

Integrated Master in Bioengineering

Uptake of synthetic musks and volatile methylsiloxanes by pea crops grown in sewage sludge-amended soils

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Resumo

As lamas de depuração são resíduos provenientes do tratamento de águas residuais, produzidas em grandes quantidades e ricas em água, matéria orgânica e outros elementos que as tornam muito atrativas para aplicação a solos agrícolas, auxiliando no tratamento de solos e cultivo de colheitas. No entanto, estas lamas também podem possuir uma vasta gama de poluentes na sua composição, o que suscita preocupações quanto a possíveis interações prejudiciais que estes compostos nocivos possam vir a ter com os solos e plantas cultivadas, nomeadamente a sua tendência de se acumularem nos solos ou serem absorvidos (sofrerem *uptake*) pelas plantas e os perigos que o posterior consumo das mesmas possa trazer. Ao mesmo tempo, entre os poluentes detetados nas lamas de depuração encontram-se contaminantes de preocupação emergente (CECs), compostos até à data pouco estudados. Assim é revelada uma necessidade de estudar as interações e possibilidade de *uptake* destes novos compostos por culturas agrícolas para compreender melhor os riscos que poderão estar associados ao cultivo e consumo de colheitas agrícolas cultivadas em solos remediados.

Neste trabalho foi avaliada a presença e absorção de 2 classes de CECs (almíscares sintéticos e metilsiloxanos voláteis) em ervilheiras (*Pisum sativum*) cultivadas em solos tratados com lamas de depuração em diferentes taxas de aplicação e níveis de fortificação de poluentes. Para tal foi utilizada uma metodologia experimental baseada na técnica de QuEChERS (*Quick, Easy, Cheap, Rugged and Safe*) para a extração das amostras seguida por quantificação em GC-MS. Os resultados experimentais focaram-se num conjunto de 6 poluentes D3, D4, D5, D6, HHCB e AHTN, e revelaram comportamentos diferentes entre as duas classes de contaminantes: os almíscares sintéticos foram absorvidos pelas raízes das plantas, tendo sido detetados em concentrações até 346 ng/g em peso seco (ps), mas não sofreram translocação para as secções superficiais das mesmas em todos os grupos de teste, enquanto os metilsiloxanos voláteis foram absorvidos, detetados em raízes em concentrações até 108 ng/g ps, e translocados em quase todas as condições testadas. Os metilsiloxanos voláteis revelaram ser mais bioacumulativos segundo a análise dos fatores de bioconcentração (BCFs), cujos valores atingiram 51,36. Comparando concentrações finais de poluentes em solos semeados e não semeados, conclui-se que a acumulação dos poluentes no solo remediado foi significativamente limitada pela presença das plantas. Para avaliar efeitos nocivos associados à ingestão das ervilhas cultivadas nos solos remediados uma análise de dose de exposição estimada vs. dose semanal tolerável foi realizada considerando os dados mais pessimistas obtidos, concluindo-se que não há riscos aparentes para a saúde humana previsíveis segundo esta métrica. A aplicação de lamas de depuração a solos agrícolas poderá então ser de elevado interesse no tratamento de solos destinados ao cultivo de colheitas semelhantes.

Palavras-Chave: Lamas De Depuração, Solos Remediados, *Uptake*, Almíscares Sintéticos, Metilsiloxanos Voláteis.

Abstract

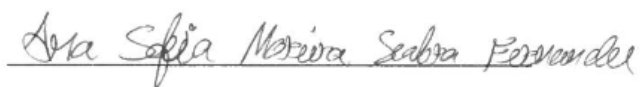
Sewage sludges are residues produced in large quantities resulting from wastewater treatment at wastewater treatment plants (WWTPs). Sludges are rich in water and organic matter along with some elements that make them very appealing for agricultural uses, such as soil treatment and conditioning and food crop growth. However, these sludges also contain a wide range of pollutants in their composition, which leads to concerns about possible harmful interactions these compounds may have with crops, namely their tendency to accumulate in soils or to be absorbed by plants (uptake) and any risky effects that consumption of said plants may produce. Furthermore, among the pollutants commonly detected in sewage sludges are several contaminants of emerging concern (CECs), pollutants that have not been greatly studied until recently. There is, therefore, a greater need to study plant-soil interactions and assess the uptake potential of these contaminants by food crops to better understand the risks that may be associated with the cultivation and consumption of crops grown in sludge amended soils.

In this work the presence and uptake of 2 CECs classes (synthetic musks, SMs, and volatile methylsiloxanes, VMSs) in pea plants (*Pisum sativum*) grown in amended soils was studied, including different application rates and sludge contaminants spiking concentrations. To do so, an extraction methodology based on QuEChERS extraction (Quick, Easy, Cheap, Rugged and Safe) followed by target quantification via GC-MS was used. The experimental results focused on a set of 6 compounds, D3, D4, D5, D6, HHCB and AHTN, and yielded different behaviours in the two contaminant classes: SMs underwent uptake by the plant roots, having been detected in concentrations as high as 346 ng/g on a dry weight basis (dw), but did not translocate to above ground sections of the plants in all test groups, while VMSs underwent uptake, having been detected in roots in concentrations up to 108 ng/g dw, and were translocated in nearly all conditions. VMSs were also revealed to be more bioaccumulative when analyzing the bioconcentration factors (BCF), which reached values as high as 51.36. Comparing the final pollutant concentrations of sown and unsown soils it was concluded that accumulation of the pollutants in the amended soils was significantly limited by the presence of plants. To assess the potential harmful effects of ingestion of the peas grown in the amended soils, a worst-case scenario analysis of estimated exposure dose vs. tolerable weekly intake was performed, concluding that no discernible risk to human health can be foreseen via this metric. The application of sludge to agricultural soils may therefore be of great interest in the treatment of soils destined to the cultivation of similar crops.

Keywords: Sludge, Amended-Soils, Plant Uptake, Synthetic Musks, Volatile Methylsiloxanes

Declaração

Declara, sob compromisso de honra, que este trabalho é original e que todas as contribuições não originais foram devidamente referenciadas com identificação da fonte.



24 de setembro de 2021

*“On the twenty-third day of the month of September
In an early year of a decade not too long before our own,
The human race suddenly encountered a deadly
Threat to its very existence.*

*And this terrifying enemy surfaced,
As such enemies often do,
In the seemingly most innocent and unlikely of places.”*

- Howard Ashman, “Prologue” from “Little Shop of Horrors”.

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Notation and Glossary

(Sorted alphabetically)

A	Slope
Acet	Acetone
ADI	Aceptable Daily Intake
ADBI	Celestolide
AHMI	Phantolide
AHTN	Tonalide
AHTN-d3	Tonalide-d3
ATII	Traseolide
BCF	Bioconcentration Factor
bw	Bodyweight
C18	Octadecyl-Silica
CECs	Contaminants Of Emerging Concern
cVMSs	Cyclic Volatile Methylsiloxanes
D3	Hexamethylcyclotrisiloxane
D4	Octamethylcyclotetrasiloxane
D5	Decamethylcyclopentasiloxane
D6	Dodecamethylcyclohexasiloxane
DCM	Dichloromethane
DPMI	Cashmeran
dw	Dry Weight
EB	Ethylene Brassylate
EED	Estimated Exposure Dose
EcoFEUP	Environmental Management System of FEUP
EI	Electron Ionization
EPS	Extracellular Polymeric Substances
EtAC	Ethyl Acetate
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
GH	Greenhouse
HBGV	Health Based Guidance Value
Hex	<i>n</i> - Hexane
HHCB	Galaxolide
HRT	Hydraulic Retention Time
K _{ow}	Octanol-Water Partition Coefficient
L3	Octamethyltetrasiloxane
L4	Decamethyltetrasiloxane
L5	Dodecamethylpentasiloxane
LogK _{ow}	Decadic Logarithm of the Octanol-Water Partition Coefficient
IVMSs	Linear Volatile Methylsiloxanes
M	Ratio of Target Mass and Internal Standard Mass
m/z	Mass-To-Charge Ratio
M4Q	Tetrakis(Trimethylsilyloxy)Silane
MA	Musk Ambrette

MK	Musk Ketone
MX	Musk Xylene
MX-d15	Musk Xylene-d15
nd	Not Detected
NOAEL	No Observed Adverse Effect Level
OC	Organic Carbon Content
OD	Outdoor
OM	Organic Matter
PCPs	Personal Care Products
PSA	Primary and Secondary Amine
Q1	QuEChERS 1
Q2	QuEChERS 2
QA/QC	Quality Assurance and Quality Control
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, Safe
r	Pearson Correlation Coefficient
Rec	Recovery Rate (%)
RF	Response Factor (Peak Area of The Compound/Peak Area of The Internal Standard)
RSD	Relative Standard Deviation
RT	Retention Time
S/N	Signal-To-Noise Ratio
s _a	Standard Deviation of the Slope
SIS	Selected Ion Storage
SMs	Synthetic Musks
SOM	Soil Organic Matter
SRT	Solids Retention Time
TOC	Total Organic Carbon
TSCF	Transpiration Stream Concentration Factor
TWI	Tolerable Weekly Intake
VMs	Volatile Methylsiloxanes
ww	Wet Weight
WWTP	Wastewater Treatment Plant

1. Introduction

1.1. Background and Presentation of the Project

Sewage sludge is the term used to refer to the solid or semi-solid by-product generated in primary, secondary, and tertiary treatment of wastewater from domestic and industrial sources, as well as street runoffs (Gardiner et al., 1995; EC, 2001a; Harrison et al., 2006). Due to the need for wastewater treatment, the production of sewage sludge is unavoidable and is expected to increase worldwide because of population growth and the progressive implementation of wastewater treatment legislation (Urbaniak et al., 2017), which brings accrued importance to sludge management options. These include three main alternatives, namely agricultural use (with or without sludge composting), incineration, and landfill (EUROSTAT, 2021), along with other usually less prominent options such as biogas-production or reuse in the construction industry (Areias et al., 2020; Cieřlik et al., 2015). Because sewage sludge is composed by organic matter, water, and several nutrients essential to crop growth (Seleiman et al., 2020), its application in the agriculture sector is an appealing option, with a series of associated agronomic and economic benefits. Its application in agricultural fields has a direct effect on the supply of nutrients to crops, some of which are considered finite resources (e.g., phosphorous), and irreplaceable for plant growth. In addition, this practice reduces the need for synthetic inorganic fertilizers and allows the improvement of soil properties, which in turn may improve the growth and productivity of crops and, consequently, result in more profitable soils and crops (Seleiman et al., 2020; Urbaniak et al., 2017; Badza et al., 2020). In fact, this is in line with the principles of circular economy (Gherghel et al., 2019).

Despite its many benefits, the adoption of agricultural reuse of sewage sludge varies greatly from country to country. Although a large percentage is reused in EU countries, such as Ireland, other countries apply less than 5% or even none of their produced sludges to soils (Hudcová et al., 2019). An important factor for this behaviour lies in the composition of these sludges. In addition to the macro and micronutrients that make sludge appealing for agricultural use, it also contains several contaminants, such as heavy metals, pharmaceutical and personal care products, pesticides, and industrial pollutants in different concentrations depending on the source of the waste and type of treatment used (EC, 2001b; Seleiman et al., 2020). The presence of these contaminants generates some concern regarding the input of these unwanted pollutants into the soil and crops, as well as environmental and health issues. This, coupled with the wide variety of pollutants in sewage sludge, some of which poorly understood regarding their interactions with soil and plants and fate in these compartments, has discouraged this practice (Reddy e Kim, 2015; Pullagurala et al., 2018). Examples of such pollutants are the so-called *Contaminants of Emerging Concern* (CECs), defined as substances currently not included in routine

environmental monitoring programs, and which may be candidates for future legislation due to their adverse effects and/or persistence (NORMAN Network, 2017). CECs comprise several classes of contaminants, including synthetic musks (SMs) and volatile methylsiloxanes (VMSs). These two classes of compounds are incorporated in consumer products, especially in personal care products. Therefore, large amounts of residues of these chemicals are discharged into sewer systems daily, reaching wastewater treatment plants (WWTPs). There, they are not totally degraded and due to their hydrophobic properties, these compounds tend to accumulate in sewage sludge. The behaviour of both classes of contaminants in amended soils has not been studied, nor their interactions with plants and plant uptake potentials, with few studies currently available. Therefore, the study of the fate of SMCs and VMSs in cropland and their overall risk to humans and the surrounding environment is extremely important.

Thus, in this work, the uptake of SMs and VMSs by pea plants (*Pisum sativum*) grown in sewage sludge-amended soils was investigated to fill some gaps regarding the uptake potential of the referred compounds by crops. To do so, greenhouse experiments were set-up, applying different sewage sludge rates and evaluating different levels of the target compounds. Subsequently, SMCs and VMSs were analysed in the different plant sections (root, peas, pods and remaining aerial sections) and in the soils. This work and its objectives are included within the scope of Project AGRONAUT- Agronomic Impact of Sludge Amendment Using a Comprehensive Exposure Viewpoint (PTDC/ASP-PLA/29425/2017; <https://sites.google.com/g.uporto.pt/agronaut/home>).

1.2. Contaminants of Emerging Concern in Sewage Sludges

“Contaminants of Emerging Concern” is a term that encompasses a wide range of pollutants, both organic and inorganic, with a common thread of being compounds previously overlooked and that have attracted more attention in recent decades due to reported negative or worrisome effects. One of largest records of CECs occurring in the environment is maintained by the NORMAN network, and by 2020 it comprises over 1000 individual substances, including flame retardants, food additives, industrial chemicals, pharmaceuticals, personal care products (PCPs), etc. (NORMAN Network, 2021). Most of these compounds enter the WWTPs through the sewage system, being only partially removed during treatment processes. Therefore, treated wastewaters and sewage sludge are primary sources of CECs into the environment (Parlapiano et al., 2021; Federle et al., 2014). Several classes of CECs have been detected in WWTP sludges over the past years, like pharmaceuticals, pesticides, synthetic musk fragrances (SMs) and volatile methylsiloxanes (VMSs) (Golovko et al., 2021; Homem et al., 2015; Capela et al., 2017). The latter two classes, SMs and VMSs, have been detected in WWTP sludges in varying

levels over the past twenty years, but their fate in sludge amended soils and their plant uptake potential is still poorly understood. For simplicity, in subsequent sections of this work the term “CECs” will be used to refer to both synthetic musks and volatile methylsiloxanes, excluding other compounds that also bear the same classification but are not objects of this study.

1.2.1. Synthetic Musks

1.2.1.1. Definition

The term musk encompasses a broad and structurally diversified spectrum of chemically defined substances, which exhibit “a common, distinct, and typical flavour” (Sommer, 2004). Musk compounds can be of natural origin, occurring in both animals and plants, or synthetically produced (Sommer, 2004; Bester, 2009). The use of synthetic musks has replaced the need for the extraction of natural musks, offering a more cost-effective source of compounds, with identical properties and performances, and allowing for high volume production that would be unsustainable to extract from natural resources (Balci et al., 2020). Musks are among the most important compounds used in the fragrance industry due to their odour and fixation properties (HERA 2004; Sommer, 2004). As a result, synthetic musks are widely used in several products, of which personal care and household cleaning products are excellent examples. Some of these products include, but are not limited to, bodywashes, hair care and styling products, creams and lotions, deodorants, detergents, fabric softeners, dishwashing liquids, and all-purpose cleaners (Roosens et al., 2007). After their domestic use by consumers, musk compounds are released into sewer systems via household effluents, reaching WWTPs (García-Jares et al., 2002). Synthetic musks have been detected in WWTP influents over the last two decades, as well as in both treated wastewaters and the resulting sewage sludges (Liu et al., 2014; Vallecillos et al., 2014). In fact, musks have been commonly detected in sewage sludge, in concentrations above 50000 ng/g for some compounds (Shek et al., 2008; Artola-Garicano et al., 2003; Sun et al., 2014). Sludge application to cropland can, therefore, represent an important source of musks for soils and plants, making the study of musk-soil and musk-plant interactions relevant and one of the focuses of this work.

1.2.1.2. Classification

Synthetic musks can be classified according to their chemical structure into four categories: nitromusks, polycyclic, macrocyclic, and alicyclic musks. The first subclass of synthetic musks to be produced was the nitromusks, which are nitrobenzene derivatives with two or three additional alkyl, keto or methoxy groups (Vallecillos et al., 2015). Nitromusks were widely used until the 1980s, when reports of their detection in fish (Yamagishi et al., 1983) and of their negative health effects emerged

and caused a decrease in their production (Sommer, 2004). Initially, it was found that musk ambrette caused photoallergic reactions (Giovinazzo et al., 1980). Since then, it has been documented that musk ambrette, as well as musk ketone and musk xylene, have other negative health effects, such as neurotoxicity, carcinogenicity, and oestrogenic activity (Hu et al., 2011; Polo et al., 2007). The last two compounds were considered highly persistent and bioaccumulative by the European Commission under REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) (EC, 2011). In response to the referred negative effects and to the documented accumulation in environmental matrices, the use of nitromusks has been progressively reduced (Bester, 2009; Sommer, 2004), with compounds being phased out of the market and legislation limiting or prohibiting their use. According to the latest revision of the European Parliament's Directive on Cosmetic Products, a set of 3 nitromusks (musks tibetene, ambrette and moskene) has been prohibited in cosmetic products in the European Union (EC, 2021), while usage of musk xylene and ketone in cosmetic products is restricted to legislated limits.

Since these restrictions on the use of nitromusks, polycyclic musks have been replacing them and emerged as the predominant class on the market (Balci et al., 2020). When compared to nitromusks, polycyclic musks have higher resistance to light and alkali environments and greater ability to bind to fabrics (Sommer, 2004). Among polycyclic musks, two compounds are especially relevant, galaxolide (HHCB) and tonalide (AHTN), which represent about 95 % of those commercially used of this subclass (Gatermann et al., 2002). Both compounds have been listed as high production volume chemicals by the Organisation for Economic Co-operation and Development and the Environmental Protection Agency (OECD, 2021; US EPA, 2021), with an annual production/import volume to the European Economic Area between 1000 and 10000 tons (ECHA, 2021a, b). Due to their high production and use in several products, galaxolide and tonalide also account for the highest detection levels in sewage sludge (average detected levels up 100000 ng/g), as reported in several recent studies (Biel-Maeso et al., 2018; Liu et al., 2014; Matamoros et al., 2012). Polycyclic musks have, however, been detected in human and other environmental matrices, including water, fish, blood, and adipose tissue (Fromme et al., 1999; Gatermann et al., 1999; Dsikowitzky et al., 2002; Kannan et al., 2005; Hutter et al., 2009; Moon et al., 2012). These findings caused some alarm when combined with information from recent reports that mention potential harmful health effects such as oestrogenic and anti-oestrogenic effects and increased photo-induced oxidative damage (Hu et al., 2011; Toivanen et al., 2008; Yamauchi et al., 2008; Fang et al., 2017). Therefore, the use of polycyclic musks in cosmetic products is, consequently, under discussion, with macrocyclic and alicyclic musks gaining additional interest as potential alternatives.

Macrocyclic musks are 15- or 17-membered ring systems that can be found in natural sources or be artificially synthesized (Vallecillos et al., 2015). They generally have more intense odours, which

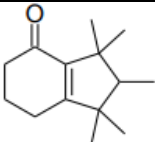
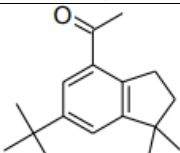
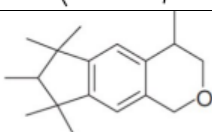
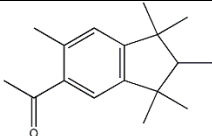
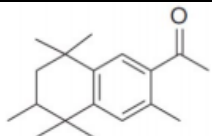
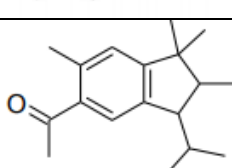
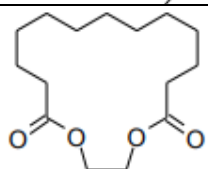
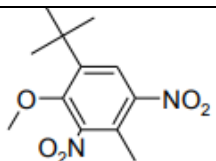
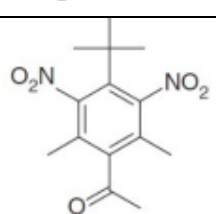
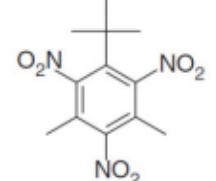
means that in comparison with polycyclic musks, smaller quantities of compounds are needed to achieve similar performance results (Li et al., 2018). They also present other compelling properties such as stability to light and alkaline conditions and higher fixation abilities, which make them of high value to the fragrance industry (Sommer, 2004). Synthetic macrocyclic musks have chemical structures that are more similar to natural ones and consequently, they seem to be more easily degradable in nature than nitro and polycyclic musks and are considered more environmentally friendly (Vallecillos et al., 2012). Despite this, they represent only a small share of the overall market (3-4%) and are currently used almost exclusively in perfumes due to the high cost related to their synthesis (Homem et al., 2015). An exception is the musk ethylene brassylate, which is relatively inexpensive due to its easy synthesis and low costs of the raw materials (Williams, 1999). The production costs of macrocyclic musks should decrease in coming years due to advances in synthetic chemistry and technology (Wong et al., 2019; Williams, 1999). The lower production costs along with macrocyclic musks' unique properties suggest that the use of these compounds may increase in coming years (Vallecillos et al., 2015).

Alicyclic musks are a relatively new category of synthetic musk odourants and are considered the fourth generation of SMs (Wong et al., 2019). They are also known as linear musks and are alkyl esters (Li et al., 2018). Due to their more recent development compared to the other classes, the use of alicyclic musks in personal care products is still very limited (Arbulu et al., 2011; Zhang et al., 2018). An increase in the use of these compounds in the coming decades is expected. In fact, due to their biodegradable properties and comparatively lower production costs than macrocyclic musks, some authors consider alicyclic musks to be the future of synthetic musk fragrances (Vallecillos et al., 2015).

1.2.1.3. Physicochemical Properties

Synthetic musks are semi-volatile, lipophilic, bioaccumulative and only partially biodegradable (Arbulu et al., 2011; Homem et al., 2015). The main physicochemical properties of the synthetic musks selected for the present work are summarized in Table 1. Among those properties are vapour pressure and boiling point, which are related to the compound's volatility. Polycyclic musks tend to have higher vapour pressures than the remaining musks, meaning they should, in theory, be the most volatile compounds among the selected musks. This is also in agreement with the presented values for boiling points. The octanol-water partition coefficient (K_{ow}) measures the hydrophobicity of a compound and can be used to assess the tendency of a pollutant to accumulate in sludge solids, with a high sorption potential attributed to compounds with $\log K_{ow}$ values above 4.0 (Rogers, 1996), which is the case of most of the selected musks. In terms of water solubility, all compounds present low values (which is in accordance with their lipophilic behaviour), despite not being necessarily indicative of a compound's polarity.

Table 1. Chemical structure and some physicochemical properties of the selected SMs.

