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ABSTRACT

Organochlorine pesticides present in sewage sludge can contaminate soil and water when they are used as either fertilizer or agricultural soil conditioner. In this study, the technique solid–liquid extraction with low temperature purification was optimized and validated for determination of ten organochlorine pesticides in sewage sludge and soil samples. Liquid–liquid extraction with low temperature purification was also validated for the same compounds in water. Analyses were performed by gas chromatography-mass spectrometry operating in the selective ion monitoring mode. After optimization, the methods showed recoveries between 70% and 115% with relative standard deviation lower than 13% for all target analytes in the three matrices. The linearity was demonstrated in the range of 20 to 70 μg L^{-1}, 0.5 to 60 μg L^{-1}, and 3 to 13 μg L^{-1}, for sludge, soil, and acetonitrile, respectively. The limit of quantification ranged between 2 and 40 μg kg^{-1}, 1 and 6 μg kg^{-1}, and 0.5 μg L^{-1} for sludge, soil, and water, respectively. The methods were used in the study of pesticide lixiviation carried out in a poly vinyl chlorine column filled with soil, which had its surface layer mixed with sludge. The results showed that pesticides are not leached into soil, part of them is adsorbed by the sewage sludge (4–40%), and most pesticides are lost by volatilization.

Introduction

Sewage sludge is a solid residue produced by the wastewater treatment system (WWTS), which may be used in agriculture as either fertilizer or soil conditioner.[1,2] However, organic chemical contaminants present in sewage sludge can be transferred to the soil, water, plant and, consequently enter the food chain.[3] The Brazilian environmental legislation recommends the monitoring of 43 organic compounds in sewage sludge, including ten organochlorine pesticides, before its application to soil.[4] Although these compounds have been banned under the Stockholm Convention since 2001, levels of these pesticides are still detected in various environmental matrices.[5–9] The organochlorine pesticides were extensively used in the past in insect control and are considered persistent, bioaccumulative in the food chain, and highly toxic for humans. Contamination by organochlorine pesticides is related to serious health problems such as neurodegenerative disorders, diabetes, changes in renal and endocrine systems, cancer, among others.[10–14]

The monitoring of these organochlorine pesticides in sewage sludge, soil and water has been performed employing different extraction techniques, including accelerate solvent extraction,[15] Soxhlet,[16] matrix solid phase dispersion (MSPD),[17] microwave,[18] pressurized liquid extraction (PLE),[19] stir bar sorptive extraction,[20] solid phase micro extraction,[21] suspended droplet micro extraction.[22] Despite these varied methodologies, the solid–liquid extraction with low temperature purification (SLE-LTP) has been a very good alternative for the extraction of organic contaminants in solid and semi-solid matrices,[23–26] because it is easier and cheaper than other techniques described in the literature. Besides, SLE-LTP has low solvent consumption, high extraction efficiency and few steps, which are characteristics desired in the development of new methods for determination of chemical contaminants.

In the SLE-LTP, a quantity of sample, water and acetonitrile are mixed and frozen below -18°C. When lowering the temperature, while the partition between water and acetonitrile occurs, chemical contaminants are extracted to organic phase. Then, after freezing the water phase, matrix components are entrapped into the structure of the ice. Thereby, this technique is denominated as low temperature purification in order to emphasize the cleanup of the extracts. On the other hand, when analyzing liquid samples, for example, water samples, this technique should be denominated only as liquid–liquid extraction with low temperature partition (LLE-LTP).

To the best of our knowledge, there are nearly 80 research articles published in the web of science about purification or partition in low temperature so far, but none of them studied organochlorine pesticides in sewage sludge, soil, and water. Therefore, this study aimed to optimize, validate, and apply the SLE-LTP and LLE-LTP for determination of ten organochlorine pesticides in sludge, soils and water samples. The analyses were performed by gas chromatography coupled to mass spectrometry (GC-MS) in selective ion monitoring mode (SIM).

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Experimental

Reagents and solutions

The analytical standards of organochlorine pesticides hexachlorobenzene, aldrin, dieldrin, endrin, dichlorodiphenyltrichloroethane (DDT), lindane, cis-chlordane, *trans*-chlordane, heptachlor, and mirex were purchased from Sigma Aldrich (St. Louis, USA), with a degree of purity of 99.9% (w/w). Stock standard solutions of each pesticide were prepared at the concentration of 500 mg L^{-1} in acetonitrile. From these solutions, an intermediate solution was prepared at 25 mg L^{-1} containing all compounds in the same solvent. Subsequently, this solution was diluted in acetonitrile to 4 mg L^{-1} to prepare the working solution. Ethyl acetate and acetonitrile high performance liquid chromatography (HPLC) grade were purchased from Vetec (Rio de Janeiro, Brazil) and Merck (Rio de Janeiro, Brazil), respectively. Sodium chloride purity of 99% (w/w) was also obtained from Vetec (Rio de Janeiro, Brazil) and anhydrous sodium sulphate from Dynamic (Diadema, Brazil). For the cleanup of the extracts the following sorbents were used: alumina (Micro Abrasives, Westfield, USA), florisil (Mallinckrodt Chemicals, St. Louis, USA), activated charcoal (Vetec, Rio de Janeiro, Brazil), octadecil C18 (Fluka, Milwaukee, USA), primary secondary amine (PSA, Sigma-Aldrich, St. Louis, USA), and silica gel 230–400 mesh (Carvalhaes, Germany).

