Analytical method development for the uptake study of different organic pollutants by crops cultivated in compost-amended soils

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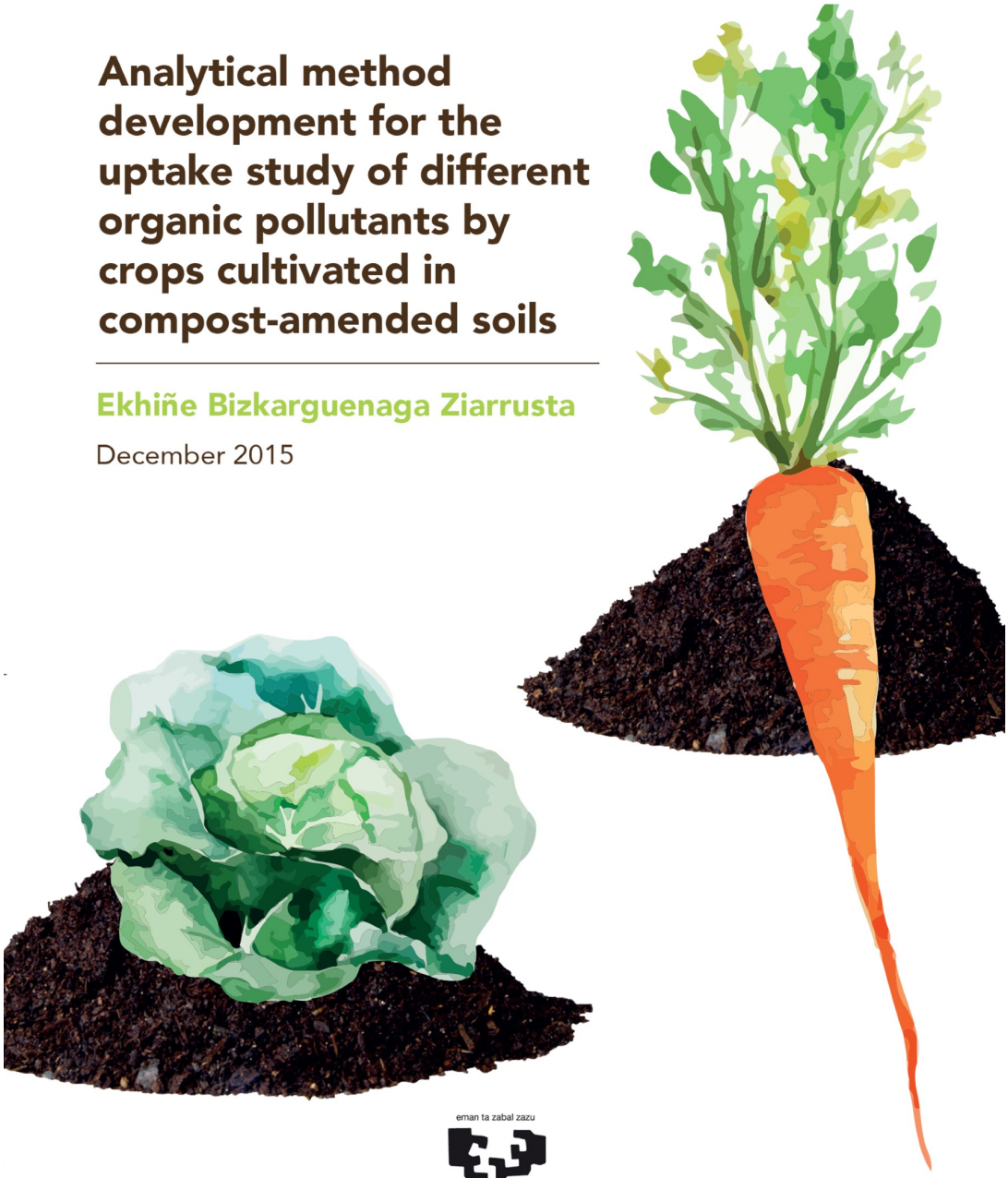
December 2015



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Analytical method development for the uptake study of different organic pollutants by crops cultivated in compost-amended soils

A thesis submitted for the international degree of
Doctor of Philosophy

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Etapa berri bati ekin aurretik lan honi bukaera ematea falta den une honetan, 4 urte hauetan zehar ezagutu, lagundu eta une orotan ondoan izan ditudan guztiei eskerrik beroenak emateko ordua iritsi da.

Lehenik eta behin, Olatzi eta Ailetteri, denbora guzti honetan zehar egin duzuen lan guztiagatik, kendu dizkizuedan lo ordu guzti horiengatik. Zuen laguntza, aholku eta gidaritza gabe lan hau ez zen posible izango. Bene-benetan eskerrik handiena aukera hau eman izanagatik. Ailette, espero que después de esta etapa llegue una época más relajada aunque conociéndote no se yo si el huracán del Caribe, como Nestor te nombró en su día, pueda calmarse. Luis Angel muchas gracias por darme la oportunidad de tomar parte en el proyecto y por los buenos momentos que hemos pasado en las excursiones realizadas al monte.

Aresatz eta Asier, nire hasierako pausuak zuen gidaritzapean eman nituen, zuoi ere eskerrik asko. Nestorri zalantzen aurrean beti laguntzeko prest agertu izanagatik. Eskerrak baita Kepa, Dani, Irantzu, Marian, Gorka, Alberto, Silvia, Juanma, momenturen baten lagungarri izan baitzarete. Mirari beti umore onez egoteagatik.

Azucena gracias por tu ayuda y consejos que nos has dado a la hora de realizar los experimentos de "uptake", y Txesko por tu tiempo invertido en el análisis de muestras realizadas en los servicios generales.

Laborategian elkarlanean ordu asko pasa ditudan Arantza eta Itsasori. Zenbat ordu eta buruhauste Manolito gobernatu nahian... batzutan pozik, bestetan triste. Patiri Manolitorekin, laborategian eta ostean eskaini didan laguntzagatik, baita tesi honen atal desberdinetan zuzenean parte hartu duten Leire M., Josu eta Eliri, tesi hau zuen lanaren fruitu ere baita.

Maitane eta Mireri, beti laguntzeko prest egon izanagatik, ez dakit nondik ateratzen dozuen denbora baina zuena pasada bat da!!! Batak tesi irakurketan besteak portadako azken ukituetan zer gehiago eska dezaket? Negar eta barre zenbat gauza ein doguzen batera. Mireia ondino Leunganeko paseoa ein barik dekogu!!!

Tesiaren irakurketa gero eta hurbilago duzuen guztioi (Azibar, Oier, Oscar, Ane, Mire, Oihana, Naiara, Unai eta Olaiari) animorik handienak.

Naiara eta Mireiari Stockholmen egindako egonaldian pasa genituen egun zoragarriengatik. Kirunararte joan eta "northern light"ak ikusteko aukerarik eduki izan ez arren, gogoratzen zarete egin genuen mendi bueltaz? Eta saunan bizitako esperientziaz? eztot aiztuko.

Joana, agradeço-te os bons momentos que passamos juntas, tanto no seminário como fora. Lembro-me da viagem que fiz a Pau e quando fui ver o teu exame de Kenpo ao Porto e tive oportunidade de conhecer a tua família. Também me recordo dos dias que fomos correr depois do trabalho nos passeios atrás da Universidade, da visita que me fizeste quando estive em Estocolmo e das aulas de yoga que nos deste tanto à Mireia como a mim. Ainda me lembro de tentar fazer o corvo no corredor Por todos os conselhos que me deste, tanto pessoais como profissionais. Muito obrigada por tudo isso e por te ter preocupado também nesta última etapa.

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Peio, Maya eta Linneari, Stockholmen burututako hiru hilabeteetan nire familia izan zinetelako. Linnea (Pernile) eta Maya zuei bioi hartu zenidaten moduagatik. Oraindik gogoratzen dut nola lehen egunean kartetako jolas bat erakusteko gai izan zineten nik suediera ulertu gabe. Egin zenituzten postre eta jaki zoragarri haiengatik. Oraindik gogoan dut izozki eta txokolatez beterik kopa...

Mintegian sartu nintzenetik egun arte ezagutzeko aukera izan dudan guztie: Josean Julen, Oskar, Arri, Jone, Ibone eta Ainara, nolako giroa zeneukaten gu sartu ginenean. Zenbat txokolate eta tertulia egin ditugun bertan. Umorea ez dadila falta hemendik aurrera ere. Keinu bat baita Zamudioko guztiei.

Laura, Olaia eta Leire K. zenbat zigarro erre eta horren harira ideia-partekatze burutu ditugun Vía Juliako sarreran. Espero egunen batean ohitura hori kentzea. Ez ideiak partekatzekoa baizik eta zigarroarena!!!

Oin sartu berri diren Iratxe, Asier, Nahia eta Leireri, ezagututeko astirik euki ez arren pazientzi eta animo guztiegaz ekiteko aholkatutendotsuet.

Eskerrik asko kuadrilako guztioi, beraien animoengatik denporaldi honetan. Hau amaitu ta pendiente dekogun bazkari edo afarie prestau behakot. Badekodaz gogoak aspaldiko

partez lasai lasai egoteko tertulien. Eider zuri eskerrak portadako ideia eta burututako lanagaitik.

Haizea zuri be esker piloa!!! Zenbet ordu pasau doguzan Dimeko liburutegien. Ziortzak dinoen moduen laster gure izena ipinikotsie!!! Eskerrik asko emondako laguntza guztiegatik. Batzutan barrea jatorta. Akordetan zara letra aukeratuten eta pruebak iten ibili ginen egune? Autoan bidean pasau doguzen orduekaitik, inglesari bildurre kentzean laguntearren. Watsapa esaldi, eta mile aholku eta aukeraz beteta dekot. Hemetik aurrera be beharko dot zure laguntza aurkezpenean. Gure txokoan inguz ensailuek!!!

Amama ta Marijo nik ja laster amaitan dot, hemetik aurrera in ahalko dodaz zuek dinozun moduen siestak!!! Haizea geratan da erreleboagaz.

Eta eskerrik handienak eta bihotz-bihotzetik, ama eta aiteri mundura ekarri joztienetik ondoan egotearren onean zein txarrean. Zenbet gorabehera euki doguzan, eta beti hor laguntzako prest. Nahiz ta sarriten diskutidu, hori ona dala usteot. Ia hementik aurrera etxean lan gehiau eiteko astie ataraten doten. Aite zugez pendiente dekoten mendire bueltea be ia posible dan aurtengo uden eitea. Hiru Erregeen Mahaia itxoten deku. Umore kontuetan klabea izan diren etxeko hiru txikiei Ane, Aimar eta Maitane ze ingo leuke izekok zuok hirurok barik? Egun txar bat dekodala, bisita bat ein ta soluzionaute. Zelan detaile txiki batek hainbeste eragin?

"Eerrauskin" garaitik oinarte aguantau dozun guztiagatik. L8, L1ko ekipoak eta fitotroneko erregadio sistema kontrolpean dekozuz jada. Mintegiko jendea be aurrez ezagutu ez arren seguruenik ikustean nor dan nor jakingozu. Zenbat poz, diskusino eta istorio partekatu doguzen eta ondino partekatu behoguzenak. Hemendik aurrera oinarte moduen edo hobeto joan daitela dana. Gauzen garrantzie baloretan erakutsi izanagatik. Ez deko zentzurik aldatu ezin dan zeozerri bueltak emoten ibilteak, saiatu soluzinoak topetan!!! Zure esaldi famatue.

Zuok guztiok emondako gauza txiki guzti ohiengatik ez balitz, hau guztia ez litzateke posible izango.

Diman Urriak 30

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LIST OF ACRONYMS

1-MP	1-methylpyperidine
10:2 FTCA	2-Perfluorodecyl ethanoic acid
10:2 FTUCA	2H-Perfluoro-2-dodecenoic acid
10:2 monoPAP	1H, 1H, 2H, 2H-perfluorododecylphosphate
2-MeO-BDE-68	2′,3,4′,5-tetrabromo-2-methoxydiphenyl ether
2H-PFOA	2H-perfluorooctanoate
3-OH-7:3-FTCA	3-hydroxy-7:3 saturated fluorotelomer carboxylate
3-OH-BDE-47	3-hydroxy- 2,2′,4,4′-tetrabromodiphenyl ether
3′-OH-BDE-28	3′-hydroxy-2,4,4′-tribromodiphenyl ether
4-MeO-BDE-103	2,2′,4′,5,6′-pentabromo-4-methoxydiphenyl ether
4-MeO-BDE-49	2,2′,4′,5-tetrabromo-4- methoxydiphenyl ether
4-nOP	4-n-octylphenol
4-NP	4-nonylphenol
4-OH-BDE-42	4-hydroxy-2,2′,3,4′- tetrabromodiphenyl ether
4-tOP	4-tert-octylphenol
4′-MeO-BDE-101	2,2′,4,5,5′-pentabromo-4'-methoxydiphenyl ether
4′-OH-BDE-49	4′-hydroxy-2,2′,4,5′-tetrabromodiphenyl ether
5-MeO-BDE-100	2,2′,4,4′,6′-pentabromo-5-methoxydiphenyl ether
5-MeO-BDE-47	2,2′,4,4′-tetrabromo-5-methoxydiphenyl ether
5-OH-BDE-47	5-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether
5:3 FTCA	3-perfluoropentyl propanoic acid
5′-MeO-BDE-99	2,2′,4,4′,5-pentabromo-5'-methoxydiphenyl ether
5′-OH-BDE-99	5′-hydroxy-2,2′,4,4′,5-penta- bromodiphenyl ether
6′-OH-BDE-99	6'-hydroxy- 2,2′,4,4′,5-pentabromodiphenyl ether
6-MeO-BDE-47	2,2′,4,4′- tetrabromo-6-methoxydiphenyl ether
6:2 diPAP	bis(1H, 1H, 2H, 2H-perfluorooctyl)phosphate
7:2 FT ketone	7:2 fluorotelomer ketone
7:2 sFTOH	7:2 secondary fluorotelomer alcohol
7:3 FTCA	3-perfluoroheptyl propanoic acid
8:2 diPAP	bis(1H, 1H, 2H, 2H-perfluorodecyl)phosphate
8:2 FTAL	8:2 fluorotelomer aldehyde
8:2 FTCA	2-perfluorooctyl ethanoic acid

8:2 FTUCA	2H-perfluoro-2-decenoic acid
8:2 monoPAP	1H, 1H, 2H, 2H-perfluorodecylphosphate
AA	acetic anhydride
ACN	acetonitrile
ADBI	celestolide
AHMI	phantolide
AHTN	tonalide
ANOVA	analysis of variance
AOX	halogenated organic compounds
AP	alkylphenol
APCI	atmospheric pressure chemical ionisation
APEO	alkylphenol ethoxylate
APFO	perfluorooctanoic acid ammonium salt
APPI	atmospheric pressure photoionisation
ASE	accelerated solvent extraction
ATII	traseolide
ATP	adenosine triphosphate
BCF	bioconcentration factor
BDE-100	2,2',4,4',5-pentabromodiphenyl ether
BDE-138	2,2',3,4,4',5'-hexabromodiphenyl ether
BDE-153	2,2',4,4',5,5'-hexabromodiphenyl ether
BDE-154	2,2',4,4',5,6'-hexabromodiphenyl ether
BDE-181	2,2´,3,4,4´,5,6-heptabromodiphenyl ether
BDE-183	2,2',3,4,4',5',6-heptabromodiphenyl ether
BDE-197	2,2´,3,3´,4,4´,6,6´- octabromodiphenyl ether
BDE-209	decabromodiphenyl ether
BDE-28	2,4,4'-tribromodiphenyl ether
BDE-47	2,2',4,4'-tetrabromodiphenyl ether
BDE-66	2,3',4,4'-tetrabromodiphenyl ether
BDE-77	3,3´,4,4´-tetrabromodiphenyl ether
BDE-85	2,2',4,4',6-pentabromodiphenyl ether
BDE-99	2,2',3,4,4'-pentabromodiphenyl ether
BFR	brominated flame retardant

BOD	biochemical oxygen demand
BPA	bisphenol A
BPB	bisphenol B
BSTFA	N,O-bis-(trimethylsilyl)trifluoroacetamide
C_{18}	octadecyl
CB-101	2,2',4,5,5'-pentachlorobiphenyl
CB-118	2,3',4,4',5-pentachlorobiphenyl
CB-138	2,2',3,4,4',5'-hexachlorobiphenyl
CB-153	2,2',4,4',5,5'-hexachlorobiphenyl
CB-180	2,2',3,4,4',5,5'-heptachlorobiphenyl
CB-28	2,4,4'-trichlorobiphenyl
CB-52	2,2',5,5'-tetrachlorobiphenyl
CBZ	carbamazepine
CCD	central composite design
CCL	contaminant candidate list
CEC	contaminants of emerging concern
CFA	clofibric acid
CI	chemical ionisation
CIS	continuous injection system
CRM	certified reference material
DBDPE	decabromodiphenylethane
DCM	dichloromethane
DEHP	di(2-ethylhexyl)phthalate
DES	diethylstilbestrol
DIP	diphenhyramine
DLLME	dispersive liquid-liquid microextraction
DOC	dissolved organic carbon
DPMI	cashmeran
dSPE	dispersive solid phase extraction
E1	estrone
E2	17ß-estradiol
E3	estriol
EDC	endocrine disrupting compound

EE2	17-α-ethinylestradiol
EFSA	european food safety authority
EI	electron impact
EPA	environmental protection agency
EQS	environmental quality standard
ESI	eletrospray ionisation
EtOAc	ethyl acetate
EU	European Union
eV	electron volt
FFD	fractionated factorial design
FOSA	Perfluorooctane sulfonamide
FOSAA	perfluorooctanesulfonamido acetate
FTOH	fluorothelomer alcohols
FUSLE	focused ultrasound solid-liquid extraction
GC	gas chromatography
GC-(NCI)-MS	gas chromatography-chemical ionisation-mass spectrometry
GC-EI-IT-MS	gas chromatography-electron impact-ion trap-mass spectrometry
GC-EI-IT-MS/MS	gas chromatography-electron impact-ion trap-tandem mass spectrometry
GC-EI-MS	gas chromatography-electron impact-mass spectrometry
GC-EI-MS/MS	gas chromatography-electron impact-tandem mass spectrometry
GC-Ion Trap	gas chromatography-ion trap
GC-IT-MS/MS	gas chromatography-ion trap-tandem mass spectrometry
GC-LRMS/MS	gas chromatography-low resolution tandem mass spectrometry
GC-MS	gas chromatography-mass spectrometry
GC-MS/MS	gas chromatography-tandem mass spectrometry
GC-NCI-MS	gas chromatography-negative chemical ionisation-mass spectrometry
GC-TOF-MS	gas chromatography time-of-flight mass spectrometry
GC-μECD	gas chromatograph with microelectron-capture detection
GPC	gel permeation chromatography
HAc	acetic acid
HBB	hexabromobenzene
HBCD	hexabromocyclodecane

HBCDD:	hexabromocyclododecanes
HHCB	galaxolide
HPLC	highperformance liquid chromatography
HRGC-IT-MS/MS	high resolution-ion trap-tandem mass spectrometry
HRGC/HRMS	high resolution gas chromatography coupled with high-resolution mass spectrometry
HS	headspace
HS-SPME	headspace solid-phase microextraction
HSSE	headspace stir-bar sorptive extraction
IBF	ibuprofen
K_{ow}	octanol-water partition coefficient
LAS	linear alkylbenzene sulphonates
LC	liquid chromatography
LC-APCI-MS/MS	liquid chromatography-atmospheric chemical ionisation-tandem mass spectrometry
LC-DAD	liquid chromatography-diode array detector
LC-ESI-MS	liquid chromatography-eletrospray ionisation-mass spectrometry
LC-ESI-MS/MS (QqQ)	liquid chromatography-eletrospray ionisation-tandem mass spectrometry
LC-ESI-QqLIT-MS-MS	liquid chromatography-electrospray tandem triple quadrupole-linear ion trap mass spectrometry
LC-MS	liquid chromatography coupled to single mass spectrometry
LC-MS/MS	liquid chromatography coupled to tandem mass spectrometry
LC-QqQ-MS/MS	liquid chromatography coupled to triple quadrupole tandem mass spectrometry
LC-UV	liquid chromatography coupled with ultraviolet spectroscopy
LD	liquid desorption
LOD	limit of detection
LOQ	limit of quantification
LVI	large volume injection
LVI-PTV	large volume injection in a programmable temperature vaporiser
LVI-PTV-GC-MS	large volume injection in a programmable temperature vaporiser coupled to gas chromatography-mass spectrometry
m-MISPE	membrane-based molecularly imprinted solid phase extraction
MA	musk ambrette
MA-HS-SPME	microwave assisted headspace solid-phase microextraction

MAE	microwave assisted extraction
MDL	method detection limit
MeO-PBDE	methoxylated polybrominated diphenyl ether
MeOH	methanol
MeTCS	methyltriclosan
MIP	molecular imprinted polymer
MK	musk ketone
MLOD	method detection limit
MQ	Milli-Q
MQL	method quantification limit
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MSPD	matrix solid phase dispersion
MSPD-GC-NCI-MS	matrix solid phase dispersion phase-gas chromatography negative chemical ionisation mass spectrometry
MSTFA	N-methyl-N-(trimethylsilyl)trifluoroacetamide
MTBE	methyl tert-butyl ether
MX	musk xylene
N-EtFOSAA	2-(N-ethylperfluorooctanesulfonamido) acetic acid
N-MeFOSAA	2-(N-methylperfluorooctanesulfonamido) acetic acid
NCI	negative chemical ionisation
NCI-MS	negative chemical ionisation-mass spectometry
NH_4OAc	ammonium acetate
NH_4OH	ammonium hydroxide
NORMAN	network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances
NP	nonylphenol
NPEO	nonylphenol ethoxylate
NPX	naproxen
NT	norethistherone
OCP	organochlorine pesticide
OECD	organisation for economic co-operation and development
OH-PBDE	hydroxilate polybrominated diphenyl ether
OP	octylphenol

OPP	organophosphorous pesticides
OT	organotins
PA	polyamide
PAH	polycyclic aromatic hydrocarbon
PAP	perfluoroalkyl phosphate
PBB	polybrominated biphenyls
PBDE	polybrominated diphenyl ether
PBEB	pentabromoethylbenzene
PC	principal component
PCA	principal component analysis
PCB	polychlorinated biphenyl
PCDD/F	polychlorinated dibenzo-p-dioxins and furans
PCN	chlorinated napthalenes
PDMS	polydimethylsiloxane
PE	phthalate esters
PES	polyethersulfone
PFAA	perfluoro alkyl acid
PFAS	per- and polyfluorinated alkyl substance
PFBA	perfluorobutanoic acid
PFBS	perfluorobutanesulfonate acid
PFC	perfluorinated compounds
PFCA	perfluoroalkyl carboxylic acids
PFDA	perfluorodecanoic acid
PFDoDA	perfluorododecanoic acid
PFDS	perfluorodecanesulfonate acid
PFHpA	perfluoroheptanoic acid
PFHxA	perfluorohexanoic acid
PFHxDA	perfluorohexanedecanoic acid
PFHxS	perfluorohexasulfonate acid
PFNA	pefluorononanoic acid
PFOA	perfluorooctanoic acid
PFODA	perfluorooctadecanoic acid
PFOS	perfluorooctane sulfonic acid

PFPA	perfluorinated phosphonic acid
PFPeA	perfluoropentanoic acid
PFSA	perfluorinated sulphonamides
PFSA	Perfluoro alkane sulfonic acid
PFTeDA	perfluorotetradecanoic acid
PFTrDA	perfluorotridecanoic acid
PFUnDA	perfluoroundecanoic acid
PG	progesterone
PHS	priority hazardous substance
PLE	presurised liquid extraction
PLE-GPC-GC-MS	pressurized liquid extraction-gel permeation chromatography-gas chromatography-mass spectrometry
PLE-IL-HS-SDME	pressurised liquid extraction coupled to automated ionic liquid-based head space single-drop microextraction
PLE-MEP	microextraction by packed sorbent following selective pressurised liquid extraction
POM	polyoxymethylene
POP	persistent organic pollutant
POSF	perfluoroctane sulfonil fluoride
PP	polypropylene
PPCP	pharmaceutical and personal care products
PS	polysstyrene
PSA	primary and secondary amine
PTFE	polytetrafluoroethylene
PTV	programmable temperature vaporiser
PUF	polyurethane foams
QqQ	triple quadrupole
QuEChERS	quick easy cheap effective rugged safe
r^2	determination coefficient
RSDs	relative standard deviations
SBSE	stir-bar sorptive extraction
SBSE-TD-GC-MS	stir-bar sorptive extraction-thermal desorption-gas chromatography-mass spectrometry
SC	Stockholm convention
SIM	selected ion-monitoring

SLE	solid liquid extraction
SPE	solid phase extraction
SPLE	selective pressurised liquid extraction
SPME	solid phase microextraction
SR	silicone rod
SRM	selected reaction monitoring
TBA	tetrabutylammonium
TBABr	tetra-n-butylammonium bromide
$TBAHSO_4$	tetra-n-butylammonium hydrogen sulphate
TBBPA	tetrabromobisphenol A
TBBPA-bis	tetrabromobisphenol A bis 2,3-dibromopropylether
TCBPA	tetrachlorobisphenol A
TCC	triclocarban
TCS	triclosan
TD	thermal desorption
TD-GC-MS	thermal desorption-gas chromatography-mass spectrometry
TDU	thermal desorption unit
THF	tetrahydrofurane
TMCS	trimethylchlorosilane
TMSH	trimethyl sulfonium hydroxide
TOC	total organic carbon
TT	testosterone
UAE	ultrasound assisted extraction
UCRM	unregulated contaminant monitoring rule
UHPLC	ultrahigh pressure liquid chromatography
UPLC-APCI-MS/MS	ultrahigh pressure liquid chromatography-atmospheric pressure chemical ionisation-tandem mass spectrometry
UPLC-ESI-MS/MS	ultrahigh pressure liquid chromatography-eletrospray ionisation-tandem mass spectrometry
UPLC-ESI-MS/MS (QqQ)	ultrahigh pressure liquid chromatography-eletrospray ionisation-tandem mass spectrometry
UPLC-QqTOF-MS	ultrahigh performance liquid chromatography and hybrid quadrupole time of flight mass spectrometry
UPLC-QTOF-MS	ultra performance liquid chromatography coupled to quadrupole time of flight mass spectrometry
US EPA	United States environmental protection agency

USA	United States of America
USAL-DSPE-DLLME	ultrasound-assisted leaching-dispersive solid-phase extraction followed by dispersive liquid–liquid microextraction
UV	ultraviolet
UWWTD	urban wastewater treatment directive
WAX	weak anion exchange
WFD	water framework directive
WHO	world health organisation
WWTP	wastewater treatment plant
μ-UHPLC-ESI-MS/MS	microultra high pressure liquid chromatography-eletrospray ionisation-tandem mass spectrometry

Two unavoidable residues from wastewater treatment plants are effluent water and sewage sludge. The latter, is considered one of the largest pollutant sink because it contains a broad range of microorganisms and organic and inorganic pollutants. Although, prior to its disposal, it is submitted to different chemical and physical treatments. However, due to the lack of effectiveness of these treatments in removing pollutants, concern about its use in agriculture as soil fertiliser is increasing. In this sense, it is important to study the behaviour of pollutants by plants cultivated in sewage or compost-amended soils.

Two main objectives were defined in the present PhD thesis. On the one hand, a great effort was made in the development of robust, selective, accurate, precise and sensitive analytical procedures for the determination of several organic pollutants, including polybrominated diphenyl ethers (PBDEs), perfluoroalkyl substances (PFASs), hormones, triclosan, bisphenol A (BPA), musk fragrances and alkyl phenols, in solid matrices, such as compost-amended soil, carrot and lettuce in order to achieve reliable data. For this purpose, focused ultrasound solid-liquid extraction combined with different clean-up strategies, such as traditional solid-phase extraction, dispersive solid-phase extraction (dSPE) or enrichment on cheap polymeric material were optimised.

Once the analytical methodologies were validated, the second objective was related with the uptake of some organic pollutants (PBDEs, PFASs, BPA and musk fragrances) by carrot and lettuce crops cultivated in fortified compost-amended soils.

Regarding the observed behaviour of the pollutants, it was concluded that pollutant properties, crop type and soil characteristics exert an important influence in the chemical translocation through the plant. In general, the higher the soil organic carbon content was, the lower the calculated bioconcentration factors. Concerning target analyte properties, water-solubility was the best property to describe the movement of the pollutants through the plant. The pollutants with higher water-solubility tended to accumulate more in the above part compartments such as leaves. As far as crop type is concerned, although differences between crops were observed, it was not possible to achieve a general conclusion.

Bearing in mind all the above mentioned, the application of sewage sludge or its derivatives as fertiliser in the agriculture could be a pathway for the entrance of the pollutants in the food chain and, therefore, it could be considered practise for human and environmental health.

Araztegietan bi hondakin nagusi daude: irteerako urak eta lohiak. Azken urteetan araztegietako lohiak, hainbat tratamendu fisiko zein kimiko jasan ondoren, nekazaritzan ongarri bezala erabili dira. Horrek kezka eragin du gizartean lohia mikroorganismo zein kutsatzaile organiko eta ez-organiko ugari metatzen dituen matrizea baita.

Tesi honetan bi helburu nagusi definitu dira. Alde batetik, metodologia analitikoen garapena burutzea konposatu organikoen (konposatu brominatu, PBDEak, konposatu perfluoratu, PFASak, fragantzia, hormona, triklosan, bisfenol A, BPA eta alkilfenol) determinazioa modu zehatz, doi, sentikor eta sendo batean gauzatzeko. Horretarako, ultrasoinu fokatu bidezko erauzketa optimizatu da garbiketa estrategia desberdinei (fase solidoko erauzketa, fase solido dispertsoko erauzketa teknika berriagoa edota aurrekontzentraziorako material polimeriko merkeen erabilera) lotuta.

Behin garatutako metodologia analitikoak berretsita, azenario eta uhaza barazkietan zenbait kutsatzaile organikoren (PBDEen, PFASen, BPAren eta fragantzien) akumulazioa aztertu da. Bigarren helburu hori burutzeko, barazkiak kutsatzaileekin aberastutako konpostarekin ongarritutako lurretan hazi dira.

Kutsatzaileen mugimenduak landarean zehar hainbat aldagaiekiko menpekotasuna erakutsi du; besteak beste, lurraren eta analitoen ezaugarriekiko eta landare motarekiko. Orokorrean, zenbat eta lurraren materia organikoaren kantitatea handiagoa izan, kutsatzailearen biokontzentrazio faktorea baxuagoa dela behatu da landarearen konpartimentu desberdinetan. Kutsatzailearen ezaugarriei dagokienez, analitoaren ur-disolbagarritasuna da bere mugimendua landarean zehar hoberen azaltzen duena. Zenbat eta analitoa uretan disolbagarriagoa izan, orduan eta gehiago mugitzen da landarean zehar eta kontzentrazio altuenak hostoetan determinatzen dira. Azkenik, landare motari dagokionez, nahiz eta desberdintasunak atzeman diren, ezin izan da ondorio orokorrik atera.

Aurretik aipatutako guztia kontuan izanda, araztegietako lohietan metatzen diren zenbait kutsatzaile landareetan meta daiteke, konposta nekazaritzan ongarri gisa erabiltzean. Ondorioz, gizakientzat zein animalientzat eskuragarri geratu daitezke eta beraien osasunerako arrisku bilakatu.

CHAPTER 1

Introduction

1.1 Wastewater treatment plants and priority and emerging organic contaminants

Water is a natural resource of Earth that all economic activities, including mining, agriculture or urban growth, require. For years, all the pollution generated in hundreds of human applications was directly thrown to water bodies, causing the contamination of water resources. Consequently, in the late 19th and early 20th centuries the first wastewater treatment plants (WWTPs) were designed in the developed countries. Moreover, the European Union (EU) Urban Wastewater Treatment Directive (UWWTD, CEC, 1991) was adopted in 1991 providing the main legislation for the control of urban pollution. The aim of the UWWTD was to avoid pollution of fresh and marine water and established certain minimum effluent standards in order to obtain this purpose. Besides, in 1997, the EU Commission adopted a draft proposal for a Council Directive establishing a framework for Community action in the field of water policy (the Water Framework Directive, WFD, CEC, 1999). The final agreement of the Directive was published on July 2000 (CEC, 2000) and the purpose was to establish a framework for the protection of freshwater, estuaries, coastal waters and ground waters in the EU, being the main requirement "to prevent deterioration of ecological quality and pollution of surface waters and to restore polluted surface waters to achieve good ecological and chemical status in all surface waters".

Apart from effluent water, other unavoidable residue in WWTPs is the sludge (semisolid slurry). The sludge production is growing all over the world from an annual production of some 5.5 million tonnes of dry matter in 1992 to 9 million tonnes by the end of 2005, and it is expected to increase up to 13 million tonnes for 2020. This increase is mainly due to the progressive implementation of UWWTD directive (CEC/1991), as well as the slow but constant rise in the number of households connected to sewers [1].

As the amount of sludge is increasing, the safe disposal of the sewage sludge is one of the major environmental concerns throughout the world. The highest sludge production in the EU was observed in Germany, UK, Spain, France and Italy. These countries contribute to almost the 73 % of the total sludge generated in EU [2]. In Germany, the Netherlands and the UK, more than the 90 % of the total population is connected to urban WWTPs, whilst lower percentages are observed in the Mediterranean and Eastern Europe countries. Under the UWWTD (CEC, 1991), towns, cities and settlements with population equivalents of more than 2000 inhabitants within the EU are compulsory required to collect and treat their urban wastewaters.

Conventional wastewater treatment consists of a combination of physical, chemical and biological processes to remove solids, organic matter and, sometimes, nutrients and pollutants from wastewater. As can be observed in **Figure 1.1**, treatment processes are classified as primary, secondary and tertiary (or advanced).

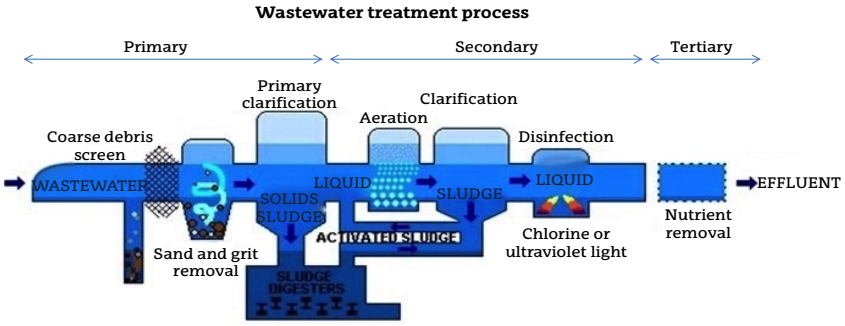

Figure1.1. Scheme of a conventional wastewater treatment plant processes.

Prior to the primary treatment, a preliminary process is normally performed. The objective of this preliminary treatment is the removal of rough solids and large materials found in raw wastewater. The removal of these materials is necessary to enhance the operation and maintenance of the WWTPs [3].

After this treatment, wastewater is submitted to the primary treatment. This treatment is designed to remove gross, suspended and floating solids from sewage. Skimming carries out the removal of floating materials, while the removal of suspended solids is performed by sedimentation. This step is also known as mechanical treatment, although chemicals are sometimes added to accelerate the process. During primary treatment, from 25 to 50 % of the incoming biochemical oxygen demand (BOD) is reduced and from 50 to 70 % of the total suspended solids and approximately the 65 % of the oil and grease are removed. Besides, compounds associated with solids such as organic pollutants, heavy metals or nitrogen, among others, are also removed in the sedimentation; however, dissolved compounds are not affected [4]. The deposited material, also known as primary sludge, is collected in tanks.

In most of the WWTPs primary treatment is followed by a secondary (biological) treatment. In this step, the dissolved organic matter, which has not been removed in the primary treatment, is eliminated. About the 85 % of the suspended solids and BOD can be removed by well running plants with a secondary treatment. This purpose is achieved using

aerobic biological treatment. Aerobic biological treatment is carried out in the presence of oxygen by microbes that metabolise the organic matter, producing more microorganisms and inorganic end products, such as carbon dioxide, ammonia and water, principally. In order to separate the effluent from the microorganism, sedimentation tanks are also used. These tanks operate in the same way as the primary sedimentation tanks and the biological solid (secondary sludge) is settled at the bottom and is normally combined with the primary sludge [5].

After secondary treatment, tertiary (advanced) treatment, which can be used to remove more than the 99 % of all the impurities from sewage, can be applied. Normally, the aim of this treatment is to improve the effluent quality before it is discharged to the receiving environment [5]. Some countries such as Finland, Germany, Denmark, Sweden and the Netherlands apply tertiary treatment in municipal wastewater at percentages higher than 80 %, while secondary treatment is predominant in Southern countries [6].

Before disposal, the sludge should be treated in order to remove microorganisms, such as bacteria and virus, and pollutants. Water removal is the primary step. For this purpose, thickening and dewatering processes are used to increase the solid content of the sewage sludge, which improves the treatability and transportability of the material, reducing the disposal costs. Common thickening processes, such as gravity thickening, dissolved air floating and gravity belt thickeners achieve a solid content of 3-6 %, while dewatering processes, including centrifuges, belt filter presses and sludge drying beds, produce sludge cakes with a solids content of 10-30 % [3, 7].

Stabilisation of sewage sludge can be achieved chemically, biologically or thermally. Anaerobic and aerobic digestions are the most commonly used methods in the EU, applied in 24 and 20 out of 28 countries, respectively. Anaerobic digestion is most commonly used in Finland, Italy, Spain, the UK and Slovakia, while aerobic digestion is more common in Poland and the Czech Republic [6]. The purpose of the digestion is to reduce the amount of organic matter and microorganisms reducing the number of diseases caused by the latter. When the digestion is carried out in the absence of oxygen but in the presence of heat, it is known as anaerobic digestion. The sludge is fermented in tanks and, thus, biogas (with a high proportion of methane that may be used to heat or to run engines) is produced. Methane generation is the main advantage of the anaerobic digestion and disadvantages are related to the long time required and its high cost. Although, at a first stage, digestion in presence of oxygen (aerobic) could be considered cheaper than the previously mentioned anaerobic digestion because the digestion occurs much faster, operating costs are, however, greater for the latter [8].

Apart from the stabilisation processes mentioned above, composting is used in 25 out of the 28 EU countries. It is an aerobic process based on mixing the sewage sludge with agricultural by-products such as straw and sawdust. Due to the digestion of sludge and plant material, heat to kill microorganisms is created. Chemical or thermal stabilisation, as well as conditioning with lime or polymers, are generally less used in sewage sludge stabilisation processes [8, 9].

WWTPs were initially designed to remove or decrease conventional pollution parameters (BOD, dissolved organic carbon (DOC), total suspended solids, etc.), but it was soon found out that the wastewater organic load included high levels of heavy metals and a variety of organic pollutants, concluding that sewage sludge acted as a sink for industrial and domestic chemicals [10, 11]. Moreover, in recent years, researchers have included as pollutants some compounds that, so far, were deemed safe or greatly considered to be totally biodegradable [12]. In fact, although degradation during wastewater and sludge treatments could remove some organic contaminants, many compounds, especially those with high lipophilic properties, tend to accumulate and can be found at different levels in biosolids [13, 14]. In other cases, and due to the use of advanced oxidation technologies implemented in WWTPs for the complete removal of organic compounds, the formation of intermediate degradation products that may be more toxic than the parental ones occurs [15, 16]. **Table 1.1** summarises the concentrations found for several organic pollutants in sludge around the world.

Due to the lack of effectiveness in removing organic pollutants shown by WWTPs, concern on the environmental exposure to such contaminants has gained attention. The presence of a large number of organic pollutants in both wastewater and sludge is enormous and different pollutant classifications are found according to the different environmental regulations.

The priority of chemical substances regulated in the environment is based on their toxicity, environmental persistence, bioaccumulation nature and widespread use. These pollutants are known as persistent organic pollutants (POPs). Among these chemicals, organic solvents, pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs), nonylphenols (NPs), perfluorooctane sulfonate acid (PFOS) and its salts, have been included according to the Stockholm Convention (SC) (see **Table 1.2**). SC was adopted in 2001 and entered in force in 2004. SC is a global treaty to protect health and environment from POPs [49].

Table 1.1. Concentrations of several organic pollutants determined in sewage sludge.

Pollutant		Country	Year	Concentration (ng/g)	Ref.
NPs, NPEOs and OPs		China	2008	2101-2546	[17]
		Greece	2008	170	[18]
		Greece	2008	12300	[18]
		Greece	2008	6140	[18]
		Spain	2009-2010	6.3-710	[19]
		Spain	2010	4183-29416	[20]
		Germany	1998-1999	2517-3675	[21]
		Greece	2006	110-2890	[22]
BPA		China	[a]	92.8	[23]
		Spain	2010	262-3590	[20]
		Greece	2008	530	[18]
		USA	2006-2007	6.48-4700	[24]
		China	2008	95-141	[17]
		China	2010-2011	0.42-152	[25]
		Greece	2006	620	[22]
Hormones	E1	China	[a]	6.8	[23]
		Spain	2010	56-73	[20]
		China	2008	11-26	[17]
	E2	Spain	2009-2010	2.2-100.4	[19]
		China	[a]	2.7	[23]
	E3	Spain	2009-2010	1.8-9.5	[19]
PPCPs	TCS	Spain	2010	1612-9093	[20]
		China	[a]	162-2506	[26]
		USA	2005-2011	10000-25000	[27]
		Greece	2008	3210	[18]
		Germany	[a]	30-280	[28]
		Brasil	2012	< LOQ	[29]
		Greece	2006	1840	[22]
	TCC	Spain	2010	47-153	[20]
		USA	2005-20111	10000-20000	[27]
Musk fragrances		UK	[a]	35-4000	[30]
		Switzerland	[a]	1400-15000	[31]
		Korea	2008	10-95800	[32]
		Austria	[a]	10-21000	[33]
		Spain	[a]	0.27-162	[34]
		Spain	[a]	< LOD-9240	[35]
		Spain	[a]	< MQL-2.0	[36]
		Spain	[a]	< LOQ-531	[37]
PBDEs		La Rioja	[a]	0.21-19.6	[38]
		Spain	2009	0.25-539	[39]
		China	2011	0.03-6.3	[40]
		USA	2005-2011	180-2400	[27]
Mono-, di-, tri-PAPs		China	2011	1.6-7.0	[41]
PFASs	PFAAs	Spain	2006	< 0.01-18.20 (PFHxS), <0.01-286.81 (PFOS), < 0.01-4.69 (PFPeA), < 0.01-2.04 (PFHxA), < 0.03-7.94 (PFOA), < 0.01-10.23 (PFNA), < 0.04-24.29 (PFDA)	[42]
		Sweeden	2009-2010	0.35-0.72 (PFHxA), 0.65-0.91 (PFOA), 16-110 (PFOS), 0.75-0.97 (PFDA)	[43]

Table 1.1. (Continuation).

Pollutant		Country	Year	Concentration (ng/g)	Ref.
PFASs	PFAAs	Spain	[a]	< 0.30-1.84 (PFHpA), < 0.6-2.82 (PFOA), < 0.3-3.03 (PFOS), < 0.45-1.02 (PFDA), < 0.21-0.44 (PFOA), <0.18-0.48 (PFUnDA), <0.3-3.03 (PFDoDA)	[44]
		Spain	2010	<MLOD-22.6 (PFBA), <MLOQ-17.2 (PFPeA), <MLOQ-4.8 (PFHxA), < MLOQ-4.5 (PFHpA), 7.0-30.3 (PFOA), 1.0-2.4 (PFNA), 6.1-23.5 (PFDA), <MLOQ-12.2 (PFUnDA), 4.0-11.3 (PFDoDA), <MLOQ-5.0 PFTrDA), <MLOQ-5.0 (PFTeDA), < MLOD-4.9 (PFHxDA), <MLOD-0.9 (PFODA), <MLOQ-7.6 (PFBS), 53.0-121.1 (PFOS), <MLOQ-7.5 (PFDS), <MLOD-10.7 (FOSA)	[45]
		USA	2007	5.4-18.2 (PFHxA), <1.8-9.7 (PFHpA), 8.4-128.0 (PFOA), 4.7-15.2 (PFNA), 3.4-43.9 (PFDA), 4.2-22.8 (PFUnDA), 2.5-19.7 (PFDoDA), <1.8-10.2 (PFTrDA), 2.6-9.6 (PFTeDA), 3.2-417.9 (PFOS)	[48]
	PFOS	The Netherland	2008-2009	0.07-48	[47]
	PFASs	USA	2004	<3-3.18 (PFHxS), 14.4-2610 (PFOS), 11.0-426 (PFDS), <6-62.1(FOSAA), <6-12.6 (N-MeFOSAA), <11- 544 (N-EtFOSAA), <6-13.3 (PFOA), <3-10.3 (PFNA), <3-72.3 (PFDA), <1.1-8.58 (PFUnDA), <3-6.51 (PFDoDA), <3-7.77 (PFTrA)	[46]

[a] Not reported

BPA: bisphenol A, E1: estrone, E2: 17-β-estradiol, E3: estriol, FOSA: perfluorooctanesulfonamide, FOSAA: perfluorooctanesulfonamido acetate, LOD: limit of detection, LOQ: limit of quantification, MQL: method quantification limit, N-EtFOSAA: 2-(N-ethylperfluorooctanesulfonamido) acetic acid, N-MeFOSAA: 2-(N-methylperfluorooctanesulfonamido) acetic acid, NP: nonylphenol, NPEO: nonylphenol ethoxylate, OP: octylphenol, PAP: perfluoroalkyl phosphate, PBDE: polybrominated diphenyl ether, PFAA: perfluoro alkyl acid,EQ:equilin PFAS: perfluoroalkyl substance, PFBA: perfluorobutanoic acid, PFBS: perfluorobutanesulfonate acid, PFDA: perfluorodecanoic acid, PFDoDA: perfluorododecanoic acid, PFDS: perfluorodecanesulfonate acid, PFHpA: perfluoroheptanoic acid, PFHxA: perfluorohexanoic acid, PFHxDA: perfluorohexanedecanoic acid, PFHxS: perfluorohexasulfonate acid, PFNA: pefluorononanoic acid, PFOA: perfluorooctanoic acid, PFODA: perfluorooctadecanoic acid, PFOS: perfluorooctane sulfonate acid, PFPeA: perfluoropentanoic acid, PFTeDA: perfluorotetradecanoic acid, PFTrDA: perfluorotridecanoic acid, PFUnDA: perfluoroundecanoic acid, PPCP: pharmaceutical and personal care products, TCC: triclocarban, TCS: triclosan.

The most important regulation concerning water management for the EU members is the WFD, adopted in 2000. WFD introduces a new legislative approach to the water management and protection, and sets out precise timetable for action, with 2015 as the target date for getting all European waters into good condition. Firstly, 33 priority pollutants were included in the Annex X of the Directive 2000/60/EC [50]. In the last revision of the Directive in 2013 [51] other 12 substances were added and their corresponding environmental quality standards (EQSs) were defined for water as well as for biota. Among other priority organic pollutants, PBDEs, some PAHs, PFOS and its derivatives or dioxins were also included. In the case of the US Environmental Protection Agency (US EPA), 126 chemicals considered as priority pollutants have been listed in their water quality regulatory program [52]. **Table 1.2** includes pollutants classified as priority according to the WFD or the US EPA.

During the last years, apart from the priority pollutants mentioned and included in **Table 1.2**, the presence of new chemicals whose presence in the environment had not been detected or whose concentrations are significantly different to those expected, has gained attention. These compounds are often referred to as "contaminants of emerging concern" (CECs) because the risk to human health and the environment associated with their presence, frequency of occurrence or source may not be known [53]. NORMAN network was launched in September 2005 with the financial support of the European Commission in order to discuss on CECs. In 2009, the NORMAN network became a self-sustaining network of reference laboratories, research centres and related organisations for the monitoring of emerging environmental substances. NORMAN network identified a list of the currently most frequently discussed emerging substances and emerging pollutants and new candidates are being negotiated (October 2013) [54]. Compounds such as musk fragrances, triclocarban (TCC), some perfluoroalkyl substances (PFASs) and their transformation products, among others, were included in the list. Apart from the compounds proposed by the NORMAN network, the European Parliament and the Council also proposed 17β-estradiol (E2), 17 α-ethinylestradiol (EE2), and diclofenac, among other chemicals, as new priority pollutants in the field of water quality by European Parliament and the Council [51, 55]. Due to the importance that emerging contaminants are taking, the US EPA defined a Contaminant Candidate List (CCL), where 116 contaminants (104 chemicals and 12 microbiological contaminants) for consideration in future regulations were gathered in the latest updated version (CCL 3) [56]. Apart from the CCL 3 list, the US EPA published the third Unregulated Contaminant Monitoring Rule (UCRM 3), requiring every 5-year monitoring of 30 unregulated contaminants to provide

national occurrence data for future regulatory consideration [57]. **Table 1.2** shows analytes included in CCL 3 and UCRM 3.

Table 1.2. Target compound classification regarding their legislative consideration.

Compounds		Priority pollutants			Emerging pollutants	
		WFD	**EPA**	**SC**	**CCL 3 (EPA)**	**UCRM 3 (EPA)**
Polybrominated diphenyl ethers (PBDEs)		X[1]		X[2]		
Musk fragrances	Tonalide (AHTN)					
	Galaxolide (HHCB)					
Alkylphenols (APs)	4-Octylphenol (4-tOPs)					
	Nonylphenol mixture (NPs)	X[3]				
Bisphenol A (BPA)		*				
Sex and steroidal hormones	Diethylstilbestrol (DES)					
	Estrone (E1)				X	X
	17β-estradiol (E2)				X	X
	Testosterone (TT)					X
	Progesterone (PG)					
Personal and pharmaceutical care products (PPCPs)	Triclosan (TCS)					
	Triclocarban (TCC)					
Perfluorinated alkyl substances (PFASs)	PFHpA					X
	PFOA			X[4]	X	X
	PFNA					X
	PFBS					X
	PFHxS					X
	PFOS and its salts	X		X[5]	X[6]	X[6]

CCL: contaminant candidate list, EPA: environmental protection agency, PFBS: perfluorobutanesulfonate, PFHpA: perfluoroheptanoic acid, PFHxS: perfluorohexanesulfonate, PFNA: pefluorononanoic acid, PFOA: perfluorooctanoic acid, PFOS: perfluorooctane sulfonate acid, SC: Stockholm convention, UCRM: unregulated contaminant monitoring rule, WFD: water framework directive. *Substance subject to review for possible identification as priority hazardous substances in ANEX III of the Directive 2008/105/EC. [1] Only tetra, penta, hexa and heptabromodiphenylether (CAS -numbers 40088-47-9, 32534-81-9, 36483-60-0, 68928-80-3, respectively). [2] Parties must take measures to eliminate the production and use of heptabromodiphenyl ether, hexabromodiphenyl ether, pentabromodiphenyl ether and tetrabromodiphenyl ether. [3] Nonylphenol (CAS 25154-52-3, EU 246-672-0) including isomers 4-nonylphenol (CAS 104-40-5, EU 203-199-4) and 4-nonylphenol (branched) (CAS 84852-15-3, EU 284-325-5). [4] PFOA and its ammonium salt (APFO) have been included in the candidate list of substances of very high concern. [5] Parties must take measures to restrict the production and use of Perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride. [6] Only for PFOS.

From the above mentioned pollutant lists, compounds such as BFRs, PFASs, pharmaceuticals and personal care products (PPCPs), hormones or NPs, among others, are identified as priority or emerging pollutants. Some of these compounds will be further commented.

Brominated Flame-Retardants (BFRs)

BFRs are a mixture of man-made chemicals that are added to a wide variety of products [58]. There are five main classes of BFRs regarding their common uses, including PBDEs in plastics, textiles and electronic castings, hexabromocyclododecanes (HBCDDs) applied in thermal insulation in the building industry, tetrabromobisphenol A (TBBPA) used in thermoplastics (mainly TVs) and polybrominated biphenyls (PBBs) in plastic foams, consumer appliances and other brominated flame retardants [59].

PBDEs include up to 209 isomers that are commercially available as three commercial mixtures of BDE congeners named after their principal component, penta-, octa- and deca-BDEs [60]. Some PBDEs, including tetra-, penta-, hexa- and heptabromodiphenylether, exhibit physicochemical properties such as environmental persistence, tendency to bioaccumulate and potential toxicity and are therefore listed as POPs. Due to their environmental persistence and tendency to bioaccumulate, exponentially increasing levels of dominating congeners of penta- and octa-BDEs have been measured in human blood and milk [61]. Because of that, the use and sale of preparations and articles containing these two flame-retardant mixtures in concentrations higher than 0.1 % (in mass) was prohibited in August 15 of 2004 in the EU [62]. Moreover, USA companies had to phase out the production of deca-BDEs by 2013 [63].

Per- and Polyfluorinated Alkyl Substances (PFASs)

Per- and polyfluorinated compounds are a family of anthropogenic chemicals that have been and are still widely used in a variety of domestic and industrial applications (production of fluorinated polymers, metal plating, photographic industry, as fat and water repellent in textile industry, and so on) due to their surface tension lowering properties in order to make products resistant against heat, oil, stains, grease and water [64].

The two most common congeners reported in the literature are PFOS salts and perfluorooctanoic acid (PFOA). These two compounds, as well as perfluoro alkyl acids (PFAAs), are stable products due to the strong C-F chemical bond. Besides, PFOA and PFOS can enter the environment in their chemical form or as product of the degradation of certain precursors used in industrial applications. Precursors such as fluorothelomer alcohols (FTOHs), perfluorinated

sulphonamides (PFSAs) and polyfluoroalkyl phosphate surfactants (PAPs), among others, can transform to stable end products such as PFAAs [65, 66]. Due to the growing concern on these chemicals, in 2000 the largest producer, the 3M Company, announced the phase out of the production of PFOS. Since then, new shorter chain PFASs and their precursors have been introduced as replacement considering that they are less persistent and toxic for humans [67]. Besides, in 2006, the US EPA announced the voluntary stewardship program to reduce by a 95 % the presence of PFOA and related chemicals in the environment by 2010 and the elimination of all of them by 2015 [68]. Furthermore, EU issued a Directive that prohibited the general use of PFOS and its derivatives from June 2008 [69]. In 2009, PFOS, its salts and perfluoroctane sulfonil fluoride (POSF) were listed as "restricted use" in Annex B of the Stockholm Convention on POPs [70] and in 2013 PFOS and its derivatives were included in the Annex I of the Directive 2013/39/EC [51] and identified as priority hazardous substances. Besides, the EU is taking into account the proposal of including PFOA and its compounds in Annex A [71]. Finally, the European Commission emitted a recommendation for the monitoring of fluorinated compounds in protein-rich food and vegetables, including not only PFOS and PFOA but also FTOHs and PAPs [72].

Pharmaceutical and Personal Care Products (PPCPs)

PPCP refers to any product used by individuals for personal health or cosmetic reasons or used by agribusiness to enhance the growth or health of livestock. As they are widely used in daily human life, they are continuously released to the environment [73]. PPCPs include prescription and over-the counter therapeutic drugs, veterinary drugs, fragrances, cosmetics and sunscreen products, among other compounds.

Within these products, synthetic musk compounds have been used as fragrance additives in soaps, shampoos, detergents, lotions and perfumes to provide odour-enhancing and blending properties since the 1930s [74]. Synthetic musks are divided in four main subgroups: nitro musks, polycyclic musks, macrocyclic musks and alicyclic musks [75, 76]. Nitro musks were the first commonly used synthetic musks, but their use was reduced in the 1950s due to evidence of toxicity to humans and the environment [77]. Nowadays, polycyclic musk fragrances are most commonly used. The most representative polycyclic musks are tonalide (AHTN) and galaxolide (HHCB), which account for the 95 % of the total market volume for polycyclic musks [78].

Among PPCPs steroid hormones are also considered as new emerging compounds to monitor in the environment. The function of steroid hormones is to control the metabolism, the immune functions, the salt and water balance, the development of sexual characteristics and the ability to withstand illness and injury. There are three classes of steroid endogenous hormones: estrogens, androgens and progestagens [79]. Estrogens such as estrone (E1), E2 and estriol (E3) are the primary female sex hormones, whilst androgens such as testosterone (TT), are more related with male hormones. Progestagens such as progesterone (PG) are hormones involved in the menstrual cycle and pregnancy [80]. Apart from natural pathways, both natural and synthetic steroid hormones are administered to humans and cattle for a variety of pharmaceutical applications. Actually, norethistherone (NT), TT and PG are widely used for a wide range of applications, such as contraception, cancer treatment and hormone replacement therapy for humans [81]. Besides, E2, TT and PG, are used for growth and reproductive control in cattle [82].

Triclosan (TCS) and TCC are also included within PPCPs. TCS was originally developed as a pesticide in Switzerland and was first seen in the USA in deodorant soaps in the 1960s [83]. Through the 1970s and 1980s, TCS was primarily used as an antiseptic agent in hospital scrub. Since then, TCC and TCS antimicrobial agents have been widely used in personal care products such as shampoos, soaps, deodorants and creams [84]. The widespread use of TCS and TCC is due to their relatively easy way of incorporation into many products because of their high boiling point. In fact, during the last decades, the use of TCS has increased dramatically. From 1976 to 2014, the United States Patent and Trademark Office issued more than 4400 patents with products containing the word "triclosan". A study conducted in the United States from 1999 to 2000 found that TCS was present in 75.7 % of all bar soaps [85].

Toxicity studies have indicated that TCS and TCC could also function as endocrine system disruptors [86-88]. They are not regulated yet, even though they are among the top 10 most commonly detected chemicals in environmental aqueous samples [86, 89, 90] and the most commonly detected PPCPs in biosolids [91].

Bisphenol A (BPA)

Bisphenol A (BPA) is a plasticiser manufactured in high quantities since 1957. Three million tons are produced worldwide each year due to its uses as monomer for the production of polycarbonates and epoxy resins, in polystyrene resins and in flame-retardants [92]. Although BPA has shown to be weakly oestrogenic [93], it is widely used in household and industry

products and, therefore, it is being continuously released into the environment. In fact, BPA was under review for possible identification as a POP or priority hazardous substance (PHS) by the WFD to control emission sources [94]. However, finally, the WFD concluded that there was not enough evidence demonstrating that BPA poses a risk to the environment. Besides, since 2006, the European Food Safety Authority (EFSA) conducted several scientific assessments on BPA and reaffirmed that there is no concern for human health from BPA, mainly because human exposure for BPA is far below the safe intake level [95]. In the final assessment on BPA published in January 2015, based on a comprehensive review of 450 recent studies related to potential health hazards associated with BPA, EFSA concluded that there is no consumer health risk from BPA exposure. Nevertheless, based on a highly precautionary approach, the European Commission, at an earlier stage, decided to restrict one specific BPA-based application, the production of BPA-based polycarbonate baby bottles since June 2011. Some European countries established further restrictions on the use of BPA-based materials in food contact plastic applications [96].

Alkylphenols (APs)

Alkylphenol ethoxylates (APEOs) belong to a group of non-ionic surfactants widely used in industrial and household applications as detergents, emulsifiers and wetting and dispersing agents [97]. They are biodegraded partially in the environment by the loss of ethoxy groups, resulting in NPs, octylphenols (OPs) and other mono-, di- and tri-ethoxylates (NP1EO, NP2EO and NP3EO, respectively). APs are widely dispersed in the environment due to the large annual worldwide productions of APEOs. NPs and OPs are more toxic than their APEO precursors [98]. In fact, linear and branched 4-nonylphenol (4-NP) are included as priority hazardous substances in WFD list and OPs as priority pollutants [51].

As can be observed in **Table 1.1**, all the above described chemicals have been detected in sewage sludge from different countries such as China, Spain, Germany, Greece, Switzerland and the UK, among others. The detected concentration ranges have also been collected and included in **Table 1.1**. NPs, BPA, TCS, TCC and musk fragrances have been detected at the mg/kg concentration level in most of the cases, while PFASs and derivatives at the low ng/g concentration level. PFOS and PFOA are the most common PFASs presented in the sludge and were detected in all the samples. The lowest concentrations were measured for hormones at very low ng/g level in all the cases.

1.2 Land application of sludge and uptake of organic contaminants by crops

Once treated, sludge can be recycled. Alternatives such as dumping at the sea, land application, landfilling and incineration have been tested in EU countries as it can be observed in **Figure 1.2**. Since the end of 1998, sea dumping of sewage sludge was banned and for 2020 it is expected that the 44 % will be recycled to land, the 32 % incinerated, the 7 % landfilled, and the 16 % used in other applications [6]. On the other hand, landfilling process is decreasing since 1992 because of the national legislations of some member states of the EU setting very strict limits for the legal amount of organic matter or total organic carbon (TOC) in sludge, which practically prohibit this application. However, incineration process, which has been almost doubled from 1992 to 2005, and biosolids reuse, which mainly includes agricultural utilisation and composting, are gaining more popularity [6].

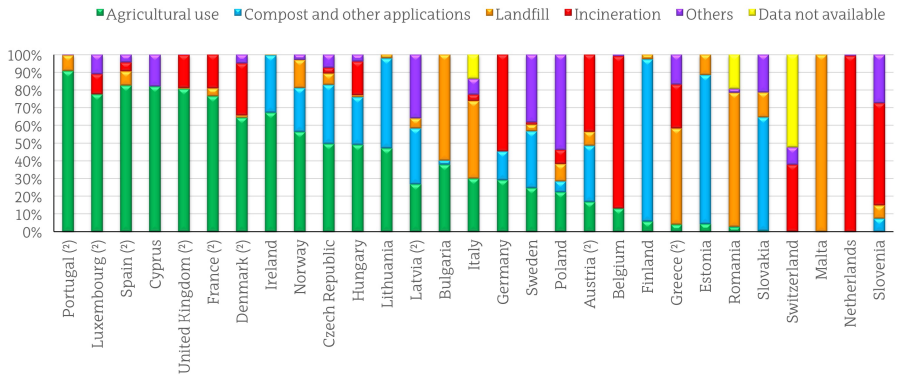

Figure 1.2. Sewage sludge disposal from urban wastewater treatment, by type of treatment in EU countries, 2011 [(1)]. [(1)] Belgium, Denmark, Spain, France, Italy, Cyprus, Luxemburg, Netherlands, Austria, Sweden, the United Kingdom, 2010. Latvia, Portugal, Finland, Switzerland, 2009. Croatia, Iceland, Turkey, Bosnia and Herzegovina, no data. (Source: Eurostat). [(2)] Based on a total excluding category of other types of treatment.

In Spain, the agricultural use of sludge is regulated by national (R.D. 1310/1990) or European legislations (86/278/EEC), mostly in relation with metal content. At the moment, efforts are being made to establish global European regulations that also deal with organic compounds. In this sense, the European Commission launched a review of the current sludge directives and prepared a working paper suggesting limit values of both metals and commonly occurring organic compounds, such as linear alkylbenzene sulphonates (LAS), di(2-ethylhexyl)phthalate (DEHP), NP and NPEO, PAHs, PCBs, polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs) and halogenated organic compounds (AOX) [99].

Under optimum conditions, sewage sludge application improves soil properties, such as the texture and the water capacity, which provide more favourable conditions for root growth and increase the drought tolerance of vegetation. Biosolids or compost application also supplies nutrients for plant growth, including nitrogen and phosphorous, as well as some micronutrients such as nickel, zinc and copper. Besides, biosolids can also serve as a cheap alternative to expensive chemical fertilisers.

However, concerns continue to rise about the potential risk of this practice since biosolids contain a broad range of toxic inorganic and organic contaminants, as well as pathogens [100]. These contaminants can be transferred from soil to plant by means of different processes: soil to root transfer, root to shoot transfer, vapour uptake from the atmosphere and particle deposition [101]. The predominance of one or other pathways is determined by the chemical and physical properties of each pollutant, such as the hydrophobicity, the water solubility and the vapour pressure, as well as by environmental conditions, including the organic content of the soil, the temperature, the pH and the plant specie, among others.

1.2.1 Soil-root transport

In general, plant roots are the most important sites for the chemical uptake from the soil [102]. The contaminants are partitioned between soil particles, interstitial water and interstitial air and the root system takes up organic chemicals from both the water and/or the air phases, being the former the most important. Therefore, factors that influence the chemical concentration in pore water also exert a control over the uptake. Organic chemicals can be sorbed to different soil components such as clay, iron oxides and organic matter, although it is the latter that usually has the strongest influence on the pore water concentration. The higher the organic matter content of the soil, the lower the chemical concentration in the pore water and, consequently, the lower the total amount of chemical taken up by the plant [103].

This uptake process involves passive and active transport. The former does not require any energy; therefore, there is no adenosine triphosphate (ATP) expenditure by the cell and the movement of the solutes occurs due to a concentration gradient. Passive transports include osmosis, dialysis and facilitated diffusion. A carrier protein facilitates the latter, apart from the concentration gradient. A carrier protein is somewhat like a revolving door allowing easy access across the membrane and the rotation back to the other side. Nevertheless, there are materials that need to be moved against the concentration gradient. This is known as active transport and requires energy and carrier proteins that are found in the plasma membrane [104].

When entering the root together with the water stream, chemical compounds are stored in underground tissues or transported upward from the root into other plant compartments through the xylem by a mass flow resulting from a pressure gradient. This driving force is created during the transpiration, where water is pulled through the root system to replace evaporative losses from stomata within the leaves [105]. In order to reach the xylem, chemicals must penetrate a number of plant tissues: the epidermis, the cortex, the endodermis and the pericycle. There are three possible pathways (see **Figure 1.3**): the apoplastic pathway via the cell walls, the symplastic pathway through the cytoplasm or the transcellular pathway, from vacuole to vacuole [106]. Casparian strip is a barrier that limits the entry of organic compounds into the xylem. Dissociated molecules and ions are transferred relatively easily, whilst substances with higher lipophilicity or strong binding capacity tend to remain in the root.