Compound CAS nr. Abbreviation Molecular formula	Class	Chemical Structure	Molecular Weight (g mol ⁻¹)	Boiling Point (°C) ^a	Vapour Pressure (mmHg at 25 °C) ^a	log K _{ow} ^a	Solubility in water (mg/L at 25 °C) ^a
Cashmeran 33704-61-9 DPMI C ₁₄ H ₂₂ O	Polycyclic Musk		206.36	277.89	4.03×10 ⁻³	4.49	5.937
Celestolide 13171-00-1 ADBI C ₁₇ H ₂₄ O	Polycyclic Musk		244.41	319.07	1.44×10 ⁻⁴	5.93	0.2202
Galaxolide 1222-05-5 HHCB C ₁₈ H ₂₆ O	Polycyclic Musk		258.44	319.14	2.52×10 ⁻⁴	5.43	0.4900
Phantolide 15323-35-0 AHMI C ₁₇ H ₂₄ O	Polycyclic Musk		244.41	311.20	4.17×10 ⁻⁴	5.3	0.7504
Tonalide 21145-77-7 AHTN C ₁₈ H ₂₆ O	Polycyclic Musk		258.44	325.92	1.60×10 ⁻⁴	5.80	0.2388
Traseolide 68140-48-7 ATII C ₁₈ H ₂₆ O	Polycyclic Musk		258.44	323.74	1.95×10 ⁻⁴	5.76	0.2562
Ethylene Brassylate EB 105-95-3 C ₁₅ H ₂₆ O ₄	Macrocyclic Musk		270.41	434.44	4.38×10 ⁻⁷	4.71	1.719
Musk Ambrette 83-66-9 MA C ₁₂ H ₁₆ N ₂ O ₅	Nitromusk		268.30	367.93	1.31×10 ⁻⁵	4.17	2.107
Musk Ketone MK 81-14-1 C ₁₄ H ₁₈ N ₂ O ₅	Nitromusk		294.34	560.94	1.13×10 ⁻¹⁴	2.11	83.59
Musk Xylene MX 81-15-2 C ₁₂ H ₁₅ N ₃ O ₆	Nitromusk		297.30	570.75	4.92×10 ⁻¹⁵	2.25	61.35

^a US EPA, 2018.

1.2.2. Volatile Methylsiloxanes

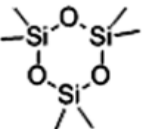
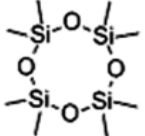
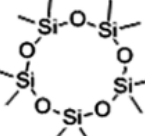
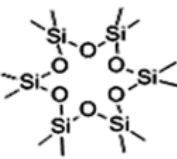
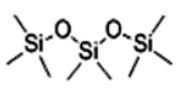
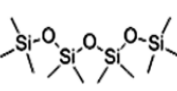
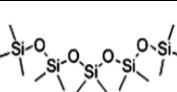
1.2.2.1. Definition and Classification

Volatile methylsiloxanes, or VMSs, are a class of organosilicon compounds, with a backbone of alternating silicon (Si) and oxygen (O) atoms in their structure, with methyl groups attached to each silicon atom (Wang et al., 2013a). They can be linear (IVMSs) or cyclic (cVMSs), with cVMSs being more used (Dewil et al., 2007). Due to their physicochemical properties (e.g., volatility, hydrophobicity and low surface tension) they have the ability to carry and release active ingredients (Alleni et al., 1997). For that reason, they are commonly added to personal care products (e.g., hair products and cosmetics), cleaning products, medical devices, rubber products, paper-coatings, and textiles (Montemayor et al., 2013; Feng et al., 2019). They are also involved in a wide range of industrial processes, such as the production of polymers or other types of silicones and are also used as dry-cleaning solvents and industrial cleaning fluids (Horii and Kannan 2008; Wang et al. 2009; Companioni-Damas et al., 2012; Dewil et al., 2006; Sanchis et al., 2013; Xu et al., 2013). This class of compounds, as well as other silicone-based compounds, are of great importance as they are essential components of a modern-day technological society (Bridges et al., 2016). Some cVMSs, such as D4, D5 and D6 have been classified as high production volume chemicals by the US Environmental Protection Agency (Hong et al., 2014). Due to their extensive use, high production and documented incomplete removal in conventional WWTPs, VMSs have been detected in several environmental matrices, including air, water, sediment, and biota, as well as in WWTP sludge (Genualdi et al., 2011; Sparham et al., 2008; Kaj et al., 2005; Sánchez-Brunete et al., 2010; Horii et al., 2019; Bletsou et al., 2013). In an effort to limit the input of VMSs into the environment, the use of two of the most highly used VMSs, D4 and D5, was recently limited to a maximum allowed concentration of 0.1% in weight in wash-off cosmetic products in the European Union (EC, 2018a). VMSs may also have negative implications to human health by accumulating in live tissues and blood (Flassbeck et al, 2001; Flassbeck et al. 2003) along with observed post-exposure damages to liver and lung tissues and estrogenic effects in mice (Lieberman et al., 1999; He et al., 2003). Consequently, VMSs have been categorized as contaminants of emerging concern (CECs), albeit more recently than synthetic musks, and therefore, they have not been so extensively studied. In fact, although some works have been published in the past years on VMSs fate in WWTPs and their emissions to the environment, few works have been developed regarding their interaction with soils and no plant uptake studies were found. Thus, the inclusion of this class of compounds in the present work is crucial to fill gaps in the referred domains.

1.2.2.2. Physicochemical Properties

Some of the properties that make VMSs appealing for such diverse applications include high thermal stability, lubricating properties, volatility, hydrophobicity, and low surface tension (Horii and Kannan, 2008; Wang et al., 2009; Lu et al., 2011; Alleni et al., 1997). They also have low solubility in water and potential to bioaccumulate, persist, and to suffer long-range transport (Capela et al., 2017), along with low degradation rates (Xu et al., 2012). Properties of VMSs may vary depending on their chemical structure. As previously referred, VMSs may be classified as linear or cyclic, with the two subclasses exhibiting slightly different behaviours, although all compounds are considered volatile (Table 2).

Table 2. Chemical structure and some physicochemical properties of VMSs.

Compound CAS nr. Abbreviation Molecular formula	Class	Chemical Structure	Molecular Weight (g mol ⁻¹)	Boiling Point (°C) ^a	Vapour Pressure (mmHg at 25 °C) ^a	Log K _{ow} ^a	Solubility in water (mg/L at 25 °C) ^a
Hexamethylcyclotrisiloxane 541-45-9 D3 C ₆ H ₁₈ O ₃ Si ₃	Cyclic		222.46	113.51	3.29	4.47	1.571
Octamethylcyclotetrasiloxane 556-67-2 D4 C ₈ H ₂₄ O ₄ Si ₄	Cyclic		296.62	159.41	1.18	5.10	4.646×10 ⁻²
Decamethylcyclopentasiloxane 541-02-6 D5 C ₁₀ H ₃₀ O ₅ Si ₅	Cyclic		370.77	196.78	0.218	5.20	5.007×10 ⁻³
Dodecamethylcyclohexasiloxane 540-97-6 D6 C ₁₂ H ₃₆ O ₆ Si ₆	Cyclic		444.92	226.77	0.0355	6.33	1.495×10 ⁻¹
Octamethyltrisiloxane 107-51-7 L3 C ₈ H ₂₄ O ₂ Si ₃	Linear		236.53	142.12	3.49	4.80	6.997×10 ⁻³
Decamethyltetrasiloxane 141-62-8 L4 C ₁₀ H ₃₀ O ₃ Si ₄	Linear		310.69	184.70	0.486	5.40	3.087×10 ⁻⁴
Dodecamethylpentasiloxane 141-63-9 L5 C ₁₂ H ₃₆ O ₄ Si ₅	Linear		384.84	217.20	0.0704	6.00	7.04×10 ⁻⁵

^a US EPA, 2018.

Linear siloxanes are considered to be more unstable than their cyclic congeners due to their ability to naturally self-degenerate into progressively more volatile and of lower molecular weight IVMSs (Ortega and Subrenat, 2009). Due to this instability, IVMSs are frequently studied as a group, and little importance is assigned to individual components, especially when compared to cVMSs. cVMSs are produced and used in larger scales than IVMSs, and their input to WWTPs is understandably higher. The combination of high vapour pressures, low water solubilities and large octanol/water partition coefficients (K_{ow}) of cVMSs also means that these compounds are more likely to adsorb to sludge extracellular polymeric substances (EPS) (Zeng et al., 2016) than to be released from WWTPs in the treated wastewater, making them obvious targets for monitoring in sewage sludge and to be studied in amended soil and plant interactions.

1.3. Sewage sludge as a source of CECs in soil

Sewage sludge is produced from primary, secondary and some tertiary wastewater treatments. Sludge can then undergo additional treatments to reduce its water content and make it safer for reuse. Water removal is essential to reduce the sludge volume, and stabilization is reached through pathogen destruction, applying anaerobic digestion, lime stabilization and composting. The term “treated sludge” may be used to refer to “sludge which has undergone biological, chemical or heat treatment, long-term storage or any other appropriate process so as significantly to reduce its fermentability and the health hazards resulting from its use” as per European Council Directive 86/278 (EC, 2018b). Additionally, sludge that has undergone stabilizing treatments can be referred to as “biosolids” if guideline requirements are fulfilled (ANZBP, 2009). During these sludge treatments, the concentrations of CECs can potentially decrease, but the removal efficiency depends on the type of process applied and the type of compounds under study. Therefore, concerns relating to the presence of CECs are still relevant even when considering biosolids and composted sludge. According to European Council Directive 86/278, sludge must be treated before agricultural use, excluding under some exceptional conditions to be established by member states, that may allow untreated sludge to be worked into or injected into soil (EC, 2018b).

As previously stated, sewage sludge contains wide variety of pollutants, including CECs. Both SMs and VMSs have been detected in sewage sludges around the world over the past two decades in varying levels. These levels are highest for the synthetic musks HHCB and AHTN and the VMSs D4, D5 and D6, which are usually detected in a range of 10 to 100000 ng/g (Homem et al., 2015; Capela et al., 2017). The variations in the concentrations of each CEC in sewage sludge depend on a wide array of factors tied to the compound’s physicochemical properties, population demographics, wastewater

origin, climatic and seasonal variations, wastewater treatment type in WWTPs, as well as WWTP design and operation parameters. One example of the latter's influence is the selection of parameters such as the hydraulic retention time (HRT) and the solids retention time (SRT). Higher SMs removal efficiencies have been associated with longer HRTs and SRTs (Clara et al., 2005), as they allowed more time for removal via adsorption to occur. This result is in line with the fact that the predominant removal mechanism of SMs and VMSs from wastewater treatment plants is the sorption to sewage sludge (Biel-Maeso et al., 2018; Bletsou et al., 2013; Horii et al., 2019).

One of the most relevant factors is the WWTP input mass loads, directly related with the overall use of a compound in consumer products, along with influent origin. For example, D5 is usually the VMS detected at the highest levels in WWTP sludges due to its greater use in consumer products (Horii and Kannan, 2008; Lu et al., 2011). The input influence is also verified when influent origin is taken into consideration. In the case of SMs, levels tend to be higher in sludges from domestic or mixed WWTPs, due to a dilution effect observed in purely industrial influents (Herren and Berset, 2000). Levels can however be very elevated in sludges from certain types of industries, such as the cosmetics industry. For example, Zeng et al. (2005) reported individual SMs levels up to 5416 ng/g in domestic WWTP sludge, 972 ng/g in sludge from food industry effluents and 169284 ng/g in sludge from cosmetic and household products industry effluents. VMSs have been detected in greater levels in sludges from domestic and mixed WWTPs as opposed to industrial WWTPs. This was verified in a study of 40 WWTPs by Lee et al. (2014), who reported average Σ VMSs concentrations of 66100 ng/g and 57600 ng/g for sludges from domestic and mixed WWTP sludges respectively, and of 16790 ng/g in industrial WWTP sludge. However, when analyzing each WWTP individually, the authors found that there was an industrial WWTP among the top 4 plants with the highest detected concentrations of siloxanes in produced sludge. The industrial WWTP in question received wastewater from textile industries, highlighting that some industrial activities may greatly contribute to emissions of VMSs into waste streams.

CECs levels in both influent/effluent and sludge are also influenced by seasonal and demographic variations (including differences in population habits and usage patterns of certain products). During warmer months, the use of PCPs by populations, and by extension the input of CECs into WWTPs, increases. This does not necessarily translate into higher CECs levels in sludges, in fact, SMs and VMSs tend to be detected in lower concentrations in sludge during warmer seasons (Biel-Maeso et al., 2018; Li et al., 2016), due to heightened volatilization in higher temperatures or due to greater degradation by more active microorganisms (Biel-Maeso et al., 2018; Lv et al., 2010; Müller et al., 2006). In spite of this, a study by Oshita et al. (2015) found slightly higher concentrations of D5 during the summer period. The differences in levels were very small (1.76 mg/L in summer and 1.20 mg/L in winter) and the study was conducted in a temperate area with smaller variations in temperature over the year, a factor that could explain the results. Since the aforementioned factors can vary greatly from one

WWTP to another, the analysis of sewage sludge becomes especially relevant whenever agricultural valorisation is of interest.

1.4. Fate of CECs in Soils

Once sewage sludge is applied to soils, the fate of the pollutants present in it can be somewhat unpredictable. Contaminants present in soils can be subjected to several processes such as sorption/desorption, degradation (biotic and abiotic), volatilization, erosion/runoff, leaching and uptake by plants, which in turn dictate the final fate of the substance. Which process or combination of processes that any contaminant will undergo depends in the first place on its physicochemical properties (e.g., water solubility, adsorption behaviour, volatility) (Graber and Gerstl, 2011) and degradation potential, as well as the surrounding environmental conditions (e.g., temperature, exposure to sunlight, microbial activity) (Bu et al., 2016). Regarding SMs and VMSs, studies on their behaviour in soils are few and tend to focus on a relatively narrow range of compounds, including galaxolide (HHCB), tonalide (AHTN), D4 and D5.

In the case of SMs, strong sorption to soil particles has been reported (Litz et al., 2007). One of the first assessments of SMs' fate in sewage sludge amended soils was done by DiFrancesco et al. (2004), who besides HHCB and AHTN, also studied cashmeran (DPMI) and musk ketone (MK) along with other fragrance materials not included in this study. In tray experiments, 4 types of soil were tested, with soil organic matter (SOM) content ranging from 0.61% to 7.01%. AHTN, HHCB, MX and MK were still detected in all soil types 3 months after the experiment began, with dissipation of AHTN and MK being particularly slow. It was also found that, in general, the greater the SOM content, the greater the retention of SMs in soil, except for MK. The authors also drew attention to the fact that the addition of sewage sludge did not significantly increase soil organic matter, and that most of the present organic carbon was derived from the tested soils. In a later study, Litz et al. (2007) shared several of the findings presented by DiFrancesco et al. (2004), namely the influence of organic carbon/SOM content on sorption and the seemingly stronger sorption of AHTN to soil when compared to HHCB. The authors also found that soil clay content may have a slightly stronger influence on AHTN sorption, but overall SOM was a more relevant factor. The adsorption of both SMs was described using Freundlich isotherms, obtaining soil adsorption rates of 68.7 – 94.0% for HHCB and 76.4 – 94.1% for AHTN. The same authors also perform dissipation studies, finding that the pollutants suffered no relevant microbial attack and the aerobic dissipation appeared to be very slow; after 24 weeks more than 73.5% of the initial AHTN and 60 – 65% of the initial HHCB remained in soils. In a more recent study (Rivier et al., 2019), the dissipation of AHTN and HHCB over a 3-month period in sludge amended soil was observed. This

outdoor pot experiment also showed higher persistence of AHTN (66 – 81% dissipation) when compared to HHCB (75 – 91% dissipation). This study also found that the tested sludge application rates (20 ton/ha and 60 ton/ha) had no discernible effect on chemical dissipation in soil, as HHCB and AHTN seemed to dissipate in the same proportion as they had been added to soil. Overall, HHCB and AHTN are considered to be very persistent in soil (Litz et al., 2007), in part due to their reported strong adsorption to SOM, which reduces their bioavailability for microbial degradation and their potential for plant uptake (Alexander, 2000). The calculated half-life times of these CECs in soils have been reported as between 10-17 months for HHCB and 2-24 years for AHTN (Litz et al., 2007). Another phenomenon to take into consideration is leaching. Leaching occurs when substances are lost from upper layers of soil and moved to deeper layers, due to being carried downward by percolating rain or other similar waters. Leaching studies are important to assess if a compound could be transported deep enough to pose a contamination risk to deeper soil layers or even groundwaters, but so far, no alarming leaching study results have been reported. Litz et al. (2007) performed leaching studies, finding that for both HHCB and AHTN the leaching capacity was negligible (<0.001%). DiFrancesco et al. (2004) found that musk ketone and musk xylene did not undergo significant leaching, and that cashmeran was the only tested fragrance material to leach at a rate greater than 1%. It was also theorized by DiFrancesco et al. (2004) that musks ketone and musk xylene had most likely been removed through transformation.

The interactions of VMSs with soil have been less studied than those of SMs. No data was found regarding the behaviour of IVMSs, but some information on cVMSs is available. It has been reported that cVMS in soil can be degraded, most likely by abiotic processes such as hydrolysis and volatilization, with biodegradation seeming unlikely (Xu, 1999; Xu and Chandra, 1999; Wang et al., 2013b). In fact, it has been shown that the level of degradation/volatilization that occurs varies according to the soil moisture content - degradation of cVMSs seems to be more significant than volatilization in less humid soils, while in those with high moisture, abiotic degradation seems to slow down, while volatilization is accelerated and becomes the predominant removal mechanism (Xu and Chandra, 1999). VMSs in soil can be hydrolyzed into silanols, which in turn can be degraded to carbon dioxide and inorganic silicate (Lehmann et al., 1994). cVMSs half-lives in soils have been estimated to be much shorter than those reported for SMs. Xu and Chandra (1999) estimated the half-lives of D5 and D6 to be around 115 minutes and 33 hours respectively, in a soil with 55% clay content and relative humidity of 32%. The authors estimated the half-life of D4 in two different soil types (clay contents of 20% and 55%) with soil humidity ranging from 32 to 100%. The estimated values ranged from 50 minutes, in the less humid, higher clay content soil, to 5 days, in the lower clay content soil at 92% relative humidity. Faced with the previous values, the authors considered the persistence of these compounds in soils to be unlikely. Not much focus has been given to the behavior of VMSs in amended soils. Wang et al. (2013b) studied the concentrations of D4-D6 in several matrices, among which several biosolid amended soil samples were

included. Of the 3 target pollutants, D6 was present in the highest levels followed by D5, while D4 was below detection level ($0.008 \mu\text{g/g dw}$) in several samples. The authors concluded their study considering D5 to not pose as a risk to plants and terrestrial animals in biosolid-amended soils due to its relatively short half-life in soil. In an environmental assessment study, estimating the partition of L3, D3, D4, D5 and D6 across several matrices (Surita and Tansel, 2014), D3 and D4 were considered to pose the greatest hazard for land application of biosolids. The authors considered D3 undergoes minimal anaerobic degradation, ranking its hazard potential as the highest in biosolids, and D4 as presenting the greatest risk of exposure in the biosolids and liquid phases.

In nearly all previous works, the influence of plants on the fate of the compounds in the soil was not taken into consideration. Although the reported phenomena could, in theory, still occur in a soil with plants, other processes not contemplated could also potentially happen, such as increased dissipation and degradation due to the microbial activity associated with plant root systems and plant uptake of CECs.

1.5. Plant Uptake of CECs

In the present context, the term uptake refers to the transfer of substances from a plant's surrounding environment into its tissues. This transfer can happen from plant roots in contact or through the stomata present on plant leaves (Colon and Toor, 2016). Root uptake of organic compounds occurs when the plant takes up contaminants from the rhizosphere by passive diffusion through the roots. Once the contaminants have entered the root hairs, they may reach the xylem through the cortex, endodermis and Casparian strip (Miller et al., 2016). If pollutants manage to reach the xylem, they may be translocated to the aerial parts of the plants, such as the leaves, by a combination of mechanisms - root pressure, capillary action, and the transpiration stream action (Keerthanan et al., 2021). Several factors dependent on both the environment and contaminants must be considered regarding plant uptake. Environmental factors that can influence plant uptake include temperature, soil composition (e.g., water and organic matter content) and type of agricultural practices, while contaminant-dependent factors include especially physicochemical properties (e.g., vapour pressure, molecular weight, water solubility and $\log K_{ow}$) (Colon and Toor, 2016). The compounds' concentration and their stability in soil can also be noteworthy factors, as compounds with half-lives shorter than 14 days are less likely to undergo uptake by plants (O'Connor, 1996). In general, hydrophobic compounds tend to remain localized in roots, while hydrophilic compounds are more easily translocated to aerial parts through transpiration stream (Wu et al., 2013). The relationship of $\log K_{ow}$ values of organic pollutants with plant uptake has been described by a normal distribution curve, according to Briggs et al.

(1982), who have reported that maximum translocation of chemicals is observed when $\text{Log } K_{ow}$ is around 1.78, at least in a simplified scenario with hydroponic cultures. However, some hydrophobic contaminants have been found to undergo translocation by binding to latex-like proteins present in the xylem sap (Goto et al., 2019).

When uptake occurs and pollutants enter the plant system, it is interesting to assess their fate within the plant, namely their potential to undergo translocation, especially when the edible crop parts are aerial and would not have direct contact with the pollutants in the soil. In fact, accumulation of contaminants is generally expected to be greater in root vegetables than other crops that are not in direct contact with soil (Colon and Toor, 2016), which has led some countries to allow the application of sewage sludge in agricultural fields only containing crops where the edible part has no direct contact with soil, such as cereals. Translocation efficiency may be quantified by the Transpiration Stream Concentration Factor (TSCF), but one of the most used metrics to describe the health risks related to plant uptake of contaminants has been the Bioconcentration Factor (BCF) described by Equation (1) (Ziarrusta, 2015):

$$BCF = \frac{\text{Concentration in dry plant tissue}}{\text{Concentration in dry soil}} \quad (1)$$

In this work concentration in dry soil is considered as the concentration at the beginning of the experiment though BCF can also be calculated with concentrations in soil measured at the end of uptake experiments (Macherius et al., 2012). BCF values below 0.01 have been considered to imply minimal contamination and very reduced risks to animals via plant consumption, however this value is somewhat arbitrary (O'Connor, 1996). This metric is primarily concerned with the degree of accumulation in plant tissues and does not take into consideration factors like the frequency of exposure via ingestion. In regulatory dietary risk assessments of chemicals, instead of metrics like BCF the competent authorities use a specific health-based guidance value (HBGV) for the chemical under assessment and compare this value with the dietary exposure of the chemical to identify health risks (FAO/WHO, 2009). Dietary exposure can be translated into parameters like the Estimated Exposure Dosage, while HBGVs like the Acceptable Daily Intake (ADI) and the Tolerable Weekly Intake (TWI) are usually derived from the No Observed Adverse Effect Levels (NOAEL) obtained via laboratory animal studies (Zarn et al., 2015).