Instrumentation

The samples were prepared using a vortex (Phoenix, São Paulo, Brazil) and a centrifuge (Kindly, São Paulo, Brazil). The extracts obtained were analyzed using a gas chromatograph (GC 7890A) coupled with a mass spectrometer (MS 5975C) (Agilent Technologies) and a DB-5 MS capillary column (Agilent Technologies), with 5% phenyl stationary phase and 95% methylpolysiloxane (30 m × 0.32 mm i.d. × 0.25 μm film thickness). Helium (99.9999% purity) was used as carrier gas at a flow rate of 1.0 mL min^{-1}. The injector was maintained at 270°C. Oven temperature programming began at 100°C (1 min), with a heating rate of 20°C min^{-1} to 200°C (2 min), followed by a heating rate of 10°C min^{-1} to 280°C (1 min). The total analysis time was 17 min. Aliquots of 1 μL were injected into the GC-MS, using a CombiPAL injector and splitless mode. The mass spectrometer detector operated in electron impact ionization at 70 eV and quadrupole type mass analyzer. The interface was kept at 280°C and the ion source at 230°C. The instrument control and data acquisition were carried out with the software (E.02.02.1431 ChemStation copyright$^{©}$ 1989–2011) from Agilent Technology. Analysis was performed in SIM mode and the selected ions for each pesticide are shown in Table 1.

Samples

Sewage sludge, soil, and water samples

For optimization and validation of the SLE-LTP method, sewage sludge samples were collected from the WWTS located in the municipality of Montes Claros (Minas Gerais, Brazil). Soil samples were collected at the depth of 0 to 20 cm in areas free from application of organochlorine pesticides. Both samples were homogenized, sieved and maintained at 4°C prior to

Table 1. Pesticide identification, retention times and selected ions in the GC-MS analysis.

Number	Pesticide	Abbreviation	Retention time	m/z
1	Lindane	LND	7.60	181, 183, 219
2	Hexachlorobenzene	HCB	7.67	282, 284, 286
3	Heptachlorobenzene	HPT	9.70	272, 273, 274
4	Aldrin	ALD	10.44	263, 265, 269
5	*Trans*-chlordane	*t*-CLD	11.73	373, 375, 377
6	*Cis*-chlordane	*c*-CLD	11.98	373, 375, 377
7	Dieldrin	DLD	12.55	263, 265, 277
8	Endrin	END	12.98	263, 317, 345
9	DDT	DDT	14.02	165, 235, 237
10	Mirex	MRX	16.10	237, 272, 274

m/z: mass/charge

extraction. Type II water obtained from Synergy$^{®}$ water purifier (Merck Millipore) was used for validation of the LLE-LTP for all studied compounds.

SLE-LTP and LLE-LTP procedure

Sludge sample

A sample of 4.00 g of sewage sludge was weighed into a 22-mL transparent vial. Then, 2 mL of Milli-Q water and 8 mL of acetonitrile were added to the sludge, and the mixture was homogenized in a vortex for 1 min. Afterwards, the vial was kept at −20°C for 1 h for phase separation by freezing water and sludge. An aliquot of 2 mL of the liquid organic phase was transferred to a falcon tube (15 mL) containing 375 mg of anhydrous sodium sulphate plus 60 mg of C18 sorbent. Then, the falcon tube was homogenized in vortex for 30 s and centrifuged at 4,000 rpm for 10 min. Finally, 1 mL of extract was transferred to a vial (2 mL) and analyzed by GC-MS.

Soil sample

The extraction of the organochlorine pesticides in the soil sample was based on the method developed for extraction of the same compounds in sewage sludge. For that reason, 4.00 g of soil were weighed into a 22-mL transparent vial and mixed with 4 mL of Milli-Q water and 8 mL of acetonitrile. The mixture was homogenized in a vortex for 5 min, and the vial was placed in a freezer at −20°C for 1 h for phase separation. An aliquot of 2 mL of the liquid organic phase was transferred to a falcon tube (15 mL) containing only 375 mg of anhydrous sodium sulphate. Then, the falcon tube was homogenized in a vortex for 30 s and centrifuged at 4,000 rpm for 10 min. Finally, 1 mL of extract was transferred to a vial (2 mL) and analyzed by GC-MS.