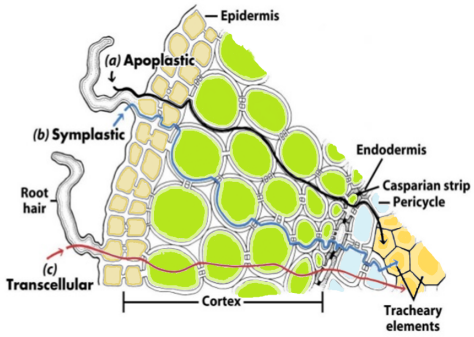

Figure 1.3. Three different pathways for solutes and water to reach from the root to the xylem: a) apoplastic, b) symplastic and c) transcellular.

The xylem tubes are made up of dead cells and conduct a sap consisting primarily of water and dissolved solutes from the root to the shoot. In contrast, phloem tubes allow the movement of sugars, especially sucrose, from "source" structures (often photosynthetic cells in the leaves) to those called "sinks" (fruits, roots). Therefore, after transport in the stem, water and solutes can be accumulated in the plant shoots, tubers and fruits [105].

Everything mentioned above is focused on neutral organic chemicals; however, chemicals such as amines, carboxylic acids, phenols and some pesticides may ionise under different soil conditions [107]. These compounds reach the xylem through the cell membranes that contain proteins known as "proton pumps" which regulate the flow of charged ions from inside to outside the cell and vice versa. An electrochemical gradient is created and the implied electrochemical force drives the movement of ions across the electrically charged membranes

[108]. Moreover, the chemical environment within the cell can vary from the external solution and the dissociation degree can increase or decrease modifying the accumulation capability of the chemical.

1.2.2 Soil-leaf uptake

Apart from the root uptake, another potential path for plant uptake of pollutants is the absorption of chemical vapour from the atmosphere or the soil particle deposition on above ground leaves.

This pathway differs from root uptake because it is mediated via a gaseous exchange instead of an aqueous solution. In fact, this pathway is likely to be important, not only for highly volatile chemicals, but also for pollutants with air-soil partition coefficients higher than water-soil partition coefficients [102]. In this case, chemicals enter the plant directly through stomata or crossing the cuticle.

Stoma is a pore found in the epidermis of leaves, stems and other organs that is used to control gas exchange (air containing carbon dioxide and oxygen enters the plant through these openings) and it is also the principal route by which water vapour from the transpiration stream is lost by the plant [101].

The cuticle is a protecting film consisting on lipid and hydrocarbon polymers (the major structural component is cutin, an insoluble cross linked polymer) impregnated with wax and a small amount of hydrophilic compounds, such as cellulose, which covers the epidermis of the leaves. It represents the initial and main barrier of penetration of foliar applied compounds. The translocation of chemicals depends on the wax and cutin amount of the leaves, as well as the chemical properties of the target analyte. Once entering the leaf, chemicals diffuse into intercellular air spaces and partition to the aqueous and lipophilic adjacent plant tissues can occur [109].

The second pathway for organic pollutant foliar uptake is the particulate deposition on plant surface. This can happen as a result of wind re-suspension or rain splash [101].

1.2.3 Plant uptake studies

Recently, in order to evaluate the risk of the presence of organic pollutants in sewage sludge or compost/biosolids, different uptake experiments have been carried out (see **Table 1.3**). Different approaches have been used in the literature, such as the use of hydroponic

or soil cultivation media.

Hydroponic is defined as a method of growing plants in the nutrient solution (homogeneous). The composition of the solution depends on the plants that are going to be cultivated. This system has several advantages. In the absence of soil requirement, this methodology is suitable for cultivations in any area where ground agriculture is impossible. Besides, this method is considered as an environmentally friendly and less polluting. In terms of yield, hydroponic cultivation usually renders better results than normal soil cultivation and the harvesting procedure is obviously easy. However, several disadvantages are also known referring to this type of cultivation. One of these disadvantages is the low buffering capacity compared with the soil, which can make plants more susceptible to a rapid death [110].

In spite of the advantages above mentioned for hydroponic cultivation, most of the uptake experiments in the literature have been carried out using soil as cultivation media (see **Table 1.3**). In this case, two alternatives are commonly used: fortified and unfortified soils. Both are applied in the literature; however, most of the studies reported are carried out using soils fortified at a known concentration level and cultivated in controlled conditions (see **Table 1.3**).

When the uptake experiments are performed using soil cultivation media, the chemical transfer is strongly influenced by soil characteristics, including cation exchange capacity, pH and TOC, among others. The latter is usually the variable that exerts the strongest influence in the uptake [111]. The higher the TOC, the stronger the compound-soil interaction is and the lower the transferred amount of pollutants into plants. This was reported in the literature when uptake of PBDEs [112], musk fragrances [113, 114], PPCPs [115] and PFAAs [116] by different crops, such as carrot, barley, meadow fescue and lettuce, among others, were carried out.

In the uptake studies found in the literature, different trends have been observed depending on the soil type, crop type and nature of the studied analyte. In this sense, it has been observed that analytes such as PBDEs [112, 117-126] and musk compounds [113, 114] tend to accumulate principally in the root of the crops. For instance, according to the literature [112, 121, 127, 128], the highest PBDE concentrations were determined in the roots of pumpkin, wheat or maize, being the accumulation for lower brominated congeners the highest. While Ding et al. [118] suggested that the contribution of soil-root-leaf pathway is totally negligible for foliar uptake; Vrkoslavová et al. [122] suggested that PBDE transport via root into plant tissues could be the primary route.

Table 1.3. Plant uptake of certain organic pollutants by different crops cultivated in hydroponic and soil cultivation medias.

Compound	Crop	Cultivation media	Fortified/Non-fortified	Ref.
PBDEs	Mangrove	Soil	Fortified	[127]
BDE-209	Rice	Soil	Fortified	[117]
PBDEs	Tree	Soil	Non-fortified	[118]
PBDEs	Ryegrass, pumpkin and maize	Soil	Non-fortified	[128]
PBDEs	Wheat	Soil	Non-fortified	[112]
PBDEs, OH-PBDEs and MeO-PBDEs	Grass	Soil	Non-fortified	[121]
PBDEs	Tobacco and nightshade	Soil	Non-fortified	[122]
PBDEs and PCBs	Maize	Hydroponic	Fortified	[135]
PBDEs, OH-PBDEs and MeO-PBDEs	Dimocarpus longan, mangifera indica, arteimiaisia sieuersina, cichorium endivia, zea mays, portuala oleracea, lactuca sativa and phaseolus vulgaris	Soil	Non-fortified	[123]
BDE-209	Ryegrass	Soil	Fortified	[124]
Polyhalogenated compounds	Rice	Soil	Non-fortified	[125]
PBDEs	Maize	Hydroponic	Fortified	[126]
TCS, HHCB, AHTN, IBF, NPX, CFA	Lettuce and spath	Hydroponic	Fortified	[136]
HHCB, AHTN, TCS	Carrot, barley, and meadow fescue	Soil	Fortified	[114]
Musks	Lettuce and carrot	Soil	Fortified	[113]
TCS	Carrot	Soil	Fortified	[137]
TCC and TCS	Cucumber, tomato, cabbage, okra, pepper, potato, beet, onion, celery, and asparagus	Hydroponic	Fortified	[138]

Table 1.3. (Continuation).

Compound	Crop	Cultivation media	Fortified/Non-fortified	Ref.
PPCP (TCS and TCC among others)	Lettuce, spinach, cucumber and pepper	Hydroponic	Fortified	[139]
PPCP (CBZ, DIP, TCC)	Pepper, tomato, collard, lettuce and radish	Soil	Fortified	[115]
PPCPs (TCS, CBZ, salbutamol, sertraline, trimethorpin)	Cabbage and Wisconsin fast plant	Hydroponic	Fortified	[139]
PPCPs (CBZ, diclofenac, fluoxetine, propranolol, sulfamethazine and TCS)	Ryegrass and radish	Soil	Fortified	[140]
NP and NP12EO	Beans	Soil	Fortified	[141]
PFAAs	Lettuce and tomato	Soil	Non-fortified	[131]
PFAAs	Radish, celery, tomato and pea	Soil	Non-fortified	[116]
PFAAs	Lettuce and strawberry	Hydroponic	Fortified	[142]
PFAAs	Lettuce	Hydroponic	Fortified	[134]
PFAAs	Grass	Hydroponic	Fortified	[143]
	Strawberry and lettuce	Hydroponic	Fortified	[144]
PFAAs	Tomato, cabbage and head zucchini	Hydroponic	Fortified	[133]
PFASs	Zea mays	Hydroponic	Fortified	[129]
PFAAs	Wheat	Soil	Non-fortified	[132]
PFOS and PFOA	Maize	Hydroponic	Fortified	[130]
Hormones	Maize	Hydroponic	Fortified	[145]
TCS and EE2	Bean	Soil	Fortified	[146]
Pharmaceuticals, hormones and parabens	Tomato, potato, carrot and corn	Soil	Non-fortified	[147]
Steroidal hormones	Lettuce	Soil	Non-fortified	[148]

AHTN: tonalide, BDE-209: decabrominated diphenyl ether, CBZ: carbamazepine, CFA: clofibric acid, DIP: diphenhyramine, EE2: ethinyl estradiol, HHCB: galaxolide, IBF: ibuprofen, MeO-PBDE: methoxylate polybrominated diphenyl ether, NP: nonylphenol, NP12EO: nonylphenol ethoxilate, NPX: naproxen, OH-PBDE: hydroxilate polybrominate diphenyl ether, PBDE: polybrominated diphenyl ether, PCB: polychlorinated biphenyl, PFAA: perfluoroalkyl acid, PFOA: perfluorooctanoic acid, PFOS: perfluorooctane sulfonate, PPCP: pharmaceutical and personal care product, TCC: triclocarban, TCS: triclosan.

Although Macherius et al. [114] worked out bioconcentration factors (the ratio between plant compartment concentration and soil concentration, BCFs) higher than Litz et al. [113] when the uptake of musk fragrances was studied by different crops, in both cases the highest concentrations were detected in the root and decreased gradually toward the plant leaves.

For analytes such as PFASs, which contain an anionic polar head and a lipophilic chain, higher translocation from root to shoot is observed [129-131]. For instance, when crops were

cultivated in soil, Blaine et al. [131] measured higher concentration in shoot or fruit than in root of radish, tomato, pea and celery for most of the PFAAs, while Wen et al. [132] determined higher concentration in root than in shoots when the uptake of PFAAs by wheat was carried out. Different studies [116, 133, 134] concluded that there is a preferential accumulation of shorter chain PFAAs over longer chain PFAAs. Further studies should be performed, however, for the better understanding of this topic.

1.3 Analysis of organic pollutants in solid matrices

1.3.1 Extraction techniques

Sample preparation is one of the steps within the analytical procedure that should not be overlooked in order to achieve high quality results in analytical chemistry. Therefore, a great effort is necessary in the development of sample preparation in order to obtain reliable data on the concentration of organic pollutants in different matrices. For this purpose, robust, selective, accurate, precise and sensitive analytical procedures are necessary.

During the last decades a variety of extraction techniques have been used for the determination of organic pollutants in solid matrices, such as sewage-sludge, soil or vegetables. Traditional Soxhlet extraction has been so far applied for the determination of organic compounds in solid matrices due to its high extraction efficiency [149]. As alternative to Soxhlet, but among what can be considered traditional extraction techniques, ultrasound energy, as well as mechanical shaking, have also been extensively used for the extraction of organic pollutants from solid matrices [22, 31, 150-152]. However, all the above-mentioned extraction techniques are time-consuming, labour intensive and require the use of large volumes of organic solvents. Despite the disadvantages mentioned above, many applications of these extraction techniques for the determination of organic compounds in solid matrices can be found in the literature [41, 149, 153, 154]. In order to overcome the above mentioned problems found for classical extraction techniques, pressurised liquid extraction (PLE), also known as accelerated solvent extraction (ASE) [155, 156], microwave assisted extraction (MAE) [157] and focused ultrasound solid-liquid extraction (FUSLE) [158] were developed in the past decades. Clear improvement of the processes based on a dramatic reduction in the extraction time, as well as in the amount of solvent required [157, 159, 160], were achieved.

FUSLE is based on the application of ultrasonic radiation using a microtip immersed directly into the extraction solvent. Ultrasound radiation, when transmitted through the medium, generates a disturbance and, due to its periodical repetition, expansion and compression cycles are created [161]. When the ultrasounds reach high enough intensity, bubbles or cavities are created during the expansion cycle. Cavitation is the process where bubbles form and then undergo implosive collapse (see **Figure 1.4**) [162]. FUSLE is one of the simplest solid-liquid extraction techniques. The mechanical effect of ultrasound induces a greater penetration of the solvent into the solid matrix and mechanical erosion of solids, including particle rupture, which improves mass transfer leading to enhanced sample extraction efficiency. The ultrasonic probes have several advantages over the ultrasonic baths. On the one hand, the energy is focused on a localised sample area, making cavitation in the liquid more efficient. On the other hand, FUSLE is more reproducible than classical ultrasonic extraction [162].

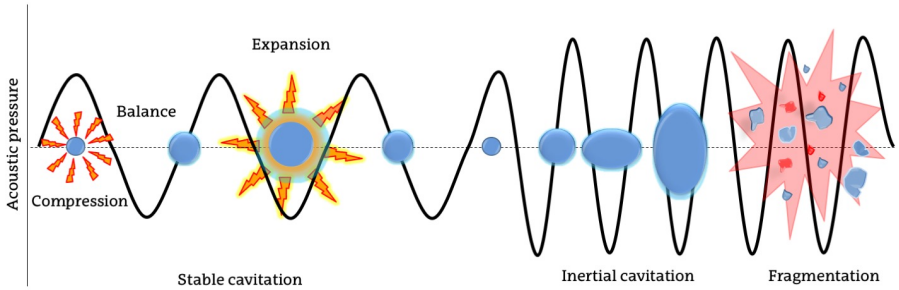

Figure 1.4. Cavitation phenomena in ultrasound sonication.

The application of FUSLE for the extraction of organic pollutants from solid samples has gained attention in the past decade. In recent literature more and more manuscripts can be found. Zabaleta et al. [163] used FUSLE for the simultaneous determination of PFASs from vegetables and soil followed by solid phase extraction (SPE) clean-up step. Martinez-Moral et al. also applied probe sonication for the determination of PFASs [44] and BPA and APs [164]. Villar et al. [165] used FUSLE for LAS extraction from sewage sludge prior to the analysis. Martinez-Moral et al. [166] also performed the determination of PBDEs in air dust using FUSLE and Errekatxo et al. [167] applied FUSLE for the simultaneous extraction of PAHs, PCBs, phthalate esters (PEs) and NPs from sediment samples.

One of the disadvantages of exhaustive extraction techniques such as FUSLE is that they are not selective and, therefore, a clean-up step is usually necessary in order to remove

compounds that can interfere in the analysis step. One of the most common clean-up approaches used for environmental sample analysis is SPE. SPE involves bringing the liquid extract into contact with a solid phase or sorbent whereby the compound is selectively sorbed onto the surface of the solid phase prior to elution [168].

Depending on the nature of the target analytes and matrix, different SPE approaches have been used in the literature for the clean-up of solid matrices (see **Table 1.4** and **Table 1.5**). In the case of slightly to non-polar organic pollutants such as PBDEs [39, 166, 169] and musk fragrances [30-32, 119], where volatile organic solvents such as acetone are used during the extraction step, normal phase SPE using Florisil, silica or alumina as sorbents are used. In this case, the extraction solvent is evaporated prior to the SPE clean-up and the extract is re-dissolved in a non-polar solvent such as n-hexane or isooctane prior to the loading [33, 119, 150]. In other cases the extract is re-dissolved in water and reverse phase SPE is used, as for instance in the case of NPs and its derivative chemicals [22], BPA [22], TCS [22] and musk fragrances [31, 170]. This last procedure is also used for more polar or ionic compounds where aqueous soluble solvents such as water, buffered solutions or methanol are used for the extraction. Since evaporation is tedious due to the lower volatility of the extraction solvent, reverse phase SPE is used after dilution in water [19, 22]. In the last decades the use of mix-mode SPE cartridges has also gained attention since a more selective preconcentration of the target analytes and, consequently, a more efficient clean-up of the extract can be achieved. For instance, mix-mode cartridges have been used for the clean-up of extracts in the case of PFASs and their precursors [41, 163, 171].

As conventional SPE uses large volumes (5-25 mL) of organic solvents for the elution step and a high amount (150-5000 mg) of sorbent material, a new clean-up procedure named dispersive SPE (dSPE) has been recently introduced by Anastassiades et al. [172] along with the Quick Easy Cheap Effective Rugged Safe (QuEChERS) extraction technique. dSPE has been usually used as the clean-up step after QuEChERS. In comparison with traditional SPE, dSPE can be considered as a "greener" technique due to the lower solvent consumption (0.5-2 mL). Besides, dSPE requires shorter extraction periods and less effort [173]. The steps involved in this clean-up procedure are shown in **Figure 1.5** and consist in four main steps:

1) After solid-liquid extraction, the supernatant is transferred to a clean-up tube where the clean-up sorbent is placed.
2) The sample and sorbent mixture are shaken. In this step, due to the interaction

between the sample matrix, the target analytes and the sorbent, the clean-up is performed.

3) The sample and sorbent mixture is centrifuged.

4) The supernatant is transferred to another test tube and evaporated prior to the analysis or directly analysed.

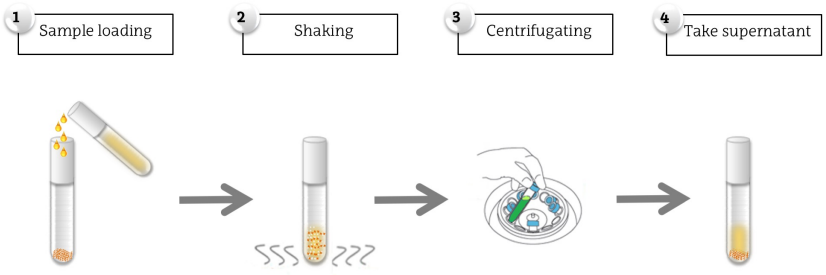

Figure 1.5. Steps involved in dSPE.

As an alternative to SPE, new inexpensive polymeric materials have also been tested in the last years, especially with preconcentration purposes in aqueous samples [174-177]. In order to use as enrichment device, the polymeric material should fulfil analytical requirements related to purity, inertness and stability. Due to their low prize, polymeric materials can be considered for single use. According to the literature, the most commonly used polymeric material for the enrichment of organic pollutants is silicone rod (SR) [178]. SR applicability is mainly focused to the analysis of non-polar organic compounds. The idea of using SR for extraction arrived in 2004 when Popp et al. [179] started to employ silicone materials in the form of rods and tubes for the enrichment of PAHs in water samples. Nevertheless, due to the low extraction efficiency shown by SR for polar compounds, some authors have already proposed new materials, such as the polyethersulfone (PES) [180], raffia [177] or polyurethane foams (PUFs) [181]. In the literature the application of PES for the enrichment of organic compounds is increasing. Villaverde-de-Sáa et al. [174] applied PES polymeric material for the extraction of PFASs from water samples and Ros et al. [175] determined BPA, APs and hormones in water samples using PES. As highlighted above, although in the literature polymeric materials have been used for the extraction and preconcentration of organic compounds from water samples, the use of such materials for the extraction of the target analytes from complex extracts could be a cheap alternative to SPE clean-up.

The above-mentioned approaches require multistep (extraction-clean-up) procedures with intermediate evaporation steps. In this sense, other alternatives have been introduced in the literature, such as matrix solid phase dispersion (MSPD), in order to perform simultaneously the extraction and clean-up steps. MSPD is a patented process first introduced in 1989 by Barker et al. [182] for the extraction of drug residues from bovine tissues. Since then, it has been proved to be an efficient and versatile technique for isolating several classes of substances from a wide variety of solid matrices [183-185]. The success of MSPD is based on the simplicity of the instrumentation required, the flexibility and the robustness compared to the previously described extraction protocols. Besides, extraction and clean-up is performed in a single step, which simplifies the method throughput. The steps involved in the process are the following (see **Figure 1.6**).

1) First, the solid sample is ground with a solid sorbent in a mortar using a pestle in order to disrupt the structure of the raw material breaking the material into smaller pieces and thus, obtaining an homogeneous sample.
2) The blend is transferred to a column.
3) Finally, analytes are eluted or fractionated with an appropriate organic solvent.

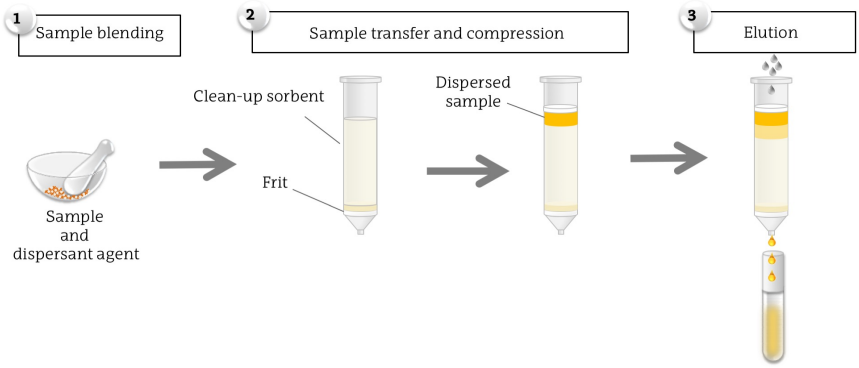

Figure 1.6. Steps involved in MSPD.

In MSPD, selectivity depends on both the nature of the sorbent material and the elution solvent employed [184]. Most of the MSPD applications have been carried out employing lipophilic sorbent materials such as C_{18}-bonded silica. However, during the last decade, the main innovation in MSPD concerns with the employment of new dispersant materials, such as carbon-based [186] and molecular imprinted polymers (MIPs) [187].

MSPD has been applied, among others, to the extraction and clean-up of PBDEs from vegetables [169], flavonoids from fruit peel [188], PCBs, PAHs, PBDEs, organochlorine pesticides (OCPs) and organophosphorous pesticides (OPPs) [189] and halogenated retardants [190] from molluscs and hormones from sludge amended soils [191].

Although mostly applied to liquid samples, microextraction techniques including solid-phase microextraction (SPME) or stir-bar sorptive extraction (SBSE) have also been used for the extraction and preconcentration of organic pollutants from solid samples [192]. SBSE is a simple solventless extraction technique introduced in 1999 by Baltussen and co-workers [193] and, among other advantages, combines the simultaneous extraction and preconcentration of the analytes in a single step, is easy to handle, requires low sample volumes (~ 20 mL), allows reutilisation, can operate overnight without any special requirements, does not present the breakthrough phenomena, is cost effective, can be easily combined with modern analytical instrumentation and has demonstrated a wide range of applications [194, 195].

SBSE consists on a magnetic stir-bar commonly coated with polydimethylsiloxane (PDMS) polymer, commercially known as "Twister". SBSE is based on the interaction between the analyte solved in the sample media and the PDMS liquid polymer. The extraction efficiency is mainly governed by the partition coefficient of the analyte between both phases. The most important variables taken into account in this technique are the extraction time, the pH of the aqueous media, the stirring speed, the addition of organic modifiers and salts, the extraction temperature, the sample volume and the volume of the polymeric material [192].

During the extraction step, the stir-bar is put in direct contact with the liquid sample by immersion or by headspace (HS) sampling (see **Figure 1.7**). In the immersion mode, which is usually abbreviated as SBSE, the polymer coated stir-bar is placed inside the vial containing the sample and the sample is stirred under controlled conditions. Most applications in the literature are performed in the immersion mode [194, 196]. HS sampling mode was extended by Bicchi et al. [197] and is known as headspace sorptive extraction (HSSE). In this case, sampling is performed by hanging the coated stir-bar in the headspace of the vial and the polymeric material is in static contact with the vapour phase of a solid or liquid matrix. The sample is usually stirred and heated in order to favour the presence of the target analytes in the vapour phase. Despite the selectivity of this procedure, fewer are the works applying HSSE [35, 36].

After the extraction by means of SBSE or HSSE, the stir-bar is removed, rinsed with distilled water in order to remove sample components which can affect in the analysis, dried by

means of clean paper tissue to remove water and submitted to desorption. The desorption can be performed thermally (TD) or chemically (using a solvent for the extraction) (see **Figure 1.7**). TD followed by gas chromatography (GC) is most commonly used in SBSE or HSSE to introduce the analytes that are accumulated in the polymer-coated stir-bar quantitatively into the chromatographic system. However, the high sensitivity of the TD requires the use of an expensive unit on the GC: the thermal desorption unit (TDU). Besides, although the TD is the most straightforward desorption mode, it is limited to thermally stable volatile and semi-volatile compounds.

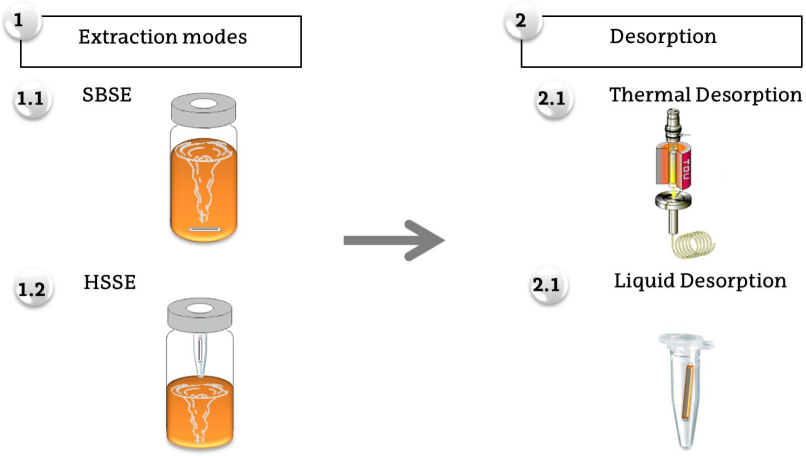

Figure 1.7. Steps involved in SBSE. Immersion (SBSE) or headspace (HSSE) preconcentration followed by either thermal (TD) or liquid (LD) desorptions.

Liquid desorption (LD) is an alternative to TD when thermally labile solutes are analysed, when the separation is carried out using liquid chromatography (LC) or when TDU coupled to GC is not available. In the LD mode, the Twister is immersed in a minimum solvent volume (solvent must guarantee the complete immersion of the coated stir-bar) for the chemical desorption of the extracted solutes. LD is sometimes accelerated by means of mechanical shaking, increasing temperature or sonication.

Recently, in order to overcome the weakness of PDMS coated stir-bar for the extraction of polar compounds, alternative coatings have been developed, but they are relatively expensive and their quality must be further assessed beyond extraction efficiency. Therefore, the development of new SBSE coatings for the simultaneous analysis of various compounds with different polarities is highly expected [196, 198, 199].

1.3.2 Analysis of organic pollutants

An important task of modern analytical chemistry is to provide accurate and reliable detection methodologies for the identification, monitoring and quantification of newly recognised contaminants in environmental samples.

Mass spectrometry (MS) coupled either to GC or LC is mostly [30, 189] used for the analysis of organic pollutants in the environment.

Gas chromatography-mass spectrometry (GC-MS) is the most ubiquitous analytical technique for the identification and quantification of organic substances in complex matrices. As summarised in **Table 1.4**, GC-MS has been widely applied for the analysis of musk fragrances [31, 33, 36], PAHs [30, 189], PPCPs [200, 201], BPA [22, 151, 202, 203], NPs [22] and hormones [17] in matrices such as sludge, soil or vegetables. **Table 1.4** also summarises the main parameters applied for GC-MS analysis of this group of compounds.

Regarding the injection mode, the split/splitless inlet is mostly used due to its simplicity and robustness (see **Table 1.4**). However, in the last decades, in order to improve the sensitivity of GC, large volume injection (LVI) in a programmable temperature vaporiser (PTV) has gained attention over the traditional split/splitless injection mode. The main difference between the split/splitless and the PTV inlets is the temperature control in the latter, which implies that it can be rapidly heated or cooled down during injection whilst the conventional split/splitless mode works isothermally. When used for the injection of large volumes, at the beginning of the injection, the temperature of the inlet is maintained low in order to eliminate the solvent while the analytes are cryofocused and, once the solvent has been eliminated through the split valve, the temperature is increased in order to transfer the target analytes to the GC column [204]. As included in **Table 1.4**, LVI-PTV has been successfully used in the analysis of musk fragrances [205] and PBDEs [38] in sewage sludge, mussels and fish matrices.

TDU coupled to a PTV is another GC inlet commonly applied when extraction is performed by means of HSSE or SBSE. A TDU consists of two PTV injectors. The coated polymer is placed in the first PTV, which is heated at temperatures in the 200-300 ºC range, while the second PTV is kept cool in order to cryofocus the thermally desorbed target analytes. Once the desorption step is over (5-10 min), the second PTV is rapidly heated and the compounds are transferred to the GC column. As included in **Table 1.4** TD-GC-MS has been successfully applied for the determination of musk fragrances [36, 206], BPA [203] and TCS [28] in sewage sludge, canned beverages, soil and sediment matrices.

Table1.4 The main parameters related to the analysis of musks, PBDEs, BPA, TCS and estrogens by means of GC.

Compound	Matrix	Extraction	Clean-up	Derivatisation	Inlet	Column	Detection	LODs (ng/g)	Ref.
Musks	Fish and mussel	QuEChERS (MQ:ACN)	dSPE (1.5 g Florisil)	-	LVI-PTV	ZB-50	GC-EI-IT-MS/MS	0.25-10 [a]	[205]
		PLE	In cell (1.0 g Florisil)						
Polycyclic musks	Sewage sludge and sediment	MA-HS-SPME (MQ)	-	-	TD	DB-5MS	GC-EI-MS	0.04-0.1	[206]
PAHs, PCBs, organochlorine pesticides, musks, polychlorinated alkanes	Sewage sludge	Soxhlet (DCM)	Silica alumina column and GPC (Bio-Beads)	-	-	HP-5MS	GC-EI-MS	-	[30]
Musk fragrances	Sewage sludge	PLE or UAE (Acetone)	SPE (500 mg RP-C_{18} Bulk Sorbent)	-	Splitless	XTI-5/30	GC-EI-MS	250 [b]	[31]
Synthetic musks	Sewage sludge	PLE (Acetone: hexane (1:1))	Silica gel column	-	Splitless	DB5	GC-EI-MS	3-10	[32]
HHCB and AHTN	Soil	PLE (hexane)	SPE (Oasis HLB)	-		HP-5MS	GC-EI-MS	0.19-0.30 [b]	[170]
Polycyclic musks	Sewage sludge	Rotation shaker followed by UAE	Alumina	-	Splitless	DB-5	GC-EI-MS	5-25	[33]
Musks	Sewage sludge	HS-SPME	-	-	Splitless	VA-5MS	GC-EI-IT-MS	0.023-0.611	[34]
Musks	Sewage sludge	HS-SBSE	-	-	TD	ZB-50	GC-EI-MS	5-30	[35]
Musks	Sewage sludge	Agitation (Hexane)	GPC (Bio-Beads SX3) SPE (Silica)	-	On column	HP-35MS	HRGC-IT-MS/MS GC-NCI-MS	100 [c]	[150]

Table 1.4. (Continuation).

Compound	Matrix	Extraction	Clean-up	Derivatisation	Inlet	Column	Detection	LODs (ng/g)	Ref.
Macrocyclic musks	Sewage sludge	HSSE	-	-	Splitless	ZB-50	GC-EI-IT-MS	0.01-0.025 [a]	[36]
Musks	Sewage sludge	PLE-IL-HS-SDME	-	-	Splitless	ZB-50	GC-MS/MS	1-3 [a]	[37]
Pharmaceuticals, fragrances and phenolic estrogens	Lettuce	MSPD	1.0 g Florisil	Post extraction + 10 µL TMSH	Splitless	TRB-5MS	GC-EI-MS	6.6-58	[200]
Musks	Oyster	MA-HS-SPME (MQ)	-	-	Splitless	DB-5MS	GC-EI-MS	0.04	[207]
Musks, UV filters, UV stabilizers	Mussels	QuEChERS (MQ:ACN)	-	-	Splitless	ZB-5	GC-EI-MS/MS	0.5-50 [a]	[208]
AHTN, HHCB	Biosolids amended soil	PLE (ACN:DCM (1:1))	Silica and alumina column	-	Pulsed splitless	DB-5MS	GC-EI-MS	0.3	[119]
AHTN, HHCB, TCS	Carrot, barley, meadow fescue and soil	QuEChERS (EtOAc:Acetone, (1:1))	-	-	-	HP-5MS	GC-EI-MS	1-60 [b]	[114]
BPA and BPB	Vegetables and fruit	QuEChERS (MQ:ACN)	DLLME	In situ + 75 µL AA	Split/Splitless	DB-5MS	GC-EI-MS	0.1-0.4	[202]
BPA	Canned beverages	SBSE	-	In situ + 200 µLAA and 300 mg Na_2CO_3	TD	HP-5MS	TD-GC-MS	0.9-2.5 [d] 4.7-12.5 [a,d]	[203]
BPA, NP, natural estrogens	Vegetables and fruit	UAE (Acetone)	-	Post extraction + 50 µL BSTFA + 50 µL pyridine	Splitless	Rxi-5MS	GC-EI-MS/MS	0.03-0.3	[151]

Table 1.4. (Continuation).

Compound	Matrix	Extraction	Clean-up	Derivatisation	Inlet	Column	Detection	LODs (ng/g)	Ref.
PPCPs	Natural soil	MAE (DCM:MeOH, (2:1))	Column (1.1 g silica gel, ~ 0.5 cm anhydrous sodium sulphate and ~ 0.5 cm activated cupper granules)	Post extraction + MSTFA and 1 % TMCS	Splitless	XTI-5	GC-EI-MS	0.02-2.51 [d]	[201]
BPA, NPs	Vegetable crops	UAE (Acetone)	-		Splitless	Rxi-5MS	GC-EI-MS/MS	0.03-0.1	[152]
NP, NPEO, BPA, TCS	Sewage sludge	Solvent extraction (agitation) (MeOH)	SPE (C_{18}, 500 mg)	Post extraction + 50 µL BSTFA and 50 µL pyridine	Splitless	DB-5MS	GC-MS	40-960	[22]
Parabens, TCS and MeTCS	Soil, sediment and sewage sludge	SBSE	-	In situ + 400 µL AA	TD	HP-5MS	TD-GC-EI-MS	0.08-1.06	[28]
PAHs, PCBs, PBDEs, OCPs, OPPs	Molluscs	MSPD	Simultaneously	-	LVI-PTV	HP-5MS	GC-EI-MS/MS GC-EI-MS	0.010-2.74	[189]
PBDEs	Molluscs	MSPD	Simultaneously (0.3 g Florisil) SPE (0.6 g deactivated silica and 4.0 g activated silica)	-	Split/Splitless	DB-5HT	GC-NCI-MS	0.01-1.6	[190]
PBDEs	Sewage sludge	PLE-MEP_S (Hex:DCM (1:1))	Simultaneously (Florisil and sodium sulphate)	-	LVI-PTV	VF-5MS	GC-IT-MS/MS	6.6-58	[38]
PBDEs, HBB, PBEB, DBDPE, HBCD, TBBPA	Sewage sludge	PLE (MQ:MeOH:Acetone (1:2:1))	SPE (2 g Silica) SPE (5g Alumina)	-	Pulsed splitless	DB-5MS	GC-NCI-MS	0.17-66	[39]

Table 1.4. (Continuation).

Compound	Matrix	Extraction	Clean-up	Derivatisation	Inlet	Column	Detection	LODs (ng/g)	Ref.
PBDEs, MeO-PBDEs and OH-PBDEs	Lettuce, carrot, soil	MSPD	Simultaneously (0.5 g C_{18}) SPE (0.5 g silica and 1.75 g acidified silica)	Post extraction + 50 µL MSTFA	Pulsed Splitless	DB-5HT	GC-NCI-MS	0.03-0.4 [a] 0.02-1.14 [c]	[169]
PBDEs	Marine biological tissues	MAE (n-pentane:DCM (1:1))	GPC (acidic silica gel column)	-		DB-5MS	GC-EI-MS	‹ 0.1 [a]	[209]
PBDEs, OH-PBDEs	Plant, soil	Soxhlet (PBDEs) Cyclohexane, methyltertbutylether (OH-PBDEs)	-	-	Split/splitless	DB-5HT (BDE-209) HP-5MS (PBDEs) Zorbax 5B-C_{18}- PBDEs)	GC-µECD (PBDEs) LC-DAD (OH-PBDEs)	6-2000 [f]	[120]

[a] Method detection limits (MDLs); [b] Limits of quantification (LOQs); [c] ng/mL; [d] ng; [e] ng/L; [f] pg.

AA: acetic anhidride, ACN: acetonitrile, AHTN: tonalide, BPA: bisphenol A, BPB: bisphenol B, BSTFA: N,O-Bis(trimethylsilyl)trifluoroacetamide, d-SPE: dispersive solid phase extraction, DBDPE: decabromodiphenylethane, DCM: dichloromethane, DLLME: dispersive liquid-liquid microextraction, EtOAc: ethyl acetate, GC-EI-IT-MS: gas chromatography-electron impact-ion trap-mass spectrometry, GC-EI-IT-MS/MS: gas chromatography-electron impact-ion trap-tandem mass spectrometry, GC-EI-MS: gas chromatography-electron impact-mass spectrometry, GC-EI-MS/MS: gas chromatography-electron impact-tandem mass spectrometry, GC-IT-MS/MS: gas chromatography-ion trap-tandem mass spectrometry, GC-MS: gas chromatography-mass spectrometry, GC-MS/MS: gas chromatography-tandem mass spectrometry, GC-NCI-MS: gas chromatography-negative chemical ionisation-mass spectrometry, GC-µECD: gas chromatograph with microelectron-capture detection, GC: gas chromatography, GPC: gel permeation chromatography, HBB: hexabromobenzene, HBCD: hexabromocyclodecane, HHCB: galaxolide, HRGC-IT-MS/MS: high resolution-ion trap- tandem mass spectrometry, HS-SPME: head space solid phase microextraction, HS-SBSE: head space stir bar sorptive extraction, HSSE: head space stir bar sorptive extraction, LC-DAD: liquid chromatography-diode array detector, LOD: limit of detection, LVI-PTV: large volume injection in a programmable temperature vaporisation injector, MA-HS-SPME: microwave assisted headspace solid phase microextraction, MAE: microwave assisted extraction, MeO-PBDE: methoxylate polybrominated diphenyl ether, MeOH: metanol, MeTCS: methyltriclosan, MQ: Milli-Q, MSPD: matrix solid phase dispersión, MSTFA: N-methyl-N-(trimethylsilyl) trifluoroacetamide, NP: nonylphenol, NPEO: nonylpheol ethoxylate, OCP: organochlorine pesticide, OH-PBDE: hydroxilate polybrominate diphenyl ether, OPP: organo phosphorous pesticide, PAH: polycyclic aromatic hydrocarbon, PBDE: polybrominated diphenyl ether, , PBEB: pentabromoethylbenzene, PCB: polychlorinated biphenyls, PLE-IL-HS-SDME: pressurised liquid extraction coupled to automated ionic liquid-based head space single-drop microextraction, PLE-MEP: microextraction by packed sorbent following selective pressurised liquid extraction, PLE: presurised liquid extraction, PPCP: personal and pharmaceutical care product, QuEChERS: Quick Easy Cheap Effective Rugged Safe, SBSE: stir bar sorptive extraction, SPE: solid phase extraction, TBBPA: tetrabromobisphenol A, TCS: triclosan, TD-GC-MS: thermal desorption-gas chromatography-mass spectrometry, TD: thermal desorption, TMCS: trimethylchlorosilane, TMSH: trimethyl sulfonium hydroxide, UAE: ultrasound assisted extraction, UV: ultra violet.

As can be observed in the examples included in **Table 1.4**, GC-MS has been mostly applied to non-polar or slightly non-polar compounds and, therefore, the most common GC columns used are 5 % phenyl-95 % dimethylpolysiloxane columns, although slightly more polar columns (50 % phenyl-50 % dimethylpolysiloxane) have also been applied for the analysis of musk compounds.

When molecules with active hydrogen atoms (alcohols, carboxylic acids, aldehydes, amines, thiols) are analysed, tailing often occurs due to the interaction between intermolecular hydrogen bonds and GC column packing material. In order to avoid this tailing and to increase volatility and thermal stability of such compounds, derivatisation is performed prior the chromatographic separation. The derivatisation can be performed ***in situ*** or post extraction. Acylation with acetic anhydride is a typical ***in situ*** derivatisation reaction. However, when derivatisation is carried out post extraction silylation, reagents such as trimethylsulfonium hydroxide (TMSH), N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) and N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) are the most common derivatisation agents for the determination of hormones [151], BPA [151, 202, 203], NPs [22], OH-PBDEs [169], TCS [28] and PPCPs [201] (see **Table 1.4**).

Concerning to the detection, GC coupled to single MS with electron impact (EI) ionisation mode is mostly used (see **Table 1.4**). However, in the literature, the application of GC coupled to tandem mass spectrometry (MS/MS) is increasing in order to gain selectivity [189, 205]. Besides, as EI ionisation mode is too harsh for some organic compounds, including PBDEs, chemical ionisation (CI) is also used [39, 169, 190].

Apart from the GC-MS, in the last decades LC coupled to single or tandem mass spectrometry (LC-MS(/MS)) has drawn attention in order to accomplish the analysis of molecules not suitable for GC-MS due to their high polarity, lack of volatility or thermal instability. In this sense, tedious and often environmentally unfriendly derivatisation steps can be avoided. However, LC-MS(/MS) suffers often matrix effect during the ionisation process, often leading to signal enhancement or suppression [210, 211].

The main parameters related with the LC-MS(/MS) analysis of some PFASs, hormones, APs, BPA and PPCPs are summarised in **Table 1.5**. Since the 1960s, the pressure limits of high performance liquid chromatography (HPLC) systems remained stagnant (at 400 bar). This pressure limit was appropriate for columns packed with particles of 3 μm or larger. However, in order to increase the resolution, the trend is to use chromatographic columns with particle sizes

lower than 2 μm, leading to what is known as ultrahigh performance liquid chromatography (UHPLC). UHPLC requires of pumps capable of providing pressures higher than 600 bar. As can be observed in **Table 1.5**, UHPLC has been used for the determination PFASs [171, 212-214], PBDEs [215], tetrabromobisphenol A bis 2,3-dibromopropylether (TBBPA-bis) [215], tetrachlorobisphenol A (TCBPA) [215], tetrabromobisphenol A (TBBPA) [215], hexabromocyclododecanes (HBCDs) [215] and PPCPs [29]. An alternative to UHPLC is the use of superficial porous particles (also known as core-shell) for column packaging which also provide an increase in resolution [216-219]. For instance, core-shell columns have been successfully applied to the analysis of PFASs [171, 213] and PAPs [41].

Regarding the stationary phase, reverse phase chromatography based on the octadecyl (C_{18}) are most commonly used for the determination of PFASs [163, 166, 171, 212-214], PAPs [41], hormones [19], APs [220], BPA [221], TCS [119] and PPCPs [29] in environmental samples as can be seen in the literature (see **Table 1.5**).

Apart from the stationary phase, LC separation is strongly dependant on the mobile phase composition. Water, methanol or acetonitrile and buffers such as ammonium acetate, formic acid or acetic acid are commonly used. Recently, in order to increase the resolution in the analysis of per- and polyfluorinated compounds ion-pair reagents such as 1-methylpyperidine have been applied [163, 222-224].

Most commonly used ionisation sources in LC-MS(/MS) are electrospray (ESI), atmospheric pressure chemical ionisation (APCI) and, in a less extent, atmospheric pressure photoionisation (APPI). The main difference between ESI and APCI is the spray formation. ESI generates ions from the mobile phase before analyte reaches the mass spectrometer. The eluent is nebulised into a chamber at atmospheric pressure in the presence of a strong electrostatic field and drying gas. The electrostatic field causes further dissociation of the analyte molecules and the drying gas shrinks the solvent droplets. The more the droplets shrink, the higher the charge concentration in the droplets and, at the moment when the repulsive forces exceed the cohesive forces, the ions in solution are transformed into ions in the gas phase which are conducted through a capillary sampling into the mass analyser. This ion source is applied to polar or ionic organic molecule analysis. Although, susceptible to signal enhancement or suppression due to the high matrix effect on ESI [225-229], this source is mostly used. As can be observed in **Table 1.5**, ESI has been used in the analysis of PFASs [163, 212, 213, 230], PAPs [231, 232], OH-PBDEs [40, 121], oestrogens [19], TCS [119], APs [220] and PPCPs [29].

Table 1.5. The main parameters related to the analysis of some PFASs, estrogens, BPA, OH-PBDEs and TCS by LC.

Compound	Matrix	Extraction	Clean-up	Detection	Mobile phase	Column	LODs (ng/g)	Ref.
PFASs	Soil, vegetables, fish	FUSLE (ACN:MQ (9:1))	SPE (150 mg WAX)	LC-ESI-MS/MS (QqQ)	Water:metanol with 2 mM NH_4OAC and 5 mM 1-MP	Zorbax Extend C_{18} (1.8 µm, 2.1 x 50 mm,)	0.2-15 [a] 0.46-2.47 [b]	[163]
PFASs	Food	Ion-pair extraction (TBA)	SPE (1.5 g Florisil and 25 mg ENVI-carb)	UPLC-ESI-MS/MS (QqQ)	Water:methanol containing 2 mM NH_4OAC	BEH C_{18} (1.7 µm, 2.1 x 50 mm)	(0.3-6.6) 10^{-3}	[212]
di-, tri-PAPs, PFPAs	Sewage sludge	Ultrasound bath (THF:HAc (1:1))	SPE (150 mg WAX in line coupled to 250 mg ENVI-carb)		Water:methanol containing 0.5 % formic acid	Kinetex C_{18} (3 µm, 4.6 x 50 mm)	0.6-5.1 [c]	[41]
PFOA and PFOS	Honey	dSPE (ACN:formic acid)		µ-UHPLC-ESI-MS/MS	Water:acetonitrile containing 1.0 % HCOOH	HALO C_{18} (2.7 µm, 0.5 x 50 mm)	0.016-0.40	[213]
PFASs	Sewage sludge	FUSLE (ACN)	Washed twice with solvent	UPLC-QTOF-MS	Water:acetonitrile containing 0.1 % HCOOH	Acquity BEH C_{18} (1.7 µm, 2.1 x 50 mm)	0.06- 0.2	[44]
PFASs	Water, sediment, fish, meat	UAE	SPE (200 mg STRATA-x)	UPLC-QqTOF-MS	Water:methanol containing 10 mM NH_4OOH	Poroshell 12 D EC-C_{18} (2.7 µm)	0.03-8 [b]	[171]
PFASs	Vegetables	Ion pair (MTBE)	SPE (sodium sulphate and florisil)	UPLC-MS/MS	Water:methanol containing 2 mM NH_4OAc	Acquity UPLC MSS3T (1.8 µm, 2.1 x 100 mm)	< 0.10 [a]	[214]
PFASs	Soil, carrot, potatoes, cucumber	UAE (ACN)	dSPE (PSA)	LC-ESI-MS/MS	Water:methanol containing 2 mM NH_4OAc	LUNA C_8 (5 µm, 2.0 x 100 mm)	0.03-0.14	[230]
OH-PBDEs	Human tissues	Solvent extraction (Hexane:DCM, (1:1))	-	LC-ESI-QqLIT-MS-MS	Water and methanol: acetonitrile containing HAc/NH_4OAc buffer to keep pH at 10	Purospher STAR RP-18	0.15-0.72 [d]	[233]
OH-PBDEs	Sewage sludge	MSPD	Simultaneously (4 g acid silica) SPE (2 g anhydrous sodium sulphate, 8 g silica gel)	LC-ESI-MS/MS	Water:acetonitrile	C_{18} (2.2 µm, 2.1 x 100 mm)	MLOQ 0.003-0.015 ng/g	[40]
PBDEs, TBBPA-bis, TCBPA, TBBPA, HBCDs	Sewage sludge	PLE (Toluene)	Simultaneously (20 g alumina)	UPLC-APCI-MS/MS UPLC-ESI/MS/MS	Methanol:water containing toluene Methanol water	BEH C_{18} (1.7 µm, 2.1 x 150 mm)	0.041-9.9 [d]	[215]

Table 1.5. (Continuation).

Compound	Matrix	Extraction	Clean-up	Detection	Mobile phase	Column	LODs (ng/g)	Ref.
BPA and its chlorinated derivatives	Sewage sludge	PLE		LC-APCI-MS/MS	Water:methanol containing 0.025% ammonia	Gemini C_{18} (3 µm, 2.0 x 100 mm)	4-8	[221]
Estrogens	Sewage sludge	MAE (MeOH)	SPE (500 mg Sep-Pak Vac C_{18})	LC-ESI-MS/MS	Water:methanol containing 0.1 % glacial acetic acid and 15 mM NH_4OAc	Pursuit XRs Ultra C_{18} (2.8 µm, 2 x 50 mm)	0.6-3.5	[19]
BPA and tebuconazole	Vegetables and juice	m-MISPE		LC-UV	Water:acetonitrile:acetic acid (47:50:3)	Octadecyl silane (5 µm, 4.6x 250 mm)	0.025-0.2 [e]	[234]
EE2 and TCS	Plant, sand, soil	Solvent extraction (agitation)		LC-UV	Water: acetonitrile	Alltech Prevall C_{18} (5 µm, 4.6 x 250 mm)	100-120 [a]	[234]
TCC, TCS	Biosolids amended soil	UAE (EtOAc)	Silica gel column	LC-ESI-MS/MS	Water:acetonitrile	SB-C_{18} (1.8 µm, 3 x 100mm)	0.12-0.47	[119]
PPCPs	Sewage sludge	QuEChERS (ACN)	dSPE (300 mg $MgSO_4$, 125 mg PSA)	UPLC-ESI-MS/MS	Water:methanol containing 0.1 % HCOOH	Acquity UPLC BEH C_{18} (1.7 µm, 2.1 x 50 mm)	0.15-3 [a]	[29]
4-tOP and 4NP	Laboratory animal feed	SBSE	-	LC-ESI-MS	Water:acetonitrile containing 0.02 % NH_4OAc	Miggtysil RP-18 GP (5 µm, 2.0 x 100 mm)	0.2-1	[220]
NPEOs	Vegetables and crops	Solvent extraction (ACN)	SPE (ENVI-carb II/PSA)	LC-ESI-MS/MS	Methanol 10 mM NH_4OAC	Phenomenex Luna C_{18} (3 µm, 2 x 150 mm)	0.1-5	[154]

[a] Method detection limits (MDLs), [b] ng/mL, [c] Method quantification limits (MQLs), [d] pg; [e] µmol/L,.

4-tOP: 4-tert-octylphenol, 4NP: 4-nonylphenol, ACN: acetonitrile, BPA: bisphenol A, dSPE: dispersive solid phase extraction, DCM: dichloromethane, EE2: ethinyl estradiol, EtOAc: ethyl acetate, FUSLE: focused ultrasound solid-liquid extraction, HAc: acetic acid, HBCD: hexabromocyclododecane, Hex: hexane, LC-APCI-MS/MS: liquid chromatography-atmospheric chemical ionisation-tandem mass spectrometry, LC-ESI-MS: liquid chromatography-eletrospray ionisation-mass spectrometry, LC-ESI-MS/MS (QqQ): liquid chromatography-eletrospray ionisation-tandem mass spectrometry, LC-ESI-QqLIT-MS-MS: liquid chromatography-electrospray tandem triple quadrupole-linear ion trap mass spectrometry, LC-UV: liquid chromatography coupled with ultraviolet spectroscopy, LOD: limit of detection, MAE: microwave assisted extraction, MeOH: metanol, m-MISPE: membrane-based molecularly imprinted solid phase extraction, MQ: Milli-Q, MSPD: matrix solid pahse dispersión, MTBE: methyl tert-butyl ether, NPEO: nonylphenol ethoxylate, OH-PBDE: hydroxilate polybrominate diphenyl ether, PAP: perfluoroalkyl phosphate, PBDE: polybrominated diphenyl ether, PFAS: perfluoroalkyl substances, PFOA: perfluorooctanoic acid, PFOS: perfluorooctane sulfonate acid, PFPA: perfluorinated phosphonic acid, PLE: pressurised liquid extraction, PPCP: pharmaceutical and personal care product, PSA: primary secondary amine, QuEChERS: Quick Easy Cheap Effective Rugged Safe, SBSE: stir bar sorptive extraction, SPE: solid phase extraction, TBA: tetrabutylammonium, TBBPA: tetrabromobisphenol A, TCBPA: tetrachlorobisphenol A, TCC: triclocarban, TCS: triclosan, THF: tetrahydrofuna, UAE: ultrasound assisted extraction, UPLC-APCI-MS/MS: ultrahigh pressure liquid chromatography-atmospheric pressure chemical ionisation-tandem mass spectrometry, UPLC-ESI-MS/MS (QqQ): ultrahigh pressure liquid chromatography-eletrospray ionisation-tandem mass spectrometry, UPLC-QqTOF-MS: ultrahigh performance liquid chromatography and hybrid quadrupole time of flight mass spectrometry, UPLC-QTOF-MS: ultra performance liquid chromatography coupled to quadrupole time of flight mass spectrometry, WAX: weak anion Exchange, µ-UHPLC-ESI-MS/MS: microultra high pressure liquid chromatography-eletrospray ionisation tandem mass spectrometry.

APCI is commonly used to analyse smaller, thermally stable polar to slightly non-polar compounds. In the APCI source, the eluent is introduced in the interface and although no potential is applied due to the heat and nebulising gas, an aerosol is formed. At the end of the heating area, a pin (corona electrode) produces an electrical discharge that forms plasma where the molecules within the aerosol are ionised in the gas phase. Although less applied than ESI, APCI has been applied for the determination of PBDEs [215] and BPA and its chlorinated derivatives [221].

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CHAPTER 2

Aims and objectives

The management of sewage sludge is becoming an issue of growing importance. For years, sewage sludge has been dumped to the sea, incinerated or used in landfilling. However, due to the problems related to the previously applied sewage sludge final destines, recycling as biosolid or compost for further use as fertiliser in the agriculture in order to improve and maintain the productivity of soils and stimulate plant growth, has become one of the most promoted uses of sewage sludge. Actually, nowadays, the 50 % of sludge is being recycled as biosolid or compost. Numerous studies have demonstrated that sewage sludge contains a number of inorganic and organic pollutants, as well as pathogens, which might be transferred from soil to plants cultivated in biosolid/compost-amended soils and exert effects on the risk for human health. Within this scenario, and with the financial support of the Spanish Ministry of Science and Education through the project CTM2011-24094, the following objectives of the present PhD work were established:

1. Optimisation of different analytical methodologies for the determination of organic pollutants in vegetables (lettuce, pepper and carrot), as well as compost-amended soils, in order to have robust and reliable analytical methods. Among the different possible target analytes, the following were selected:

    - ✓ Polybrominated diphenyl ethers (PBDEs)
    - ✓ Musk fragrances
    - ✓ Hormones, alkylphenols (APs), bisphenol A (BPA) and triclosan (TCS)
    - ✓ Per- and polyfluorinated alkyl substances (PFASs)

2. Study of the uptake and translocation of organic pollutants by carrot and lettuce crops cultivated in different compost-amended soils. In order to understand the influence of the nature of the analyte, the crop or the soil type in the uptake of pollutants, the following experiments were designed:

    - ✓ Uptake of Penta-BDE mixture, BDE-99 (2,2',3,4,4'-pentabromodiphenyl ether), BDE-138 (2,2',3,4,4',5'-hexabromodiphenyl ether) and BDE-209 (decabromodiphenyl ether) by lettuce (***Batavia Golden Spring*** and ***Summer Queen*** varieties) and carrot (***Chantenay*** and ***Nantesa*** varieties) crops cultivated in three compost-amended soils (soil 2.1, soil 2.4 and substrate) with different total organic content (TOC).

- ✓ Uptake of perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS) and perfluorooctanesulfonamide (FOSA) by lettuce (***Batavia Golden Spring*** variety) and carrot (***Chantenay*** and ***Nantesa*** varieties) cultivated in two compost-amended soils (soil 2.4 and substrate) with different TOC.
- ✓ Uptake of 8:2 diPAP by lettuce (***Batavia Golden Spring*** variety) and carrot (***Chantenay*** variety) cultivated in two compost-amended soils (soil 2.4 and substrate) with different TOC.
- ✓ Uptake of tonalide (AHTN), galaxolide (HHCB) and BPA by carrot (***Chantenay*** variety) cultivated in compost-amended soil 2.4.

CHAPTER 3

Focused ultrasound solid-liquid extraction for the determination of PBDEs in vegetables and compost-amended soil

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3.1 Introduction

Polybrominated diphenyl ethers (PBDEs) are the most frequently used brominated flame retardants (BFRs). There are three commercial technical mixtures of PBDEs: penta-BDE, octa-BDE and deca-BDE, which are composed of a mixture of congeners and named according to their average bromine content. Congeners 2,4,4'-tribromodiphenyl ether (BDE-28), 2,2',4,4'-tetrabromodiphenyl ether (BDE-47), 2,2',3,4,4'-pentabromodiphenyl ether (BDE-99), 2,2',4,4',5-pentabromodiphenyl ether (BDE-100), 2,2',4,4',5,5'-hexabromodiphenyl ether (BDE-153), 2,2',4,4',5,6'-hexabromodiphenyl ether (BDE-154), 2,2',3,4,4',5',6-heptabromodiphenyl ether (BDE-183) and decabromodiphenyl ether (BDE-209), which are relevant for dietary exposure, are considered as primary interest congeners by the Panel on Contaminants in the Food Chain (CONTAM Panel) due to their occurrence in the composition of the technical BDE mixture, in the environment and in food [1].

PBDEs have been used in a wide array of products, including building materials, electronics, furnishings, motor vehicles, airplanes, plastics, polyurethane foams, textiles and so on. Some of them may be covalently bound into materials during production, but most of them are simply additives. Consequently, they can be released from these products during their production, use, disposal and recycling processes and, as a consequence, PBDEs can leach into the environment and reach animals and humans through water, food chain and dust [2-4]. Although their acute toxicity is low, recently, concerns over the persistence, ability to bioaccumulate and potential for toxicity of the most widely used BFRs, have led to increasing regulation and restrictions on their production and use [5-7]. For example, in 2008 the use of deca-BDEs was banned in electrical and electronic applications in the European Union (EU), while penta- and octa-BDEs have been added to the Persistent Organic Pollutants (POPs) list of the Stockholm Convention (http://chm.pops.int/Programmes/NewPOPs/The9newPOPs/tabid/672/%20language/en-US/Default.aspx) [8].

In spite of the processes that influent water is submitted to in wastewater treatment plants (WWTPs), potential harmful substances, including PBDEs, are present in both effluent water and sewage sludge, which are a mirror of the chemical and products consumed in modern society [9]. PBDEs are routinely detected in sewage sludge in the low mg/kg range and values have been reported from Sweden [4, 10, 11], USA [12, 13], Germany [14], The Netherlands [15], China [16], Australia [17] and Kuwait [18].

Meanwhile, agricultural application of sewage sludge has become the most widespread

method for disposal of sludge since it is the most economical outlet for sludge and offers the opportunity to recycle plant nutrients and organic matter to soil for crop production [19]. At present, around 40 % of the sewage sludge produced in Europe is used as a fertiliser in agriculture [20]. In general, the EU considers that the re-use of sludge should be encouraged since it represents a long-term solution, provided that the quality of the sludge re-used is compatible with public health and environmental protection requirements [21]. However, concern has increased due to the presence of heavy metals, organic contaminants and pathogenic bacteria in sewage sludge. According to Clarke and Smith [22], PBDEs are included as emerging organic contaminants to be studied in biosolids with agricultural purposes since the contamination of sludge and effluents with PBDEs could have potential implications for disposal and beneficial reuse strategies. One way to study the introduction of organic contaminants to humans via the food chain is to study the uptake of such pollutants by different crop plants. Within this scenario, the measurement of PBDEs in sludge amended soil and crops have gained importance [23, 24].

Effective sample pre-treatment, including extraction and clean-up procedures, are compulsory prior to the instrumental analysis with the aim of identification and accurate determination of PBDEs in a variety of solid matrices. Different solid-liquid extraction techniques such as the classical Soxhlet, which requires 4-24 h extraction, has been used for years [25-27]. Several faster extraction techniques have been developed to reduce both the extraction time and the solvent consumption, including microwave assisted extraction (MAE) [28-30] and pressurised liquid extraction (PLE) [31-33]. Recently, focused ultrasound solid-liquid extraction (FUSLE) has gained interest due to its simplicity, low cost and the improved efficiency and reproducibility compared to classical ultrasound baths. FUSLE has been recently applied to extract PBDEs from solid matrices such as dust [34] but no applications for the analysis of PBDEs in vegetables and amended soil are found in the literature.

Due to the lack of selectivity of the above mentioned extraction techniques, a clean-up step is also necessary before the analysis step. In the case of PBDEs for almost all the matrices solid phase extraction (SPE) or Gel Permeation Chromatography (GPC) have been mostly used [2, 6, 29, 35-39].

In the present work, FUSLE combined with SPE clean-up was optimised for the determination of PBDEs in vegetables (lettuce, carrot and pepper) and compost-amended soil. The FUSLE extraction was also compared with MAE.

3.2 Experimental section

3.2.1 Cleaning protocol

All laboratory material was washed with a common detergent, rinsed with abundant Elix water (Millipore,Bedford, MA, USA), sonicated in an acetone bath and maintained there for 24 h. Afterwards the material was rinsed with Milli-Q water (‹0.05 μS/cm, Milli-Q model 185, Millipore). All glassware material, except for the volumetric one, was dried in an oven at 120 °C for at least 4 h. In the case of test tubes, the same procedure was employed but later the material was dried in a muffle oven at 400 °C for at least 4 h in order to remove all PBDE traces and decrease blank signal.

3.2.2 Reagents and materials

PBDEs (in cyclohexane) at 10 ng/μL concentration level were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The dilutions at lower concentrations were daily prepared according to the experimentation. All the chemical standards were stored in the dark at 4 °C and the stock solutions at -20 °C. Reference material (PBDEs SQC072 in sediment) was supplied by Sigma-Aldrich (Milwaukee, USA).

Isooctane, n-hexane, acetone, dichloromethane (DCM) and toluene (all HPLC grade) were purchased from LabScan (Dublin, Ireland) and copper (powder Cu) from Merck (Darmstadt, Germany). For filtration, 0.45 μm, 25 mm polyamide filters (Macherey Nagel, Düren, Germany) were used. LC-Florisil (2 and 5 g) and LC-silica (2 g) cartridges were purchased from Supelco (Walton-on-Thomas, UK) in order to carry out the clean-up step.

H_2 gas was used as carrier gas in the detection step and it was obtained by the Hydrogen Generator AD-1020 (CINEL Strumenti Scientifici, Vigonza, Padova, Italy).

3.2.3 Instrumentation

Samples were frozen and freeze-dried at low temperature (~ -50 °C) using a Cryodos-50 laboratory freeze-dryer from Telstar Instrument (Sant Cugat del Valles, Barcelona, Spain). For sample extraction, a Sonoplus HD 3100 ultrasonic homogeniser (Bandelin Electronic, Berlin, Germany) equipped with a MS 73 titanium microtip and a Mars X CEM (Matthews, NC, USA) microwave oven, which belongs to Central Analysis Service of Advanced Research Facilities of the University of the Basque Country, were used. All the fractions were evaporated in a Turbovap LV Evaporator (Zymark, Hopkinton, MA, USA) using a gentle stream of nitrogen (Carburos,

Barcelona, Spain). The SPE clean-up step was performed using Visiprep SPE manifold which was provided by Supelco (Bellefonte, PA, USA). The extracts were analysed on an Agilent 6890N gas chromatography (GC) coupled to an Agilent 5975 N mass spectrometer (MS) (Agilent Technologies, Avondale, PA, USA).

3.2.4 Spiking of samples

All matrices were freeze-dried (see **section 3.2.3**), homogenised in a glass mortar and fortified with target analytes at two concentration levels: 6 ng/g and 58 ng/g. A known amount of matrix was weighed, covered with acetone, spiked with PBDEs and stirred during 12 h. After that, acetone was evaporated and the sample was aged for 2 weeks. When pepper matrix was spiked, instead of acetone, n-hexane was added since when acetone was used a non-homogeneous fortified sample was obtained.

3.2.5 Focused ultrasound solid-liquid extraction

PBDEs were extracted from amended soil, carrot, lettuce and pepper using an adaptation of a previously published method [40]. A sample aliquot of 0.5 g was weighed, 10 mL of acetone was added and the vessel was immersed in an ice-water bath (~ 0 °C) for extraction. In the case of amended soil samples, 0.5 g of activated copper (previously treated with HNO_3 30 %, rinsed with ultrapure water and DCM, and dried at 50 °C) was added to eliminate sulphur from the soil, which might interfere during the chromatographic analysis [6, 27, 41]. According to Errekatxo et al. [40], samples were exposed to ultrasonic energy at 20 % power and 7 cycles during the optimised extraction time (2 min). Non-fortified extracts were processed in parallel for blank analysis. The supernatant was filtered through a polyamide filter and the extract was evaporated to ~ 1 mL in a Turbovap LV Evaporator using a nitrogen blown-down after the addition of isooctane. Isooctane addition was carried out in order to prevent analyte losses during evaporation and guarantee that the concentrated extract was enriched in a non-polar solvent before SPE clean-up [40, 42].

3.2.6 Microwave assisted extraction

The MAE method was based on EPA 3546 method [43]. Briefly, 0.5 g of dried sample was weighed and transferred to the Teflon microwave vessel, 10 mL of acetone was added and the following extraction conditions were studied:

a) Oven set to a power of 1200 W, ramped to 115 °C within 15 min and held for 10 min.

b) Oven set to a power of 1200 W, ramped to 90°C within 15 min and held for 10 min.

When the irradiation period was completed, samples were removed from the microwave cavity and were allowed to cool down to room temperature before opening. The supernatant was filtered through a polyamide filter and the extract was treated according to strategies described in **section 3.2.7**. In the case of the amended soil, ~0.5 g of activate copper were also weighed and added in the Teflon vessel.

