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Original Research

Organochlorine Pesticides in Selected Sewage Sludge in South Africa : Assessment and Method Validation

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Abstract

The purpose of this study was to determine the concentrations of organochlorine pesticides (OCPs) in sewage sludge from three wastewater treatment plants (WWTPs), with a view to study their contribution to the environmental pollution of the Amathole District in Eastern Cape, South Africa. Analyses were performed by gas chromatography joined with micro electron capture detector (μECD). The limits of detection (LODs) of the tested congeners varied from 0.04 ng/g α-Lindane (α-BHC) to 0.49 ng/g (endosulfan sulfate), and the limits of quantification ranged from 0.22 ng/g (aldrin) to 2.17 ng/g δ-lindane (δ-BHC). Total concentrations of the 17 congeners for different seasons in the sludge ranged from 191 to 947 ng/g dw. For the six predominant congeners, the total highest concentration levels were found in this order dichlorodiphenyl trichloroethane (DDT), dichlorodiphenyl dichloroethane (DDD), α-BHC, γ-BHC, aldrin and endosulphate 1, and with values of 1512, 1330, 1095, 998, 994 and 547 ng/g respectively. For this result the contamination levels of some congeners for South African sludge can be categorized as high compared with European countries, but moderate to lower compared to other countries worldwide. Some congener (α-BHC, γ-BHC, aldrin, endosulphate 1, DDD and DDT) contents in the samples exceeded limits set by the European Commission for 1000 ng/g set for Σ DDT and 500 ng/g for other pesticide use of sludge in agriculture.

Keywords: sewage sludge, wastewater treatment plants, gas chromatography/μECD, organochloride pesticides, South Africa

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Introduction

Organochlorine pesticides (OCPs) are harmful chemicals used to destroy or control pesticides that are destroying food or crop production. The widespread use of pesticides to increase agricultural productivity has played a major role in recent decades. The chemicals have been applied for many years to avert, repel or mitigate the effects of pests [1-3]. One common nature of OCPs are their persistence in the environment, and many countries have banned their use because of their genotoxic and carcinogenic effects. Their metabolites are still present in the environs owing to their persistence, and are more harmful than their parent compounds. The toxicity, bioaccumulation and longer half-lives of the compounds present a high level of risk to the environment [4-5]. Organochlorine pesticides (OCPs) move in the environment through non-point sources such as agricultural runoff and vapourization following their field application. OCPs are highly lipophilic and tend to adsorb solid particles of sewage in wastewater treatment plants (WWTPs). Therefore, sewage sludge can be one of the major sources of these ecological pollutants when they are applied as landfill or agricultural manure. In addition, their concentration in sewage sludge can be indicative of the environmental release of the compounds [6-7].

The majority of organochlorides that are banned in South Africa are still in use, causing various hazard effects to ecosystems. A study from the Jukskei River catchment area in Gauteng, South Africa showed a significant high level concentration of OCPs in all the sampling points [8-9]. There were also some studies monitoring OCPs in sewage sludge in Europe [4, 10]. The European Union has developed the draft of a "working document on sludge" to promote the use of sewage sludge in agriculture with limit values set for several heavy metals and some organic compounds, including seven PCB congeners (maximum limit of 800 ng/g). For OCPs a value of 1,000 ng/g was established as the highest contaminant limit of ΣDDTs, and 500 ng/g for other pesticides [11-13]. Due to the ecological risk of the aforementioned compounds to both human and aquatic organisms, it is therefore very important to constantly examine the pesticide residues in the environments [14-15]. There is also a need for development and validation of methods with higher sensitivity, reliability, and accuracy. Most common methods for the identification and quantification of pesticide residues were based on using gas chromatography with electron capture detector (ECD), mass spectrometer (MS) and Flame ionization detector (FID) [7, 10]. μ-ECD is very sensitive for the analysis of halogenated compounds [16-18].