Water sample

Since the extraction of the organochlorine pesticides in soil samples has been developed, the extraction of the same compounds in water sample was performed in the same way. A sample of 4 mL of water and 8 mL of acetonitrile were added into a 22-mL transparent vial. The vial was placed in a freezer at −20°C for 1 h for phase separation. An aliquot of 2 mL of the liquid organic phase was transferred to a falcon tube (15 mL) containing 375 mg of anhydrous sodium sulphate. Then, the falcon tube was homogenized in a vortex for 30 s and

centrifuged at 4,000 rpm for 10 min. Finally, 1 mL of extract was transferred to a vial (2 mL) and analyzed by GC-MS.

Validation

Selectivity, linearity, limit of quantification (LOQ), accuracy and precision were evaluated for organochlorine pesticides in the three studied matrices.[27,28] The selectivity of the method was investigated by evaluating organochlorine pesticide-free sewage sludge, soil and water (blank) in six independent replicates.

The linearity was evaluated by spiking sludge and soil extracts at seven concentration levels, with three independent replicates. The extracts were obtained after performing SLE-LTP in a pesticide-free sample (blank). Standard solutions of the ten pesticides in acetonitrile, at six levels of concentration, were also analyzed by GC-MS in triplicate. Concentrations were equally spaced in the range evaluated for each calibration curve. The least square linear regression was applied to the experimental data to estimate the regression parameters. The outliers were treated and confirmed by the Jacknife test with maximum exclusion of 22.2% of the data. Regression analysis was evaluated by the parameters normality (Ryan and Joiner test), homoscedasticity (Brown and Forsythe test), and independence (Durbinand Watson test). Analysis of variance (ANOVA) was applied to each calibration curve to check the lack of linear fit.

The LOQs were determined by spiking samples of sludge, soil and water with the pesticides in the lowest acceptable concentration. The samples were submitted to the SLE-LTP or LLE-LTP and after the chromatographic analyses of the extracts, the LOQs were considered as ten times the signal/noise ratio.

The accuracy was evaluated by experiments of fortification/recovery. Three levels of concentration were evaluated in three replicates. For each level, recovery values were considered acceptable when they were between 70% and 120% for sludge and soil samples. For the water sample, recovery percentages were acceptable between 80% and 110%.

Precision, under repeatability conditions, was evaluated by the relative standard deviation (RSD) obtained in the experiments of fortification/recovery of organochlorine pesticides in sludge, soil and water. The assays were carried out in seven replicates of a pesticide concentration in each matrix. The acceptability criterion for RSD was $\leq 20\%$ for the matrices analyzed.

Matrix effect was assessed during the analysis of the sludge extracts by GC-MS. Standard solutions of the pesticides prepared in pure solvent (acetonitrile) and sewage sludge extracts, at three different concentrations, were injected into GC-MS. The means of the areas obtained in the matrix and solvent were compared by the t-test for each pesticide. Percentages of the matrix effect was assessed by relating the areas of the analytes in pure solvent to areas obtained from sludge extracts $[\% = (\bar{A}_{solvent} - \bar{A}_{matrix}) \times 100/\bar{A}_{solvent}]$.

Application of the methods

Once the methods were validated, an experiment was carried out at laboratory scale for its application to real samples. In this experiment, we evaluated the mobility of organochlorine pesticides into soil amended with sludge under irrigation. The main purpose was to closely mimic and recreate the conditions taking place during real farming practices. Thus, a PVC (poly vinyl chlorine) column, 5 cm in diameter, was sectioned in six parts of 5 cm each, which were called rings. The rings were affixed to form a column, which kept a perfect vertical position. Afterwards, the first four rings of the column were filled with soil, from bottom to top. The fifth ring was filled with soil amended with sewage sludge previously contaminated with 100 $\mu g\ kg^{-1}$ of the organochlorine pesticide. Distilled water was added to the sixth ring to saturate the system in order to moisturize the soil near to field capacity. A PVC cap with a central orifice was attached to the base of the column to drain the leachate water. Subsequently, a 50 mm rain was simulated on the top of the column by sprinkling deionized water every three days. The volume of the leachate water was collected into plastic bottles placed below the column. Then, 4 mL of this sample were submitted to the LLE-LTP and analyzed by GC-MS to quantify organochlorine pesticides. The experiments in the soil column were carried out in triplicate.

After 60 days of monitoring the pesticides, the soil column was again sectioned and the soil from each ring was submitted to the SLE-LTP. The sample of soil amended with sludge, from the fifth ring, was analyzed using the validated methodology for soil.

