8	6.5
S9	2.8	n. d.	n. d.	8.1	n. d.	3.6	< 3	1.7	1.5	< 5
S10	2.8	26.9	n. d.	346.8	14.1	1.2	< 3	< 1	n. d.	n. d.
S11	3.4	n. d.	13.2	40.9	n. d.	4.2	< 3	n. d.	n. d.	n. d.
S12	3.5	n. d.	n. d.	161.1	7.4	1.9	< 3	n. d.	n. d.	n. d.
S13	1.1	n. d.	3.5	1.7	n. d.	6.3	< 3	n. d.	< 1	n. d.
S14	1	n. d.	n. d.	102	n. d.	1	< 3	< 1	< 1	n. d.
Df / %	–	36	57	100	36	–	100	64	64	43

TOC: total organic carbon; BP-3: benzophenone-3; CAF: caffeine; TCC: triclocarban; KTZ: ketoconazole; Df: detection frequency; n.d.: not detected.

S8, S12 and S13) and untreated (S3, S4, S5, S10 and S11) sewage denote unambiguous anthropogenic inputs of such emerging contaminants in SSES. In addition, the detection of residues and moderate to low concentrations observed in S2, S6, S7, S9 and S14 indicate some environmental mobility of hydrophobic compounds deposited in sediments from SSES as was previously reported by dos Santos *et al*.[50] and Abreu *et al*.[26] Similarity, the temporal significant decrease ($P < 0.05$) in concentrations of benzophenone-3, caffeine, triclocarban and ketoconazole, in all sampled sites, can be related to natural mobility in surface sediment layers, which can be influenced by seasonal hydrodynamic flows in SSES.

Benzophenone-3 (BP-3), used in sunscreen products and as a food additive, was detected in concentrations from < 2 to 102.7 ng g^{-1}. BP-3 is one of the most widely detected UV sunscreens in environmental matrices, and lower range of concentrations were detected in USA (6.9-10.8 ng g^{-1}),[40] Brazil (< 3-17 ng g^{-1})[4] and China (0.6-3 ng g^{-1}).[51] In fact, the patterns influencing environmental inputs of BP-3 are not well known. On the other hand, data on ecotoxicology effects of BP-3 indicates that this endocrine disruptor may induce alterations in estrogen and testosterone balance leading to alterations in birth weight and gestational age in humans. In addition, decrease in egg production, hatching, testosterone, and steroidogenic genes were reported for other vertebrate animals exposed to BP-3.[52] However, based on continuous release of BP-3 to water bodies as well as its physicochemical properties further studies should be performed to investigate levels and effects induced by BP-3 worldwide.

Triclocarban, a bactericide widely used in many personal care products, have been frequently detected in environmental matrices around the world. In this study, detected concentrations ranged from < 1 to 470.2 ng g^{-1}. Similar range of concentration was found in sediments from Brazil (< 1-1318 ng g^{-1}),[4] and lower levels were detected in samples from China (1.73-43.9 ng g^{-1}),[53] India (4.3-26.3 ng g^{-1})[54] and Saudi Arabia (nd-10 ng g^{-1}).[55] The prevalent TCC levels seen in S1, S4, S5, S8, S10, S12 and S14 during the 2015 campaign are in the same range enough to induce deleterious effects on aquatic organisms.[53] Thus, special attention should be given to this compound in future studies in the region.

The ketoconazole concentrations (< 5 to 16.9 ng g^{-1}) measured in SSES were lower than have been found in sediment samples from Thailand (6-59 ng g^{-1})[56] and northern Brazil (< 5-277 ng g^{-1}).[4] This antifungal compound presents a very broad-spectrum fungicidal property, being prescribed for a wide range of fungal infections, and marketed in different formulations.

Similarity, caffeine levels detected in the present study were lower (< 1-28.3 ng g^{-1}) compared to other recent studies.[4] Anyway, the occurrence of such emerging contaminants in surface sediment from SSES indicates anthropogenic inputs probably related to poor sewage treatment processes adopted in the region.[30]

Conclusions

Analysis employing an effective QuEChERS multiresidue extraction combined with determination by LC-MS/MS showed the environmental occurrence of benzophenone-3, caffeine, ketoconazole and triclosan in surface sediment samples from SSES. The method proved to be adequate for the analysis of 10 ECs, enabling the quantification of ECs in concentrations up to 1 ng g^{-1} and presenting the advantages as the use of a small volume of organic solvent, easiness to perform and rapidity. Based on the results, the diffused contamination sources of emerging contaminants to SSES are related to effluents of treated and untreated sewage which are directly and continuously released into the estuary. Moreover, the poor sewage treatment processes adopted in the region contributed to the environmental occurrence of such molecules in the analyzed samples. Besides, it is important to highlight that some compounds were detected in concentrations capable to induce deleterious effects on aquatic organisms. Considering interannual variability in measured concentrations, additional studies should be held seeking better understand the simultaneous influence of releases, oceanographic factors and TOC amounts on EC levels associated to surface sediments.