The aim of this work was to develop and validate methods using GC- μECD detectors for determining pesticide residues in the environment. These methods were applied to determine the concentration of OCP residues in the sludge collected from three WWTPs in Eastern Cape, South Africa. To the best of our knowledge, there is no data on the concentration levels of OCPs in the sewage sludge in this area. The objectives of this study were to investigate the levels and compositions of organochlorine pesticides in the sludge of three WWTPs around Amathole municipality in Eastern Cape, South Africa. Screen sewage sludge samples to determine the existence of the most persistent organic pollutants, test the extraction method for efficiency and reproducibility, quantify the selected organic contaminants and compare the values obtained in this work to the current European commission Sludge Guidelines and make recommendations [19]. Due to a lot of farming activities, the area around this site was suspected to be contaminated by pesticide residues as a result of leakage and improper disposal of pesticides and containers. No study had been conducted to investigate the contamination status at this site.

Material and Method

A mixed standard of the organochlorine (α, β, γ, and δ - hexachlorocyclohexane (HCH), heptachlor, heptachlor epoxide, dieldrin, aldrin, endrin, endrin aldehyde, endosulfan I, endosulfan II, endosulfan sulfate, p,p'-DDE, p,p'-DDD, p,p'-DDT, and methoxychlor) was purchased from Sigma-Aldrich in a solvent mixture, with each of concentration 2000 μg/ml in (acetone:toluene). The internal standard used was pentachloronitrobenzene (99%) from Aldrich (Germany). Other solvents of residue-free purity (99.1%) purchased from Merck Co. (Germany) and used for this study were acetone, methanol, ethyl acetate, hexane, dichloromethane, sodium chloride and sodium sulfate. Milli-Q water purification system from Millipore (Bedford, MA, USA) was used to prepare all aqueous solutions. A stock solution containing the mixed analytes (20 mg/mL) was prepared by dissolving 0.5 mL of standard solution in a 50 mL volumetric flask with acetone:toluene (1:1) vol/vol and stored at -4ºC.

Sampling

Wastewater sludge samples were collected from three wastewater treatment plants (WWTPs) operating with a conventional activated sludge process – all located in the Amathole District municipality in Eastern Cape, South Africa. The sampling map and locations of the three plants are shown in Fig. 1. The samples analyzed were of urban and agricultural origins with animal rearing, citrus orchard and other crop-farming activities and animal-rearing origin. Sampling was carried out in spring 2015 (October and November), summer 2016 (January and February), autumn 2016 (April and May) and winter 2016 (July and August) in order to reflect the seasonal variability in sludge contaminant concentrations as much as possible. In each