3.2.7 Clean-up of the extracts

Different strategies were studied for the clean-up of the extracts. Strategies a, b and c were applied in the case of FUSLE extracts cleaning-up, while c and d protocols were used with the same objective after MAE.

a) 200 µL of the concentrated extract was loaded onto a 2-g Florisil cartridge, previously conditioned using 5 mL of n-hexane, and the target analytes were eluted with 18 mL of a (80:20, v:v) n-hexane: toluene mixture.

b) 200 µL of the concentrated extract was loaded onto a 2-g silica cartridge, previously conditioned using 5 mL of n-hexane, and the target analytes were eluted with 18 mL of a (80:20, v:v) n-hexane:toluene mixture.

c) 200 µL of the concentrated extract was loaded onto a 5-g Florisil cartridge, previously conditioned using 15 mL of n-hexane, and the target analytes were eluted with 25 mL of a (80:20, v:v) n-hexane:toluene mixture.

d) 200 µL of the concentrated extract was loaded onto a 10-g Florisil cartridge, previously conditioned using 20 mL of n-hexane, and the target analytes were eluted with 40 mL of a (80:20, v:v) n-hexane:toluene mixture.

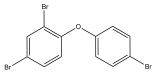
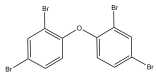
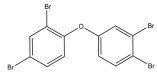
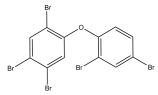
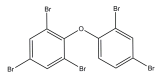
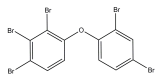
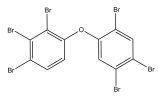
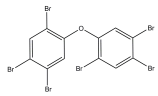
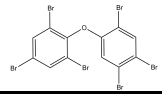
In all the cases the eluate was evaporated to dryness and then reconstituted in 120 µL of n-hexane before gas chromatography-mass spectrometry (GC-MS) analysis.

All the extracts were introduced into a HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm). PBDEs were separated using the following oven temperature programme: 60 °C (hold 1 min), temperature increase at 7.0 °C/min up to 300 °C, where it was finally held for 15 min (carrier gas H_2 at 1.3 mL/min flow-rate).

The MS was operated in the electron impact ionisation mode (EI) and the energy of the electrons was kept at 70 eV. The interface temperature was set at 310 °C and the ionisation source and the quadrupole temperatures at 230 °C and 150 °C, respectively. Measurements were

performed in the selected-ion-monitoring (SIM) mode and the ions monitored for each analyte are listed in **Table 3.1**. The first ion was used as quantifier and the second one as qualifier.

Table 3.1. Chemical structure, CAS number, log P_w and the ions monitored for each analyte studied. First ion was used as quantifier and the second one as qualifier.

Polibrominated diphenyl ethers (PBDEs)	Structure	CAS number	m/z	ACD/LogP[a]
2,4,4′-tribromodiphenyl ether (BDE-28)		41318-75-6	406/408	6.70
2,2′,4,4′-tetrabromodiphenyl ether (BDE-47)		5436-43-1	486/484	7.39
2,3′,4,4′-tetrabromodiphenyl ether (BDE-66)		189084-61-5	486/484	7.47
2,2′,3,4,4′-pentabromodiphenyl ether (BDE-99)		CASID30342	404/564	8.19
2,2′,4,4′,5-pentabromodiphenyl ether (BDE-100)		189084-64-8	404/564	8.03
2,2′,4,4′,6-pentabromodiphenyl ether (BDE-85)		182346-21-0	404/564	8.02
2,2′,3,4,4′,5′-hexabromodiphenyl ether (BDE-138)		182677-30-1	484/644	7.73
2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153)		68631-49-2	484/644	8.98
2,2′,4,4′,5,6′-hexabromodiphenyl ether (BDE-154)		207122-15-4	484/644	8.83

[a] Values reported in The Free Chemicals Data Base: http://www.chemspider.com/.

3.3 Results and discussion

3.3.1 Optimisation of the clean-up step

In order to optimise the clean-up step for PBDEs, non-fortified carrot was extracted with

10 mL of acetone using FUSLE (2 min at 20 % power and 7 cycles) and the extract was spiked at 240 ng/mL concentration level. Both Florisil and silica cartridges were tested due to their wide applicability to lipid removal from the samples [5, 6, 35]. In the case of Florisil, 2-g and 5-g cartridges were evaluated, while only 2-g cartridges were used in the case of silica (see **section 3.2.7**). The study was repeated in triplicate for each of the cartridges used and the results can be observed in **Figure 3.1**. In the case of 2-g Florisil cartridges, recoveries exceeding 100 % were obtained for most of the PBDEs studied, especially for the lighter congeners, with recovery values up to 156 %. The results obtained for 2-g Florisil cartridges clearly indicate the presence of co-eluting interferences. In the case of 5-g Florisil and 2-g silica cartridges, similar results (according to analysis of variance, ANOVA test performed at 95 % of confidence level) were obtained for BDE-28, BDE-47, BDE-66, BDE-99, BDE-100 and BDE-154 ($F_{Experimental}$ = 1.13-6.44 ‹ $F_{Critical}$ = 10.13), while better recoveries were obtained for 5-g Florisil in the case of the heavier PBDEs (BDE-85, BDE-138 and BDE-153) ($F_{Experimental}$ = 10.93-13.13 › $F_{Critical}$ = 10.13). Finally, 5-g Florisil cartridges were chosen as optimum sorbent and used in further experiments.

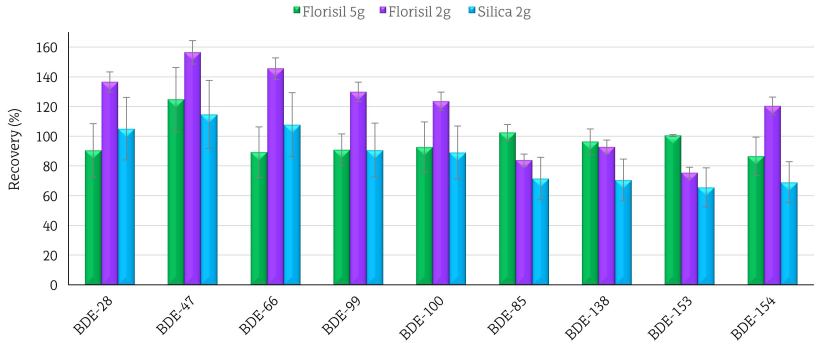


Figure 3.1. Recoveries (%) obtained for spiked carrot extracts (n=3) when 2-g and 5-g Florisil cartridges and 2-g silica were used as sorbents.

Further experiments were performed in order to fix the elution volume when 5-g Florisil cartridges were used (previous experiments had been performed with 25 mL of a (80:20, v:v) n-hexane: toluene mixture). Aliquots were separately collected every 3 mL. According to the results in **Figure 3.2**, 15 mL volume was enough for quantitative recovery (99-106 %) of target analytes. Therefore 15 mL of the elution solvent were used in further experiments.

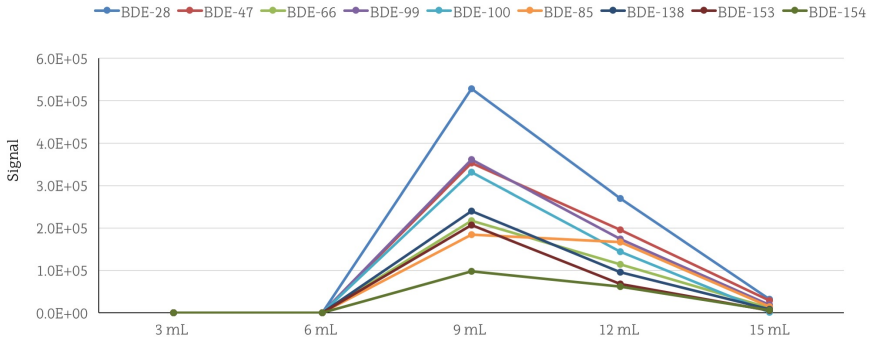


Figure 3.2. Solvent elution profile for 5-g Florisil cartridges using 3-15 mL of n-hexane:toluene (80:20, v/v) as elution solvent mixture.

3.3.2 FUSLE vs MAE

FUSLE and MAE were applied and compared in terms of the analysis of PBDEs in vegetables (lettuce, carrot and pepper) and amended soil. In a first attempt, FUSLE extraction conditions optimised previously in a published work for the determination of different organic contaminants including polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), phthalate esters (PEs) and alkylphenols (APs) [40] were used. MAE method was based on EPA 3546 [43].

In the case of FUSLE extraction, although in the Errekatxo et al. [40] method 2 min FUSLE extractions were enough for quantitative extractions, 1 x 2 min, 2 x 2 min, 3 x 2 min, 1 x 3 min, 2 x 3 min and 3 x 3 min extraction periods were also evaluated here in order to see whether extraction recoveries could be improved in order to obtain exhaustive extraction. For that purpose, new 10 mL solvent fractions were used and collected in different test tubes. The recoveries obtained are included in **Figure 3.3**. According to the ANOVA of the results, no significant differences were observed ($F_{Experimental}$ = 1.00-3.03 < $F_{Critical}$ = 4.07) and, therefore, one single 2-min extraction was finally chosen as optimum.

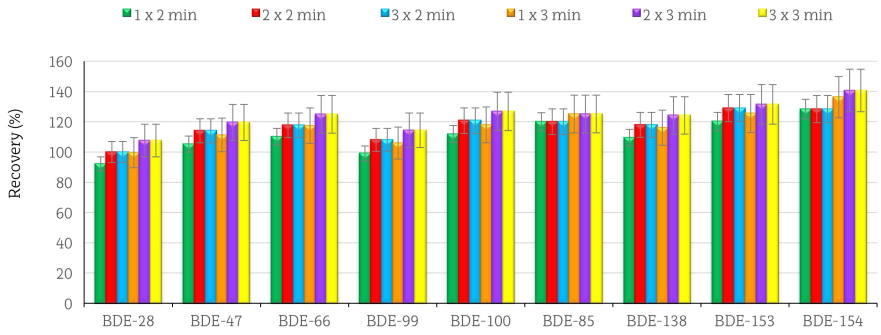


Figure 3.3. Recoveries (%) obtained for spiked pepper samples (n=3) at different extraction times.

This FUSLE method was applied to the four matrices of interest and taking into account the results included in **Figure 3.4 a, b, c** and **d** for carrot, lettuce, pepper and amended soil, respectively, satisfactory results (~ 100 %) in terms of the whole protocol extraction efficiency were obtained in most of the cases except for BDE-28 in amended soil, which showed an extraction yield of 69 %.

Fortified samples were analysed also by means of MAE based on EPA 3546 method. Firstly, extraction conditions described in **section 3.2.6** (protocol a) and clean-up with 5-g Florisil cartridges were tested. According to the ANOVA of the results, comparable recovery values were obtained by means of FUSLE and MAE in the case of carrot (**Figure 3.4 a**) and pepper (**Figure 3.4 c**) ($F_{Experimental}$ = 1.15-8.03 ‹ $F_{Critical}$ = 10.12, $F_{Experimental}$ = 1.14- 6.55 ‹ $F_{Critical}$ = 10.12 for carrot and pepper, respectively), although repeatability was significantly lower for MAE when applied to pepper matrix ($F_{Experimental}$ = 25-1181 › $F_{Critical}$ = 9) based on a F-test of the results. In the case of lettuce (**Figure 3.4 b**) and amended soil (**Figure 3.4 d**) matrices, recoveries obtained for MAE exceeded 100 % for most of the congeners studied, indicating the lower selectivity obtained with MAE extractions. It should be underlined that the clean-up step was optimised using FUSLE extracts and not MAE extracts, which were more colourful than the former, indicating the extraction of more interferences. Thus, in order to improve the results for MAE, two other new set of experiments were performed in the case of amended soil:

a) Extraction at 115 °C and clean-up with 10-g Florisil.

b) Extraction at 90 °C and clean-up with 5-g Florisil

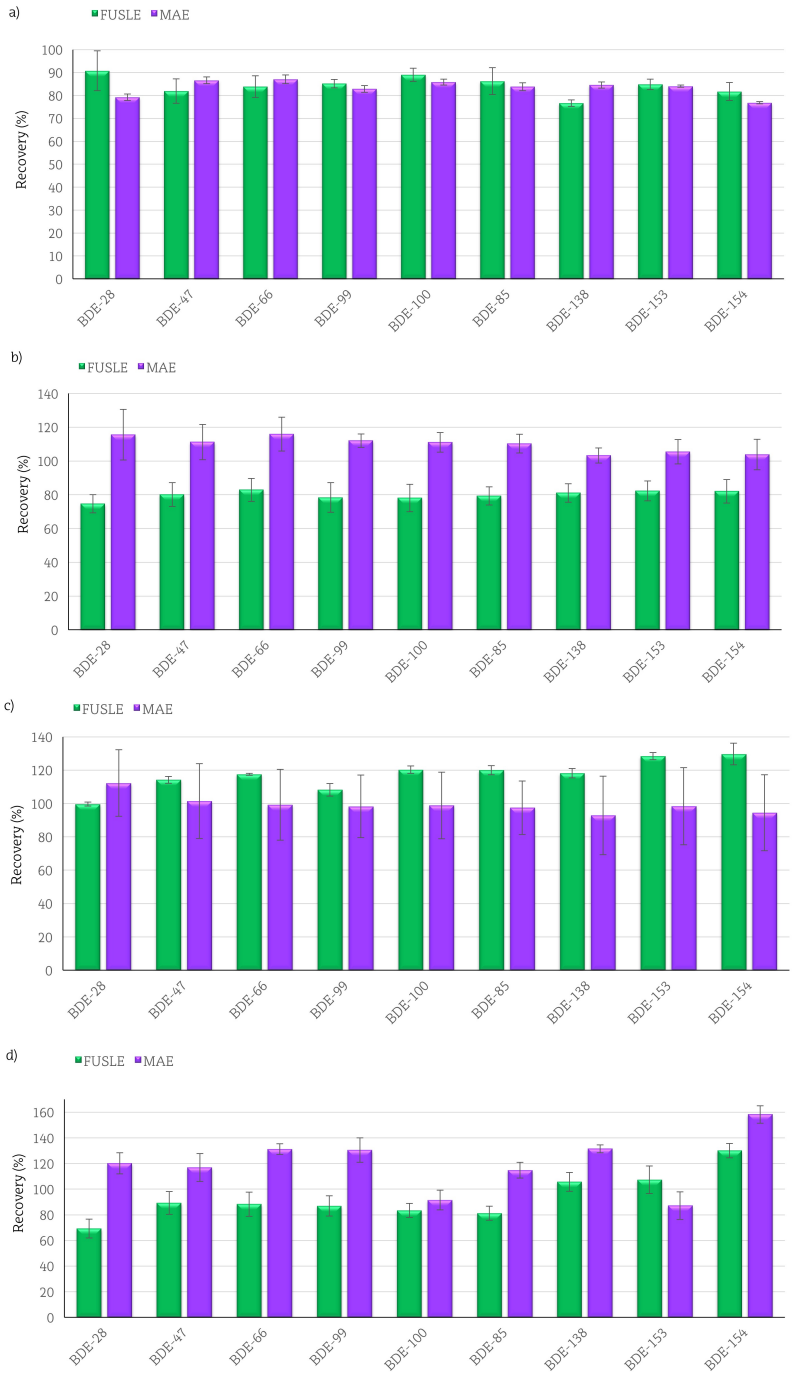


Figure 3.4. Recoveries (%) obtained for FUSLE and MAE (n=3) in the case of a) carrot, b) lettuce, c) pepper and d) compost-amended soil using 5-g Florisil clean-up.

The best results (see **Figure 3.5**) were obtained when milder MAE conditions followed by 5-g Florisil clean-up were applied and the use of 10-g Florisil cartridges did not imply any improvement of the results. However, even under the mildest conditions tested, extraction yields exceeded 100 % for most of the congeners and further MAE optimisation should be performed for application to amended soil and lettuce.

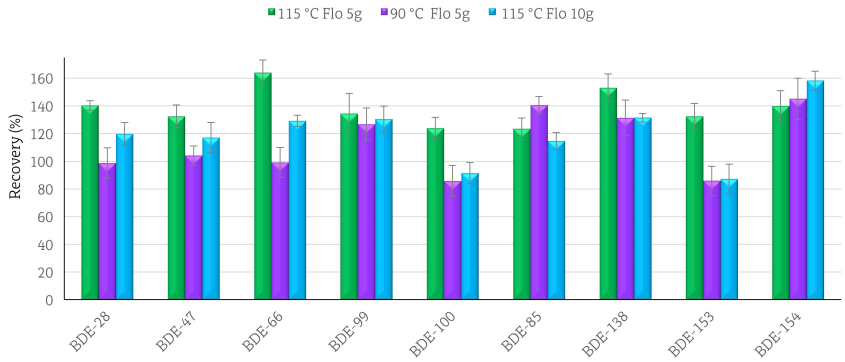


Figure 3.5. Recoveries (%) obtained for different MAE extraction temperatures (90 and 115 °C) using 5-g and 10-g Florisil clean-up cartridges for amended soil matrix (n=3).

3.3.3 Validation of the method

In the absence of a certified reference material (CRM) for PBDEs in vegetables or soil, two approaches were followed for method validation. On the one hand, fortified samples of the four matrices studied were analysed under optimal conditions. On the other hand, the developed method was applied to CRM SQC072 certified sediment for both PBDEs and PCBs.

In terms of the recovery obtained from fortified samples, FUSLE combined with 5-g Florisil clean-up provided acceptable results (see **Table 3.2**) for the four matrices studied at two spiking levels (6 and 58 ng/g). Recovery ranges of 97-122 %, 103-124 %, 98-122 % and 73-120 % were at the lowest concentration level (6 ng/g) and 87-125 %, 71-83 %, 100-130 % and 69-130 % at the highest level (58 ng/g) in the case of carrot, lettuce, pepper and amended soil, respectively. The average values (n=5) obtained for CRM SQC072 under optimised conditions are compared to the certified values in **Figure 3.6**. Although not included in the present work, the results for PCBs were also included. In terms of recovery, the recovery values were within the 86-120 % for PBDEs and in the 85-115 % for PCBs, except for CB-28 which showed recoveries up to 142 %. It could be concluded that results obtained under optimal conditions are in good agreement with the certified values, both for the target PBDEs and for PCBs.

Table 3.2. Average (n=3) recovery (%) and RSD (%) at high (58 ng/g) and low concentration (6 ng/g) and MDL values obtained for PBDEs in spiked matrices (carrot, lettuce, pepper and compost-amended soil).

Analyte	r^2 (MDLs-1 ng/μL)	RSD (%, n=3) in two ranges 6/ 58 ng/g				Recovery (%) in two ranges 6/ 58 ng/g				MDLs (ng/g) at 6 ng/g			
		Carrot	Lettuce	Pepper	Amended soil	Carrot	Lettuce	Pepper	Amended soil	Carrot	Lettuce	Pepper	Amended soil
BDE-28	0.996	18/3	21/7	13/1	15/11	102/90	103/75	105/100	73/69	2	1	3	1
BDE-47	0.996	20/3	10/9	13/2	16/10	108/125	107/80	101/114	93/89	3	1	2	2
BDE-66	0.994	23/14	10/8	13/1	14/11	122/89	105/83	98/117	91/88	2	1	1	2
BDE-99	0.996	20/2	13/11	23/3	30/9	99/91	113/78	117/108	94/87	3	3	3	2
BDE-100	0.989	19/9	12/10	13/2	23/7	117/93	111/78	106/120	88/83	3	1	2	3
BDE-85	0.997	17/11	14/7	23/2	24/7	97/102	110/79	122/120	107/81	2	2	3	2
BDE-138	0.996	23/5	11/7	16/2	24/7	108/96	124/71	116/108	120/106	2	1	2	5
BDE-153	0.993	12/3	11/7	15/2	22/10	101/101	120/82	117/128	106/107	2	1	3	1
BDE-154	0.994	15/4	18/8	10/5	14/4	100/87	118/82	115/130	130/108	2	1	1	3

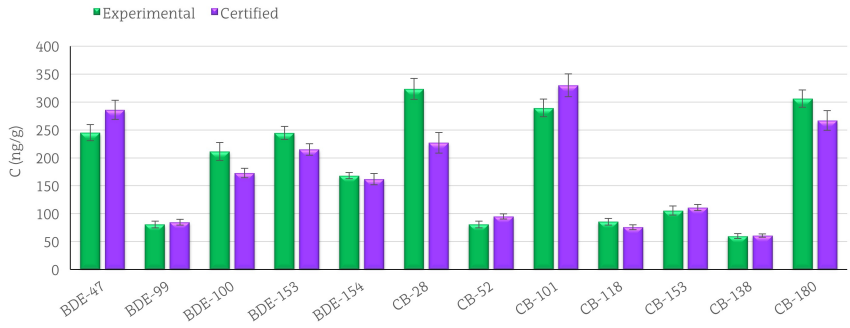


Figure 3.6. Comparison of the results obtained using the present methodology (n=5) and the certified values of the reference material SQC072 for PBDEs and PCBs.

Method detection limits (MDLs) and relative standard deviations (RSDs) were also determined for the four matrices tested. Instrumental calibration curves were performed from the MDLs up to 1 ng/μL and squared determination coefficients (r^2) values higher than 0.993 were obtained (see **Table 3.2**) for all the congeners. MDLs were calculated according to US Environmental Protection Agency (EPA) Method (http://www.epa.gov/waterscience/methods/det/rad.pdf) and matrices (n=7) were spiked at 6 ng/g. The results for splitless injection are included in **Table 3.2** and were in the 1-5 ng/g range. In order to improve the MDLs, large volume injection-programmable temperature vaporiser (PTV-LVI) (20 μL) in a PTV system was also assayed. Although the signal for LVI-PTV injection of the standards increased ten times compared to splitless injection, no improvement was observed for real samples. The MDL values obtained in the present work were compared with other values found in the literature (see **Table 3.3**). In this sense, MDL values are in the same order of magnitude as those obtained by Shin et al. [28], Hale et al. [44] and Park et al. [45]. Better limits of detection (LODs) defined as three times the signal-to-noise ratio (S/N) were obtained in other works [6, 30, 32, 46-49]. For instance, in the case of FUSLE applied to dust samples [34] it should be highlighted that tandem mass spectrometry (MS/MS) was used. Besides, and as recommended by the US EPA, we think that MDLs calculated using real samples give a more realistic value of the detection limit. Tandem mass spectrometry [32-34, 48], ion trap [46] or negative chemical ionisation-mass spectrometry (NCI-MS) [6, 49] provided, in general, the best values. In terms of precision, RSD values were in the 1-30 % range for the fortified samples and in the 3-12 % for CRM SQC072. The latter were in good agreement with the RSD of the certified values which were in the 5-8 % range for both PBDEs and PCBs.

The present method was applied to the determination of PBDEs in soil, carrot, lettuce and pepper from local markets and concentrations were always lower than the MDL values.

Table 3.3. MDLs (ng/g) and LODs (ng/g) found in the literature for PBDEs in different solid samples.

Sample	Extraction	Analysis	MDL (ng/g)	Reference
Soil and vegetables	FUSLE	GC-MS	1-5	In this work
Sediment	SPLE	GC–NCI–MS	1-46 10^{-3} [a]	[6]
Sediment	Soxhlet	GC–NCI–MS	3-50 10^{-3} [a]	[6]
Sediment	USAL-DSPE-DLLME	GC–MS/MS	0.02-0.08[a]	[34]
Soil	ASE	GC–NCI–MS	1-2[b]	[48]
Biosolid	ASE	GC–NCI–MS	2-10[b]	[48]
Corn	ASE	GC–NCI–MS	1-5[b]	[48]
Soil	Soxhlet	GC-Ion Trap	1 10^{-3} [a]	[50]
Vegetables	Soxhlet	GC-Ion Trap	1 10^{-3} [a]	[50]
Cow milk	Soxhlet	GC-Ion Trap	0.8 10^{-3} [a]	[50]
Foodstuff	Soxhlet	GC-MS	5-40 10^{-3} [a]	[51]
Foodstuff	MSPD	GC-LRMS/MS	0.01-0.55 10^{-3} [c]	[52]
Soil	Soxhlet, ASE and MAE	HRGC/HRMS	1.52-24.8 10^{-3} [c]	[32]
Soil and vegetables	Soxhlet	GC–NCI–MS	5.3-6 10^{-3}	[53]
Sewage sludge	MAE	GC–NCI–MS	1.8-6.1	[30]
Soil	PLE	GC-TOF-MS	0.1–1	[49]
Dust	FUSLE	GC-MS/MS	0.05-0.8[a]	[36]

[a] LODs were calculated based on three times the signal-to-noise ratio. [b] Method quantitation limit. [c] LODs, the calculation mode was not reported. SPLE: Selective Pressurised Liquid Extraction, USAL-DSPE-DLLME: Ultrasound-Assisted Leaching-Dispersive Solid-Phase Extraction followed by Dispersive Liquid–Liquid Microextraction, ASE: Accelerated Solvent Extraction, MSPD: Matrix Solid-Phase Dispersion, MAE: Microwave Assisted Extraction, PLE: Pressurised Liquid Extraction, GC–NCI–MS: Gas Chromatography Negative Chemical Ionisation Mass Spectrometry, GC–MS/MS: Gas Chromatography–tandem Mass Spectrometry, HRGC/HRMS: High-Resolution Gas Chromatography coupled with High-Resolution Mass Spectrometry, GC-TOF-MS: Gas Chromatography Time-Of-Flight Mass Spectrometry.

3.4 Conclusions

2-min FUSLE extraction combined with 5-g Florisil clean-up was optimised for the determination of PBDEs in vegetables and compost-amended soil. FUSLE turned out to be an alternative to more expensive extraction techniques such as MAE or PLE, providing good MDLs (1-5 ng/g), precision (1-30 %) and recoveries (71-130 % for vegetables and 69-130 % for amended soil).

MAE and FUSLE were compared as alternatives extraction techniques and although, similar recoveries were obtained in the case of carrot (77-91 % for FUSLE and 77-87 % for MAE) and pepper (100-130 % for FUSLE and 93-109 % for MAE) matrices, recoveries higher than 100 % were attained for lettuce and amended soil in the case of MAE.

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CHAPTER 4

Development of stir-bar sorptive extraction-thermal desorption-gas chromatography-mass spectrometry for the analysis of musks in vegetables and compost-amended soils

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4.1 Introduction

In recent years, some of the focus of the scientific community has shifted from priority to what are commonly called emerging pollutants [1]. Although some of these chemicals have been used for over a century, it is only within the last 20 years that their environmental impacts have been considered. As a result, there is little information available on occurrences and toxicological data, and few or no regulations or guidelines have been established so far. Examples of emerging contaminants include pharmaceuticals and personal care products (PPCPs), detergents, flame retardants or disinfection by-products, among others. Within the PPCPs, synthetic musk compounds are commonly used as fragrance additives in soaps, shampoos, detergents, lotions and perfumes [2-5]. These compounds have been described as a new group of bioaccumulative and persistent xenobiotics. Due to their bioaccumulative properties and health adverse reactions, which led to the prohibition of musk tibetene, musk moskene and musk ambrette, their use declined in the 90s [4, 6, 7]. At present, other two nitromusks, musk ketone and musk xylene are still permitted but with restrictions [8, 9]. On the other hand, there was a parallel increase in the use of polycyclic musks, a second group of synthetic musks, which comprises several high volume use products, such as tonalide (AHTN) and galaxolide (HHCB). Although these compounds are still largely used in personal care products and so on, research indicates that the musks fragrances are environmentally persistent, can accumulate in human bodies and they are suspected hormone disruptors [10]. Once used, these products are washed down the drain and ended up in wastewater treatment plants (WWTPs) [8, 11, 12]. Purification of wastewater produced in different human activities (i.e. household, industry, hospitals) usually takes place in WWTPs. After wastewater treatment, non-degraded compounds, together with their degradation products, are discharged by WWTP effluents into surface waters.

Another potential route of introduction of pollutants and their metabolites into the environment is sewage sludge, since potentially harmful substances, such as organic contaminants, metals and pathogens, can still be found in both effluent water and sewage sludge [13]. In this sense, sludge-amended soils can be considered a way for the introduction of the previously mentioned harmful substances into the food chain through the uptake of crops. The introduction of organic contaminants by the food chain can be studied performing an uptake analysis by different crop plants. If any of the experiments on plant uptake or bioaccumulation of organic pollutants from crops should have any significance, the analysis should be performed

using reliable analytical procedures.

Soxhlet extraction [14], accelerated solvent extraction (ASE) [15, 16], microwave assisted extraction (MAE) [17] and sequential dispersion extraction [18] have been used in the literature for the analysis of musk in solid samples, such as sludge or sediments. Since extraction techniques such as Soxhlet, ASE or MAE are not selective, further clean-up steps are usually necessary and, thus, intermediate evaporation steps are usually incorporated in order to reduce extract volumes. These extraction-evaporation-clean-up-evaporation sequences can introduce losses of analytes such as synthetic musks due to their semi-volatility.

Besides, some of above mentioned traditional extraction procedures, are still used even though they are time-consuming, labour-intensive, complicated, expensive and they produce considerable quantities of waste. Within this scenario, in the last years, new microextraction techniques have been developed in order to improve sample pre-treatments. Introduced in 1999 for analyte pre-concentration from aqueous samples, stir-bar sorptive extraction (SBSE) device consists on a magnetic stir-bar, a coating of extraction phase on the outside, and a thin glass layer between the two [19, 20]. For the extraction, the bar is allowed to stir the sample solution to speed up the partitioning of the analytes between the matrix and the coating. Once the extraction process is finished, the stir-bar is usually thermally desorbed. As far as we know, even though some studies have demonstrated the feasibility of SBSE for the extraction of alcohols, aldehydes, ketones, acids and terpenes [21], esters [22], phenols and lactones [23] from food and soil samples [24], SBSE has not yet been applied in the analysis of musks from vegetables or soil.

Thus, the main objective of this work is the development of a new preconcentration method based on sorptive microextraction using polydimethylsiloxane (PDMS) coated stir-bars for the analysis of musks compounds in vegetables and compost-amended soils. This procedure minimises the consumption of sample, solvents and time, in order to study not only the presence of target analytes in compost-amended soil but also the potential uptake of musks by different crops (lettuce, pepper and carrot).

4.2 Experimental section

4.2.1 Cleaning procedure

All the glassware was cleaned with abundant water and soaked into clean acetone (LabScan, HPLC grade, 99.8 %) for at least 45 min. No detergent was used during the cleaning of

the amber vials in order to avoid possible interferences produced by the detergent residues. Afterwards, the material was rinsed with Elix water (Millipore, Bedford, MA, USA) and Milli-Q water (< 0.057 S/cm, Milli-Q model 185, Millipore, MA, USA). The glassware was dried in an oven at 100 ºC for an hour and, finally, at 400 ºC for 3 h for further clean-up.

4.2.2 Reagents and materials

The studied polycyclic musks 6,7-dihydro-1,1,2,3,3-pentamethyl-4(5H)-indanone (Cashmeran, DPMI, 89.5 %), 4-acetyl-6-tert-butyl-1,1-dimethyl indan (Celestolide, ADBI, 99.8 %), 6-acetyl-1,1,2,3,3,5-hexamethyl indan (Phantolide, AHMI, 93.1 %), 5-acetyl-1,1,2,6-tetramethyl-3-isopropylindane (Traseolide, ATII, 83.2 %), 1,3,4,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(g)-2-benzopyran (Galaxolide, HHCB, 53.5 %) and 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene (Tonalide, AHTN, 97.9 %) were purchased from LGC Standards GmbH (Augsburg, Germany). The studied nitro musk fragrances 1-tert-butyl-2-methoxy-4-methyl-3,5-dinitrobenzene (Musk Ambrette, MA, 99 %) and 4-aceto-3,5-dimethyl-2,6-dinitrotertbutylbenzene (Musk Ketone, MK, 98 %) were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The mass-labelled surrogate standard musk xylene [$^{2}H_{15}$]-MX was purchased from Dr. Ehrenstorfer GmbH at 100 mg/L in acetone. Stock solutions for each compound were dissolved in isopropanol in order to prepare 100 mg/L dilutions.

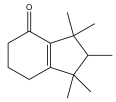
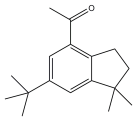
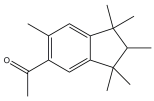
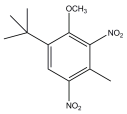
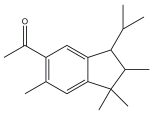
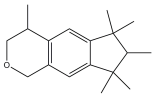
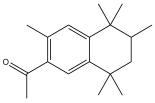
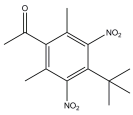
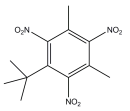
The CAS number of each chemical, together with the structure, boiling points and the octanol water partition coefficient (log K_{ow}) values, are shown in **Table 4.1**.

Ethyl acetate (HPLC grade, 99.8 %), methanol (MeOH, HPLC grade, 99.9 %), isopropanol (HPLC grade, 99.8 %) dichloromethane (DCM, HPLC grade, 99.8 %) and acetone (HPLC grade, 99.8%) were obtained from Labscan (Dublin, Ireland) and acetonitrile (ACN, HPLC, 99.9 %) from Sigma Aldrich (Steinheim, Germany).

The PDMS stir-bars employed (so called twisters supplied by Gerstel, Mülheim an der Ruhr, Germany) were 20 mm x 0.5 mm (long x film thickness) size. Prior to use, twisters were chemically cleaned in an ACN: MeOH (1:1, v:v) mixture under ultrasound energy during 30 min. Finally, the stir-bars were conditioned in a thermal condition unit at 280 ºC for 2 h under a nitrogen atmosphere.

Agitation was carried out using a 15 position magnetic stirrer (Ika Werke, Staufen, Germany).

Table 4.1. Target analyte names and abbreviations and other analytically relevant data.

Analyte	Cas number	Structure	m/z quantifier (qualifiers)	Log K_{ow} [a]	Boiling point (°C at 760 mmHg)
Cashmeran (DPMI)	33704-61-9		191 (206, 192)	4.9	286.1
Celestolide (ADBI)	13171-00-1		229 (244, 173)	6.6	309.1
Phantolide (AHMI)	15323-35-0		229 (244, 187)	6.7	336.6
Musk Ambrette (MA)	83-66-9		253 (268, 254)	3.7	369.3
Traseolide (ATII)	68140-48-7		215 (258, 173)	6.3	350.0
Galaxolide (HHCB)	1222-05-5		243 (258, 213)	5.9	326.3
Tonalide (AHTN)	1506-02-1		243 (258, 159)	5.7	356.8
Musk Ketone (MK)	81-14-1		279 (294, 280)	4.3	369.0
Mass-labelled Musk Xylene ([$_2$H^{15}]-MX)	877119-10-3		246 (261)	[b]	[b]

[a] Experimental values, obtained from Database of physico-chemical properties. Syracuse Research Corporation:http://www.syrres.com/esc/physdemo.htm [b] value not available

The vegetables (carrot, pepper and lettuce) were bought in a local supermarket. The different soils (soil 2.1 and soil 2.4) were obtained from LUFA Speyer (Speyer, Germany). Different

physico-chemical properties of the soils (organic material, granulometry, pH, etc) can be checked in the next link: http://www.lufa-speyer.de/images/stories/bodanalyse.pdf. The universal substrate was acquired from a commercial agricultural house.

Optimisation procedure by means of experimental designs was performed using Statgraphics Centurion program (XV.I version, StatPoint, Inc., USA).

4.2.3 Sample pre-treatment

The vegetable samples were cut in small pieces and freeze-dried in a Cryodos-50 laboratory freeze-dryer from Telstar Instrumat (Sant Cugat del Valles, Barcelona, Spain). In the case of substrate, it was sieved using an Octagon siever (Endecotts, London, England). Afterwards, the matrices used for optimisation and validation purposes were cleaned by soaking in heated (60 ºC) DCM during 30 min. The soils (2.1 and 2.4) and the universal substrate were mixed with the compost acquired from a WWTP in Calahorra (Spain) in a soil:compost ratio of 95:5.

For optimisation and validation purposes, matrices were fortified as follows: a known amount of matrix was weighed, covered with acetone (approximately 1.5 mL of acetone per gram of fortified sample), fortified with target analytes and stirred during 24 h. After that, acetone was evaporated under mild conditions (no heating or nitrogen blowdown were used) and the sample was aged for one week.

4.2.4 Stir-bar sorptive extraction

0.5 g of the solid sample (soil or vegetable) was directly weighed in an amber vessel and 9 mL of water: MeOH (80:20, v/v) mixture was added. A clean PDMS stir-bar was introduced in the slurry in order to preconcentrate the analytes. The extraction was carried out in a temperature controlled water bath at 40 ºC and stirred to 600 rpm during 3 h. Once the sorption step was over, the stir-bars were removed and rinsed with Milli-Q water in order to remove the solid residues and dried carefully with a paper tissue before the desorption step.

4.2.5 Thermal and liquid desorption

The stir-bars were thermally desorbed at a desorption temperature of 300 ºC during 10 min using a commercial thermal desorption unit (TDU) (Gerstel) connected to a CIS-4 injector (Gerstel). The TDS-2 unit was equipped with a TDSA autosampler (Gerstel) able to handle 98 coated stir-bars. All glass tubes containing the stir-bars were placed in a tray that was assembled

in the TDSA autosampler. The program of the CIS-4 injector was fixed as follows: a desorption flow of 75 mL/min, a vent pressure of 7.2 psi and a cryo-focusing temperature of -30 ºC. At a vent time of 0.01 min, the split valve was closed for 1.51 min and the temperature program of the injector was programmed as follows: 12 ºC/s to 300 ºC, where it was held for 3 min (cleaning step).

In the case of the liquid desorption (LD), the PDMS stir-bars were introduced into 0.5 mL amber safe-lock tubes (Eppendorf, Hamburg, Germany) containing 300 µL of ethyl acetate and soaked during 15 min. Finally, 20 µL of the extract were injected in the gas chromatographic-mass spectrometric (GC–MS) system using large volume injection (LVI) coupled to a programmable temperature vaporiser (PTV). LVI was carried out using the MPS 2 autosampler with a 100 µL syringe according to a previous work of our research group [25]. During LVI, the inlet temperature was held at 20 ºC by cooling with a N_2, while the column head pressure was fixed to 5 psi and the flow rate through the split vent was set at 75 mL/min in order to purge out most of the solvent. At a vent time of 0.5 min the split valve was closed for 1.5 min and the temperature program of the injector was programmed as follows: 12 ºC/s to 300 ºC, where it was held for 3 min (cleaning step).

4.2.6 GC-MS analysis

Both the TDU and the PTV were installed in an Agilent 6890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph. The chromatographic column used was an HP5-MS (30 m x 0.25 mm, 0.25 µm, Agilent) and the oven temperature was programmed as follows: start at 60 ºC (3 min), a temperature increase at 30 ºC/min to 190 ºC and a second increase at 5 ºC/min to 290 ºC, where it was finally held for 5 min. Helium (99.9995 %, Carburos Metálicos, Barcelona, Spain) was used as carrier gas at a constant flow of 1.3 mL/min. The transfer line, ion source and quadrupole analyser temperatures were maintained at 300, 230 and 150 ºC, respectively. Detection was carried out using an Agilent 5975 electron ionisation MS system (Agilent Technologies, Palo Alto, CA, USA). The ions monitored for each analyte are summarised in **Table 4.1**. The first ion was used as quantifier and the rest of ions (in brackets) as qualifiers.

4.3 Results and discussion

4.3.1 Cleaning protocol evaluation

One of the main issues in the analysis of musk compounds from environmental samples are blanks, as concentrations in the samples may be in the ng/L range, and products used in the

laboratory might contain the analytes at higher concentrations. In this sense, first of all, the cleaning protocols of stir-bars were evaluated and improved before optimisation of the extraction conditions.

Thermally desorbed and chemically desorbed twisters needed to be conditioned before further use due to the noise observed in the chromatograms obtained in the scan mode. In this sense, three different solvents or solvent mixtures, DCM:MeOH (1:1) (v:v), ACN:MeOH (1:1) (v:v) and pure ACN were tested. Stir-bars were then sonicated for 30 min and half of them (n=2 for each solvent) were thermally conditioned at 280 ºC under a N_2 flow. The rest of the stir-bars (n=2 for each solvent) were not thermally conditioned. The best results were obtained when the PDMS stir-bars were cleaned with a (1:1, v:v) ACN:MeOH solvent mixture and not significant differences were observed between thermally and not thermally cleaned PDMS stir-bars.

4.3.2 Optimisation of the PDMS-coated SBSE

During sorptive microextraction procedures there are several variables that can affect the extraction efficiency. Thus, procedure variables such as the sample extraction mode, the salting out effect, the addition of MeOH, the stirring rate, the extraction temperature and the extraction time profile were evaluated using GC-MS analysis.

4.3.2.1 Headspace vs immersion mode

In a preliminary series of experiments, the extraction efficiency of target analytes was compared at different extraction temperatures (60 ºC, 80 ºC and 100 ºC) in the case of headspace (HS) mode. Assays were performed with vials containing a slurry of the sample (0.5 g of fortified solid matrix + 6 mL of Milli-Q water) during 1 h. Thereafter, the stir-bars were desorbed during 15 min in an ultrasonic bath, with 300 µL of ethyl acetate. Better responses were obtained for all the analytes at 80 ºC. Hence, 80 ºC was considered for further experiments in the case of HS extraction mode.

Three different approaches were studied in order to evaluate the performance of extraction mode into PDMS-coated stir-bars:

a) The HS mode, where the PDMS stir-bar was placed in the headspace of a vial which contained the 0.5 g of the fortified solid matrix. Extraction was carried out at 80 ºC without agitation during 1 h.

b) The HS mode with the vial containing a slurry of the sample (0.5 g of fortified solid

matrix + 6 mL of Milli-Q water). Extraction was carried out at 80 ºC during 1 h.

c) The immersion mode. A PDMS-coated stir-bar was immersed in a vial containing a slurry of the sample (0.5 g of fortified solid matrix + 6 mL of Milli-Q water). Extraction was carried out under agitation (600 rpm) at room temperature during 1 h.

Extraction efficiency, defined as the amount of analyte extracted to the PDMS phase, was calculated by comparing the responses (n=3) obtained after the TD of stir-bars that had been in contact with the fortified samples (1 ng/µL extract concentration level) and the responses obtained after the direct introduction of the analytes in the CIS-4 unit. Glass wool spiked at 300 ng of the target analytes was introduced in the thermal desorption tubes and desorbed as optimised. The extraction efficiencies obtained are included in **Figure 4.1.** The immersion mode provided the best results for all the target compounds and, thus, further optimisation was performed in the immersion mode.

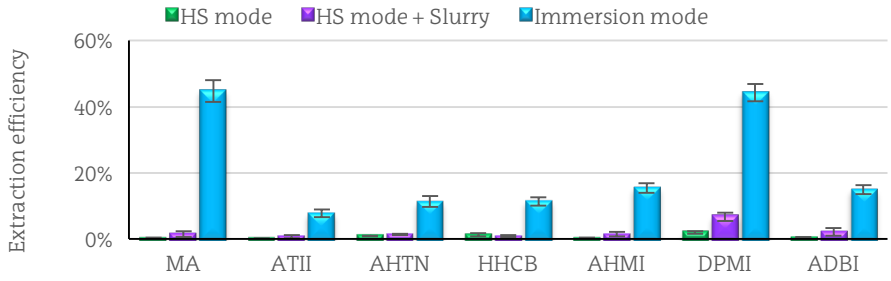


Figure 4.1. Extraction efficiency (n=3, 95 % of confidence level) for HS, HS slurry and immersion mode for PDMS extraction of musks from carrot.

4.3.2.2 *Optimisation of the extraction solvent volume*

The volume of extraction solvent used to prepare the slurry was also optimised. Due to the form and size of our vessels the volumes studied were 6 mL, 9 mL and 12 mL. Milli-Q was used with this purpose.

According to the results obtained in the analysis of variance (ANOVA), comparable results were obtained for all the compounds ($F_{Experimental}$ = 1.3-7.0 ‹ $F_{Critical}$ = 19). However, since not significant differences were observed, we decided to choose 9 mL due to better relative standard deviation obtained for DPMI and ADBI. Besides, not homogeneous stirring was observed in the case of 6 mL.

4.3.2.3 Optimisation of the NaCl and MeOH addition and the stirring speed by means of a central composite design

In the case of the addition of NaCl (0-30 %), the addition of MeOH (0-20 %) and the stirring speed (360-840 rpm) a central composite design (CCD) was built in order to study the influence of those three variables using freeze-dried carrot samples fortified at 1 µg/g extract concentration level under constant extraction time (1 h) and temperature (room temperature) and 9 mL Milli-Q addition.

The results obtained were fitted to a non-linear equation and the response surfaces obtained for HHCB are included in **Figure 4.2** (similar results were obtained for most of the analytes). According to the results obtained, it was observed that in the case of NaCl only the linear coefficient was significant and it was negative for all the target analytes except DPMI. In the case of DPMI interaction between NaCl and MeOH was also significant. For hydrophobic analytes (log K_{ow} › 3.0) the addition of NaCl does not improve, or even reduces, the extraction efficiency, due to the increase in the viscosity of the sample, leading to slower extraction kinetics. On the basis of the salting out effect, the results obtained in the present work are in good agreement with the literature [20]. Therefore, NaCl was fitted at 0 % for further experiments.

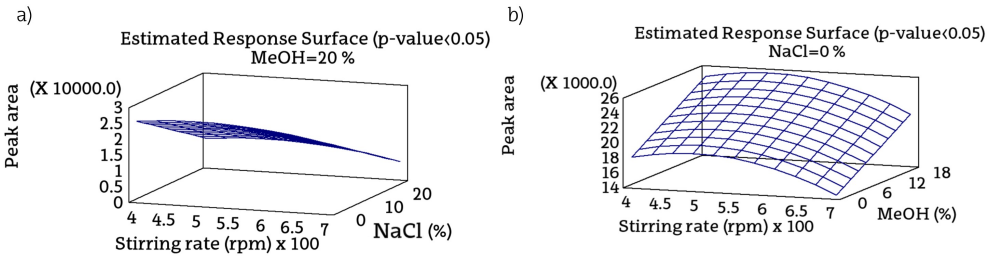


Figure 4.2. Response surfaces obtained for the CCD designed during the optimisation of the extraction step for HHCB a) fixed parameter: 20 % MeOH and b) fixed parameter: 0 % NaCl.

In the case of MeOH the linear coefficient was only significant and positive. The effect of MeOH is more complex to explain. According to the literature for compounds with high log K_{ow} (higher than 5.0), MeOH avoids adsorption of the analytes onto the glass of the vial, while for compounds with lower log K_{ow} (lower than 3.0) MeOH increases solubility of the compound in the solution, decreasing the partition into the PDMS phase [20]. However, this discussion is valid for water samples, where analytes are preconcentrated from the water sample into the PDMS

polymer. In the case of solid matrices, the analytes need to be extracted from the solid matrix into the aqueous solution before preconcentration in the PDMS polymer and it is, at this first stage, where the addition of MeOH enhances extractability of the target analytes**.** Thus, the addition of MeOH improved extraction yields of musks from the solid matrix into the PDMS-coated stir-bar.

Stirring rate was not significant and thus, it was fixed at 600 rpm.

4.3.2.4 *Optimisation of the extraction temperature*

Extraction temperature was studied in the literature during SBSE optimisation and two opposite effects have been observed. While at elevated temperatures the extraction equilibrium is reached faster, K_{PDMS-W} partition coefficient and extraction efficiency become lower [20, 25].

In the present work, 8 g of freeze dried carrot sample were spiked at 800 ng/g concentration level of musks and different extraction temperatures were evaluated. Aliquots of 0.5 g were extracted at room temperature, 40 ºC and 60 ºC for 1 h. After the extraction, the samples were chemically desorbed and analysed by GC-MS. In the case of the experiments performed at 40 ºC and 60 ºC, a water bath was used in order to control the temperature. The results obtained as an average of normalised chromatographic peak areas are included in the **Figure 4.3**. Although comparable results were obtained for 40 ºC and 60 ºC ($F_{Experimental}$ = 1.6-5.0 ‹ $F_{Critical}$ = 8.0 according to ANOVA) in the case of AHTN, HHCB, AHMI and ADBI, 40 ºC were finally chosen due to lower temperature values are kept constant more easily in the water bath. Besides, DPMI showed significantly higher responses at 40 ºC.

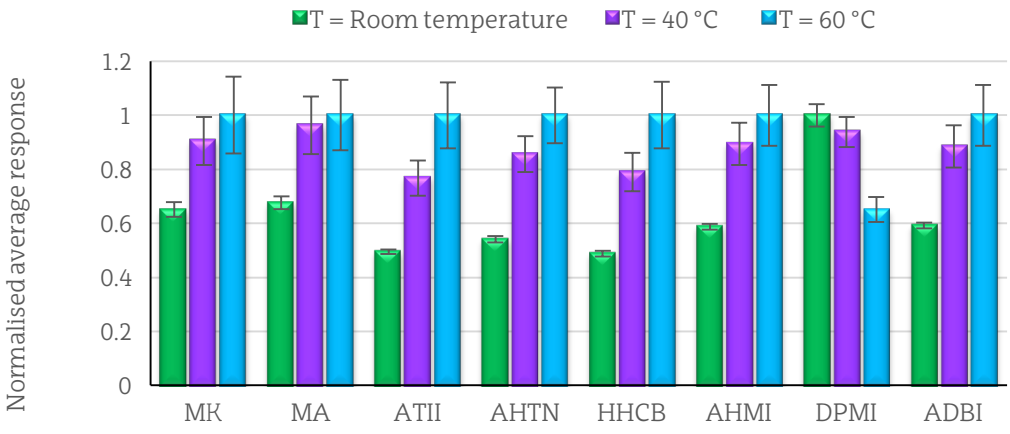


Figure 4.3. Influence of temperature in SBSE for target analytes in carrot samples.

4.3.2.5 Extraction time profile

Once the extraction conditions were carefully selected, the kinetic profile of the extraction process was studied to distinguish the kinetic sorption process from the equilibrium one. The extraction time profiles of the analytes studied were performed by stirring spiked (1 µg/g for 0.5 g samples) carrot samples at different extraction periods (15-690 min) under the conditions optimised before. Equilibrium was reached after 180 min for all the analytes (see **Figure 4.4**). Therefore, 3 h were chosen as optimum extraction time in order to guarantee equilibrium conditions and, thus, small changes in time would not affect precision. Although 3 h could seem a long period, it should be taken into account that 15 samples could be extracted simultaneously using a 15-position agitator.

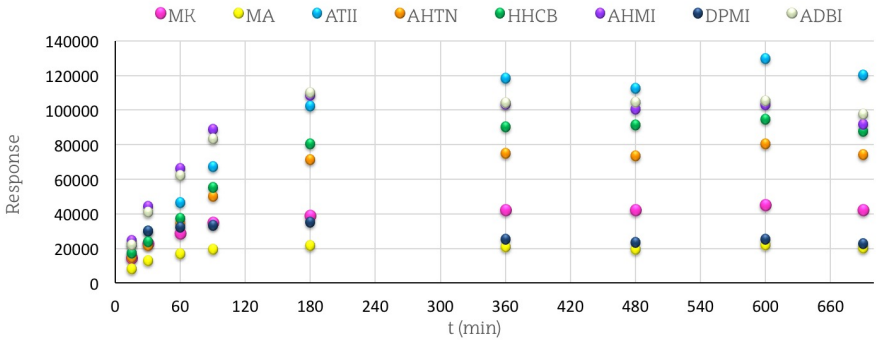


Figure 4.4. Extraction time profile for the synthetic musks studied in carrot sample.

4.3.3 Optimisation of the desorption step

4.3.3.1 Evaluation of the thermal desorption

Since too many variables are involved in the desorption step, an initial screening of their influence was carried out following a fractionated factorial design (FFD). The variables and the ranges studied were the following: desorption time (5-20 min), desorption temperature (250-300 ºC), vent flow (50-100 mL/min), vent pressure (3-11.4 psi) and cryo-focusing temperature ((-50 ºC)-50 º C). A FFD (Resolution V) where desorption temperature (E) was defined as a function of the rest of the optimised variables was used with this purpose.

According to the analysis of effects of the results obtained (see **Table 4.2**) desorption temperature and time had no significant effect for all the analytes. Besides, due to the previous

experience of the group in thermal desorption of stir-bars, it is known that higher temperature and long times are better for the complete desorption of the analytes in order to avoid carryover effect, and therefore, desorption temperature and time were fixed at 300 ºC and 10 min, respectively. In the case of cryo-focusing temperature, lower values rendered higher signals in most of the cases, except for ATII (positive effect) and MK (not significant effect). In the case of the vent flow variable, no clear tendency could be withdrawn. Finally, the last three parameters (cryo-focusing temperature, vent flow and vent pressure) were further evaluated by means of a CCD.

Table 4.2. Analysis effects obtained from the fractionated factorial design used in the optimisation of the desorption step.

	ADBI	**AHMI**	**AHTN**	**ATII**	**DPMI**	**HHCB**	**MA**	**MK**
Vent Flow (A)	NS	-	NS	NS	+	NS	NS	+
Vent Pressure (B)	NS	NS	NS	NS	-	NS	NS	NS
Cryo-focusing temperature (C)	-	-	-	+	-	-	-	NS
Desorption time (D)	NS	NS	NS	NS	NS	NS	NS	NS
Desorption temperature (E)	NS	NS	NS	NS	NS	NS	NS	NS
AD	NS	NS	NS	-	NS	NS	NS	NS
BE	NS	NS	NS	-	NS	NS	NS	NS
AC	NS	NS	NS	+	-	NS	NS	NS
R^2	0.87	0.81	0.86	0.91	0.99	0.86	0.83	0.82

NS≡Not significant, + ≡possitive effect and - ≡negative effect.

The results of the CCD are included in **Figures 4.5 a** and **b** for ATII and AHMI, respectively. According to the values of the significant parameters (p-value ‹ 0.05) the following effects were observed: low cryo-focusing temperatures yielded the best signals for most of the compounds (DPMI, ADBI, AHMI, HHCB, MA, MK and AHTN). All the compounds showed the same effect for vent pressure, low values rendering the highest signals. In the case of vent flow, no

significant effect was observed for none of the analytes. Consequently, the operative conditions for the desorption step were defined as follows: a cryo-focusing temperature of -30 ºC, a vent pressure of 7.2 psi and a vent flow of 75 mL/min.

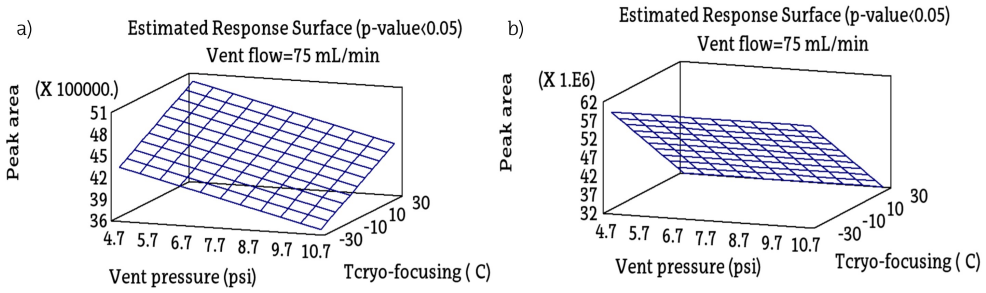


Figure 4.5. Response surface for a) ATII and b) AHMI during the optimisation of the thermal desorption.

4.3.3.2 Evaluation of the liquid desorption efficiency

Liquid desorption efficiency of stir-bars was evaluated using 300 µL (minimum solvent amount to cover the stir-bars) of ethyl acetate (suitable for LVI-PTV–GC–MS analysis) and soaked during 15 min in an ultrasound bath and calculated as the amount of each fraction related to the total amount desorbed after two consecutive extractions (n=3). Desorption efficiency higher than 96 % and precision ‹ 15 % was obtained in the first solvent fraction for all the analytes, which assured a quantitative desorption in an unique step. Thus, stir-bars were desorbed with 300 µL of ethyl acetate and assisted with an ultrasonic bath for 15 min in successive experiments.

4.3.4 Method validation and application to real samples

4.3.4.1 Apparent recovery

In order to obtain the recovery percentages, assays were performed using 0.5 g of different matrices (pepper, carrot, lettuce and amended soils: 2.4, 2.1 and substrate) fortified at the two concentration levels: i) high concentration level: 20 ng/g of DPMI, 4 ng/g of ADBI, 3 ng/g of AHMI, MA, ATII and MK and 10 ng/g in the case of HHCB and AHTN and ii) low concentration level: 0.025 ng/g for MA and MK, 0.05 ng/g in the case of ATII, AHMI and ADBI, 0.5 ng/g for AHTN and 1 ng/g for DPMI and HHCB. Fortification mode was explained in the experimental section. 4 and 7 aliquots of each matrix were extracted in the case of high and low concentration levels, respectively, under optimised conditions and analysed by TD-GC-MS (scan and SIM modes) under

optimised conditions as well, using $^{2}H_{15}$-MX as surrogate.

Two different quantification approaches were studied for sample quantification:

a) Instrumental calibration: Glass wool spiked at different amounts of analytes 3 - 22.5 ng and 15 ng of $^{2}H_{15}$-MX was introduced in the thermal desorption tubes and desorbed as optimised.

b) Matrix-matched calibration: Analyte-free matrices were spiked at increasing concentrations of 0.25-6 ng/g for MA, MK, AHMI and ATII, 1.5-8 ng/g for ADBI, 5-50 ng/g for DPMI and 5-100 ng/g for AHTN and HHCB and 4 ng of $^{2}H_{15}$-MX and were submitted to SBSE-TD-GC-MS under optimised conditions.

When instrumental calibration was used a high matrix effect was observed (recoveries exceeding 120 %) for all the matrices except for carrot, which rendered acceptable results (82-122 %). Thus, quantification using instrumental calibration was discarded in successive experiments since the use of surrogates did no guarantee matrix effect correction. In the present work, matrix-matched calibration rendered satisfactory recoveries (see **Figures 4.6 a** and **b**) for all of the analytes and matrices studied at the two concentration levels evaluated. Similar results were found in the literature where matrix-matched calibration and/or standard addition approaches were applied to quantify polycyclic or macrocyclic musk fragrances from solid samples as sewage slugde [26, 27] and sediments [28].

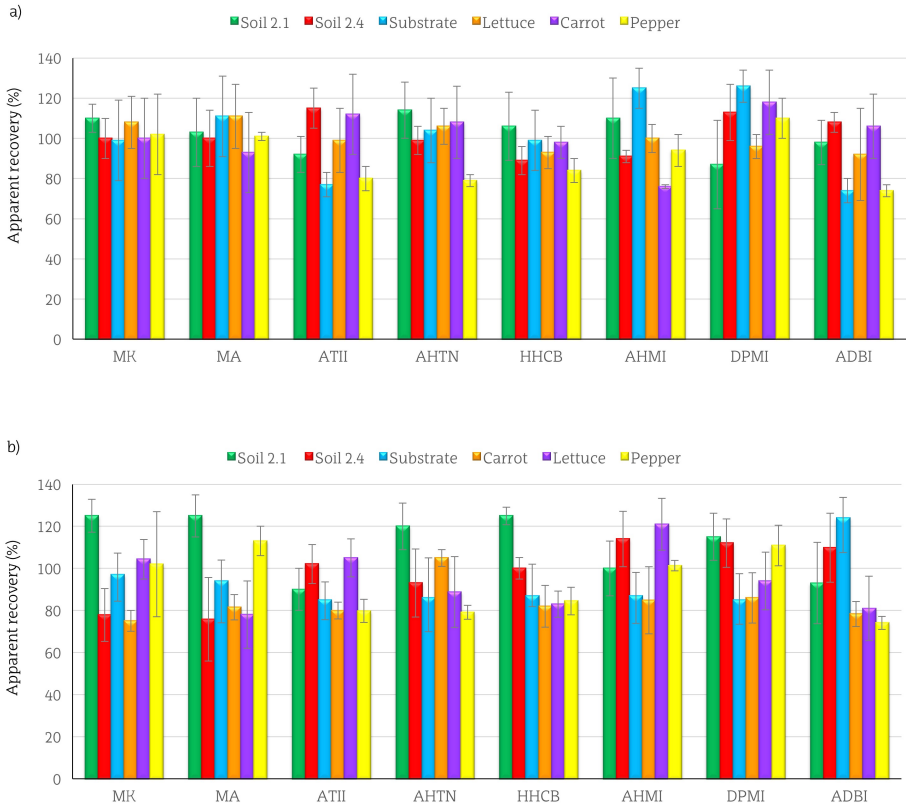


Figure 4.6. Average recoveries (%) and their deviation using matrix-matched calibration and $^{2}H_{15}$-MX as surrogate for a) low (n=7, 95 % confidence level) and b) high (n=4, 95 % confidence level) concentration levels.

4.3.4.2 *Method detection limits*

Method detection limits (MDLs, see **Table 4.3**) of each analyte were determined according to the US Environmental Protection Agency revised guidance for MDL calculation (http://www.epa.gov/waterscience/methods/det/rad.pdf) using seven replicates of the matrices of interest spiked at different levels. Each of the samples were firstly pre-cleaned (see **4.2.3 section**) and fortified at levels of 0.025 ng/g for MA and MK, 0.05 ng/g in the case of ATII, AHMI and ADBI, 0.5 ng/g for AHTN and 1 ng/g for DPMI and HHCB.

Table 4.3. MDLs (95 %, ng/g) (n=7) for the 6 different matrices in the analysis of vegetables and amended soil by SBSE-TD-GC-MS.

Analyte	MDLs (95 %, ng/g)					
	Soil 2.4	**Soil 2.1**	**Substrate**	**Carrot**	**Lettuce**	**Pepper**
MK	0.01	0.02	0.01	0.01	0.02	0.01
MA	0.01	0.01	0.01	0.02	0.01	0.01
ATII	0.05	0.04	0.02	0.01	0.04	0.01
AHTN	0.3	0.2	0.3	0.6	0.3	0.3
HHCB	0.6	1.1	0.8	0.1	0.1	0.1
AHMI	0.01	0.06	0.02	0.04	0.02	0.01
DPMI	0.8	0.6	0.5	0.8	0.8	0.7
ADBI	0.03	0.03	0.05	0.01	0.03	0.01

Better MDLs (0.01-1.1 ng/g) for soil samples were obtained in the present work compared with MDLs obtained in Osemwengie study (10-40 ng/g) where PLE-GPC-GC-MS was applied to the analysis of musk in sewage biosolids [29].

4.3.4.3 Precision

The precision was evaluated in terms of repeatability on relative chromatographic peak areas (with respect to internal standard) of matrices fortified at 3 ng/g for AHMI, MA, ATII and MK, 4 ng/g for ADBI, 20 ng/g for DPMI and 10 ng/g for HHCB and AHTN (high concentration level) and at the same concentration levels used for the MDLs determination (low concentration level) spiked matrices using 4 or 7 replicates, respectively. Samples were analysed in the same day and by the same analyst. RSD values within 1 % and 23 % were obtained for all the analytes and matrices as shown in **Table 4.4**. A chromatogram of the target compounds obtained for carrot sample in the evaluation of precision at the low concentration level (MDLs determination) is included in **Figure 4.7**.

Table 4.4. RSDs (n=4) obtained for the analysis performed in the same day.

Analyte	RSDs (%), (Low[a] n=7 /High[b] n= 4)					
	Pepper	**Lettuce**	**Carrot**	**Soil 2.1**	**Soil 2.4**	**Substrate**
MK	19/12	12/10	22/10	22/18	10/6	13/10
MA	21/20	13/12	16/13	10/1	12/5	16/6
ATII	15/5	13/12	18/12	17/9	12/6	14/5
AHTN	7/5	22/11	12/6	7/5	22/5	23/6
HHCB	8/4	13/11	14/8	20/7	13/6	17/6
AHMI	8/6	12/4	13/8	23/20	5/4	10/6
DPMI	16/15	18/13	14/11	12/8	18/7	23/7
ADBI	12/7	6/3	12/7	22/8	6/4	14/6

[a] MDL concentration levels, [b] MA, MK, AHMI and ATII (3 ng/g), ADBI (4 ng/g), DPMI (20 ng/g), AHTN and HHCB (10 ng/g).

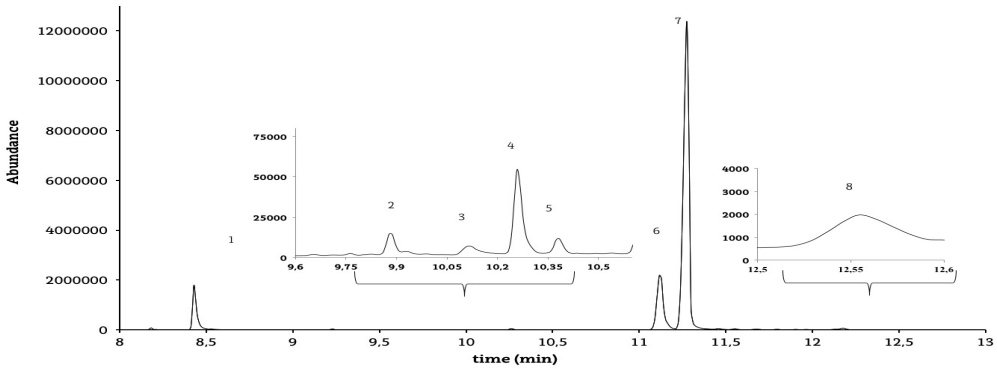


Figure 4.7. SBSE-TD-GC-MS chromatogram obtained for carrot sample fortified with the target compounds at the low concentration level (MDLs determination, see section 4.3.4.2) and analysed under the optimal conditions in the SIM mode. (1) DPMI, (2) ADBI, (3) AHMI, (4) MA, (5) ATII, (6) HHCB, (7) AHTN and (8) MK.

4.3.4.4 Application of the developed method to real samples

The optimised extraction method was applied to the analysis of three vegetables (carrot, pepper and lettuce) samples bought in a local market and mixtures of compost:soils (soil 2.1, soil 2.4 and substrate) (5:95).