Lastly, it should be noted that, overall, some of the apparently relevant factors in the uptake studies are related to the characteristics of the studied plant, namely the root lipid content and carbohydrate content of root cell walls, transpiration rate, shape and size of leaves and the permeability of root cell membranes (Colon and Toor, 2016; Chen et al., 2009; Zhang et al., 2017; Zhang and Zhu, 2009). The uptake of organic contaminants can therefore vary greatly from one plant species to another, and selected crop characteristics should be taken into account when assessing the uptake risk of

pollutants. In subsection 1.5.1. the characteristics of the selected crop, *Pisum sativum*, are briefly summarized.

Uptake studies of the selected CECs are scarce and have not yet been conducted with the selected crop. In the few studies available, only some synthetic musks were assessed. These works mention that the main factors governing the uptake process of the selected compounds are their hydrophobicity and the organic matter content of the soils. In fact, uptake and translocation of SMs are dynamic, time dependent processes, not likely to reach a state of equilibrium (Macherius et al., 2012). Detailed information on those uptake studies may be consulted in section 2.

1.5.1. Selected Crop Characteristics

Pisum sativum is a widely popular cool-season crop, with a few different varieties available, such as garden peas, fields peas and vegetable peas. Growth times vary depending on the season, with major producers located in Canada, parts of Europe, United States of America, and Australia (Khan et al., 2016). The crop is mainly produced for consumption of seeds (peas). Besides its clear value as a food source, the crop can serve as a forage crop for livestock and as a rotational crop, meaning that it is frequently introduced into crop rotations to improve soils and break up disease and pest cycles (Khan et al., 2016).

Pisum sativum is also a green manure crop, meaning specimens can fixate atmospheric nitrogen, making it available for the following crop. This ability is tied to root-soil interaction. Pea plants have extensive root systems, branched out in both width and depth, with the larger taproot branching into thinner structures until the root-hairs. Root-hairs can develop growths due to symbiotic relations with soil biota, namely the bacteria *Rhizobium leguminosarum*, that allow for nitrogen fixation, enriching the soil (Pavek, 2012). Regarding the remaining parts of the plant, most varieties are 30-150 cm in height (Khan et al., 2016), with smooth, hollow stems (Figure 1). Due to the stems being relatively lightweight, specimens possess thin vine-like structures called tendrils, that offer additional support by curling themselves around neighbouring plants or man-made trellises. The leaves are made up of two large leaflike stipules in the shape of a ruff, terminal tendrils, and a varying number of leaflets, that in many modern cultivars are converted into additional tendrils (McGee cited by Pavek, 2012). Pea plants also have flowers from which the peapods sprout and are considered a strictly self-fertilizing species (Cousin, 1997). All the aerial sections of the plant, except for the temporary flowers, are labeled in Figure 1.



Figure 1. *Pisum sativum* L. a - stem, b - tendrils, c - leaves, d - immature pods.

Pisum sativum L. plants are infected with plant pathogens with varying degrees of impact on crop yield, from foliar diseases like bacterial blight (caused by *Pseudomonas syringae* pv. *Pisi*), powdery mildew (*Erysiphe pisi*), to seed and root rots, viral diseases, and insect pests like aphids (*Acyrtosiphon pisum*) (Pavek, 2012). Despite these vulnerabilities, the crop remains extremely popular and the demand for peas is likely to increase according to global population growth (Khan et al., 2016).

2. State of the Art

Few plant uptake studies focusing on the selected CECs have been published so far. Of the very few available uptake studies, all addressed the uptake of SMs, and none assessed the uptake of VMSs. In fact, most uptake studies focus on other classes of contaminants, such as heavy metals, pesticides, and pharmaceutical compounds. To investigate the uptake of CECs from soil, studies have been conducted using amended soils (applying sludge, compost or biosolids), soils irrigated with reclaimed wastewaters or by directly spiking the soil with the desired contaminants before sowing. Since this work focuses on the uptake of contaminants from sewage sludge amended soils it would be more logical to review only works carried out with similarly amended soils. However, due to the scarcity of plant uptake studies, the literature review was expanded to also include soils treated with biosolids/composted sludge or done with direct spiking of non-amended soil, totaling 7 studies, whose results are summarized in Table 3. These studies were conducted under different conditions, with one study being carried out with both greenhouse and field experiments (Litz et al., 2007), one with just outdoor pot experiments (Rivier et al., 2019), and the remaining in controlled environments exclusively. It should be noted that, although field experiments more closely resemble the conditions of an agricultural field, subjected to climatic variations and exposure to the elements, they include many more variables that need to be considered. Due to the simplified conditions, greenhouse experiments provide easier-to-interpret results and can provide valuable information, especially in the early stages of study. However, bioavailability of some compounds may be higher than in open field environments (Keerthanan et al., 2021).

The first study of SMs' plant uptake found in literature was carried out by Litz et al. (2007), who investigated galaxolide (HHCB) and tonalide (AHTN) with carrots and lettuce in sludge amended soils. The lettuce and carrot crops were selected as representatives of "worst-case scenarios", being crops with high mass of leave surface and with high oil/fat content in roots, respectively. Lettuce experiments were conducted both in greenhouse, evaluating different types of soils, and in field conditions. Higher levels of SMs were detected in the outdoor plants, but the authors theorized that this could be due to rain splash, a factor that is not present in a greenhouse environment, combined with the high plant biomass close to the soil surface. Higher concentrations of AHTN were detected across all lettuce samples, which the authors could not explain without further investigation. The authors, however, note the differences in vapor pressure and solubility between the two musks (HHCB is slightly more volatile and soluble) as a possible contributing factor, especially when applied to plants with high surface biomass. In the greenhouse experiments, lettuce and carrot cultures were grown in 3 different soil types - a gleyic Podzol soil rich in organic carbon content (OC) (5.8% OC, 3.5% clay), a Luvisol soil richer in clay content (3.1% OC, 29.5% clay) and a Cambisol soil (1.2% OC, 3.6% clay), which had also been used for

the outdoor experiments. Once again, levels of AHTN in lettuce were higher than those of HHCB but the same was not observed in carrot roots, in which higher levels of HHCB were detected in samples grown in the tested soils. The highest levels of AHTN and HHCB were detected in the roots of carrots grown in the clay rich Luvisol soil, probably due to closer contact between the roots and finer particles of this soil. Overall, the use of different types of soil led to different results, even using the same plant. In fact, soil particle size and organic matter content are key factors in this type of study. Litz et al. (2007) concluded that in soils with higher organic matter content there was less uptake of CECs into plant tissues, which was also later verified by Macherius et al. (2012) and Ziarrusta (2015). Since higher levels of organic matter are associated with greater sorption of SMs to soils, it would be expected that plant uptake is lower due to the decrease in the pollutants' bioavailability. The same authors also concluded that plant uptake by volatilization of SMs from soil did not play an important role in the results.

Macherius et al. (2012) also studied the uptake of HHCB and AHTN by carrots, along with barley and meadow fescue plants. In their experiments with spiked soils (4300 ng/g of AHTN and 1000 ng/g of HHCB), AHTN and HHCB were detected in all analyzed plant parts being determined at higher levels in the root peel, followed by the root core and finally the leaves. The calculated BCFs were different for HHCB (0.89 in roots and 0.76 in leaves) and AHTN (0.50 in roots and 0.18 in leaves), with higher values obtained for HHCB. Ziarrusta (2015) also reported higher accumulation of HHCB than AHTN in carrots, which was explained by the higher water solubility of HHCB, one of the factors Litz et al. (2007) had proposed to explain similar results. Both Macherius et al. (2012) and Ziarrusta (2015) observed that the levels of HHCB and AHTN in carrot peels seemed to reflect the concentrations of the pollutants in the culture soils, finding higher concentrations in samples from soils with higher initial pollutant levels. Macherius et al. (2012) also observed that the differing levels of HHCB and AHTN were in line with the already differing levels of the individual pollutants in the soils. The concentrations in the carrot root cores and leaves obtained by Macherius et al. (2012) were more similar between the two musks, indicating similar translocation rates for both compounds. The results reported for the carrot experiments were then combined with those obtained in the previous study by Litz et al. (2007), indicating a strong relationship between BCFs and the soil total organic carbon (which is related to the SOM content). This was later also verified by Ziarrusta (2015), pointing out once again that uptake/translocation is lower in soils with higher SOM. Macherius et al., (2012) were also able to verify the relationship between $\text{Log } K_{ow}$ values and BCFs originally proposed by Briggs et al. (1982), despite it having originally been described for much simpler conditions (hydroponic cultures instead of a complex soil-plant system), concluding that BCFs were higher the highest the $\text{Log } K_{ow}$ value of a compound. However, because the $\text{Log } K_{ow}$ values of the two musks are in such a narrow range ($5.43 < \text{Log } K_{ow} < 5.9$) the authors warn that further studies are needed to confirm the relationship for other compounds. As the concentrations of both musks were almost identical, translocation from root to leaves was

considered to be a rate limited process (volatilization from soil to root surface was once again dismissed). Regarding the results obtained with barley and meadow fescue plants, the most relevant conclusions are related to the higher levels of SMs in the roots of these plants when compared to the carrots roots. The explanation provided by the authors ties with the higher overall surface area to volume ratio of the more ramified barley roots when compared with carrot roots, which may facilitate the transfer of pollutants by diffusion. Translocation of SMs in these plants was relatively low.

Rivier et al. (2019) analyzed grains of barley sown into sludge amended soil in outdoor pot experiments. The authors reported HHCB and AHTN comparable to control grains or below detection limits. However, the absence of the two target SMs in the grains does not rule out the presence of any non-targeted metabolites of AHTN or HHCB. Chen et al. (2010) performed experiments with another cereal, wheat, regarding AHTN uptake. They found AHTN concentrations in wheat seedlings to be higher in roots, followed by stems and lastly leaves, which is similar to what was observed by Macherius et al. (2012) with carrots and by Zhang et al. (2019) with the ornamental plant *Bougainvillea spectabilis*. Uptake in the seedlings increased with the AHTN levels in soils and fitted a linear equation. It should however be noted that seedlings are very young plants and that an increase in biomass due to plant growth has led to lower detected levels over time (Macherius et al., 2012).

To the authors' best knowledge, the most recent study on the topic (Ramos et al., 2021) studied the uptake of SMs and UV-filters by tomatoes (*Solanum lycopersicum* L. cv. Micro-Tom) grown in sludge-amended soils via pot experiments carried out in a climatic chamber. It is the only study to include SMs other than HHCB and AHTN, presenting results for a total of 8 musks (DPMI, ADBI, AHMI, ATII, HHCB, AHTN, MX, MK). HHCB was the SM detected in the highest levels (35 – 68 ng/g dw) followed by DPMI, AHTN and ATII (up to 5 ng/g dw) in tomato fruits. The reported levels in the tomato fruits were higher than the ones reported in barley grains. Therefore, even when the edible plant part is not in direct contact with soil, the risk may still vary from crop to crop, most likely due to plant biological and chemical characteristics. All the other polycyclic musk compounds were not detected in the analyzed tissues but were also not present in the amended soils at the beginning of the experiment in high levels (nd – 1.62 ng/g dw), which is in line with the usual detection patterns of these pollutants in WWTP sludges. Of the two nitromusks selected for the study, musk xylene (MX) was not found in either control soil, sludge amended soil or in plant parts, but musk ketone (MK) was detected in the tomatoes despite not having been detected in either soil at the beginning or at the end of the experiment. The only explanation for this result is the occurrence of an undetected contamination during the experiment.

Table 3. Plant uptake studies of synthetic musks.

Compounds	Plant	Experimental Conditions	Initial Pollutant Concentration in Soil (ng/g dw)	Detected Levels in Plant Samples (ng/g dw)	Reference
HHCB, AHTN	Lettuce (<i>Lactuca sativa</i>); carrot roots and leaves (<i>Daucus carota</i>).	Sludge-amended soil experiments in greenhouse environment and outdoor field plots. 3 types of soil tested: Cambisol (1.20% OC; 5.40 pH), Gleyic Podzol (5.80 % OC; 5.10 pH) and Luvisol (3.10% OC; 6.10 pH).	HHCB:30000 AHTN:30000	<u>HHCB:</u> Lettuce: Cambisol soil (GH): 110 Gleyic Podzol soil (GH): 105 Luvisol soil (GH): 78 Cambisol soil (OD): 198 Carrot Leaves: Gleyic Podzol soil (GH): 107 Luvisol soil (GH): 1070 Carrot Root: Gleyic Podzol soil (GH): 3180 Luvisol soil (GH): 14300 <u>AHTN:</u> Lettuce: Cambisol soil (GH): 275 Gleyic Podzol soil (GH): 252 Luvisol soil (GH): 209 Cambisol soil (OD): 738 Carrot Leaves: Gleyic Podzol soil (GH): 115 Luvisol soil (GH): 902 Carrot Root: Gleyic Podzol soil (GH): 2700 Luvisol soil (GH): 12800	Litz et al., 2007.
AHTN	Wheat seedlings (<i>Triticum aestivum</i>).	Spiked soil experiments in culturing boxes. Soil with 3.86% OM, 48.90% clay and pH 6.23.	Control soil: 0 Soil A: 2500 Soil B: 10000 Soil C: 20000	<u>Control soil:</u> Root: ≈ 5560 Stem: nd Leaves: nd <u>Soil A:</u> Root: ≈ 15740 Stem: ≈ 960 Leaves: ≈ 960 <u>Soil B:</u> Root: ≈ 25000 Stem: ≈ 4630 Leaves: ≈ 1850 <u>Soil C:</u> Root: ≈ 73150 Stem: ≈ 15740 Leaves: ≈ 3700 (Values taken from image)	Chen et al., 2010.
HHCB, AHTN	Carrot roots and leaves (<i>Daucus carota</i> ssp. <i>sativus</i> , cvs Napoli, Amager, Rothild and Nutri-Red), Barley root (<i>Hordeum vulgare</i> , cv Edel), Meadow Fescue root (<i>Festuca pratense</i> , cv Fure)	Spiked soil experiments in greenhouse environment. Soil with 0.90% TOC and pH 5.5, mixed with a commercially available, slow-release fertilizer (3 g/kg soil).	HHCB: ≈1000 AHTN: ≈4300	<u>HHCB:</u> Carrot root peel: 4350 Carrot root core: 220 Carrot root (total): 860 Carrot leaf: 740 Barley root: 810 Meadow fescue root: 1770 <u>AHTN:</u> Carrot root peel: 13410 Carrot root core: 300 Carrot root (total): 2320 Carrot leaf: 820 Barley root: 6900 Meadow fescue root: 12660	Macherius et al., 2012.

Table 3. Plant uptake studies of synthetic musks (Cont.).

Compounds	Plant	Experimental Conditions	Initial Pollutant Concentration in Soil (ng/g dw)	Detected Levels in Plant Samples (ng/g dw)	Reference
HHCB, AHTN	Carrot root and leaves (<i>Daucus carota</i> ssp. <i>sativus</i> , cv Chantenay)	Compost amended-soil pot experiments in greenhouse environment. Soil with 2.30 ± 0.50% TOC, and pH 7.20.	<u>High Level (ww): *</u> HHCB: 5000 AHTN: 5000 <u>Low Level (ww): *</u> HHCB: 0.80 – 1.9 AHTN: 0.80 – 1.9	<u>HHCB (Low Level):</u> Peel: 30 Leaves: 10.5 Core: < 1.2 <u>HHCB (High Level):</u> Peel: 1321.5 Leaves: 34.5 Core: 51 <u>AHTN (Low Level):</u> Peel: 20.5 Leaves: 10 Core: < 1.5 <u>AHTN (High Level):</u> Peel: 1024 Leaves: 27 Core: 38	Ziarrusta., 2015.
HHCB, AHTN	Barley grains (<i>Hordeum vulgare</i> L., cv. Heder)	Sludge-amended soil experiments in outdoor pots. Soil with 4.40% OM, 26.0% clay, pH 5.10.	<u>Soil: Sludge A: *</u> HHCB: 139.40 AHTN: 49.20 <u>Soil: Sludge B: *</u> HHCB: 344.38 AHTN: 86.09 <u>Soil: Sludge C: *</u> HHCB: 188.59 AHTN: 69.70	<u>HHCB:</u> Control soil: 0.5±0.2 Soil: Sludge A: 0.5±0.3 Soil: Sludge B: < 0.2 Soil: Sludge C: < 0.2 <u>AHTN:</u> Control soil: < 0.2 Soil: Sludge A: 0.9±0.1 Soil: Sludge B: < 0.2 Soil: Sludge C: < 0.2	Rivier et al., 2019.
HHCB	<i>Bougainvillea spectabilis</i> (roots, stems and leaves).	Spiked soil experiments in pots, conducted in calorstats. Soil with 3.86% OM and pH, 7.79.	Soil A: 50000 Soil B: 100000	<u>Soil A:</u> Root: 31.16 Stem: 11.83 Leaf: 20.76 <u>Soil B:</u> Root: 75.18 Stem: 30.45 Leaf: 31.22	Zhang et al., 2019.

Table 3. Plant uptake studies of synthetic musks (Cont.).

Compounds	Plant	Experimental Conditions	Initial Pollutant Concentration in Soil (ng/g dw)	Detected Levels in Plant Samples (ng/g dw)	Reference
DPMI, ADBI, AHMI, ATII, HHCB, AHTN, MX, MK	Tomato fruit (<i>Solanum lycopersicum</i> L. cv. Micro-Tom)	Biosolid amended soil experiments conducted in a climatic chamber. Soil with 1.50% OC and 4.80% clay; perlite added to soil.	<u>Control soils:</u> DPMI: nd ADBI: nd AHMI: nd ATII: nd HHCB: 2.0 ± 0.6 AHTN: 1.5 ± 0.1 MX: nd MK: nd <u>Amended soils:</u> DPMI: 1.36 ± 0.05 ADBI: 1.62 ± 0.01 AHMI: 1.04 ± 0.01 ATII: 2.52 ± 0.02 HHCB: 341 ± 5 AHTN: 88 ± 1 MX: nd MK: nd	<u>DPMI:</u> Control soils: nd Amended soils: 5 ± 4 <u>ADBI:</u> Control soils: nd Amended soils: nd <u>AHMI:</u> Control soils: nd Amended soils: nd <u>ATII:</u> Control soils: 2.4 ± 0.2 Amended soils: 2.6 ± 0.3 <u>HHCB:</u> Control soils: 35 ± 12 Amended soils: 68 ± 32 <u>AHTN:</u> Control soils: 3 ± 1 Amended soils: 4 ± 2 <u>MX:</u> Control soils: nd Amended soils: nd <u>MK:</u> Control soils: nd Amended soils: 1.3 ± 0.7	Ramos et al., 2021.

dw – dry weight; ww – wet weight; OC – Organic carbon content; GH – Greenhouse; OD – Outdoor; OM – Organic matter; TOC – Total organic carbon

*Converted values.

Regarding the uptake of VMSs, the knowledge gap is even greater, and no studies on plant uptake have been found at the time of writing this document. Some studies reporting the detection of VMSs in vegetation, including grasses, mosses, and lichens (Sanchís et al. 2015), pine needles (Ratola et al., 2015), and marine vegetation (Rocha et al., 2019) have been published in recent years, but in all of these works the focus was on assessing the impact of contamination by air or sea water, rather than the uptake of VMSs from soils. In the case of pine needles, it was reported that they trapped the siloxanes present in the gas-phase of air (Ratola et al., 2015), which within the current scope could correspond to the occurrence of foliar uptake. Because volatilization from soil can be a predominant removal mechanism in the case of VMSs (Xu and Chandra, 1999), it would be reasonable to assume that foliar uptake could play an important role in the accumulation of the more volatile compounds. However, because it is also interesting to understand if root uptake occurs in crops, limiting the release of the target compounds to air can be useful for understanding the results, as the amounts detected in the aerial parts of the plants would be originated by root uptake, followed by translocation. It should be noted that this simplification, while helpful for filling knowledge gaps, may not accurately represent

what happens in an agricultural field or greenhouse. Therefore, for a broader understanding, different types of experiments will be needed.

Due to the very limited information at the time of writing, it is difficult to draw clear conclusions regarding plant uptake of CECs. Regarding the possible uptake of VMSs by plants it is known that these compounds have been previously detected in plant samples, but the absence of precedents regarding uptake from amended soils does not allow for any *a priori* predictions of any results this work may yield. That same lack of information does, however, greatly justify the objectives of this dissertation and its parent project. Despite the knowledge gaps for SMs being less glaring, the inclusion of this class of compounds may also yield relevant conclusions. Some trends have emerged from the previous few studies done with these compounds: higher concentrations of SMs tend to be reported in plant parts that are in direct contact with soil and lower concentrations in parts like fruits and grains; uptake tends to be hindered by higher soil organic carbon content and consequent reduced bioavailability of pollutants due to greater sorption to soil; and translocation from roots to other plant sections has appeared to be a rate limited process. From data such as the results provided from Litz and al. (2007), it can also be inferred that those levels of SMs may vary from one crop to another due to factors inherent to the plants, like their chemical composition and overall morphology. Because there is no known precedent from uptake studies of the chosen CECs by the selected crop, uptake and translocation of SMs may diverge from previously reported data for other crops.

3. Technical Description

3.1. Reagents and Materials

Individual standards of volatile methylsiloxanes with purity levels equal to or above 97% (L3: octamethyltrisiloxane, L4: decamethyltetrasiloxane, L5: dodecamethylpentasiloxane, D3: hexamethylcyclotrisiloxane, D4: octamethylcyclotetrasiloxane, D5: decamethylcyclopentasiloxane, D6: dodecamethylcyclohexasiloxane) were purchased from Sigma-Aldrich (St. Louis, MI, USA). A commercial mix of musk compounds, containing cashmeran (DPMI), celestolide (ADBI), phantolide (AHMI), traseolide (ATII), tonalide (AHTN), galaxolide (HHCB), musk ambrette (MA), musk ketone (MK), musk xylene (MX) and ethylene brassylate (EB) at 10 mg/L in cyclohexane was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Internal standards musk xylene-d15 (MX-d15) 100 mg/L in acetone and tonalide-d3 (AHTN-d3) 100 mg/L in isooctane were purchased from Dr. Ehrenstorfer (Augsburg, Germany), while the internal standard tetrakis(trimethylsiloxy)silane (M4Q) was purchased from Sigma-Aldrich (St. Louis, MI, USA) with 97% purity. For sewage sludge spiking, musk galaxolide (HHCB) at 48-52% purity in liquid form and tonalide (AHTN) at 98% purity in pellet form were also purchased from Sigma-Aldrich (St. Louis, MI, USA). All commercial solutions and standards were stored in the dark, in amber glass vials, at -20°C . Analytical grade solvents, hexane (Hex), ethyl acetate (EtAc), dichloromethane (DCM) and acetone (Acet) were purchased from VWR BDH Prolabo (Fontenay-sous-Bois, France). The sorbents used in the extraction procedures included sodium chloride (NaCl) purchased from Merck (Darmstadt, Germany), anhydrous magnesium sulphate (MgSO_4), baked for 12 h at 450°C , from Panreac AppliChem (Barcelona, Spain), primary and secondary amine exchange bonded silica sorbent (PSA) and octadecyl-silica (C18) from Supelco (Bellefonte, PA, USA). Nitrogen (99.999%) used in the evaporation step and helium (99.999%) used in the GC-MS system were supplied by Air Liquide (Maia, Portugal).