Results and discussion

Chromatographic analysis

For the optimization of the chromatographic analysis, a standard solution of the organochlorine pesticides prepared in sludge extract at 50 $\mu g\ L^{-1}$ was introduced into the split/splitless injector liner packed with glass wool to reduce matrix interferences during chromatographic analysis. However, most pesticides showed low intensity signals and there was an inexplicable absence of the signal for END (Fig. 1). Then, we performed a new analysis with removal of the glass wool and, consequently, the signals of the pesticides in the chromatogram increased from 9 to 120 times. In addition, the signal for END was detected in the chromatogram for the first time (Fig. 1). The most obvious reason for this result is that organochlorine pesticides adsorb strongly in silanol groups of the glass wool. Similar result was reported in a previous study on organophosphorus pesticides using GC-MS.[29]
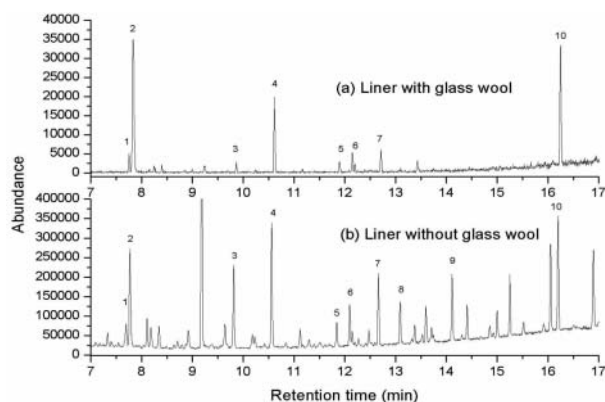

Figure 1. Total ion chromatograms (TIC) of a sewage sludge extract spiked with ten organochlorine pesticides at 50 $\mu g\ L^{-1}$: (a) split/splitless injector liner packed with glass wool; and (b) without glass wool. The peak identifications are described in Table 1.

Extraction methods

For the optimization of the SLE-LTP procedure, we first considered the methods of detection of organic chemical contaminants in sewage sludge reported in the literature.[30–32] After evaluating the best contaminant extraction conditions in these previous studies, we proposed a strategy for the extraction of organochlorine pesticides in sewage sludge. One important point to considerer is that the first procedure performed, which was similar to that described in the procedure section, but without using the sorbent, provided satisfactory pesticide recoveries, ranging from 71 to 124%. For this reason, only two parameters of the SLE-LTP were evaluated for sewage sludge, which have not been thoroughly investigated in previous works.

Due to the complexity of the matrix, the first additional parameter evaluated was the step of cleanup of extracts with sorbents before chromatographic analysis, which was based on dispersive solid phase extraction (d-SPE). For this purpose, 2 mL of extracts obtained after freezing the sample were transferred to a falcon tube, containing anhydrous sodium sulphate and 60 mg of sorbent. The sorbents investigated were alumina, C18, activated charcoal, florisil, PSA and silica gel and the results can be seen in Figure 2.

Activated charcoal provided clearer extracts, because it removed a greater deal of matrix interferences. However, we found that the charcoal reduced significantly the chromatographic response of the pesticides. This result is in accordance with a previous study[33] reporting that graphitized black carbon was not suitable for cleanup in the QuEChERS method. On the other hand, C18 was the best option because it showed recoveries higher than 70% for all pesticides and extracts clean enough to allow all identifications. These results are in agreement with those found by Sanchez-Brunete et al.[17] in the extraction of 16 pesticides by MSPD, using cleanup with C18
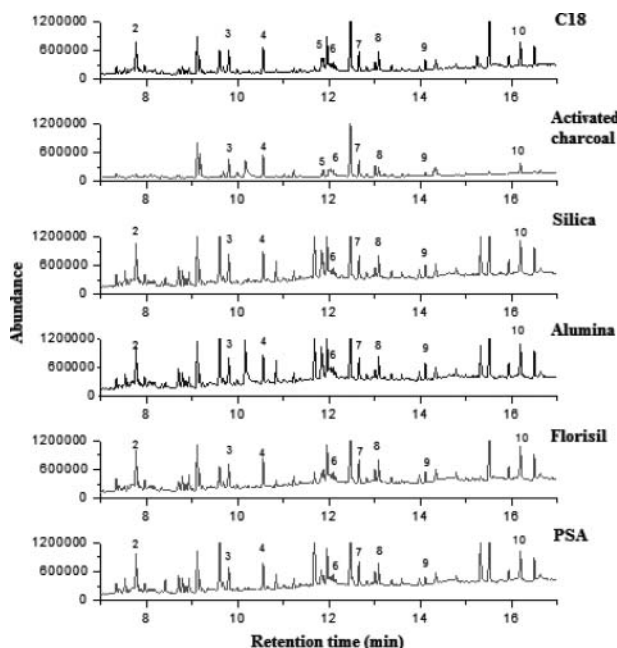
and GC-MS analyses. The other sorbents evaluated were not efficient in the cleanup of extracts (Fig. 2).