Acknowledgments

The authors acknowledge the financial support granted by the Brazilian agencies FAPERGS, CAPES (Finance Code 001), FINEP and fellowships granted by CAPES. Primel, E. G. (DT 305716/2020-4) and Castro, I. B. (PQ 302713/2018-2) received a productivity research fellowship from the CNPq.

Author Contributions

MJSC was responsible for conceptualization, formal analysis, investigation, methodology, validation, writing original draft; SCB for conceptualization, formal analysis, investigation, methodology, validation, writing original draft; EGP for writing review and editing, resources, project administration; IBC for conceptualization, methodology, writing original draft, review and editing, and project administration.

References

1. Fernandes, M.; da Fonseca, E. M.; Lima, L. S.; Sichel, S. E.; Delgado, J. F.; Correa, T. R.; Aguiar, V. M. C.; Baptista Neto, J. A.; *Reg. Stud. Mar. Sci.* **2020**, *35*, 101143. [Crossref]
2. Celis-Hernandez, O.; Cundy, A. B.; Croudace, I. W.; Ward, R. D.; Busquets, R.; Wilkinson, J. L.; *Water Res.* **2021**, *189*, 116610. [Crossref]
3. Rosenfeld, P. E.; Feng, L. G. H. In *Risks of Hazardous Wastes*; Rosenfeld, P. E.; Feng, L. G. H., eds.; William Andrew Publishing: Boston, 2011, p. 215.
4. Chaves, M. J. S.; Barbosa, S. C.; Malinowski, M. M.; Volpato, D.; Castro, Í. B.; Franco, T. C. R. S.; Primel, E. G.; *Sci. Total Environ.* **2020**, *734*, 139374. [Crossref]
5. Pusceddu, F. H.; Sugauara, L. E.; de Marchi, M. R.; Choueri, R. B.; Castro, Í. B.; *Mar. Pollut. Bull.* **2019**, *142*, 576. [Crossref]
6. Castro, Í. B.; *Trends Environ. Anal. Chem.* **2019**, *24*, e00068. [Crossref]
7. Cesar, A.; Pereira, C. D. S.; Santos, A. R.; Abessa, D. M. S.; Fernández, N.; Choueri, R. B.; DelValls, T. A.; *Braz. J. Oceanogr.* **2006**, *54*, 55. [Crossref]
8. Beretta, M.; Britto, V.; Tavares, T. M.; da Silva, S. M. T.; Pletsch, A. L.; *J. Soils Sediments* **2014**, *14*, 1278. [Crossref]
9. Fernandes, M. J.; Paíga, P.; Silva, A.; Llaguno, C. P.; Carvalho, M.; Vázquez, F. M.; Delerue-Matos, C.; *Chemosphere* **2020**, *239*, 124729. [Crossref]
10. Deere, J. R.; Moore, S.; Ferrey, M.; Jankowski, M. D.; Primus, A.; Convertino, M.; Servadio, J. L.; Phelps, N. B. D.; Hamilton, M. C.; Chenaux-Ibrahim, Y.; Travis, D. A.; Wolf, T. M.; *Sci. Total Environ.* **2020**, *724*, 138057. [Crossref]
11. Ngo, T. H.; Van, D.-A.; Le Tran, H.; Nakada, N.; Tanaka, H.; Huynh, T. H.; *Environ. Sci. Pollut. Res.* **2020**, *28*, 12082. [Crossref]
12. Díaz-Cruz, M. S.; Molins-Delgado, D.; Serra-Roig, M. P.; Kalogianni, E.; Skoulikidis, N. T.; Barceló, D.; *Sci. Total Environ.* **2019**, *651*, 3079. [Crossref]
13. Soares, K. L.; Sunyer-Caldú, A.; Barbosa, S. C.; Primel, E. G.; Fillmann, G.; Diaz Cruz, M. S.; *Chemosphere* **2021**, *267*, 129085. [Crossref]
14. Shi, C.; Gui, W.; Chen, J.; Zhu, G.; *Bull. Environ. Contam. Toxicol.* **2010**, *84*, 236. [Crossref]
15. Anastassiades, M.; Lehotay, S. J.; Štajnbaher, D.; Schenck, F. J.; *J. AOAC Int.* **2003**, *86*, 412. [Crossref]
16. Carmona, E.; Andreu, V.; Picó, Y.; *Sci. Total Environ.* **2014**, *484*, 53. [Crossref]