3.2. Standards Preparation

Individual stock solutions of each of the VMSs, including the internal standard M4Q, were prepared in hexane at 1 g/L. From those individual stock solutions, a mix stock solution containing all the target analytes was prepared in hexane at 5 mg/L. A diluted M4Q individual stock solution, with a final concentration of 5 mg/L, was also prepared in the same solvent. A stock solution containing all the internal standards was prepared at 500 $\mu\text{g/L}$ in hexane from the commercial standards of MX-d15 and AHTN-d3 and M4Q. This solution was used for soil, sewage sludge, and plant extraction experiments. For leachate extractions, a similar internal standard solution was prepared from the same preparations but using analytical grade acetone instead of hexane. For plant extraction method validation purposes, two

mix solutions containing all target compounds (250 and 500 µg/L) and internal standards (500 µg/L) were prepared in hexane from the mix stock solution of VMSs, the commercial mix of SMs and the individual internal standard solutions. For the assays using fortified sewage sludge, a mix solution with HHCB, AHTN, D4, D5 and D6 in acetone (45 mg/L) was prepared from the commercial individual standards. Nine calibration standards in hexane, with concentrations of each analyte ranging from 5 to 500 µg/L (internal standard concentration of 250 µg/L) were also prepared. All the previously mentioned preparations were stored in amber glass vials, and stored at – 20 °C, protected from the light.

3.3. Sewage Sludge Preparation

Anaerobic digested sewage sludge was collected from a Portuguese WWTP, projected to serve 300 000 inhabitants and which receives mostly domestic wastewaters. A large sample (≈8 kg) of sludge was transported in a cooler to the lab in polypropylene bags. Then, it was divided into four portions (2 kg) and each one spiked with different volumes of the mix solution in acetone (HHCB, AHTN, D4, D5 and D6): unspiked, 1500, 3000 and 6000 ng/g ww. The sewage sludge was mixed to obtain homogeneity and the containers were left open overnight in a clean air chamber to allow the acetone to evaporate.

3.4. Experimental Plant Uptake Set-up

Plant uptake experiments were set-up in a greenhouse at Campus Agrário de Vairão, located in Vila do Conde, Porto. A total of 102 plastic frustum-shaped pots (14 cm in height, 9 cm radius at top and base radius of 6.5 cm) were used to test 17 different experimental conditions in triplicate, as summarized in Figure 2. Besides the presence/absence of plants, the other main conditions tested were 4 different application rates (4, 9, 18 and 30 ton/ha) of both the unspiked sludge and the spiked sludges at 3 different target concentration levels (1500, 3000 and 6000 ng/g ww of D4-D6, HHCB and AHTN). Additionally, control pots without the addition of sludge to the soil were also prepared for both the plant-free and sown soil scenarios. To prepare the required pots, 1,5 kg of soil were weighted in each of the pots, while the necessary quantities of sludge for each of the chosen application rates were weighted. To mix the sludge with the soil, about 2/3 of the soil was added to each pot and along with the required amount of sludge. This was manually mixed and then the rest of the soil was gradually added. The sludge-soil mixture was homogenized until all visible soil was uniform in appearance and seemed slightly humid to the touch. Figure 2 describes the distribution of plant-free and sown pots according to the tested sludge application rates and sludge spiking concentrations. Each combination of these factors received a code (“F” indicative of spiking level followed by an “L” indicative of the sludge application rate) used to identify different test groups more easily.

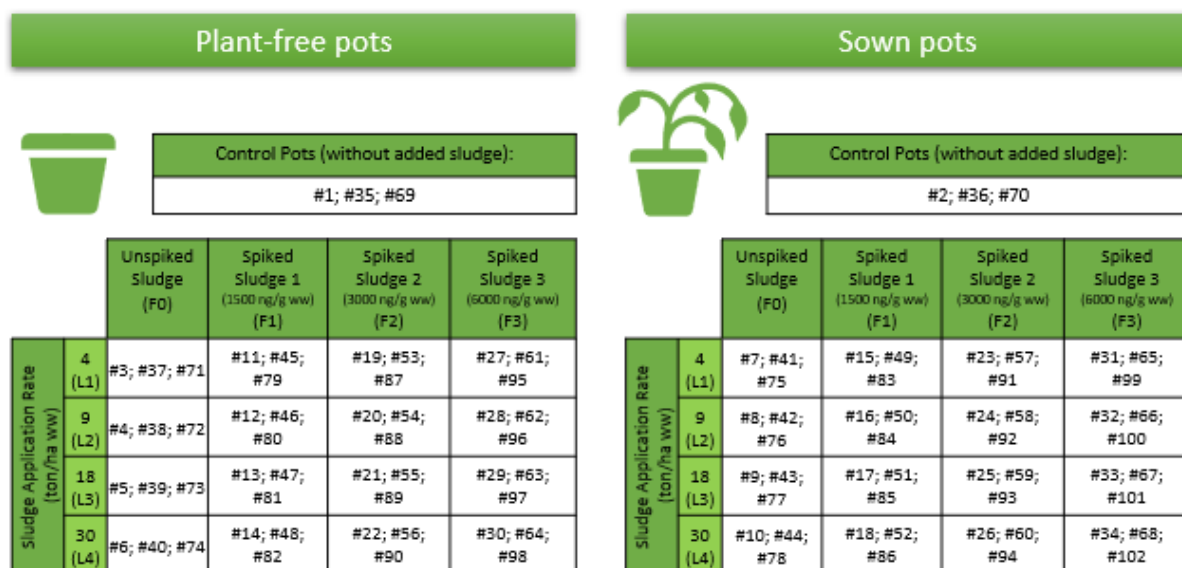


Figure 2. Distribution of pots numbered 1 through 102 according to assigned treatment.

Once the preparation of the pots was complete, they were distributed across 3 mesh tables (1 replicate in each table) and a plastic container was placed under each pot to collect any possible leachates after watering. In the pots designed to contain plants, 3 pea seeds were placed in their center, in a triangular pattern. These seeds were then covered with a 3 cm layer of soil and slightly pressed down to allow seeds to have a good contact to soil substrate before all pots were watered and covered with tinfoil, while germination took place. The excess water that had drained into the outer plastic containers was transferred into 50 mL polypropylene tubes that were then labelled and stored in a freezer at -20 °C until analysis.

In the following week, cross shaped cuts were made in the center of the tin foil coverings to allow for oxygen entrance, while minimizing possible volatilization losses of the target compounds from the soil. At the beginning of the third week of plant growth, a wooden stake was inserted in the center of the pots to give plants a physical support as their tendrils were developing. During cultivation, plants were usually watered once a week, with 100 mL of water. Water was always added in two parts with an hour's interval in between to avoid leaching. As the plants began to yield mature pods, starting from 68 days after sowing, these were collected and stored as described ahead. When a plant showed only mature or aborted pods, meaning that more peas or pods would not form or mature further, the plant was harvested by cutting it just above the root, except when only one plant remained/had sprouted in the pot. Pots were collected and transported to the lab as soon as the last plant had been harvested. It should be noted that whenever a pot used for plant growth was collected, its corresponding control pot (differing only in plant presence/absence) would also be collected and transported to the lab. The experiment lasted a total of 86 days, when the last remaining pots were collected. However, the first

peapods and plants were harvested just 68 days after sowing, meaning that plant material collection took place over a period spanning roughly 3 weeks. Photographs of several of the experiment set-up steps as well as of the plant sample preparation and extraction procedures were taken throughout the work and are available in Annex 1.

3.5. Sample Preparation

For each pot, all pea plants, excluding roots, were harvested, placed in polypropylene bags, and transported to the lab for pretreatment. The collected plants were carefully rinsed with ultrapure water to remove dust and left to dry for 30 minutes at a clean air cabinet and then divided into parts (peas, pods, leaves, tendrils, and stems). The stems were cut into smaller portions (1-2 cm in length) to facilitate further sample homogenization, as well as the leaves that were cut into small pieces of about 1 cm². The harvested pea pods were opened with a scalpel, the peas removed and both parts stored separately. In the case of roots, due to the difficulty of removing them from the pots without breaking them, the pots were transported to the lab after harvesting. There, their contents were turned into a tray and the soil manually decompressed so that all the roots could be collected. Then, roots were rinsed with ultrapure water to remove soil particles and left to dry at room temperature in a clean air cabinet. The soil obtained from each pot was sieved (mesh 2 mm), allowing the recovery of root fragments that might have broken, and soil samples were collected in polypropylene bags for later analysis. The same soil pretreatment procedure was followed in the control pots. All plant sections were stored in polypropylene bags at -20 °C until analysis. Before extraction, each plant section (roots, pods and peas) was ground still frozen in a commercially available Q.5321 Qilive mill (France). To avoid sample degradation due to overheating, samples were ground in short pulses with minute-long intervals between them. To analyze the aerial part of the plant, the stems, tendrils and leaves sections were combined and then ground as explained before. The average moisture content of each plant compartment was calculated by drying 2 g of sample in a 210 JP Selecta 100 W laboratory oven (Barcelona, Spain), in triplicate, at 105 °C for 15 hours and then weighting the samples again. The calculated average moisture contents (in percentages) were as follows: 80.9 ± 0.6 (roots), 83.0 ± 0.9 (stems), 80 ± 3 (tendrils), 86 ± 1 (leaves), 83 ± 3 (aerial parts), 70 ± 3 (peas) and 83.3 ± 0.3 (pods).

3.6. Extraction Procedure for Plant Samples

The extraction procedure used for the analysis of the different plant sections was based on the QuEChERS method, originally described by Anastassiades et al. (2003). In short, 2 g of pretreated plant sample was weighted for 50 mL cylindrical polypropylene tubes with a conical bottom and then, 125 ng

of internal standards (MX-d15, AHTN-d3 and M4Q) was added to each tube. The tubes were vortexed for about 30 seconds and allowed to stabilize for 1-hour before 10 mL of DCM:Hex (1:1 v/v) was added. The tubes were vortexed for 1 min and then placed in a 420 W JP Selecta ultrasonic bath (Barcelona, Spain) for 15 min. Once removed from the bath, 1 g of NaCl and 4 g of MgSO_4 (QuEChERS 1, Q1) was added to each tube. The addition of these salts/desiccant agents will enhance extraction efficiency and ensure the phase separation between the organic solvent and the water present in the samples. After this addition, the tubes were vortexed for 1 min, centrifuged for 10 min at 4000 rpm (2670 g) (Hettich GmbH & Co., Tuttlingen, Germany) and the resulting supernatant was collected into 15 mL cylindrical polypropylene tubes with a conical bottom, already containing 300 mg of PSA and 1 g MgSO_4 (QuEChERS 2, Q2). This is a clean-up step based on dispersive solid-phase extraction, used to remove undesired sample interferences. The mixture was vortexed for 1 minute and centrifuged for 10 minutes under the conditions mentioned above. The resulting supernatant was collected into 12 mL amber glass vials and concentrated to 1 mL under a gentle stream of nitrogen. The concentrated extract was transferred into an amber glass microvial, and the volume reduced to 500 μL under a gentle stream of nitrogen for further analysis in the GC-MS system.

3.7. Leachate Extraction Method

For analyzing the leachates obtained after watering the pots, a procedure previously developed in the research group was performed. Before the extraction all the samples were centrifuged for 5 min at 4000 rpm (2670 g) to remove suspended particles. Then, 30 mL of the centrifuged sample was placed into a 50 mL polypropylene tube with conical bottom and 125 ng of internal standard (MX-d15, AHTN-d3 and M4Q) in acetone was added. Tubes were vortexed for 1 min and left to stabilize for 30 minutes. Then, 10 mL of Hex was added, and the mixture was vortexed for 1 min, followed by an ultrasonic bath (420 W for 10 min) extraction step. To separate phases, a second centrifugation was performed (5 min, 4000 rpm), the supernatant transferred to 12 mL amber glass vials and concentrated to 1 mL under a gentle stream of nitrogen. The concentrated extract was transferred into an amber glass microvial, and the volume reduced to 500 μL under a gentle stream of nitrogen for further analysis in the GC-MS system.

3.8. Soil and Sewage Sludge Extraction Method

The same extraction procedure was used to analyze soil and sewage sludge samples. Briefly, 2.5 g of soil or sludge were weighted into a 50 mL polypropylene tube with conical bottom and 500 ng of internal standard (MX-d15, AHTN-d3 and M4Q) was added. After 1-hour of stabilization, 2.5 g of MgSO_4

were added and the mixture vortexed for 1 minute. Then, target analytes were extracted with 5 mL of Hex, followed by 5 mL of Hex:DCM (1:1 v/v) and then 5 mL of Hex:EtAc (1:1 v/v). To perform the extraction, the mixture was vortexed for 1 min and ultrasonicated for 10 min in an ultrasound bath (420 W). The organic phase was separated by centrifugation at 4000 rpm (2670 g) for 5 min. The resulting supernatants were combined in a 15 mL polypropylene tube, to which 300 mg of MgSO_4 , 300 mg of PSA, and 50 mg of C18 were added. The mixture was vortexed for 1 minute and centrifuged for 10 minutes under the conditions mentioned above. The supernatant was collected into 12 mL amber glass vials and concentrated to 1.5 mL under a gentle stream of nitrogen. The concentrated extract was transferred into an amber glass microvial, and the volume reduced to 1 mL for further analysis in the GC-MS system. The extraction method had been previously validated for both matrices and the resulting MDLs are available in Annex 5 in Tables A4 and A5.

3.9. Instrumental Analysis

The extracts were analysed using a Varian Ion Trap GC-MS system (Walnut Creek, CA, USA), equipped with a 4000-GC gas chromatograph, a 240-MS ion trap mass spectrometer, a CP-1177 split/splitless injector adapted with a Merlin microseal system and an CP-8410 autosampler. The selected chromatographic column was a low-bleed Agilent DB-5 MS ultra-inert column (30 m $\times$ 0.25 mm I.D., 0.25 μm film thickness). Helium (99.999%) at 1.0 mL/min was used as carrier gas. The GC oven temperature program was defined as 35 $^{\circ}\text{C}$ (5 min), raised at a rate of 6 $^{\circ}\text{C}/\text{min}$ until 155 $^{\circ}\text{C}$, followed by a rate of 5 $^{\circ}\text{C}/\text{min}$ to 175 $^{\circ}\text{C}$ (hold for 6 min), 5 $^{\circ}\text{C}/\text{min}$ to 210 $^{\circ}\text{C}$, and lastly 35 $^{\circ}\text{C}/\text{min}$ to 300 $^{\circ}\text{C}$ (held for 5.43 min), in a total runtime of 50 minutes. 1 μL was injected in splitless mode. The manifold, ion trap, transfer line and injector temperatures were 50, 220, and 250 $^{\circ}\text{C}$, respectively. The mass spectrometer was operated in electron ionization (EI) mode (70 eV) with a filament current of 50 μA . The quantification of the target compounds was performed in EI mode using time-scheduled selected ion storage (SIS) and the VARIAN, INC. Mass Spectrometry Workstation software, version 6.9.3. The main parameters used for the quantification are described in Table A1 in Annex 2. Examples of Chromatograms are presented in Annex 3.

3.10. Quality Assurance and Quality Control (QA/QC)

Due to the ubiquity of SMs and VMSs in daily use products, measures were taken during plant cultivation, sample handling and extraction procedures to avoid contaminations. At the greenhouse site, all involved personnel were instructed to avoid the use of cosmetics and fragrances and to only handle the plant pots while wearing nitrile gloves. Likewise, in the laboratory, the use of detergents, cosmetics

and personal care products was avoided by all personnel. When treating plant or soil samples, nitrile gloves were always used and changed between handling samples from different pots to avoid cross-contamination. In the laboratory, samples were handled in a clean air cabinet. Glass materials were rinsed with acetone and non-calibrated items were baked at 400 ° C for at least one hour. At least 2 procedural blanks were performed with every batch of extractions and used to correct the results.

3.11. Waste Management

The residues generated in this work were mainly organic solutions containing hexane, dichloromethane and ethyl acetate and trace amounts of SMs and VMSs and also residues of sorbents used in the extraction procedures (MgSO_4 , NaCl, C18 and PSA) along with plant sections, soil and sludge. All residues were collected in proper labelled closed containers and stored, protected from light and ignition sources, for further treatment by the Environmental Management System of FEUP – EcoFEUP. Acetone used to rinse material was collected to allow later recovery by distillation for other uses.

4. Results and Discussion

4.1. Validation of the analytical method for the determination of SMCs and VMSs in pea plants

The method developed for the SMCs and VMSs analysis in pea plants was based on QuEChERS followed by GC-MS analysis. The validation of the proposed methodology was performed by determining quantification parameters such as the linearity response of the GC-MS instrumental approach and its associated statistical criteria, detection and quantification limits, and by assessing precision and accuracy for all the selected plant compartments (peas, pods, aerial part - leaves, tendrils, stems and roots).

The linearity response was assessed by the direct injection of nine calibration standards in the GC-MS system. The concentrations of the target compounds in these standards ranged from 5 to 500 µg/L, while the internal standards (MX-d15, AHTN-d3 and M4Q) were kept at a concentration of 250 µg/L. Calibration curves were obtained by correlating the mass ratio of each target and its corresponding internal standard ($\text{mass}_{\text{target compound}}/\text{mass}_{\text{internal standard}}$) with the response factors ($\text{RF} = \text{Peak area}_{\text{target compound}}/\text{Peak area}_{\text{internal standard}}$). Linear responses were found for all the compounds within the studied range and all calibration curves and aforementioned parameters are available in Annex A4. The associated correlation coefficients (r) were all above 0.995 and relative standard deviations (RSD) of the slopes were lower than 5% for all compounds.

Instrumental (IDL) and method detection (MDL) limits were calculated for each target compound in each analyzed compartment, based on a signal-to-noise ratio (S/N) of 3, while instrumental (IQL) and method quantification (MQL) limits based on $S/N=10$. Those values may be consulted in Table 4. Instrument detection limits ranged from 0.001 pg (D5) to 7.50 pg (MA). Method detection limits, presented in ng of compound per g of plant material (ww, wet weight) varied between 0.002 (D5 in aerial part) and 6.54 (EB in pods).

The precision measures the level of proximity between results for the same sample. It was evaluated by the relative standard deviation (%RSD) of three replicates of the same sample, spiked at two different levels (125 ng/g ww and 250 ng/g ww) and performed on the same day (intra-day precision). The inter-day precision was also assessed through the RSD of three assays performed on different days, at a spike level of 250 ng/g ww and only for the peas compartment. The accuracy, which measures the degree of proximity between the obtained and the expected result, was assessed by recovery tests performed using spiked samples at two different levels (125 ng/g ww and 250 ng/g ww). Those results are summarized in Table 5. Internal standard recoveries, while not presented in said Table were as high as 84% (MX-d15), 86% (AHTN-d3) and 59% (M4Q).

Table 4. Instrument Detection and Quantification limits and Method Detection and Quantification Limits of the target pollutants.

Compound	Linearity Range (µg/L)	IDL (pg)	IQL (pg)	MDL (ng/g ww)				MQL (ng/g ww)			
				Peas	Pods	Aerial Part (Leaves, Tendrils and Stems)	Root	Peas	Pods	Aerial Part (Leaves, Tendrils and Stems)	Root
DPMI	5 – 500	0.79	2.63	0.31	0.44	0.42	0.24	1.04	1.46	1.39	0.79
ADBI	5 – 500	0.94	3.13	0.41	0.40	0.32	0.37	1.35	1.33	1.06	1.24
AHMI	5 – 500	0.45	1.52	0.36	0.38	0.30	0.33	1.20	1.27	1.01	1.09
MA	5 – 500	7.50	25.00	1.91	3.20	2.10	2.34	6.38	10.65	6.99	7.81
ATII	5 – 500	1.67	5.56	0.65	0.88	0.58	0.71	2.15	2.95	1.92	2.38
HHCB	5 – 500	0.79	2.63	0.62	0.69	0.45	0.51	2.08	2.30	1.50	1.71
MX	5 – 500	3.75	12.50	1.71	2.13	1.39	1.31	5.72	7.10	4.63	4.36
AHTN	5 – 500	0.22	0.74	0.68	0.77	0.51	0.54	2.28	2.58	1.68	1.81
MK	5 – 500	1.88	6.25	0.67	0.86	0.58	0.56	2.23	2.86	1.92	1.86
EB	5 – 500	5.00	16.67	3.85	6.54	5.01	4.02	12.84	21.80	16.69	13.39
D3	5 – 500	0.16	0.52	0.54	0.78	0.50	0.47	1.79	2.61	1.65	1.56
L3	5 – 500	0.47	1.56	0.30	0.48	0.28	0.30	0.98	1.61	0.94	1.01
D4	5 – 500	0.18	0.61	0.22	0.30	0.21	0.23	0.75	1.00	0.69	0.77
L4	5 – 500	0.41	1.35	0.29	0.42	0.27	0.28	0.96	1.40	0.91	0.94
D5	5 – 500	0.001	0.003	0.002	0.003	0.002	0.002	0.007	0.009	0.006	0.007
L5	5 – 500	0.46	1.52	0.25	0.35	0.23	0.28	0.84	1.18	0.75	0.94
D6	5 – 500	0.05	0.17	0.13	0.19	0.11	0.14	0.43	0.62	0.37	0.47

Regarding accuracy, most target compounds yielded high recovery rates (above 70%) across all matrices and spiking levels. Notable exceptions were D3 and EB which featured lower recoveries (from 30% to 60%) across more than one plant compartment. Regarding precision, the RSD values were mostly lower than 10% for the target analytes, both in the intra-day and inter-day assessments. In conclusion, the proposed extraction method appears to be valid for its intended use due to all the parameters discussed above.

Table 5. Precision (inter and intra-day) and Accuracy for all the compounds analyzed at different spike levels (n=3).