The second parameter investigated was rest time of sewage sludge samples spiked with the working standard solution of the pesticides. Figure 3 shows that rest times between 0 h and 168 h had similar recoveries for most of the pesticides. Only ALD showed a significant reduction in the concentration in the studied period, decreasing around 30% of the initial value after 168 h of spiking. Although DDT has also shown a significant reduction in the concentration, its variation was lower than ALD. In this way, 3 h was chosen as the time for spiking sludge samples in the validation step, because 90% of the compounds showed stable recovery from that spiking time.

After optimization of the pesticide extraction in sewage sludge, the best condition for extraction was assessed for the same pesticides in soil samples. However, the pesticide recoveries ranged from 65 to 80%, which were lower than the recoveries in the sewage sludge sample. In order to improve the results obtained during extraction, it was needed to increase the homogenization time of the vial containing soil, water and acetonitrile from 1 min to 5 min. The increase in homogenization time provided satisfactory results for all the compounds under study, with recoveries around 93–114%. The best explanation for the high recovery values observed in the test is the higher adsorption of the pesticides in the soil, which may cause an incomplete removal of the pesticides at only 1 min of homogenization.

Another problem observed during optimization was the volume of water for freezing the soil. The sludge sample required only 2 mL water for homogeneous freezing, but in this condition the soil sample did not freeze. This result clearly indicates that the freezing of the sample depends on the moisture of the sample. While the moisture of the soil was only 2% (w/w), the moisture of the sludge sample was 10% (w/w), i.e., five times greater, thus, the soil needed a higher volume of water (4 mL) for freezing.

The best condition established for extraction of the pesticides in soil samples was used for extraction of the pesticides in water sample, obviously removing the soil mass of the procedure. Bearing in mind that acetonitrile and water is a homogeneous system, it was not needed to homogenize the vial in vortex before freezing. In these conditions, the pesticide recoveries ranged from 93–125%.

Validation of SLE-LTP and LLE-LTP

A validation protocol of the analytical procedures was carried out in order to establish the performance characteristics of the
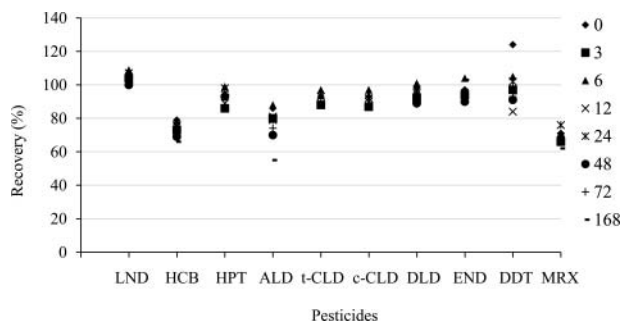
Figure 2. Total ion chromatograms of sewage sludge extracts subjected to cleanup with different sorbents. Concentration of pesticides in extract was 500 μg L^{-1}. The peak identifications are shown in Table 1.

Figure 3. Recovery percentages of organochlorine pesticides using the SLE-LTP in sewage sludge samples with different spiking times (0, 3, 6, 12, 24, 48, 72 and 168 h).

proposed methods, ensuring the adequate identification and quantification of the organochlorine pesticides.

Selectivity

The selectivity of the method was evaluated by comparing the chromatograms from pesticide-free sludge, soil, and water extracts (blank) with a standard solution of the pesticides in acetonitrile at 50 μg L^{-1}. Figure 4 shows that the interference peaks were not at the same retention time of the target analytes, demonstrating the selectivity of the method for the three analyzed matrices.

Linearity and matrix effect

For the quantification of the pesticides, one must take into account that soil and sludge are complex matrices containing a large amount of compounds that can interfere in the analyte signal, causing a matrix effect. Therefore, in order to compensate this effect, matrix-matched standard calibration was used for quantification of the target compounds.

Linearity was evaluated using seven concentration levels in the range from 10 to 70 μg L^{-1} for the pesticides in sludge extracts, except for lindane, which ranged from 20 to 70 μg L^{-1}, because this compound was not detected at concentrations below 20 μg L^{-1}. For the analytical curve of the soil extracts, the concentration ranged from 0.5 to 60 μg L^{-1}, at seven concentration levels, with the lowest level being equal to the LOQ of each compound. On the other hand, the analytical curves prepared in acetonitrile ranging from 3 to 13 μg L^{-1} were used for quantification of pesticides in water samples, at six concentration levels. The triplicate of each calibration point provided information on the inherent variability of response measurements (pure error). Calibration data were obtained by linear regression and the determination coefficients (R^2) were greater than 0.99 for most of the pesticides in three analytical curves (Table 2). The lack of fit was not

significant ($P > 0.05$) for the analytical curves of sludge and soil extracts and the solvent acetonitrile. Linearity was assessed by the ordinary least squares method (OLSM). The outliers were confirmed by the Jacknife residual test, with maximum exclusion of 22.2% in 21 replicates for sludge and soil. A maximum of 22.2% was also removed in 18 replicates prepared in acetonitrile. The Ryan–Joiner test indicated a normal distribution of the regression residuals, with correlation coefficients calculated above the critical values and deviation from normality non-significant for the ten pesticides in sludge and soil extracts and in acetonitrile. The homoscedasticity of residuals was confirmed by the Levene's test, demonstrating the homogeneous distribution for the three analytical curves. Independence of regression residuals with distribution of points without positive or negative trend was confirmed by the Durbin–Watson test. The results of the tests confirmed the OLSM suitability to ten pesticides. This complete system for linearity evaluation followed a procedure based on an acceptable and consistent statistics proposed by Souza and Junqueira.[34]