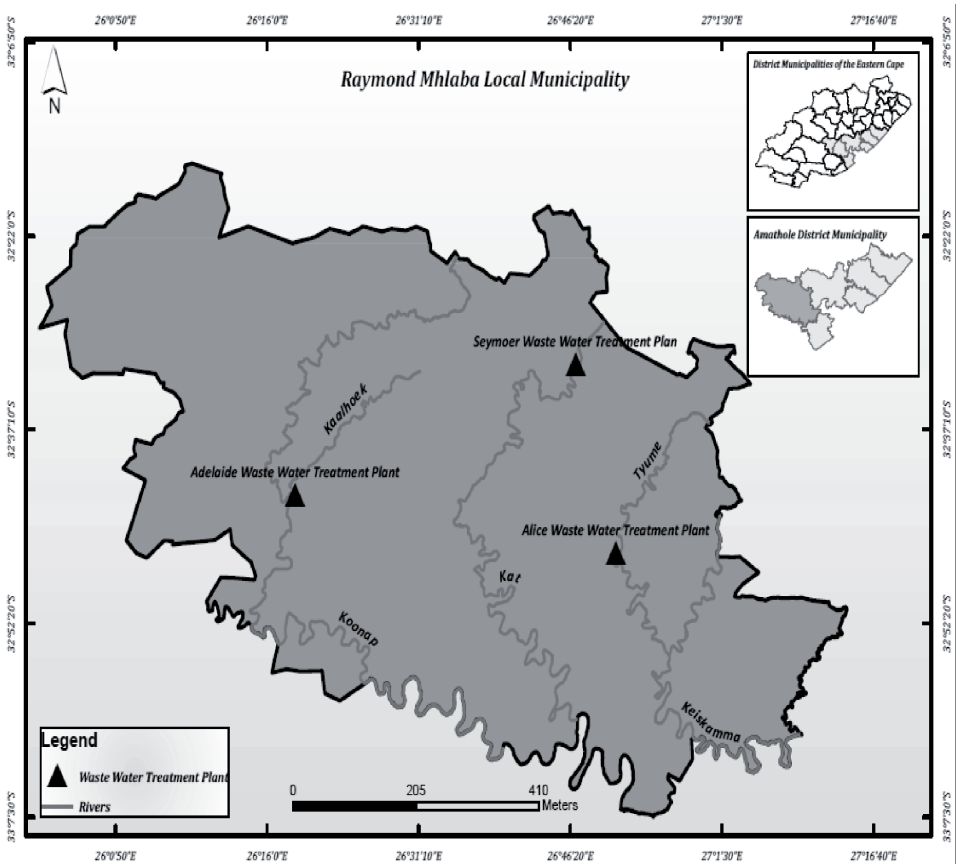

Fig. 1. Map of the sampling sites with the representation of the sampling points of the WWTPs and surrounding rivers in Eastern Cape, South Africa.

season, grab samples of sludge (n = 3) were collected from the WWTPs twice monthly over a period of two months into 2 L wide-mouth bottles from the dewatered activated sludge cake, which is the final residue of wastewater treatment processes. The sludge samples were composited in a stainless steel bucket, transported in a cooler box with ice to the laboratory and stored in a refrigerator at 4ºC until they were analysed. The collected sludge samples were dried at room temperature, ground to homogenize and maintained at -4ºC prior to extraction.

Extraction Procedure

The extraction of OCPs in the sewage sludge was carried out as follows. Weight 2 g of the dried sludge was extracted with 20 mL dichloromethane-acetone (1:1 v/v) for 30 min using ultrasonication and performed twice. The solvent extracts were pooled together and the activated Cu was added to remove element sulfate, then follow by dehydrated with anhydrous sodium sulfate. A rotary evaporator was further used to concentrated the extracts to about 1-2 mL, and later placed in a glass column (12 mm i.d.) loaded with 10 g activated Florisil (60–100 mesh) for purification, and subsequently eluated with 10 mL hexane containing 10% acetone (v/v). The eluate was further concentrated to 0.1 mL under a gentle stream of pure nitrogen and injected into GC analysis.

GC/μECD Analysis

The OCP was determined using GC/μ-ECD (Agilent Technologies, CA, USA) equipped with a double-detector FID and GC/μ-ECD. 1 μL of the purified sample extracts were injected into a fused-silica capillary column (30 m × 0.32 mm id) coated with 0.25 μm chemically bonded HP-5 phase. 99.9% purity of helium gas was used as the carrier gas at a linear velocity of 2.4 mL /min and auxiliary gas (N_2) at a linear velocity of 40 mL min^{-1}. The oven was held at 100ºC for 1 min, and ramped up at 20ºC per min to 180ºC, at 5ºC/min to 270ºC, and at 20ºC/min to 320ºC. The detector was operated at 320ºC and injector temperature at 250ºC.

Results and Discussion

Method Validation

To perform the quality assurance step a recovery study of the analytes was carry out under the same conditions as those expressed for the samples. The sludge samples were spiked and the recovery

Table 1. Mean recoveries (%) and standard deviation (n = 3) and repeatability of the target compounds in spiked water and sludge samples.

Congeners	Recoveries of the studied OCPs in sewage sludge			Repeatability RSD (%) n = 6
	50 ng/g	100 ng/g	200 ng/g	
α-BHC	102±3.4	105.1±3.9	99.6±4.1	5.7
β-BHC	99.5±5.4	90.2 ±2.8	88.4±3.1	8.9
γ-BHC	93.2±4.8	97.6±2.9	87.6±4.1	5.2
δ-BHC	88.5±3.1	93.5±3.5	96.8±5.1	3.5
HEPTACHLOR	104.5±2.9	101.2±2.8	93.6±3.8	7.8
ALDRIN	98.5±3.9	88.9±3.1	93.5±3.9	9.6
HEPTACHLOR EPOXIDE	88.5±1.9	81.6±4.2	85.2±4.1	5.2
ENDOSULFAN 1	91.5±3.8	95.8±5.3	99.1±4.9	4.9
4,4 –DDE	107.4±3.7	103±4.4	98.5±2.8	7.8
DIELDRIN	83.6±4.1	86.5±3.5	93.6±4.4	6.5
ENDRIN	88.2±2.5	84.6±3.3	91.2±3.1	6.1
4, 4, DDD	102.4±4.2	105±5.1	95.6±4.7	5.9
ENDOSULFAN 11	82.5±3.1	86.5±3.2	89.9±2.6	5.2
4, 4, DDT	80.1±3.9	85.4±2.1	88.5±2.2	4.1
ENRIN AIDEHYDE	95.7±1.8	91.7±2.8	86.8±2.1	3.9
ENDOSULFAN SULFATE	92.6±1.7	97.5±3.2	94.3±2.6	6.7
METHOXYCHLOR	97.5±2.3	88.5±3.6	90.5±3.8	8.5