Compound	Intra-day Precision (%RSD, n=3)								Inter-day Precision (%RSD, n=3)	Accuracy (% Recovery, n=3)							
	125 ng/g ww				250 ng/g ww				250 ng/g ww	125 ng/g ww				250 ng/g ww			
	Peas	Pods	Aerial Part	Roots	Peas	Pods	Aerial Part	Roots	Peas	Peas	Pods	Aerial Part	Roots	Peas	Pods	Aerial Part	Roots
DPMI	8.1	3.3	15.2	14.3	7.1	5.3	14.5	14.5	9.1	107.1	99.0	78.3	72.9	103.9	113.9	73.0	102.4
ADBI	2.4	1.0	1.5	2.7	6.5	1.1	2.3	1.8	8.5	81.7	83.6	86.4	89.7	104.2	80.4	88.3	91.0
AHMI	3.3	1.5	1.7	1.1	8.7	1.3	3.0	2.3	5.9	84.3	86.5	88.5	91.8	93.4	82.6	90.2	92.2
MA	3.8	6.6	3.9	0.9	2.5	2.7	5.4	1.4	13.2	100.8	102.9	105.9	106.2	134.8	109.6	109.9	107.0
ATII	0.6	2.2	1.0	0.7	7.4	0.8	2.4	2.5	5.4	86.9	87.7	89.7	90.3	100.6	84.2	93.2	91.9
HHCB	1.4	1.8	6.1	1.4	8.0	0.6	3.8	1.8	3.3	82.8	83.1	88.6	88.4	85.9	80.8	92.4	91.0
MX	3.8	7.8	3.8	1.3	2.1	0.2	2.0	1.7	2.9	92.4	98.1	97.6	95.4	94.6	98.9	98.5	97.9
AHTN	4.5	3.2	5.6	1.0	8.4	3.2	2.7	2.0	9.1	68.8	71.4	78.6	79.4	84.0	74.8	86.9	90.2
MK	2.0	2.9	4.1	2.3	3.1	1.6	9.5	0.7	7.0	97.9	111.9	103.4	97.0	118.3	104.3	101.3	100.5
EB	0.6	2.2	16.3	2.7	7.1	2.5	3.7	4.3	9.0	79.7	69.7	54.2	76.7	108.0	71.7	59.4	89.9
D3	3.5	9.5	7.9	6.5	6.6	13.1	11.9	7.2	6.4	65.4	57.8	68.3	78.3	89.7	66.1	62.9	76.9
L3	0.8	4.3	6.1	7.3	2.5	4.6	6.4	0.6	2.8	87.6	88.8	75.6	86.6	96.5	91.5	81.4	86.3
D4	3.8	9.4	14.7	9.2	8.6	7.9	11.0	4.7	7.9	87.8	95.5	82.0	87.7	87.0	88.2	86.7	95.0
L4	1.4	4.5	6.3	3.0	1.5	3.7	3.2	1.6	2.3	102.6	96.9	91.9	93.5	99.9	96.7	95.0	98.9
D5	2.6	8.5	10.9	2.3	3.9	4.5	3.1	1.5	3.9	87.4	88.6	85.1	89.6	88.0	91.0	92.1	97.7
L5	2.0	8.6	2.0	0.9	1.7	1.7	1.6	0.9	2.4	106.6	98.5	106.7	100.7	108.4	106.9	103.8	102.3
D6	2.7	10.7	3.3	0.2	2.2	4.3	3.9	2.2	2.9	84.0	91.1	86.0	92.6	95.1	100.3	92.6	97.0

4.2. Determination of CECs in the initial soil, applied sewage sludge and leachates

Before the start of the experiments, samples of the initial substrate (soil, amended soil with different application rates of unspiked or spiked sewage sludge) were collected and saved for later analysis. Triplicates of these samples were extracted and quantified and their resulting concentration values were used to estimate the initial concentration of each contaminant in each pot according to test group. These estimated concentrations are presented in Table A5 in Annex 6. In the soil used for the control pots and to prepare the soil:sludge mixes, only 4 CECs (D3, D4, D5 and HHCB) were detected among the 17 target contaminants. The pollutant present in the highest concentration was D3 (4.9 ± 0.5 ng/g dw), followed by D4 and D5 (both present at around 2 ng/g dw) and lastly by the musk compound HHCB (1.2 ± 0.1 ng/g dw). All 4 pollutants are therefore present in low concentrations and nearly all (except for D3) are considered high production volume chemicals. As far as it was known, there was no prior application of sewage sludge, so the detection of the CECs can be mainly attributed to their ubiquity in daily life, coming from several sources of contamination.

Sewage sludge presents a wider range of pollutants, as it would be expected. Among all the selected target compounds, only 6 synthetic musks were not detected. Three of them (MA, MK, MX) are classified as nitromusks, which have their input into WWTPs greatly limited, and one (EB) is classified as a macrocyclic musk compound, more prone to degradation. In those assays, it was not possible to quantify DPML with reliability due to the presence of an interferent with a similar retention time and mass spectrum. This problem persisted in the later stages of the work, so it was decided not to quantify this compound. The most representative SMs in the sewage sludge sample were HHCB (57297 ± 4169 ng/g dw) and AHTN (8127 ± 425 ng/g dw), followed by ADBI (195 ± 6 ng/g dw). A wider variety of VMSs was present in this sludge. Not only the cVMSs D3-D6 were detected in higher levels than in the soil - 16 ± 7 ng/g dw (D3) to 9340 ± 459 ng/g dw (D5) - but also the lVMSs L3-L5, ranging from 5 ± 1 ng/g (L3) to 327 ± 11 ng/g (L5).

As previously mentioned, the irrigation process was carried out in order to minimize the formation of leachates. The few leachates collected (formed after the first watering process) did not reveal the presence of the target compounds. It was then considered that losses via leaching would not be significant.

4.3. Plant culture development overview

Over the 86 days during which the experiment took place, plant growth was monitored by evaluating several parameters (e.g., plant height, occurrence of flowering, etc.). The recorded data and

its evaluation were carried out by another researcher related to the Project AGRONAUT – Agronomic Impact of Sludge Amendment Using a Comprehensive Exposure Viewpoint, in which this work is also integrated, and therefore it was not explored in this dissertation. It should be noted that not all sown pots yielded plants. In pots #16 and #52 none of the seeds germinated and, consequently, there were no plants to collect. It is unlikely that the lack of germination occurred due to the pot treatments (groups F1L2 and F1L4) because in both cases, the other replicates produced mature plants. The lack of development is, therefore, most likely due to the seeds having been damaged in some way, which may also have happened in several other pots, where only one or two of the three sown seeds germinated. During the growing period, there were several days of unseasonal heat, which led to premature wilting of certain plant specimens, which was also reflected in the decrease of collected biomass at the end of the experiment. For this reason, it was also impossible to allow pods to reach full maturation (i.e., the point at which they would be collected for consumption) without some plant parts, mainly leaves and tendrils, beginning to dry. The dry parts of each of the plants, when present, were separated from the remaining live sections and stored separately, but extraction procedures were not carried out with the dry parts of the plants.

4.4. Determination of CECs in *Pisum sativum* specimens grown in amended soil

4.4.1. Synthetic Musks

The only two musk compounds detected in the collected plant samples were HHCB and AHTN, i.e. the two contaminants present at higher levels in sewage sludge. In controls (#2, #36, #70), the only SM detected in plants was HHCB, but only in roots and aerial parts. This result is somewhat expected, as only HHCB was present in the control soil and at very low levels; the absence of AHTN in these plants also indicates that foliar uptake did not occur, either through the potential AHTN volatilization from neighboring pots (which were rotated throughout the experiment as previously explained), or by external sources of contamination (e.g., staff working in the greenhouse). The detection of HHCB in the aerial parts of the control plants could be due to root uptake followed by translocation or to foliar uptake. However, not all plant groups presented HHCB in their aerial parts as will be discussed later, so foliar uptake occurring in only some of the pots seems unlikely.

Regarding the detection of SMs in plants grown in the amended soil pots, HHCB and AHTN were detected in differing patterns. HHCB was detected in all plant compartments in at least one test group, although generally at levels lower than those found in the roots, but AHTN was only detected in roots. Overall, average AHTN levels in roots ranged from below the detection limit to 283 ± 83 ng/g dw. These

values are lower than the ones reported by Macherius et al. (2012) and Chen et al. (2010) for barley and wheat roots, but in both cases initial concentrations of the target pollutants in soil were higher. HHCB was detected in roots in levels ranging from 6 ± 2 ng/g dw to 346 ± 13 ng/g dw. The most similar values found in literature are those reported in carrot root cores by Macherius et al. (2012), who obtained an initial pollutant concentration in the soil similar to the treatment group F3L4. However, not many conclusions can be drawn as the roots differ quite noticeably, especially in chemical composition due to carrot roots serving as a storage organ.

To facilitate the comparison of root uptake of HHCB and AHTN, Figure 3 shows their average concentrations (n=3) according to the treatment group. The translocation of HHCB into above ground plant compartments is illustrated by Figure 4 and will be discussed further ahead. In both images error bars representing the standard deviation are presented. The high deviations in measurements across some sample groups is most likely due to the inherent variability of the plant specimens. Additionally, the full results of the uptake experiment of SMs are available in Annex 6 in Table A6.

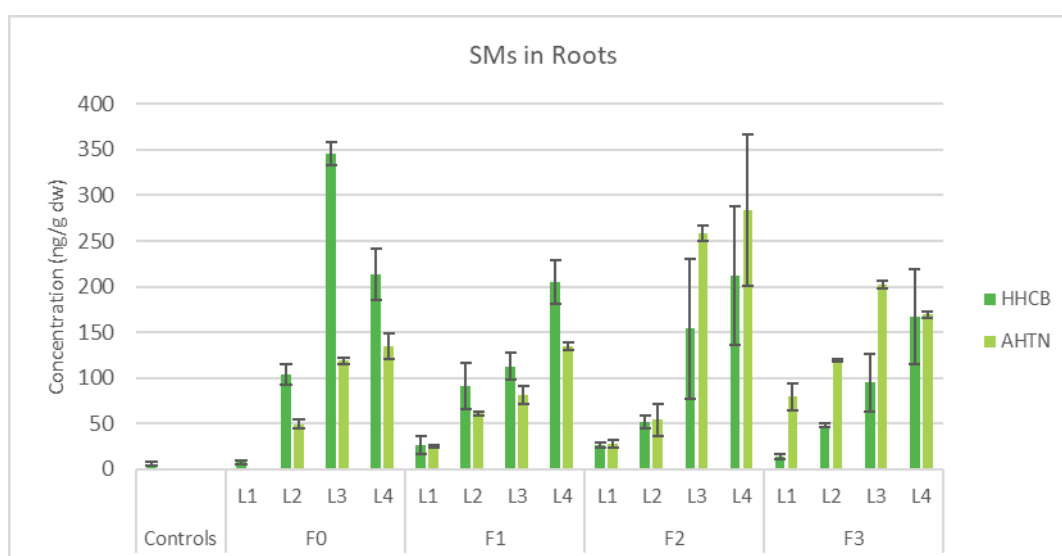


Figure 3. Average concentrations (n=3) of HHCB and AHTN in roots of *Pisum sativum* specimens grown in amended soils according to sludge application rate and sludge spiking level. Error bars represent the standard deviation.

As can be seen in Figure 3, within each sewage sludge spiking level (F0-F3), root uptake of both compounds seems to increase according to the application rate. This could indicate that root uptake somewhat reflects the pollutant concentration in soil, as proposed by Ziarrusta (2015). However, the influence of spiking levels in the sewage sludge for the same application rate (L1-L4), does not seem to be so clear in root uptake. This may mean that the amount of sewage sludge that is applied to crops and, consequently, the change in the resulting amended soil organic matter content, as well as its composition in terms of minerals, nutrients, etc., may have a greater impact in the uptake of these pollutants than its initial concentrations in the amended soils. Anyway, when comparing the

concentrations of HHCB and AHTN in the roots, when the spike levels are lower (F0 and F1), the concentration of HHCB tends to be higher than that of AHTN, but at higher levels (F2 and F3) this behaviour seems to reverse, as shown in Figure 3. In fact, there is no clear explanation for this. However, it is possible that this can be explained by the interaction between the soil and the target pollutants, namely through the determination of sorption capacities and kinetics that are directly influenced by the initial concentrations of the target analytes and that will consequently influence the bioavailability of the compounds to the root uptake. Therefore, additional assays are needed to justify these results.

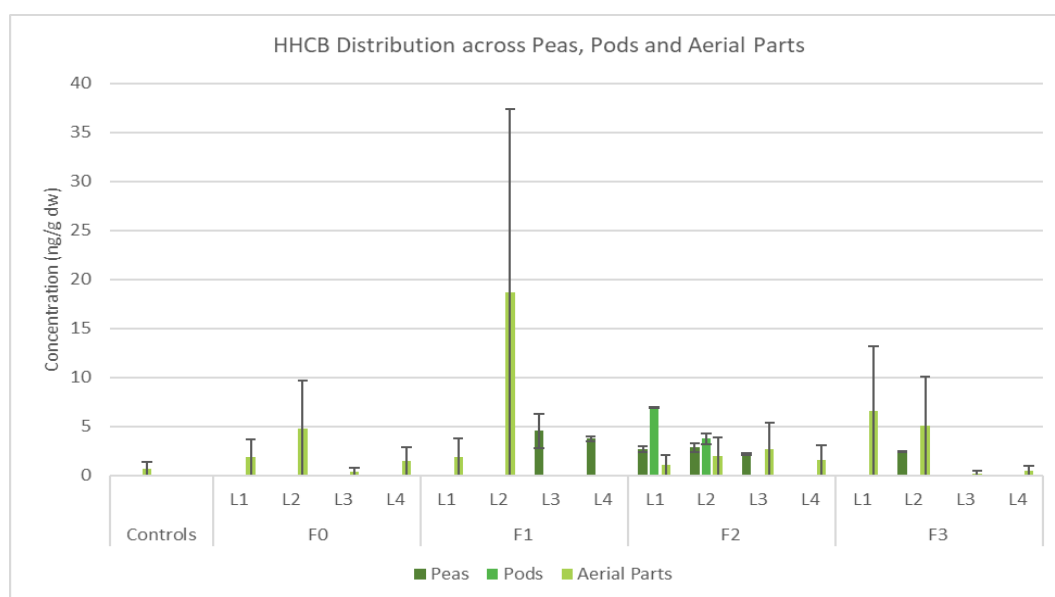


Figure 4. Average concentrations ($n=3$) of HHCB in peas, pods and aerial plant parts of *Pisum sativum* specimens grown in amended soils according to sludge application rate and sludge spiking level. Error bars represent the standard deviation.

Focusing now on the above ground parts of the plant, HHCB has been detected in peas at concentrations ranging from 2.16 ± 0.07 ng/g dw to 5 ± 2 ng/g dw, but only in some of the treatment groups (F1L3, F1L4, F2L1, F2L2, F2L3 and F3L2), being below the detection limits in the pots that received the other treatment options. In pods, HHCB was only detected in plants from treatment groups F2L1 and F2L2. HHCB is expected to move first to the pods and then to the peas, which begin to mature after their inclosing initially flat pods. Peas and pods have differences in their composition (e.g., water content), which may help explain why hydrophobic pollutants such as HHCB may accumulate more in the less water-rich peas. The groups in which HHCB was detected in peas and pods were not necessarily the ones with the highest pollutant concentration in the amended soil or in roots. Furthermore, no relationship was found between those concentrations detected in peas and pods and other soil dependent variables, such as OM%. This is in line with the possibility of the transfer of pollutants from

roots to above ground sections of plants being a rate limited process, as proposed by Macherius et al. (2012).

In contrast to peas and pods, HHCB was detected in all aerial part samples except in those from pot groups F1L3 and F1L4. The absence in these groups is somewhat unexpected because in theory, HHCB would have to travel through the aerial parts to reach the peas and pods (botanically fruit and seeds). The plants from these test groups were among the most developed biomass wise so it could be theorized that the greater biomass of the aerial parts could have had a diluting effect in HHCB concentrations to the point of them being below detection levels. As stated before, AHTN was not detected anywhere but the roots, indicating that translocation of the contaminant to above ground compartments did not occur. This may be due in part to the plant's characteristics, seeing as there is precedent of translocation occurring into grains (Rivier et al., 2019) and fruits (Ramos et al., 2021) of other plants, even when AHTN levels in the soil were lower. It should be noted that in the aforementioned works, the concentrations of SMs in the roots were not determined, so it is unclear whether translocation in those crops, and by extension in *Pisum sativum*, is dependent on root concentration or limited by factors such as the presence of the Casparian strip, which forces the compounds to move through the cells (simplastic transport) before the core portions of the root and the xylem can be reached. HHCB is slightly less hydrophobic and more soluble than AHTN. Therefore, this may also be a reason why only HHCB underwent translocation in the plants, in addition to plant-specific factors such as lipid content. Since there is no precedent of uptake studies in peas with similar compounds, further investigation is required to fully understand the differences in HHCB and AHTN translocation in the chosen crop.

4.4.2. Volatile Methylsiloxanes

Overall, VMSs have less clear distribution patterns than SMs. Once again, not all of the compounds present in the amended soils were detected in plants. In fact, only cVMSs have been detected. IVMSs were determined in lower levels in the initial amended soils and they are less stable than cVMSs, which may explain these results. The exact fate of IVMSs is difficult to predict due to the scarcity of information regarding their behaviours in soils, but due to their physicochemical properties and greater instability than cVMSs, hydrolysis in soil or volatilization from wet soil could be likely mechanisms (Gaj, 2018).

All of the target cVMSs were detected in the control plants. D4 and D5 were present in the aerial parts and roots, while D3 and D6 were only detected in peas and aerial parts, respectively. The detection of D6 in the aerial parts, despite the absence of D6 in roots may indicate that some deposition/uptake of volatilized cVMSs from neighbouring pots may have occurred in the above ground

sections of the plants, especially in the leaves. The presence of D3 in peas, without being detected in other plant sections, can probably be explained by an external contamination of this sample during handling or extraction, since peas are the only analysed compartment without direct contact with soil or air during plant growth.

In the remaining experiments, cVMSs were detected in nearly all analyzed compartments. Figures 5 to 8 show the concentrations of each cVMS in each plant compartment, according to treatment groups. The full data is presented in Annex 6 in Table A7.

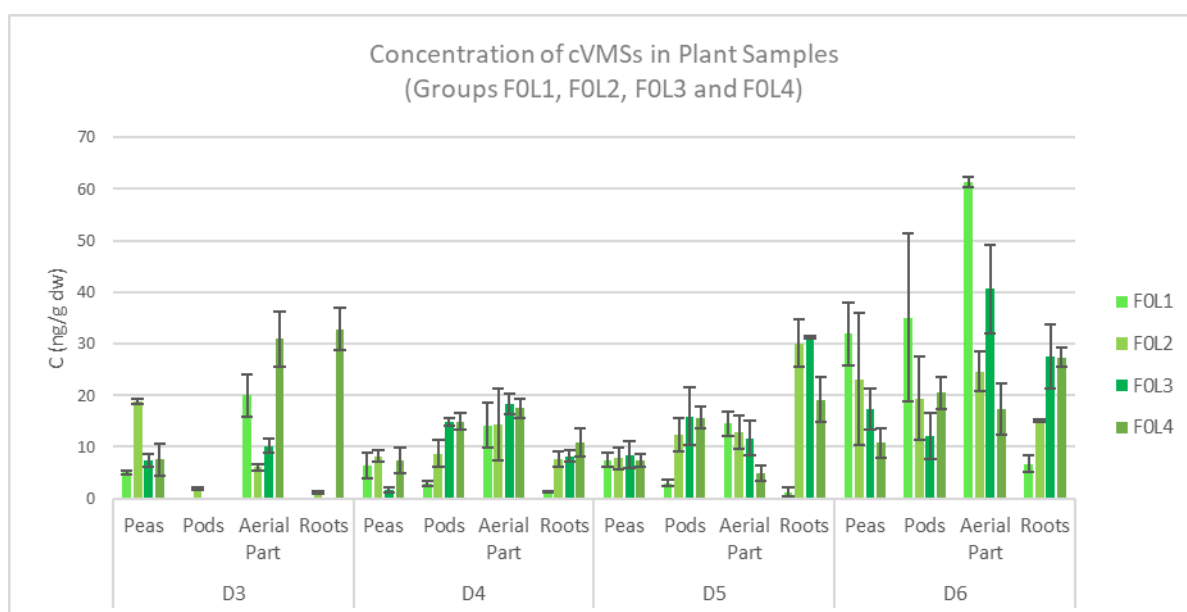


Figure 5. Concentrations of cVMSs in the different plant compartments of test groups FOL1-L4.

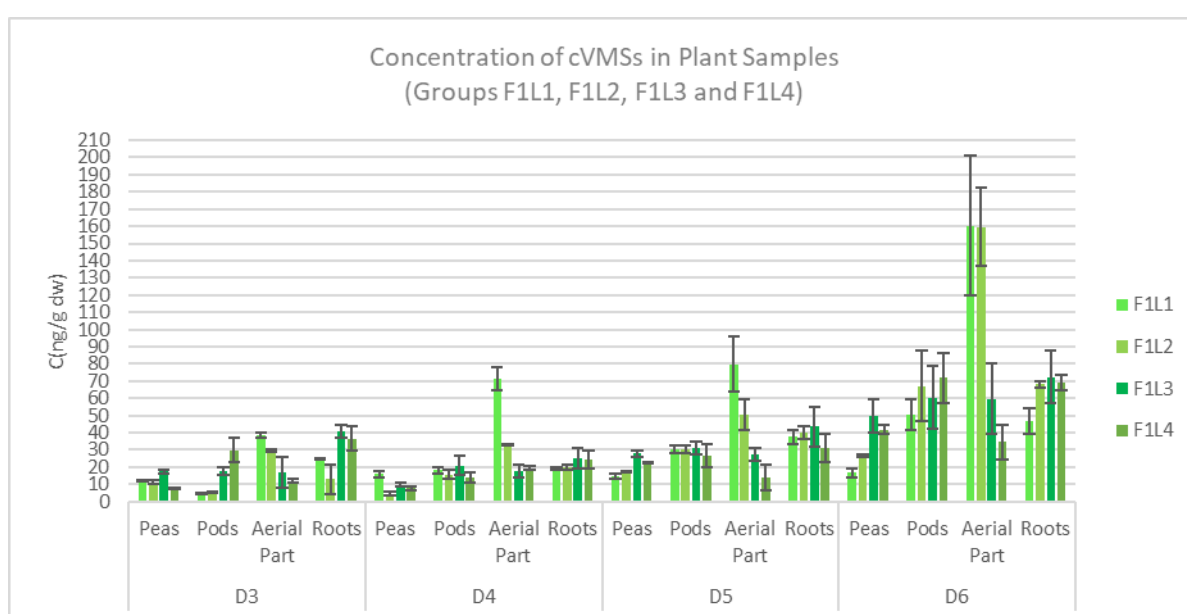


Figure 6. Concentrations of cVMSs in the different plant compartments of test groups F1L1-L4.