A representative example of the statistical tests is shown in Figure 5 for the linear regression of the *t*-CLD when it was prepared in sludge extract, soil extract and acetonitrile. Similar results were found for the other nine pesticides.

Limits of quantification, accuracy and precision

It is important to notice that the limits established referred to concentrations in sewage sludge, soil, and water samples prior to the extraction procedure and, consequently, they could be described as either LOQ or method quantification limits.

LOQ values for pesticides in sludge samples ranged from 2 to 40 μg kg^{-1}, corresponding to 1 to 20 μg L^{-1} (Table 2). Note that these values are lower than recommended by the environmental protection agency (EPA/Victoria), which establishes the following limits: 50 μg kg^{-1} for most of the compounds; 500 μg kg^{-1} for DDT; and no specific values for END and MRX.[35] Satisfactory
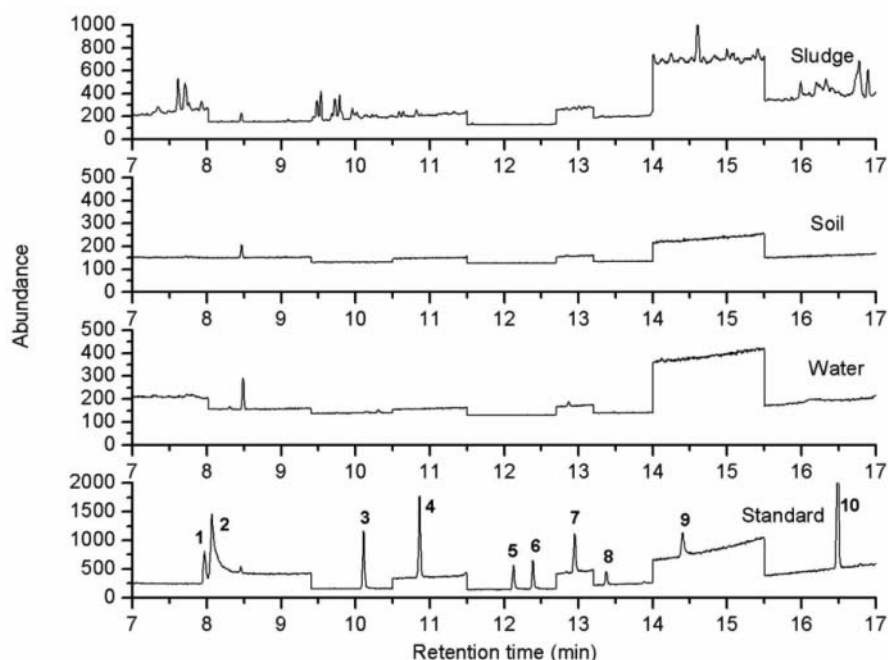

Figure 4. SIM mode chromatograms of sludge, soil, and water extracts obtained by SLE-LT and LLE-LTP in pesticide-free samples (blank). SIM mode chromatogram obtained from the standard solution of the pesticides in acetonitrile in the concentrations from 22 to 40 μg L^{-1}. The peak identifications are shown in Table 1.

Table 2. LOQ, accuracy and precision for SLE-LTP and LLE-LTP of ten organochlorine pesticides in sewage sludge, soil, and water.