The average concentrations (n=3) obtained for all the analytes are shown in **Table 4.5**. It was observed that AHTN appeared in most of the matrices with the highest concentration in pepper. The second most common synthetic musk present was HHCB that it was found in lettuce and in all the soils. MK was also detected in lettuce and all the amended soils but at lower concentrations.

The concentration values obtained in soil samples are similar in some cases or lower than to the concentrations (HHCB 5-18 ng/g, AHTN 1.9-4.0 ng/g) obtained by Osemwengie in biosolids [29] and the concentrations (AHTN 8-18 ng/g, HHCB 22-38 ng/g and 0.3-2 ng/g) obtained by Difrancesco et al. in sludge samples [30]; nevertheless it must be said that the complexity of the matrices studied by them is significantly higher.

Table 4.5. Average concentrations (ng/g) (n=3) and standard deviation obtained in the analysis of real samples of pepper, carrot, lettuce and amended soils (soil 2.1, soil 2.4 and substrate).

Analyte	Concentration values (ng/g), n=3					
	Pepper	**Carrot**	**Lettuce**	**Compost: Soil 2.4 (5:95 %)**	**Compost: Soil 2.1 (5:95 %)**	**Compost: Substrate (5:95 %)**
MK	‹MDL	‹MDL	0.030±0.003	7.7±0.1	7.8±0.2	6.5±1.1
MA	0.07±0.01	‹MDL	‹MDL	‹MDL	‹MDL	‹MDL
AHTN	3.7±0.1	‹MDL	0.4±0.02	‹MDL	‹MDL	‹MDL
HHCB	‹MDL	‹MDL	0.4±0.02	4.6±0.6	5.3±0.3	3.8±0.5
AHMI	‹MDL	‹MDL	‹MDL	0.0652±0.005	0.0713±0.001	0.0264±0.002
DPMI	‹MDL	‹MDL	‹MDL	‹MDL	‹MDL	‹MDL
ATII	‹MDL	‹MDL	‹MDL	‹MDL	‹MDL	‹MDL
ADBI	‹MDL	0.04±0.004	‹MDL	‹MDL	‹MDL	‹MDL

4.4 Conclusions

As an ending consequence of a thorough optimisation and validation procedure, the combination of SBSE and TD followed by GC-MS allows precise simultaneous determination of 8 musks fragrances in vegetables (carrot, lettuce and pepper) and three different compost-

amended soil samples at the low concentration levels found in these solid matrices. Although both instrumental and matrix-matched calibrations are comparable in the case of carrots, matrix-matched calibration approach was necessary in order to quantify target analytes in lettuce, pepper and soils. The good MDL, apparent recovery and repeatability values obtained, together with the simplicity and the quite high grade of automation, make of this procedure a right tool for quality analysis of musks in a wide range of different real solid samples.

4.5 References

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CHAPTER 5

Simultaneous determination of endocrine disrupting compounds in carrot, lettuce and compost-amended soil by means of focused ultrasound solid-liquid extraction and dispersive solid-phase extraction as simplified clean-up

Journal of Chromatography A 1389 (2015) 8–18

5.1 Introduction

During the last decade, a number of naturally occurring and synthetic chemicals referred as endocrine-disrupting compounds (EDCs) have been shown to cause disorder in the endocrine system, causing dangerous consequences to humans and wildlife, even when they are present in the aquatic environment at concentration levels as low as pg-ng/L [1]. The group of molecules identified as EDCs is highly heterogeneous and includes persistent organic pollutants (POPs), pesticides, some heavy metals, musk fragrances, industrial chemicals and their by-products, including polychlorinated biphenyls (PCBs), among others [1]. A large number of chemicals with endocrine disrupting activity, including the above mentioned, have been released into the environment, and nowadays, there is a public and worldwide concern on this topic. Within this scenario, different response actions have been taken; for instance, the European Union (EU) included some EDCs in the list of the so-called priority contaminants EC ENV 191000/01 and the US EPA created the Endocrine Disruptor Screening Program (http://www.epa.gov/endo). The situation is constantly evolving because some EDCs were banned decades ago and others more recently, with significant differences between countries.

It has been widely reported that the occurrence of EDCs is especially significant in places near influents and effluents of wastewater treatment plants (WWTPs) [2, 3] due to the lack of removal during the wastewater treatment. Once EDCs are discharged into the urban wastewater collection system, they may be transferred to the residual sewage sludge during wastewater treatment [4]. Therefore, sewage sludge and sludge/compost-amended soils are also new sources of EDC pollution [4, 5] and food intake is one of the other pathways to human exposure to EDCs. Another pathway of introduction of EDCs in the food chain is related to migration from food containers and packaging [6, 7]. A special EU legislation concerning the products which can migrate from plastic containers to food is established by directive CD 2004/19/EC.

The analysis of EDCs is usually a challenge due to their low concentrations (in the environment and foodstuffs) and complicated sample clean-up procedures based on classical approaches which are generally carried out. According to the literature, solid-liquid extraction (SLE) by stirring or shaking [8, 9], ultrasound assisted extraction (UAE) [10, 11], microwave assisted extraction (MAE) [12, 13], pressurised liquid extraction (PLE) [14, 15] and, recently, QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) [16–18] are the most commonly used

procedures applied for the extraction of EDC compounds in solid samples.

On the other hand, another alternative consists on the use of focused ultrasound solid-liquid extraction (FUSLE). FUSLE offers a simple extraction procedure requiring a low amount of sample (0.01-1.0 g), solvent (5-15 mL) and short (from seconds to few minutes) extraction times [13, 19]. Besides, the microtip of the focused ultrasound is placed directly in the extracts and this, together with the higher-power ultrasound, makes the power of the focused ultrasound technique 100 times higher than the traditional ultrasonic bath methods [20].

None of the extraction techniques applied in the determination of EDCs, as described above, are selective and, therefore, a clean-up step is usually needed. Clean-up of the samples is generally performed using classical approaches, such as solid-phase extraction (SPE) [8, 9, 11, 19]. Conventional column-based SPE uses plastic or glass columns containing a high amount (150-5000 mg) of sorbent material and high organic solvent consumption (column conditioning and elution steps). A new clean-up procedure named dispersive solid phase extraction (dSPE) was recently introduced by Anastassiades et al. [21] along with the QuEChERS extraction method and it has usually been used as the clean-up step of QuEChERS procedure. In comparison with traditional SPE, dSPE saves time, effort, money and solvent consumption [16–18, 22].

Liquid chromatography–mass spectrometry (LC-MS) technique, which allows the determination of EDCs without the need of a prior derivatisation step, has been extensively applied during the last years [23, 24]. LC separations of EDCs are usually carried out using columns packed with C_{18}-modified silica gel particles [23]. However, considerable interest is currently devoted to columns packed with core-shell particles and several recent reviews, have summarised the advantages and applications of these columns [25].

Within this scenario, the aim of the present work was to develop a method based on FUSLE coupled to dSPE protocol as a simplified clean-up strategy and liquid chromatography–triple quadrupole-tandem mass spectrometry (LC-QqQ-MS/MS) analysis for the simultaneous determination of 11 key EDCs, including natural hormones (diethylstilbestrol, DES; estrone, E1; 17ß-estradiol, E2; norethindrone, NT; progesterone, PG and testosterone, TT) and xenobiotic hormone-mimicing compounds (nonylphenol mixture, NPs; 4-tert-octylphenol, 4-tOP; 4-n-octylphenol, 4-nOP; bisphenol A, BPA; and triclosan, TCS) in vegetables (lettuce and carrot), as well as in amended soil. As far as we know, the application of a combined method using FUSLE and dSPE to this group of analytes has not been carried out before in the selected matrices. The

developed method was finally applied to the analysis of both real vegetable (carrot and lettuce) bought in a local market and compost-amended soil samples.

5.2 Experimental section

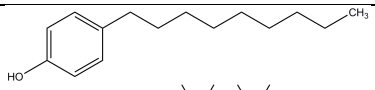
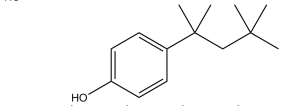
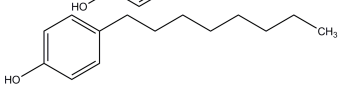
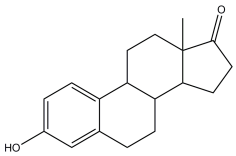
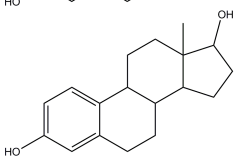
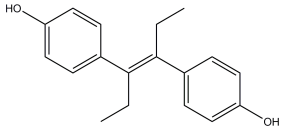
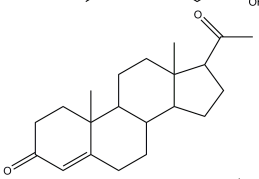
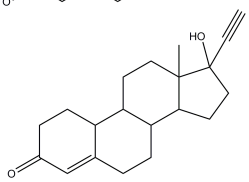
5.2.1 Reagents and materials

All glassware materials (balloons for sample freeze-drying and extraction vessels) were washed with a common detergent, rinsed with abundant Elix water (Millipore, Bedford, MA, USA), sonicated in an acetone (LabScan, HPLC grade, 99.8 %) bath and maintained there for 24 h. Afterwards, the material was rinsed with Milli-Q water (< 0.05 μS/cm, Milli-Q model 185, Millipore) and dried in an oven at 120 °C for at least 4 h. An additional step was employed for glass extraction vessels, which were also dried in a muffle oven at 400 °C for at least 4 h. All plastic material was discarded after use. The chemical standards (names and abbreviations) used in this work are listed in **Table 5.1** including the supplier of each product and some physicochemical properties. In the case of surrogate analogues, [$^2H_3$]-17β-estradiol ([$^2H_3$]-E2, 98 %), [$^2H_4$]-nonylphenol ([$^2H_4$]-NP, 97 %) and [$^2H_9$]-progesterone ([$^2H_9$]-PG, 98 %) were purchased from Sigma Aldrich (Steinheim, Germany). [$^2H_{16}$]-Bisphenol A ([$^2H_{16}$]-BPA, 99.9 %) was obtained from Supelco (Walton-on-Thames, UK).

Individual stock solutions for each target compound, as well as the surrogate analogues, were dissolved in methanol (MeOH, 99.9 %, Alfa-Aesar, Karlsruhe, Germany) to prepare stock solutions in the 1000-5000 mg/L concentration range, except E1, which was dissolved in dichloromethane (DCM, HPLC grade, 99.8 % LabScan, Dublin, Ireland). 75 mg/L solutions were prepared in MeOH monthly and dilutions at lower concentrations were prepared daily. All the standards and stock solutions were stored at -20 °C.

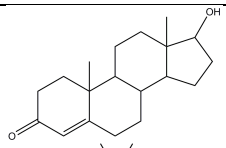
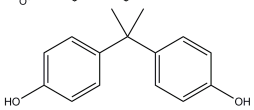
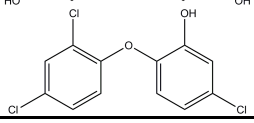
Vegetables (carrot and lettuce) were obtained from a local market and cut into small pieces before freeze-drying. A Cryodos-50 laboratory freeze-dryer from Telstar Instrument (Sant Cugat del Valles, Barcelona, Spain) was used with this objective. The compost was acquired from Calahorra WWTP (Spain). Soil 2.4 used in all the experiments was supplied by LUFA Speyer (Speyer, Germany).

Table 5.1. Names, abbreviations, structure, suppliers, purity, Log K_{ow} and pKa of the target analytes.

Analytes	Chemical structure	Supplier	Purity (%)	Log K_{ow}[a]	pKa[a]
Nonylphenols (technical mixture) (NPs)		Riedel-de Háen	94.0	5.8	10.7
4-tert-octylphenol (4-tOP)		Supelco	99.4	5.4	10.7
n-octhylphenol (4-nOP)		Sigma-Aldrich	99.0	5.5	10.4
Estrone (E1)		Riedel-de Häen	99.5	3.4	10.8
17β-estradiol (E2)		Sigma-Aldrich	99.0	3.9	10.7
Diethylstilbestrol (DES)		Sigma-Aldrich	99.9	5.1	9.1
Progesterone (PG)		Sigma-Aldrich	99.6	3.9	18.9
19-norethindrone (NT)		Sigma-Aldrich	99.5	3.0	17.6

[a] Values reported in The Free Chemicals Data Base: http://www.chemspider.com/.

Table 5.1 (Continuation).

Analytes	Chemical structure	Supplier	Purity (%)	Log K_{OW}[a]	pKa[a]
Testosterone (TT)		Sigma-Aldrich	99.9	3.3	19.1
Bisphenol-A (BPA)		Sigma-Aldrich	99.0	3.6	9.6
Triclosan (TCS)		Sigma-Aldrich	≥97.0	4.7	7.9

[a] Values reported in The Free Chemicals Data Base: http://www.chemspider.com/.

For the extraction two different vessels were used: 50-mL polypropylene conical tubes (PP, internal diameter 27.2 mm x 117.5 mm length) obtained from Deltalab (Barcelona, Spain) and 50-mL Pyrex round bottom centrifuge tubes (internal diameter 29 mm x 125 mm length) from Thomas Scientific (Swedesboro, NJ, USA). Extractions were carried out using a Bandelin ultrasonic homogeniser (20 kHz; Bandelin Electronic, Berlin, Germany) equipped with a 3-mm titanium microtip. For the filtration of the supernatant two different filters were tested: polyamide (PA, 0.45 μm, 25 mm, Macherey-Nagel, Germany) and polytetrafluoroethylene (PTFE, 0.45 μm, 25 mm, Teknokroma, Barcelona, Spain). Fractions were evaporated in a Turbovap LV Evaporator (Zymark, Hopkinton, MA, USA) using a gentle N_2 (99.999 %, Messer, Tarragona, Spain) blow down.

For the clean-up step Envi-Carb graphitised carbon (100 m^2/g specific surface area, 120/400 mesh, Supelco, Bellefonte, USA), charcoal activated powder (reagent grade, Scharlab, Barcelona, Spain), primary and secondary amine (PSA, 570 m^2/g specific surface area, Agilent Technologies, Palo Alto, CA, USA) and C_{18} (500 m^2/g specific surface area, Agilent Technologies) were used as dispersive sorbents. Additionally, magnesium sulphate anhydrous ($MgSO_4$, extra pure, Scharlau, Barcelona, Spain) from Scharlau was also used in the dSPE step. For centrifugation, 1.5-mL Eppendorf tubes were purchased from Eppendorf (Berzdorf, Germany) and 10-mL PP tubes were provided by Deltalab. When Eppendorf tubes were used in dSPE a 24 Place Microlitre centrifuge (230 V/50-60 Hz) obtained from Heraeus Instrument (Hanau, Germany) was used. In the case of 10 mL PP tubes, a 6 places centrifuge (230 V/50-60 Hz)

obtained from Eppendorf was used.

PP microfilters (0.2 µm, 13 mm) provided by Pall (NY, USA) and PTFE hydrophilic filters (0.2 µm, 13 mm) from Teknokroma were tested to filter extracts before LC-MS/MS analysis.

MeOH (HPLC grade, 99.9 %), acetonitrile (ACN, HPLC grade, 99.9 %) and n-hexane (HPLC grade, 99.9 %) were supplied by LabScan. Milli-Q water and MeOH (Romil-UpS) were used as mobile phase eluents and ammonium hydroxide (25 % as NH_4OH, Panreac, Reixac, Barcelona, Spain) for mobile phase modifications. High purity nitrogen gas (› 99.999 %) supplied by Messer (Tarragona, Spain) was used as collision gas and nitrogen gas (99.999 %) provided by AIR Liquid (Madrid, Spain) was used as both nebuliser and drying gas.

5.2.2 Sample treatment and focused ultrasound solid-liquid extraction

For optimisation and validation experiments samples were prepared as follow. The matrix was freeze-dried, homogenised in a glass mortar and fortified with the target analytes at the proper concentration level. Hence, a known amount of matrix was weighed, covered with acetone, spiked with the target analytes and stirred during 24 h. After that, acetone was evaporated under mild conditions (no heating or nitrogen blow-down were used) and the sample was aged for 1-2 weeks, according to previously published methods [26, 27]. According to optimisation, a sample aliquot of 0.5 g (carrot and amended soil) or 0.25 g (lettuce), was weighed and 10 mL of acetone: hexane (70:30, v:v) solvent mixture and surrogate standards ($[^2H_{16}]$-BPA, $[^2H_4]$-NP, $[^2H_3]$-E2 and $[^2H_9]$-PG) at 50 ng/g concentration level were added. The vessel (PP 50-mL) was immersed in an ice-water bath (~ 0 °C), according to results obtained previously by the research group [13, 19]. The extraction was performed at 33 % power and a pulsed time on of 0.8 s and a pulsed time off of 0.2 s during 5 min. 0.5 g of lettuce (0.5 g of lettuce sample correspond to a higher volume of matrix compared with carrot) were not properly extracted in 10 mL of solvent. According to the manufacturer recommendations FUSLE microtip should be 1 cm immersed in the extractant. Thus, 0.25 g of sample were used in the case of lettuce in order to avoid the use of larger (› 10 mL) amounts of extraction solvents during the FUSLE procedure. The supernatant was filtered through 0.45 µm PTFE filter (selected according blank signals) and the extracts evaporated to dryness and reconstituted in different solvents depending on the clean-up approach selected.

5.2.3 Clean-up step

For dSPE five different sorbent combinations were tested:

a) Envi-Carb graphitised carbon (75 mg).
b) Envi-Carb graphitised carbon (75 mg), charcoal powdered carbon (30 mg), PSA (50 mg) and $MgSO_4$ (150 mg).
c) Envi-Carb graphitised carbon (75 mg), charcoal powdered carbon (30 mg) and $MgSO_4$ (150 mg).
d) Envi-Carb graphitised carbon (75 mg), Florisil (50 mg) and $MgSO_4$ (150 mg).
e) Envi-Carb graphitised carbon (75 mg), C_{18} (50 mg) and $MgSO_4$ (150 mg).

In the case of dSPE clean-up approach a) a modification of the method published by Powley et al. [28] was applied. In this sense, 75 mg (according to the optimised sorbent amount) of Envi-Carb were added to a 1.5-mL PP Eppendorf tube. In the case of the clean-up approaches b, c, d and e), the dispersive sorbents were added to 10-mL PP test tubes instead of 1.5-mL Eppendorf tubes. In all the cases, the eluates were evaporated to dryness and then reconstituted in 1.5 mL of ACN before loading into the clean-up tubes, except in the case of Florisil (approach d), which was reconstituted in 1.5 mL of isooctane before the clean-up step. Then, the extracts were vortexed for 40 s and centrifuged (4000 rpm during 10 min at 4 °C). In all the cases, the eluates were collected and evaporated to dryness under a gentle stream of nitrogen at 35 °C and reconstituted in 250 µL of MeOH (99.9 %). Finally, the reconstituted extracts were filtered through 0.2 µm PTFE filters (selected according to the blank signal evaluation) before the LC-MS/MS analysis.

5.2.4 LC-MS/MS analysis

Samples were analysed in an Agilent 1260 series HPLC equipped with a degasser, a binary pump, an autosampler and a column oven and coupled to an Agilent 6430 triple quadrupole (QqQ) mass spectrometer equipped with both electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) sources (Agilent Technologies). The quantitative analysis of the target compounds was performed in the dynamic selected reaction monitoring (dynamic SRM) acquisition mode. High purity nitrogen gas (99.999 %) was used as nebuliser, drying and collision gas. MS/MS ionisation parameters were set as follows: a N_2 flow rate of 12 L/min, a capillary voltage of 5000 V, a nebuliser pressure of 45 psi (310.3 KPa) and a source temperature of 350 ºC.

Separation of analytes was carried out using two different analytical columns: an Agilent Zorbax Extend-C_{18} (2.1 mm, 50 mm, 1.8 μm) column (pH range 2.0-11.5) and an Ace Ultra Core Super C_{18} core-shell column (2.1 mm, 50 mm, 2.5 μm) column (pH range 2.0-11.0). A Zorbax Eclipse XDB-C_{18} pre-column (2.1 mm, 5 mm, 1.8 μm) and ViciJour Sure-guard disposable in line filter (24.4 mm, 10.0 mm, 0.5 μm) were used, respectively. The column temperature was set to 35 ºC for Agilent Zorbax Extend-C_{18} column and at 30 ºC in the case of Ace Ultra Core Super C_{18} column. The injection volume and flow rate were set at 10 μL and 0.3 mL/min, respectively.

Under optimised conditions a binary mixture consisting of water:MeOH (95:5, v:v) (mobile phase A) and of MeOH:water (95:5, v:v) (mobile phase B), both containing 0.05 % of NH_4OH were used for gradient separation of target analytes. Linear gradient was as follows: 30 % B maintained for 4 min, increased to 60 % B in 3 min and to 80 % B in 10 min, where it was maintained constant for 10 min. Initial gradient conditions (30 % B) were then achieved in 3 min, where it was finally held for another 10 min (post-run step).

Both negative and positive voltages were simultaneously applied in a single injection according to the target analytes. Fragmentor voltage and collision energy were optimised for ESI source in the 20-200 V and 5-60 eV ranges by injection of individual compounds. Besides, cell accelerator voltage was optimised in the 1-7 V range and multiplier voltage from 0 V to 200 V. Optimum conditions are summarised in **Table 5.2**.

Instrumental operations, data acquisition and peak integration were performed with the Masshunter Workstation Software (Version B.06.00, Agilent Technologies).

5.3 Results and discussion

5.3.1 Optimisation of the LC-MS/MS

Optimisation of the LC-MS/MS parameters was done at a concentration level of 500 ng/mL for all the analytes.

Table 5.2. Precursor and product ions (first ion was used as quantifier and the second one as qualifier) at optimal fragmentor voltage (V) and collision energy (eV) values. Polarity and cell accelerator voltage values are also included. Calibration ranges, determination coefficients (r^2), instrumental limits of detection (LODs) and limits of quantification (LOQs) for the target analytes are also included.

Analyte	Precursor ion	Product ions	Polarity	Fragmentor voltage (V)	Collision Energy (eV)	Cell accelerator (V)	Calibration range (ng/mL)	r^2	LOD (ng/mL)	LOQ (ng/mL)
NPs	219.0	133.0 147.4	Negative	120	25/25	3	8-425	0.9967	8.0	12.4
4-tOP	205.2	132.9 134.2	Negative	120	20/15	3	0.5-450	0.9996	0.5	0.6
4-nOP	205.1	106.1 119.0	Negative	120	15/20	1	0.4-448	0.9999	0.5	0.7
TCS	286.9/288.9	34.6	Negative	80/80	10/10	1	2-424	0.9986	2.5	2.8
BPA	227.1	212.0 132.7	Negative	110	10/20	5	0.5-460	0.9999	0.5	0.7
DES	267.1	251.0 236.9	Negative	140	20/20	4	0.5-462	0.9999	0.5	0.9
E1	269.5	145.3 143.2	Negative	180	35/40	3	0.4-403	0.9998	0.5	0.8
E2	271.5	145.3 183.3	Negative	140	35/40	3	0.9-461	0.9996	1.0	3.0
NT	299.2	109.0 145.0	Positive	120	20/15	5	0. 5-459	0.9997	0.5	0.6
TT	289.3	97.0 109.0	Positive	120	20/25	5	0.5-458	0.9999	0.5	1.2
PG	315.2	97.2 109.2	Positive	110	20/20	2	0.3-444	0.9936	0.3	0.6
[$^2H_4$] NP	223.1	110.1	Negative	120	15	1				
[$^2H_{16}$] BPA	241.5	142.4	Negative	120	25	3				
[$^2H_3$] E2	271.5	185.1	Negative	140	35	1				
[$^2H_9$] PG	324.3	100.2	Positive	60	20	2				

According to the results obtained by Iparraguirre et al. [29] and Salgueiro-González et al. [30], the chromatographic sensitivity of the present target analytes improves at basic pHs. For this reason, in a first approach, Zorbax Extend-C_{18} column (which stands pHs up to 11.5) and a partially porous Ace Ultra Core Super C_{18} core-shell column (2.5 μm particle size) were evaluated for the separation (the chromatographic program was included in **section 5.2.4**) of the 11 target analytes using a mobile phase consisting of 95:5 (v:v) water:MeOH (A) and 5:95 (v:v) water:MeOH (B) with 0.05 % of NH_4OH in both cases, A and B. As can be observed in **Figure 5.1** Ace Ultra Core Super C_{18} column significantly improved the chromatographic behaviour of the analytes in terms of resolution of the peaks. Moreover, lower maximum pressures were achieved, 205 bars (2973 psi) versus 415 bars (6019 psi) at 0.3 mL/min flow rate. Thus, Ace Ultra Core Super C_{18} column was used in further experiments.

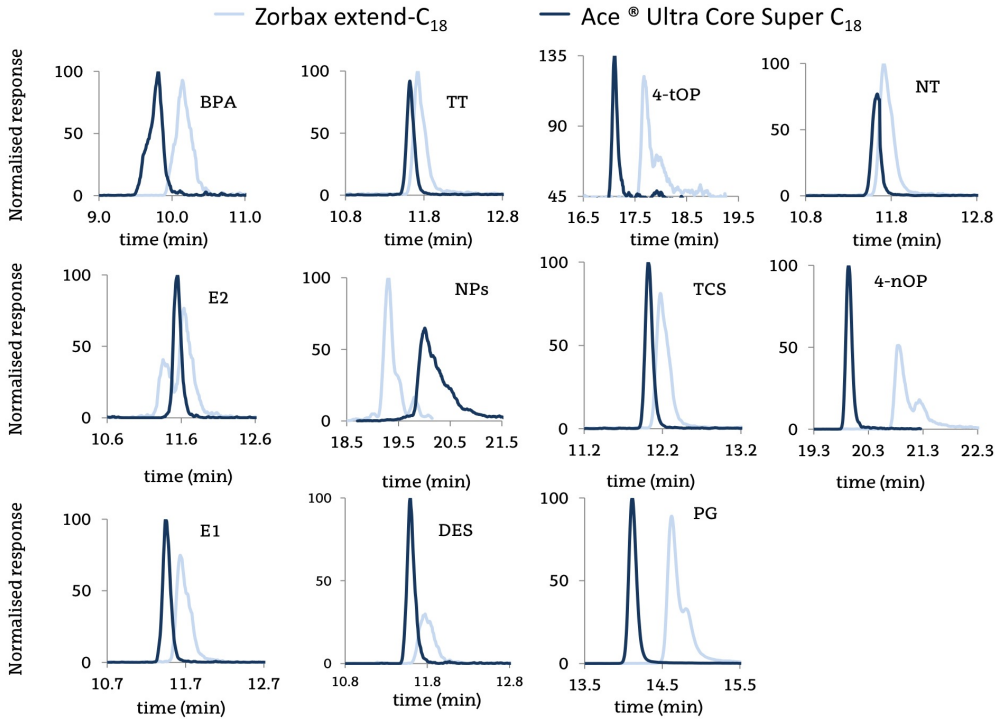


Figure 5.1. The influence of the column particle type on the target analyte peak shape.

Different column temperatures were also optimised: 30 ºC, 35 ºC and 40 ºC (higher temperatures were not recommended by the manufacturer). 30 ºC improved the chromatographic signal and peak shape for TCS, BPA, DES, E1 and E2 (see **Figure 5.2 a** for BPA).

On the other hand, APs, TT, NT and PG showed similar responses at all the temperatures tested (see **Figure 5.2 b** for 4-tOP). Due to the improvement in resolution for most of the target analytes, 30 ºC was chosen.

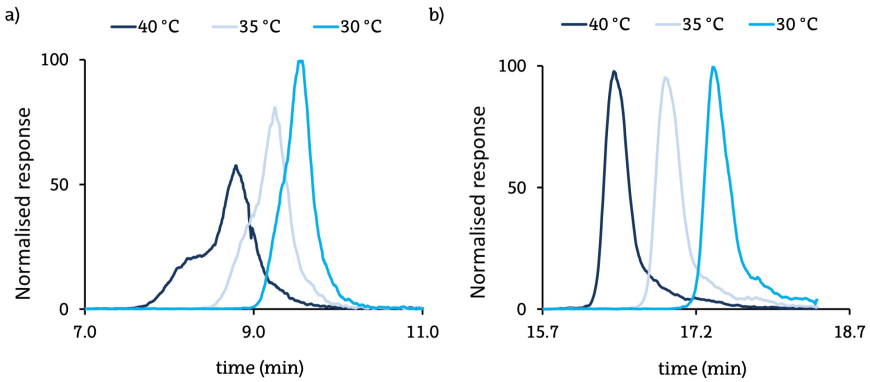


Figure 5.2. Peak shape and signal improvement when different column temperatures were tested for a) BPA and b) 4-tOP.

Since ESI and APCI interfaces were previously evaluated in our research group [29] for LC-MS/MS detection of some of the present target analytes and lower instrumental limits of detections (LODs) were obtained for the majority of them using ESI, only ESI was optimised in the present work.

Due to the dependency on column flow, ESI was firstly optimised at 3 different flow values (0.2, 0.3 and 0.4 mL/min) following two approaches. On the one hand, an experimental design approach (a central composite design, CCD, built by Statgraphic program, Statgraphics centurion XV) was used and capillary voltage (3000-6000 V), nebuliser pressure (30-50 psi) and drying gas (N_2) flow (8-12 L/min) variables were optimised at a fixed flow of 0.2 mL/min. On the other hand, Agilent Source Optimiser program was also used to optimise the same variables and an extra parameter, drying gas temperature (250-350 ºC) at the three different flow rates.

Response surfaces were built using Excel 2010 software including only significant parameters ($p_{value} < 0.05$) obtained from the Statgraphic program. Relative standard deviations (RSDs %) of the central point experiments (n=4) were in the range of 1-6 %. Drying gas flow had a positive effect for the majority of analytes (see **Figure 5.3 a** for NPs). Positive (4-tOP, 4-nOP, BPA, E1 and E2) or negative (TCS, DES, NT and TT) effects were observed in the case of capillary voltage (see **Figure 5.3 b** and **5.3 c** for E2 and TT, respectively). Finally, the nebuliser pressure was significant for six of the target analytes (see **Figure 5.3 b** for E2 and **Figure 5.3 c** for TT).

In parallel to the CCD, ESI was also automatically optimised by means of the optimisation software function. The same tendency was observed (see **Figure 5.4** for E2 and TT).

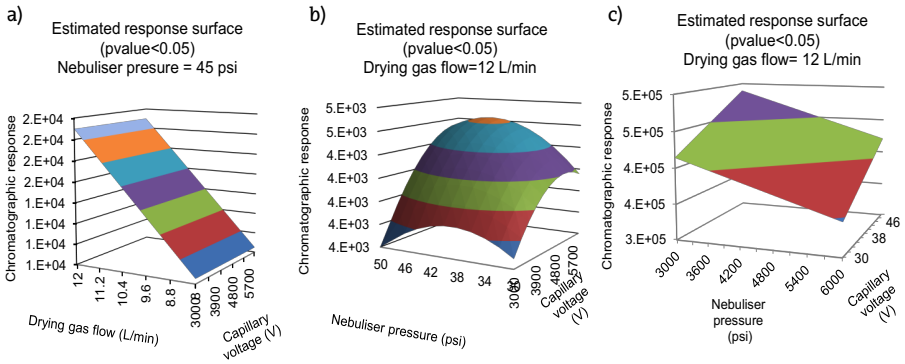


Figure 5.3. ESI optimisation: a) drying gas flow effect for NPs, b) positive effect of capillary voltage for E2 and c) negative effect of capillary voltage for TT.

Consensus optimum values were fixed: capillary voltage at 5000 V, drying gas flow at 12 L/min and nebuliser pressure at 45 psi. In the case of drying gas temperature, 350 ºC was selected as optimum value. Although Agilent Source Optimiser software does not consider the interaction between the variables, this program is less time consuming and it is fully automatic. Thus, it was decided to use the automatic optimisation software function for the evaluation of ESI parameters at the remaining flow rates (0.3 and 0.4 mL/min). Similar optimum values were obtained in the case of both 0.3 and 0.4 mL/min flow rates.

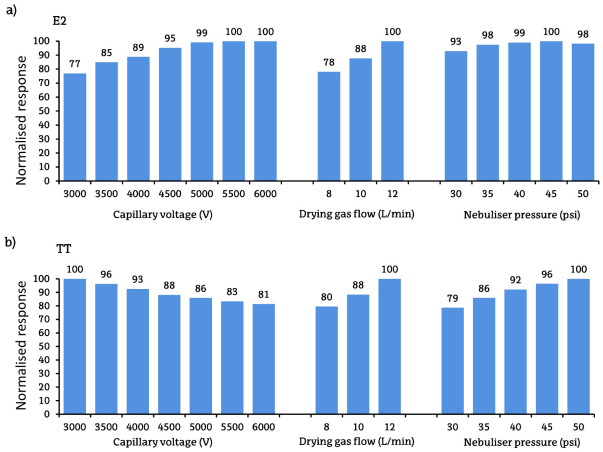


Figure 5.4. ESI optimisation (capillary voltage, drying gas flow and nebuliser pressure) at 0.2 mL/min flow rate by means of the optimisation software: a) for E2 and b) for TT.

The influence of the mobile phase flow in the separation of the analytes was finally evaluated at 0.2, 0.3 and 0.4 mL/min under the previously optimised electrospray parameters. Similar responses were obtained at 0.2 and 0.3 mL/min for NPs, 4-tOP and BPA, while 0.3 mL/min improved the peak intensity of DES, E1, E2 and 4-nOP. When 0.4 mL/min was used the chromatographic signal for the majority of the analytes did not improve, except for TCS, PG and NT, which were, in a less extend, more sensitive at 0.4 mL/min. Although a faster analysis was achieved at 0.4 mL/min, 0.3 mL/min was chosen as the optimum flow rate to obtain a compromise between peak shape, analysis time and sensitivity for all the target analytes.

The parameters related to the mass detector (i.e., fragmentor voltage, collision energy, collision cell accelerator, multiplier voltage and cycle time) were studied using the previously optimised instrumental parameters of ESI and flow rate. Both, target analytes and surrogates were considered.

Optimum values for each target analyte are summarised in **Table 5.2**. Two transitions were chosen when possible; the most sensitive transition was chosen as the quantifier and the second one as the qualifier.

Under optimised conditions, instrumental calibration curves were built with standard solutions (in MeOH) from 0.40 ng/mL to 500 ng/mL range using 10 standard solutions. As can be observed in **Table 5.2**, the linearity was kept in a wide concentrations range and determination coefficients (r^2) in the range of 0.9936-0.9999 were obtained for the external calibration. Instrumental LODs and quantification (LOQs) were estimated and defined as the average response (n=3) of the lower concentration level of the calibration curve for each analyte plus three and ten times the standard deviation, respectively. As can be observed in **Table 5.2**, the LODs and LOQs obtained were below 2.5 ng/mL and 3.0 ng/mL, respectively, except in the case of NPs, which showed 8.0 ng/mL (LOD) and 12.4 ng/mL (LOQ) values. It should be taken into account that NPs is a mixture of isomers and not a single analyte.

5.3.2 Optimisation of FUSLE

5.3.2.1 Improvement of NP blanks

Blank contamination problems are often encountered during the analysis of APs, especially NPs [29–31]. As blank signal subtraction does not always correct efficiently NP blank problems, the source of a possible NP contamination was firstly evaluated in the present work in terms of the extraction material used.

Two extraction vessels were used and compared: a 50-mL PP conical tube and a 50-mL Pyrex round bottom centrifuge tube. Blank samples were extracted by means of the procedure outlines in the experimental section (see **section 5.2.2**) and comparable results in terms of NP peak signals (n=2) were obtained for both extraction vessels ($F_{Experimental}$ = 1.9 < $F_{Critical}$ = 7.7, according to the analysis of variance, ANOVA) discarding the PP material as the NP source. Taking into account that similar blanks were obtained and, since according to Capelo et al. [20], smaller diameters avoid dead cavitations zones and it has been proved that conical bottom vessels are more appropriate than the spherical bottom vessels, PP conical tubes were chosen.

On the other hand, the two filtering steps included in the present method were also individually checked based on the filter problems previously observed in the literature [31]: (***i***) the supernatant FUSLE extracts filtered through the 0.45 μm filters (see **section 5.2.2**) and (***ii***) the final MeOH solvent filtered through the 0.2 μm filters previous to the injection in the LC-MS/MS system (see **section 5.2.3**). In this sense, 0.45 μm PA filters vs. 0.45 μm hydrophilic PTFE filters and 0.2 μm hydrophobic PP vs. 0.2 μm PTFE hydrophilic filters were tested. Using the 0.45 μm PTFE and the 0.2 μm PTFE filters NP blank signal was reduced in a 94 % and in a 91 %, respectively. Adsorption of the target analytes in the filters was not observed in the present work.

5.3.2.2 Composition of the extraction solvent

For the optimisation of extractant nature, 0.5 g (dry weight) of both carrot and amended soil were extracted according to the method developed by Zabaleta et al. [26]. Samples were fortified at 250 ng/g concentration level (according to the fortification procedure included in **section 5.2.2**) and used during the whole FUSLE optimisation. All the optimisation of the FUSLE parameters below was performed with a clean-up with 25 mg Envi-Carb (described in the **experimental section**). The experiments were always performed in triplicate.

In a first approach, different polarity solvents and combinations of some of them were studied according to the literature [19, 20, 32]: pure MeOH, ACN, DCM, MeOH:acetone (1:1, v:v) and hexane:acetone (1:1, v:v) mixtures.

Figure 5.5 shows that the responses obtained (normalised to the highest signal) were significantly higher when hexane:ace (1:1, v:v) mixture and ACN were used for the majority of the analytes in the case of carrot. NPs, 4-nOP and TCS did not show significant differences ($F_{Experimental}$ = 1.0-6.4 < $F_{Critical}$ = 18.5, according to ANOVA) between hexane:acetone (1:1, v:v) and ACN solvents. Moreover, the evaporation step to concentrate hexane:acetone (1:1, v:v) was

much faster than ACN, 33 min and 120 min, respectively. Similar results were observed in the case of the amended soil. Thus, hexane:acetone (1:1, v:v) mixture was chosen as extraction solvent in the case of both carrot and amended soil matrices. Similar results were obtained by Andreu et al. [15] for sludge amended soil sample extraction. Besides, acetone and hexane have been extensively used for the extraction of the present target analytes [10, 13, 15].

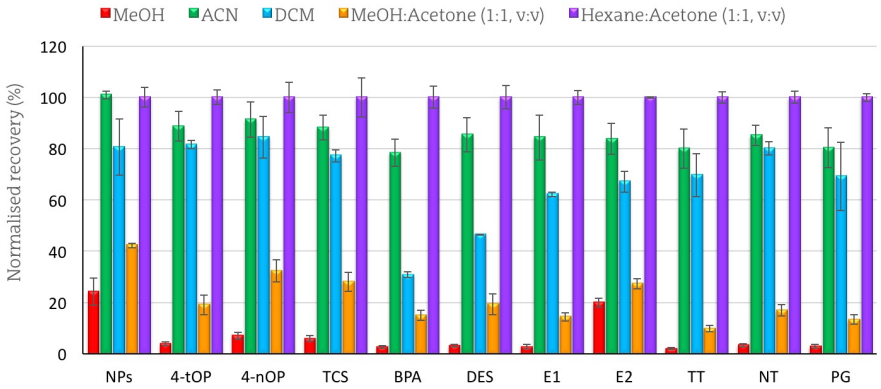


Figure 5.5. Influence of the solvent nature in the extraction yield during FUSLE extraction of carrot. Signals were normalised to the highest chromatographic response (n=3, 95 % confidence level).

Therefore, four different hexane:acetone solvent mixture compositions (20, 30, 50 and 80 % of hexane) were tested. The ratio hexane:acetone did not have any effect on 4-nOP, BPA and DES ($F_{Experimental}$ = 1.3-2.0 ‹ $F_{Critical}$ = 2.3, according to ANOVA). For the rest of the analytes, no significant differences were observed between hexane: acetone 30:70, 50:50 and 80:20 (v:v) ratios ($F_{Experimental}$ =1.6-4.7 ‹ $F_{Critical}$= 5.1). Besides, the yield of the extraction decreased (15-85 %) for the 20:80 hexane:acetone (v:v) case and it was, therefore, discarded. Besides, with the increment of hexane proportion, large evaporation times were needed. Hence, hexane: acetone 30:70 (v:v) was finally chosen as the extractant for further experiments.

5.3.2.3 Evaluation of the extraction and sonication times and amplitude

Once the solvent type was fixed, the influence of the extraction time, sonication time and amplitude was studied for carrot. A cycle is a sum of the period of time that sonication is on (defined as sonication time) and the period of time that sonication is off. A CCD was built using Statgraphics in order to optimise extraction time (0.5-5 min), amplitude (10-56 %) and sonication time (0.2-0.8 s). The responses obtained were adjusted to the nonlinear **Equation 5.1:**

$$Y=B_0+B_1X_1+B_2X_{2+}B_3X_{3+}B_{11}X_1^2+B_{22}X_2^{2+}B_{33}X_3^2+B_{12}X_1X_2+B_{13}X_1X_3+B_{23}X_2X_3 \quad \textit{Equation 5.1}$$

where, Y is the chromatographic response, B are the adjusting parameters and X the studied variables: 1=extraction time, 2= amplitude, 3= sonication time. Response surfaces were built using Excel 2010 software including only significant coefficient parameters (p_{value} < 0.05) obtained from Statgraphics program. RSDs % of the central experiments (n=4) were in the range of 3-10 %.

Although, the linear term B_3 (sonication time) was not significant for all the analytes, the quadratic B_{33} term clearly indicated that the best responses were obtained when high sonication time values were used (0.8 s) (**Figure 5.6 a** and **Figure 5.6 b** for DES and E1, respectively). Extraction time parameter did not show any significant effect, except in the case of TT and DES, for which the B11 extraction time quadratic term was significant and the best responses were obtained when high extraction times were used (5 min) (see **Figure 5.6 a**). The amplitude did not affect the response of any of the analytes and an intermediate value (33 %) was chosen.

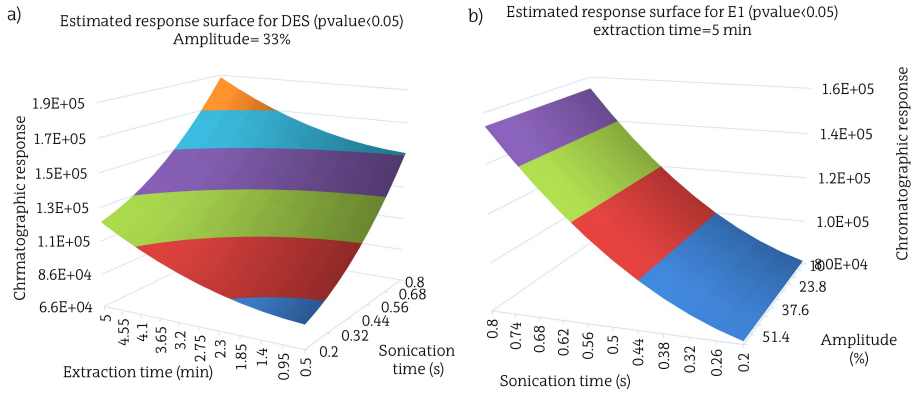


Figure 5.6. Response surfaces for a) DES when amplitude was fixed at 33 % and b) E1 when extraction time was fixed at 5 min.

Due to the lack of a certified reference material (CRM) and in order to determine whether exhaustive extraction was carried out under optimised conditions, up to three consecutive extractions using fresh extraction solvent each time were performed on the same carrot and amended soil samples. Recoveries lower than 10 % were obtained in the second extraction, except for NPs and 4-tOP (16-23 % for carrot and amended soil). Thus, only a single extraction was selected and carried out in further experiments for all the matrices.

5.3.3 Optimisation of the clean-up step

The most common sorbents used in the dSPE protocols are PSA (to remove various polar acids, polar pigments some sugars and fatty acids), graphitised carbon black (in order to remove sterols and pigments such as chlorophyll) and C_{18} (to remove non-polar interfering substances like lipids) [33]. $MgSO_4$ is also normally added in order to eliminate any water residue from the organic solvent.

The amount (25 mg, 50 mg and 75 mg) of Envi-Carb graphitised carbon was tested, as a first approach. The recovery of the target analytes was similar in most of the cases ($F_{Experimental}$ = 1.0-5.1 ‹ $F_{Critical}$ = 9.6, according to the ANOVA), except in the case of PG ($F_{Experimental}$ = 11.2 › $F_{Critical}$ = 9.6) where the recovery decreased significantly when the amount of Envi-Carb increased from 25 to 75 mg. Significantly cleaner carrot extracts were obtained when 75 mg sorbent amount was used and, thus, the latter was chosen as optimum.

Although good extraction efficiencies were achieved using 75 mg Envi-Carb for most of the analytes, four different mixtures of Envi-Carb-sorbent combinations were also tested: i) Envi-Carb graphitised carbon-charcoal powdered carbon-PSA, ii) Envi-Carb graphitised carbon-charcoal powdered carbon, iii) Envi-Carb graphitised carbon-Florisil and iv) Envi-Carb graphitised carbon-C_{18}. The suitability of the clean-up approaches was carried out by comparing the mixtures with the Envi-Carb clean-up in terms of both, recovery of the target analytes and the cleanliness of the final extracts. **Table 5.3** shows the clean-up recovery which was calculated by comparing the responses obtained when the extracts were spiked at 500 ng/mL before the clean-up (RSDs = 10-33 %) and after clean-up step (RSDs = 2-12 %) for all the cases. In general terms, Envi-Carb graphitised carbon and the combination of Envi-Carb and C_{18} showed the best efficiencies for all the target analytes (see **Table 5.3**).

Envi-Carb, activated charcoal powder carbon and PSA mixture was discarded due to the low recoveries obtained (0.1-19 %). In the case of PSA sorbent, the strong affinity observed could apparently be due to the hydrogen bonds formed between hydroxyl groups of our target analytes (see structures in **Table 5.1**) and the amino groups of PSA. A mixture of Envi-Carb and activated carbon without PSA was also tested. After removing PSA, low recoveries in the range of 2-34 % were also obtained, except for NPs (55 %) and 4-tOP (68 %). Thus, this sorbent combination was also discarded for further analysis.

Regarding to the low recoveries (see **Table 5.3**) obtained in the presence of charcoal powdered carbon (surface area › 100 m^2/g) compared to the values obtained by Envi-Carb (100 m^2/g), the

difference in the interaction mechanisms of both has been previously reported [34]. Charcoal powdered carbon can also contain several functional groups such as hydroxyl, carbonyl and carboxylic, thus, analytes with –OH or $–NH_2$ groups could be irreversibly adsorbed in the charcoal powder carbon by both hydrogen bonding and dispersive interactions.

Table 5.3. The influence of different dSPE sorbents combinations in the clean-up recovery (%) of carrot extracts.

Analyte	Clean-up recovery (%, n=4)				
	Envi-Carb graphitised carbon	**Envi-Carb, activated charcoal powder carbon and PSA**	**Envi-Carb and activated charcoal powder carbon**	**Envi-Carb and Florisil**	**Envi-Carb and C_{18}**
NPs	74	19	55	129	76
4-tOP	88	18	68	76	78
4-nOP	87	1	5	71	62
TCS	84	0.9	6	67	80
BPA	85	13	34	14	79
DES	86	0.1	5	17	83
E1	75	0.4	3	14	74
E2	80	0.8	4	13	62
TT	68	0.6	3	14	63
NT	75	5	24	20	68
PG	45	0.4	2	12	40

In the case of Envi-Carb and Florisil combination, the FUSLE extract was re-dissolved in isooctane. Low recoveries (12-20 %) were obtained, except for NPs (129 %), 4-tOP (76 %), 4-nOP (71 %) and TCS (67 %). In fact, although Florisil sorbent has been widely used [13, 19, 29] in the classical SPE clean-up of the target analytes, to the best of our knowledge, there are not methods published using Florisil in dSPE protocols. Thus, this clean-up approach was discarded.

Finally, as can be observed in **Table 5.3**, higher (4-tOP, 4-nOP and E2) and comparable (NPs, TCS, DES, BPA, E1, TT, NT and PG) clean-up recoveries were obtained in the case of Envi-

Carb and Envi-Carb and C_{18} combinations, respectively.

Consecuently, Envi-Carb and Envi-Carb-C_{18} combination were considered in further experiments. The carrot extracts from both clean-up procedures were analysed (LC-MS/MS) and compared in the SCAN mode and slightly cleaner chromatograms were observed using Envi-Carb-C_{18} mixture. In order to verify these results, the same extracts were also analysed by GC-MS according to Errekatxo et al. [19] method and once again cleaner chromatograms (see **Figure 5.7**) were observed using Envi-Carb-C_{18} mixture.

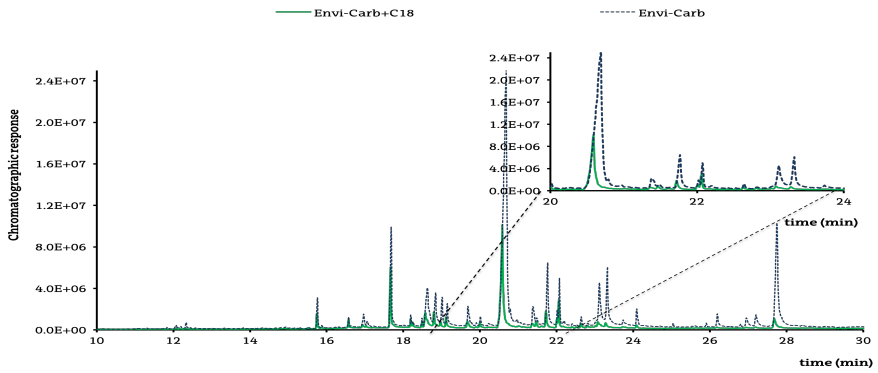


Figure 5.7. GC-MS chromatograms adquired in the SCAN mode for the carrot extract using both clean-up procedures: Envi Carb + C_{18} and Envi Carb.

On the other hand, in the case of amended soil samples, only Envi-Carb dSPE protocol was selected. Although a slightly matrix effect (60 %) in the detection was observed in the case of PG, recoveries close to 100 % in the range of 82-125 % were obtained in the case of FUSLE amended soil extracts injected without any clean-up procedure and using Envi-Carb dSPE protocol. However, Envi-carb dSPE protocol was used in order to avoid the frequent cleaning of the ESI interphase and the blocking of the different connector tubing in the LC system after repeated injections of compost-amended soil extracts when no clean-up was applied. Extracts were colourless after the Envi-carb cleaning.

Finally, the clean-up step recovery of both dSPE procedures optimised for carrot and amended soil was tested. In this sense, after removing the first ACN extract, 1.25 mL of acetone were added and vortexed once again for 40 s. Efficiencies in the 40-83 % were obtained for the dSPE protocol for carrots using only ACN, and those values did not significantly improve after the addition of acetone. Therefore, a single elution using ACN was chosen for carrots. In the case

of amended soil extracts, however, the addition of 1.25 mL of acetone significantly improved the efficiencies (15-50 %), while the addition of another extra 1.25 mL acetone aliquot had not a significant effect (efficiencies lower than 7 % in the third extract). Therefore, the target analytes were efficiently extracted in ACN plus acetone in the case of amended soil samples cleaned-up using dSPE with Envi-Carb.

Matrix effects occurring at LC-MS/MS detection were evaluated by comparing the responses obtained for carrot and amended soil samples which were spiked at 500 ng/mL after clean-up step and a standard solution of MeOH with the same analyte concentration. As can be observed in **Table 5.4**, recoveries close to 100 % were obtained for the majority of the analytes in the case of both Envi-Carb-C_{18} (for carrot) and Envi-Carb (for amended soil) optimum dSPE clean-up approaches indicating a lack of detection matrix effect.

Table 5.4. Matrix effect during detection of Envi-Carb–C_{18} approach for carrot and compost-amended soil samples in the case of Envi-Carb.

Analyte	**Matrix effect in the detection (%)**	
	Envi-Carb (amended soil)	**Envi-Carb-C_{18} (carrot)**
NPs	125±6	108±7
4-tOP	108±8	110±6
4-nOP	111±9	104±2
TCS	114±9	91±3
BPA	109±9	93±4
DES	113±11	107±12
E1	101±3	93±8
E2	101±3	95±7
TT	85±2	87±7
NT	89±8	83±5
PG	64±14	112±10

Envi-Carb-C_{18} and Envi-Carb dSPE approaches were finally chosen as optimum clean-up procedures of carrot and amended soil, respectively, in terms of clean-up efficiency, cleanliness of the extracts and matrix effect in the detection.

5.3.4 Method validation and application to real samples

5.3.4.1 Recoveries using external calibration, surrogate correction and matrix-matched calibration

Method validation was performed for FUSLE extraction with a posterior clean-up with Envi-Carb-C_{18} in the case of carrot and lettuce samples. FUSLE followed by Envi-Carb clean-up approach was used in the method validation for amended soil. Carrot, lettuce and amended soil samples were fortified at two levels (see concentration levels at **Table 5.5**) for each analyte.

Recovery (n=4) was calculated with an external calibration approach without surrogate corrections. According to the results (see **Table 5.5**), no quantitative recoveries were obtained for the majority of the analytes and matrices and, thus, it was concluded that there was a matrix-effect in the dSPE clean-up, since no matrix effects were previously observed in the detection step and quantitative extraction had been observed for FUSLE. Apparent recoveries were also calculated after correction with the corresponding surrogate and using a matrix-matched calibration approaches. Matrix-matched calibration was performed with samples spiked at the lower concentration level used in the validation (see **Table 5.5**). Although, good apparent recoveries using surrogate corrections were obtained (in the 70–130 % range for most of the target analytes and matrices, see **Table 5.5)**, matrix-matched approach was needed for the quantification of TT and 4-tOP in lettuce. In the case of amended soil samples, matrix-matched was needed for the proper quantification of most of the analytes (NPs, TCS, BPA, DES, TT, E1 and E2). Thus, quantification using instrumental calibration was discarded in successive experiments since the use of surrogates did not always guarantee matrix effect correction for all the analytes and matrices at the two concentration levels evaluated.

Table 5.5. Fortified concentration (ng/g) level, recovery (%), apparent recovery using labelled standards and matrix-matched calibration for carrot, lettuce and amended soil samples (n=4). Relative standard deviation (%) of the apparent recoveries included in brackets.

Analyte	Carrot matrix						
	Low level				High level		
	Fortified at (ng/g)	Recovery (%)	Recoveries using labelled standards	Recoveries with matrix-matched	Fortified at (ng/g)	Recoveries using labelled standards	Recoveries with matrix-matched
NPs[a]	25	11	124 (18)	379 (21)	100	129 (9)	172 (20)
4-tOP[a]	5	51	104 (10)	103 (19)	25	135 (14)	122 (17)
4-nOP[a]	5	30	76 (7)	82 (15)	25	80 (5)	91 (22)
TCS[a]	5	49	90 (12)	89 (18)	25	89 (13)	90 (19)
BPA[b]	5	64	110 (16)	94 (12)	25	133 (28)	104 (20)
DES[b]	5	38	99 (13)	100 (17)	25	125 (11)	93 (20)
E1[c]	5	28	108 (8)	89 (12)	25	143 (18)	86 (27)
E2[c]	5	24	98 (22)	104 (23)	25	141 (22)	98 (20)
TT[c]	1	14	84 (18)	85 (17)	5	109 (19)	99 (18)
NT[c]	1	34	103 (13)	60 (20)	5	145 (22)	95 (23)
PG[d]	1	12	90 (11)	69 (5)	5	72 (9)	95 (10)
Analyte	**Lettuce matrix**						
	Low level				High level		
	Fortified at	Recovery	Recoveries using labelled standards	Recoveries with matrix-matched	Fortified at	Recoveries using labelled standards	Recoveries with matrix-matched
NPs[a]	50	7	126 (10)	67 (12)	150	126 (19)	48 (24)
4-tOP[a]	15	75	165 (9)	68 (13)	45	117 (9)	87 (8)
4-nOP[a]	15	50	93 (11)	106 (6)	45	106 (6)	113 (4)
TCS[a]	15	58	112 (6)	95 (10)	45	95 (9)	97 (4)
BPA[b]	15	74	125 (11)	88 (9)	45	122 (5)	77 (2)
DES[b]	15	26	122 (14)	66 (16)	45	127 (8)	68 (4)
E1[c]	15	39	122 (9)	92 (10)	45	134 (12)	102 (4)
E2[c]	15	10	107 (24)	75 (13)	45	66 (8)	101 (2)
TT[c]	8	17	80 (14)	91 (15)	25	54 (9)	71 (8)
NT[c]	8	24	98 (16)	68 (12)	25	64 (10)	40 (7)
PG[d]	1	2	97 (8)	65 (24)	5	74 (3)	130 (5)
Analyte	**Compost-amended soil matrix**						
	Low level				High level		
	Fortified at	Recovery	Recoveries using labelled standards	Recoveries with matrix-matched	Fortified at	Recoveries using labelled standards	Recoveries with matrix-matched
NPs[a]	50	156	226 (15)	90 (10)	150	113 (15)	89 (9)
4-tOP[a]	15	50	80 (4)	95 (15)	45	91 (10)	82 (11)
4-nOP[a]	15	42	70 (4)	92 (14)	45	87 (7)	81 (11)
TCS[a]	15	28	58 (10)	78 (19)	45	80 (13)	75 (15)
BPA[b]	15	39	54 (9)	69 (16)	45	80 (7)	73 (13)
DES[b]	15	5	63 (7)	64 (25)	45	37 (20)	87 (17)
E1[c]	15	9	58 (13)	76 (24)	45	88 (13)	70 (16)
E2[c]	15	7	49 (18)	74 (17)	45	84 (14)	72 (14)
TT[c]	8	13	55 (13)	74 (23)	25	104 (15)	72 (14)
NT[c]	8	30	125 (9)	65 (16)	25	105 (6)	80 (10)
PG[d]	1	13	118 (27)	95 (19)	5	91 (11)	67 (18)

Analytes were corrected with [a] [$^2H_4$]-NP, [b] [$^2H_{16}$]-BPA, [c] [$^2H_3$]-E2 and [d] [$^2H_9$]-PG surrogates.

5.3.4.2 Method detection limits and precision of the method

Method detection limit (MDL) of each analyte was determined according to EPA guidance for MDL calculation (http://www.epa.gov/waterscience/methods/det/rad.pdf) for carrot, lettuce and amended soil samples by spiking seven replicates of each blank matrix with each analyte at the lowest concentration used in the validation of each matrix (see **Table 5.5**). The MDLs were then calculated according to **Equation 5.2** and they are summarised in **Table 5.6**.

$$MDL = t_{(n-1,\ 1-\alpha=0.99)} \times S_d \quad \textbf{Equation 5.2}$$

where t= 3.143 corresponds to the Student´s t-value for a 99 % confidence level and 6 degrees of freedom and S_d is the standard deviation of the replicate analyses.

Table 5.6. MDLs obtained by FUSLE-dSPE-LC–ESI-MS/MS method in the case of carrot, lettuce and amended soil samples.

Analyte	Carrot		Lettuce		Amended soil	
	Spiked at ng/g	MDLs (ng/g)	Spiked at ng/g	MDLs (ng/g)	Spiked at ng/g	MDLs (ng/g)
NPs	25	100	50	152	40	31
4-tOP	5	3	14	6	11	1
4-nOP	5	1	14	3	11	0.9
TCS	5	2	14	3	11	3
BPA	5	2	14	4	11	2
DES	5	2	14	2	11	1
E1	5	0.9	13	6	11	2
E2	5	1	14	1	11	3
TT	1	0.1	7	0.5	7	2
NT	1	0.2	7	0.8	7	3
PG	1	0.1	2	0.2	2	2

MDL values in the range of 0.1-6 ng/g were obtained for the majority of the analytes in the case of the three matrices (except for NPs). Similar MDLs were obtained by Chiu et al. [8] for estrogens (2-5 ng/g). Although, better detection limits (0.04-0.6 ng/g) were obtained by Lu et al. [10], it should be highlighted that LOD values (3 times signal to noise ratio, S/N) instead of MDLs were reported. Higher MDLs (31, 100 and 152 ng/g in the case of amended soil, carrot and lettuce

samples, respectively) were determined for the NP technical mixture.

The precision of the method in terms of repeatability, expressed as RSD, was evaluated for FUSLE-Envi-Carb-C_{18} (carrot and lettuce) and FUSLE-Envi-Carb (amended soil) coupled to LC-ESI-MS/MS at two concentration levels and the results obtained are also summarised in **Table 5.5.** RSDs between 2-27 % were obtained within a day for all the matrices and analytes. Similar results (RSDs=10-31 %) were obtained by Navarro et al. [13] when FUSLE-SPE-GC-MS was applied to the analysis of hormones and APs. Besides, Peysson et al. [17] and Pouech et al. [18] reported RSD values below 30 % when QuEChERS followed by dSPE was carried out for the determination of hormones and BPA, among other compounds, in sewage sludge and rat samples, respectively. The reproducibility was calculated using ten replicates which were analysed in different days. Similar RSD values (13-29 %) were obtained among the days for most analytes in the case of reproducibility assays.

5.3.4.3 Application of the developed method to real samples

The optimised method was applied in the analysis of carrot, lettuce and amended soil samples (n=3). Only BPA was detected at 9±1 ng/g and 11±2 ng/g in carrot and lettuce samples, respectively. BPA was not detected in method blank samples. The rest of the analytes were below MDLs. Lower BPA concentration values (around 2 ng/g) from Indian lettuce samples were reported by Lu et al. [10]. However, further research about real vegetable and amended soil samples is needed to take conclusions about the concentrations obtained.

European Food Safety Authority (EFSA) established the maximum acceptable and tolerable daily intake of BPA at 50 μg/Kg of body weight/day according to CD 2004/19/EC. On the other hand, World Health Organization (WHO) recommended a minimal consumption of 400 g of fresh fruit and vegetables (http://www.who.int/dietphysicalactivity/publications/f%26v_promotion_effectiveness.pdf). Considering an average humidity percentage of carrot and lettuce (91-93 %) and average concentration of BPA detected in the present work, the daily intake of BPA through the consumption of vegetables for a 60 kg adult would be 2.0 μg/kg of body weight/day. Hence, a 4 % of the maximum daily intake could arise from the vegetables consumption. Further research should also be carried out for the confirmation of this hypothesis.

5.4 Conclusions

To the best of our knowledge, the combination of FUSLE-dSPE-LC-(ESI)-MS/MS allows, for the first time, the accurate and precise simultaneous determination of 11 EDCs in vegetable (carrot and lettuce) and amended soil samples. The method requires a low amount of solvent (10 mL) and short (5 min) extraction times. A simplified dSPE clean-up strategy was also applied saving time (~60 s), effort, money and solvent consumption, compared with other solid-liquid extraction methods such as SPE. Florisil, charcoal powder carbon and PSA sorbent were discarded due to the low recoveries obtained, probably because of the formation of hydrogen bonds between the sorbents and the hydroxyl groups of target analytes. Although both, Envi-Carb-C_{18} and Envi-Carb dSPE approaches provided satisfactory results in terms of recoveries for most of the target analytes and matrices, the cleanest extracts and lower matrix effect was observed in the case of Envi-Carb-C_{18} sorbent combination for carrot samples. Good apparent recoveries (70-130 %) for most of the analytes and matrices were obtained when an external calibration approach was used after correction with deuterated analogues. Matrix-matched calibration approach was also needed for the proper quantification of some of the analytes in the matrices evaluated.

5.4.1 References

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CHAPTER 6

Enrichment of perfluorinated alkyl substances on polyethersulfone using 1-methylpyperidine as ion-pair reagent for the clean-up of carrot and compost-amended soils extracts

Talanta 143 (2015) 263-270

6.1 Introduction

Per- and polyfluoroalkyl substances (PFASs) include a broad range of ionic and neutral compounds. Among them, perfluoroalkyl acids (PFAAs), which take a prominent place in the literature, are a family of about 30 chemicals consisting on an aliphatic carbon chain (typically from C4 to C16) attached to one hydrophilic end group, such as carboxylic, sulfonic, sulfinic, phosphonic or phosphinic acids. PFAAs are resistant against hydrolysis, photolysis, microbial degradation and metabolisation [1]. Moreover, long chain PFAAs have been found to be highly persistent, toxic and bio-accumulative in wildlife and humans [2,3]. Due to the carbon-fluorine (C-F) covalent bond stability, one of the strongest found in organic chemistry, numerous PFASs, including PFAAs, have some unique chemical properties such as an extremely high thermal and chemical stability [4,5]. Besides, due to their surface tension lowering properties, PFASs have been and are still widely used in many industrial applications and consumer products, including stain repellents, metal plating foams, paints, alkaline cleaners, polishes, non-stick cookware, semiconductors, photographic films, pesticide formulations, denture cleaners, electronic adhesives, food packaging, etc.

Once they enter the body, PFASs are poorly eliminated. Longer carbon chain PFASs (C › 8) have been reported to bioaccumulate more than short chain ones [6-8]. The slow elimination rates of PFASs [9-10] suggest that "continued exposure could increase body burdens to levels that would result in adverse outcomes" (USEPA 2009, http://www.epa.gov/oppt/existingchemicals/pubs/pfcs_action_plan1230_09.pdf), which has led the European Union, North America and the major world manufacturers, such as 3M and DuPont, to impose restrictions on the production and use of long chain PFAAs (such as perfluorooctane sulfonate acid, PFOS, and perfluorooctanoic acid, PFOA). In the year 2000 the largest global producer of PFASs, the 3M company, announced the phase out of the production of PFOS and related chemicals. Since then, new shorter-chain PFAAs (C4-C7) and their precursors are being introduced as replacements considering that the latter are less bio-accumulative or toxic in living beings [11,12].

The US Environmental Protection Agency (USEPA) recently included PFOA and linear L-PFOS in its pared-down third drinking water contaminant candidate list 3 of 32 compounds for further regulatory studies (USEPA 2011, http://www.epa.gov/safewater/ccl/pdfs/ccl3_docs/pre-fr_ccl3.pdf). The agency also included six PFAAs (perfluoro-1-butanesulfonate, L-PFBS,

perfluoro-1-hexanesulfonate, L-PFHxS, L-PFOS, perfluoro-n-heptanoic acid, PFHpA, PFOA and perfluoro-n-nonanoic acid, PFNA) in its final list of 32 contaminants for the unregulated contaminants monitoring rule 3 (USEPA 2011b, http://water.epa.gov/lawsregs/rulesregs/sdwa/ucmr/ucmr3/upload/UCMR3_FinalRule.pdf). Besides, directive 2013/39/EU of the European Parliament lays down the environmental quality standards (EQSs) for a group of priority substances, where PFOS has been included.