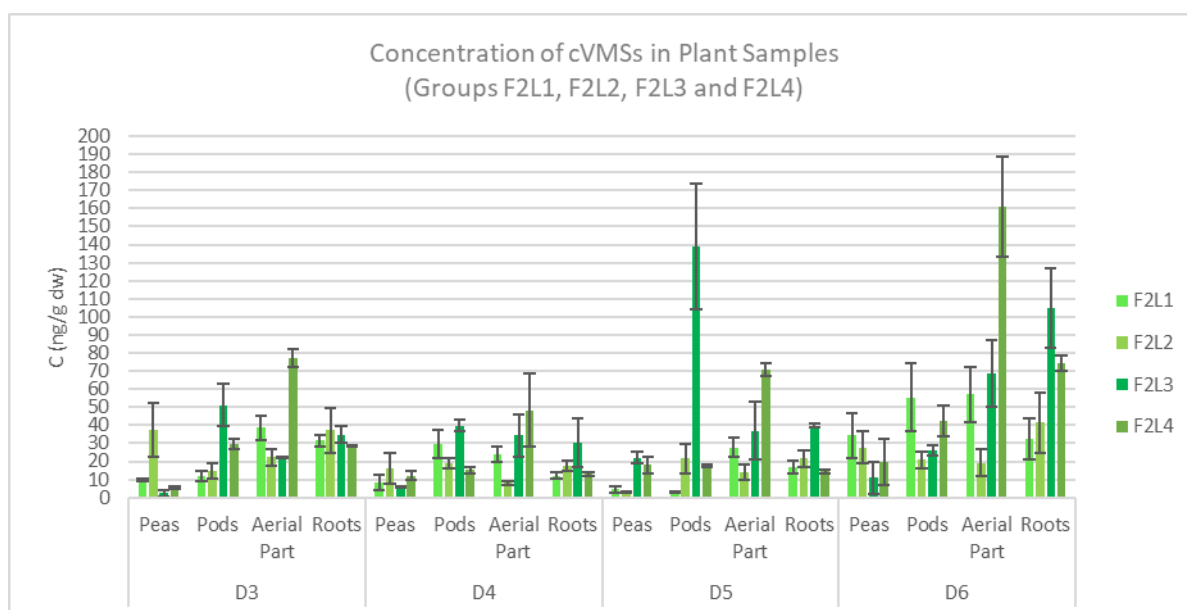


Figure 7. Concentrations of cVMSs in the different plant compartments of test groups F2L1-L4.

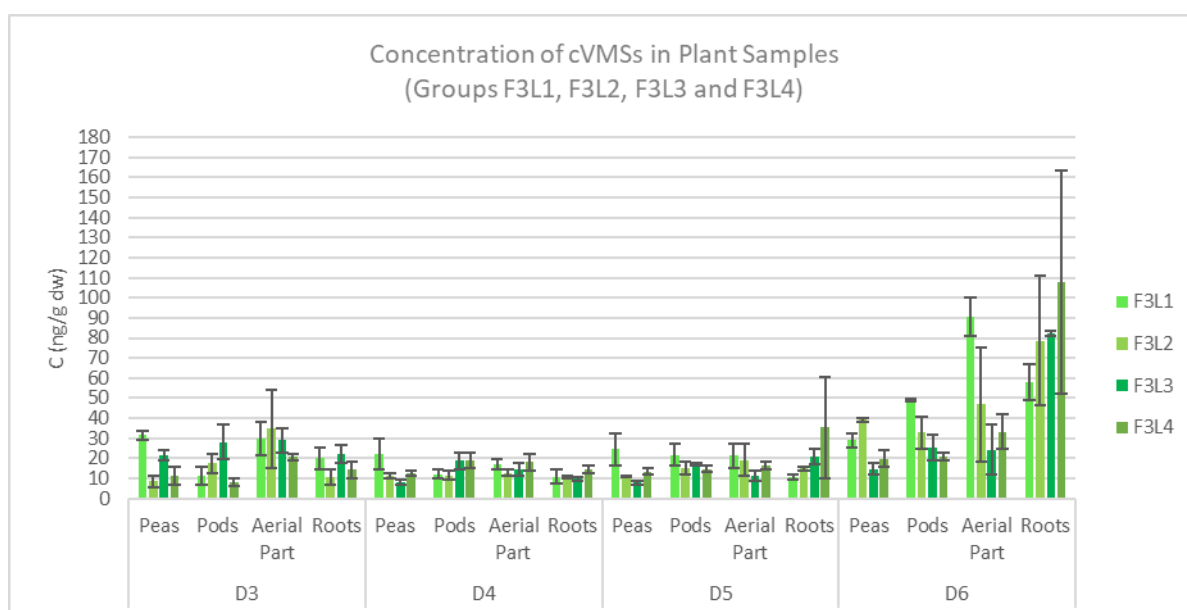


Figure 8. Concentrations of cVMSs in the different plant compartments of test groups F3L1-L4.

Overall, D3 does not seem to show a standard behaviour that can be related to the different sewage sludge application rates tested. In fact, this compound was not present in the fortification solution used and therefore, its behaviour was expected to be similar in treatments with the same application rate (e.g. F0L1~F1L1~F2L1~F3L1). This is also the most volatile cVMS and, therefore, some losses by volatilization along with slight variations in the initial concentration in the amended soils may in part explain the results.

D6 was generally detected in higher concentrations than the remaining cVMSs across all plant compartments. This is the most hydrophobic VMS among the selected compounds and it is also the least volatile. It is also present in significantly lower levels than D5 in all amended soils. In conclusion, D6 seems more prone to uptake and translocation likely due to its physicochemical properties, conferring more affinity to plant tissues. In turn, D4 and D5 which have more similar Log K_{ow} values seem to have been uptaken/translocated in more similar patterns and concentrations than D6, despite the differences in initial amended soil concentration being greater than the ones between D5 and D6.

While application rate seems to have a slight influence on root uptake similar to what was observed with SMs, no clear pattern between application rate and concentration in the above ground sections is discernible. Regarding the effect of spiking rates, D4-D6 levels in above ground plant samples seem to plateau below 40 ng/g dw in the highest spike level groups, excluding a few outliers. Considering the similar concentrations of D4 and D5 in plant samples, despite their very different concentrations in the amended soils, it is possible to infer that translocation of cVMSs in *Pisum sativum* is also a rate limited process.

To the best of the author's knowledge, this was the first plant uptake study performed with VMSs. Although the mechanisms involved are not clear, the main conclusion of this study is that cVMSs can undergo uptake and translocation in plants cultivated in sludge-amended soils. The extent to which this uptake occurs and can negatively impact health will be discussed further ahead, as well as the partition of VMSs in the soil-plant system.

4.5. CEC Mass Balances

Finally, a mass balance of 9 of the initial target compounds (D3, D4, L4, D5, L5, D6, ADBI, HHCB and AHTN) was performed using the initial estimated input concentration in each pot, and the detected concentrations of each target in the plant samples and in the soil samples collected at the end of the experiment. The analysis of these soil samples was carried out by other researcher of the AGROANUT project and, for that reason, they will not be discussed in detail outside the context of the mass balance. As stated in Section 3, for each sown pot there was a corresponding unsown pot prepared within the same conditions. Soil samples from these pots were also analyzed to compare the effect of plant presence in pollutant concentrations in the soils. Overall, there was a noticeable difference in CECs concentrations in soils of sown and unsown pots at the end of the experiment, meaning the presence of the plants played a key role in dissipation of the contaminants from soils. The greater dissipation should not be just due to plant uptake, since the partition to plant compartments percentages alone do not justify the differences between the two groups. In Table 6, the ranges of average partition percentages

of each CEC to the soil and plant tissues across all test groups are presented. The full mass balances for each compound and test group are available in Annex 6 in Tables A8-A16.

In all sown pots, each of the evaluated CECs was detected at a lower concentration at the end of the experiment than the initial estimated input concentrations (except for two instances where external contamination with cVMSs likely occurred) due to uptake and other losses occurring in the soil. In the plant-free pots, the same did not happen, and 5 VMSs (D4, L4, D5, L5 and D6) were detected in higher concentrations than the estimated initial values in some test groups. These results are likely due to contaminations in the pots that may have occurred over the course of the experiment, for example by deposition of VMSs in the amended soil surface when the tinfoil covers were removed during watering. Contaminations with musk compounds seem to have been comparatively much more contained, with just one group presenting a higher quantity of AHTN in the final unsown amended soil than the initial estimate. Contaminations with synthetic musks were comparatively less significant probably due to the limitations in use of fragrances and cosmetics products by all of the involved during the experiment; in contrast VMSs are much more ubiquitous in daily life so it is reasonable that contaminations involving them would be harder to avoid. Despite this, cVMSs seem to have been more persistent.

Table 6. Average (n=3) range of partition of CECs to soil and plant compartments.

Compound	Sown Pots						Plant-Free Pots	
	% Peas	% Pods	% Aerial Parts	% Roots	% Soil	% Losses	% Soil	% Losses
D3	0.00 – 0.33	0.00 – 0.32	0 – 0.58	0.00 – 0.95	0.00 – 73.34	25.15 – 99.94	24.29 – 90.71	9.29 – 75.71
D4	0.00 – 0.45	0.00 – 0.29	0.01 – 1.63	0.01 – 0.80	1.60 – 109.12	-10.50 – 98.33	21.75 – 159.86	-59.86 – 78.25
L4	0.00	0.00	0.00	0.00	0.00	100.00	68.34 – 158.04	-58.04 – 31.66
D5	0.00 – 0.08	0.00 – 0.08	0.01 – 1.69	0.00 – 0.39	3.56 – 53.11	44.81 – 96.37	70.92 – 140.29	-40.29 – 29.08
L5	0.00	0.00	0.00	0.00	0.00 – 42.76	57.24 – 100.00	91.77 – 160.96	-60.96 – 8.23
D6	0.00 – 0.61	0.00 – 0.55	0.01 – 2.75	0.02 – 0.90	21.85 – 115.43	-19.94 – 77.90	68.23 – 359.97	-259.97 – 31.77
ADBI	0.00	0.00	0.00	0.00	0.00 – 23.00	77.00 – 100.00	14.18 – 57.81	42.19 – 85.82
HHCB	0.00 – 0.001	0.00 – 0.002	0.00 – 0.15	0.00 – 0.42	0.00 – 30.70	69.27 – 99.43	0.00 – 63.93	36.07 – 100.00
AHTN	0.00	0.00	0.00	0.00 – 0.19	36.76 – 66.05	33.83 – 63.19	35.30 – 104.66	-4.66 – 104.66

In sown pots, D6 and AHTN dissipated, on an average of all test groups, to around 50% of their initial estimated quantity in the 86-day test period. This period is smaller than the previously reported half-life values of AHTN in amended soils without plants (2-24 years) (Litz et al., 2007), but greater than the estimated 33 hours half-life of D6 proposed by Xu and Chandra (1999). L5 and ADBI were not detected in plant compartments but were detected in the final amended sown soils of some test groups in no clear pattern. L4 was only included in the mass balance because of the unsown pot data, where it didn't completely dissipate in some groups.

D6 seems to be the most persistent compound in amended soil. When comparing different treatment groups, in higher initial soil pollutant concentrations, the percentages of partition to plants were visibly lower than in the lower initial pollutant concentration groups, supporting the hypothesis that plant uptake of D6 from soil may be rate limited. The same phenomenon can also be observed with the other spiked compounds, D4, D5, HHCB and AHTN.

4.6. Bioconcentration Factors

BCF values of D3, D4, D5, D6, HHCB and AHTN were calculated for each of the plant matrices across each of the treatment groups, using the detected concentrations of each target in the plant tissues and the estimated initial concentrations in pot soil on a dry mass basis. The range of BCF values for each plant compartment are presented in Table 7 and the full values are available in Annex 8 in Tables A17 and A18. In cases where the initial CEC concentration in the pot soil was 0, BCFs were not calculated.

Table 7. Range of calculated Bioconcentration Factors (BCFs) of CECs in plant compartments.

Compound	Pea	Pod	Aerial Parts	Roots
D3	0.49 – 7.52	0.00 – 10.24	0.00 – 15.24	0.00 – 8.22
D4	0.00 – 6.04	0.00 – 6.88	0.10 – 27.10	0.08 – 7.30
D5	0.00 – 0.69	0.00 – 1.44	0.04 – 51.36	0.05 – 2.00
D6	0.11 – 7.79	0.08 – 12.32	0.12 – 39.31	0.39 – 11.44
HHCB	0.00 – 0.02	0.00 – 0.01	0.00 – 0.53	0.06 – 4.65
AHTN	0.00	0.00	0.00	0.00 – 1.72

When comparing the BCF values, the most bioaccumulative appears to be D6, which is in line with the mass balance results. The BCFs of D5 were below 1 in nearly all test groups and plant compartments and D5 appeared to be less bioaccumulative than D4 and D6. One exception occurred in the aerial parts of the control group, where D5 was detected at a very high concentration (103 ± 37 ng/g) when compared to its concentration in the initial soil (2 ± 1 ng/g dw) likely due to contaminations, so the resulting BCF was much higher than all other values calculated for this compound and a clear outlier. According to the results of Table 7, VMSs appear to be more bioaccumulative than SMs, especially when compared to AHTN, which did not accumulate anywhere but in roots and had been previously shown to be less bioaccumulative in plants than HHCB (Macherius et al., 2012). Most BCF values listed in Table 7 are above the 0.01 threshold, a value from which the existence of risks to animals via plant consumption is no longer considered minimal. However, this metric does not include relevant factors such as the frequency of intake and is therefore, a superficial conclusion.

One advantage that BCF possesses, despite its limitations, is allowing simple comparisons of plant uptake between soils with different pollutant concentrations. BCFs tended to be lower in groups with

higher initial concentrations of CECs in the soil. The same was verified by Ziarrusta (2015) with carrots when comparing a low pollutant concentration group of plant samples and a higher pollutant concentration in soil. That same work, along with Macherius et al. (2012), also presents similar patterns across the plant compartments; both report BCFs to be higher in roots followed by leaves, similar to what was observed in this work regarding HHCB across all treatment groups, even if in this work some BCF values were higher than the range of 0.02 to 1.5 described in the aforementioned studies, probably due to the different crop used. The same is not verified with cVMSs, where the BCFs values tended to be higher in the aerial parts rather than in the roots. Because there are no plant uptake studies of VMSs available in the literature, there are also no BCF values to compare with ones obtained in an uptake context to aid in justifying this phenomenon. Likely causes could be accrued transport of VMSs through the plant's aerial parts or contaminations of the plant specimens by deposition of the pollutants in the above ground sections, which could help explain why VMSs seemed to accumulate more in the pods rather than peas, which are in no direct contact with atmospheric air.

4.7. CECs exposure via pea consumption

To assess any potential risk to human health that the consumption of peas from amended soils may yield, a simple analysis of tolerable weekly dosage (TWI) vs estimated exposure dosage (EED) was conducted, assuming a worst-case scenario, i.e. the highest recorded, of D3, D4, D5, D6 and HHCB in the analyzed peas. Because AHTN was not detected in any pea samples, the calculations for this compound were performed considering the LOD as the worst-case scenario, replacing the value of concentration. For the calculations, an average adult bodyweight of 70 kg was considered. Weekly intake of peas was estimated assuming at least 1 ingestion of 86 g at least once every two days (US EPA, 2011), which is equivalent to an intake of 0.301 kg of peas per week. TWI was estimated based on acceptable daily intake (ADI), which in turn was estimated based on NOAEL values found in literature for rats, assuming an adjustment factor of 100 (10× for intraspecies variability and 10× for extrapolation of data from rats to humans). The calculated EED values are presented in Table 8 along with the estimated ADI and TWI values as well as the considered NOAEL ingestion values and the selected concentrations of contaminant in peas.

Table 8. CECs' estimated exposure doses, tolerable weekly intakes and data utilized in their calculations.

Compound	Concentration in Peas (mg/kg ww)	NOAEL ingestion (mg/kg bw/day)	Acceptable daily intake (ADI) (mg/kg bw/day)	Tolerable weekly intake (TWI) (mg/kg bw/week)	Estimated exposure dose (EED) (mg/kg bw/week)
HHCB	1.34×10^{-3}	150 ^a	1.5	10.5	5.77×10^{-6}
AHTN	6.80×10^{-4}	5 ^b	0.05	0.35	2.92×10^{-6}
D3	1.10×10^{-2}	2000 ^{c,*}	2	14	4.73×10^{-5}
D4	6.58×10^{-3}	960 ^d	9.6	67.2	2.83×10^{-5}
D5	8.24×10^{-3}	1000 ^e	10	70	3.54×10^{-5}
D6	1.47×10^{-2}	1000 ^f	10	70	6.32×10^{-5}

bw – bodyweight; ww – wet weight;

*Estimated based on DL₅₀, NOAEL value not available.^aECHA, 2008a; ^bECHA, 2008b; ^cECHA, 2021c; ^dECHA, 2021d; ^eECHA, 2021e; ^fECHA, 2021f.

All the calculated EED values were very low and well below the corresponding TWI as can be observed in Table 8. By this metric, the likelihood of any of the presented CECs posing health risks to consumers seems low, in contrast with some of the results yielded by the BCF analysis. This metric reveals VMSs exposure via ingestion to be greater than that of SMs, which is in line with the BCF values presented in the previous subsection. While also edible depending on the variety, pea pods were not included in this assessment because the required reference values were not available. Judging by the low EED obtained with peas however, similarly low risk from pod consumption could be expected.

According to this metric, sewage sludge application to soils intended for the cultivation of the chosen crop appears to yield no adverse effects to consumers, at least when applied in similar rates to the ones tested, which in turn had been chosen partially based on common application rates in agriculture, and in which the contaminants concentration don't exceed the ones presented in this work.

5. Conclusions

An 86-day long greenhouse experiment studied the uptake of synthetic musks (SMs) and volatile methylsiloxanes (VMSs) from sewage sludge amended soils, using the crop *Pisum sativum*. Upon sample analysis and data treatment, 6 of the original 17 target compounds were detected in plant samples. Of these, 2 were the SMs HHCB and AHTN, which showed somewhat different behaviours. The more soluble and less hydrophobic HHCB was detected in some of the above ground parts of the plant, including peas and pods in small concentrations (up to 6.90 ± 0.02 ng/g dw), while AHTN was only uptaken by the roots without quantifiable translocation to other plant sections. The likely source of this difference in behaviour ties with the compounds themselves, namely their physicochemical properties and differing sorption to soils, which is also the likely reason AHTN was eventually detected in higher levels in roots (up to 283 ± 83 ng/g dw) of test groups with more pollutant saturation. In line with the literature, HHCB proved to be less persistent in soils when compared to AHTN via mass balances and to be more bioaccumulative in most plant tissue samples with BCF values reaching 4.65. The absence of quantifiable AHTN in above ground sections of the plant is most likely due to crop characteristics.

To the author's best knowledge this was the first plant uptake experiment to include VMSs. These compounds proved to undergo root uptake in concentrations up to 108 ± 56 ng/g dw (D6) and were translocated to all analyzed plant sections even if contaminations may have happened in some samples. D6 proved to be the VMS to undergo uptake to the greatest extent and to have been the most bioaccumulative, with calculated BCF values as high as 39.31. It was also, of all the target compounds, the most persistent in soil, both in sown and unsown soils.

In higher pollutant soil concentrations, the concentrations of the spiked CECs in the plants did not increase in line with the pollutant concentrations in the soil. BCF values also tended to be lower in these test groups, instead of increasing or remaining similar to the lower pollutant concentration groups. These results point to uptake and translocation in pea plants likely being rate limited processes to some extent.

The presence of plants in the soil systems appears to have aided in pollutant dissipation from the amended soils but seeing as the CECs' partition percentages to plant compartments are so small (below 3%), uptake alone cannot account for dissipation. Therefore, other phenomena associated with the root and rhizosphere like microbial degradation are likely largely responsible for the difference in dissipation rates observed between sown and unsown soils.

Lastly, the application of sewage sludge to agricultural soils destined to the cultivation of pea plants does not appear to have health related drawbacks to consumers, even when the worst-case scenarios of

each CEC intake were considered. This result is a promising point in favour of application of WWTP sludge to crop soils but due to the limited data on the topic and variability inter and intra plant species more research is still needed.

6. Limitations and Future Works

The analysis of volatile and semi-volatile compounds at trace levels is a complex task with any matrix, but especially in a matrix as complex as a higher plant like *Pisum sativum*. Plant specimens have innate variability, and no two plants will grow or react to stress in the exact same manner. This variability was visible in very high standard-deviations observed in some plant groups, which may have been attenuated with a greater plant sample size of each group. This would have complicated the logistics of this work in other ways, namely by prolonging the already large amount of time needed for sample treatment. In future plant uptake assays, opting to test fewer conditions and having more pots in each group could be considered, helping to soften the intraspecies variability. This could also yield extra biomass, which could be used to determine some useful parameters which were not evaluated in this work (e.g., root lipid content). In sewage sludge and pot soil samples there is the issue of a probable inhomogeneous mixing due to the difficulty homogenizing these matrices.

Due to the great number of plant and soil samples which had to be analysed, some omissions had to be made. Firstly, soil samples from each pot were not collected at the beginning of the experiment so the mass balances had to be done with estimated concentrations that didn't take into consideration the losses that occurred during the manual mixing of sludges with soils. Despite the surface of the pots having been covered, contaminations seem to have occurred, at least with VMSs (e.g., dust deposition). In an initial phase of the experiment planning, the placement of air samplers above the pots had been considered to track any volatilization losses or other contaminations sources via the air but ended up not being included due to the greenhouse's design and the already large volume of other matrices samples. The addition of these analysis could have been useful in the interpretation of some results but would have prolonged the time needed to complete this work even further. The GC-MS utilized for the analysis and quantification of extracts experienced some malfunctions and once repaired needed to be shared by several parallel projects, greatly delaying the attainment of results.

Finally, regarding future work, as mentioned before, the cultivated plants were also utilized for a parallel work, focusing on the effect of sewage sludge on crop growth and yield. Combining those results with the ones obtained in this study would allow for a more in-depth analysis of the advantages and drawbacks of the use of WWTP sludge on agricultural soils for the cultivation of peas and closely related crops, and such work is planned for the immediate future. Due to the knowledge gaps regarding CEC uptake by crops, further uptake studies with different crops could aid in determining clearer patterns in contaminant-plant interactions.

Some of the results yielded by this work were not fully comprehended due to limitations regarding the interaction of CECs with soil over time. The determination of sorption capacities and kinetics of the CECs to soil could help interpret some of the results yielded by this work, namely those of AHTN and HHCB uptake in roots. Additionally, this type of experiments should be planned in the future to help bridge the knowledge gaps regarding the behaviour of CECs in soils.

While the results regarding the dissipation of CECs from the sown sludge amended soils seem encouraging, this experiment did not take into consideration the effects of repeated applications to the same soil. Seeing as data regarding repeated applications of sludge to soils are somewhat sparse it would make sense to complete the assessment of the risk of sludge application in agricultural soils with experiments including this variable at some point in the future.

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Annex 1 Pictures from experiment set-up, sample treatment and extraction procedures

The following photographs were taken over the course of the experimental work to complement the description of the experimental set-up as well as of the procedures described in section 3. Pictures detailing the more relevant steps of the experimental work were chosen and are presented in chronological order.

Experimental set-up:



Figure A1. Sample of the initial WWTP sludge collected from a local WWTP. Figure A2. Spiked sludges before transport to greenhouse site. Figure A3. Sludge weighting in plastic containers. Figure A4. Manual mixing of sludge and soil.



Figure A5. Placement of seeds in a pot. Figure A6. Sowing of seeds in a pot. Figure A7. Pots covered with tinfoil after initial watering (day 0). Figure A8. Visible seedlings after germination (day 7). Figure A9. Experiment set-up (day 7). Figure A10. Experiment set-up after placement of stakes (day 30).



Figure A11. Pots at the beginning of plant collection (day 68).

Sample handling and treatment:



Figure A12. Harvested *Pisum sativum* specimen (without root). Figure A13. Plant specimen from Figure A12 separated into compartments (clockwise from top left: pods, leaves, cut stems, dead leaves and tendrils, peas; center: tendrils). Figure A14. Recovered *Pisum sativum* root before rinsing.