	Sludge				Soil				Water		
	Recovery (%) ± RSD				Recovery (%) ± RSD				Recovery (%) ± RSD		
	LOQ	10^a μg L^{-1}	25^a μg L^{-1}	50^b μg L^{-1}	LOQ	6^a μg L^{-1}	13^b μg L^{-1}	20^a μg L^{-1}	LOQ	1^a μg L^{-1}	7^b μg L^{-1}	13^a μg L^{-1}
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LND	20	97 ± 0.45	94 ± 2	113 ± 4	3	85 ± 4	108 ± 3	70 ± 9	0.5	108 ± 5	110 ± 8	104 ± 6
HCB	1	78 ± 3	79 ± 7	84 ± 3	0.5	91 ± 12	90 ± 3	91 ± 9	0.5	104 ± 1	101 ± 6	107 ± 6
HPT	4	97 ± 0.9	96 ± 3	103 ± 2	1	78 ± 7	76 ± 4	76 ± 7	0.5	95 ± 2	98 ± 8	97 ± 3
ALD	4	80 ± 0.6	81 ± 4	94 ± 3	1	95 ± 4	82 ± 2	76 ± 5	0.5	103 ± 2	101 ± 6	104 ± 4
t-CLD	2	94 ± 3	96 ± 4	104 ± 1	1	84 ± 10	101 ± 1	108 ± 5	0.5	102 ± 4	106 ± 6	95 ± 4
c-CLD	2	90 ± 4	96 ± 3	106 ± 1	1	101 ± 13	102 ± 2	109 ± 10	0.5	108 ± 9	102 ± 7	99 ± 4
DLD	8	99 ± 0.4	99 ± 2	108 ± 2	1.5	116 ± 6	104 ± 3	99 ± 2	0.5	91 ± 4	103 ± 12	105 ± 5
END	8	102 ± 2	99 ± 0.8	106 ± 0	1.5	114 ± 7	104 ± 3	102 ± 6	0.5	99 ± 4	104 ± 13	111 ± 12
DDT	4	84 ± 3	86 ± 2	98 ± 5	3	115 ± 6	105 ± 8	98 ± 7	0.5	100 ± 4	91 ± 11	93 ± 7
MRX	4	70 ± 2	72 ± 9	78 ± 0	0.5	102 ± 4	112 ± 1	110 ± 5	0.5	107 ± 3	106 ± 5	106 ± 5

LOQ in μg L^{-1}, ^{a}average of three replicates and ^{b}average of seven replicates.

results were also achieved for all compounds under study in the soil sample, with LOQ between 1 and 6 μg kg^{-1}, corresponding to 0.5 to 3 μg L^{-1} (Table 2). All LOQ values were below the maximum residue limit (MRL) recommended by the Brazilian legislation (CONAMA Resolution n° 420 from 2009)[36] which establishes limits only for LND, HCB, ALD, DLD, END, and DDT, corresponding to 20, 5, 3, 200, 400 and 550 μg kg^{-1}, respectively. The best LOQs were obtained for the water sample and estimated at 0.5 μg L^{-1}. Considering that certain pesticides showed no adequate performance characteristics at low concentrations in water sample, and in order to simplify the subsequent routine quality controls, the LOQ was established as a single value for all compounds. This LOQ was lower than MRL for HCB (1 μg L^{-1}), ALD and DLD (0.7 μg L^{-1}), cis and trans chlordane (2 μg L^{-1}) and very close to the MRL of HPT (0.4 μg L^{-1}) and LND (0.2 μg L^{-1}). These limits are established by the EPA in water for human consumption.[37] Taking into consideration that LOQ values were

satisfactory, we choose not to concentrate the water extracts because the LLE-LTP would be easier, simpler and faster.

Accuracy evaluates the closeness of agreement between the measured values and the true value. In this study, accuracy was evaluated using recovery experiments of pesticides at three concentration levels in sludge, soil, and water (Table 2). For the sludge sample, the pesticide concentrations in extracts were 10, 25 and 50 μg L^{-1}, corresponding to 20, 50 and 100 μg kg^{-1}. These values were chosen because they represent values close to LQ, MRL, and the concentration of method optimization, respectively. For LND, accuracy was evaluated only for two concentrations because the LQ was higher than 10 μg L^{-1}. The recovery percentages ranged between 70% and 108%, according to IUPAC recommendations.[27] The values obtained in this study are similar to reported recoveries using MSPD, Soxtec, Soxhlet, and PLE for analyses of organochlorine pesticides in sewage sludge.[17,38] For soil samples, accuracy was evaluated at 6, 13 and 20 μg L^{-1},
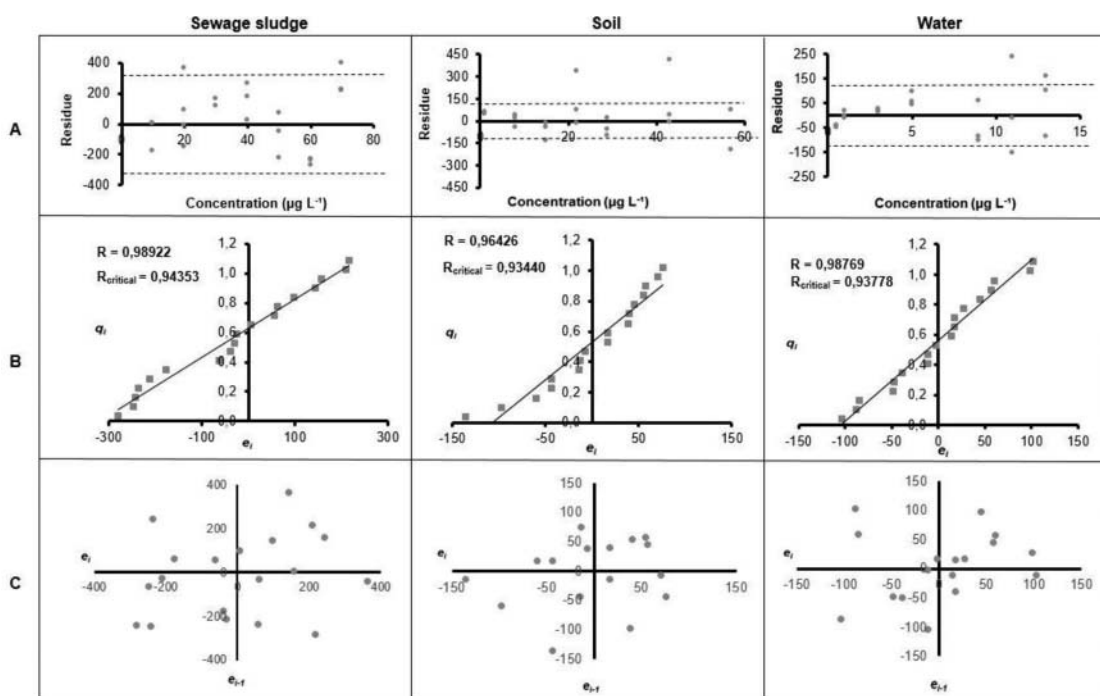