Because of their widespread use, analytical approaches for PFAS determination in different matrices, such as air [13,14], water [4,15], sediments [16,17], biota [18], food [19,20], sludge [21,22], crops [23,24] and human plasma [25], are continuously being developed. Alkaline digestion [26], acid digestion [27], ion-pair extraction [28], solvent extraction [19], pressurised liquid extraction (PLE) [17], focused ultrasound solid-liquid extraction (FUSLE) [29,30] and matrix solid-phase dispersion (MSPD) [18] are the most commonly used extraction techniques for the PFAS analysis in solid matrices.

Due to the lack of selectivity of the above mentioned extraction techniques, matrix effects are a challenge in the analysis of PFASs in solid samples. As reported previously in the literature, ion suppression or enhancement can occur during the analysis of PFASs by liquid chromatography tandem mass spectrometry (LC-MS/MS) [16]. Therefore, extracts require further clean-up to remove co-extracted lipids and other potential matrix components that interfere with the ionisation of the target compounds. According to the literature, individual or serial weak anion exchange (WAX) solid phase extraction (SPE) and Envi-Carb (dispersive graphitised carbon) are the most widely used clean-up strategies for quantification of PFASs in food [17,22,31,32]. Other sorbent mixtures such as Florisil and Envi-Carb mixture [33] and CUQAX256 SPE cartridge (mixed mode C_8+quaternary amine) [34] have also been used.

For the analysis of PFASs, LC coupled to triple-quadrupole (QqQ) MS is the most common instrument [35,36] and gas chromatography (GC) is less frequently used because a derivatisation step is necessary for carboxylic and sulfonic acid analysis [37,38].

Within this context, the aim of the present work was to develop an alternative cheap and easily achievable clean-up approach for the enrichment of perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFSAs) and perfluorooctane sulfonamide (FOSA) using a polymeric material and an ion-pair reagent after the extraction by means of FUSLE from carrot and amended soil samples. For the polymeric material, silicone rod (SR) and

polyethersulfone (PES) were tested and 1-methylpyperidine (1-MP) and tetrabutylammonium (TBA) salts were studied as ion-pair reagents. Finally, the method was applied for the determination of PFASs in uptake experiments where carrots were cultivated in a compost-amended soil polluted with PFOS. To the best of our knowledge, neither sorptive extraction nor polymeric materials such as SR and/or PES have been previously applied to the clean-up extracts of PFASs from solid samples.

6.2 Experimental section

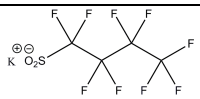
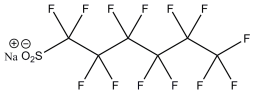
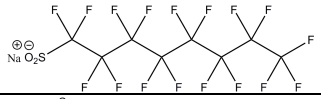
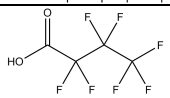
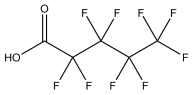
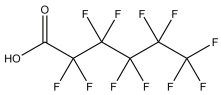
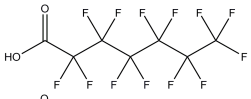
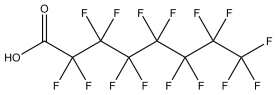
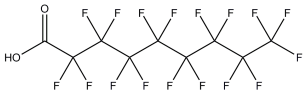
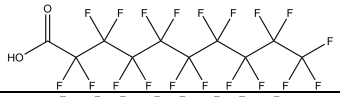
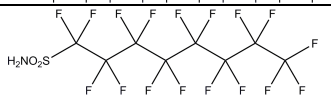
6.2.1 Chemicals and materials

A mixture of native perfluorinated standards containing potassium perfluoro-1-butanesulfonate, L-PFBS; sodium perfluoro-1-hexanesulfonate, L-PFHxS; potassium perfluoro-1-octanesulfonate, L-PFOS; perfluoro-n-butanoic acid, PFBA; perfluoro-n-pentanoic acid, PFPeA; perfluoro-nhexanoic acid, PFHxA; perfluoro-n-heptanoic acid, PFHpA; perfluoro-n-octanoic acid, PFOA; perfluoro-n-nonanoic acid, PFNA; perfluoro-n-decanoic acid, PFDA and isotopically mass-labelled compounds at 5 µg/mL and 2 µg/mL in methanol (MeOH), respectively, were obtained from Wellington Laboratories (Guelph, Ontario, Canada). FOSA was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The abbreviation, the structure, the purity of the standards and the acidity constant (as pK_a) values as well as their corresponding surrogates are also included in **Table 6.1**.

MeOH (HPLC grade, 99.9 %) was supplied by LabScan (Dublin, Ireland) and acetonitrile (ACN, HPLC grade, 99.9 %) from Sigma Aldrich (Steinheim, Germany). Ultra-pure water was obtained using a Milli-Q water purification system (‹ 0.05 µS/cm, Milli-Q model 185, Millipore, Bedford, MA, USA).

Ion-pair reagent tetra-n-butylammonium bromide (TBABr, › 98 %) was obtained from Sigma Aldrich, whereas tetra-n-butylammonium hydrogen sulphate ($TBAHSO_4$, › 98 %) and 1-MP (purity › 98 %) were provided by Merck (Schuchardt OHG, Germany). Formic acid (HCOOH, 98-100 %) was purchased from Scharlau (Barcelona, Spain).

Table 6.1. Structures, their corresponding surrogate, purity (%) and pKa values of the target analytes.

Analyte		Surrogate	Structure	Purity (%)	pKa
PFSAs	L-PFBS	MPFHxS		›98.0	0.14 [a]
	L-PFHxS	MPFHxS		›98.0	0.14 [a]
	L-PFOS	MPFOS		›98.0	0.14 [a]
PFCAs	PFBA	MPFBA		›98.0	0.2-0.4 [b]
	PFPeA	MPFHxA		›98.0	0.5 [b]
	PFHxA	MPFHxA		›98.0	0.9 [b]
	PFHpA	MPFOA		›98.0	-
	PFOA	MPFOA		›98.0	2.8 [b]
	PFNA	MPFNA		›98.0	2.57 [c]
	PFDA	MPFDA		›98.0	2.6 [b]
	FOSA	MPFHxS		›97.5	6.52 [a]

[a] [39], [b] [40], [c] [41]

For the mobile phase composition MeOH from Fisher Scientific (Loughborough, UK) was used. Ammonium acetate (NH_4OAc, ≥ 99 %) was purchased from Sigma Aldrich. For

chromatographic separation of PFASs, Zorbax Eclipse XDB-C_{18} guard column (2.1 mm x 5 mm, 1.8 μm) followed by Zorbax Extend-C_{18} Rapid Resolution HT (2.1 mm x 50 mm, 1.8 μm) column were provided by Agilent Technologies (Palo Alto, CA, USA). Sure-Guard in-line filter (24.4 mm, 10 mm, 0.5 μm) obtained from VICI Jour (Schenkon, Switzerland) followed by ACE UltraCore 2.5 SuperC_{18} core-shell column (2.1 mm x 50 mm, 2.5 μm) purchased by Advanced Chromatography Technologies (Aberdeen, Scotland) were also tested for target analyte separation.

For extraction, 50-mL polypropylene conical tubes (PP, internal diameter 27.2 mm x 117.5 mm length) obtained from Deltalab (Barcelona, Spain) and a Bandelin ultrasonic homogeniser (20 kHz; Bandelin Electronic, Berlin, Germany) equipped with a 3 mm titanium microtip were used. Desorption was made using a Digital Ultrasonic Cleaner (2500 mL, Axtor by Lovango, Barcelona). Fractions were evaporated in a Turbovap LV Evaporator (Zymark, Hopkinton, MA, USA) using a gentle stream of nitrogen (› 99.999 % of purity) supplied by Messer (Tarragona, Spain). After the extraction step, the supernatant was filtered through polyamide filters (0.45 μm, 25 mm, Macherey-Nagel, Germany). GHP (hydrophilic polypropylene) microfilters (0.2 μm, 13 mm, Pall, USA) were used to filter extracts to polypropylene plastic vials (Waters Corporation, Milford, MA, USA) before LC-MS/MS analysis.

The PES sorbent was acquired from Membrane (Wuppertal, Germany) in a tubular format (0.7 mm external diameter, 1.43 g/mL density). The commercial silicone elastomer (SR, in flexible rod form, 0.97 g/mL density) was purchased from Goodfellow (PA, USA). Pieces of the polymers (1.9-cm length for PES and 1-cm for silicone rods) were cut using a sharp blade and accurately weighed (**~** 100 mg) and conditioned in MeOH for at least 24 h. The SR was also thermally conditioned at 120 ° C for 180 min under a nitrogen stream (ca. 30 mL/min, purity › 99.999 %) supplied by Messer. Thereafter, the materials were dried using a lint-free tissue and kept in closed glass vessels until use. Sorbents were discarded after use. Agitation was carried out using 30-mL polystyrene vessels (PS, 60 x 27 mm) provided by ServiQuimia (Tarragona, Spain) in a 15-position magnetic stirrer (Kika Werke, Staufen, Germany).

For uptake experiments, soil 2.4 was supplied by LUFA Speyer (Speyer, Germany), compost was acquired from Calahorra wastewater treatment plant (WWTP, Spain) and both carrot seeds (***Chantenay*** variety) and carrots (used for method optimisation/ validation) were obtained in a local market.

6.2.2 Cultivation of carrot exposed to compost-amended soil fortified with PFOS

Carrot (***Chantenay*** variety) seeds were washed with Milli-Q water. Afterwards, seeds were germinated for approx. 10 days in petri dishes that were covered with moistened paper filters. Once almost all the seeds were germinated, 6 seedlings were transplanted in each pot containing compost-amended soil (soil and compost mixture 95:5, w:w). Previously, a known amount of compost was weighed, covered with acetone, fortified with PFOS to obtain 500 ng/g concentration in the soil compost mixture, stirred for 24 h and aged for a week. This concentration was selected because according to a review previously published by Clarke and Smith [42], perfluorinated chemicals were detected at concentrations of 100 ng/g level or higher in sludge. Pots were maintained in environmental controlled conditions in a greenhouse (temperature was set to 25 ºC during the day and at 18 ºC during the night with a 14-h day length and a relative humidity of 50 % during the day and 60 % overnight) and they were watered regularly with distilled water or Hoagland nutrient solution [43]. Plants were harvested after a period of at least 3 months and all the pots were collected simultaneously. Carrots were carefully washed with tap water and peeled with a vegetable peeler (depth ca. 2 mm). All plant material was freeze-dried (leaves, peel and core), packed in plastic bags and stored in a freezer until analysis.

6.2.3 Sample treatment and focused ultrasound solid-liquid extraction

Taking into account the vapour pressure of PFOS ($3.31\ 10^{-4}$ Pa at 20 ºC) [44] and that the Organisation for Economic Co-operation and Development (OECD, 2002) classified PFOS as an involatile chemical [45], carrots were frozen and freeze-dried before the extraction step using a Cryodos-50 laboratory freeze-dryer from Telstar Instrument (Sant Cugat del Valles, Barcelona, Spain) due to the negligible possibility of volatilisation. Compost-amended soil samples were dried at room temperature for 24-48 h. For optimisation and validation purposes a known amount of matrix was weighed, covered with acetone, fortified with the target analytes and stirred during 24 h. After that, acetone was evaporated and the sample was aged for one week.

Extraction was carried out under conditions optimised in a previous work [29]. 0.5 g of sample was placed together with 7 mL of an ACN:Milli-Q water (9:1, v:v) mixture in a 50 mL vessel and surrogate standards (MPFHxS, MPFOS, MPFBA, MPFHxA, MPFOA, MPFNA, MPFDA, MPFUnDA, MPFDoDA) were added (20 µL of a 0.5 ng/µL solution). The FUSLE step was performed for 2.5 min in duplicate, with a sonication time of 0.8 s and at 10 % of power (80 W).

Extractions were carried out at 0 °C in an ice-water bath. After the extraction step, the supernatant was filtered through polyamide filters and FUSLE extract was evaporated to ~ 1 mL under a nitrogen stream using a Turbo Vap LV Evaporator.

6.2.4 Clean-up evaluation

6.2.4.1 Clean-up using polymeric material

Under optimised conditions the clean-up using a polymeric material and an ion-pair reagent was performed as follows. The extract evaporated to ~ 1 mL was diluted with 5 mL of 5 mM aqueous solution of 1-MP and poured to 30 mL polystyrene vessel containing a magnetic stirrer and the sorbent material. The vessels were closed and extraction was performed at room temperature with a stirring rate of 750 rpm for 3 h. After the extraction time was over, the sorbent material was removed and rinsed with Milli-Q water in order to eliminate residues, dried with a clean tissue and placed inside a 1-mL Eppendorf tube. For desorption, 500 µL of MeOH were added and the polymeric material was sonicated for 15 min in an ultrasonic bath. After that, the extract was evaporated down to 200 µL and samples were filtered through a 0.2 µm GHP filters before LC-MS/MS analysis.

6.2.4.2 Clean-up using solid-phase extraction

This clean-up was performed according to a method published by Zabaleta et al. [29]. The extract evaporated to ~ 1 mL was diluted in 6 mL of Milli-Q water. The 200-mg Waters Oasis-WAX cartridges were conditioned with 5 mL of MeOH and 5 mL of Milli-Q water. After the sample loading, both 1 mL of HCOOH (2 %) and 1 mL of Milli-Q:MeOH (95:5, v:v) mixture were added with cleaning purposes and the cartridges were dried for 1 h under vacuum. Then, the analytes were eluted using 4 mL of MeOH with 2.5 % NH_4OH and collected in a single vial. After elution, the extract was concentrated to dryness under a gentle stream of nitrogen at 35 °C and reconstituted in 200 µL of LC-MS grade MeOH. Finally, the reconstituted extract was filtered through a 0.2 µm GHP filter before LC-MS/MS analysis.

6.2.5 LC-MS/MS analysis

An Agilent 1260 series HPLC chromatograph equipped with a degasser, a binary pump, an autosampler and a column oven coupled to an Agilent 6430 triple quadrupole (QqQ) mass spectrometer equipped with both electrospray (ESI) and atmospheric pressure chemical

ionisation (APCI) sources (Agilent Technologies, Palo Alto, CA, USA) was employed for the separation and quantification of PFASs. Under optimum conditions, 5 µL of sample volume were injected on a Sure-Guard in-line filter followed by ACE UltraCore 2.5 SuperC_{18} reverse phase column for separation of the target analytes at a flow rate of 0.3 mL/min. Separation was carried out at 35 °C by gradient elution using a binary mobile phase consisting of water:MeOH (95:5, v:v) mixture (solvent A) and MeOH:water (95:5, v:v) as solvent B, both containing 2 mmol/L NH_4OAc and 5 mmol/L 1-MP. The gradient profile started with 90 % A (hold time 0.3 min) and continued with a linear change to 80 % A up to 1 min, to 50 % A up to 1.5 min and to 20 % A up to 5 min (hold time 5 min) followed with a linear change to 0 % A up to 13 min and a hold time until 16 min. Initial conditions were regained at 17 min followed by equilibration until 26 min.

Quantification was performed in the selected reaction monitoring (SRM) acquisition mode. High purity nitrogen (› 99.999 %) supplied by Messer was used as collision gas and nitrogen (99.999 %) purchased from Air Liquide (Madrid, Spain) was used as nebuliser and drying gas. ESI in the negative mode was carried out using a capillary voltage of 3000 V, a drying gas flow rate of 10 L/min, a nebuliser pressure of 50 psi and a drying gas temperature of 350 °C. Optimal fragmentor electric voltage and collision energy values for the target analytes and surrogates were optimised in a previous work [29] and are included in the **Table 6.2**.

Table 6.2. Precursor and product ions (first ion was used as quantifier and the second as qualifier) at optimum fragmentor voltage(V) and collision energy (eV) values for the target analytes.

Analytes	Precursor ions (m/z)	Product ion (m/z)	Fragmentor voltage (V)	Collision Energy (eV)
PFBA	213	169	60	5
PFHxPA	399	79	100	10
PFPeA	263	219/175	60	5
L-PFBS	299	99/80	100	30
PFHxA	313	269/119	60	5
PFOPA	499	79	150	20
PFHpA	363	319/169	60	10
L-PFHxS	399	99/80	150	20
PFOA	413	369/169	60	5
PFDPA	599	79	100	5
PFNA	463	419/169	60	5
LPFOS	499	99/80	150	45
FOSA	498	498/78	220	5
PFDA	513	469/269	100	5
MPFBA	217	172	60	5
MPFHxA	315	270	60	5
MPFHxS	403	103	150	30
MPFOA	417	372	60	5
MPFOS	503	99	60	45
MPFNA	468	423	60	5
MPFDA	515	470	100	5

Instrumental operations, data acquisition and peak integration were performed with the Masshunter Workstation Software (Quantitative Analysis, Version B.06.00, Agilent Technologies).

6.3 Results and discussion

6.3.1 Optimisation of the polymeric based preconcentration and clean-up step

For the optimisation, the signals of PFHxA, PFHpA, PFOA, PFNA, PFDA, L-PFBS, L-PFHxS, L-PFOS and FOSA were followed. Although PFBA and PFPeA were in the reagent mixture, they were not considered due to the unsuitable chromatographic peak shape obtained (see **Figure 6.1**), probably because of the bad retention of the short chain PFCAs in reverse phase columns. Better results might be obtained using an ion-exchange column as reported in the literature [46].

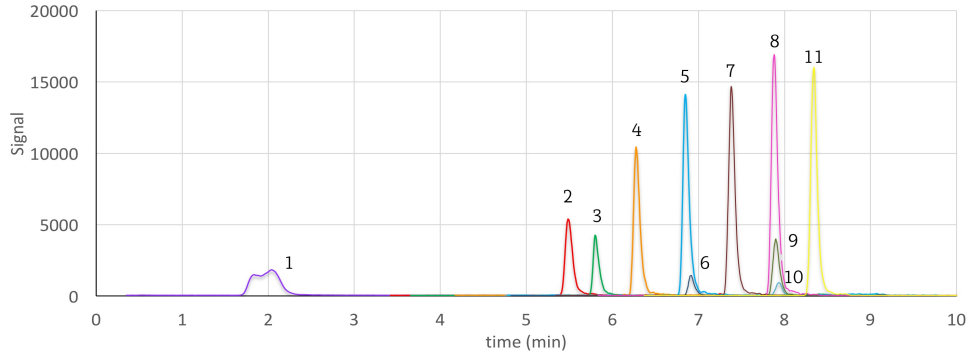


Figure 6.1. Chromatogram of a 5 ng/mL PFAAs and FOSA solution: (1) PFBA, (2) PFPeA, (3) PFBS, (4) PFHxA, (5) PFHpA, (6) PFHxS, (7) PFOA, (8) PFNA, (9) PFOS, (10) FOSA and (11) PFDA.

With the aim of selecting the most suitable polymeric material, 7 mL of Milli-Q water spiked at 100 ng/mL (PFHxA, PFHpA, PFOA, PFNA, PFDA, L-PFBS, L-PFHxS, L-PFOS and FOSA) and adjusted to pH 9.5 were poured in a 30-mL PS vessel containing a magnetic stirrer and the ion-pair reagent (1-MP at 50 mM or TBA at 56 mM). The sorbent material, polydimethylsiloxane (PDMS) in the SR format (3 pieces of 1-cm length) or PES (3 pieces of 1.9-cm length) tubular tubes, was introduced inside the sample, the vial was capped and the extraction was performed overnight at room temperature.

Keeping in mind that extraction into the polymeric materials is larger when analytes are in their neutral form and that acidic compounds such as PFAAs are in the anionic at any pH interval, the addition of different ion-pair reagents was also studied. In the literature, one of the most widely used ion-pair reagent for the determination of PFASs is TBA. Alzaga et al. [37] and Villaverde-de-Saá et al. [47] employed TBA as ion-pair reagent to preconcentrate PFASs in aqueous matrices and improved the solid-phase microextraction (SPME) and solid (polymeric material) liquid extraction extractability. Regarding the polymeric material, PES was firstly proved as a good alternative for the extraction of polar analytes [48] and, later, for PFASs in water samples [47]. However, in the present work, the purpose was not mere preconcentration but also to find an alternative clean-up step for FUSLE extracts of solid samples. On the other hand, Ullah et al. [49] studied the use of 1-MP as organic modifier in the mobile phase, and they observed that 1-MP acts as an ion-pairing reagent and it masks the negative charges of the phosphonate group leading to an increased retention of perfluoroalkylphosphonates (PFPAs) on a C_{18} stationary phase by means of hydrophobic interaction. Thus, both polymeric materials (PES vs SR) and ion-pair reagents (TBABr, $TBAHSO_4$ and 1-MP) were evaluated and compared according to the extraction efficiencies.

The best extraction efficiencies (see **Figure 6.2.**) were obtained for the majority of the target analytes by means of PES and 1-MP combination, except in the case of PFCAs for PFDA, which showed better extraction yields when TBABr was used as ion-pair reagent and SR as sorbent material. It should be underlined that, while PES polymeric material worked better with 1-MP, TBABr gave better results with SR. Besides, the relative standard deviations (RSDs %) were worse for SR combined with TBABr even when the extraction efficiency was higher (i.e. PFDA).

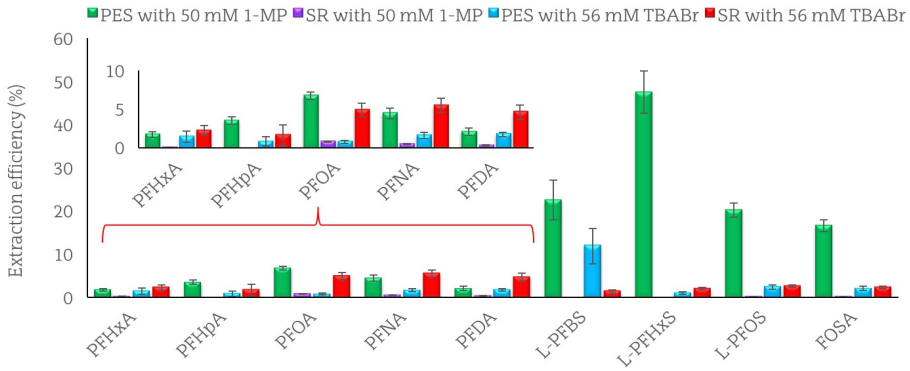


Figure 6.2. Extraction efficiency of the enrichment approach for the different polymeric materials (PES and SR) and ion-pair reagents (1-MP and TBA).

At the same time, the size of the counter-ion (bromide and hydrogen sulphate) was also studied when TBA was used as ion-pair reagent because analyte retention can be controlled by alteration of the concentration and the nature of the counter-ion [50]. When TBABr was used instead of $TBAHSO_4$, signal enhancement was observed since, according to the analysis of variance (ANOVA) not comparable results were achieved ($F_{Experimental}$ from 29 to 1292 › $F_{Critical}$=8). This could be attributed to the larger competition between the ionic part of the analyte and the hydrogensulphate than with the bromide ions. Bearing in mind all the above-mentioned, the rest of the experiments were performed using 1-MP and PES as ion-pair reagent and sorbent material, respectively.

Since the extraction efficiencies obtained were in the range of 2-7 % for PFCAs, 20-48 % for PFSAs and 17 % for FOSA and were far from 100 %, a mass balance of the preconcentration step was carried out in order to determine the analyte distribution. Once the enrichment step was over, the extraction media (water) was analysed according to the method published by Ullah et al. [49] in order to estimate the amount of the analyte remaining in the aqueous phase. The walls of the extraction vessel were sonicated with 7 mL of MeOH that were transferred to a test tube and evaporated down to 200 µL and analysed by means of LC-MS/MS. Less than 11 % of the total amount of PFASs was adsorbed in the wall (see **Figure 6.3**). Both acidic families showed the same trend; the longer the length of the fluorocarbon chain, the higher the affinity between the target analyte and the wall of the extraction vessel but, in all the cases it could be considered negligible (less than 10 %) compared to the distribution in the other two phases. However, when the fraction remaining in the aqueous media was considered, the concentrations observed were not negligible and certain tendencies were observed. As PFCAs are more hydrophilic than PFSAs, they showed a higher affinity to the aqueous media (see **Figure 6.3)**. While the fraction in the polymeric phase was high (approx. 33 %) for the long chain PFCAs (PFNA and PFDA), short chain PFCAs showed a higher affinity for the aqueous media. The same trend was observed by Villaverde-de-Saá et al. [47] during the preconcentration of water samples.

Subsequently, the concentration of 1-MP and the amount of PES were optimised. The concentration of the ion-pair reagent seemed not to be significant in the extraction yield of PFASs because comparable results ($F_{Experimental}$ =1.1-3.9 ‹ $F_{Critical}$ = 5.8) were achieved except for PFDA ($F_{Experimental}$ =14.2 › $F_{Critical}$ = 5.8) and FOSA ($F_{Experimental}$ =9.9 › $F_{Critical}$ = 5.8) according to the ANOVA of the results. In this case the higher the amount of the ion-pair reagent, the higher the extraction efficiency. Additionally, the lowest RSD values were obtained for 5 mM 1-MP in most

of the cases and, therefore, 5 mM 1-MP was used in the next set of experiments. Regarding the amount of PES, 2, 3, 6 and 9 pieces of 1.9-cm PES fibers were tested. Although, similar extraction efficiencies (see **Figure 6.4**) were obtained for 6 and 9 fibers of PES for most of the analytes, 9 pieces were selected as optimum amount due to the better reproducibility results obtained in this case for some analytes and the improvement observed for analytes (PFHxA and PFHpA) whose extraction efficiency was low.

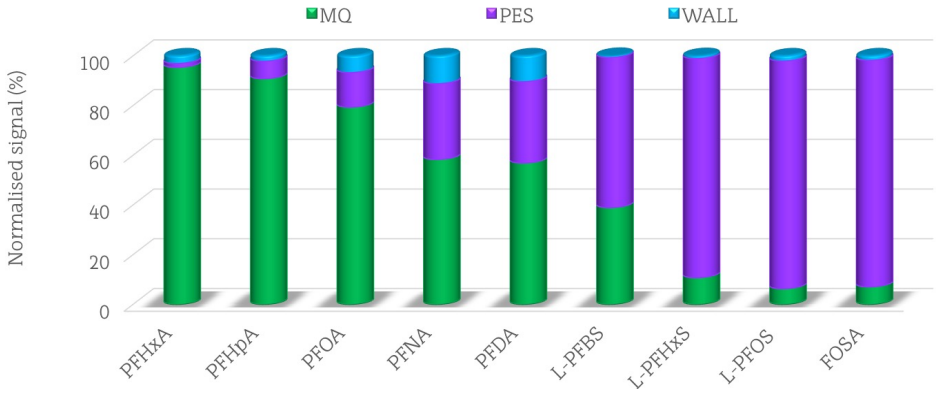


Figure 6.3. Mass balance approach of the clean-up step using PES polymeric material.

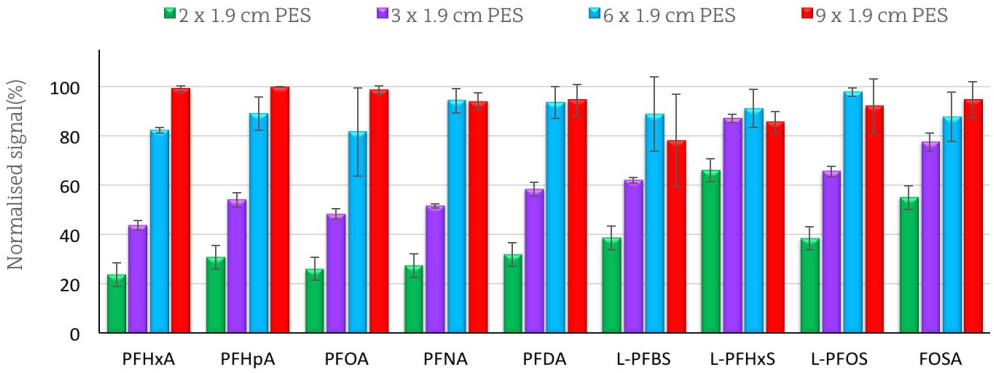


Figure 6.4. Signals normalised to the highest response of the enrichment approach for different amount of PES polymeric material.

The extraction time profiles of the target analytes studied were obtained by stirring (750 rpm) 7 mL of spiked Milli-Q water (100 ng/mL) with 5 mM of 1-MP as ion-pair reagent at pH 9.5 at room temperature and 9 fibers of 1.9 cm of PES. Assays were performed in duplicate (n=2)

at ten different extraction periods between 15 min and 24 h. The results obtained for some of the analytes studied are shown in **Figure 6.5**. All the compounds reached the equilibrium state after 180 min extraction.

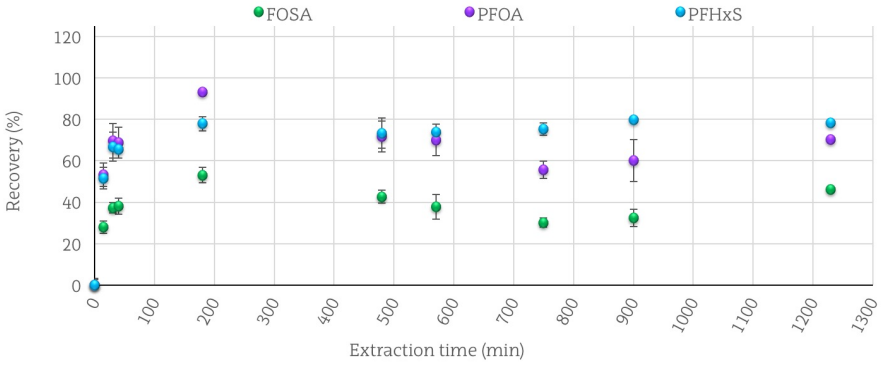


Figure 6.5. Extraction time profiles for FOSA, PFOA and PFHxS under the optimised PES clean-up conditions.

All the optimisation above was performed using Milli-Q water but since the objective of the present work was the use of PES and 1-MP for the clean-up of FUSLE extracts of amended soils and carrots, the extraction efficiency obtained in the presence of the matrix was also estimated. Clean-up efficiencies in the range of 56-98 % and 18-69 % were obtained for amended soil and carrot, respectively. Thus, the use of labelled standards and matrix-matched calibration approaches were considered for the proper quantification of the target analytes.

6.3.2 Method validation

Zorbax Extend-C_{18} Rapid Resolution HT as well as ACE UltraCore 2.5 SuperC_{18} columns were tested for the separation of PFASs. The best sensitivity in terms of the improvement on the peak signal was obtained using ACE UltraCore 2.5 SuperC_{18} column and, thus, limits of quantification (LOQs) were estimated from the calibration curve at low concentration levels based on 10 times signal-to-noise ratio (S/N = 10) (**Table 6.3**). The values obtained (0.007-0.08 ng/g) were better than the values reported by Zhang et al. (0.6-5 ng/g) [22] and by Moreta and Tena (1.4-7.5 ng/g) [30]. Instrumental calibration curves were performed from LOQ up to 1200 ng/g and determination coefficient (r^2) values higher than 0.9875 were obtained (see **Table 6.3**) for all the compounds when instrumental calibration approach with isotopically labelled analogue correction was used.

Table 6.3. LOQs (ng/g) using two different columns, MDL values at 50 ng/g and RSD (%) at high (290 ng/g) and low concentration (50 ng/g) levels for PFSAs, PFCAs and FOSA in fortified amended soil and carrot.

Analyte	LOQs (ng/g) Zorbax Extend-C_{18} Rapid Resolution HT	LOQs (ng/g) ACE UltraCore 2.5 SuperC_{18}	MDLs (ng/g) at 50 ng/g	r^2 using instrumental calibration with labelled analogue correction (LOQs 1 ng/µL)		RSD (% , n=3) at two levels 50/ 290 ng/g	
			Amended soil	Carrot		Amended soil	Carrot
PFHxA	0.23	0.080	0.95	1.83	0.9995	8/8	19/11
PFHpA	0.08	0.007	0.57	0.24	0.9999	9/6	11/4
PFOA	0.08	0.007	0.60	0.20	0.9996	5/3	15/8
PFNA	0.08	0.007	0.86	0.16	0.9995	5/4	15/6
PFDA	0.08	0.010	0.54	0.11	0.9996	5/7	17/6
L-PFBS	0.23	0.080	2.85	0.78	0.9996	16/6	8/23
L-PFHxS	0.23	0.080	2.19	0.99	0.9999	5/3	11/6
L-PFOS	0.08	0.007	0.31	0.42	0.9997	5/2	13/6
FOSA	0.16	0.060	0.86	1.66	0.9875	11/11	11/13

Method detection limits (MDLs) and relative standard deviations (RSDs) were also determined for the two tested matrices. MDLs were calculated according to US Environmental Protection Agency Method (http://www.epa.gov/waterscience/methods/det/rad.pdf) and matrices (n=7) were spiked at 50 ng/g. The MDL values obtained in this work were in the range of 0.3-2.9 ng/g and 0.1-1.8 ng/g for amended soil and carrot, respectively. Similar MDL values were reported by Zabaleta et al. [29] (1-7 ng/g amended soil and 0.3-3.2 ng/g for carrot) when FUSLE extraction followed by SPE clean-up on Oasis WAX was performed or by Bossi et al. (3-77 ng/g) [51] when ion-pair extraction by means of TBA was carried out in fish, birds and marine mammals. However, better MDL values were reported by Ullah et al. [34] (1.8-20 pg/g) when extraction with ACN/water and clean-up on mixed mode co-polymeric sorbent (C_8+quaternary amine) using SPE, was employed for dietary samples such as meat, fish and vegetables, and by Vestergren et al. [33] (0.3-2.4 pg/g) when ion-pair extraction and subsequent SPE clean-up on Florisil and graphitised carbon was performed in dietary samples (baby food, vegetables, fish and meat). However, it should be highlighted that procedural blanks were performed for assessment of MDLs by Vestergren et al. [33].

Absolute recoveries at the lower concentration level (50 ng/g) were in the range of 4-30 % and 1-46 % when external instrumental calibration was used for amended soil and carrot, respectively, and no correction with the corresponding labelled standards was performed (see **Table 6.4**). However, values close to 100 % were obtained for almost all the analytes when the signals were corrected with the corresponding labelled standard (77-140 % amended soil and 102-129 % carrot), except for PFHpA (294 % for amended soil and 179 % for carrot) and L-PFBS (63 % for amended soil and 31 % for carrot) (**Table 6.4**). When using a matrix-matched calibration approach without labelled analogue correction, acceptable apparent recoveries were also obtained for PFHpA (93 % and 95 % for amended soil and carrot, respectively) and L-PFBS (83 % and 137 % for amended soil and carrot, respectively).

Recoveries without correction were also determined at the higher concentration level (290 ng/g) for amended soil (1.3-94 %) and carrot (0.9-28 %) and the results were similar to those obtained at the lower fortification level (see **Table 6.4**). Correction with labelled analogues was suitable for all the analytes (58-136 % for amended soil and 79-132 % for carrot), except for PFHpA (165 % and 162 % in the case of amended soil and carrot) and L-PFBS (16 % and 28 % for amended soil and carrot). For these analytes matrix-matched calibration was once again necessary (see **Table 6.4**). In terms of precision, RSD values were in the 2-27 % range for the fortified samples for both matrices.

Table 6.4. Recoveries (%) and apparent recoveries (%) at high (290 ng/g) and low (50 ng/g) concentration levels for PFCAs, PFSAs and FOSA in fortified soil and carrot samples.

Analyte	Recoveries (%) ± s using instrumental calibration without/with labelled analogue correction at 50 ng/g		Recoveries (%) ± s using matrix-matched calibration without labelled analogue correction at 50 ng/g		Recoveries (%) ± s using instrumental calibration without/with labelled analogue correction at 290 ng/g		Recoveries (%) ± s using matrix-matched calibration without labelled analogue correction at 290 ng/g	
	Amended soil	Carrot	Amended soil	Carrot	Amended soil	Carrot	Amended soil	Carrot
PFHxA	4 ± 2/ 106 ± 7	1.1 ± 0.3/ 104 ± 9	111 ± 38	90 ± 7	1.3 ± 0.3/ 70 ± 4	0.9 ± 0.3/ 115 ± 13	80 ± 22	119 ± 25
PFHpA	10 ± 7/ 294 ± 26	1.9 ± 0.4/ 179 ± 18	93 ± 29	95 ± 6	3.0 ± 0.5/ 165 ± 18	1.3 ± 0.3/ 162 ±11	92 ± 18	121 ± 22
PFOA	18 ± 9/ 120 ± 6	2.9 ± 0.7/ 113 ± 3	88 ± 30	95 ± 14	7 ± 1/ 86 ± 10	1.7 ± 0.3/ 119 ± 5	102 ± 16	123 ± 23
PFNA	20 ± 10/ 119 ± 5	5 ± 1/ 119 ± 2	82 ± 27	93 ± 13	15 ± 2/ 89 ± 10	2.9 ± 0.6/ 132 ± 7	110 ± 18	119 ± 27
PFDA	8 ± 6/ 77 ± 4	6 ± 1 / 102 ± 3	85 ± 27	69 ± 2	18 ± 4/ 58 ± 8	4 ± 1/ 112 ± 6	112 ± 25	128 ± 34
L-PFBS	18 ± 9/ 63 ± 14	5 ± 1/ 31 ± 2	83 ± 27	137 ± 19	7 ± 1/ 16 ± 5	1.9 ± 0.4/ 28 ± 37	90 ± 16	131 ± 31
L-PFHxS	30 ± 16 / 85 ± 5	17 ± 2/ 111 ± 3	76 ± 22	116 ± 11	31± 6 / 60 ± 13	8 ± 3/ 122 ± 9	84 ± 21	130 ± 46
L-PFOS	12 ± 11/ 107 ± 10	40 ± 5 / 121 ± 2	89 ±31	87 ± 12	50 ± 15/ 71 ± 5	28 ± 9/ 130 ± 8	71 ± 22	125 ± 40
FOSA	19 ± 13/ 140 ± 22	47 ±2/ 129 ± 12	84 ± 26	88 ± 31	94± 18/ 136 ± 23	16 ± 6/ 79 ± 7	96 ± 19	126 ±42

In the absence of a certified reference material (CRM), inter-method comparability was carried out. The same soil, carrot leaves, carrot peel and carrot core samples obtained from the uptake experiment of PFOS described in the **section 6.2.2** were analysed in triplicate by the method presented here, as well as by FUSLE extraction technique followed by a completely independent clean-up step previously developed in our laboratory [29]. Excellent agreement between the two sets of results (see **Table 6.5**) was obtained for carrot peel, core and leaves and amended soil according to ANOVA ($F_{Experimental}$ =2.59, 5.06, 5.82 and 2.34 ‹ $F_{Critical}$ =7.71, respectively). Concentrations similar to the soil level were determined in leaves; therefore, bioconcentration factors (BCF, the concentration measured in plant compartment divided by soil concentration) close to one were obtained. However, concentrations in the carrot peel and core were 10 times lower. Since concentration in the blank experiments showed concentration for PFOS lower than the MDLs in the different carrot compartments, it could be concluded that translocation of PFOS occurred from soil and not due to foliar deposition from the air. Further experiments should be performed, however, in order to understand PFASs uptake in vegetables.

Table 6.5. Inter-method comparison of the PFOS concentration (ng/g) in different compartments of carrots (peel, core and leaves) exposed to compost-amended soil fortified with PFOS using PES-ion pair and Oasis-WAX clean-up procedures.

	C ng/g, (RSD%) by means of PES	**C ng/g, (RSD%) by means of Oasis WAX [29]**
Peel	72 (6)	69 (12)
Core	62 (3)	62 (3)
Leaves	669 (6)	736 (5)
Amended soil	653 (6)	561 (17)

6.4 Conclusions

A new clean-up approach for the simultaneous determination of a suite of PFAAs and FOSA in carrot and amended soil samples based on the enrichment on PES polymer using a 1-MP as ion-pair reagent of FUSLE extracts followed by LC-MS/MS determination was developed and validated. In this study, different polymeric materials combined with different ion-pair reagents were tested. The best results in terms of apparent recoveries, RSD values and MDLs were obtained by means of PES and 1-MP ion-pair reagent with respect to quantification of all the target compounds, internal calibration (instrumental calibration with isotopically mass-labelled analog correction) was used except for PFHpA and L-PFBS since no suitable

mass-labelled compounds were available and, therefore, matrix-matched calibration was necessary. Moreover, this method gives the possibility to analyse more than ten samples simultaneously (depending on the stirring device available in the laboratory) and it is extremely simple and cheap (reduced cost of the PES polymer; c.a. 0.05 €/unit) and implies a low solvent volume consumption. Besides, comparable results were obtained in terms of accuracy when the developed method was compared to a more expensive clean-up approach using SPE cartridges (c.a. 200 €/30 unit). To the best of our knowledge, this is the first time that the combination of 1-MP as ion-pair reagent and PES has not only been used in the preconcentration of PFASs but also with clean-up purposes. PFOS uptake in carrot was performed in the present work and the highest concentrations were observed in the leaves of the carrot, higher than in the peel and in the core compartments. Further experiments with different PFASs, crops and types of soils should be carried out in future research for the better understanding of PFASs uptake.

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CHAPTER 7

Uptake of polybrominated diphenyl ethers by carrot and lettuce crops grown in compost-amended soils

Environmental Science and Pollution Research

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7.1 Introduction

The development of new resources and technologies has allowed the identification of an increasing number of compounds that pose an environmental risk to the human livings [1]. These so-called "emerging" compounds, which are not always metabolised, are discharged into sewers and wastewater treatment plants (WWTPs) [2]. Although, the WWTPs were initially designed to remove conventional pollution parameters from the wastewater stream, it was soon found out that the wastewater organic load included high levels of a variety of hazardous organic pollutants and, thus, additional treatment steps and control measures became necessary [3]. Meanwhile, WWTP effluents and sewage sludge are potential sources of pollutants and their metabolites introduction into the environment [4]. The spectrum of organic pollutants of anthropogenic origin occurring in sewage sludge is extremely large and constantly changing, depending on the locality, season and technology used in the WWTPs [5, 6]. The body of the published work on the presence of organic pollutants in sludge covers organochlorine pesticides [7], dioxin-like compounds [8 ,9] and, more recently, chlorinated napthalenes (PCNs) [10], polycyclic aromatic hydrocarbons (PAHs) [10], synthetic musks [10], oestrogens [11], organotins (OTs) [12, 13], polybrominated diphenyl ethers (PBDEs) [14] and nonylphenol (NP) [15].

Due to their intensive use in commercial, consumer and household products as flame-retardants (PBDEs) are now global contaminants. Concentration levels that vary from several nanograms to micrograms per gram have been reported worldwide [16 -20]. Besides, PBDEs are readily biomagnified in food webs due to their high lipophilicity with potential toxic effects for the living organisms. In this sense, in 2013 the European Water Framework Directive (WFD, 2013/39/EC) referenced PBDEs as priority hazardous substances.

Although debromination is probably the most important biotransformation pathway for PBDEs, they can also be biotransformed to hydroxylated (OH-PBDEs) or methoxylated polybrominated diphenyl ethers (MeO-PBDEs). Both groups were identified as natural compounds produced by some marine invertebrates [21] and on the other hand, recent studies also demonstrated that MeO-PBDEs can be biotransformed to OH-PBDEs, generating greater amounts of OH-PBDEs. Given that some endpoint, OH-PBDEs often exhibit greater toxicity than PBDEs, it is prudent to consider OH-PBDEs as chemicals of concern, despite their seemingly "natural" origin [22]. Recent PBDEs exposure studies have detected OH-PBDEs and MeO-PBDEs in matrices, such as marine organisms [23-25], humans [26, 27] and soils and plants [28-30].

The total amount of sewage sludge produced in the European Union (EU) is expected to be 13 million tons in 2020, being Germany, the United Kingdom, France, Italy and Spain the largest producers. At present, there are mainly three disposal methods for sewage sludge: agricultural application as fertiliser, landfilling and incineration. The use of sludge on agriculture constitutes around the 40 % of the total sewage sludge compost produced in Europe, which means that agricultural application has become the most widespread method for disposal since it is the most economical outlet [31]. The European Commission (Council Directive 86/278/EEC) regulated the use in agriculture of residual sludge from domestic and urban wastewater. Once in the agro-ecosystem, organic compounds can follow different pathways: (i) loss to the atmosphere and to surface water by erosion caused by air or rain, (ii) degradation in the soil, potentially generating even more toxic compounds, (iii) transport through the soil to the groundwater, endangering drinking water resources and (iv) transfer to plants or livestock and soil invertebrates by direct soil digestion [32]. Therefore, organic contaminants such as PBDEs can be transferred to humans via agricultural products. Their bioavailability depends on several factors, including their structure, their physicochemical properties (i.e., hydrophobicity and the water solubility), environmental conditions, including organic matter of the cultivation media, temperature and pH, among others, and the cultivated plant [33].

Within this context, and taking into account that plants form an essential basis of the animal and human diet, an evaluation of the uptake and accumulation of potential harmful organic contaminants in plants is of importance for risk assessment. Although previous studies on organic contaminants have investigated the uptake and accumulation by plants (corn, carrot, lettuce, barley, maize, wheat, among other plants) of some pesticides or veterinary drugs [34], polychlorinated biphenyls (PCBs) and PBDEs [3, 35-38], musk fragrances and triclosan [39] and perfluorinated compounds (PFCs) [40-44], further data are required for a science-based decision on the risk of the application of sewage sludge compost for agriculture purposes [45]. In this sense, the main objective of the present work was the evaluation of plant uptake of PBDEs in different crops (lettuce and carrot) cultivated in compost-amended soils, naturally or artificially contaminated with different PBDE congeners. Both crops were selected in order to see the differences between carrot, in which the uptake could be more controlled by root uptake mainly because the root (the edible part) is in direct contact with the contaminated soil, and lettuce, where the edible part (leaves) is not in direct contact with the polluted soil. The evaluation of the formation of any debrominated, hydroxylated or methoxylated biotransformation product in the compost-amended soil-plant system was also carried out.

7.2 Experimental section

7.2.1 Reagents and materials

The names of the target analytes and their corresponding surrogates, the abbreviations, the molecular weight and the octanol/water partition coefficient (as log K_{ow}) are summarised in **Table 7.1**.

Pentabromodiphenyl ether technical mixture (penta-BDE, technical mix. 99 %), containing a mixture of tri-, tetra-, penta- and hexa-BDE congeners, was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). BDE-138, ~1000 mg/L, was ordered to Isostandards Materials (Madrid, Spain). BDE-209 (98 % purity) was supplied by Sigma Aldrich (Steinheim, Germany) and BDE-99 (› 98 % of purity) was provided by Wellington Laboratories (Guelph, Canada). All the chemical standards were used to fortify the compost-amended soils and served as reference compounds for the analytical measurements. BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183 and BDE-197 individual standards (50 µg/mL each) in isooctane were purchased from AccuStandard (New Haven, CT, USA). The solution of MeO-PBDEs (5-MeO-BDE-47, 6-MeO-BDE-47, 4-MeO-BDE-49, 2-MeO-BDE-68, 5'-MeO-BDE-99, 5-MeO-BDE-100, 4'MeO-BDE-101 and 4-MeO-BDE-103, 5 µg/mL each) in nonane/toluene was obtained from Wellington Laboratories. Individual solutions of 3´-OH-BDE-28, 5-OH-BDE-47 and 3-OH-BDE-47 at 50 µg/mL each and 4´-OH-BDE-49, 4-OH-BDE-42 and 5´-OH-BDE-99 at 10 µg/mL each, all of them in acetonitrile, were purchased from AccuStandard.

As surrogate standards BDE-77 and BDE-181 in isooctane (50 µg/mL each) were acquired from AccuStandard, $^{13}C_{12}$-BDE-209, 25 µg/mL in toluene, from Wellington Laboratories, and $^{13}C_{12}$- TCS, (100 µg/mL in nonane, 99 %) from Cambridge Isotope Laboratories (Andover, MA, USA).

Acetone (HPLC grade, 99.8 %) was used in the fortification step. n-Hexane (HPLC grade, 95 %), dichloromethane (DCM, HPLC grade) and toluene (HPLC grade) were used in the matrix solid-phase dispersion (MSPD) protocol. Samples were reconstituted in isooctane (HPLC grade) or ethyl acetate (EtOAc, HPLCC grade). All of them were purchased from LabScan (Dublin, Ireland).

Table 7.1. Selected features of the target and the surrogate compounds including names and abbreviations, molecular weight, octanol/water partition coefficient (log K_{OW}) (Chemspider, www.chemspider.com) and figures of merit of the method (MSPD-GC-(NCI)-MS) used for the analysis of amended soil, carrot and lettuce matrices. Quantification limits (LOQs) of the GC-MS/MS methodology are also included.

Analyte	Molecular weight	Log K_{ow}	Apparent recovery (%) ± standard deviation at 25 ng/g			MQL dw (ng/g) for MSPD-GC-(NCI)-MS			LOQ (ng/mL) of the GC-MS/MS method	Target ions (m/z) quantifier, qualifiers (GC-(NCI)-MS)	Transitions used for the GC-MS/MS in the SRM mode and the quadrupole energies (eV)
			Soil	Carrot	Lettuce	Soil	Carrot	Lettuce			
2,4,4´-tribromodiphenyl ether (BDE-28) [1]	407.6	5.88	95±4	94±2	102±8	0.01	0.01	0.02	0.10	79.0, 81.0, 160.7	405.9›246.1 (20) 407.8›248.0 (25)
2,2´,4,4´-tetrabromodiphenyl ether (BDE-47) [1]	485.8	6.77	92±3	91±1	96±6	0.02	0.03	0.05	0.11	79.0, 81.0, 160.7	486.0›326.0 (30) 488.0›327.9 (30)
2´,3,4´,5-tetrabromo-2 methoxydiphenyl ether (2-MeO-BDE-68) [1]	516.6	6.39	99±5	102±5	114±10	0.1	0.2	0.3	0.55	79.0, 81.0, 160.7	515.3›421.4 (18) 355.7›312.9 (20)
2,2´,4,4´- tetrabromo-6-methoxy diphenyl ether (6-MeO-BDE-47) [1]	516.6	6.39	103±4	102±2	113±7	0.1	0.1	0.2	0.35	79.0, 81.0, 160.7	515.3›421.5 (18) 355.7›313.0 (20)
2,2´,4,4´,6-pentabromodiphenyl ether (BDE-100) [1]	564.7	7.32	89±2	95±4	95±6	0.08	0.1	0.2	0.22	79.0, 81.0, 160.7	404.0›297.0 (50) 566.0›405.7 (15)
2,2´,4,4´-tetrabromo-5-methoxydiphenyl ether (5-MeO-BDE-47) [1]	516.6	6.39	114±5	106±6	114±8	0.1	0.2	0.3	0.75	79.0, 81.0, 160.7	515.7›355.7 (18) 355.7›340.9 (20)
2,2´,4´,5-tetrabromo-4 methoxy diphenyl ether (4-MeO-BDE-49) [1]	516.6	6.39	104±3	102±4	117±7	0.2	0.2	0.3	0.84	79.0, 81.0, 160.7	515.4›500.4 (18) 355.7›341.0 (18)
2,2´,4,4´,5-pentabromodiphenyl ether (BDE-99) [1]	564.7	7.32	89±3	92±3	93±5	0.1	0.1	0.2	0.43	79.0, 81.0, 160.7	403.9›296.7 (50) 566.0›406.0 (30)
2,2´,4,4´,6´pentabromo-5 methoxy diphenyl ether (5-MeO-BDE-100) [1]	595.5	-[a]	100±3	99±2	109±7	0.1	0.1	0.2	0.42	79.0, 81.0, 160.7	593.6›435.8 (20) 433.6›418.6 (10)
2,2´,4´,5,6´-pentabromo-4-methoxy diphenyl ether (4-MeO-BDE-103) [1]	595.5	-[a]	90±3	97±4	105±3	0.2	0.2	0.3	0.67	79.0, 81.0, 160.7	593.5›578.5 (20) 433.6›418.6 (8)
2,2´,4,4´,5,6´-hexabromodiphenyl ether (BDE-154) [1]	643.6	8.09	81±3	90±5	88±5	0.06	0.1	0.1	0.30	79.0, 81.0, 160.7	484.0›482.8 (40) 643.8›483.4 (10)
2,2´,4,4´,5-pentabromo-5´-methoxy diphenyl ether (5´-MeO-BDE-99) [1]	595.5	-[a]	93±6	93±3	105±5	0.1	0.1	0.2	0.52	79.0, 81.0, 160.7	433.6›418.5 (14) 593.3›433.6 (10)
2,2´,4,5,5´-pentabromo-4´-methoxy diphenyl ether (4´-MeO-BDE-101) [1]	595.5	-[a]	87±1	93±2	100±8	0.2	0.3	0.4	0.78	79.0, 81.0, 160.7	593.7›433.7 (20) 433.6›418.8 (10)

Table 7.1 (Continuation).

Analyte	Molecular weight	Log K_{ow}	Apparent recovery (%) ± standard deviation at 25 ng/g			MQL dw (ng/g) for MSPD-GC-(NCI)-MS			LOQ (ng/mL) of the GC-MS/MS method	Target ions (m/z) quantifier, qualifiers (GC-(NCI)-MS)	Transitions used for the GC-MS/MS in the SRM mode and the quadrupole energies (eV)
			Soil	Carrot	Lettuce	Soil	Carrot	Lettuce			
2,2´,4,4´5,5´-hexabromodiphenyl ether (BDE-153) [1]	643.6	8.09	77±3	85±6	83±5	0.05	0.06	0.1	0.19	79.0, 81.0, 160.7	484.0›323.9 (50) 644.0›483.1 (10)
2,2',3,4,4',5'-hexabromodiphenyl ether (BDE-138) [1]	643.6	7.73	80±4	90±6	89±5	0.1	0.2	0.3	0.62	79.0, 81.0, 160.7	-[b]
2,2´,3,4,4´,5´,6-heptabromodiphenyl ether (BDE-183) [2]	722.5	8.85	93±3	103±2	100±6	0.1	0.1	0.3	0.64	79.0, 81.0, 561.4	-[b]
2,2´,3,3´,4,4´,6,6´-octabromodiphenyl ether (BDE-197) [2]	801.4	9.62	96±6	101±3	108±8	0.1	0.2	0.3	0.72	408.5, 79.0, 81.0	-[b]
Decabromodiphenyl ether (BDE-209) [3]	949.2	11.16	129±6	115±4	110±9	0.04	0.03	0.09	-[c]	486.5, 484.5, 482.5	-[b]
3´-hydroxy-2,4,4´-tribromodiphenyl ether (3'-OH-BDE-28) [4]	423.6	-[a]	98±9	108±7	85±3	0.2	0.2	0.4	0.99	79.0, 81.0, 342.0	-[b]
5-hydroxy-2,2´,4,4´-tetrabromodiphenyl ether (5-OH-BDE-47) [4]	501.5	-[a]	103±18	113±10	111±5	0.5	0.6	1.1	1.01	79.0, 81.0, 420.0	-[b]
4-hydroxy-2,2´,4,5´-tetrabromodiphenyl ether (4-OH-BDE-49) [4]	501.5	-[a]	100±15	102±4	90±1	0.7	0.6	1.4	1.58	79.0, 81.0, 422.0	-[b]
3-hydroxy-2,2´,4,4´-tetrabromodiphenyl ether (3-OH-BDE-47) [4]	501.5	-[a]	88±19	113±8	109±13	0.3	0.3	0.5	1.36	79.0, 81.0, 420.0	-[b]
4-hydroxy-2,2´,3,4´-tetrabromodiphenyl ether (4-OH-BDE-42) [4]	501.5	-[a]	106±11	117±6	128±5	0.3	0.4	0.7	1.81	79.0, 81.0, 420.0	-[b]
5´-hydroxy-2,2´,4,4´,5-pentabromo diphenyl ether (5'-OH-BDE-99) [4]	580.4	-[a]	100±8	109±10	114±7	0.5	0.5	1.1	1.80	79.0, 81.0, 498.0	-[b]
3,3´,4,4´-tetrabromodiphenyl ether (BDE-77)	485.8	-[a]								79.0, 81.0, 325.7	-[d]
2,2´,3,4,4´,5,6-heptabromodiphenyl ether (BDE-181)	722.5	-[a]								79.0, 81.0, 561.4	-[d]
[$^{13}C_{12}$]-decabromodiphenyl ether ($^{13}C_{12}$-BDE-209)	959.2	-[a]								494.5, 492.5, 496.5	-[d]
[$^{13}C_{12}$]-triclosan ($^{13}C_{12}$-TCS)	284.2	-[a]								228.0, 265.1, 267.5	-[d]

[a] not reported, [b] analysed in the SIM mode, [c] not analysed, [d] not included, [1]: BDE-77 is used as surrogate, [2]: BDE-181 is used as surrogate, [3]: $^{13}C_{12}$-BDE-209 is used as surrogate, [4]: $^{13}C_{12}$-TCS is used as surrogate.

Silica gel (Merck, Darmstadt, Germany) was activated in an oven at 130 ºC overnight. Acidified silica (10 % H_2SO_4, w/w) was prepared with concentrated H_2SO_4 (Merck, 95-97 %). Glass cartridges (10 mL capacity) were purchased from Normax (Marinha Grande, Portugal). Frits were provided by International Sorbent Technology (Mid Glamorgan, UK). Octadecyl-functionalised silica (C_{18}) was supplied by Sigma-Aldrich and was used in the MSPD extraction.

N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) was used as derivatisation reagent (Sigma-Aldrich).

A Cryodos-50 laboratory freeze-dryer from Telstar Instrument (Sant Cugat del Valles, Barcelona, Spain) was used to freeze-dry the samples.

MSPD fractions were evaporated in a Turbovap LV Evaporator (Zymark, Hopkinton, MA, USA) using a gentle stream of N_2 (› 99.999 %, Messer, Tarragona, Spain).

The compost was acquired from Calahorra WWTP (Spain). Soil 2.1 and 2.4 were supplied by LUFA Speyer (Speyer, Germany). The properties of the different soils and compost used in this work are summarised in **Table 7.2**. The universal substrate and vegetable (lettuce and carrot) seeds were obtained from a commercial agricultural house (www.mvgarden.com). Two different varieties of carrots (***Daucus carota ssp. sativus***, ***Nantesa*** and ***Chantenay***) and lettuces (***Lactuca sativa***, ***Batavia Golden Spring*** and ***Summer Queen***) were used in the uptake experiments.

Hoagland nutritive solution, containing potassium nitrate (KNO_3, 99.0 %), calcium nitrate tetrahydrate ($Ca(NO_3)2\ 4H_2O$, 98.0 %), ammonium phosphate monobasic ($(NH_4)H_2PO_4$, 96.0-102.0 %), magnesium sulphate heptahydrate ($MgSO_4$ 7H2O, 99.0-100.5 %), manganese chloride tetrahydrate ($MnCl_2\ 4H_2O$, 98.0-102.0 %), boric acid (H_3BO_3, 99.8 %), zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$, 99.0-104.0 %), copper sulphate pentahydrate (CuSO4 5H2O, 99.0-100.5 %) and sodium molybdate dehydrate ($Na_2MoO_4\ 2H_2O$, 98.0-100.5 %), all of them acquired from Panreac (Castellar del Vallès, Spain), was prepared monthly according to Epstein and Bloom's work [46]. Briefly, an appropriate amount of the different salts were weighed, followed by pH adjustment at 5.5 with NaOH (reagent grade, ≥ 98 %, Panreac) and/or HCl (ACS reagent, 37 %, Panreac) in order to prepare 25 L.

7.2.2 Crop cultivation

7.2.2.1 Compost fortification and plant cultivation

A known amount of compost (approx. between 100-1200 g according to the experiment

set) was weighed, covered with acetone (approx. 1L) until all the compost was covered, fortified with the corresponding analyte concentration and stirred for 24 h. After that, the slurry was placed under a fume hood for solvent evaporation and the fortified compost sample was aged for one week. The compost was always thoroughly manually mixed with the different soils.

Table 7.2. Characterisation according to physicochemical parameters of the different soils. Soils 2.1 and 2.4 were characterised by LUFA Speyer (Speyer, Germany) and substrate and compost by Neiker Tecnalia (Bizkaia, Spain).

Parameter	Soil 2.1	Soil 2.4	Universal substrate	Compost
Total organic carbon (TOC %)	0.7±0.1	2.3±0.5	53±9	55±8
N %	0.05±0.01	0.20±0.04	0.35±0.05	2.4±0.3
pH	5.1±0.3	7.2±0.2	5.7±0.2	7.9±1.5
Cation Exchange Capacity (meq/100 g)	4.3±0.5	31±5	48±4	50±9
particles‹ 0.002mm	2.8±1.1	26±2	4.5±0.5	10±1
0.002-0.05mm	10.2±1.8	41±1	27±3	40±4
0.05-2mm	87±2	34±2	53±6	42±3
› 2mm	-[a]	-[a]	16±2	8.9±1.5
soil type	Sand	Sandy loam	Sandy loam	Loam
Water Holding Capacity (g/100g)	31±2	44±1	-[b]	-[b]
Water per Volume (g/1000ml)	1471±28	1288±36	-[b]	-[b]

[a] no particles > 2 mm, [b] not determined

Carrot and lettuce seeds were sonicated with Milli-Q water for 10 min, followed by several rinses with Milli-Q water previous to the germination experiments. In the case of carrot, petri dishes were covered with moistened filter paper and the seeds were evenly distributed in the petri dish for germination. Afterwards seeds were covered with another piece of moistened filter paper. Germination took place in approx. 14 days. In the case of lettuce seeds (approx. 6-10 seeds in three different positions) were placed in the pots without the previously germination step described above.

All of the pots contained a (95:5) soil:fortified compost mixture (2 kg for all the

experiments except in the case of the uptake experiments of BDE-99 by the ***Summer Queen*** lettuce where pots contained 350 g). Pots were located in close proximity to each other and maintained in controlled environmental conditions in a greenhouse (temperature was set to 25 ºC during the day and at 18 ºC during the night with a 14-h day length and a relative humidity of 50 % during the day and 60 % overnight) and they were regularly watered with distilled water and Hoagland nutritive solution.

Plants were harvested during a period of approx. 4-5 weeks for lettuce and approx. 12-15 weeks for carrot. After harvesting, the carrots and lettuces (all plants in a pot were collected and pooled to one sample) were removed from the pots. Carrot samples were separated into root peel, root core and leaves compartments. Carrots were peeled with a vegetable peeler (~2 mm depth). Lettuce hearts and leaves were analysed together. Each portion was subsequently washed carefully three times with deionised water prior to storage. Carrot and lettuce samples were freeze-dried at low temperature (~ -50 ºC) before treatment. Both, vegetables and air-dried compost-amended soil samples were stored at -20 °C before extraction.

The plant uptake and maintenance were done with the support of the General Analysis Service (SGIker) of the University of the Basque Country (UPV/EHU) in the unit called "Growing plant material under controlled conditions: phytotron and greenhouse".

7.2.2.2 Carrot experiments

In the case of the PBDE uptake by carrot, different experiments were carried out using two varieties of carrot (***Chantenay*** and ***Nantesa***), three types of soils (soil 2.1, soil 2.4 and substrate), two different PBDE isomers (BDE-209 which was studied for its high environment presence and BDE-138 due to it has not been previously studied) and two levels of concentration (low and high). In all the cases, it was the compost and not the soil that contained the target analytes. In all the experiments performed, apart from the congener used in the fortification step, the possible formation of other congeners was tested. The conditions of the experiments were the following:

a) ***Chantenay*** and ***Nantesa*** carrot species cultivated in amended soils (soil 2.1, soil 2.4 and substrate) fortified with BDE-138 in order to adjust to a nominal concentration of 120 ng/g in the soil:compost mixture (95:5). Two replicates of all the experiments were performed.
b) ***Chantenay*** and ***Nantesa*** carrot species cultivated in a (95:5) soil 2.4:compost mixture containing BDE-209 at two concentration levels: 7-20 ng/g (low level) and 7500 ng/g

(high level). One replicate was performed for the low-level experiments and two for the high-level experiments.

c) ***Chantenay*** carrot specie cultivated in a (95:5) substrate:compost mixture containing BDE-209 at two concentration levels: 20 ng/g (low level) and 7500 ng/g (high level). One replicate was performed for the low-level experiments and two for the high-level experiments.

In all the cases, non-fortified experiments (n=1) were run in parallel. In the case where BDE-209 was detected in the compost without the need of fortification (low level experiments), non-fortified experiments (n=1) were also run in the absence of the compost. In all the experiments, the analysis of the pooled samples was performed in triplicate by gas chromatography-negative chemical ionisation-mass spectrometry (GC-(NCI)-MS) (see **section 7.2.3.2**). The peel, leaves and core concentrations were measured.

7.2.2.3 Lettuce experiments

In the case of the PBDE uptake by lettuce, different experiments were carried using two types of lettuce species (***Batavia Golden Spring*** and ***Summer Queen***) and different PBDE isomers at different concentrations. Penta-BDE mixture was studied in order to see the effect of nature of the BDEs, BDE-209 was once again evaluated in the case of lettuce due to its high presence in the environment and BDE-99 expecting a higher accumulation than that observed for BDE-138 due to its lower bromination and higher accumulation ability. All of them were selected in order to determine metabolite presence coming from different bromination level PBDEs congeners. Soil 2.4 was used for all the experiments. Besides, in all the experiments performed, apart from the congener used in the fortification step, the possible formation of other congeners was tested. In all the cases it was the compost and not the soil that contained the target analytes.