determined from the concentration differences between the spiked samples and the unspiked samples with 3 replicate analyses. Extractions were carried out at three fortification levels: 50, 100 and 200 ng/g. The spiked sludge was allowed to be kept for 14 h to enable the spiked compounds to assimilate with the sample matrix. To limit the effects of enzymatic activities and microbial biotransformation it was kept in a refrigerator during this period. To measure the reproducibility/ precision of the method, sample analyses and recovery study were carried out in triplicate. Table 1 shows the mean recovery values with the relative standard deviations. For every batch of sample analysis, blank analyses were also carried out along with measuring the likely influences from external sources during analysis. The method linearity was studied in the range 5-320 µg/L. Calibration curves showed satisfactory correlation coefficients (greater than 0.99). To determine the repeatability of the chromatograph, this was performed by injecting 7 times a standard solution of 0.4 µg/mL with an automatic injector (Fig. 2). The peak areas obtained values ranging from 0.40 to 4.04%, whereas the relative standard deviations (RSD) generated for the retention times ranged from 0.01 to 0.03% (Table 2). For determining the chromatographic within-laboratory reproducibility, this was estimated during different days during two repeated weeks and was found to be lower than 10%, expressed as RSD, for all OCPs. Results are shown in Table 1. In all the fortification levels, recoveries were in the range of 80.1-107.4%, whereas RSD values ranged from 1.7 to 5.4%, thus satisfying the requirements of the European Union Guideline [20]. The values obtained are comparable to the recoveries reported by other authors using supercritical fluid extraction [21], pressurized liquid extraction [22] or Soxhlet extraction [23-26] for the analysis of OCPs in environmental samples.

Limits of Detection (LOD) and Quantification (LOQ)

Limits of detection (LODs) and quantification (LOQs) measured as the least amount of target analyte that yields a chromatogram peak with a signal-to-noise ratio of three and ten times the background chromatographic noise, respectively, were determined using extracts from blank samples. Low limits were obtained due to the selectivity of the analytical procedure and the high sensitivity of GC–µECD, permitting the determination of OCPs at the levels found in sludge samples. The LODs and LOQs corresponding to the different OCPs are shown in Table 2. These values range from 0.04 to 0.49 ng/g for LODs and from 0.22 to 2.17 ng/g for LOQs and are in the lower end of those reported by other authors [24, 27-30].