17. Vulliet, E.; Berlioz-Barbier, A.; Lafay, F.; Baudot, R.; Wiest, L.; Vauchez, A.; Lestremau, F.; Botta, F.; Cren-Olivé, C.; *Environ. Sci. Pollut. Res.* **2014**, *21*, 11370. [Crossref]
18. Berlioz-Barbier, A.; Vauchez, A.; Wiest, L.; Baudot, R.; Vulliet, E.; Cren-Olivé, C.; *Anal. Bioanal. Chem.* **2014**, *406*, 1259. [Crossref]
19. Dimpe, K. M.; Nomngongo, P. N.; *TrAC, Trends Anal. Chem.* **2016**, *82*, 199. [Crossref]
20. Kim, L.; Lee, D.; Cho, H.-K.; Choi, S.-D.; *TrAC, Trends Anal. Chem.* **2019**, *22*, e00063. [Crossref]
21. Santos, L. H. M. L. M.; Ramalhosa, M. J.; Ferreira, M.; Delerue-Matos, C.; *J. Chromatogr. A* **2016**, *1437*, 37. [Crossref]
22. Yao, L.; Dou, W.-Y.; Ma, Y.-F.; Liu, Y.-S.; *Chemosphere* **2021**, *282*, 131047. [Crossref]
23. Necibi, M.; Lanceleur, L.; Mzoughi, N.; Monperrus, M.; *Bull. Environ. Contam. Toxicol.* **2016**, *97*, 659. [Crossref]
24. Nannou, C. I.; Boti, V. I.; Albanis, T. A.; *Anal. Bioanal. Chem.* **2019**, *411*, 1383. [Crossref]
25. Begliomini, F. N.; Maciel, D. C.; de Almeida, S. M.; Abessa, D. M.; Maranho, L. A.; Pereira, C. S.; Yogui, G. T.; Zanardi-Lamardo, E.; Castro, Í. B.; *Environ. Pollut.* **2017**, *226*, 494. [Crossref]
26. Abreu, F. E. L.; Lima da Silva, J. N.; Castro, Í. B.; Fillmann, G.; *J. Hazard. Mater.* **2020**, *398*, 122937. [Crossref]
27. de Souza, A. C.; Taniguchi, S.; Lopes Figueira, R. C.; Montone, R. C.; Bícego, M. C.; Martins, C. C.; *J. Hazard. Mater.* **2018**, *360*, 428. [Crossref]
28. Pereira, C. D. S.; Maranho, L. A.; Cortez, F. S.; Pusceddu, F. H.; Santos, A. R.; Ribeiro, D. A.; Cesar, A.; Guimarães, L. L.; *Sci. Total Environ.* **2016**, *548-549*, 148. [Crossref]
29. Gimiliani, G. T.; Fornari, M.; Redígolo, M. M.; Bustillos, J. O. W. V.; Abessa, D. M. S.; Pires, M. A. F.; *Case Stud. Chem. Environ. Eng.* **2020**, *2*, 100020. [Crossref]
30. Fontes, M. K.; de Campos, B. G.; Cortez, F. S.; Pusceddu, F. H.; Nobre, C. R.; Moreno, B. B.; Lebre, D. T.; Maranho, L. A.; Pereira, C. D. S.; *Sci. Total Environ.* **2021**, *757*, 143808. [Crossref]
31. Ohoro, C. R.; Adeniji, A. O.; Okoh, A. I.; Okoh, O. O.; *Int. J. Environ. Res. Public Health* **2019**, *16*, 3026. [Crossref]
32. Kristensen, E.; Andersen, F. Ø.; *J. Exp. Mar. Biol. Ecol.* **1987**, *109*, 15. [Crossref]
33. Cerqueira, M. B. R.; Caldas, S. S.; Primel, E. G.; *J. Chromatogr. A* **2014**, *1336*, 10. [Crossref]
34. Instituto Nacional de Metrologia, Qualidade e Tecnologia (INMETRO); *DOQ-CGCRE-008, Orientação sobre Validação de M*étodos *Analíticos*; 2020. [Link] accessed in July 2022
35. *Statistica*, 13.0; StatSoft, Tulsa, OK, USA, 2015.
36. SANTE/12682/2019: *Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed*; 2019. [Link] accessed in July 2022
37. Trufelli, H.; Palma, P.; Famiglini, G.; Cappiello, A.; *Mass Spectrom. Rev.* **2011**, *30*, 491. [Crossref]
38. Łozowicka, B.; Rutkowska, E.; Jankowska, M.; *Environ. Sci. Pollut. Res.* **2017**, *24*, 7124. [Crossref]
39. Carmona, E.; Andreu, V.; Picó, Y.; *J. Pharm. Biomed. Anal.* **2017**, *146*, 117. [Crossref]
40. He, K.; Hain, E.; Timm, A.; Tarnowski, M.; Blaney, L.; *Sci. Total Environ.* **2019**, *650*, 3101. [Crossref]