The conditions of the experiments were the following:

a) ***Batavia Golden Spring*** lettuce specie cultivated in (95:5) soil 2.4:compost mixture fortified with penta-mix at a nominal concentration of 6000 ng/g. A single pot was fortified in this set of experiments.

b) ***Batavia Golden Spring*** lettuce specie cultivated in a (95:5) soil 2.4:compost mixture fortified with BDE-209 at two nominal concentrations of 25 ng/g (low concentration level) and 7500 ng/g (high concentration level). In the case of the low concentration level, a single pot was used. Two pots were cultivated for the high concentration experiments.

c) ***Summer Queen*** lettuce specie cultivated in the (95:5) soil 2.4:compost mixture fortified with BDE-99 at a nominal concentrations of 500 ng/g (low concentration level) and 5000 ng/g (high concentration level). Two pots were cultivated at both concentration levels and in this case, BDE-209 was not present in the compost used.

In all the cases non-fortified experiments were run in parallel. In the case where BDE-209 was present in the compost without the need of fortification (low-level experiments), non-fortified experiments were also run in the absence of the compost. In all the experiments, the analysis of the pooled lettuce (all lettuces from the same pot were pooled) was performed in triplicate by means of GC-NCI-MS (see **section 7.2.3.2**) and the hearts and leaves of the lettuces were analysed together.

7.2.3 Sample preparation and analysis

7.2.3.1 Matrix Solid-Phase Dispersion

Previously optimised MSPD extraction [47] was used for the simultaneous extraction/clean-up of the samples. Briefly, 0.5 g of samples (compost:soil mixture and carrot) and 0.25 g in the case of lettuce were dispersed with 0.5 g of C_{18} dispersing adsorbent using a glass mortar with a pestle to achieve a complete homogenisation. 0.5 g of silica followed by 1.75 g of acidified silica (10 % H_2SO_4, w:w) was added into an empty glass cartridge (10-mL capacity), fitted with a single bottom frit. Then, the dispersed sample mixture spiked with the surrogate standards was placed over the clean-up sorbents followed by a second frit. The cartridge was tightly compressed using a stick. Target analytes were eluted in two different fractions; in the first fraction PBDEs and MeO-PBDEs were collected in 10 mL of a 75:25 (v:v) n-hexane:DCM mixture, while OH-PBDEs were collected in a subsequent eluate of 20 mL of pure DCM. The first eluate was concentrated to dryness under a nitrogen stream using a Turbovap LV Evaporator and reconstituted to a final volume of 100 µL of isooctane. The second eluate was also evaporated to dryness, reconstituted in EtOAc and submitted to a derivatisation [47] step for the analysis of OH-PBDEs (final volume of 100 µL of isooctane).

7.2.3.2 Gas chromatography-(negative chemical ionisation)-mass spectrometry analysis

All the extracts were analysed according to Iparraguirre et al. [47]. An Agilent 7890A GC equipped with an Agilent 7683B automatic sampler and a split/splitless injection port was coupled to an Agilent 5975C MS (Agilent Technologies, Palo Alto, CA, USA) using negative chemical ionisation (NCI) with methane (CH_4) as reagent gas. All standard solutions and sample

extracts were injected in the pulsed splitless mode with an injection volume of 2 µL at 300 ºC with a pulse pressure set at 25 psi for 1.5 min. The chromatographic analysis was carried out with 15 m x 0.25 mm, 0.1-µm film thickness DB-5HT capillary column (J & W Scientific, Folsom, CA, USA). The oven temperature program was set as follows: the initial temperature was set at 80 ºC (hold time 1.5 min) followed by a temperature increase of 60 ºC/min to 220 ºC, where it was kept for 10 min, then ramped to 270 ºC at 5 ºC/min, with a final ramp at 10 ºC/min to 300 ºC, where it was finally held for 5 min. Carrier gas was He at 1 mL/min flow-rate. Transfer line, MS source and MS quadrupole temperatures were set at 310 ºC, 230 ºC and 150 ºC, respectively. Measurements were performed in the selected ion monitoring (SIM) mode and the ions monitored for each analyte are showed in **Table 7.1**.

7.2.3.3 Gas chromatography - triple quadrupole tandem mass spectrometry analysis

The extracts were also submitted to gas chromatography coupled to triple quadrupole tandem mass spectrometry (GC-MS/MS) analysis in order to evaluate the potential presence of degradation and/or transformation PBDEs products. An Agilent 7890A GC equipped with an Agilent 7693 automatic sampler and a split/splitless injection port coupled to an Agilent 7000 triple quadrupole mass spectrometer (Agilent Technologies, Avondale, PA, USA) was used. All the extracts were injected in the pulsed splitless mode with an injection volume of 2 µL at 300 ºC with a pulse pressure set at 25 psi for 1.5 min. The analytes were introduced into a HP-5 capillary column (30 m × 0.25 mm, 0.25 µm, Agilent Technologies). The oven temperature program was set as follows: the initial temperature was set at 80 ºC (hold time 1.5 min) followed by a temperature increase of 30 ºC/min to 220 ºC where it was kept for 1 min, then ramped to 250 ºC at 5 ºC/min (hold time 1 min) with a final ramp at 5 ºC/min to 300 ºC, where it was finally held for 5 min. Helium was used as carrier gas at a flow rate of 1.3 mL/min. The mass spectrometer worked in the electron impact (EI) ionisation mode with an electron energy of 70 eV. The interface temperature was set at 310 ºC, while the temperature of the ionisation source and the detector were maintained at 230 °C and 150 °C, respectively. In the case of PBDEs and MeO-PBDEs, measurements were performed in the selected reaction-monitoring (SRM) mode where the collision gas was N_2 at 1.5 mL/min. The ion transitions for each analyte are shown in **Table 7.1**. Both, first and second quadrupole energies (eV) applied are also included. For the derivatised OH-PBDEs, measurements were performed in the SIM mode and the ions monitored were m/z 136.8 and 138.8 for all the analytes. The determined instrumental limits of quantification (LOQs) for the target analytes are also included in **Table 7.1**.

7.2.3.4 Quality control

The analytical figures of merit of the MSPD-GC-NCI-MS method were presented elsewhere [47] for soil, carrot and lettuce samples and are summarised in **Table 7.1**.

The overall quality and accuracy of the analysis was also periodically monitored. The results of three parallel extractions were processed once a week and averaged for quantification using internal standard calibration. Standard solutions of PBDEs, MeO-BDEs and OH-BDEs mixtures (0.5-50 ng/μL) were prepared in order to check the linearity of the method (r^2 = 0.9876-0.9998). After correction with the corresponding surrogate, acceptable apparent recoveries in the 77-130 % range for all the analytes and matrices were obtained. RSDs in the range of 9-30 % were also obtained in the case of both, compost-amended soil and vegetables (carrot and lettuce) samples. At least, two procedural blanks were always processed every 12-15 control samples and concentrations lower than the method quantification limits (MQL) which were calculated using 10 times the signal-to-noise (S/N) ratio of matrices spiked at a low level (5 ng/g) were obtained.

7.2.3.5 Bioconcentration metrics and statistical analysis

The corresponding bioconcentration factors (BCFs) were calculated for the different compartments of each crop in accordance with the **Equation 7.1** on the basis of the dry weight of the material analysed. In the case of carrot, where three different compartments were analysed (peel, core and leaf), total BCFs (BCF_{Total}) were calculated taking into account the BCF of each compartment (BCF_{Peel}, BCF_{Core} and BCF_{Leaves}), as well as the compartment mass and the total mass of the carrot as shown in **Equation 7.2**, where m_{Peel} is the mass of the carrot peel, m_{Core} the mass of the core, m_{Leaves} the mass of the leaves and m_{Plant} the total mass of the carrot.

$$BCF = \frac{Concentration\ in\ dry\ plant\ tissue\ (\frac{ng}{g})}{Concentration\ in\ dry\ soil\ (\frac{ng}{g})} \qquad \textbf{Equation 7.1}$$

$$BCF_{Total} = \frac{1}{m_{Plant}} (BCF_{Peel}\, m_{Peel} + BCF_{Core}\, m_{Core} + BCF_{Leaves}\, m_{Leaves}) \qquad \textbf{Equation 7.2}$$

In the case of lettuce, since the leaves and heart were analysed together (see **section 7.2.2.3**), only $BCF_{Lettuce}$ was calculated.

Statistical analysis of the data was completed in the case of carrot using The Unscrambler software (version 7.6 Camo, Norway). As The Unscrambler software does not take into account the standard deviation of the responses, the lower and the upper values of the

confidence interval were added for calculation (each sample is named with two numbers). Prior to any data treatment, the responses were normalised and centred before the principal component analysis (PCA). The validation of PCA was leverage correction [48].

7.3 Results and discussion

7.3.1 Stability of PBDEs in the amended soil

The stability of BDE-138 and BDE-209 was studied in amended soil 2.4 by means of GC-NCI-MS analysis. Independent experiments were considered for both target analytes. Compost was fortified at 4800 ng/g for BDE-138 and BDE-209. The compost was mixed with soil 2.4 at a 95:5 soil:compost mixture and aliquots of 5 g were wrapped in aluminium foil and kept in the dark for 90 days, under the same conditions used during the plant uptake experiments (see **Section 7.2.2.1**). Samples were analysed at 0, 1, 4, 11, 15 and 90 days after exposure and the results are shown in **Figure 7.1**. According to the one way analysis of variance (ANOVA, $F_{Experimental} = 2.55 < F_{Critical} = 3.11$ and $F_{Experimental} = 1.31 < F_{Critical} = 3.11$ for BDE-138 and BDE-209, respectively), no significant degradation of the target analytes was observed during the experiment period. However, Xia et al. [49] observed a minimal apparent degradation in soils amended with biosolids containing penta-BDEs for 33 years. Andrade et al. [50] examined PBDEs in soils from 30 mid-Atlantic US fields and observed a lesser predominance of BDE-209 relative to BDE-47 and BDE-99 in biosolid-applied soils than in the biosolid itself. Besides, Gerecke et al. [51] observed reductive debromination of BDE-209 under anaerobic conditions. In the case of Hale and co-workers [3], they observed that the relative contributions of BDE-206, BDE-207 and BDE-208 compared to BDE-209 in both, biosolids-amended clay and sandy soil, were higher than in the commercial deca-BDE mixture but did not exceed the applied ratios in the biosolids. In all the studies mentioned above, degradation of BDE-209 was postulated. As an anaerobic soil [52] was used for cultivation media, debromination of the studied analytes could be therefore expected in the present work but it did not occur. Nevertheless, as reported by Vrkoslavová et al. [33] for tobacco and nightshade, no differences between the initial and final concentration of PBDEs after a cultivation period neither in planted nor in unplanted pots was observed.

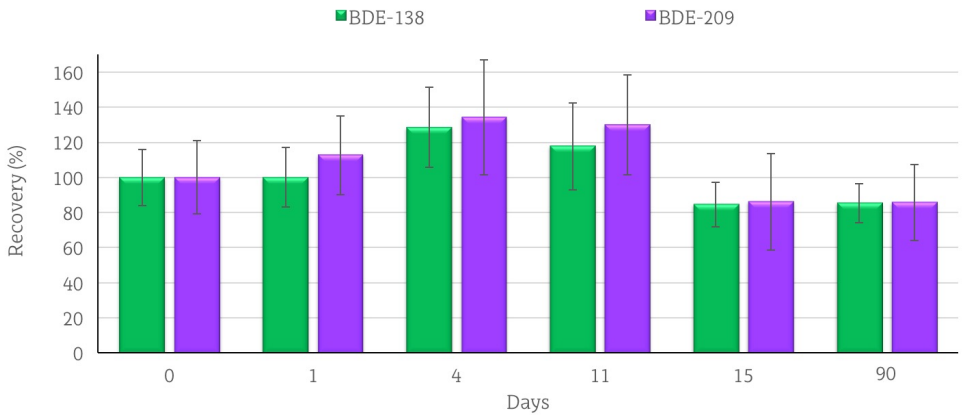

Figure 7.1. Stability of BDE-138 and BDE-209 in compost-amended soil 2.4 during 90-day degradation experiment.

7.3.2 Plant uptake results

7.3.2.1 Uptake by carrot

The average concentrations of the target analytes (n=3, ng/g) and their relative standard deviations (in brackets) for the amended soil and carrot (peel, core and leaves compartments), as well as the average BCFs and their relative standard deviations (in brackets), are included in **Table 7.3**. In the case of the relative standard deviation of BCFs, this was calculated by error propagation since the BCF is calculated as the ratio of the plant tissue concentration and the soil concentration (see **section 7.2.3.5**). The target analytes were always under the MQLs in the unfortified samples (see **Table 7.1**).

A PCA of the data included in **Table 7.3** was performed. The type of soil, the carrot specie and the type of analyte (as log K_{ow}, see **Table 7.1**), the concentrations found in the soil and the BCFs were included in the PCA. 4 principal components (PCs) were enough to explain up to 93 % of the total variance of the data, where the first PC (PC1) explained up to the 48 % of the variance, the second PC (PC2) up to the 22 %, the third PC (PC3) up to the 13 % and the fourth PC (PC4) up to the 10 %. **Figures 7.2 a, b** and **c** show the bi-plots of the PC1 versus PC2, the PC1 versus PC3 and the PC1 versus PC4, respectively.

Table 7.3. Bioconcentration factors obtained for the peel (BCF_{Peel}), core (BCF_{Core}), the leave (BCF_{Leaves}) and the total plant (BCF_{Total}) obtained for the different soil types, the different carrot (***Daucus carota ssp. Sativus***) species (***Nantesa*** and ***Chantenay***) and the two target analytes (BDE-138 and BDE-209) studied. Concentration (ng/g) for the soil and the different carrot compartments (peel, core and leave) are also given. The sample name indicates the values given for the lower and the upper values of the confidence interval introduced in The Unscrambler program.

Soil	Variety	Analyte	Pots	C_{Soil} (ng/g)	C_{Peel} (ng/g)	C_{Core} (ng/g)	C_{Leaves} (ng/g)	BCF_{Peel}	BCF_{Core}	BCF_{Leaves}	BCF_{Total}	Sample name
Soil 2.1	Chantenay	BDE-138	Pot 1	111 (18)	2 (14)	< MDL	< MDL	0.020 (23)	[a]	[a]	0.0031 (29)	1, 22
Soil 2.1	Chantenay	BDE-138	Pot 2	99 (5)	2 (15)	< MDL	< MDL	0.025 (16)	[a]	[a]	0.0038 (24)	2, 23
Soil 2.1	Nantesa	BDE-138	Pot 1	68 (13)	4 (24)	< MDL	< MDL	0.059 (27)	[a]	[a]	0.0091 (33)	3, 24
Soil 2.1	Nantesa	BDE-138	Pot 2	96 (9)	7 (23)	< MDL	< MDL	0.075 (25)	[a]	[a]	0.0115 (30)	4, 25
Soil 2.4	Chantenay	BDE-138	Pot 1	124 (12)	1 (25)	< MDL	< MDL	0.011 (28)	[a]	[a]	0.0017(33)	5, 26
Soil 2.4	Chantenay	BDE-138	Pot 2	81 (20)	2 (15)	< MDL	< MDL	0.020 (25)	[a]	[a]	0.0030 (31)	6, 27
Soil 2.4	Nantesa	BDE-138	Pot 1	144 (15)	4 (14)	< MDL	< MDL	0.024 (20)	[a]	[a]	0.0037 (27)	7, 28
Soil 2.4	Nantesa	BDE-138	Pot 2	93 (11)	2 (4)	< MDL	< MDL	0.018 (12)	[a]	[a]	0.0028 (21)	8, 29
Substrate	Chantenay	BDE-138	Pot 1	120 (36)	4 (30)	< MDL	< MDL	0.033 (47)	[a]	[a]	0.0051 (50)	9, 30
Substrate	Chantenay	BDE-138	Pot 2	91 (15)	2 (15)	< MDL	< MDL	0.017 (21)	[a]	[a]	0.0026 (28)	10, 31
Substrate	Nantesa	BDE-138	Pot 1	73 (15)	1 (26)	< MDL	< MDL	0.014 (30)	[a]	[a]	0.0021 (35)	11, 32
Substrate	Nantesa	BDE-138	Pot 2	133 (15)	1 (29)	< MDL	< MDL	0.007 (32)	[a]	[a]	0.0011 (37)	12, 33
Soil 2.4	Chantenay	BDE-209	Pot 1	7 (2)	< MDL	< MDL	10 (6)	< MDL	[a]	1.43937 (6)	0.4765 (13)	13, 34
Soil 2.4	Chantenay	BDE-209	Pot 2	7199 (22)	63 (3)	< MDL	29 (39)	0.009 (22)	[a]	0.00409 (45)	0.0027 (27)	14, 35
Soil 2.4	Chantenay	BDE-209	Pot 3	6726 (30)	7 (10)	< MDL	19 (30)	0.001 (31)	[a]	0.00281 (42)	0.0011 (38)	15, 36
Soil 2.4	Nantesa	BDE-209	Pot 1	20 (42)	< MDL	< MDL	10 (0.4)	< MDL	[a]	0.50510 (42)	0.1672 (44)	16, 37
Soil 2.4	Nantesa	BDE-209	Pot 2	7086 (12)	16 (13)	< MDL	14 (10)	0.002 (17)	[a]	0.00198 (16)	0.0010 (15)	17, 38
Soil 2.4	Nantesa	BDE-209	Pot 3	6154 (12)	24 (10)	< MDL	16 (7)	0.004 (15)	[a]	0.00253 (14)	0.0014 (14)	18, 39
Substrate	Chantenay	BDE-209	Pot 1	20 (25)	< MDL	< MDL	14 (8)	< MDL	[a]	0.73380 (26)	0.2429 (29)	19, 40
Substrate	Chantenay	BDE-209	Pot 2	11374 (25)	146 (14)	< MDL	13 (5)	0.013 (29)	[a]	0.00113 (30)	0.0023 (29)	20, 41
Substrate	Chantenay	BDE-209	Pot 3	10757 (25)	86 (31)	< MDL	37 (17)	0.008 (40)	[a]	0.00348 (30)	0.0024 (27)	21, 42

[a] not calculated since C_{Core} and C_{Leaves} were lower tan MDLs.

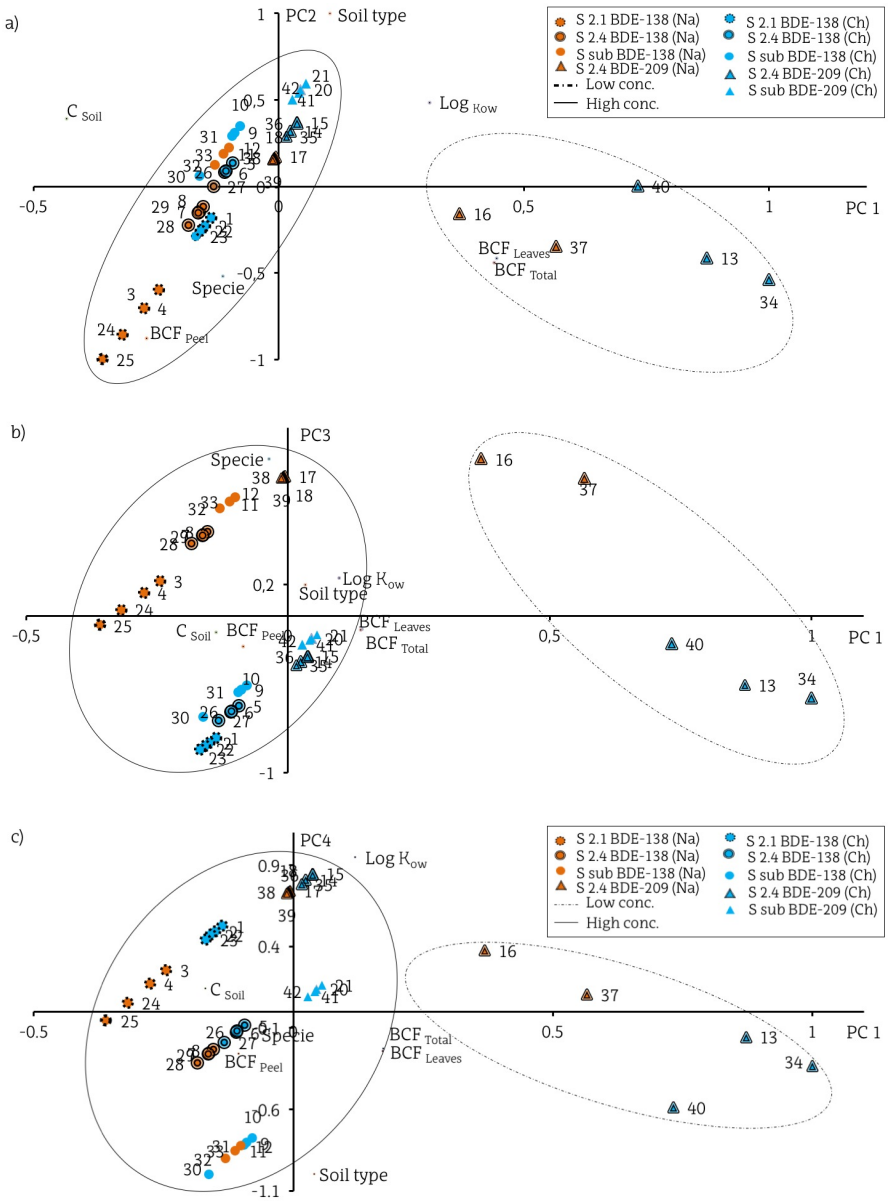

Figure 7.2. Bi-plots of the scores and loadings representing a) the first principal component (PC1) versus the second principal component (PC2), b) the PC1 versus the third principal component (PC3) and c) the PC1 versus the fourth principal component (PC4). Abbreviations: S=Soil; Sub=substrate; Na=Nantesa carrot variety; Ch=Chantenay carrot variety and Conc. = concentration level.

As can be clearly observed from **Figure 7.2 a**, while BDE-138 accumulated exclusively in the peel, BDE-209 accumulated mainly in the leaves (BCF_{Leaves}) when present at a low concentration. Therefore, all the BDE-138 samples lay close to BCF_{Peel} and all the samples polluted with BDE-209 at a low level were close to the variable BCF_{Leaves}, since, when BDE-209 was present at a low concentration it only accumulated in the leaves at a much higher percentage than when this pollutant was present at a high concentration level. Accumulation in the leaves could be due to translocation after root uptake or by foliar uptake from the air. According to the values obtained from the blanks, no appreciable contribution from foliar uptake was observed, similar to the results obtained in the literature [29, 38]. In general terms, when present at a high concentration, BDE-209 was also detected in the peel, at a higher concentration than in the leaves. BCF_{Peel} for BDE-138 was larger than that for BDE-209, around 10 times larger when the target analytes were cultivated in soil 2.4 (both for ***Nantesa*** and ***Chantenay*** species) and two times larger in the case of the substrate. BCF_{Total} was highly correlated to the BCF_{Leaves} due to the high values obtained for the latter when BDE-209 was present at a low concentration. The results for BDE-138 were more similar to those obtained by other authors [33, 35, 38], where the concentrations in the root were higher than in the leaves.

In the case of **Figure 7.2 b**, PC3 was directly related to the plant species. All the experiments performed with the ***Chantenay*** specie laid closer to BCF_{Peel}, no matter the values belonged to BDE-138 or BDE-209 (at high concentration). However, from the raw data it was not so easy to draw a conclusion. In the case of BDE-138, for soils 2.1 and 2.4, higher BCF_{Peel} values were observed in ***Nantesa*** than in ***Chantenay***, the opposite behaviour was observed in the substrate soil.

In the case of **Figure 7.2 c**, the influence of the soil type could be deduced around PC4. In the case of BDE-138, a higher BCF_{Peel} was observed for soils 2.1 and 2.4 in the ***Nantesa*** specie, while this difference was not significant in the case of the ***Chantenay*** specie according to the one way ANOVA ($F_{Experimental}$ =1.21 ‹ $F_{Critical}$=9.55). In the case of BDE-209, the grouping due to the soil type could also be observed but only for the amended soils at the high pollution level; however, the one way ANOVA showed that there were no significant differences ($F_{Experimental}$=2.55 ‹ $F_{Critical}$=3.10). Other works have shown that the bioavailability of BDE-209 was controlled by the soil TOC [51] and lower BCFs were obtained the higher the TOC in the soil for wheat uptake. However, no conclusive results could be obtained in the present work.

As a general conclusion of the PBDE uptake in carrot, it could be underlined that the BCF was strongly dependent on the nature of the BDE-isomer, not only in the BCF values obtained but

also in which part of the plant the analyte was accumulated. While BDE-138, with a lower log K_{ow} value, accumulated in the peel of the carrot, BDE-209 accumulated in the leaves with a much higher BCF when present at a low concentration and in the peel and the leaves (with a higher accumulation in the peel) when present at a high concentration.

In order to understand these different translocation behaviour, the following should be understood. Uptake has been shown to consist of two processes: i) establishing equilibrium between the concentrations of the compound of interest in the solution within the root and the solution surrounding the root and ii) sorption of the chemical onto the lipid and lipid-like solids of the roots. Adsorption onto root is important for compounds that are lipophilic and could be affected by the stage of the plant growth because chemical and physical properties of roots change significantly over the life of the plant. Older roots tend to be thicker and have less surface area per unit mass. As roots age, some of their capabilities for exudation, water uptake and assimilation of contaminants may diminish. Environmental factors such as moisture stress or nutrient supply may have a profound effect on root development and function at all stages of growth [54, 55]. Within this context, this hypothesis cannot be confirmed in the present work, since appreciable physical differences were not observed at the end of the uptake experiments. Further evaluation should be carried out by means of cellular morphology study in order to contrast this hypothesis.

Other important predictions emerge from a plant defence mechanism. There is a tolerance to organic pollutants by plant regarding to the detected concentration and specific compound. When the compounds are not detected by the plant system as a dangerous contaminant, the activation of the plant protection mechanism is limited. It is the case of the "low" concentration levels at which a different behaviour of plant translocation could probably happen. In fact, the presence of contaminants may not be detected by the plant and can imply the possible translocation to the different compartments: root, shoot and/or leaves [54, 55]. Besides, at these concentration levels, some organic compounds are beneficial for the plant. However, at higher concentrations, they exert damaging effects to plants depending on the specific compound and plant specie. In these cases, the protection mechanisms are centred around binding and chelating agent formation, being pollutants sequestrated and stored in the vacuoles that are located in the root in the case of carrot specie [56]. The translocation results observed for carrot in the present work, could apparently be explained according to this last consideration; however, further experiments should be carried out in order to confirm this hypothesis.

7.3.2.2 Uptake by lettuce

The average concentrations (in ng/g) and the relative standard deviations (in brackets) for the amended soil and lettuce, as well as the average BCFs and relative standard deviations (in brackets) are included in **Table 7.4**. In the case of the unfortified samples, the target analytes were always under the MQL (see **Table 7.1**) of the method applied. In the case of penta-BDE mixture, isomers BDE-28, BDE-47, BDE-100, BDE-99, BDE-154, BDE-153 and BDE-183 were identified and measured at a quantifiable level.

According to the results in **Table 7.4**, the BCF in lettuce was strongly affected by the nature of the target analyte (expressed as log K_{ow}), while the concentration of the pollutant in the amended soil had a much lower significance. The influence of the type of lettuce was not so clear since only BDE-99 was studied in two different types of lettuce. In this case, the highest BCF values were obtained for the ***Batavia Golden Spring*** specie. However, further experiments should be performed in order to obtain more significant conclusions in terms of the lettuce specie.

Table 7.4 Average concentration (in ng/g) and the relative standard deviations (in brackets) for the amended soil 2.4 and lettuce (***Lactuca sativa***), as well as the average bioconcentration factors (BCF) and relative standard deviations (in brackets) during the uptake experiments of penta-BDE mixture, BDE-209 and BDE-99.

Variety	**Analyte**	**Pots**	**C_{Soil} (ng/g)**	**$C_{Lettuce}$ (ng/g)**	**$BCF_{Lettuce}$**
Batavia Golden Spring	BDE-28	Pot 1	649 (14)	155 (1)	0.239 (14)
	BDE-47		2247 (14)	458 (3)	0.204 (15)
	BDE-100		42 (2)	8 (6)	0.202 (7)
	BDE-99		803 (18)	152 (12)	0.190 (21)
	BDE-154		61 (11)	1 (8)	0.018 (14)
	BDE-153		182 (1)	11 (5)	0.059 (5)
	BDE-183		27 (18)	14 (0.1)	0.509 (18)
	BDE-209		6 (9)	3 (35)	0.409 (36)
Batavia Golden Spring	BDE-209	Pot 1	25 (29)	19 (8)	0.741(30)
	BDE-209	Pot 2	8670 (31)	4648 (23)	0.536 (39)
	BDE-209	Pot 3	4384 (12)	2503 (3)	0.571 (13)
Summer queen	BDE-99	Pot 1	523 (15)	48 (15)	0.092(22)
	BDE-99	Pot 2	326 (24)	26 (9)	0.080 (26)
	BDE-99	Pot 3	2985 (4)	336 (12)	0.113 (12)
	BDE-99	Pot 4	2991 (2)	180 (9)	0.060 (9)

The highest influence was obviously the type of analyte. Actually, if the BCF value was plotted against the log K_{ow} value (see **Figure 7.3**), two different tendencies could be observed. While BDE-183 and BDE-209 showed BCF values close to 0.5 or higher, the BCF values for the rest of the target analytes were lower than 0.239 in all the cases. Moreover, for BDE-28, BDE-47, BDE-100, BDE-99, BDE-154 and BDE-153 with log K_{ow} values in the 5.88-8.09 range, a decrease in the BCF value was observed the higher the log K_{ow} value. This tendency was not observed, however, in the case of target analytes with log K_{ow}› 8.5 (BDE-183 and BDE-209). Huang et al. [35] observed that lower brominated PBDEs were more liable to be taken up by plants (pumpkin, maize and ryegrass) but, in their work, this behaviour was constant for high brominated (9-10) congeners unlike the present work. However, Wang and co-workers [38] obtained an increase in the root concentration in maize with the increase of the log K_{ow} for BDE-15, BDE-28 and BDE-47. It should be underlined that in the latter work the uptake was not from a fortified amended soil but from a spiked solution and, therefore, a different behaviour could be expected since the soil organic matter also plays a role in the uptake [35]. In the work by Mueller and co-workers [37] for the uptake of BDE-47, BDE-99 and BDE-100 for penta-BDE mixture to radish and zucchini, a similar uptake of the three congeners was observed in the root but translocation to the shoot in zucchini plants dramatically increased with the increase of bromine atoms. If translocation was due to passive movement in the transpiration stream of the plant, one might expect that lower brominated congeners with greater water solubility would be more easily transported into above ground tissues. However, other variables such as species specific root morphology, physiology, water and nutrient requirements and acquisition mechanisms must also play a role [37]. Vrkoslavová and co-workers [33] also observed that BCFs of BDE-47, BDE-99, BDE-100 and BDE-209 were negatively correlated with their log K_{ow}. In the case of the work by Inui and co-workers [57] for dioxin-like compound uptake by zucchini, cultivars "Black Beauty" and "Gold Rush" accumulated preferentially some penta-, hexa- and even pentachlorinated biphenyl congeners, whereas "Patty Green" accumulated preferentially tetrachlorinated congener CB-77. Further research should be performed since the mechanism of uptake and translocation remains still unknown and is influenced by several factors [33]. On the one hand, desorption from the soil can control the sorption process [57, 58]. On the other hand, root exudates could alter the bioavailability [37, 59] by forming a more hydrophilic complex that is better transported by the plant. Another possibility for the better desorption of hydrophobic compounds from soil is the presence of POP-binding proteins in the plants [57] and phloem proteins could be responsible for the accumulation of hydrophobic compounds by facilitating their transport. Other variables that could play an important role are plant physiology, lipid composition, water content and

transpiration rates [60, 61].

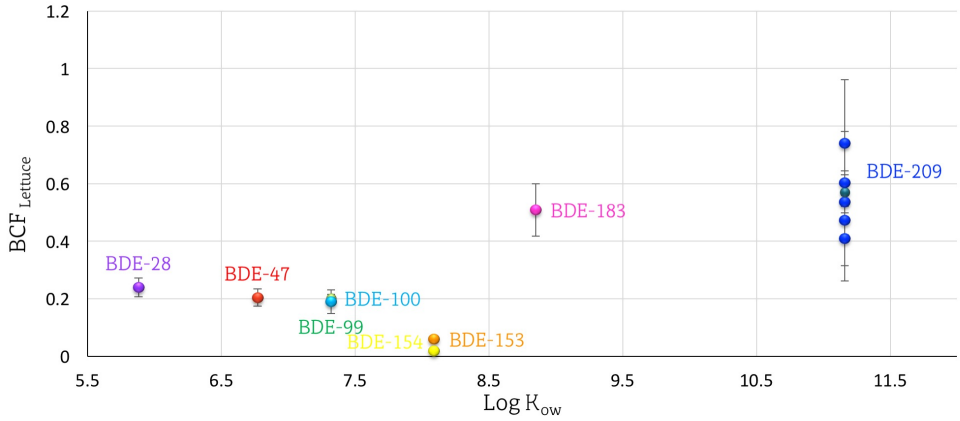

Figure 7.3. Bioconcentration factors (BCFs) of the different congeners in lettuce (***Batavia Golden Spring*** and ***Summer Queen*** varieties) versus the octanol-water partition coefficients (log K_{ow}).

Since the uptake of both BDE-99 and BDE-209 in lettuce was performed at different amended soil concentrations, the concentrations found in the lettuce were plotted against the concentration in the amended soil. As can be observed in **Figures 7.4 a** and **b** for BDE-99 and BDE-209, respectively, a good correlation was observed, indicative that the BDE-99 and BDE-209 present in the lettuce, came from the root uptake from the soil and the subsequent translocation. Similar results were reported by Li et al. for BDE-209 in wheat [53].

Finally, compared to the BCF values obtained for BDE-209 in carrot, uptake in lettuce was in general higher. The lettuce BCFs obtained for BDE-209 were in the same order of magnitude than those obtained for wheat straw (0.32-1.13) [53]; however, the values obtained for BCF_{Root} in the case of carrot samples were much lower than those in wheat (1.94-6.69) [53] and in roots (0.13-0.57) and shoots (0.04-0.12) of radish, alfalfa, squash, pumpkin, maize and ryegrass [62]. In the work by Hale and co-workers [3] no accumulation of PBDEs in corn was observed. Since the application of dewatered biosolid cakes disperses small organic-rich conglomerates rather than an homogeneous layer, the authors postulated that the aggregates in soil may delay plant uptake of PBDEs.

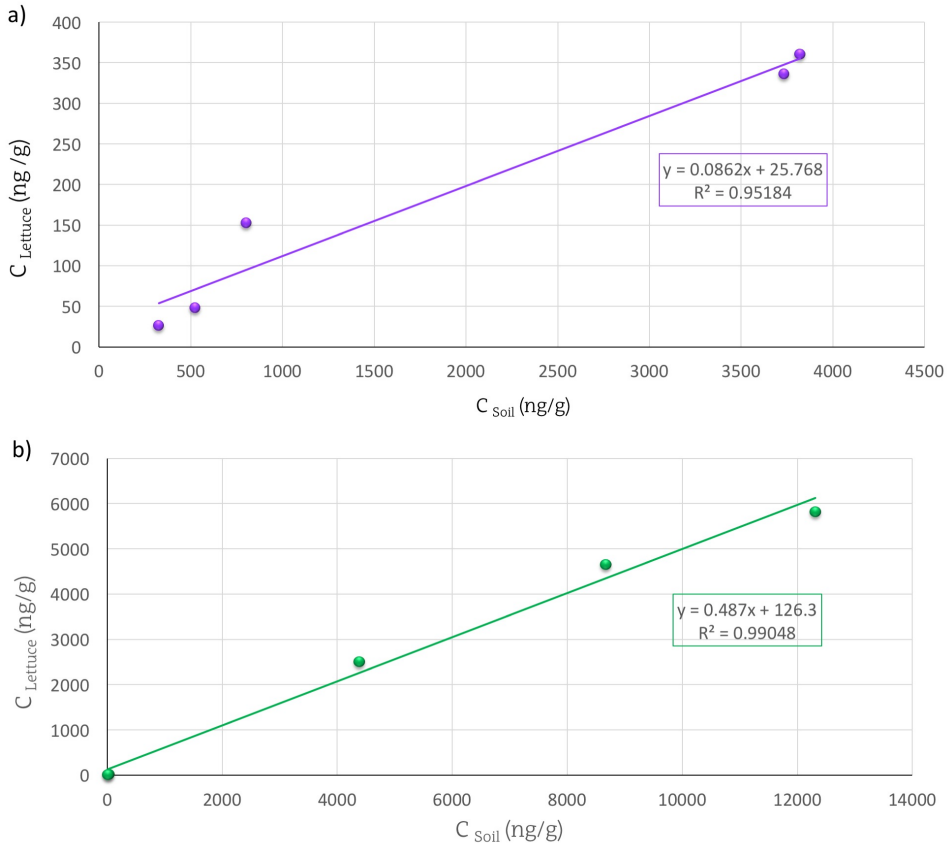

Figure 7.4. Concentration in lettuce versus the concentration in the compost-amended soil 2.4 for a) BDE-99 and b) BDE-209.

7.3.3 Degradation and metabolisation

In all the experiments mentioned above, the presence of lower brominated congeners was followed. Besides, in the experiments performed in lettuce for the penta-BDE mixture, BDE-209 and BDE-99 and in the case of carrot for BDE-209, transformation products, including OH- and MeO-PBDEs, were also studied by means of both, GC-(NCI)-MS and GC- MS/MS analysis. Neither debromination nor the formation of OH- and MeO-PBDEs was observed in the present work. However, Huang and co-workers [62] observed the metabolisation of BDE-209 into lower brominated congeners (di- to nona-) and OH-PBDEs in both the soil and the plant tissues (radish, alfalfa, squash, pumpkin, maize and ryegrass). In the latter work, 3-hydroxy-2,4-dibromodiphenyl ether (3'-OH-BDE-7) was the OH-PBDE congener detected in most of the cases. However, the

standard of this compound was not available in the present work. Similar to the present work, 3-hydroxy-2,2´,4,4´-tetrabromodiphenyl ether (3-OH-BDE-47) was not detected by Huang et al. [62]. Besides, these transformation products were detected in the root and shoot of the studied plants and in our work the whole lettuces (hearts and leaves together) were studied. Wang and co-workers [38] also detected lower brominated congeners in the uptake of BDE-15, BDE-28 and BDE-47 in maize and Chow et al. [63] in the case of BDE-209 in rice.

7.4 Conclusions

Uptake of different PBDE isomers into carrot and lettuce from different types of compost-amended soils was studied in the present work. Degradation of PBDEs in soil in the absence of the plants was not observed. BDE-209 showed a singular behaviour in the case of the uptake by carrot. While translocation to the leaves was predominant when BDE-209 was present at the low concentration, when BDE-209 was at high concentration level in the soil:compost mixture high concentration in the peel was detetermined, similar behaviour was observed for BDE-138. The results in blanks reject a foliar uptake. In the case of lettuce samples, PBDEs with a log K_{ow} ‹ 8.5 showed lower BCFs the higher the bromination level, while for PBDE isomer with log K_{ow} › 8.5 (BDE-183 and BDE-209) higher BCFs were observed. Further research should be carried out in order to understand the influence of the log K_{ow} in the uptake of non-polar contaminants. The behaviour of BDE-99 in lettuce also guaranteed that root uptake of the target analytes occurred rather than foliar uptake. While the uptake in lettuce was in general higher than in carrot, the influence of the variety of lettuce or carrot was not conclusive, similar to the effect of the different type of soils. Finally, no metabolisation of the target analytes studied was observed.

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CHAPTER 8

Uptake of perfluorooctanoic acid, perfluorooctane sulfonate acid and perfluorooctane sulfonamide by carrot and lettuce grown in compost-amended soils

Chemosphere (Under review)

8.1 Introduction

Within the wide number of emerging contaminants described in the literature, perfluoroalkyl substances (PFASs) have gained importance in the last decade [1, 2]. According to their properties, water and grease repellence, and their highly chemical and biological stability, common applications include nonstick cookware, breathable membranes for clothing, stain-resistant carpets and fabrics, components of fire fighting foams and surfactants [3]. The most commonly monitored PFASs in environmental matrices are perfluorooctane sulfonate (PFOS), which was banned by the Stockholm Convention in 2009, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonamide (FOSA), among others [4, 5]. Due to the growing concern on this class of chemicals, PFOS and its derivatives have been listed as priority hazardous substances in the field of water policy under the Directive 2013/39/EU [6].

Due to their wide use and persistence, PFASs are widely dispersed in the environment, which can explain their occurrence in different environmental compartments, such as air [7], water [8], sediment [9] and sludge [10], among others. Sewage sludge is widely recognised as a major sink of some PFASs [9]. Besides, land application of biosolids or compost (a biosolid and vegetable waste such as sawdust mixture) from municipal, agricultural and industrial wastewater treatment plants (WWTPs) is becoming an increasingly important global practice due to the recognised improvements (i.e., increased organic matter content, aggregate stability, porosity, water filtration rate and increased fertility and productivity) biosolids or compost can bring to soil. Therefore, the use of biosolids or compost as fertilisers in agriculture may present an exposure route of PFASs into the soils [4], which may be then transferred to the food chain by plant uptake and exert a potential health risk. It is necessary, therefore, to understand the behaviour of PFASs in the soil-plant system as a result of biosolid or compost application. Few investigations have reported this issue [11-17].

Another way to study the bioavailability of pollutants is the use of passive sampling. The diffusion driving forces and separation mechanisms of passive sampling are based on the difference in the chemical potentials of trapped and non-trapped analytes. The devices for passive sampling are usually based on diffusion through a well-defined diffusion barrier or permeation through a membrane [18]. Although passive sampling of gaseous and aqueous samples has been used for many years, the application to soil samples is relatively new [19]. The monitoring of volatile and semivolatile organic compounds in soil is normally done by measuring

the concentration of the target analytes in the soil-gas phase [20]. Besides, other passive sampling approaches, including solid-phase microextraction [21], semipermeable membrane devices [22] or C_{18} adsorbent tubes placed in polyethylene (PE) bags [19], have been used for the passive sampling of organic compounds in solid matrices such as fish tissue, soil and sediment. Over the past decades, passive probes, such as polymer-coated glass fibers [23], polyoxymethylene (POM) films [24] and PE devices [25], have also been employed for measurement of chemicals in surface sediment pore-water.

Within this context, the main objective of the present work was to investigate the uptake and distribution of PFOA, PFOS and FOSA from different compost-amended soils by two varieties (***Chantenay*** and ***Nantesa)*** of carrot (***Daucus carota ssp sativus***) and one variety (***Golden Spring)*** of lettuce (***Lactuca sativa***). Both crops were selected in order to see the differences between a root vegetable, where the fruit is underground in direct contact with the polluted soil, and a leaf vegetable, where the edible part is not in direct contact. Besides, different polymeric materials, including polyethersulfone (PES), silicone rod (SR) and POM, were deployed in the amended soils during the crop cultivation period, in order to see whether the amount sorbed in the polymeric material could be correlated with the concentrations observed in the plant compartments.

8.2 Experimental section

8.2.1 Chemical reagents and laboratory materials

The solid reagents PFOS (98 % purity) and PFOA (96 % purity) were purchased from Sigma Aldrich (Steinheim, Germany) and FOSA (97.5 % purity) from Dr. Ehrenstorfer GmbH (Augsburg, Germany). A mixture containing 5 µg/mL of perfluoro-n-butanoic acid (PFBA), perfluoro-n-pentanoic acid (PFPeA), perfluoro-n-hexanoic acid (PFHxA), perfluoro-n-heptanoic acid (PFHpA), PFOA, perfluoro-n-nonanoic acid (PFNA), perfluoro-n-decanoic acid (PFDA), perfluorobutane sulfonate (L-PFBS), perfluorohexane sulfonate (L-PFHxS) and L-PFOS in methanol (MeOH) and the surrogate mixture containing 2 µg/mL of isotopically mass-labelled sodium perfluoro-1-hexane[$^{18}O_2$]sulfonate (MPFHxS), sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate (MPFOS), perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid (MPFBA), perfluoro-n-[1,2-$^{13}C_2$]-hexanoic acid (MPFHxA), perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid (MPFOA), perfluoro-n-[1,2,3,4,5-$^{13}C_5$]nonanoic acid (MPFNA), perfluoro-n-[1,2-$^{13}C_2$]decanoic acid (MPFDA), perfluoro-n-[1,2-$^{13}C_2$]undecanoic acid (MPFUnDA) and perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid (MPFDoA) in MeOH were purchased from

Wellington Laboratories (Guelp, Ontario, Canada).

In the case of vegetables, a Cryodos-50 laboratory freeze-dryer from Telstar Instrument (Sant Cugat del Valles, Barcelona, Spain) was used to freeze-dry the samples. For extraction, 50-mL polypropylene conical tubes (PP, internal diameter 27.2 mm x 117.5 mm length) obtained from Deltalab (Barcelona, Spain) and a Bandelin sonifier ultrasonic homogeniser (20 kHz; Bandelin Electronic, Berlin, Germany) equipped with a 3-mm titanium microtip were used. After the extraction step, the supernatant was filtered through polyamide filters (0.45 μm, 25 mm, Macherey-Nagel, Germany). Evolute-WAX (primary/secondary amine modified polystyrene-divinylbenzene incorporating non-ionisable hydroxyl groups, 200 mg) solid phase extraction (SPE) cartridges were purchased from Biotage (Uppsala, Sweden). MeOH (HPLC grade, 99.9 %) was supplied by LabScan (Dublin, Ireland) and acetonitrile (ACN, HPLC grade, 99.9 %) from Sigma Aldrich (Steinheim, Germany). Ultra-pure water was obtained using a Milli-Q water purification system (< 0.05 μS/cm, Milli-Q model 185, Millipore, Bedford, MA, USA). GHP (hydrophilic polypropylene) microfilters (0.2 μm, 13 mm, Pall, USA) were used to filter extracts before liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.

For the mobile phase composition, MeOH obtained from Fisher Scientific (Loughborough, UK) was used. Ammonium acetate (NH_4OAc, ≥ 99 %) was purchased from Sigma Aldrich and 1-methylpyperidine (1-MP, purity > 98 %) was provided by Merck (Schuchardt OHG, Germany). For chromatographic separation of PFASs, a sure-Guard in-line filter (24.4 mm, 10 mm, 0.5 μm) obtained from VICI Jour (Schenkon, Switzerland) followed by an ACE UltraCore 2.5 SuperC_{18} core-shell (2.1 mm x 50 mm, 2.5 μm) column purchased by Advanced Chromatography Technologies (Aberdeen, Scotland) were used.

All the reagents used for the Hoagland nutritive solution preparation, potassium nitrate (KNO_3, 99.0 %), calcium nitrate tetrahydrate ($Ca(NO_3)_2$ $4H_2O$, 98.0 %), ammonium phosphate monobasic ($(NH_4)H_2PO_4$, 96.0-102.0 %), magnesium sulphate heptahydrate ($MgSO_4$ $7H_2O$, 99.0-100.5 %), manganese chloride tetrahydrate ($MnCl_2$ $4H_2O$, 98.0-102.0 %), boric acid (H_3BO_3, 99.8 %), zinc sulphate heptahydrate ($ZnSO_4$ $7H_2O$, 99.0-104.0 %), copper sulphate pentahydrate ($CuSO_4$ $5H_2O$, 99.0-100.0 %) and sodium molybdate dehydrate (Na_2MoO_4 $2H_2O$, 98.0-100.0 %) were purchased from Panreac (Castellar del Vallès, Spain). The Hoagland solution was prepared monthly according to Epstein and Bloom's work [26]. Briefly, an appropriate amount of the different salts were weighed, followed by pH adjustment at 5.5 with NaOH (reagent grade, ≥ 98 %, Panreac) and/or HCl (ACS reagent, 37 %, Panreac) in order to prepare 25 L.

Lettuce (***Lactuca sativa***, ***Batavia Golden Spring*** variety) and carrot (***Daucus carota ssp. sativus, Chantenay*** and ***Nantesa***varieties) seeds and the universal substrate were obtained from a commercial agricultural house (www.mvgarden.com). The compost was acquired from Calahorra WWTP (Spain) and soil 2.4 was supplied by LUFA Speyer (Speyer, Germany) (detailed information in **Table 8.1**).

Table 8.1. Characterisation according to the physicochemical parameters of the different soils. Soil 2.4 characterised by LUFA Speyer (Speyer, Germany) and substrate and compost by Neiker Tecnalia (Bizkaia, Spain).

Parameter	Soil 2.4	Universal substrate	Compost
TOC %	2.3±0.5	53±9	55±8
N %	0.20±0.04	0.35±0.05	2.4±0.3
pH	7.2±0.2	5.7±0.2	7.9±1.5
Cation Exchange Capacity (meq/100 g)	31±5	48±4	50±9
particles‹ 0.002mm	26±2	4.5±0.5	10±1
0.002-0.05mm	41±1	27±3	40±4
0.05-2mm	34±2	53±6	42±3
› 2mm	-[a]	16±2	8.9±1.5
soil type	sandy loam	sandy loam	Loam
water Holding Capacity (g/100g)	44±1	-[b]	-[b]
water per Volume (g/1000ml)	1288±36	-[b]	-[b]

[a] no particles › 2 mm, [b] not determined

PES sorbent was acquired from Membrane (Wuppertal, Germany) in a tubular format (0.7 mm external diameter, 1.43 g/mL density). The commercial silicone elastomer in flexible rod form (SR, 0.97 g/mL density) was purchased from Goodfellow (PA, USA). POM, 1.41 g/mL density film, was supplied by CS Hyde Company (Illinois, USA).

8.2.2 Polymeric material conditioning

Pieces of the polymers were cut using a sharp blade and accurately weighed; 30 mg (three pieces of 1.5 cm each one) for PES, 50 mg (one piece of 2 × 2.5 cm) for POM and 40 mg (one

piece of 0.2 cm) for SR. PES was soaked twice for 15 min and conditioned for 24 h in MeOH (HPLC grade, 99.9 %). The same procedure was performed for POM but using ethyl acetate (HPLC grade, 99.9 %) as solvent. SR pieces were soaked twice for 15 min with MeOH and conditioned, afterwards, in a thermal condition unit at 120 °C for 3 h under a nitrogen stream (›99.999% of purity) supplied by Messer (Tarragona, Spain).

8.2.3 Compost fortification and plant cultivation

A known amount of compost was weighed, covered with acetone (HPLC grade, 99.8 %), fortified with the corresponding analyte (PFOA, PFOS and FOSA) in order to achieve 500 ng/g nominal concentration level in the soil:compost (95:5) mixture and stirred for 24 h. After that, it was placed under a fume hood for solvent evaporation and the sample was aged for one week. Then, the compost was thoroughly manually mixed with the soil.

In the case of carrot, uptake was studied for two varieties (***Chantenay*** and ***Nantesa***) in two soil types (substrate and soil 2.4). Previous to the germination step, seeds were sonicated with Milli-Q water. Petri dishes were covered with moistened filter paper and the seeds were evenly distributed in the Petri dish (see **Figure 8.1**). Once the germination had occurred (12-14 days), 6 seedlings were transplanted in each pot (see **Figure 8.1**) containing fortified (n=2) and non-fortified (n=1) soil:compost (95:5) mixture (2 Kg). Carrots were cultivated under controlled environmental conditions in a greenhouse. The conditions were the following: temperature was set at 25 °C during day and at 18 °C during the night with a 14 h day length and a relative humidity of 50 % (during the day) and 60 % (overnight). They were regularly watered with distilled water and Hoagland (see **section 8.2.1**) nutritive solution. Previously conditioned (see **section 8.2.2**) pieces of the polymers (PES, POM and SR) were placed in the pots where carrots were cultivated. Plants in each pot were harvested after approx. 12-14 weeks, washed carefully with deionised water and pooled to one sample. Samples were separated into root peel (~2 mm depth obtained by peeling with a vegetable peeler), root core and leaves compartments and analysed in triplicate.

The same protocol applied for carrot experiments was carried out when uptake of lettuce (***Batavia Golden Spring*** variety) was studied for PFOA and PFOS in soil 2.4 and for FOSA in both soils (soils 2.4 and substrate). The differences between carrot and lettuce experiments were the harvesting period (4-5 weeks in the case of lettuce), the amount of amended soil (350 g) and the compartments analysed. In this case, lettuces were divided in the heart (the part which is in direct contact with the soil) and the leaves (the edible part).

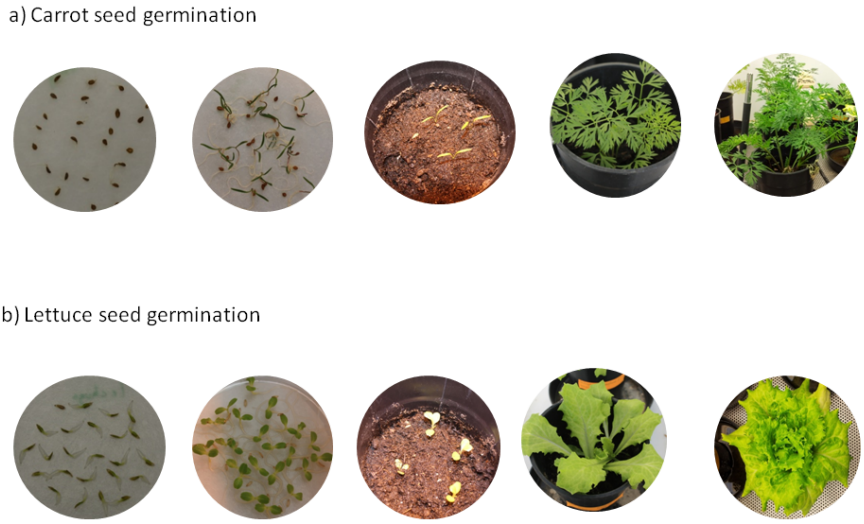

Figure 8.1. Carrot (a) and lettuce (b) evolution during seeds germination step and plant growing period.

Vegetables (carrot and lettuce) and compost-amended soil samples were freeze-dried (~ -50 °C) and air-dried, respectively. All the samples were stored at -20 °C, prior to be analysed in triplicate for the determination of the target analytes and their potential degradation products.

8.2.4 FOSA degradation experiment

Compost was fortified with FOSA in order to achieve a 500 ng/g concentration in the (95:5) soil:compost mixture. The degradation experiments were performed in two different amended soils (soil 2.4 and substrate) without crop cultivation but under the same conditions as plant uptake experiments (see **Section 8.2.3**). Samples from different pots containing 100 g of fortified soil:compost (95:5) mixture were collected in different (n= 6) time periods (from 6 h to 108 days).

8.2.5 Sample treatment and analysis

8.2.5.1 Vegetables, amended soil and polymeric materials

Focused ultrasound solid-liquid extraction (FUSLE) followed by SPE clean-up approach and LC-MS/MS analysis method was performed according to a previous work [27].

After crop harvesting, the polymers were also removed from each pot, rinsed with Milli-Q water in order to eliminate residues, dried with a clean paper tissue and placed inside

1.5-mL Eppendorf tubes. For analyte desorption, 500 µL of MeOH were added and the polymeric materials were sonicated during 30 min using an ultrasound bath (USB Axtor by Lovango, Barcelona, Spain). Finally, the extracts were filtered through a 0.2 µm GHP filter before LC-MS/MS analysis as described in a previously published work of the research group [27].

8.2.6 Quality control

Apparent recoveries in the range of 74-93 %, 69-90 % and 56-106 % for PFOA, PFOS and FOSA as well as method detection limits (MDLs) between 0.7-5.3 ng/g, 1.0-8.3 ng/g, 1.3-12.4 ng/g for PFOA, PFOS and FOSA in the compost-amended soil, carrot and lettuce matrices according to the previously published work [27]. The MDLs were determined according to the USEPA (http://www.epa.gov/waterscience/methods/det/rad.pdf) by fortification of five replicates of each blank matrix with each analyte at a low concentration (20 ng/g). Then, the MDL was worked out as MDL = t (n – 1, 1 –α = 0.95) × sd, where t=2.13 corresponds to the Student's t-value for a 95 % confidence level and four degrees of freedom and sd refers to the standard deviation of the replicate (n=5) analyses. During the sample treatment, procedural blanks and control samples (samples fortified at known concentration) were analysed every 15 samples. While values lower than MDLs were obtained in the case of blanks, apparent recoveries were in agreement with values obtained in a previous work [27].

8.2.7 Bioconcentration metrics

To enable meaningful comparison among soils and crops, bioconcentration factors (BCFs) were calculated for the different compartments of each crop. The corresponding BCF (the ratio between the concentration measured in plant compartment and the concentration measured in the compost-amended soil) values were calculated on the basis of the dry weight of the materials analysed. Total BCFs (BCF_{Total}) for each pot were calculated according to **Equations 8.1 and 8.2**, for carrot and lettuce, respectively, considering the mass of the different harvested plant compartments and the total mass of the crop.

$$BCF_{Total\ Carrot} = \frac{1}{m_{Plant}} (BCF_{Peel}\, m_{Peel} + BCF_{Core}\, m_{Core} + BCF_{Leaves}\, m_{Leaves}) \quad \textit{Equation 8.1}$$

$$BCF_{Total\ Lettuce} = \frac{1}{m_{Plant}} (BCF_{Heart}\, m_{Heart} + BCF_{Leaves}\, m_{Leaves}) \quad \textit{Equation 8. 2}$$

where m_{Peel} is the mass of the carrot peel, m_{Core} is the mass of the carrot core, m_{Leaves} is the mass of the carrot leaves, m_{Plant} is the sum of all the weighed compartments in each crop, m_{Heart} is the mass of the lettuce heart, BCF_{Peel} is the peel bioconcentration factor, BCF_{Core} is the

core bioconcentration factor, BCF_{Leaves} is the leaves bioconcentration factor and BCF_{Heart} is the lettuce heart bioconcentration factor.

8.3 Results and discussion

8.3.1 Uptake by carrot

The average (n=3) concentration (in ng/g) of PFOA, PFOS and FOSA measured in the different plant compartments of the carrots and the amended soil in both cases, before ($C_{soil, t=0}$) and after (C_{soil}) the harvesting period, the relative standard deviations (in brackets), as well as the average of the BCF_{Total} and its relative standard deviations (in brackets and calculated by error propagation) of each experimental set are given in **Table 8.2.**

Regarding PFOA, while values lower than MDLs were obtained in unfortified compost-amended soils, no significant difference was observed between the initial (t=0) concentrations and the concentration measured at the harvesting moment in the fortified compost-amended soil mixture. Recoveries in the range of 83-127 % were calculated for the soil:compost mixture samples in all the sets of the experiments; therefore, it could be concluded that neither degradation nor drainage losses during watering occurred within the cultivation period. With respect to the variables soil type and plant species, different tendencies were observed. While the soil type seemed to have an influence on the BCFs, the influence of the carrot specie seemed to be lower. Actually, when the BCF_{Peel} was plotted against the TOC content of the two soils studied (see **Figure 8.2 a**), it could be observed that the higher the TOC value, the lower the BCF_{Peel} was obtained. This observation was confirmed according to the two-way analysis of variance (ANOVA, $F_{Experimental}=1.09 < F_{Critical}=9.28$ and $F_{Experimental}=10.35 > F_{Critical}=10.16$ for species and soil type, respectively), since significant differences between the ability of PFOA uptake was observed depending on the soil TOC. Theobald et al. [28] observed the same tendency when the distribution in sediments of acidic PFASs in the North and Baltic's Sea was carried out. However, as can be observed from **Figure 8.2 a,** and, taking into account the ANOVA, the influence of the specie is soil dependent. While for the experiments performed in soil 2.4 the carrot specie had no significant effect (ANOVA, $F_{Experimental}=1.93 < F_{Critical}=18.51$), in the case of the substrate, the difference was significant (ANOVA, $F_{Experimental}=121 > F_{Critical}=19$) and the uptake in the ***Chantenay*** variety was higher than in the ***Nantesa*** variety. A similar behaviour was observed in the literature for different organic pollutants [11, 29-31].

Table 8.2. Average (n=3) concentration (in ng/g) and the relative standard deviations (in brackets) for the carrot (***Chantenay*** and ***Nantesa***) root peel, root core and leaves cultivated in two different compostaamended soil types (soil 2.4 and substrate), as well as the total BCF (BCF_{Total}) values.

Soil	Variety	Pots	$C_{t=0\ soil}$ (ng/g)			C_{soil} (ng/g)			C_{Peel} (ng/g)			C_{Core} (ng/g)			C_{Leaves} (ng/g)			BCF_{Total}		
			PFOA	PFOS	FOSA	PFOA	PFOS	FOSA	PFOA	PFOS	FOSA	PFOA	PFOS	FOSA	PFOA	PFOS	FOSA	PFOA	PFOS	FOSA
Soil 2.4	Chan	Pot 1	a	a	a	< MDL	a	a	< MDL	a	a	< MDL	a	a	< MDL	a	a	< MDL	b	b
Soil 2.4	Chan	Pot 2	495 (8)	a	a	524 (2)	a	a	291 (2)	a	a	143 (1)	a	a	754 (2)	a	a	0.805 (2)	b	b
Soil 2.4	Chan	Pot 3	560 (10)	a	a	553 (21)	a	a	261 (4)	a	a	154 (5)	a	a	1210 (4)	a	a	0.941 (16)	b	b
Soil 2.4	Nan	Pot 1	a	a	a	< MDL	a	a	< MDL	a	a	< MDL	a	a	< MDL	a	a	< MDL	b	b
Soil 2.4	Nan	Pot 2	500 (20)	a	a	439 (11)	a	a	181 (7)	a	a	141 (1)	a	a	562 (6)	a	a	0.692 (6)	b	b
Soil 2.4	Nan	Pot 3	470 (10)	a	a	427 (7)	a	a	262 (14)	a	a	148 (6)	a	a	1468 (4)	a	a	1.349 (9)	b	b
Subs	Chan	Pot 1	a	a	a	< MDL	a	a	< MDL	a	a	< MDL	a	a	< MDL	a	a	b	b	b
Subs	Chan	Pot 2	425 (5)	a	a	414 (2)	a	a	123 (5)	a	a	147 (20)	a	a	829 (20)	a	a	0.933 (14)	b	b
Subs	Chan	Pot 3	390 (20)	a	a	327 (5)	a	a	187 (5)	a	a	96 (10)	a	a	923 (8)	a	a	1.026 (7)	b	b
Subs	Nan	Pot 1	a	a	a	< MDL	a	a	< MDL	a	a	< MDL	a	a	< MDL	a	a	b	b	b
Subs	Nan	Pot 2	470 (12)	a	a	599 (23)	a	a	73 (3)	a	a	33 (4)	a	a	594 (2)	a	a	0.276 (5)	b	b
Subs	Nan	Pot 3	530 (7)	a	a	518 (6)	a	a	78 (9)	a	a	49 (4)	a	a	412 (1)	a	a	0.329 (2)	b	b
Soil 2.4	Chan	Pot 1	a	a	a	< MDL	a	a	< MDL	a	a	< MDL	a	a	< MDL	a	a	b	b	b
Soil 2.4	Chan	Pot 2	a	480 (7)	a	432 (23)	a	a	170 (2)	a	a	225 (7)	a	a	720 (15)	a	b	0.863 (19)	b	b
Soil 2.4	Chan	Pot 3	a	410 (15)	a	402 (6)	a	a	173 (2)	a	a	256 (7)	a	a	777 (6)	a	b	1.071 (7)	b	b
Soil 2.4	Nan	Pot 1	a	a	a	< MDL	a	a	< MDL	a	a	< MDL	a	a	< MDL	a	b	b	b	b
Soil 2.4	Nan	Pot 2	a	350 (20)	a	324 (11)	a	a	188 (6)	a	a	150 (4)	a	a	486 (12)	a	b	0.829 (9)	b	b
Soil 2.4	Nan	Pot 3	a	320 (17)	a	298 (29)	a	a	170 (9)	a	a	174 (13)	a	a	674 (2)	a	b	1.017 (21)	b	b
Subs	Chan	Pot 1	a	a	a	< MDL	a	a	< MDL	a	a	< MDL	a	a	< MDL	a	b	b	b	b
Subs	Chan	Pot 2	a	480 (18)	a	348 (14)	a	a	68 (4)	a	a	64 (2)	a	a	322 (4)	a	b	0.387 (10)	b	b
Subs	Chan	Pot 3	a	380 (9)	a	371 (15)	a	a	62 (12)	a	a	77 (4)	a	a	512 (10)	a	b	0.463 (12)	b	b
Subs	Nan	Pot 1	a	a	a	< MDL	a	a	< MDL	a	a	< MDL	a	a	< MDL	a	b	b	b	b
Subs	Nan	Pot 2	a	415 (12)	a	401 (21)	a	a	75 (5)	a	a	83 (2)	a	a	320 (2)	a	b	0.321 (13)	b	b
Subs	Nan	Pot 3	a	505 (20)	a	625 (4)	a	a	60 (5)	a	a	64 (9)	a	a	389 (20)	a	b	0.181 (11)	b	b
Soil 2.4	Chan	Pot 1	a	a	a	< MDL	a	a	< MDL	a	a	< MDL	a	a	< MDL	a	a	b	b	b
Soil 2.4	Chan	Pot 2	a	a	562 (11)	144 (10)	a	41 (21)	a	43 (22)	63 (28)	a	a	38 (23)	a	a	447 (3)	a	b	0.78 (8)	0.13 (7)
Soil 2.4	Chan	Pot 3	a	a	530 (7)	132 (27)	a	33 (39)	a	43 (1)	63 (3)	a	a	48 (7)	a	a	218 (25)	a	b	0.60 (14)	0.18 (9)
Soil 2.4	Nan	Pot 1	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b	b	b	b
Soil 2.4	Nan	Pot 2	a	a	555 (9)	155 (14)	a	a	a	73 (3)	a	a	a	a	a	a	341 (9)	a	b	0.41 (6)	b
Soil 2.4	Nan	Pot 3	a	a	565 (15)	184 (9)	a	a	a	60 (3)	a	a	a	53 (4)	a	a	125 (20)	a	b	0.43 (5)	b
Subs	Chan	Pot 1	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b	b	b	b
Subs	Chan	Pot 2	a	a	550 (8)	344 (13)	a	30 (12)	a	18 (5)	a	a	a	41 (5)	a	a	349 (5)	a	b	0.25 (3)	b
Subs	Chan	Pot 3	a	a	544 (12)	322 (11)	a	26 (10)	a	15 (10)	a	a	a	18 (4)	a	a	252 (12)	a	b	0.17 (2)	b
Subs	Nan	Pot 1	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b	b	b	b
Subs	Nan	Pot 2	a	a	547 (20)	609 (4)	a	a	a	38 (6)	a	a	a	54 (3)	a	a	654 (16)	a	b	0.28 (3)	b
Subs	Nan	Pot 3	a	a	551 (15)	512 (19)	a	a	a	54 (1)	a	a	a	33 (1)	a	a	566 (14)	a	b	0.24 (4)	b

Subs: Substrate; Chan:Chantenay, Nan: Nantesa. [a] not detected, [b] Not calculated since C_{Peel}, C_{Core} or C_{Leaves} were not detected or were lower than MDLs.