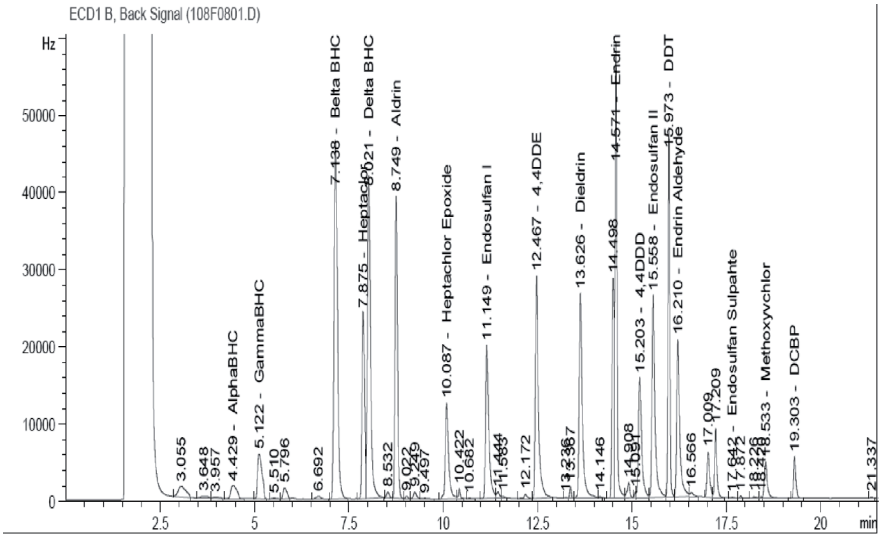

Fig. 2. Chromatogram of 0.4 µg/mL injected seven times for repeatability.

Concentration of OCPs in Sewage Sludge Samples

Sludge samples collected from three municipal wastewater treatment plants (MWWTPs) in the Eastern Cape Province located in urban and rural sites, were analyzed with a validated method. The concentrations of OCPs found in sewage sludge are expressed as ng/g dry weight. Table 3 shows the highest concentration of OCPs expressed as the summation of the 17 compounds found in WWTP1 (947 ng/g) and the lowest concentration in WWTP3 (191 ng/g). WWTP1 is a rural area where a lot of farming activities are carried out, especially citrus orchards, therefore the agricultural activities may be the reason for the high concentration in this site. The lowest concentration was found in an urban area with fewer farming activities around the treatment plant. Total OCP concentrations are higher than the values reported by other researchers [18, 31], although a similar range of values was found in an

Table 2. Calibration data and repeatability of the studied OCPs.

Congeners	Calibration data equation	R^2	RSD (t_r)	LOD ng/g	LOQ ng/g	Area RSD%
α-BHC	y = 13833x-4389	0.9951	0.01	0.04	0.28	2.45
β-BHC	y = 99379x-15367	0.9928	0.01	0.28	0.31	1.56
γ-BHC	y = 64859x – 46461	0.9956	0.02	0.08	1.19	1.36
δ-BHC	y = 325750x - 194418	0.9948	0.02	0.05	2.17	1.87
HEPTACHLOR	y = 303236x-29840	0.9949	0.01	0.31	0.32	0.89
HEP EPOXIDE	y = 40636x-4826	0.9944	0.03	0.15	2.11	4.04
ALDRIN	y = 107562x – 13109	0.9938	0.01	0.21	0.22	1.42
ENDOSULFAN	y = 192696x – 91905	0.9962	0.02	0.33	1.31	0.40
4,4 –DDE	y = 334989x - 254724	0.9958	0.02	0.48	0.38	0.55
DIELDRIN	y = 155561x – 1507	0.9937	0.01	0.43	0.54	1.79
ENDRIN	y = 326806x - 235886	0.9969	0.03	0.09	1.53	1.11
4, 4, DDD	y = 119133x - 126127	0.9969	0.02	0.21	0.28	1.50
ENDOSULFAN 11	y = 351175x - 348939	0.9945	0.03	0.45	1.21	3.12
4, 4, DDT	y = 32161x – 2506	0.9978	0.03	0.31	0.33	3.08
ENRIN AIDEHYDE	y = 58541x – 65935	0.9991	0.02	0.35	0.27	1.55
ENDOSULFAN SULFATE	y = 14084x – 9954	0.9994	0.02	0.49	1.31	1.83
METHOXYCHLOR	y = 8430x – 9942	0.9924	0.01	0.38	1.38	3.41

Table 3. Concentrations of OCPs (ng/g) in sewage sludge collected in three activated sludge WWTPs for a whole year.