Figure A15. Rinsed roots before freezing. Figure A16. Ground frozen root. Figure A17. Ground frozen peas. Figure A18. Ground frozen pods. Figure A19. Composite ground sample of remaining aerial parts (frozen leaves, stems and tendrils).

Plant Extraction procedure:

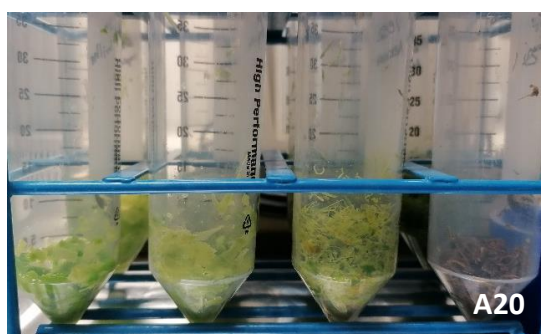


Figure A20. Samples after internal standard addition (left to right: peas, pods, aerial compartments, roots). Figure A21. Falcon tubes in ultrasound bath.

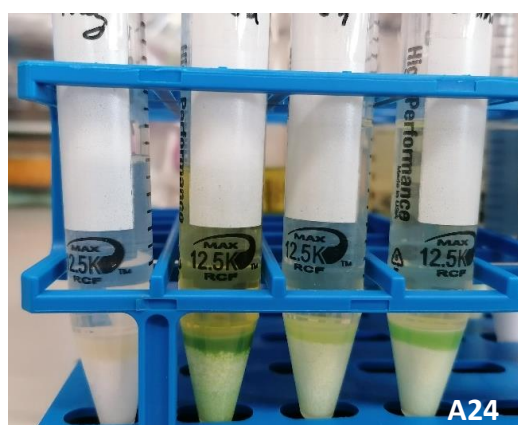


Figure A22. Samples after extraction solvent addition and ultrasound bath. Figure A23. Samples after addition of Q1, vortex and centrifugation. Figure A24. Supernatant from Figure A23 after addition of Q2, vortex and centrifugation. Figure A25. Evaporation step in 12mL vials. Figure A26. Prepared extracts, concentrated to 500 μ L.

Annex 2 SIS mode parameters for the detection and quantification of the different target compounds by GC-MS

Table A1. SIS mode parameters for the detection and quantification of the different target compounds in plant extracts by GC-MS.

Segment Description		Identification and Quantification Parameters		
Time Range (min)	Mass Ranges (m/z)	Target Compound	Retention Time (min)	Qualifier and Quantifier ^(a) Ions (m/z)
0.00 – 7.00	Ionization Off	-	-	-
7.00 – 9.00	132-135, 190-194, 206-210	D3	7.86	133.1, 191.2, 207.1
9.00 – 11.50	130-136, 204-210, 220-224	L3	9.86	133.1, 206.2, 221.2
11.50 – 14.50	190-196, 264-270, 280-285	D4	13.40	193.1, 265.3, 281.2
14.50 – 16.50	190-195, 206-212, 294-298	L4	15.41	191.2, 207.1 , 295.5
16.50 – 18.00	248-255, 266-272, 354-360	D5	17.58	251.2, 267.1 , 355.3
18.00 – 19.00	146-150, 280-286, 367-372	M4Q	18.39	147.2, 281.2 , 369.3
19.00 – 21.00	146-150, 280-286, 367-372	L5	19.83	147.2, 281.2 , 369.3
21.00 – 24.50	324-328, 340-345, 428-434	D6	21.66	325.3, 341.2 , 429.3
24.50 – 27.50	131-138, 161-165, 204-208	DPMI	26.08	135.2 , 163.2, 206.2
27.50 – 31.30	170-175, 229-232, 243-246	ADBI	30.67	173.1, 229.2 , 244.2
31.30 – 33.00	168-175, 184-190, 228-232	AHMI	31.76	187.2, 229.2 , 230.1
33.00 – 37.00	114-139, 151-175, 182-192, 211-215, 242-255, 280-296	MA	34.06	115.2, 134.1, 253.1
		MX-d15	34.50	122.2, 136.2, 294.2
		ATII	34.82	131.2, 173.1, 215.2
		HHCB	34.96	171.1, 213.2, 243.2
		MX	35.04	128.2, 243.3, 282.1
		AHTN-d3	35.19	160.2, 190.2, 246.2
		AHTN	35.23	159.2, 187.2, 243.3
37.00 – 39.60	126-131, 241-249, 277-282	MK	38.97	128.2, 247.2, 279.2
39.60 – 41.50	150-157, 207-215, 224-229	EB	40.33	155.2, 211.2, 227.2
41.50 – 50.00	Ionization Off			

^(a)Qualifier ions in **bold**.

Annex 3 Chromatograms

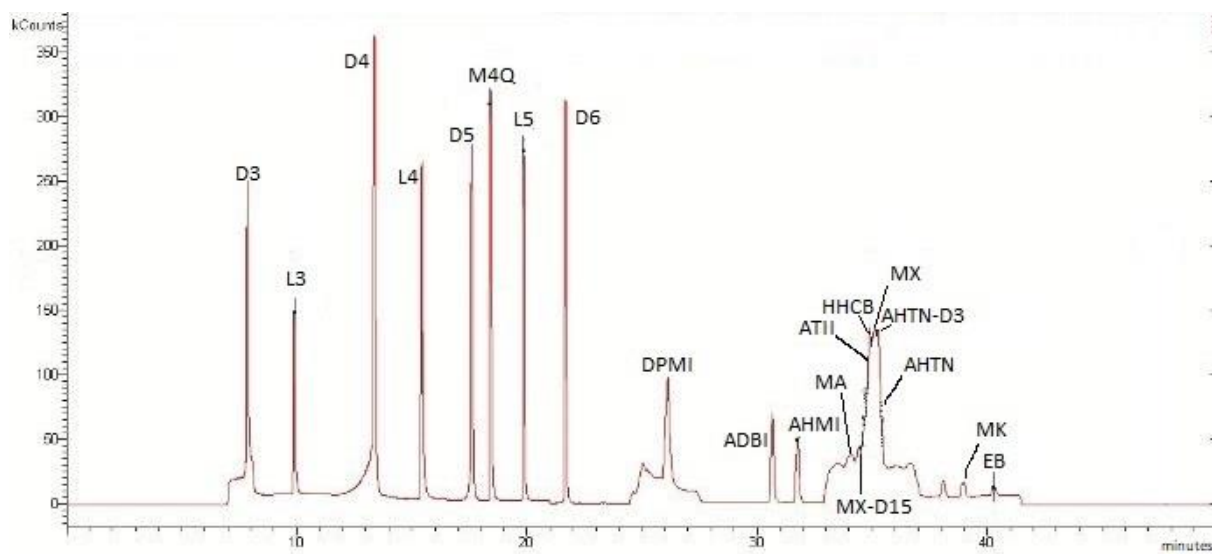


Figure A27. Chromatogram of a calibration standard (200 µg/L).

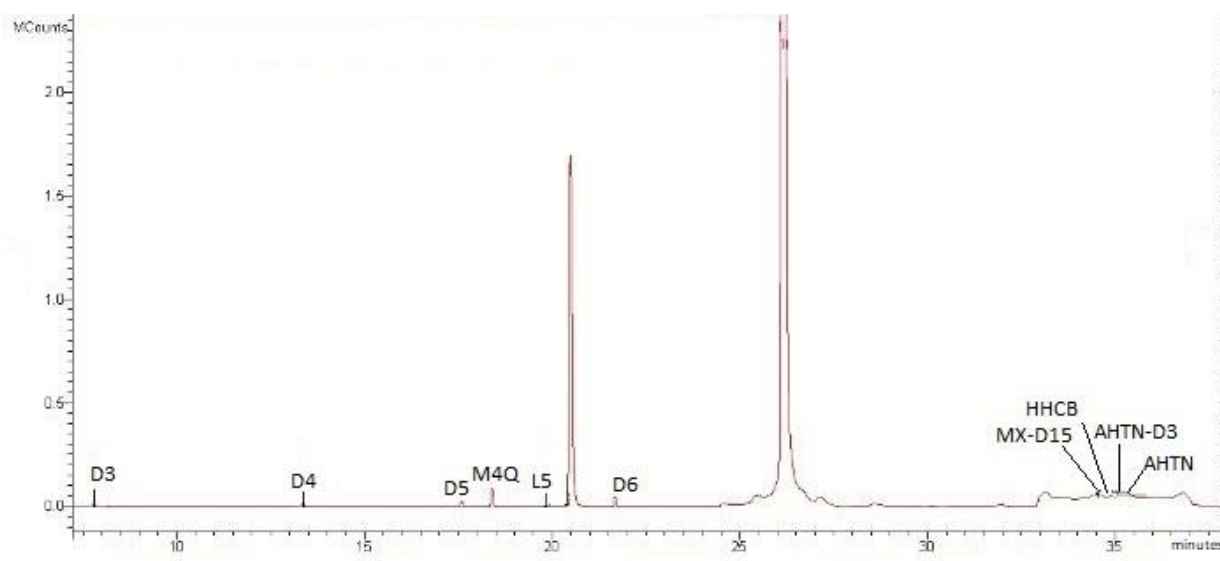


Figure A28. Chromatogram of a *Pisum sativum* root sample (test group F3L4).

Annex 4 Calibration Curves

Calibration curves for each target compound were obtained from the Response Factors (RF) of each calibration standard presented in Table A2. The validation parameters of each curve were then calculated and are summarized in Table A3. Finally, the graphic representations of the calibration curves are presented in Figures A29 to A45.

Table A2. Response factors (RF) obtained for each compound at different concentration levels, used for the construction of the calibration curves.

C (µg/L)	Response Factors (RF)																
	D3	L3	D4	L4	D5	L5	D6	DPMI	ADBI	AHMI	MA	ATII	HHCB	MX	AHTN	MK	EB
5	0.097	0.010	0.137	0.024	0.064	0.017	0.089	0.044	0.021	0.025	0.015	0.020	0.029	0.026	0.089	0.019	0.003
10	0.116	0.023	0.184	0.046	0.086	0.035	0.092	0.056	0.044	0.052	0.029	0.037	0.056	0.034	0.135	0.036	0.006
25	0.172	0.060	0.267	0.116	0.129	0.083	0.183	0.099	0.109	0.135	0.056	0.092	0.146	0.091	0.182	0.086	0.010
50	0.249	0.128	0.390	0.265	0.199	0.192	0.237	0.194	0.261	0.306	0.134	0.221	0.319	0.204	0.311	0.202	0.024
100	0.437	0.291	0.626	0.530	0.324	0.394	0.405	0.325	0.480	0.566	0.246	0.414	0.623	0.410	0.538	0.415	0.047
200	0.809	0.550	1.235	1.089	0.639	0.828	0.729	0.624	1.018	1.176	0.532	0.871	1.304	0.905	1.044	0.893	0.102
300	1.189	0.828	1.805	1.637	0.953	1.273	1.035	1.099	1.752	1.952	0.902	1.495	2.109	1.514	1.718	1.493	0.175
400	1.544	1.105	2.281	2.064	1.188	1.617	1.289	1.488	2.422	2.668	1.259	2.028	2.910	2.181	2.286	2.099	0.239
500	1.955	1.449	2.982	2.728	1.561	2.194	1.716	1.833	2.995	3.295	1.482	2.540	3.650	2.639	2.809	2.574	0.295

Table A3. Calibration curve equations and their respective validation parameters.

Compound	Calibration curve equation $RF = (a \pm s_a).M$	r (> 0.995)	s_a/a ($< 5\%$)
D3	$RF = (0.98 \pm 0.03).M$	0.9986	2.83%
L3	$RF = (0.71 \pm 0.01).M$	0.9996	1.39%
D4	$RF = (1.48 \pm 0.05).M$	0.9984	3.06%
L4	$RF = (1.34 \pm 0.02).M$	0.9997	1.37%
D5	$RF = (0.78 \pm 0.02).M$	0.9987	2.75%
L5	$RF = (1.06 \pm 0.02).M$	0.9993	2.03%
D6	$RF = (0.85 \pm 0.03).M$	0.9973	3.95%
DPMI	$RF = (0.91 \pm 0.02).M$	0.9990	2.42%
ADBI	$RF = (1.47 \pm 0.04).M$	0.9987	2.67%
AHMI	$RF = (1.63 \pm 0.03).M$	0.9993	1.92%
MA	$RF = (0.75 \pm 0.02).M$	0.9988	2.64%
ATII	$RF = (1.25 \pm 0.03).M$	0.9989	2.45%
HHCB	$RF = (1.79 \pm 0.03).M$	0.9993	1.93%
MX	$RF = (1.30 \pm 0.04).M$	0.9983	3.08%
AHTN	$RF = (1.41 \pm 0.03).M$	0.9994	1.90%
MK	$RF = (1.27 \pm 0.03).M$	0.9987	2.67%
EB	$RF = (0.145 \pm 0.004).M$	0.9988	2.64%

RF – response factor; M – ratio of target mass and internal standard mass; r – correlation coefficient; a – slope; s_a – standard deviation of the slope; s_a/a – relative standard deviation of the slope.

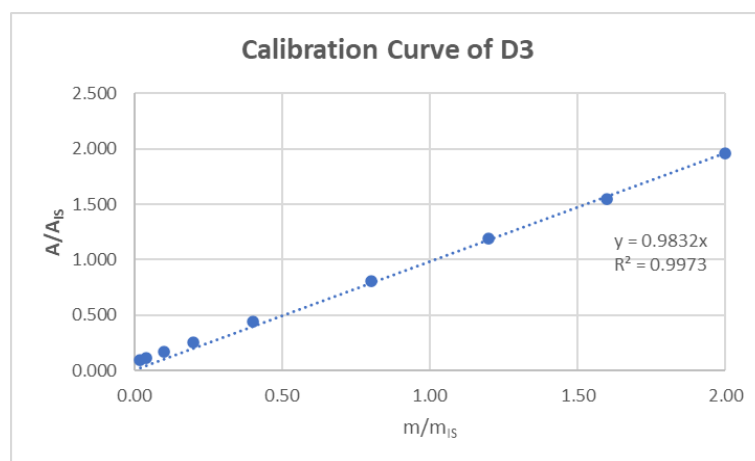


Figure A29. Graphic representation of the calibration curve of D3.

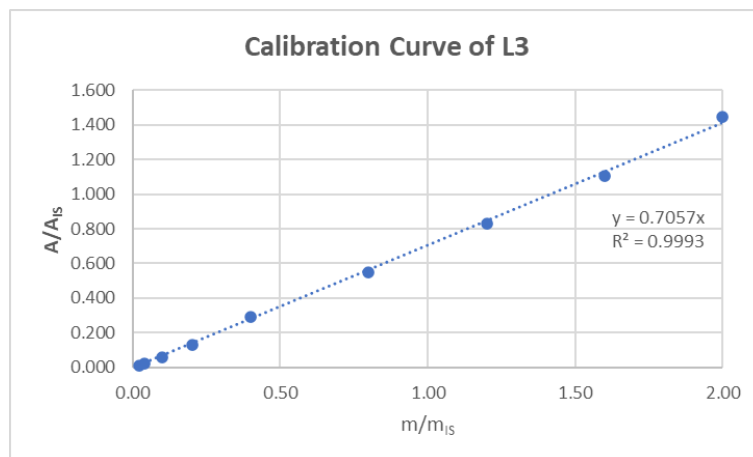


Figure A30. Graphic representation of the calibration curve of L3.

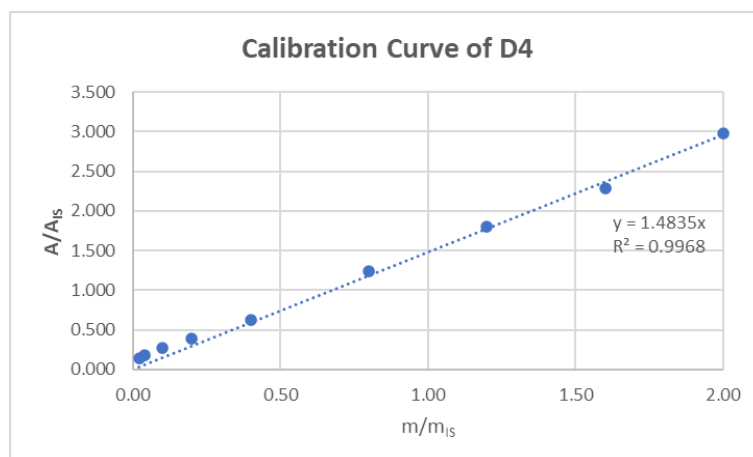


Figure A31. Graphic representation of the calibration curve of D4.

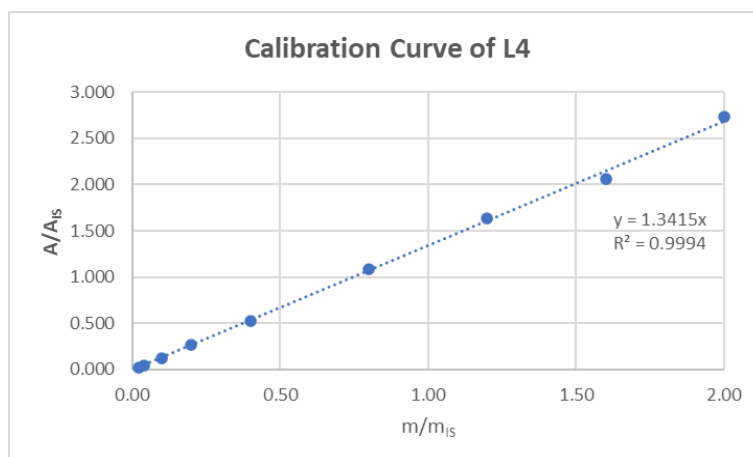


Figure A32. Graphic representation of the calibration curve of L4.

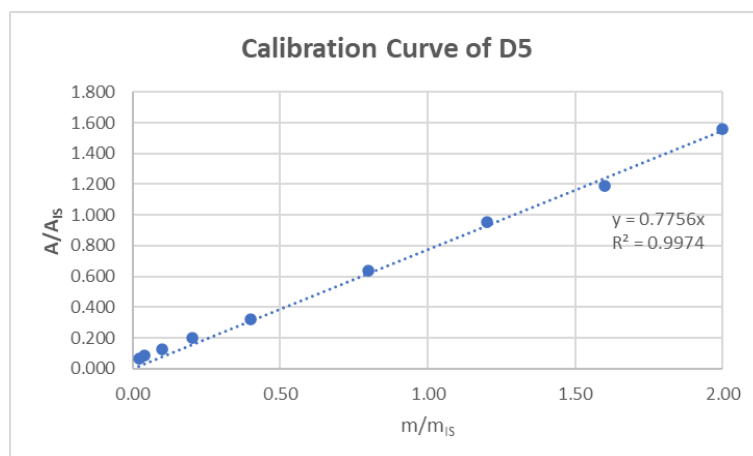


Figure A33. Graphic representation of the calibration curve of D5.

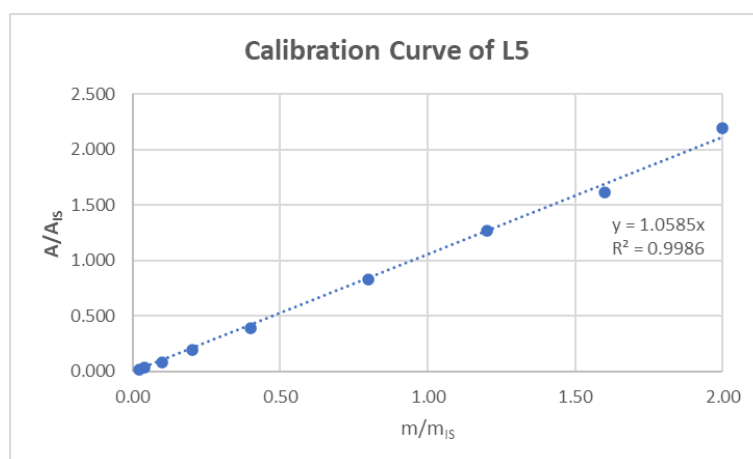


Figure A34. Graphic representation of the calibration curve of L5.

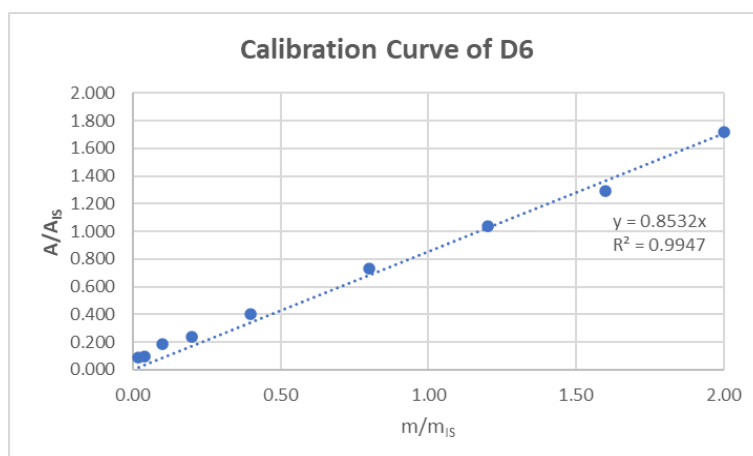


Figure A35. Graphic representation of the calibration curve of D6.

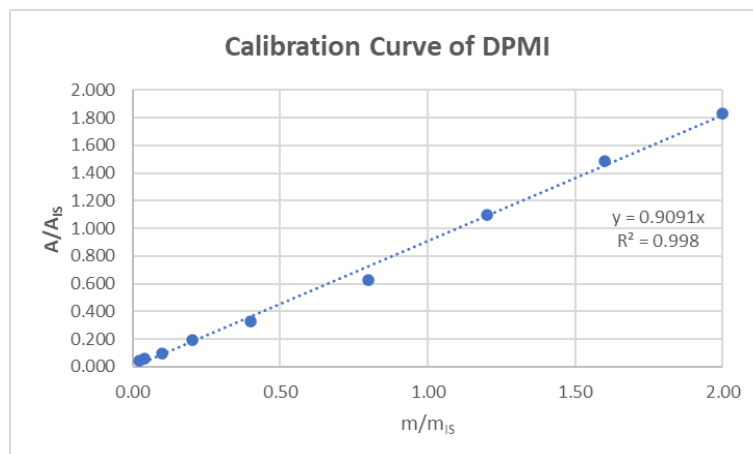


Figure A36. Graphic representation of the calibration curve of DPMI.