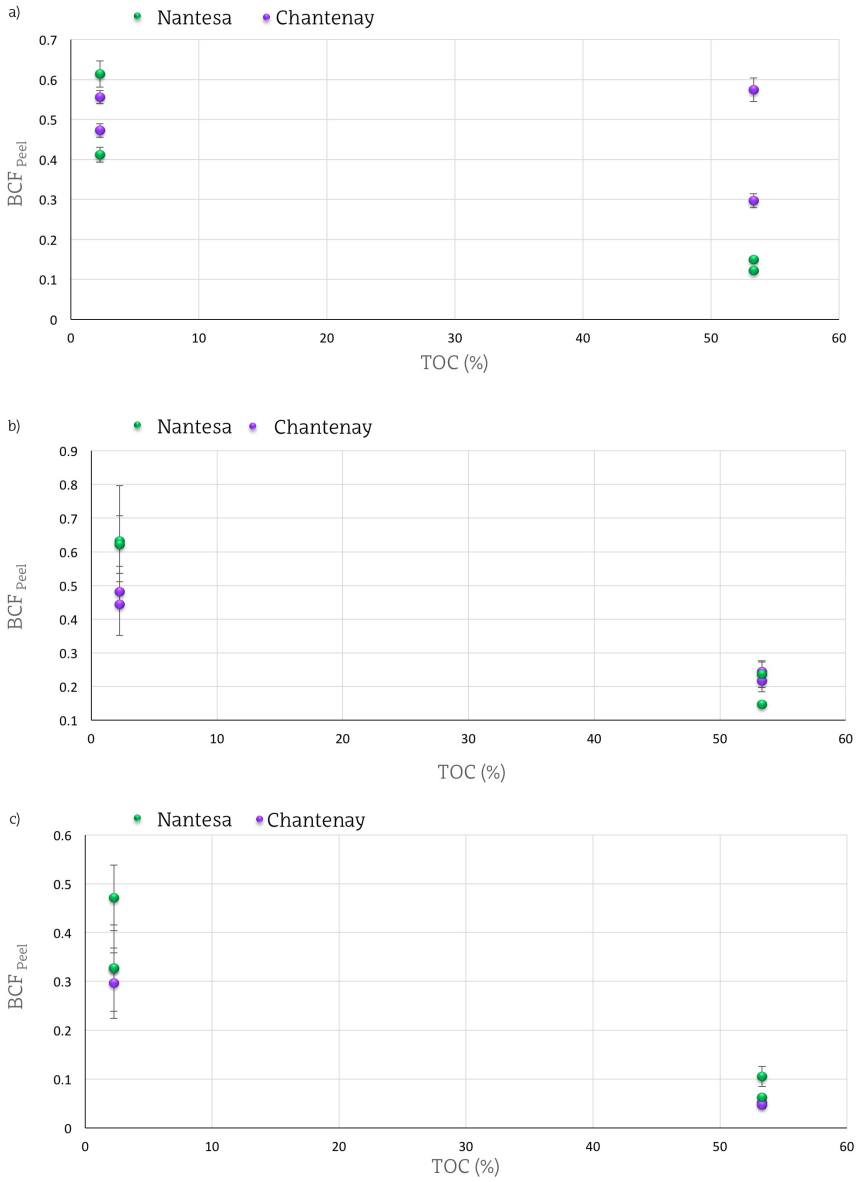

Figure 8.2. BCF_{Peel} against soil TOC for (a) PFOA, (b) PFOS and (c) PFOS coming from FOSA degradation uptake experiments.

Concerning the different compartments, while values lower than MDLs were obtained in the unfortified set of experiments, for fortified experiments the BCF_{Peel} (0.12-0.61) and BCF_{Core} (0.05-0.36) were in the same order of magnitude. The highest BCF values (0.80-3.34), higher than

1.0 in most of the cases, were obtained for the leaves. Since PFOA was not detected in the leaves of the control carrots, it could be concluded that pollutant concentrations measured in carrot leaves resulted from the uptake through the root to the aboveground parts, followed by a gradual transfer from roots to stems and then to leaves by means of a transpiration stream and not due to a foliar uptake from the air. Lunney et al. [32] reported similar observations for the uptake of polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane, as well as for PBDEs [29]. Relating to translocation, a similar tendency was observed by Blaine et al. [17] when acidic PFASs were determined in root crops such as radish, and by Lechner at al. [16] when carryover of PFOA and PFOS from soil to carrot, potatoes and cucumbers was studied.

Concerning PFOS, values lower than MDLs were obtained in unfortified amended soil experiments, whereas, in the fortified ones, recoveries (considering both, before ($C_{soil,\ t=0}$) and after (C_{soil}) the harvesting period amended soil concentration values) between 60-119 % were determined. Recoveries lower than 75 % were attributed to PFOS mobilization during soil irrigation and not to degradation, since, according to the literature [33], PFOS is resistant against thermal and chemical degradation. Besides, no degradation products were detected in the present work. Concentrations of PFOS in the different carrot compartments and BCF_{Total} were also included in **Table 8.2.** Similar to PFOA, BCF_{Peel} for PFOS was plotted against the TOC of the soils (**Figure 8.2 b**) and a two-way ANOVA of the data was performed ($F_{Experimental}$=2.24 ‹ $F_{Critical}$=9.28 for plant species and $F_{Experimental}$=28.72 › $F_{Critical}$=10.13 for soil TOC). It could be concluded that the higher the TOC, the lower the BCF_{Peel} value obtained. However, no influence of the carrot specie on the uptake could be derived for soil 2.4 and substrate. Similar to PFOA, the highest BCF values were found in the leaves. The BCF values obtained for PFOA and PFOS were in the same order of magnitude in leaves and peel.

In the case of FOSA, degradation occurred and FOSA was detected neither in the carrot nor in the soil after the carrot cultivation (see **Table 8.2**). However, high concentrations of PFOS were detected, indicative of the transformation of the FOSA precursor in soil to the stable PFOS end-product, similar to the results found in the literature [34]. The behaviour of the PFOS coming from the FOSA degradation was similar to the behaviour of PFOS uptake from the amended soils fortified directly with PFOS. The highest concentrations were measured in the leaves, followed by peel and core (see **Table 8.2**)**.** Moreover, according to the two-way ANOVA ($F_{Experimental}$ = 47.97 › $F_{Critical}$ = 10.13 and $F_{Experimental}$ = 1.03 ‹ $F_{Critical}$ = 9.28 for soil TOC and plant species, respectively), significant differences in BCF_{Peel} were observed depending on the type of soil (see **Figure 8.2 c**) and BCF_{Peel} decreased with the increase in the TOC of the soil. However, the plant species had no

significant effect.

Figure 8.3 plots the BCF_{Peel} values obtained for PFOS coming from the degradation of FOSA and BCF_{Peel} values coming directly from amended soils fortified with PFOS. The good correlation obtained (r^2› 0.87) and the slope close to 1 (slope=0.8016) are indicative of the similar uptake behaviour observed for the PFOS coming from the FOSA degradation and the PFOS coming from a soil directly fortified with PFOS.

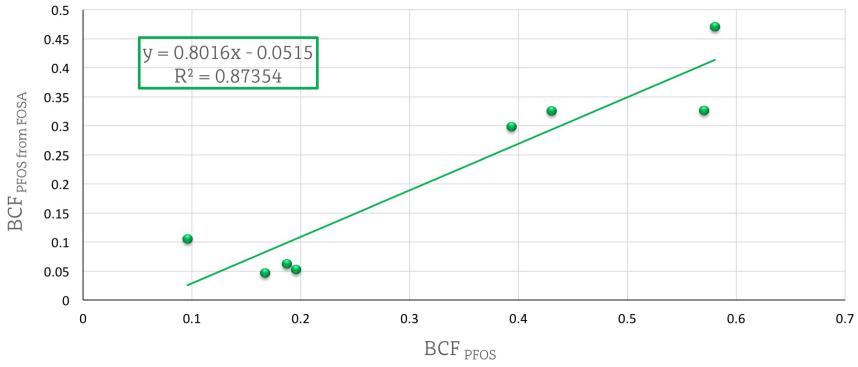

Figure 8.3. BCF_{Peel} values for PFOS from amended soil fortified with FOSA (PFOS*) against BCF_{Peel} values for PFOS from amended soil fortified with PFOS.

8.3.2 Uptake by lettuce

The average concentration (in ng/g) of the target analytes measured in the different lettuce compartments and the amended soil in both cases, before ($C_{soil,\ t=0}$) and after (C_{soil}) the harvesting period, the relative standard deviations (in brackets), as well as the average BCF_{Total} and their relative standard deviations (in brackets and calculated by error propagation) of each experimental set are given in **Table 8.3.**

In the case of PFOA, the amended soil concentration values (see **Table 8.3**) before ($C_{soil,\ t=0}$) and after (C_{soil}) the harvesting period were similar, indicative of a lack of degradation or drainage through watering. The concentrations of PFOA in the heart and the leaves were from two to four times higher than in the amended soil and the reproducibility between the two pots was good. Concentrations measured in the leaves and the heart were not at the same level, the heart compartment (BCF_{Heart}= 4.06-4.46) tended to accumulate twice the leaves (BCF_{Leaves}=1.43-2.07). Values at the same level were obtained by Blaine et al. [12] when lettuce was cultivated in an industrially impacted and in a municipal soil.

Table 8.3. Average (n=3) concentration (in ng/g) and the relative standard deviations (in brackets) for the lettuce (***Batavia Golden Spring*** variety) leaves and heart and BCF_{Total} values.

Soil	Pots	$C_{t=0\ soil}$ (ng/g)			C_{Soil} (ng/g)			C_{Heart} (ng/g)			C_{Leaves} (ng/g)			BCF_{Total}		
		PFOA	**PFOS**	**FOSA**	**PFOA**	**PFOS**	**FOSA**	**PFOA**	**PFOS**	**FOSA**	**PFOA**	**PFOS**	**FOSA**	**PFOA**	**PFOS**	**FOSA**
Soil 2.4	Pot 1	[a]	[a]	[a]	< MDL	[a]	[a]	< MDL	[a]	[a]	< MDL	[a]	[a]	[b]	[b]	[b]
Soil 2.4	Pot 2	540 (10)	[a]	[a]	557 (8)	[a]	[a]	2264 (2)	[a]	[a]	1151 (6)	[a]	[a]	3.19 (7)	[b]	[b]
Soil 2.4	Pot 3	580 (15)	[a]	[a]	633 (17)	[a]	[a]	2827 (7)	[a]	[a]	907 (6)	[a]	[a]	2.45 (13)	[b]	[b]
Soil 2.4	Pot 1	[a]	[a]	[a]	[a]	< MDL	[a]	[a]	< MDL	[a]	[a]	< MDL	[a]	[b]	[b]	[b]
Soil 2.4	Pot 2	[a]	505 (12)	[a]	[a]	481 (6)	[a]	[a]	1588 (16)	[a]	[a]	53 (13)	[a]	[b]	0.60 (15)	[b]
Soil 2.4	Pot 3	[a]	510 (13)	[a]	[a]	555 (12)	[a]	[a]	1780 (1)	[a]	[a]	101 (20)	[a]	[b]	0.65 (7)	[b]
Soil 2.4	Pot 1	[a]	[a]	[a]	[a]	< MDL	< MDL	[a]	< MDL	< MDL	[a]	< MDL	< MDL	[b]	[b]	[b]
Soil 2.4	Pot 2	[a]	[a]	520 (8)	[a]	113 (23)	530 (20)	[a]	48 (11)	404 (7)	[a]	< MDL	< MDL	[b]	0.047 (25)	0.083 (21)
Soil 2.4	Pot 3	[a]	[a]	496 (9)	[a]	79 (14)	354 (14)	[a]	42 (19)	342 (14)	[a]	< MDL	< MDL	[b]	0.060 (23)	0.108 (20)
Subs	Pot 1	[a]	[a]	[a]	[a]	< MDL	< MDL	[a]	< MDL	< MDL	[a]	< MDL	< MDL	[b]	[b]	[b]
Sub	Pot 2	[a]	[a]	500 (14)	[a]	480 (17)	89 (10)	[a]	87 (6)	122 (4)	[a]	< MDL	< MDL	[b]	0.034 (19)	0.172 (11)
Subs	Pot 3	[a]	[a]	510 (16)	[a]	477 (6)	134 (2)	[a]	75 (19)	101 (18)	[a]	< MDL	< MDL	[b]	0.023 (20)	0.109 (18)

Subs: substrate. [a] not detected, [b] not calculated since C_{Heart} or C_{Leaves} were not detected or were lower than MDLs.

Regarding PFOS, no significant differences in the amended soil concentration were observed before ($C_{soil, t=0}$) and after (C_{soil}) harvesting (see **Table 8.3**). The reproducibility between the two pots was also satisfactory and, while concentrations in the heart of the lettuce were three times the concentrations in the amended soil, the concentrations in the leaves were negligible (3-5 % of the concentration measured in the heart). Therefore, while BCF_{Heart} were in the 3.03-3.21 range, BCF_{Leaves} were lower than 0.2. As in the case of PFOA, similar BCF_{Leaves} were also obtained for PFOS (municipal soil) by Blaine et al. [12].

In the case of FOSA, cultivation was carried out in both soil 2.4 and substrate. Soil-type dependent partial degradation of FOSA to PFOS was observed (see **Table 8.3** and **Figure 8.4**) and the higher the TOC the higher the FOSA degradation was observed. Concentrations of FOSA and PFOS in the leaf compartment were lower than the MDL values of the method and, therefore, BCF_{Heart} and BCF_{Total} were only calculated. In the case of FOSA, according to one-way ANOVA, no influence of the soil type in the accumulation was observed ($F_{Experimental}=1.34 < F_{Critical}=5.99$) with BCF_{Heart} in the range of 0.75-1.37. However, in the case of PFOS from FOSA degradation, according to one-way ANOVA the soil influence on the accumulation was observed ($F_{Experimental}=16.67 > F_{Critical}=5.99$), with higher accumulation the lower the TOC value, similar to the carrot results obtained in the present work and the literature [11, 29-31].

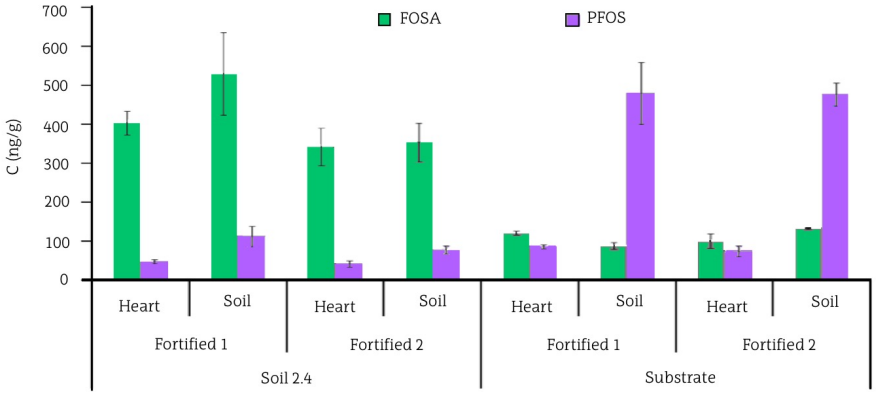

Figure 8.4. Average (n=3) concentrations (ng/g) observed for PFOS and FOSA in the compost-amended soils fortified with FOSA and in the lettuces hearts grown in the amended soil.

While in the experiments for carrot similar BCF values were obtained for PFOA and PFOS, differences were observed for lettuce. The different behaviour observed for PFOA, PFOS and FOSA in directly fortified experiments could be attributed to their different hydrophilic head and

different water solubility (11000, 7500 and 2.90 10^{-5} mg/L**,** for PFOA, PFOS and FOSA, respectively) [35, 36], as well as their soil to water partitioning coefficient (log K_{OC}) values (2.4, 3.7 and 4.1 cm^3/g for PFOA, PFOS and FOSA, respectively) [37]. If translocation was due to a passive movement in the transpiration stream of the plant, one might expect that pollutants with greater water solubility would be more easily transported into above ground tissues; this behaviour was observed for BCF_{Leaves} and BCF_{Heart} of the three target analytes with PFOA › PFOS › FOSA. Blaine et al. [17] observed the same tendency for different acidic PFASs. However, higher BCF_{Root} for PFOS than for PFOA was obtained by Felizeter et al. [13] when uptake of acidic PFASs by hydroponically grown lettuce was performed. Trapp et al. [38] speculate that plants growing in soil outdoors would have a different translocation factor from roots into stems and leaves, than plants growing in hydroponic solutions.

8.3.3 FOSA degradation

FOSA was totally degraded in the presence of carrot; however, in the presence of the lettuce, the degradation was partial and dependent on the TOC value of the soil. The higher the TOC value the higher FOSA degradation observed (see **Figure 8.4**). The differences in the degradation level could be attributed to the crop type or to the cultivation period. Therefore, FOSA degradation experiments (108 days) without the presence of the crop were performed and no degradation was observed. Comparable FOSA concentrations were obtained according to ANOVA for soil 2.4 and substrate ($F_{Experimental}$ = 4.52 ‹ $F_{Critical}$= 4.75) during the degradation experiment. Consequently, it could be concluded that degradation of FOSA occurred due to the presence of the crops. The same tendency was observed by Günter et al. [39] for the enhanced degradation of aliphatic hydrocarbons during ryegrass growth and by White et al. [40] who reported significant decreases in concentration of p,p'-dichlorodiphenyldichloroethylene (p,p'-DDT) in the presence of pumpkin, zucchini and spinach, either in the rhizosphere or near root, compared to the concentrations in the bulk soil.

8.3.4 Accumulation in the polymeric materials

Although PFOA, PFOS and FOSA were detected in all the three polymeric materials (PES, POM and SR) tested in the carrot experiments, repeatability for SR was very poor (RSDs › 100 %), while for PES and POM acceptable RSD values (‹ 30 %) were obtained.

Good correlation between BCF in the polymeric material ($BCF_{Polymer}$, PES and POM) and $BCF_{Peel,,}$ as well as BCF_{Core}, was observed but not for BCF_{Leaves}. The equations and the determination

coefficients (r^2) obtained when the BCF_{Peel} was compared to the $BCF_{Polymer}$ inserted in the pots are included in **Table 8.4**. The accumulation in PES was higher and more highly correlated to the accumulation in the peel of the carrot than for the POM material. From the slopes of the equations obtained (see **Figure 8.5**), it could be concluded that the accumulation on the PES was similar for PFOA and PFOS (both from the PFOS and FOSA experiments). This fact was consistent with the BCF_{Peel} values obtained for both PFOS (both coming from the degradation of FOSA or not) and PFOA in carrot, which were similar. However, the highest correlation was obtained for the experiments where the amended soil had been fortified with PFOS. It could be concluded that the results obtained for PES and PFAS accumulation in carrots are promising and could help in the simulation of the uptake of contaminants by crops.

Table 8.4. Determination coefficients (r^2) for the correlation between carrot BCF_{Peel} and polymeric materials BCF (PES and POM) for PFOA, PFOS and PFOS* (PFOS from FOSA degradation). The slope and the intercept of the linear equations and the standard deviations (in brackets) are also given.

Analyte	**Polymeric material**	**r^2**	**slope**	**b_0**
PFOA	PES	0.63	59.4 (18.4)	5.1 (7.6)
PFOA	POM	0.25	0.61 (0.43)	-0.036 (0.18)
PFOS	PES	0.85	53.8 (9.3)	16.3 (3.5)
PFOS	POM	0.87	1.48 (0.24)	-0.12 (0.09)
PFOS from FOSA degradation	PES	0.63	57.3 (18.0)	4.5 (4.7)
PFOS from FOSA degradation	POM	0.42	0.18 (0.09)	0.073 (0.023)

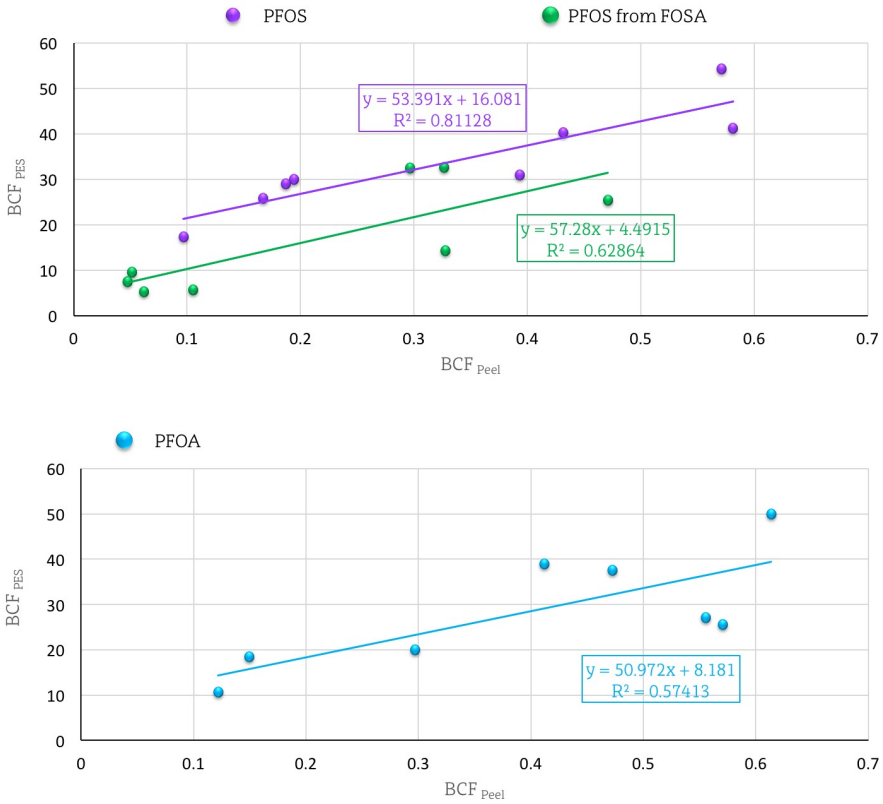

Figure 8.5. BCF_{PES} values for PFOS, PFOS from FOSA degradation and PFOA against their BCF_{Peel} values.

8.4 Conclusions

While some PFAS crop accumulation studies are available in the literature, this study provides the first evidence of biotransformation of FOSA and subsequent plant uptake of this chemical and its metabolite (PFOS) in a soil plant environment. Biotransformation of FOSA to PFOS only occurs in the presence of a crop and is dependent either on the crop type or on the cultivation period.

From this study it is clear that PFAS transport in the water phase of xylem or phloem sap is possible. The results presented demonstrate and conclude that uptake, translocation and storage of PFOA, PFOS and FOSA appear to be highly dependent on the crop type, as well as on the soil characteristics used as cultivation media. While similar accumulation of PFOA and PFOS occurs in carrot, higher accumulation occurs for PFOA in lettuce, probably related to its higher

water solubility.

The results obtained for the PES polymer and carrot uptake are promising since the polymeric material could be used as a first approach to estimate the accumulation potential of the target analytes. To the best of our knowledge, this is the first time that polymeric materials are used for the simulation of the uptake of PFASs in crops such as carrots.

Summing up, it can be said that under the tested environmental conditions, target compounds such as PFOA, PFOS and FOSA can be taken up by plants from soil and, therefore, if plants are grown in soils contaminated with PFOA, PFOS or FOSA, these can enter the food chain turning into a significant public health risk.

8.5 References

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CHAPTER 9

Uptake of 8:2 perfluoroalkyl phosphate diester and its degradation products by carrot and lettuce grown in compost-amended soils

Chemosphere (Under review)

9.1 Introduction

During the last decades perfluoroalkyl acids (PFAAs) have been detected in different environmental compartments including water [1], sewage sludge [2], food [3] and air [4]. These chemicals present a wide range of applications in consumer products due to their biological and chemical stability, as well as their water and grease repellence and surface tension lowering properties. In this sense, PFAAs are commonly used in non-stick cookware, breathable membranes for clothing, stain-resistant carpets and fabrics, components of fire fighting foams, surfactants, shampoos, paints or inks, among others. The high concentrations of PFAAs often reported in effluents from wastewater treatment plants (WWTPs) [5, 6] and their capacity to accumulate in the sludge [7, 8] suggest that these matrices can contribute to their presence in the environment. However, the source and origin of PFAAs found in the environment are still not well known. Apart from the direct release of PFAAs from industrial emissions and commercial products, indirect sources, such as the transformation of precursor compounds, including fluorotelomer alcohols (FTOHs), perfluorinated alkyl sulfonamides or polyfluoroalkyl phosphates (PAPs), through different reactions (i.e., atmospheric oxidation, metabolisation or hydrolysis) can lead to the formation of PFAAs [9, 10].

As mentioned before, PAPs are one of the families of compounds considered as PFAA precursors. They belong to a group of hydrophobic phosphates attached to partially fluorinated alkyl chains and are commercially produced as a mixture of several polyfluorinated chain lengths (i.e., 4:2, 6:2, 8:2 and 10:2) and can have one (monoPAP), two (diPAP) or three (triPAP) polyfluorinated tails. In recent years, PAPs were primarily applied in food contact paper industries to replace the previously phased out levelling and wetting agent such as perfluorooctane sulfonate acid (PFOS) [11]. In the case of diPAPs, they have been reported to be present in matrices such as household dust [12], human serum [13, 14], drinking water [15] or even sewage sludge [14, 16].

Several works in the literature have reported concentrations for different PAPs. While Liu and co-workers [16] found different 8:2 monoPAP (1H, 1H, 2H, 2H-perfluorodecylphosphate) and 10:2 monoPAP (1H, 1H, 2H, 2H-perfluorododecylphosphate) at the low ng/g presence but did not determine any diPAPs, Deon and co-workers [14] found mainly 6:2 diPAP (bis(1H, 1H, 2H, 2H-perfluorooctyl)phosphate) and 8:2 diPAP (bis(1H, 1H, 2H, 2H-perfluorodecyl)phosphate) at the ng/g levels. Loi et al. [17] obtained similar results in sewage sludge samples from Hong Kong

where 6:2 diPAP and 8:2 diPAP were present at concentrations as high as PFOS. Moreover, recent studies reported in the literature have shown that PAPs could be bio-transformed into PFAAs in sludge [18] and in biota such as rat [19] and rainbow trout [20]. In the experiments performed with rats, elevated levels of perfluorooctanoic acid (PFOA) were measured in blood and 7:3 FTCA (3-perfluoroheptyl propanoic acid), 8:2 FTCA (2-perfluorooctyl ethanoic acid) as well as 8:2 FTUCA (2H-perfluoro-2-decenoic acid) were also detected. Besides, Yoo et al. [21] determined that PFOA was the major homologue followed by perfluorodecanoic acid (PFDA) when the determination of FTOHs and perfluoro alkyl substances (PFASs) in plants from biosolid-amended field, was performed. However, when the experiments were carried out in presence of rainbow trout, 8:2 FTCA, 10:2 FTCA (2-Perfluorodecyl ethanoic acid), 8:2 FTUCA and 10:2 FTUCA (2H-Perfluoro-2-dodecenoic acid) were the major products, while small amounts of PFOA and PFDA were also detected.

Sludge or sludge derived compost are used as soil fertilisers in agriculture since their application improves soil properties, such as the water capacity and the texture, and supplies nutrients. However, concerns about this practice continue rising mainly because biosolids contain a broad range of toxic organic and inorganic chemicals, as well as pathogens [22]. Therefore, the use of biosolids as fertilisers may present an exposure pathway of contaminants since translocation through the plant and accumulation in the edible part of vegetables can lead transfer to the food chain. Therefore, uptake experiments to evaluate the risk of PFAAs due to the degradability of precursors such as PAPs, FTOHs and saturated (FTCAs) and unsaturated (FTUCAs) fluorotelomer carboxylates, among others, have gained scientific attention.

In the last years, several works have been carried out to determine FTOHs and their degradation products in biosolid-amended soils and plants. Yoo et al. [21] reported a quantitative determination of PFAAs and FTOHs in grass plants from biosolid-amended fields. In the previously mentioned work, most PFAAs were detected quantitatively in the grass plants cultivated in soil that received multiple sludge applications. However, FTOHs were quantifiable in a few grass plant samples and at very low concentration comparing with PFAAs. Moreover, it was observed that the shortest chain PFAAs, the highest grass accumulation factor. Zhang et al. [23] also found FTOHs and their possible degradation products, including PFAAs (C4-C9), FTCAs and FTUCAs in soils and plants. Besides, the concentrations of some intermediate degradation products in plants were higher than in soil. This implied that not only soil microbes, but also plant may have the ability to degrade FTOHs. The same conclusion was achieved by Lee et al. [24] when the decline of 6:2diPAP and its metabolites upon soil amendment with biosolids that

had been sown with ***Medicago truncatula*** plants was evaluated. In this case, it was reported that the decline of 6:2diPAP could be due to both, plant uptake and biotransformation contribution over time. The latter one was further evidenced by the degradation of 6:2 diPAP to its corresponding FTOH intermediates and PFAAs.

The use of passive sampling devices could be an easy approach to determine the bioavailability of organic pollutants and its degradation products. Passive sampling is based on the ability of the chemical to bond to the surface of a passive sampler (an adsorbent). In the past, passive sampling has been mostly applied to the analysis of water and air [25] samples, but nowadays the application for soil analysis is gaining attention [26]. For that purpose, different devices have been used, including semipermeable membrane devices [27] and different polymers such as polyoxymethylene (POM) [28], polyethylene (PE) [29] and polyethersulfone (PES) [30], among others.

Within this context, the main objective of the present work was to investigate the uptake and distribution of 8:2 diPAP and its degradation products by carrot (***Daucus carota ssp sativus, Chantenay*** variety) and lettuce (***Lactuca sativa, Golden Spring*** variety) from two different compost-amended soils. Both crops were selected to see the differences between a root vegetable, where the fruit is underground in direct contact with the polluted soil, and a leaf vegetable, where the edible part is not in direct contact with the polluted soil. The use of different sorbent materials to determine the concentration availability of the target analytes was also evaluated.

9.2 Experimental section

9.2.1 Chemicals, reagents and laboratory material

The solid reagent 8:2 diPAP and potassium perfluoro-1-butanesulfonate (L-PFBS), sodium perfluoro-1-hexanesulfonate (L-PFHxS), L-PFOS, perfluoro-n-butanoic acid (PFBA), perfluoro-n-pentanoic acid (PFPeA), perfluoro-n-hexanoic acid (PFHxA), perfluoro-n-heptanoic acid (PFHpA), PFOA, perfluoro-n-nonanoic acid (PFNA), PFDA, sodium 1H, 1H, 2H, 2H-perfluorooctyl phosphate (6:2 mono-PAP), 8:2 mono-PAP, 6:2 di-PAP, 2-perfluorohexyl ethanoic acid (6:2 FTCA), 8:2 FTCA, 2H-perfluoro-2-octanoic acid (6:2 FTUCA), 8:2 FTUCA, 7:3 FTCA, 3-perfluoropentyl propanoic acid (5:3 FTCA), the surrogate mixture sodium perfluoro-1-hexane [$^{18}O_2$] sulfonate (MPFHxS), sodium perfluoro-1-[1,2,3,4-$^{13}C_4$] octanesulfonate (MPFOS), perfluoro-

n-[$^{13}C_4$] butanoic acid (MPFBA), perfluoro-n-[1,2-$^{13}C_2$] hexanoic acid (MPFHxA), perfluoro-n-[1,2,3,4-$^{13}C_4$] octanoic acid (MPFOA), perfluoro-n-[1,2,3,4,5-$^{13}C_5$] nonanoic acid (MPFNA), perfluoro-n-[1,2-$^{13}C_2$] decanoic acid (MPFDA), perfluoro-n-[1,2-$^{13}C_2$] undecanoic acid (MPFUdA) and perfluoro-n-[1,2-$^{13}C_2$] dodecanoic acid (MPFDoA), sodium bis (1H, 1H, 2H, 2H-[1,2-$^{13}C_2$] perfluorodecyl) phosphate (M4-8:2di-PAP), 2H-perfluoro-[1,2-$^{13}C_2$]-2-decenoic acid (M8:2 FTUCA), 2-perfluorohexyl-[1,2-$^{13}C_2$]-ethanoic acid (M6:2 FTCA), 2-perfluorooctyl-[1,2-$^{13}C_2$]-ethanoic acid (M8:2 FTCA) and 2-perfluorodecyl-[1,2-$^{13}C_2$]-ethanoic acid (M10:2 FTCA) solutions were purchased from Wellington Laboratories (Ontario, Canada). The purity of all the target analytes was higher than 98 %.

In the case of vegetables, a Cryodos-50 laboratory freeze-dryer from Telstar Instrument (Sant Cugat del Valles, Barcelona, Spain) was used to freeze-dry the samples. For extraction, 50-mL polypropylene conical tubes (PP, internal diameter 27.2 mm x 117.5 mm length) obtained from Deltalab (Barcelona, Spain) and a Bandelin ultrasonic homogeniser (20 kHz; Bandelin Electronic, Berlin, Germany) equipped with a 3-mm titanium microtip were used. After the extraction step, the supernatant was filtered through polyamide filters (0.45 μm, 25 mm, Macherey-Nagel, Germany). Evolute-WAX (primary/secondary amine modified polystyrene-divinylbenzene incorporating non-ionisable hydroxyl groups, 200 mg) solid-phase extraction (SPE) cartridges were purchased from Biotage (Uppsala, Sweden). Methanol (MeOH, HPLC grade, 99.9 %) was supplied by LabScan (Dublin, Ireland) and acetonitrile (ACN, HPLC grade, 99.9 %) from Sigma Aldrich (Steinheim, Germany). Ultra-pure water was obtained using a Milli-Q water purification system (‹ 0.05 μS/cm, Milli-Q model 185, Millipore, Bedford, MA, USA). GHP (hydrophilic polypropylene) microfilters (0.2 μm, 13 mm, Pall, USA) were used to filter extracts before liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.

For the mobile phase composition, MeOH from Fisher Scientific (Loughborough, UK) was used. Ammonium acetate (NH_4OAc, ≥ 99 %) was purchased from Sigma Aldrich and 1-methylpyperidine (1-MP, purity › 98 %) was provided by Merck Schuchardt OHG (Hohenbrunn, Germany). For chromatographic separation of PFASs and PAPs, a sure-Guard in-line filter (24.4 mm, 10 mm, 0.5 μm) obtained from VICI Jour (Schenkon, Switzerland) followed by an ACE UltraCore 2.5 SuperC_{18} core-shell (2.1 mm x 50 mm, 2.5 μm) column purchased from Advanced Chromatography Technologies (Aberdeen, Scotland) were used.

The compost was acquired from Calahorra WWTP (Spain) and soil 2.4 was supplied by LUFA Speyer (Speyer, Germany). The universal substrate and the vegetables, lettuce (***Lactuca sativa, Batavia Golden Spring*** variety) and carrot (***Daucus carota ssp. sativus***, ***Chantenay***

variety) seeds were obtained from a commercial agricultural house (www.mvgarden.com/). The properties of the different soils and compost are summarised in **Table 9.1**.

Table 9.1. Characterisation according to the physicochemical parameters of the different soils. Soil 2.4 characterised by LUFA Speyer (Speyer, Germany) and substrate and compost by Neiker Tecnalia (Bizkaia, Spain).

Parameter	Soil 2.4	Universal substrate	Compost
TOC %	2.3±0.5	53±9	55±8
N %	0.20±0.04	0.35±0.05	2.4±0.3
pH	7.2±0.2	5.7±0.2	7.9±1.5
Cation Exchange Capacity (meq/100 g)	31±5	48±4	50±9
Particles ‹ 0.002mm	26±2	4.5±0.5	10±1
0.002-0.05mm	41±1	27±3	40±4
0.05-2mm	34±2	53±6	42±3
› 2mm	-[a]	16±2	8.9±1.5
Soil type	Sandy loam	Sandy loam	Loam
Water Holding Capacity (g/100 g)	44±1	-[b]	-[b]
Water per Volume (g/1000 ml)	1288±36	-[b]	-[b]

[a] no particles › 2mm, [b] not determined

All the reagents used for the Hoagland nutritive solution preparation, potassium nitrate (KNO_3, 99.0 %), calcium nitrate tetrahydrate ($Ca(NO_3)_2$ $4H_2O$, 98.0 %), ammonium phosphate monobasic ($(NH_4)H_2PO_4$, 96.0-102.0 %), magnesium sulphate heptahydrate ($MgSO_4$ 7H2O, 99.0-100.5 %), manganese chloride tetrahydrate ($MnCl_2$ $4H_2O$, 98.0-102.0 %), boric acid (H_3BO_3, 99.8 %), zinc sulphate heptahydrate ($ZnSO_4$ $7H_2O$, 99.0-104.0 %), copper sulphate pentahydrate ($CuSO_4$ $5H_2O$, 99.0-100.0 %) and sodium molybdate dehydrate (Na_2MoO_4 $2H_2O$, 98.0-100.0 %) were purchased from Panreac (Castellar del Vallès, Spain). The Hoagland solution was prepared monthly according to Epstein and Bloom's work [31]. Briefly, an appropriate amount of the different salts was weighed, followed by pH adjustment at 5.5 with NaOH (reagent grade, ≥ 98 %, Panreac) and/or HCl (ACS reagent, 37 %, Panreac) in order to prepare 25 L.

PES sorbent was acquired from Membrane (Wuppertal, Germany) in a tubular format

(0.7 mm external diameter, 1.43 g/mL density). The commercial silicone elastomer in flexible rod form (SR, 0.97 g/mL density) was purchased from Goodfellow (PA, USA). POM, 1.41 g/mL density film, was supplied by CS Hyde Company (Illinois, USA). 1.5 mL Eppendorf tubes were obtained from Eppendorf (Hamburg, Germany).

9.2.2 Polymeric material conditioning

Pieces of the polymers were cut using a sharp blade and accurately weighed; 30 mg (three pieces of 1.5 cm each one) for PES, 50 mg (one piece of 2 × 2.5 cm) for POM and 40 mg (one piece of 0.2 cm) for SR. PES was soaked twice for 15 min and conditioned for 24 h in MeOH (HPLC grade, 99.9 %). The same procedure was performed for POM but using ethyl acetate (HPLC grade, 99.9 %) as solvent. SR pieces were soaked twice for 15 min with MeOH and conditioned afterwards in a thermal condition unit at 120 °C for 3 h under a nitrogen stream (›99.999% of purity) supplied by Messer (Tarragona, Spain).

9.2.3 8:2 diPAP degradation experiments

Compost was fortified with 8:2 diPAP as described in **section 9.2.4** in order to achieve a 500 ng/g concentration in the soil: compost mixture (95:5). The degradation experiments were performed in two different amended soils (soil 2.4 and substrate) without crop cultivation. 8 different small pots containing 100 g of the (95:5) soil: compost mixture were placed under the following controlled environmental conditions: temperature was set to 25 °C during the day and at 18 °C during the night with a 14-h day length and a relative humidity of 50 % during the day and 60 % overnight. After that, samples were collected in 8 time periods (one pot per period) from 3 h to 108 days period of time. All the pots were analysed in triplicate.

9.2.4 Compost fortification and plant cultivation

A known amount of compost was weighed, covered with acetone, fortified with the corresponding analyte and stirred for 24 h. After that, the mixture was placed under a fume hood for solvent evaporation. Then, the compost was thoroughly manually mixed with the soil at a 95:5 soil: compost ratio and aged for one week.

Carrot and lettuce seeds were sonicated with Milli-Q water previous to the germination step. Petri dishes were covered with moistened filter paper and the seeds were evenly distributed in the Petri dish for germination (12-14 days). After germination, the carrot seedlings (approx. 6) were transferred to the 2-kg pots while lettuce seedlings were transferred to 350-g

pots containing a (95:5) soil: compost mixture. The plants were placed under controlled environmental conditions (see **section 9.2.3**), regularly watered with distilled water and Hoagland nutritive solution. Plants were harvested during a period of approx. 1 month for lettuce and approx. 3 months for carrot. Two pot replicates were used for the fortified samples, while a single pot was used for the control (unfortified) samples, which were randomly distributed among the fortified samples. After harvesting, the carrots and lettuces were carefully washed with deionised water. Lettuces were separated in the leaves (the edible part) and the heart (the part that it is in direct contact with the soil). Carrot samples were separated into root peel, root core and leaf compartments. Root peel was obtained after peeling with a vegetable peeler (depth of ~2 mm). Vegetables (carrot and lettuce) were freeze-dried (-50 °C) and soil samples air-dried before treatment. All the samples were stored at -20 °C before the extraction. The different compartments of carrots and lettuces from the same pot were pooled together and analysed in triplicate.

Previously conditioned pieces of the polymers (PES, POM and SR) were also placed in the two pots where carrots were cultivated.

9.2.5 Sample treatment and analysis

9.2.5.1 Vegetables and amended soils

Focused ultrasound solid-liquid extraction (FUSLE) of the vegetables and amended soils followed by SPE clean-up and LC-MS/MS analysis were performed according to a previously published work [32]. Briefly, 0.5 g of samples (carrot, lettuce and compost-amended soil) were weighed and extracted in 7 mL of ACN:Milli-Q (9:1). The supernatant was filtered through 0.45 µm polyamide filters, evaporated down to approx. 1 mL under a nitrogen (99.999 %) stream, dissolved in 6 mL of water and submitted to SPE clean-up step using mix mode Evolute WAX cartridge.

9.2.5.2 Polymeric materials

After crop harvesting, the polymers were removed from each pot, rinsed with Milli-Q water in order to remove residues, dried with a clean paper tissue, placed inside 1.5-mL Eppendorf tubes and sonicated for 2 x 15 min in an ultrasound bath (USB Axtor by Lovango, Barcelona, Spain) using 500 µL of MeOH. Finally, the extracts were filtered through a 0.2-µm GHP filter before LC-MS/MS analysis described in a previously published work [32].

9.2.6 Quality control

Apparent recoveries (%) as well as method detection limits (MDLs) for each analyte in all the studied matrices are included in **Table 9.2**. The MDLs were determined by fortification of five replicates of each blank matrix with each analyte at a low concentration (20 ng/g), according to the USEPA (http://www.epa.gov/waterscience/methods/det/rad.pdf). Then, the MDL was worked out as MDL = t ($_{n-1,1-\alpha=0.95}$) × sd, where t=2.13 corresponds to the Student's t-value for a 95 % confidence level and four degrees of freedom and sd refers to the standard deviation of the replicate analyses (n=5). During the sample treatment, procedural blanks and control samples (samples fortified at a known concentration) were analysed every 15 samples. While values lower than MDLs were obtained in the case of blanks, apparent recoveries of the control samples were in good agreement with the values obtained in the previous method validation [32].

Table 9.2. Apparent recoveries (%) and method detection limits (MDLs) for each analyte in all the studied matrices.

		Compost amended soil		Carrot		Lettuce	
Analyte	**Surrogate**	**Apparent recovery (%)**	**MDLs (ng/g)**	**Apparent recovery (%)**	**MDLs (ng/g)**	**Apparent recovery (%)**	**MDLs (ng/g)**
6:2 diPAP	[b]	90 (5)	1	100 (12)	1.4	132 (8)	3
8:2 diPAP	[b]	73 (19)	1	92 (6)	3.2	105 (13)	3
6:2 monoPAP	M4-8:2 diPAP	80 (20)	1	110 (14)[a]	1.1	70 (4)[a]	2
8:2 monoPAP	M4-8:2 diPAP	75 (10)	1	86 (11)[a]	1.0	68 (7)[a]	1
6:2 FTCA	M6:2-FTCA	75 (1)	1	72 (14)	0.5	101 (27)	4
FHpPA	[b]	83 (18)	0.1	46 (8)	0.4	93 (25)	3
6:2 FTUCA	M6:2-FTCA	115 (15)[a]	2	100 (30)[a]	0.3	97 (28)	4
FPePA	[b]	116 (8)	0.1	42 (9)	0.5	93 (25)	2
8:2 FTUCA	[b]	98 (5)	1	86 (13)	0.3	104 (17)	3
8:2 FTCA	[b]	75 (5)	0.4	81 (14)	0.3	105 (20)	3

[a] Internal calibration. [b] Calculated using matrix-matched calibration approach.

9.3 Results and discussion

9.3.1 Degradation of 8:2 diPAP

The main degradation pathway of 8:2 diPAP reported in the literature [33-36] is likely to be microbial hydrolysis of phosphate ester bonds to produce 8:2 monoPAP followed by FTOH formation, which may further oxidise to produce the perfluorocarboxylic acids (PFCAs).

In the present work, the stability of 8:2 diPAP was studied for two amended soil types (soil 2.4 and substrate) in the absence of the crop and before the plant uptake experiments. The two soils were chosen due to their differences in the TOC content. Substrate, which is often used in crop and plant cultivation, has a higher TOC (see **Table 9.1**). The two amended soils were watered during the degradation study period in order to simulate the same conditions used for the uptake experiments, being the absence of the crop the only difference.

Figure 9.1 shows the concentration profiles for 8:2 di PAP and some of the different degradation products monitored in the case of 2.4 soil type. As it can be clearly observed from **Figure 9.1 a**, depletion of 8:2 diPAP occurred in the first 6 h and then the concentration remained almost constant. Actually, it could be concluded that degradation had started either during the fortification or aging period of the compost since, on the one hand, the concentration at zero time was much lower than the nominal concentration (500 ng/g) and some of the typical degradation products of 8:2 diPAP, such as 8:2 monoPAP (**Figure 9.1 b**) and 8:2 FTCA (Figure 9.1 **c**) were observed from the beginning (t=0 h). Both, 8:2 monoPAP and 8:2 FTCA could be considered as transition metabolites since their concentrations decreased during the exposition period, while the concentrations of 8:2 FTUCA (**Figure 9.1 c**), 7:3 FTCA (**Figure 9.1 c**), PFHxA (**Figure 9.1 d**), PFHpA (**Figure 9.1 d**) and PFOA (**Figure 9.1 d**) increased. The major degradation product was PFOA. Similar degradation products were observed for the biodegradation of 8:2 FTOH (2-perfluorooctyl ethanol) in activated sludge [29] and in soil [27], although in the latter one, determined metabolites such as 8:2 fluorotelomer aldehyde (8:2 FTAL), 7:3 fluorotelomer unsaturared carboxylate (7:3 FTUCA) and 3-hydroxy-7:3 saturated fluorotelomer carboxylate (3-OH-7:3-FTCA) and also novel metabolites previously not identified in any environmental samples as 7:2 secondary fluorotelomer alcohol (7:2 sFTOH), 7:2 fluorotelomer ketone (7:2 FT ketone) and 2H-perfluorooctanoate (2H-PFOA) were determined.

Taking into account the results of the present work and the ones obtained by Wang et al. [33], it could be proposed that 8:2 diPAP degraded to 8:2 monoPAP, that further depleted to 8:2 FTOH (not monitored in the present work) and then the degradation products of 8:2 FTOH were observed. A possible biodegradation pathway for 8:2 diPAP in compost-amended soils (see **Figure 9.2**) is proposed taking into account both the pathway proposed by Wang et al. [33] and the analytes determined in the present work. Compounds inside boxes are the metabolites determined in the present work. It should be highlighted, however, that degradation products (ie. 8:2 FTAL, 7:3 FTUCA, 3-OH-7:3-FTCA, 7:2 sFTOH, 7:2 FT ketone and 2H-PFOA) in addition to non extractable degradation products covalently bound to matrix components) could be

present but were not monitored in the present work due to the lack of standards in our laboratory.

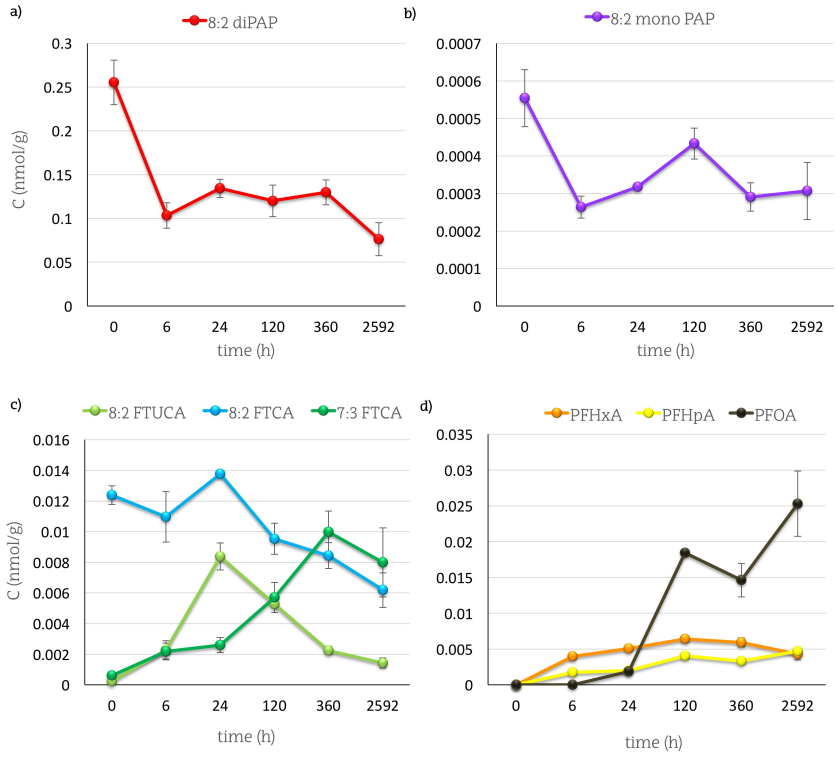

Figure 9.1. Concentration profiles for 8:2 diPAP and its metabolites (8:2 monoPAP, 8:2 FTUCA, 8:2 FTCA, 7:3 FTCA, PFOA, PFHpA and PFHxA) monitored in compost-amended soil 2.4.

Similar results in terms of the degradation products generated and the trends observed were obtained for the substrate. However, and probably due to the higher TOC value of the substrate, the final concentration of 8:2 diPAP was lower (156 ng/g), while the PFOA final concentration was higher (30 ng/g).

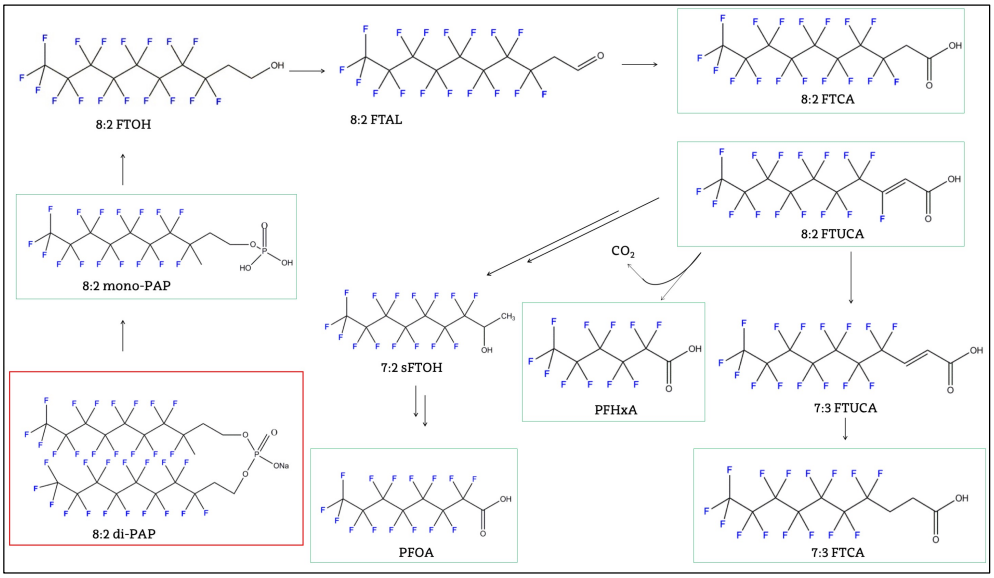

Figure 9.2. Potential biodegradation pathway of 8:2 diPAP considering both the degradation pathway reported by Wang et al. [33] and the analytes determined in the present work in the compost-amended soils.

9.3.2 Uptake by carrot of 8:2 diPAP and its degradation products

The average (n=3) concentration measured in the amended soil and in the three carrot compartments (peel, core and leaves), as well as the total bioconcentration factors (BCF_{Total}) and their corresponding relative standard deviations (RSDs %) were reported in **Table 9.3** for each experimental set. BCF_{Total} was calculated as shown in **Equation 9.1**, where the bioconcentration factors in peel (BCF_{Peel}), core (BCF_{Core}) and leaves (BCF_{Leaves}) were calculated as the concentration ratio between the concentration found in the plant tissue and the concentration in the amended soil. m_{Peel}, m_{Core} and m_{Leaves} are the mass of the peel, core and leaf compartments, respectively, and m_{Plant} the total mass of the carrot.

$$BCF_{Total} = \frac{1}{m_{Plant}} (BCF_{Peel}\, m_{Peel} + BCF_{Core}\, m_{Core} + BCF_{Leaves}\, m_{Leaves}) \quad \textit{Equation 9. 1}$$

Concentrations in the carrot compartments (peel, core and leaves) grown in unfortified amended soils were lower than the MDLs.

In the case of the fortified samples, it should be underlined that the concentration in the (95:5) soil:compost mixture was determined at the time that plants were harvested. This was decided because when degradation experiments were performed, it was observed that 8:2 diPAP concentration in soil after approx. 6 h remained constant.

Table 9.3. Average (n=3) concentration (in ng/g) of 8:2 diPAP and its metabolites, as well as the relative standard deviations (in brackets), for the carrot (***Chantenay*** variety) root peel, root core and leaves cultivated in two different soil types (soil 2.4 and substrate) and the total BCF (BCF $_{Total}$) values.

Analytes	C (ng/g) and BCF $_{Total}$		Soil 2.4		Substrate	
			Pot 1	Pot 2	Pot 1	Pot 2
8:2 diPAP	C (ng/g)	Soil	168 (13)	198 (17)	200 (24)	109 (8)
		Peel	6.6 (4)	4.9 (8)	6.1 (7)	4.6 (19)
		Core	‹ MDL	‹ MDL	‹ MDL	‹ MDL
		Leaves	7.8 (8)	5.5(8)	6.1 (4)	5.3 (10)
	BCF $_{Total}$		0.037 (4)	0.025(5)	0.026 (2)	0.043(5)
7:3 FTCA	C (ng/g)	Soil	8.1 (6)	9.0 (10)	6.2(3)	5.3 (23)
		Peel	‹ MDL	‹ MDL	‹ MDL	‹ MDL
		Core	‹ MDL	‹ MDL	‹ MDL	‹ MDL
		Leaves	‹ MDL	‹ MDL	‹ MDL	‹ MDL
	BCF $_{Total}$		-[a]	-[a]	-[a]	-[a]
PFNA	C (ng/g)	Soil	1.6 (6)	1.5 (4)	1.3 (5)	1.1 (4)
		Peel	1.1 (5)	1.1 (2)	1.0 (5)	1.0 (2)
		Core	1.3 (4)	1.1 (3)	1.0 (7)	1.2 (15)
		Leaves	1.1 (2)	1.2 (5)	1.2 (5)	1.1 (2)
	BCF $_{Total}$	BCT $_{Total}$	0.74 (2)	0.74 (3)	0.80 (4)	1.05 (11)
PFOA	C (ng/g)	Soil	102 (16)	75 (9)	65 (3)	52 (8)
		Peel	70 (20)	70 (5)	20 (14)	19 (10)
		Core	96 (2)	67 (19)	21 (21)	12 (10)
		Leaves	93 (16)	157 (9)	89 (9)	46 (13)
	BCF $_{Total}$	BCT $_{Total}$	0.86 (8)	1.43 (7)	0.55 (9)	0.39 (8)
PFHpA	C (ng/g)	Soil	20 (15)	9 (6)	5 (8)	3 (8)
		Peel	7 (14)	5 (1)	5 (3)	5 (11)
		Core	11 (4)	4 (17)	3 (15)	3 (19)
		Leaves	18 (19)	21 (11)	44 (9)	19 (12)
	BCF $_{Total}$	BCT $_{Total}$	0.64 (11)	1.23 (8)	2.41 (7)	2.52 (10)
PFHxA	C (ng/g)	Soil	11 (20)	6 .4 (7)	7.9 (10)	4.5 (2)
		Peel	20 (18)	10.2 (2)	4.8 (20)	4.1 (12)
		Core	25 (21)	6 (18)	2.9 (28)	3.1 (24)
		Leaves	102 (14)	144 (16)	61 (10)	44(10)
	BCF $_{Total}$	BCT $_{Total}$	4.6 (11)	11 (15)	2.1 (9)	2.8 (9)
PFPeA	C (ng/g)	Soil	6.4 (28)	4.4 (6)	3.6 (11)	2.2 (2)
		Peel	20 (23)	8.7 (7)	5.1 (24)	4.8 (5)
		Core	19 (8)	6.3 (14)	3.7 (11)	4.0 (14)
		Leaves	93 (20)	115 (11)	51 (24)	65 (8)
	BCF $_{Total}$	BCT $_{Total}$	8.0 (14)	12.4 (10)	4.1 (19)	7.9 (7)
PFBA	C (ng/g)	Soil	2.6 (12)	2.0 (7)	1.8 (3)	1.4 (9)
		Peel	28 (20)	15.0 (1)	5.7 (8)	6.3 (11)
		Core	22.6 (3)	10 (17)	4.5 (13)	3.9 (15)
		Leaves	116 (4)	130 (10)	123 (9)	36(12)
	BCF $_{Tota}$	BCT $_{Total}$	23.3 (4)	33 (9)	17 (8)	8.6 (9)

-[a] Concentration were lower than the MDL and therefore BCF $_{Total}$ was not calculated

Concentrations lower than the nominal (500 ng/g) for both the soil 2.4 (168-198 ng/g)

and the substrate (108-200 ng/g) (see **Table 9.3**) were determined. Moreover, 7:3 FTCA and PFCAs such as PFNA, PFOA, PFHpA, PFHxA, PFPeA and PFBA were detected in the compost: soil mixture when plants were sampled (see **Table 9.3**). In this case, 8:2 monoPAP, 8:2 FTCA and 8:2 FTUCA, which had been observed in the 8:2 diPAP degradation experiment in the absence of crop, were not detected; however, PFNA (1.1-1.6 ng/g), PFPeA (2.2-6.0 ng/g) and PFBA (1.4-2.6 ng/g) were detected at low concentrations. Once more, PFOA was the major degradation product.

Concentrations measured in both types of soils (soil 2.4 and substrate) used for cultivation media were comparable for 8:2 diPAP ($F_{Experimental}$ = 1.72 ‹ $F_{Critical}$ = 5.99), PFHxA ($F_{Experimental}$ = 1.80 ‹ $F_{Critical}$ = 5.99) and PFPeA ($F_{Experimental}$ = 5.86 ‹ $F_{Critical}$ = 5.99) according to the one-way analysis of variance (ANOVA). However, the concentrations in the amended soil 2.4 were higher than in the amended substrate soil for PFNA, PFOA, PFHpA and PFBA ($F_{Experimental}$ = 15.84, 6.66, 10.11, 6.51 › $F_{Critical}$ = 5.9, respectively).

Several PFCAs, as well as 8:2 diPAP, were measured in the different carrot plant compartments. The concentrations (nmol/g) in the amended soils and in all the analysed compartments for all the experiments and all the detected compounds are included in **Figure 9.3.** Although 7:3 FTCA was detected in the soils, it was not translocated through the plants since values lower than MDLs were achieved in carrot plant compartments. According to the literature [23, 24], the occurrence of these analytes in the carrot compartments may be due to plant metabolisation of 8:2 diPAP and/or due to the uptake of PFCAs present in the amended soil due to the degradation of 8:2 diPAP.

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Regarding 8:2 diPAP, concentrations lower than MDLs were determined in core, while concentrations lower than in the compost-amended soil were observed in peel and leaves. Therefore, low BCF factors were determined in both, peel (0.025-0.042) and leaves (0.028-0.049). This could mean that either this target compound is extremely retained to the soil and translocation through the plant almost does not occur or the carrot metabolises it. When the uptake of 6:2 diPAP and 8:2 diPAP was studied with alfalfa (***Medicago truncatula***) by Lee at al. [24], uptake of diPAPs was observed in plants sampled from two types of biosolid-amended soil after 1.5 months, but subsequent analysis of plants sampled at 3.5 and 5.5 months revealed

either no detection or a decline in the diPAP concentrations. Taking into account that our carrots were harvested after 3 months, it could be concluded that the same tendency as Lee et al. [24] was observed here and that the carrots tend to metabolise 8:2 diPAP.

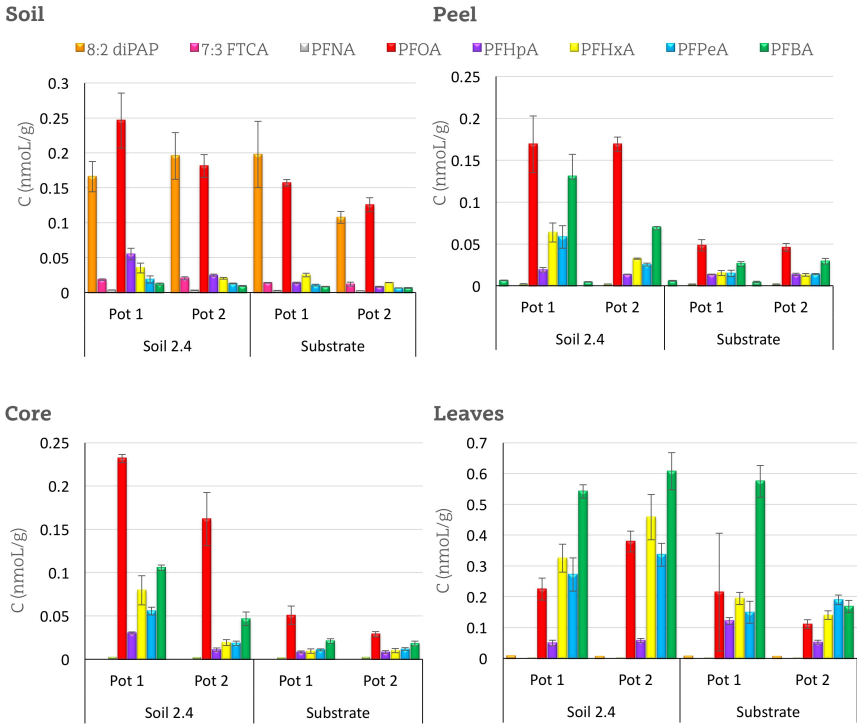

Figure 9.3. Concentration (nmol/g) of 8:2 diPAP and its degradation products measured in the different carrot plant compartments (peel, core and leaves) and compost-amended soils (Soil2.4 and substrate).

Concerning PFCAs, the results indicated that in all the cases the highest concentrations were determined in leaves instead of peel and core for all the quantified analytes. In relation to PFOA, it was observed that, while in the peel and in the core, concentrations higher than the other PFCAs concentrations were determined (see **Figure 9.3**), in the leaves, the highest concentrations were achieved for PFBA followed by PFPeA and PFHxA.

This might be because of pollutants are in direct contact with the peel and core. On the other hand, in the case of the leaves, pollutants must be translocated through the plant via transpiration of contaminated soil-water through transport (from the root to the other plant compartments) tissues, like the xylem. This translocation could be favoured by analyte solubility

and the higher the solubility, the higher the translocation. In this sense, **Figure 9.4** shows the variation of (a) BCF_{Core}, (b) BCF_{Leaves}, (c) BCF_{Peel} and (d) BCF_{Total} with respect to the number of carbon atoms (related with the compounds solubility) and the type of soil. As can be observed, all the BCF values decreased as the number of carbon atoms in the carbon chain increased independently of the type of soil and similar to the results obtained in the literature [24]. In other words, the higher the solubility, the higher the BCF values obtained. In a previous work of the research group [30] it was also observed that the BCFs obtained for PFOA, PFOS and FOSA were related with their water solubility. In the case of the 4 carbon-chain length PFCA (PFBA), BCF was also dependent of the type of soil and BCF decreased when the TOC of the soil increased, similar to previous results in the literature [37]. This conclusion was also obtained by Yoo et al. [21] when the uptake by grass of perfluorinated chemicals and FTOHs in plants cultivated in biosolid amended soils was evaluated.