Congeners	WWTP1				WWTP2				WWTP3				Σ congeners
	OCT-NOV	JAN-FEB	APR-MAY	JY-AUG	OCT-NOV	JAN-FEB	APR-MAY	JY-AUG	OCT-NOV	JAN-FEB	APR-MAY	JY-AUG	
α-BHC	160	98	158	131	115	32	68	78	98	43	48	66	1095
β-BHC	12	n.d.	n.d.	8	34	12	15	41	n.d.	n.d.	n.d.	n.d.	122
γ-BHC	270	23	150	76	105	66	87	77	77	n.d.	42	21	994
δ-BHC	15	9	12	11	9	11	13	17	n.d.	n.d.	n.d.	n.d.	97
HEPTA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
ALDRIN	121	67	88	107	141	120	105	110	58	23	25	33	998
HEP EPO	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
END 1	99	51	88	66	40	49	62	72	12	n.d.	n.d.	8	547
4,4 –DDE	34	45	32	41	33	23	43	21	32	12	23	29	368
DIELDRIN	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
ENDRIN	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
4, 4, DDD	108	77	108	132	145	133	139	169	99	56	89	75	1330
END 11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
4, 4, DDT	128	220	165	131	150	101	144	120	108	57	89	99	1512
ENRIN AIDE	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
END SUL	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
METHO	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
Σ 17 OCPs	947	590	801	703	772	547	676	705	484	191	316	331	

Results are the mean of four samplings, n.d.: < method detection limit.

urban sludge as published by [7, 32-35]. In our study, α-BHC, Aldrin, DDE, DDT and DDD are the five most ubiquitous compounds with frequency of detection of 100%. Other compounds that were detected with their percentage frequency are β-BHC (50%), γ-BHC (92%), δ-BHC (67%), and endosulphate I (84%). Conversely, other organochlorine pesticides (including heptachlor, heptachlor epoxide, dieldrin, endrin, endosulphate- II, endrin aldehyde, endosulfan sulphate, and methoxychor) were not detectable. These results are consistent with those of related studies on sewage sludge [32-35]. The highest concentrations of DDT, DDD and DDE detected ranged from 12 to 220 ng/g. For both the α-BHC and the aldrin concentrations in the sewage sludge, the levels varied from 32-160 ng/g and 23–121 ng/g respectively. For β, γ, and δ-BHC, concentrations were in the range of 9-270 ng/g and endosulphate I was detected in concentrations ranging from 8 to 99 ng/g (Table 3). The use of OCPs has been banned in many countries, including South Africa, where DDT is still used by government official for malaria vector control in some parts of the country, and DDT sticks strongly to sludge particles and does not move quickly to wastewater [8-9]. These groups of OCPs may still be used secretly under an unknown trade name in crop farming due to their low cost and effectiveness for pest control [36-39]. Among the six predominant congeners, the total highest concentration levels were found in the order: ΣDDT, ΣDDD, Σα-BHC, Σγ-BHC, Σaldrin and Σendosulfate 1 with values 1512, 1330, 1095, 998, 994 and 547 ng/g respectively (Table 3). The European commission sludge guideline to promote the use of sewage sludge in agriculture with limit values set for OCPs with a value of 1,000 ng/g was established as the highest contaminant limit of ΣDDTs, and 500 ng/g for other pesticides. The results showed that the six predominant congeners exceed the set limit. Other authors reported similar values and higher values of total congeners in other countries of the world [11, 31].

Conclusions

This work presents a validated, simple and rapid method for simultaneous quantification of 17 compounds of organochlorine pesticides in sludge samples. A good validation of method with good recovery of the congeners in the sludge matrixes was obtained. The present study

assessed the concentrations and distribution levels of OCPs in sewage sludge samples from three WWTPs in the Amathole District, Eastern Cape, South Africa, and added to the scarce knowledge of their environmental distribution in sludge from South Africa. Due to a variety of potential inputs from several sources, OCP contaminants were ubiquitously distributed in the sludge samples investigated. Total OCP (17 compounds) concentrations ranged from 191 to 947 ng/g, which were higher than the proposed EU limit. In relation to similar studies on sewage sludge worldwide, contamination levels of OCPs were at comparable levels with those reported for other WWTPs. DDT is the congener with highest concentration throughout the year. Hence, the OCP concentrations in sludge in South Africa were generally higher than for some EU countries, which are believed to be linked to the protracted use of DDTs in South Africa. It is concluded that WWTPs remain potential sources of toxic contaminants to the local environment. Therefore, stringent control measures should be considered for protecting or restoring sewage sludge quality. As the current study is not representative of sewage sludge production in South Africa, there is a need to implement research and monitoring program priorities for OCPs and other emerging pollutants in sewage sludge.

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Conflict of Interest

The authors wish to declare that there is no conflict of interest regarding the publication of this manuscript.

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