Figure 5. Results of the linearity statistics tests for t-CLD in sludge and soil extracts and acetonitrile. (A) Residual plots for outlier diagnose by Jacknife standardized residuals test, (B) Normality test of the residues: e_i = residual, R = Ryan–Joiner correlation coefficient, q_i = normal value expected, (C) Plots of residuals autocorrelation: e_i = residual.

corresponding to 12, 26 and 40 μg kg^{-1}. The lowest concentration represents a value close to pesticide LOQs. The results were also satisfactory since the recovery rate ranged between 70% and 116%. For the water sample, the accuracy was performed for concentrations lower than those of soil and sludge, since the MRLs are also very low in this matrix. For concentrations of 1, 7 and 13 μg L^{-1}, the recovery was satisfactory, as it ranged between 91% and 111%.

Precision tests evaluate the agreement between the results. Precision was evaluated under repeatability conditions in the concentrations of 50 μg L^{-1}, 13 μg L^{-1}, and 7 μg L^{-1} of the pesticides in sludge, soil, and water, respectively. The results were satisfactory, as the RSD was below 13% for the three matrices (Table 2).

Application of the procedure developed

The development of SLE-LTP and LLT-LTP for determination of ten organochlorine pesticides in soil, sludge, and water matrices was needed because, so far, there is no report in the literature of a simple, easy, fast, and cheap method for studies on leaching of compounds in the environment. The techniques developed allowed monitoring target pesticides in water samples obtained from a leaching column during 60 days, totaling 20 samples collected. None of the pesticides were quantified in the water samples, though this result is expected because these compounds show low solubility in water, between 0.0032 and 7.3 mg L^{-1}.

Analyses of the soil samples showed that there were pesticides only on the surface layer of the leaching column, i.e., the fifth ring from bottom to top. This result clearly indicates that organochlorine pesticides are not leached into soil; consequently, this experiment demonstrated that these compounds do not contaminate groundwater. The concentrations of the pesticides ranged from 4 to 40 μg kg^{-1} in the surface layer and higher adsorption of the pesticides in this layer can be attributed to the incorporation of the sewage sludge to the soil. Generally, sewage sludge shows lipophilic compounds in its composition, which increases retention of the organochlorine pesticides. Although organochlorine pesticides were retained in the surface layer, their initial concentration decreased between 40% and 96% after 60 days. One possible explanation of this effect could be based on the assumption that part of the pesticides volatilized in the experimental conditions. Previous studies demonstrated that organochlorine pesticides volatize from soil in conditions of higher moisture, organic matter, and temperature.[39–43] It is noteworthy that the room temperature ranged from 25 to 37°C during the experiments, according to the National Institute of Meteorology.

Conclusion

In this study, SLE-LTP and LLE-LTP were validated for the extraction of ten organochlorine pesticides of sewage sludge, soil, and water samples. The validated methods are simpler, easier and of lower cost, with pesticide extraction at rates higher than 70% and RSD lower than 13%. The LOQs were lower than the MRLs established by national and international legislation. Application of the methods in leaching studies of the target pesticides in column showed that 4 to 40% of the pesticides are retained into soil amended with sewage sludge and they are not leached. Most pesticides were lost by volatilization due to conditions of high moisture, organic matter, and temperature of the experiments. For these reasons, SLE-LTP and LLE-LTP represent good alternatives for the monitoring of organochlorine pesticides in agricultural lands that use sewage sludge.

Acknowledgments

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