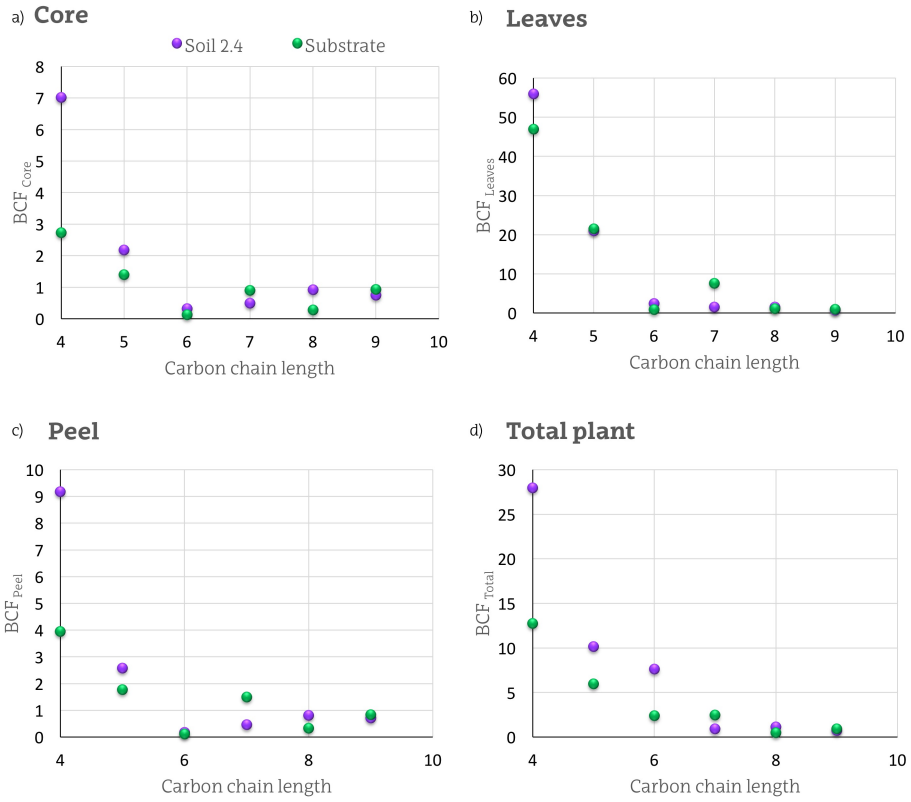

Figure 9.4. Correlation between carbon chain length and carrot compartment BCFs (BCF_{Core}, BCF_{Leaves}, BCF_{Peel} and BCF_{Total}) of PFCAs.

In a previous work of the research group [30], the uptake by carrot of PFOA from amended soils polluted with this compound was studied. When the results obtained for PFOA coming from 8:2 di-PAP degradation in the present work were compared with the results obtained in that previous work, several similarities were observed. In both cases the highest concentration was detected in the leaves, concluding that PFOA translocation through the plant occurred. In terms of the magnitude of the BCFs observed, while in the case of the soil 2.4 the values obtained were comparable ($F_{Experimental}$ = 2.08< $F_{Critical}$ = 5.99), 2 times lower BCF_{Total} were obtained (0.39-0.55) for the substrate when PFOA was coming from 8:2 diPAP degradation compared with the values obtained when compost was directly fortified with PFOA (0.933-1.026). The different tendency observed in the latter could be attributed to how strongly the 8:2 diPAP is bound by the soil components and how readily it is degraded.

As it can be observed in **Table 9.3**, the concentrations of PFOA (8:2 diPAP major metabolite) are in the range of 75-102 ng/g and 52-65 ng/g when the experiments are carried out in presence of soil 2.4 and substrate soil, respectively. It could be thought that 8:2 diPAP could bind covalently to soil humic matter and could strongly be sorbed, becoming less accessible to microorganisms when substrate was used as cultivation media. Therefore, degradation as well as transport is limited the higher the TOC of soil [38].

Moreover, regarding to the results obtained in a previous work [30] of the research group when PFOA, PFOS and FOSA uptakes by carrot were studied, FOSA degradation was also soil dependent. The higher the TOC values, the higher the degradation of FOSA to PFOS was observed, but it could be thought that also the higher sorption and therefore lower BCFs. Similar conclusions were reported by Selim et al. [39] when solute transport in soils was studied and by Koskinen et al. [40] when change in sorption and bioavailability of imidacloprid metabolites was studied in soil.

Besides, the carrot plant uptake tendency of all the PFASs substances studied in the previous work [30] of the research group and in the present work was compared taking into account the water solubility of the target compounds. The higher the solubility, the higher the accumulation of the pollutants was observed (see **Figure 9.5**). The same tendency was observed by Lee et al. [24] when the fate of diPAPs and their metabolites in alfalfa (***Medicago truncatula***) plants were studied.

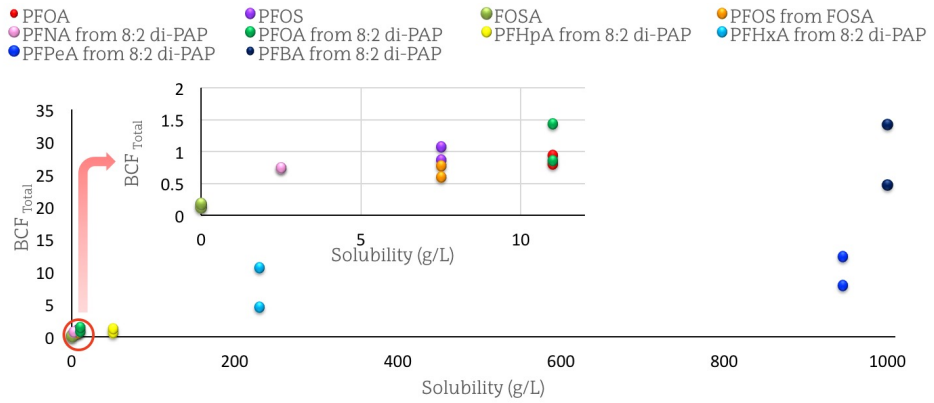

Figure 9.5. Total BCFs of 8:2 diPAP and its degradation products in carrot, as well as BCF_{Total} obtained in a previous work of the research group [30] when compost was fortified with PFOS, FOSA and PFOA against their water solubility.

9.3.3 Uptake by lettuce of 8:2 diPAP and its transformation products

The average (n=3) concentrations (in ng/g) of the target analytes measured in the amended soil and in the different lettuce compartments (heart and leaves), the relative standard deviations (in brackets), as well as the average BCF_{Total} (calculated using **Equation 9.2**) and their relative standard deviations (in brackets and calculated by error propagation) of each experimental set are given in **Table 9.4**. Concentrations in the lettuce compartments grown in unfortified amended soils were lower than MDLs.

$$BCF_{Total} = \frac{1}{m_{Plant}} \left(BCF_{Heart}\, m_{Heart} + BCF_{Leaves}\, m_{Leaves}\right) \quad \textit{Equation 9.2}$$

where the BCFs in lettuce heart (BCF_{Heart}) and lettuce leaf (BCF_{Leaves}) were calculated as the concentration ratio between the concentration found in the plant tissue and the concentration in the amended soil. m_{Heart} and m_{Leaves} are the mass of the lettuce heart and leaves compartments while and m_{Plant} is the total mass of the lettuce.

Similar to the results obtained for carrot, it was observed that the 8:2 diPAP concentrations measured in the fortified amended soil (137-249 ng/g and 204-219 ng/g for soil 2.4 and substrate, respectively) after harvesting were lower than the nominal concentration. But as in the degradation process, the concentration of 8:2 diPAP after 6 h remained constant, these final concentrations were selected as the best option for the calculation of BCFs. In this set of experiments, only PFOA biotransformation metabolite was detected in soil and higher

concentrations of PFOA were detected in the substrate soil (47-57 ng/g) than in soil 2.4 (10-17 ng/g). It could be mentioned that the opposite tendency was observed comparing with PFOA concentration in soil where carrot had been cultivated. Therefore, it could be concluded that the influence of the crops is something that it should be taken into account in this type of experiments [38].

Table 9.4. Average (n=3) concentration (in ng/g) of 8:2 diPAP and its metabolite (PFOA), as well as the relative standard deviations (RSD %, in brackets), for the lettuce (***Batavia Golden Spring*** variety) heart and leaves cultivated in two different soil types (soil 2.4 and substrate) and the total BCF (BCF_{Total}) values.

Analytes	**C (ng/g) and BCF_{Total}**		**Soil 2.4**		**Substrate**	
			Pot 1	**Pot 2**	**Pot 1**	**Pot 2**
8:2 diPAP	C (ng/g)	Soil	137 (29)	249 (4)	219 (21)	204 (19)
		Heart	17 (7)	11 (29)	8 (25)	37 (2)
		Leaves	‹ MDL	‹ MDL	‹ MDL	‹ MDL
	BCF_{Total}	BCF_{Total}	0.0068 (7)	0.0025 (29)	0.0025 (25)	0.012 (2)
PFOA	C (ng/g)	Soil	17 (2)	10 (5)	55 (28)	47 (3)
		Heart	26 (13)	15 (3)	15 (22)	15 (2)
		Leaves	‹ MDL	‹ MDL	‹ MDL	‹ MDL
	BCF_{Total}	BCF_{Total}	0.093 (13)	0.089 (3)	0.019 (22)	0.022 (2)

In terms of the concentration found in the two compartments of the lettuce, it was determined that concentrations of 8:2 diPAP and PFOA in the edible part of the lettuce (the leaves) were below MDLs. However, both, 8:2 diPAP and PFOA were detected in the heart of the lettuce, which is in direct contact with the fortified soil. In the case of the lettuce, the rest of the degradation products were neither detected in the lettuce compartments.

The bioconcentration factors for the lettuce heart (BCF_{Heart}) were calculated and a lack of homogeneity among pots was observed for 8:2 diPAP within the same soil. Therefore, no conclusions on the influence of the soil TOC in the uptake of this compound could be drawn. However, BCF_{Heart} values for PFOA were comparable between the two pots for the same type of soil ($F_{Experimental}$ = 5.42 and 1.26 ‹ $F_{Critical}$ = 18.51 for soil 2.4 and substrate respectively) but it could be concluded that the BCF_{Heart} values were higher for soil type 2.4. The higher the TOC value of

the soil, the lower the analytes translocation from the soil to the plant was observed, similar to the literature [30-38].

In a previous work [30] carried out in the research group, PFOA, PFOS and FOSA uptake by lettuce when compost-amended soil (soil 2.4) was fortified directly with the above-mentioned target analytes was studied. Regarding PFOA, higher BCF_{Heart} (4.06-4.46) as well as BCF_{Leaves} (1.43-2.07) were determined in the previous work. The differences between BCFs could be attributed to the PFOA availability. When compost is directly fortified with the target analyte, this is available for the plant during the growing plant stages, while when soil is fortified with 8:2 diPAP, PFOA is not detected at the first stages.

Similar to the results obtained for carrot, when the results from the present and the previous [30] work of the research group are put together (see **Figure 9.6**), it is observed that the higher solubility of the compounds, the higher BCFs determined for lettuce. Moreover, the origin of the pollutant is also an important factor as it can be observed in **Figure 9.6** for PFOA and PFOS. Lower BCFs were always determined when the pollutant was coming from a precursor such as 8:2 diPAP (for PFOA) and FOSA (for PFOS). The same was observed for carrot.

In relation with the different crops studied (lettuce and carrot), no trend could be drawn. Depending on the analyte and its origin (directly fortified or precursor degradation) the tendency obtained is different. As an example, when plants were cultivated in compost directly fortified with PFOA, higher BFC_{Total} (2.45-3.19) for lettuce than for carrot (0.81-0.94) were calculated. However, when PFOA coming from 8:2 diPAP transformation, BCF_{Total} (0.86-1.43) for carrot were higher than the BFC_{Total} (0.089-0.093) obtained for lettuce.

9.3.4 Accumulation in the polymeric materials

8:2 diPAP was not detected in any of the polymeric materials tested, while PFOA was detected in PES but not in SR and POM, when carrot and lettuce experiments were performed in the fortified amended soils. However, values lower than MDLs were obtained for non-fortified experiments in both studied crops.

The PFOA concentrations in the PES material were very similar in both types of soils (153-165 ng/g and 98-211 ng/g for soil 2.4 and substrate, respectively) but no correlation with the concentrations found in the carrot compartments was observed. In a previous work of the research group, correlations between the PFOA concentrations in the carrot peel and the concentrations found in the PES material were observed [30] but in that case the amended soil

was fortified directly with PFOA, while PFOA in the present study is a degradation product of 8:2 diPAP. One of the facts that could have an influence in the results is that the PFOA in the plant tissue can have two different origins, the degradation of 8:2 diPAP in the soil and the metabolisation of 8:2 diPAP within the plant tissue [23, 24].

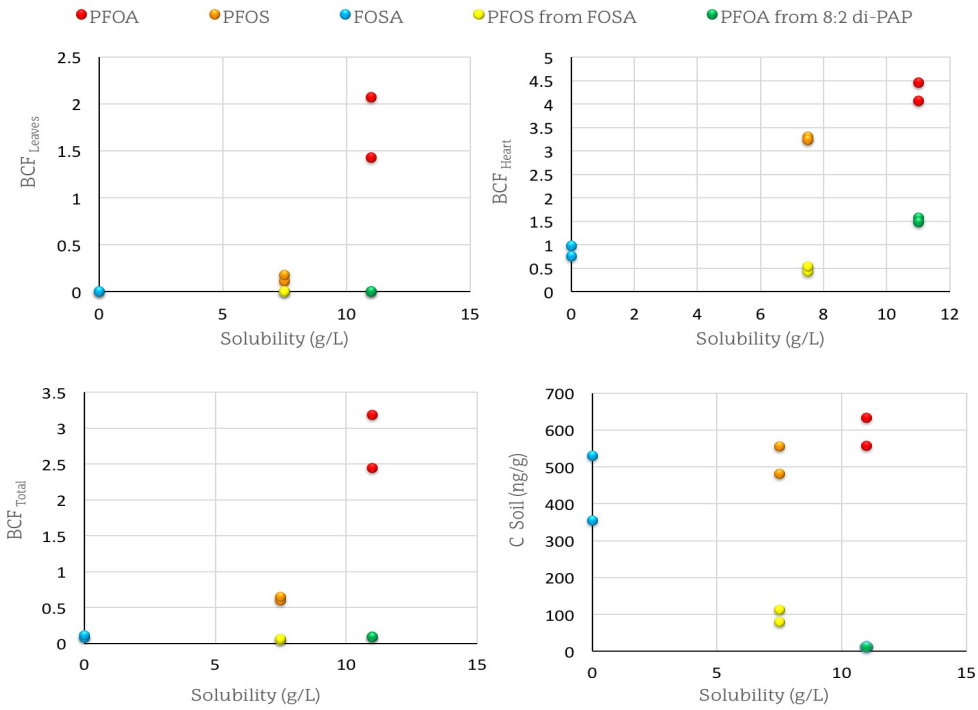

Figure 9.6. BCF_{Leaves}, BCF_{Heart}, BCF_{Total} and C_{Soil} (ng/g) against solubility of the target analytes (PFOA, PFOS, FOSA, 8:2 diPAP and their degradation products) obtained in the present work and in a work performed by our research group [30] for lettuce.

In the case of lettuce experiments, higher PFOA concentrations were determined in PES deployed in the substrate (148-189 ng/g) soil than in the PES deployed in soil 2.4 (47-98 ng/g). The same trend was observed when PFOA concentration was determined in the cultivation media as it can be observed in **Table 9.4**.

9.4 Conclusions

As far as we know, this is the first work in the literature where the uptake of 8:2 diPAP precursor has been studied. Biotransformation of 8:2 diPAP in the presence and the absence of crop in both studied soils (soil 2.4 and substrate) was observed, being PFOA the major transformation product, although compounds such as 8:2 FTCA, 8:2 FTUCA, PFBA, PFPeA, PFHxA, PFHpA and PFNA were also detected.

In the case of carrot, the BCF_{Total} values observed for 8:2 diPAP were lower than 0.004 and therefore a low accumulation of this compound in carrot is expected. However, the BCF_{Total} values for the transformation products were higher, in the 0.64-32.60 range. Actually, a correlation between the carbon chain length and BCFs of PFCAs coming from 8:2 diPAP degradation was observed. BCFs decreased when carbon chain length increased. Similar to previous results of the research group, water solubility of the target analytes seems to play an important role for the contaminants translocation within the carrot. Regarding lettuce, both the 8:2 diPAP and the detected transformation product PFOA, were accumulated in the heart but were not translocated through the plant.

9.5 References

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CHAPTER 10

Uptake of tonalide, galaxolide and bisphenol A by carrot and simulation with polymeric materials

Journal of Agricultural and Food Chemistry (Under review)

10.1 Introduction

Under the Urban Wastewater Treatment Directive (UWWTD), towns and cities within the 28 European Union members are required to collect and treat their urban wastewater. The reuse of the sludge is also encouraged, and final disposal to surface waters has been banned. However, wastewater treatment plants (WWTPs), also called "biological treatments", are demonstrated not to be effective enough in contaminant removal [1]. Not all the chemicals entering the WWPTs are completely degraded and are either removed by sorption and deposition to the final sludge, by volatilisation or by discharge onto a surface water body [1]. In some cases, transformation products generated during the influent treatment are even more toxic than their precursors [2].

In this sense, contaminants of emerging concern (CECs) have been detected in effluent discharges from municipal and/or industrial WWTPs, including pharmaceuticals and personal care products (PPCPs) [3- 8]. Among the PPCPs, both synthetic musk fragrances and bisphenol A (BPA) have been considered 'emerging' compounds and have also been detected in sewage sludge, biosolids and/or compost [9].

Polycyclic musks account for approximately 85 % of the worldwide production of fragrances. The principal source of synthetic musks to the environment is thought to be WWTP effluents and sludges. Tonalide (AHTN) and galaxolide (HHCB), the most commonly used polycyclic synthetic musks, have been detected in sewage sludge at high concentration levels, 0.03–16 mg/kg dry weight and 0.1–81 mg/kg dry weight for AHTN and HHCB, respectively [9].

On the other hand, WWTP studies have also detected BPA in raw water, sewage sludge and effluents [10]. BPA concentrations in sludge are very variable, with values ranging from low μg/kg to mid mg/kg [9].

Land application of sewage sludge and/or sludge-derived compost has been adopted worldwide as an option for sludge management. Crops grown in soils amended or irrigated with wastewater containing CECs are exposed to contaminant uptake, which become, therefore, an entrance of pollutants in the food chain. Within this context, and taking into account that plants form an essential basis of animal and human diet, an evaluation of the uptake and accumulation of potential harmful organic contaminants in plants is of importance for risk assessment. Although previous studies on organic contaminants have investigated the uptake

and accumulation by plants (corn, carrot, lettuce, barley, maize, wheat, among other plants) of some pesticides or veterinary drugs [11], polychlorinated biphenyls (PCBs) [12-14], polybrominated diphenyl ethers (PBDEs) [14-16], musk fragrances and triclosan [17], organophosphate compounds [18] or perfluoroalkyl substances (PFASs) [19-24], few are the works that used polymeric materials for the uptake simulation of the contaminants [25].

Therefore, the aim of this study was to investigate the impact of compost-amended soil using naturally and/or artificially contaminated compost with BPA, HHCB and AHTN on the bioconcentration of these compounds in the soil–carrot system. Besides, different polymeric materials, including polyethersulfone (PES), silicone rod (SR) and polioxymethylene (POM) were also deployed in the compost-amended soil pots during the carrot cultivation period, in order to correlate the bioconcentration of the target analytes in the carrot compartments and in the materials and, thus, evaluate the potential carrot uptake simulation. Finally, the results obtained in the present work were compared with the results obtained for PBDEs [26] and PFASs [27] in previous works of the research group in order to understand which type of pollutant presents higher bioconcentration factors (BCFs) and present, therefore, a potential higher risk for human health.

10.2 Experimental section

10.2.1 Reagents and materials

HHCB (53.5 % purity) and AHTN (97.9 % purity) musk fragrances were purchased from LGC Standards GmbH (Augsburg, Germany) and musk xylene (nitro-musk) used as surrogate was purchased from Dr. Ehrenstorfer GmbH, Augsburg, Germany. BPA (99.0 %) and [$^{2}H_{16}$]-BPA (99.9 %) used as surrogate were obtained from Supelco (Walton-on-Thames, UK).

HPLC grade acetone (99.8 %), methanol (MeOH, 99.9 %), acetonitrile (ACN, 99.9 %) and ethyl acetate (EtOAc, 99.9 %) were supplied by LabScan (Dublin, Ireland).

Milli-Q water (‹0.05 µS/cm, Milli-Q model 185, Millipore) and MeOH (Romil-UpS, Cambridge, UK) were used as mobile phase and ammonium hydroxide (25 % as NH_4OH, Panreac, Reixac, Barcelona, Spain) for mobile phase modifications. High purity nitrogen gas (›99.999 %) supplied by Messer (Tarragona, Spain) was used as collision gas and nitrogen gas (99.999 %) provided by AIR Liquid (Madrid, Spain) was used as both nebuliser and drying gas.

PES sorbent was acquired from Membrane (Wuppertal, Germany) in a tubular format (0.7 mm external diameter, 1.43 g/mL density). The commercial SR (0.97 g/mL density) was purchased from Goodfellow (PA, USA). POM (1.41 g/mL density film) was supplied by CS Hyde Company (Illinois, USA). PES tubes were cut in 1.9-cm length (approx. 30 mg) pieces. Bars of about 0.2 cm (approx. 40 mg) were cut in the case of SR. POM material was cut into pieces of 2 x 2.5 cm (approx. 50 mg). All polymeric materials were washed and conditioned with EtOAc.

The PDMS stir-bars employed (so called twisters) supplied by Gerstel, (Mülheim an der Ruhr, Germany) were 20 mm × 0.5 mm (long x film thickness) size. Prior to their use, a chemical cleaning step was firstly performed in an ACN:MeOH (1:1, v:v) mixture under ultrasound energy during 30 min and conditioned in a thermal condition unit at 280 °C for 2 h under a nitrogen atmosphere.

For the focused ultrasound solid-liquid extraction (FUSLE), 50-mL polypropylene conical tubes (PP, internal diameter 27.2 mm × 117.5 mm length) were obtained from Deltalab (Barcelona, Spain). Extractions were carried out using a Bandelin sonifier ultrasonic cell disruptor/homogeniser (20 kHz; Bandelin Electronic, Berlin, Germany) equipped with a 3-mm titanium microtip. For the filtration of the supernatant polytetrafluoroethylene filters (PTFE, 0.45 m, 25 mm, Teknokroma, Barcelona, Spain) were used. Fractions were evaporated in a Turbovap LV Evaporator (Zymark, Hopkinton, MA, USA) using a nitrogen (99.999 %) blow down.

Envi-Carb graphitised carbon (100 m^2/g specific surface area, 120/400 mesh, Supelco,) and C_{18} (500 m^2/g specific surface area, Agilent Technologies, Palo Alto, CA, USA) were used as dispersive sorbents. Additionally, anhydrous magnesium sulphate ($MgSO_4$, extra pure, Scharlau, Barcelona, Spain) was also used in the dispersive solid phase extraction (dSPE) clean-up step. For centrifugation, 1.5-mL Eppendorf tubes were purchased from Eppendorf (Berzdorf, Germany). A 24-Place Microlitre centrifuge (230 V/50–60 Hz) was obtained from Heraeus Instrument (Hanau, Germany).

The compost (55 ± 8 % Total Organic Carbon, TOC, 2.4 ± 0.3 % total N and pH 7.9) was acquired from Calahorra WWTP (Spain). LUFA Speyer (Speyer, Germany) supplied the sandy loam soil 2.4 (2.3 ± 0.5 % TOC, 0.20 ± 0.04 % total N and pH 7.2) used in the experiments. Carrot seeds were obtained from a commercial agricultural house (www.mvgarden.com). ***Chantenay*** carrot (***Daucus carota ssp sativus***) variety was used in the uptake experiments.

All the reagents used for the Hoagland nutritive solution preparation, potassium nitrate (KNO_3, 99.0 %), calcium nitrate tetrahydrate ($Ca(NO_3)_2$ $4H_2O$, 98.0 %), ammonium

phosphate monobasic ($(NH_4)H_2PO_4$, 96.0-102.0 %), magnesium sulphate heptahydrate ($MgSO_4$ $7H_2O$, 99.0-100.5 %), manganese chloride tetrahydrate ($MnCl_2$ $4H_2O$, 98.0-102.0 %), boric acid (H_3BO_3, 99.8 %), zinc sulphate heptahydrate ($ZnSO_4$ $7H_2O$, 99.0-104.0 %), copper sulphate pentahydrate ($CuSO_4$ $5H_2O$, 99.0-100.0 %), sodium molybdate dehydrate (Na_2MoO_4 $2H_2O$, 98.0-100.0 %) and NaOH (reagent grade, ≥ 98 %) and HCl (ACS reagent, 37 %), both of them used for pH adjustment, were purchased from Panreac (Castellar del Vallès, Spain).

10.2.2 Compost fortification and carrot cultivation

10.2.2.1 BPA

In the case of BPA, previously sieved (~2 mm) compost was spiked at two concentration levels. For this purpose, an amount of compost was weighed, covered with acetone, fortified with the corresponding analyte concentration and stirred for 24 h. After that, it was placed under a fume hood for solvent evaporation and the sample was aged for one week. The compost was then thoroughly mixed with soil 2.4 at a 5:95 ratio. The fortification procedure with BPA was carried out in order to adjust to the nominal concentrations of 500 ng/g (low level) and 5000 ng/g (high level) in the soil:compost mixture (95:5).

The pots (n=2, for both low and high concentration levels) with 2 kg of the soil 2.4: compost mixture were sown with previously germinated (~14 days) carrot seeds. For germination, petri dishes were covered with moistened filter paper and the seeds were evenly distributed in the petri dish. Afterwards, seeds were covered with another piece of moistened filter paper. The number of plants per pot was 3–4.

Control (n=1) plants of carrots grown in the non-fortified compost-amended soil 2.4 mixture were placed in between the fortified amended soil pots.

The cultivation of the carrot was performed under controlled greenhouse conditions. Temperature was set at 25 °C during the day and at 18 °C during the night with a 14-h day length and a relative humidity of 50 % and 60 % during the day and overnight, respectively, and they were regularly watered with distilled water and Hoagland nutritive solution. Hoagland nutritive solution was prepared monthly according to Epstein and Bloom's work [28]. Briefly, an appropriate amount of the different salts were weighed, followed by pH adjustment at 5.5 in order to prepare 25 L.

The previously conditioned pieces of the polymers (PES, POM and SR) were also placed in the pots where carrots were cultivated.

Carrots were harvested during a period of three months reflecting the minimum time to produce relatively mature crops and all plants per pot were collected and pooled to one sample. Each plant was divided into roots (peel and core) and leaves. Fresh weight of all plant fractions was recorded. Carrots were peeled with a vegetable peeler (~ 2 mm depth).

10.2.2.2 AHTN and HHCB

For AHTN and HHCB experiments, a 95:5-soil 2.4:compost mixture containing AHTN and HHCB at an average nominal concentration of 5000 ng/g (high level) was obtained after compost fortification as indicated above for BPA. As the compost already contained the musk compounds at a low concentration level (16-38 ng/g), fortification in the latter case was unnecessary. Two pots of carrots grown at both the high (n=2) and the low (n=2) concentration levels were cultivated as above mentioned for BPA experiments.

However, since the musk were present in the compost, control (n=1) plants of carrots for musk experiments were grown in the soil 2.4 without the compost addition, and the pots were once again placed between the fortified or naturally contaminated compost-amended soil pots to investigate possible foliar uptake of the target compounds.

10.2.3 Sample treatment

Carrot samples (root peel, root core and leaves) were freeze-dried using a Cryodos-50 laboratory freeze-dryer (Telstar Instrumat, Sant Cugat del Valles, Barcelona, Spain). In the case of the compost-amended soil, this was air-dried for approx. 48 h. Both were stored at -20 °C until analysis. Analysis were performed in triplicate in all the cases.

10.2.3.1 Musk fragrances

In the case of AHTN and HHCB, the extraction of the samples was carried out in accordance with a modification of a method previously published by our research group [29]. 0.5 g of sample (95:5 % of soil 2.4: compost mixture or carrot) were weighed and 9 mL of Milli-Q:MeOH (80:20, v:v) mixture and 20 µL of surrogate [H_{15}]-MX (10 ng/µL) were added. The PDMS-stir-bar was also added and the extraction was performed for 3 h at a temperature of 40 °C using a 15-position magnetic stirrer at 600 rpm (RT 15 power, Kika Werke, Staufen, Germany). After that, the stir-bar was collected and cleaned with Milli-Q water, dried with a paper tissue to remove solid waste and placed in an Eppendorf for desorption. The analytes were then desorbed in 300 µL of EtOAc in an ultrasonic bath (15 min). The extracts were finally

analysed by gas chromatography-mass spectrometry (GC-MS).

In the case of polymeric materials (PES, POM and SR) for musk uptake, these were cleaned with Milli-Q water, dried with a clean paper tissue and desorbed under the same conditions as the stir-bars.

10.2.3.2 BPA

According to the previously optimised method [30], a sample aliquot of 0.5 g (carrot and compost-amended soil) was weighed and [$^{2}H_{16}$]-BPA surrogate standard at 50 ng/g concentration level and 10 mL of acetone:hexane (70:30, v:v) solvent mixture were added. The samples were then immersed in an ice-water bath (~0 °C) and the extraction was performed at 33 % power and a pulsed time on of 0.8 s and a pulsed time off of 0.2 s during 5 min by means of FUSLE. The supernatant was filtered through 0.45 µm PTFE filter and the extracts evaporated to dryness, reconstituted in 1.5 mL of ACN and submitted to the clean-up approach by means of dSPE. Sorbents used were Envi-Carb (75 mg), C_{18} (50 mg) and $MgSO_4$ (150 mg) mixture for carrot and Envi-Carb (75 mg) for compost-amended soil extracts. The extracts were vortexed for 40 s and centrifuged (4000 rpm during 10 min at 4 °C). In all the cases, the eluates were collected and evaporated to dryness under a gentle stream of nitrogen at 35 °C and reconstituted in 250 µL of MeOH (99.9 %). Finally, the reconstituted extracts were filtered through a 0.2 µm PTFE filter before liquid chromatography-tandem mass spectrometry (LC–MS/MS) analysis.

10.2.4 GC-MS analysis

An Agilent 6890N gas chromatograph (GC) equipped with an Agilent 7683B autosampler and a split/splitless inlet coupled to an Agilent 5975N (Agilent Technologies, Palo Alto, CA, USA) mass spectrometer (MS) was used for musk analysis. All standard solutions and extracts were injected (injection volume of 2 µL) at 300 °C in the splitless mode. The chromatographic analysis was carried out with an Agilent HP-5MS capillary column (30 m x 0.25 mm, 0.25 µm). The oven temperature program was as follows: the initial temperature was set at 60 °C followed by a temperature increase of 30 °C/min to 200 °C and 3 °C/min until a temperature of 220 °C. Finally, the ramp temperature increased at 30 °C/min to a final temperature of 300 °C, which was held for 5 min. Hydrogen was used as a carrier gas at a flow rate of 1.3 mL/min. Detection was performed using electron impact (EI) ionisation at 70 eV. The interface temperature was set at 310 °C and the temperatures of the ion source and the quadrupole at 230 °C and 150 °C, respectively. The measurements were performed in the

selective ion monitoring (SIM) mode and the ions monitored for each analyte were 243 (258, 213), 243 (258, 159) and 246 (261) for AHTN, HHCB and [$^2H_{15}$]-MX, respectively. The first ion was used as quantifier and the ions included in brackets as qualifiers.

10.2.5 LC-MS/MS analysis

BPA extracts were analysed in an Agilent 1260 series HPLC coupled to an Agilent 6430 triple quadrupole (QqQ) mass spectrometer equipped with electrospray ionisation (ESI) source (Agilent Technologies). The quantitative analysis of the target compounds was performed in the dynamic selected reaction monitoring (dynamic SRM) acquisition mode. High purity nitrogen gas (99.999 %) was used as nebuliser, drying and collision gas. MS/MS ionisation parameters were set as follows: a N_2 flow rate of 12 L/min, a capillary voltage of 5000 V, a nebuliser pressure of 45 psi and a source temperature of 350 °C. Separation was carried out using an Ace Ultra CoreSuper C_{18} core–shell column (2.1 mm, 50 mm, 2.5 µm) and a ViciJour Sure-guard disposable in line filter (24.4 mm, 10.0 mm, 0.5 µm). The column temperature, the injection volume and flow rate were set at 30 °C, 5 µL and 0.3 mL/min, respectively. Under optimised conditions [30] a binary mixture consisting of water:MeOH (95:5) (mobile phase A) and of MeOH:water (95:5) (mobile phase B), both containing 0.05 % of NH_4OH, was used. Linear gradient was as follows: 30 % B maintained for 4 min, increased to 60 % B in 3 min and to 80 % B in 10 min, where it was maintained constant for 10 min. Initial gradient conditions (30 % B) were then achieved in 3 min, where it was finally held for another 10 min (post-run step). Negative voltage was applied. Fragmentor voltage and collision energy values used were optimised elsewhere [30]. Precursors and product ions for BPA and surrogate [$^2H_{16}$]-BPA were the following: BPA (227.1, 212.0 and 232.0) and [$^2H_{16}$]-BPA (241.0 and 223.5), where the first ion was the precursor, the second the product used as quantifier and the third the product used as qualifier. Instrumental operations, data acquisition and peak integration were performed with the Masshunter Workstation Software (Version B.06.00, Agilent Technologies).

10.2.6 Quality control

During sample treatment, procedural blanks (n=2) and control samples (n=3, samples fortified at a known concentration) were analysed (each 12-15 samples). In this sense, method detection limit (MDL) values lower than 1.7 and 2.0 ng/g for BPA, 1.0 and 1.2 ng/g for HHCB and 1.3 and 1.5 ng/g for AHTN in the case of compost-amended soil and carrot, respectively, were obtained. Apparent recoveries were in agreement with the values obtained in the previously published works [29, 30] for all the compounds and both matrices (carrot and compost-

amended soil).

10.2.7 Bioconcentration factor

BCFs were calculated for the different compartments of the carrot. The corresponding BCF values were calculated in accordance with **Equation 10.1** on the basis of the dry weight of the material analysed. Root (BCF_{Root}) and total BCFs (BCF_{Total}) were calculated for each pot considering the total mass of the different harvested plant compartments and the total mass of the crop as shown in the **Equations 10.2** and **10.3**, respectively.

$$\text{BCF} = \frac{\text{Concentration in dry plant tissue } (\frac{\text{ng}}{\text{g}})}{\text{Concentration in dry soil } (\frac{\text{ng}}{\text{g}})} \qquad \textit{Equation 10.1}$$

$$BCF_{Root} = \frac{1}{m_{Plant}} \left(BCF_{Peel}\, m_{Peel} + BCF_{Core}\, m_{Core}\right) \quad \textit{Equation 10. 2}$$

$$BCF_{Total} = \frac{1}{m_{Plant}} \left(BCF_{Peel}\, m_{Peel} + BCF_{Core}\, m_{Core} + BCF_{Leaves}\, m_{Leaves}\right) \quad \textit{Equation 10.3}$$

where the bioconcentration factors in peel (BCF_{Peel}), in core (BCF_{Core}) and in leaves (BCF_{Leaves}) were calculated as the concentration ratio between the concentration found in the plant tissue and the concentration in the compost-amended soil. m_{Peel}, m_{Core} and m_{Leaves} are the mass of the peel, core and leaf compartments respectively, and m_{Plant} the total mass of the carrot.

10.3 Results and discussion

10.3.1 Target analyte concentrations in the compost-amended soil

The concentration of the target substances in the (95:5) soil 2.4:compost mixture at the high concentration level was examined before carrot cultivation (day 0) and after 123 days of plant cultivation (harvesting period) and the values obtained were included in the **Figure 10.1**. The concentrations detected at day 0 differed from the calculated nominal concentrations (5000 ng/g) in the case of the three analytes (BPA, AHTN and HHCB). This could be due to losses occurring during the compost fortification.

Concentration detected at day 0 was lower than after 123 days for AHTN, which was probably due to a lack of homogeneity of AHTN distribution within the compost-amended soil. However, in general, the concentrations of both polycyclic musk compounds HHCB and AHTN

were found to be fairly stable over the cultivation period of 123 days and no significant differences ($F_{Experimental}$=1.0-12.4 ‹$F_{Critical}$=18.5) according to the analysis of variance (ANOVA) at the 95 % confidence level were observed, indicative of a lack of degradation of musk compounds during the cultivation period. The resistance against degradation of AHTN and HHCB from soils has also been reported in the literature [17, 31] and similar results were obtained by Macherius et al. [17] who reported a dramatically decrease in the spiked fragrance concentrations prior to the cultivation experiments.

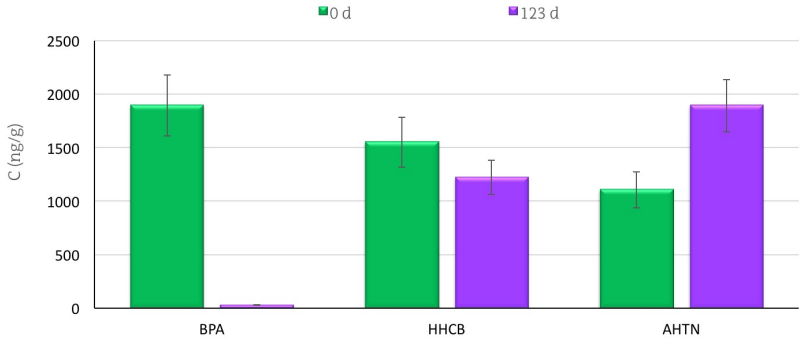

Figure 10.1. Average (n=3) concentrations (ng/g) of the target substances in the (95:5) soil 2.4:compost mixture at the high concentration level evaluated before carrot cultivation (day 0) and after 123 days of plant cultivation (harvesting period).

In the case of BPA, however, a dramatically degradation (82-83 %) was observed after the harvesting period (see **Figure 10.1**) at the high concentration level evaluated. Similar results were obtained in the case of the low level (~ 500 ng/g) concentration with a percentage degradation of 88-89 % after 123 days. A half-life value of 36 days for BPA in an agricultural soil confirmed our observations of higher biodegradability for this compound [32]. Besides, Langdon et al. [33] found BPA half-fives ranging from 18 to 102 days in soil under laboratory conditions. The fact that BPA was degraded rapidly in aqueous and soil environments taken from diverse locations in the United States and Europe [34], as well as in studies conducted in Japan [35] suggests that BPA degrading microorganisms are widely distributed in the nature.

Due to the degradation, BPA was detected in none of the different carrot compartments (root peel, root core and leaves) and, thus, its uptake could not be studied. Further research should be performed in order to see the presence of BPA degradation products both in the amended soil and carrot compartments.

10.3.2 Uptake of musk by carrot

The average (n=3) concentration (in ng/g) of the musks measured in the different carrot compartments, the relative standard deviations (in brackets), as well as the average BCF_{Total} and their relative standard deviations (in brackets and calculated by error propagation) of each experimental set are given in **Table 10.1**. In the case of control samples (carrots cultivated without the compost addition) the concentrations of both analytes were below the MDLs in all the carrot compartments. Reproducibility (2-29 %) between the two pots was satisfactory for both analytes in both sets of experiments (low and high concentration levels).

Table 10.1. Average (n=3) concentrations (ng/g, n=3) of the musk fragrances in the different compartments of the plant (root peel, leaves and root core) at the two concentration levels evaluated. Bioconcentration factors (BCFs) of the analytes in the different compartments and the total BCFs (BCF_{Total}) are also included together with their relative standard deviations (RSDs %, in brackets).

Experiment	Compartment	C_{HHCB} (ng/g)	C_{AHTN} (ng/g)	BCF_{HHCB}	BCF_{AHTN}	BCF Total HHBC	BCF Total AHTN
Pot 1 (low level)	Peel	27 (2)	18 (20)	1.54 (15)	0.67 (24)	0.34 (19)	0.19 (20)
	Leaves	7 (2)	8 (21)	0.40 (15)	0.28 (25)		
	Core	‹MDL	‹MDL	-[a]	-[a]		
Pot 2 (low level)	Peel	33 (20)	23 (24)	1.25 (28)	0.59 (28)	0.42 (19)	0.23 (19)
	Leaves	14 (17)	12 (17)	0.51 (26)	0.32(28)		
	Core	‹MDL	‹MDL	-[a]	-[a]		
Pot 3 (high level)	Peel	1026 (13)	868 (12)	0.84 (25)	0.46 (20)	0.17 (21)	0.09 (17)
	Leaves	26 (9)	18 (19)	0.02 (23)	0.01 (25)		
	Core	34 (12)	25 (9)	0.03 (24)	0.01 (19)		
Pot 4 (high level)	Peel	1617 (10)	1180 (6)	0.87 (17)	0.42 (15)	0.13 (14)	0.06 (12)
	Leaves	43 (3)	36 (18)	0.02 (14)	0.01 (22)		
	Core	68 (5)	51 (5)	0.04 (15)	0.02 (15)		

-[a] Concentrations were lower than the MDLs and, therefore, BCF_{Total} was not calculated.

Concerning the different compartments, the concentrations of both HHCB and AHTN in the carrot root peel were similar to the concentrations in the compost-amended soil

considering both the low and the high concentration levels evaluated. On the other hand, while values lower than MDLs were obtained in the case of core samples at the low level, BCFs in the root peel compartment (BCF_{Peel}=0.59-1.54) tended to be twice or three the BCFs in the leaves (BCF_{Leaves}=0.28-0.51). At the high concentration level, BCF_{Leaves} (0.010-0.023) significantly decreased compared with the values obtained for the low concentration level.

Since the concentrations in the leaves for both the low and the high concentration level were similar, aerial deposition could be suspected; however, this hypothesis was discarded since the musk compounds studied were not detected in the leaves of the control plants (without compost addition) grown among the exposed ones. Thus, the presence of the compounds in the leaves could be the result of their translocation from the root compartments. Similar results were reported in the literature when uptake investigations were carried out for a set of PCBs and PBDEs [14], veterinary pharmaceuticals [11] and musk fragrances [31], which were affected by the concentration in the amended soil. Taking into account the results obtained for the leaves, it could be concluded that translocation was lower the higher the concentration of the musk fragrances. Similar results for the leaves were observed in the uptake experiments of decabromodiphenyl ether (BDE-209) by carrot conducted in our research group [26], which showed a lesser translocation to the leaves when present at a high concentration level in the amended soil.

Concerning to the bioconcentration of the both musk compounds, despite similar structural properties and log K_{ow} values of AHTN (5.75) and HHCB (5.43), the concentrations measured in root and leaves differed significantly. HHCB tended to accumulate twice more than AHTN in all the plant compartments for both the low and high concentration levels evaluated, which could be related with the highest water solubility of HHCB (24 10^3 μg/L) compared with AHTN (18 10^3 μg/L).

The total organic carbon (TOC) influence on the uptake of HHCB and AHTN was also taken into account. In this sense, the data (BCF_{Root} and BCF_{Leaves}) obtained in the present work were compared with those provided by Litz et al. [31] and Macherius et al. [17] and combined in **Figures 10.2 a** and **b**. A 5.03 % TOC value was considered in our case, according to the compost: soil mixture composition (5:95 %). A high correlation between the BCF_{Root} (linear correlation) and BCF_{Leaves} and the soil TOC can be observed. The higher the TOC is the lower the root and leaves translocation of the analytes. Other works [36, 37] have shown that the analyte bioconcentration and translocation was influenced by the soil TOC and lower BCFs were obtained the higher the TOC in the soil.

The BCFs obtained for the musk compounds were also compared (see **Figures 10.3 a** and **b**) with the values obtained in previous works [26, 27, 38] from our research group for the same amended soil and carrot specie for other target analytes, including two PBDEs, BDE-138 (7.73 and 19 µg/L of log K_{ow} and water solubility, respectively) and BDE-209 (11.16 and 0.14 µg/L of log K_{ow} and water solubility, respectively), and several PFASs, perfluorooctanoic acid (PFOA, 6.30 and 11 10^6 µg/L of log K_{ow} and water solubility, respectively), perfluorooctane sulfonate (PFOS, 6.28 and 75 10^5 µg/L of log K_{ow} and water solubility, respectively), perfluorootanesulfonamide (FOSA, 7.58 and 0.029 µg/L of log K_{ow} and water solubility, respectively), perfluoro-n-nonanoic acid (PFNA, 7.27 and 2.5 10^6 µg/L of log K_{ow} and water solubility, respectively), perfluoro-n-heptanoic acid (PFHpA, 5.33 and 5.1 10^7 µg/L of log K_{ow} and water solubility, respectively), perfluoro-n-hexanoic acid (PFHxA, 4.37 and 2.3 10^8 µg/L of log K_{ow} and water solubility, respectively), perfluoro-n-pentanoic acid (PFPeA, 3.40 and 9.5 10^8 µg/L of log K_{ow} and water solubility, respectively) and perfluoro-n-butanoic acid (PFBA, 2.43 and 10 10^8 µg/L of log K_{ow} and water solubility, respectively).

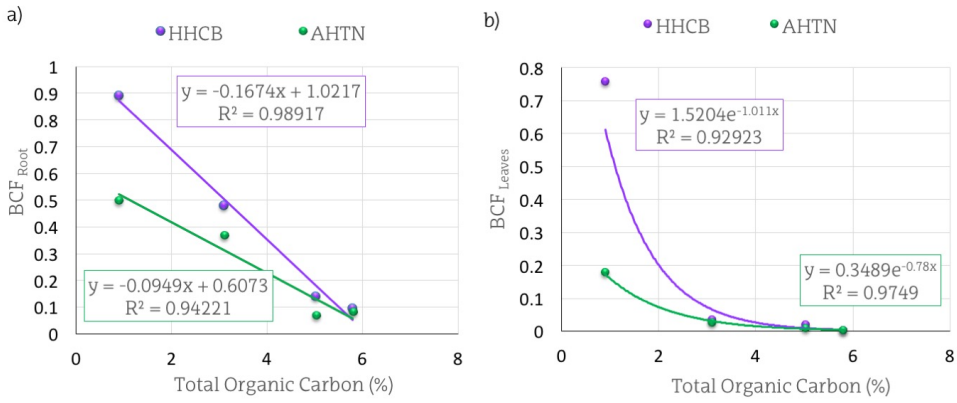

Figure 10.2. Correlation between the data for HHCB and AHTN uptake obtained from our experiments and those provided by Litz et al. [31] and Macherius et al. [17], including a) BCF_{Root} and b) BCF_{Leaves}.

According to the results obtained, an exponential correlation between the logarithm of water solubility and BCF_{Total} (see **Figure 10.3 a**), as well as BCF_{Peel} (determination coefficients of r^2=0.32), BCF_{Core} (r^2=0.74) and BCF_{Leaves} (r^2=0.60) was observed for all the analytes. The accumulation was higher with the water solubility increment, observing a dramatically bioconcentration increased for the analytes (PFHxA, PFPeA and PFBA) with a logarithm water solubility higher than 8 µg/L. In order to confirm these results, we zoomed at the region with the lower water solubility logarithm values (see **Figure 10.3 b**) observing the similar behaviour for

all the analytes.

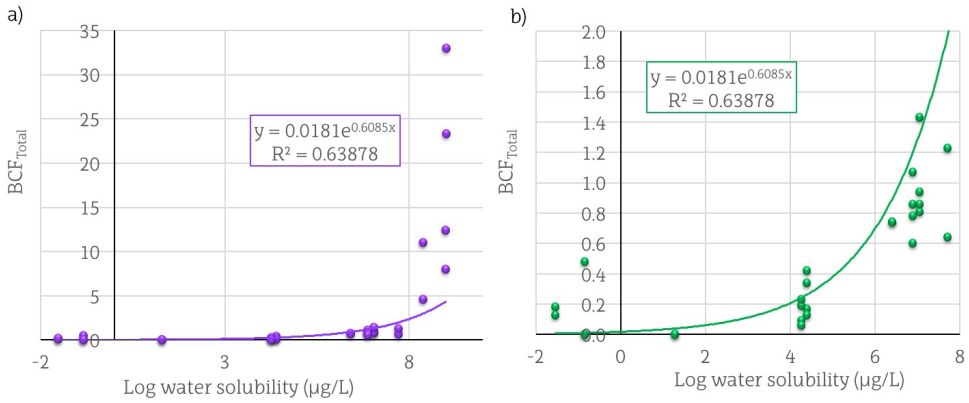

Figure 10.3. Logarithm of water solubility versus the BCF$_{Total}$ of a) BDE-138, BDE-209, PFOA, PFOS, PFOSA, PFNA, PFHpA, PFHxA, PFPeA and PFBA and b) all the analytes except PFHxA, PFPeA and PFBA**.**

BCF$_{Total}$ values were also plotted against the log K$_{ow}$ of the analytes (see **Figure 10.4 a**) and, although, in general terms it could be observed that the higher the log K$_{ow}$ value the lower the BCF$_{Total}$ were obtained, when we zoomed (see **Figure 10.4 b**) at the region with the higher log K$_{ow}$ values, it could be observed that the correlation decreased. Therefore, it could be concluded that water solubility could explain better the uptake of the compounds studied than log K$_{ow}$. This could be explained because some of the analytes included, PFASs, have both a hydrophobic chain and a hydrophilic head and therefore log K$_{ow}$ is not a very representative parameter for them.

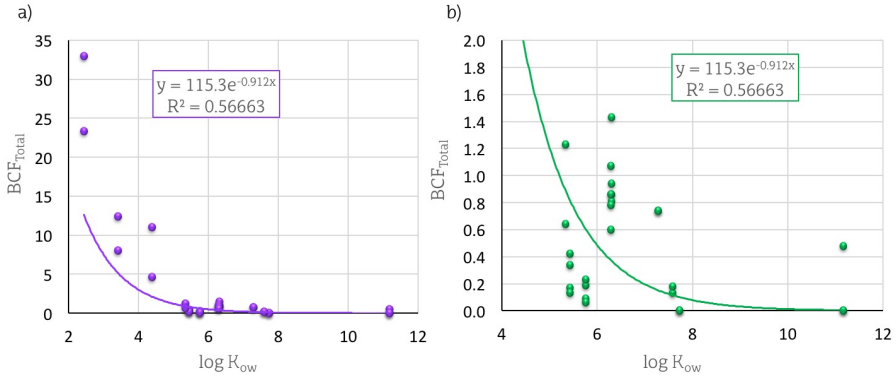

Figure 10.4. Log K$_{ow}$ of the analytes versus the total BCFs including a) BDE-138, BDE-209, PFOA, PFOS, PFOSA, PFNA, PFHpA, PFHxA, PFPeA and PFBA analytes and b) all the analytes except PFHxA, PFPeA and PFBA.

In summary, although the influence of the analyte nature (expressed as log K_{ow}) was not conclusive, water solubility influence was highly correlated with the bioconcentration of the analytes when different target analyte families, including musk fragrances, PBDEs and PFASs were compared.

10.3.3 Polymeric materials

Three different polymeric materials (PES, POM and SR) were deployed in all carrot pots in order to compare the measured concentrations in the carrot and the polymeric materials. Although AHTN and HHCB were detected in all the materials, due to the high uncertainty (RSDs ≥40 %) associated with the results for POM, it was discarded. On the other hand, acceptable precision values (RSDs=15-27 %) were obtained for both PES and SR.

Although the higher concentrations were detected by means of SR, the accumulation in PES was more highly correlated to the accumulation in the root peel of the carrot than for the SR material. Actually, SR was discarded due to the lack of correlation (r^2‹0.5) between the values for the polymeric material and the carrot peel. The equations and r^2 obtained when BCF_{Peel} was compared to the BCFs in the PES polymeric material (BCF_{PES}) are included in **Figure 10.5** for the two musk compounds studied. It could be concluded that the results obtained for PES and musk accumulation in carrots could help in the simulation of the uptake of contaminants by root crops. Similar correlation (r^2=0.63-0.85) values were obtained for the compounds PFOS and PFOA [27].

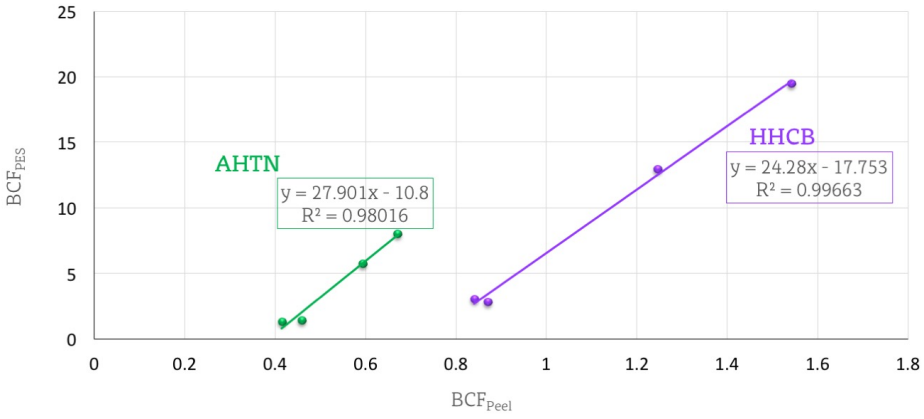

Figure 10.5. BCF_{Peel} against BCF_{PES} for the two musk fragrances.

10.4 Conclusions

Uptake study of AHTN and HHCB was performed in the present study by carrot crop. For both analytes, the highest BCFs were determined in carrot root peel. However, in spite of the similar structural properties of both musk fragrances, HHCB accumulation was twice comparing to the AHTN accumulation.

Regarding BPA, this target compound was dramatically degraded during the cultivation period. Therefore, BPA was not detected in carrot plant compartments such as peel, core and leaves.

Results obtained in the present work as well as results obtained for the carrot uptake of PBDEs, PFASs and 8:2 diPAP in previously performed works in our research group were gathered. It was concluded that the translocation from soil to plant is closely related with the water solubility of the target compounds. The higher the solubility is, the higher BCFs were determined.

Good correlation between the BCF_{Peel} and BCF_{PES} were achieved for AHTN and HHCB. In this sense, it could be concluded that the PES polymeric material it can be use for the uptake simulation of different pollutants by root crops such as carrot. The same trend was observed in a previous research carried out in our laboratory for PFOS and PFOA.

10.5 References

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CHAPTER 11

Conclusions

The results obtained during the research summarised in the present PhD thesis memory have allowed the accomplishment of the previously established objectives. This chapter summarises the main conclusions from this PhD thesis.

Four analytical methods were optimised and validated for the determination of different organic pollutants, such as polybrominated diphenyl ethers (PBDEs), musk fragrances, hormones, alkylphenols (APs), triclosan (TCS), bisphenol A (BPA) and perfluoroalkyl substances (PFASs) in vegetables (carrot and lettuce), as well as compost-amended soils.

In three out of four analytical methods, focused ultrasound solid-liquid extraction (FUSLE) was applied, mainly because short extraction period and small quantities of organic solvent (7-10 mL) are necessary by means of this technique. Regarding the extraction solvent nature, it should be highlighted that depending on the target analyte properties one or another solvent was selected: acetone for the extraction of PBDEs, hexane: acetone mixture for hormones, TCS, APs and BPA and acetonitrile: water mixture for PFASs. Through out the use of these extraction solvents, apart from the target analytes, other matrix components were also extracted. Therefore, due to the lack of selectivity of the FUSLE methods developed, different techniques based on sorptive extraction on polymeric sorbents were evaluated for the clean-up step. While solid-phase extraction (SPE) is a well-establised technique, dispersive solid-phase extraction (dSPE) and the low cost commercial polymeric material polyethersulfone (PES) are recently implemented methodologies. One or another strategy has been selected according to the analyte properties.

As an alternative to the traditional SPE, dSPE was applied for vegetable and soil samples. This clean-up strategy provides the opportunity to mix in an easy way sorbents with different nature. In the present work, i) Envi-Carb, ii) Envi-Carb, activated charcoal powder and primary and secondary ammine (PSA), iii) Envi-Carb and activated charcoal powder, iv) Envi-Carb and Florisil and v) Envi-Carb and octadecyl (C_{18}) mixtures were tested. Low extraction efficiencies were achieved except in the i) and v) cases which could be due to the high interaction between the sorbents and the target analytes. Concerning the elution step, low solvent volumes (1.5 mL) were used. Moreover, different strategies were applied depending on the matrix. While in the case of vegetable samples elution was performed using acetonitrile (ACN) organic solvent, for compost-amended soil ACN followed by acetone was necessary in order to improve significantly the efficiencies. Therefore, it could be concluded that dSPE technique is not only analyte dependent but also matrix dependent.

As an alternative to SPE and dSPE, PES combined with 1-methylpyperidine as ion-pair reagent has demonstrated to be an excellent material for the microextraction of PFASs such as perfluorocarboxylic acids (PFCAs) and perfluoro sulphonate acids (PFSAs) from solid samples. However, the determination of perfluorophosphonic acid (PFPA) was not possible due to the obtained low extraction efficiency values. Again, as with dSPE, the use of organic solvents was minimal (500 μL for desorption) and the robustness of the technique was demonstrated in several solid matrices. Despite requiring long extraction times and not being an exhaustive procedure, its simplicity allows the simultaneous performance of multiple extractions at a very low cost.

In the case of musk fragrances, although preliminary experiments were performed using FUSLE, irreproducible results were obtained, probably due to the open vessels used when performing FUSLE and the required evaporation steps in such procedures. As an alternative, polydimethylsiloxane-stir-bar sorptive extraction (PDMS-SBSE) procedure was validated, being the extraction and the clean-up steps simultaneously performed. Stir-bar thermal desorption (TD) was optimised in order to achieve the best limits of the detection (LODs), but since the analyte concentrations in the plant uptake experiments were high enough, liquid desorption (LD) of the stir-bar was alternatively used for the analyte desorption. In this sense, the use of a TD unit coupled to GC was not compulsory.

Once the analytical methodologies were optimised and validated, plant uptake studies of different pollutants including PBDEs (2,2´,4,4´,5-pentabromodiphenyl ether, BDE-99, 2,2',3,4,4',5'-hexabromodiphenyl ether, BDE-138, decabromodiphenyl ether, BDE-209 and penta-BDE mixture), PFASs (perfluorooctanoic acid, PFOA, perfluorooctane sulfonate, PFOS, perfluorooctane sulfonamide, FOSA and bis(1H, 1H, 2H, 2H-perfluorodecyl)phosphate, 8:2 iPAP), BPA and musk fragrances (tonalide, AHTN and galaxolide, HHCB) by carrot and lettuce crops cultivated in different compost-amended soils were carried out.

It could be highlighted that in the case of both musk fragrances (AHTN and HHCB), as well as for 8:2 diPAP, the theoretical and the initial (before harvesting period) fortified analyte concentrations were not the same. This could be due to evaporation losses during compost fortification step or because the analytes tend to stick on the glass material used in that step. However, no degradation was suspected since the concentrations remained constant during the cultivation periods.

In almost all the uptake studies performed, degradation of the target compound was

studied and, some of their potential degradation products were also monitored. In the case of PBDEs (BDE-138 and BDE-209), PFOA, PFOS, AHTN and HHCB no degradation was observed during the cultivation period. On the other hand, while FOSA was totally or partially degraded to PFOS in the presence of carrot and lettuce, respectively, no FOSA degradation was observed in the absence of crops, concluding that the presence of the vegetable roots could facilitate the degradation of FOSA. Regarding 8:2 diPAP, PFOA was the major degradation product, but there were also detected other intermediates (2-perfluorooctyl ethanoic acid, 8:2 FTCA, 2H-perfluoro-2-decenoic acid, 8:2 FTUCA, 3-perfluoroheptyl propanoic acid, 7:3 FTCA) or final products (PFOA, perfluoro-n-heptanoic acid, PFHpA, perfluoro-n-hexanoic acid, PFHxA, perfluoro-n-butanoic acid, PFBA) products were also detected. The presence of these degradation products was not only dependent on the crop presence, but also on the crop type. While in the case of lettuce PFOA was the only metabolite detected, in the case of carrot 7 metabolites were detected. Concerning BPA, although this target analyte was degraded around 80 % in the compost-amended soil, no degradation products were determined owing to the lack of the standards of the potential degradation products previously reported in the literature. In the future, these samples should be processed using high resolution mass spectrometry (HRMS) in order to identify potential BPA degradation products.

In all the performed studies, foliar uptake of different organic compounds was discarded taking into account the values obtained from the blank samples (plants cultivated in absence of pollutants). Regarding the bioconcentration factors (BCFs) determined in the present thesis work, it was concluded that the organic matter amount (TOC) of the cultivation media, the studied crops, as well as the pollutant characteristics influence in the target analyte uptake.

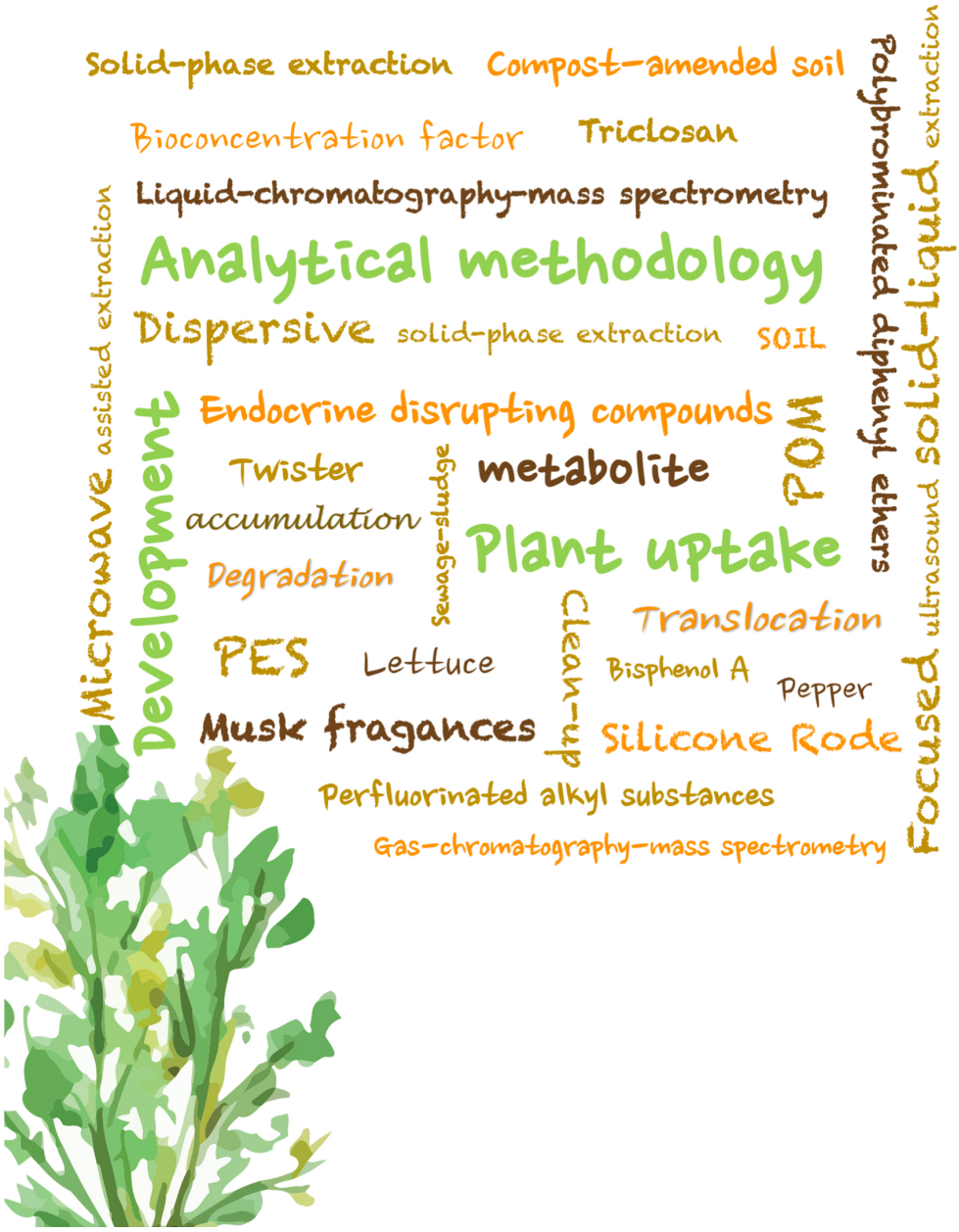
Concerning the soil characteristics, in general, lower translocation of the compounds through the plant was observed with higher TOC. In the case of the crop type, no general conclusions could be observed. Whereas some of the target analytes accumulated more in carrot (i.e. BDE-209 and PFOA), other compounds (PFOS) shown similar accumulation tendencies in both crops. Moreover, it could be mentioned that, while the uptake in carrot of PFOS and PFOA was similar no matter they came from a soil directly fortified with those analytes or from the degradation of FOSA or 8:2 diPAP, different BCFs were obtained for lettuce. When PFOS and PFOA come from the degradation of other analytes (i.e. FOSA and 8:2 diPAP) their concentrations vary in the cultivation media until they become constant. If cultivation period is short, like in the case of lettuce, the degradation products (PFOS and PFOA) concentration are not constant. In this sense, it is difficult to compare the bioconcentration

factors obtained in both cases, the experiments where PFOS or PFOA were directly fortified or were degradation products of FOSA and 8:2 diPAP degradation products. For carrot, the cultivation period is longer and concentrations of the degradation products reach equilibrium and are, therefore, more comparable to the BCFs obtained when the target analytes were not coming from a degradation process.

In the case of PBDEs, the higher the bromination degree, the lower the observed translocation. The same tendency was noted with increasing the carbon chain length of PFASs. Although in general terms it was observed that the higher the log K_{ow} value the lower the BCF_{Total} obtained, it could be concluded that water solubility could explain better than log K_{ow} parameter the uptake of the compounds studied. This could be explained because some of the studied analytes, such as PFASs, have both a hydrophobic chain and a hydrophilic head and, thus, log K_{ow} is not a very representative parameter. From the different uptake experiments carried out in the present work, it could be also concluded that the most polar pollutants are more susceptible to enter the food chain through vegetables grown in compost-amended soils.

In addition, different polymeric materials were tested to simulate the uptake of plants and some promising results were achieved in the case of PES material. Since good correlation between carrot peel and PES BCFs were determined for PFOA, PFOS, AHTN and HHCB, the use of such polymeric materials for uptake simulation could facilitate preliminary uptake studies avoiding laborious and expensive uptake experiments.

Bearing in mind all the results obtained in the present PhD thesis, it can be concluded that under the tested environmental conditions, target compounds such as PFASs, musk fragrances and PBDEs can be taken up by plants from naturally or artificially contaminated soils. Hence, these pollutants can enter to the food chain, turning into a potential human health risk the use of sludge and/or compost for soil amendment and their agriculture application should be supervised and regulated.


Solid-phase extraction
Compost-amended soil
Bioconcentration factor
Triclosan
Liquid-chromatography-mass spectrometry
Analytical methodology
Dispersive
solid-phase extraction
SOIL
Endocrine disrupting compounds
Twister
metabolite
POM
accumulation
Plant uptake
Degradation
Translocation
PES
Lettuce
Bisphenol A
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