41. Matongo, S.; Birungi, G.; Moodley, B.; Ndungu, P.; *Chemosphere* **2015**, *134*, 133. [Crossref]
42. Zhang, P.; Zhou, H.; Li, K.; Zhao, X.; Liu, Q.; Li, D.; Zhao, G.; Wang, L.; *RSC Adv.* **2018**, *8*, 4703. [Crossref]
43. Kumirska, J.; Łukaszewicz, P.; Caban, M.; Migowska, N.; Plenis, A.; Białk-Bielińska, A.; Czerwicka, M.; Qi, F.; Piotr, S.; *Chemosphere* **2019**, *232*, 232. [Crossref]
44. Biel-Maeso, M.; Corada-Fernández, C.; Lara-Martín, P. A.; *Chemosphere* **2017**, *185*, 1001. [Crossref]
45. Kumirska, J.; Migowska, N.; Caban, M.; Łukaszewicz, P.; Stepnowski, P.; *Sci. Total Environ.* **2015**, *508*, 498. [Crossref]
46. Artifon, V.; Castro, Í. B.; Fillmann, G.; *Environ. Sci. Pollut. Res.* **2016**, *23*, 16047. [Crossref]
47. Artifon, V.; Zanardi-Lamardo, E.; Fillmann, G.; *Sci. Total Environ.* **2019**, *649*, 1620. [Crossref]
48. Batista, R. M.; Castro, I. B.; Fillmann, G.; *Sci. Total Environ.* **2016**, *566-567*, 446. [Crossref]
49. Segura, P. A.; Takada, H.; Correa, J. A.; El Saadi, K.; Koike, T.; Onwona-Agyeman, S.; Ofosu-Anim, J.; Sabi, E. B.; Wasonga, O. V.; Mghalu, J. M.; dos Santos, A. M.; Newman, B.; Weerts, S.; Yargeau, V.; *Environ. Int.* **2015**, *80*, 89. [Crossref]
50. dos Santos, D. M.; Turra, A.; de Marchi, M. R. R.; Montone, R. C.; *Environ. Sci. Pollut. Res.* **2016**, *23*, 16152. [Crossref]
51. Tang, Z.; Han, X.; Li, G.; Tian, S.; Yang, Y.; Zhong, F.; Han, Y.; Yang, J.; *Ecotoxicol. Environ. Saf.* **2018**, *164*, 540. [Crossref]
52. Ghazipura, M.; McGowan, R.; Arslan, A.; Hossain, T.; *Reprod. Toxicol.* **2017**, *73*, 175. [Crossref]
53. Chen, Z.-F.; Wen, H.-B.; Dai, X.; Yan, S.-C.; Zhang, H.; Chen, Y.-Y.; Du, Z.; Liu, G.; Cai, Z.; *J. Hazard. Mater.* **2018**, *357*, 376. [Crossref]
54. Vimalkumar, K.; Arun, E.; Krishna-Kumar, S.; Poopal, R. K.; Nikhil, N. P.; Subramanian, A.; Babu-Rajendran, R.; *Sci. Total Environ.* **2018**, *625*, 1351. [Crossref]
55. Picó, Y.; Alvarez-Ruiz, R.; Alfarhan, A. H.; El-Sheikh, M. A.; Alshahrani, H. O.; Barceló, D.; *Sci. Total Environ.* **2020**, *701*, 135021. [Crossref]
56. Juksu, K.; Zhao, J.-L.; Liu, Y.-S.; Yao, L.; Sarin, C.; Sreesai, S.; Klomjek, P.; Jiang, Y.-X.; Ying, G.-G.; *Sci. Total Environ.* **2019**, *690*, 1110. [Crossref]

Submitted: May 3, 2022
Published online: August 2, 2022