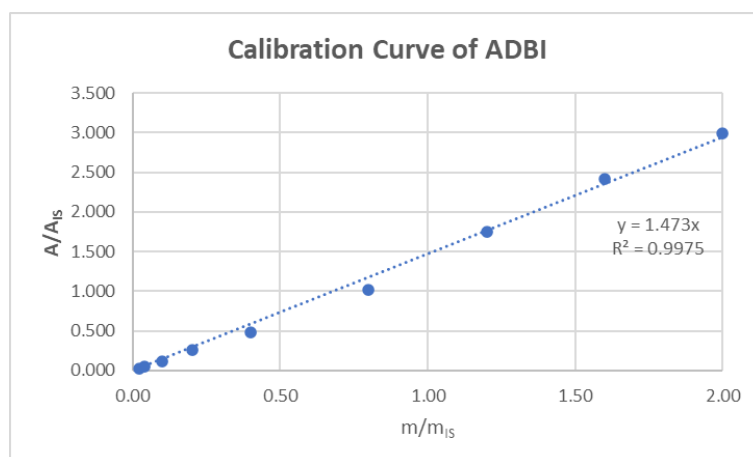


Figure A37. Graphic representation of the calibration curve of ADBI.

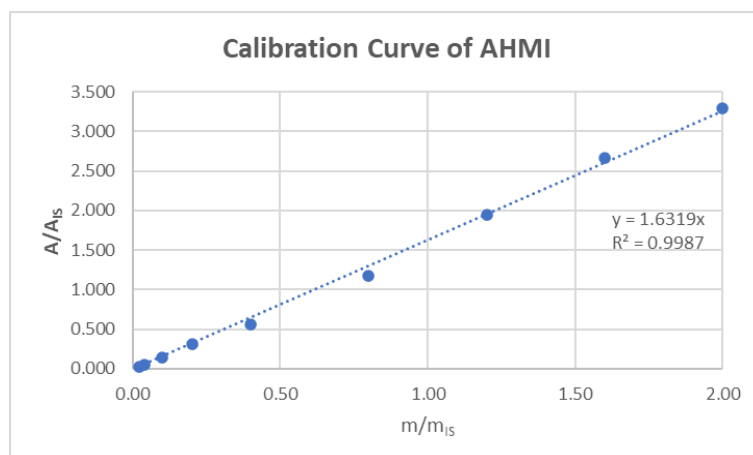


Figure A38. Graphic representation of the calibration curve of AHMI.

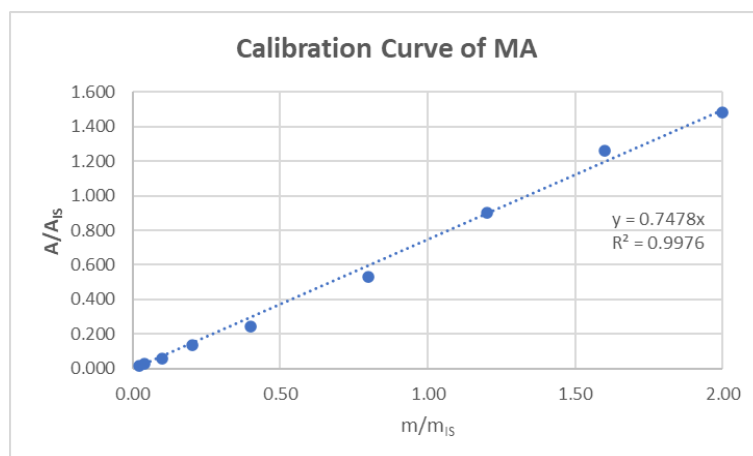


Figure A39. Graphic representation of the calibration curve of MA.

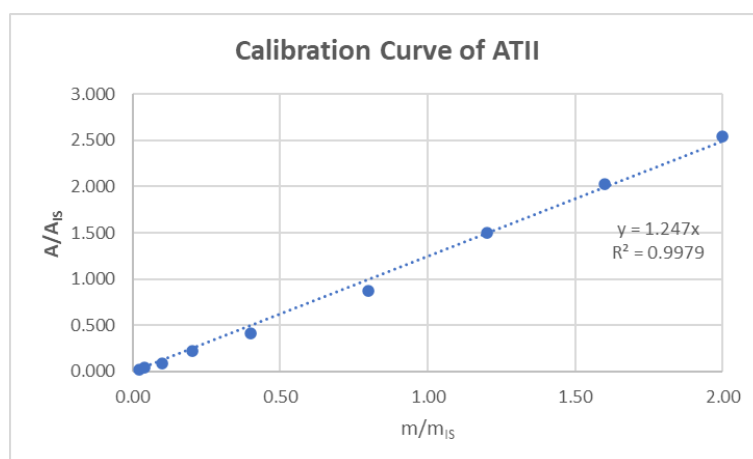


Figure A40. Graphic representation of the calibration curve of ATII.

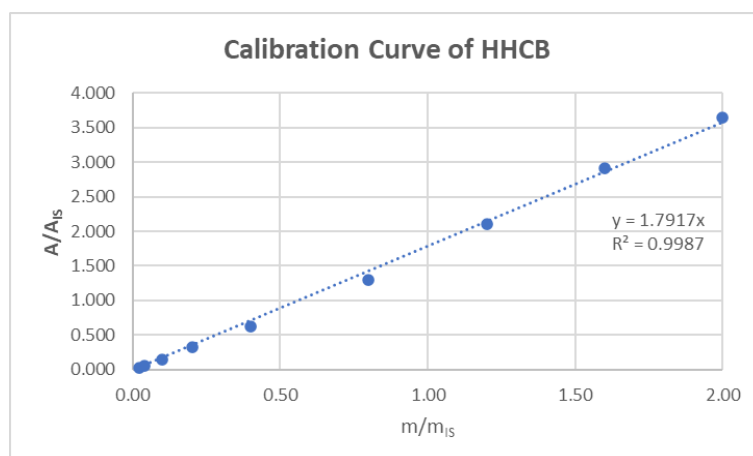


Figure A41. Graphic representation of the calibration curve of HHCB.

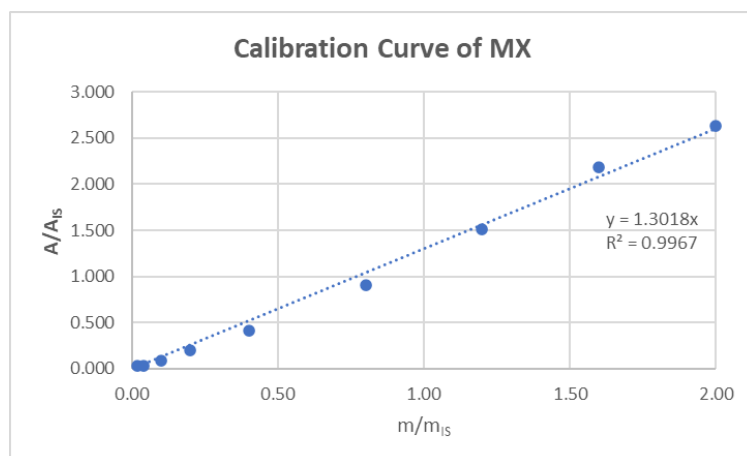


Figure A42. Graphic representation of the calibration curve of MX.

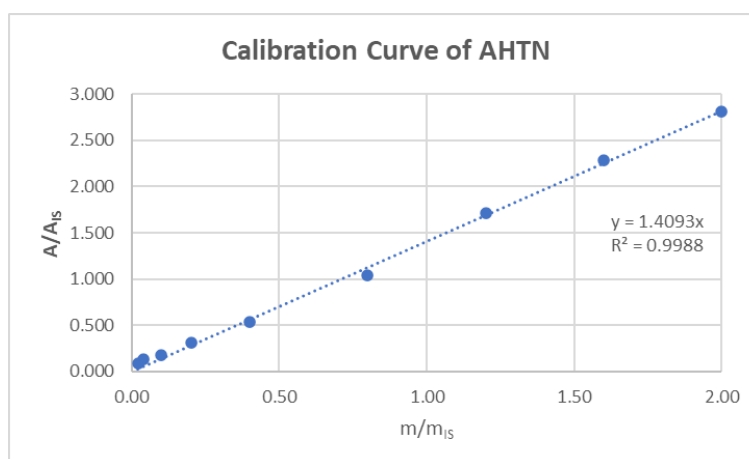


Figure A43. Graphic representation of the calibration curve of AHTN.

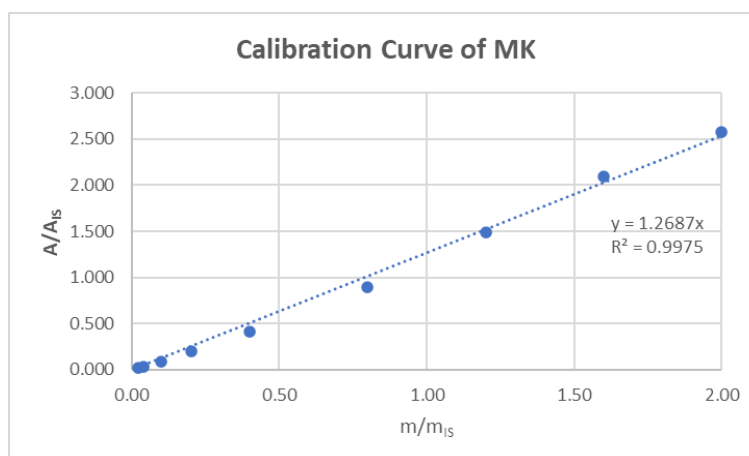


Figure A44. Graphic representation of the calibration curve of MK.

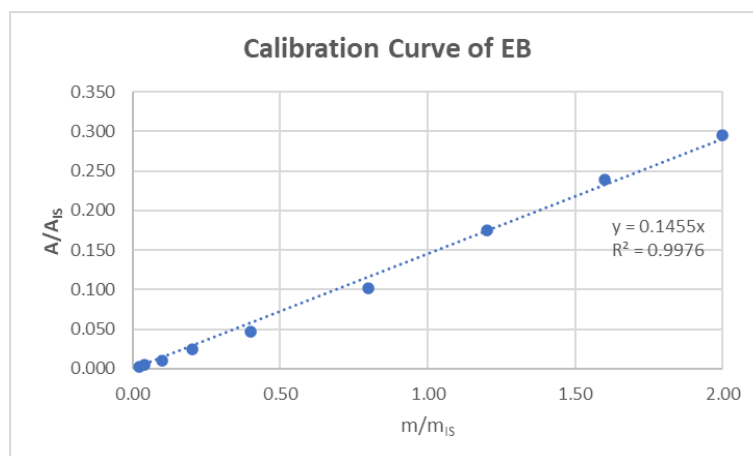


Figure A45. Graphic representation of the calibration curve of EB.

Annex 5 Method Detection Limits of Sludge and Soil Extraction Procedures

Table A4. Method Detection Limits and Method Quantification Limits of the extraction method used in soil and sewage sludge samples according to matrix type.

Compound	Soil		Sewage Sludge	
	MDL (ng/g dw)	ML (ng/g dw)	MDL (ng/g dw)	ML (ng/g dw)
DPMI	2.16	7.21	9.78	32.61
ADBI	0.13	0.42	2.49	8.31
AHMI	0.37	1.24	5.95	19.83
MA	0.07	0.25	0.16	0.55
ATII	0.48	1.62	6.79	22.62
HHCB	0.28	0.94	0.13	0.42
MX	0.71	2.38	4.37	14.56
AHTN	0.41	1.36	2.12	7.06
MK	0.21	0.71	3.10	10.33
EB	0.07	0.23	88.50	294.99
D3	0.34	1.13	0.77	2.57
L3	0.21	0.71	1.04	3.47
D4	0.06	0.19	0.25	0.82
L4	0.12	0.39	0.46	1.54
D5	0.03	0.10	0.00	0.00
L5	0.14	0.47	0.58	1.94
D6	0.03	0.11	0.08	0.26

Annex 6 Average Concentrations of CECs in Plant and Soil Samples

In this section data concerning the detected concentrations of the target CECs in plant, sludge and soil samples are presented across 3 Tables. Table A5 lists the initial pollutant concentrations in the original soil and in the unspiked sludge, along with the estimated initial concentrations of pollutants in pots according to test group. Targets which were not detected in either soil or sludge are not included. Table A6 presents the average (n=3) wet and dry weight concentrations of SMs HHCB and AHTN in each of the analyzed plant sections along with the respective standard deviations. In Table A7 the same is presented for the cVMSs D3-D6.

Table A5. Concentrations (ng/g dw) of target CECs in starter soil and unspiked sludge at the beginning of the experiment (n=3) and estimated concentrations of CECs in pots according to test group.

Matrix/ Test Group	CEC concentration (ng/g dw)									
	D3	L3	D4	L4	D5	L5	D6	ADB1	HHCB	AHTN
Soil t ₀	4.9 ± 0.5	0	2.2 ± 0.6	0	2 ± 1	0	0	0	1.2 ± 0.1	0
Unspiked Sludge	16 ± 7	5 ± 1	216 ± 10	60 ± 49	9340 ± 459	327 ± 11	2009 ± 67	195 ± 6	57297 ± 4169	8127 ± 425
F0L1	4.9 ± 0.5	0.010 ± 0.002	2.6 ± 0.6	0.1 ± 0.1	21 ± 1	0.68 ± 0.02	4.2 ± 0.1	0.41 ± 0.01	121 ± 9	17.0 ± 0.9
F0L2	4.9 ± 0.5	0.022 ± 0.004	3.1 ± 0.6	0.3 ± 0.2	43 ± 2	1.42 ± 0.05	8.7 ± 0.3	0.85 ± 0.03	250 ± 18	35 ± 2
F0L3	5.0 ± 0.5	0.043 ± 0.009	4.1 ± 0.6	0.5 ± 0.4	83 ± 4	2.8 ± 0.1	17.4 ± 0.6	1.69 ± 0.05	498 ± 36	70 ± 4
F0L4	5.1 ± 0.5	0.07 ± 0.01	5.2 ± 0.6	0.9 ± 0.7	135 ± 7	4.7 ± 0.2	29 ± 1	2.78 ± 0.09	817 ± 59	116 ± 6
F1L1	4.9 ± 0.8	0.010 ± 0.002	2.6 ± 0.2	0.12 ± 0.01	21 ± 2	0.68 ± 0.05	4.2 ± 0.3	0.405 ± 0.008	120 ± 4	16.9 ± 0.2
F1L2	4.9 ± 0.8	0.022 ± 0.004	27 ± 2	0.15 ± 0.02	75 ± 6	1.6 ± 0.1	38 ± 3	1.16 ± 0.02	259 ± 10	69.3 ± 0.8
F1L3	5.0 ± 0.8	0.043 ± 0.009	52 ± 5	0.30 ± 0.03	148 ± 12	3.1 ± 0.2	77 ± 5	2.30 ± 0.04	516 ± 19	138 ± 2
F1L4	5.0 ± 0.9	0.07 ± 0.01	85 ± 2	0.50 ± 0.06	242 ± 20	5.1 ± 0.4	126 ± 9	3.78 ± 0.07	846 ± 31	227 ± 3
F2L1	5 ± 1	0.010 ± 0.002	19 ± 8	0.07 ± 0.02	42 ± 11	0.7 ± 0.3	24 ± 9	0.7 ± 0.2	135 ± 24	42 ± 8
F2L2	5 ± 1	0.021 ± 0.004	37 ± 10	0.14 ± 0.04	85 ± 23	1.5 ± 0.5	51 ± 18	1.4 ± 0.3	281 ± 49	88 ± 17
F2L3	5 ± 1	0.041 ± 0.008	71 ± 19	0.28 ± 0.08	166 ± 45	3 ± 1	101 ± 36	2.7 ± 0.7	558 ± 98	175 ± 34
F2L4	5 ± 1	0.07 ± 0.01	115 ± 31	0.5 ± 0.1	272 ± 74	5 ± 2	166 ± 59	4 ± 1	917 ± 161	288 ± 56
F3L1	4.9 ± 0.5	0.010 ± 0.002	29 ± 2	0.08 ± 0.01	56 ± 2	0.87 ± 0.05	41 ± 2	1.01 ± 0.05	166 ± 4	65.6 ± 0.5
F3L2	4.9 ± 0.5	0.021 ± 0.004	57 ± 3	0.17 ± 0.02	116 ± 4	1.8 ± 0.1	87 ± 4	2.1 ± 0.1	345 ± 9	137 ± 1
F3L3	5.0 ± 0.5	0.042 ± 0.008	112 ± 7	0.34 ± 0.03	228 ± 7	3.6 ± 0.2	172 ± 9	4.2 ± 0.2	687 ± 17	273 ± 2
F3L4	5.1 ± 0.5	0.07 ± 0.01	183 ± 11	0.56 ± 0.05	374 ± 12	6.0 ± 0.3	283 ± 14	6.9 ± 0.3	1128 ± 28	448 ± 4

Table A6. Average (n=3) detected concentrations of SMs in plant samples and respective standard deviations (ng/g ww and ng/g dw).

Test Group	Compound	Peas		Pods		Aerial Parts		Roots	
		ng/g ww	ng/g dw	ng/g ww	ng/g dw	ng/g ww	ng/g dw	ng/g ww	ng/g dw
Controls	HHCB	nd	nd	nd	nd	0.6 ± 0.1	3.7 ± 0.7	1.1 ± 0.4	6 ± 2
	AHTN	nd	nd	nd	nd	nd	nd	nd	nd
F0L1	HHCB	nd	nd	nd	nd	0.6 ± 0.3	3 ± 2	1.4 ± 0.5	8 ± 2
	AHTN	nd	nd	nd	nd	nd	nd	nd	nd
F0L2	HHCB	nd	nd	nd	nd	1.3 ± 0.8	8 ± 5	20 ± 2	104 ± 12
	AHTN	nd	nd	nd	nd	nd	nd	9.4 ± 0.9	49 ± 5
F0L3	HHCB	nd	nd	nd	nd	0.55 ± 0.06	3.2 ± 0.4	66 ± 2	346 ± 13
	AHTN	nd	nd	nd	nd	nd	nd	22.6 ± 0.7	119 ± 4
F0L4	HHCB	nd	nd	nd	nd	0.6 ± 0.2	4 ± 1	41 ± 5	214 ± 28
	AHTN	nd	nd	nd	nd	nd	nd	26 ± 3	135 ± 14
F1L1	HHCB	nd	nd	nd	nd	1.3 ± 0.2	5 ± 2	5 ± 2	26 ± 10
	AHTN	nd	nd	nd	nd	nd	nd	4.8 ± 0.3	25 ± 2
F1L2	HHCB	nd	nd	nd	nd	3 ± 3	19 ± 19	17 ± 5	92 ± 25
	AHTN	nd	nd	nd	nd	nd	nd	11.6 ± 0.4	61 ± 2
F1L3	HHCB	1.3 ± 0.5	5 ± 2	nd	nd	nd	nd	22 ± 3	113 ± 15
	AHTN	nd	nd	nd	nd	nd	nd	16 ± 2	82 ± 10
F1L4	HHCB	1.10 ± 0.09	3.7 ± 0.3	nd	nd	nd	nd	39 ± 5	205 ± 24
	AHTN	nd	nd	nd	nd	nd	nd	25.7 ± 0.9	135 ± 5
F2L1	HHCB	0.80 ± 0.09	2.7 ± 0.3	1.152 ± 0.004	6.90 ± 0.02	0.5 ± 0.2	3 ± 1	5.0 ± 0.5	26 ± 3
	AHTN	nd	nd	nd	nd	nd	nd	5.3 ± 0.7	28 ± 4
F2L2	HHCB	0.8 ± 0.1	2.8 ± 0.4	0.62 ± 0.09	3.7 ± 0.5	0.7 ± 0.3	4 ± 2	10 ± 1	51 ± 7
	AHTN	nd	nd	nd	nd	nd	nd	10 ± 3	54 ± 17
F2L3	HHCB	0.64 ± 0.02	2.16 ± 0.07	nd	nd	1.5 ± 0.5	9 ± 3	29 ± 15	154 ± 77
	AHTN	nd	nd	nd	nd	nd	nd	49 ± 2	259 ± 8
F2L4	HHCB	nd	nd	nd	nd	1.0 ± 0.3	6 ± 2	40 ± 14	212 ± 76
	AHTN	nd	nd	nd	nd	nd	nd	54 ± 16	283 ± 83

Table A6. (Cont.) Average (n=3) detected concentrations of SMs in plant samples and respective standard deviations (ng/g ww and ng/g dw).

Test Group	Compound	Peas		Pods		Aerial Parts		Roots	
		ng/g w	ng/g dw	ng/g ww	ng/g dw	ng/g ww	ng/g dw	ng/g ww	ng/g dw
F3L1	HHCB	nd	nd	nd	nd	1 ± 1	8 ± 7	2.5 ± 0.6	13 ± 3
	AHTN	nd	nd	nd	nd	nd	nd	15 ± 3	79 ± 15
F3L2	HHCB	0.73 ± 0.02	2.45 ± 0.05	nd	nd	1.1 ± 0.9	6 ± 5	9.2 ± 0.5	48 ± 3
	AHTN	nd	nd	nd	nd	nd	nd	22.8 ± 0.3	119 ± 2
F3L3	HHCB	nd	nd	nd	nd	0.37 ± 0.04	2.2 ± 0.2	18 ± 6	95 ± 32
	AHTN	nd	nd	nd	nd	nd	nd	38.7 ± 0.8	203 ± 4
F3L4	HHCB	nd	nd	nd	nd	0.80 ± 0.08	4.6 ± 0.5	32 ± 10	167 ± 52
	AHTN	nd	nd	nd	nd	nd	nd	32.3 ± 0.7	169 ± 4

Table A7. Average (n=3) detected concentrations of VMSs in plant samples and respective standard deviations (ng/g ww and ng/g dw).

Test Group	Compound	Peas		Pods		Aerial Part		Roots	
		ng/g ww	ng/g dw	ng/g ww	ng/g dw	ng/g ww	ng/g dw	ng/g ww	ng/g dw
Controls	D3	1.7 ± 0.8	6 ± 3	nd	nd	nd	nd	nd	nd
	D4	nd	nd	nd	nd	9 ± 1	54 ± 8	3 ± 1	16 ± 6
	D5	nd	nd	nd	nd	18 ± 6	103 ± 38	0.8 ± 0.3	4 ± 2
	D6	nd	nd	nd	nd	20 ± 3	116 ± 19	nd	nd
F0L1	D3	1.5 ± 0.1	5.0 ± 0.3	nd	nd	3.4 ± 0.7	20 ± 4	nd	nd
	D4	1.9 ± 0.7	6 ± 2	0.49 ± 0.07	2.9 ± 0.4	2.4 ± 0.7	14 ± 4	0.24 ± 0.01	1.26 ± 0.05
	D5	2.2 ± 0.4	8 ± 1	0.5 ± 0.1	3.0 ± 0.7	2.5 ± 0.4	14 ± 2	0.39 ± 0.03	2.0 ± 0.2
	D6	9 ± 2	32 ± 6	6 ± 3	35 ± 16	10.5 ± 0.2	61 ± 1	1.3 ± 0.3	7 ± 2
F0L2	D3	5.6 ± 0.2	18.8 ± 0.6	nd	nd	1.0 ± 0.1	6.1 ± 0.7	nd	nd
	D4	2.4 ± 0.3	8 ± 1	1.4 ± 0.4	9 ± 3	2 ± 1	14 ± 7	1.5 ± 0.3	8 ± 1
	D5	2.3 ± 0.6	8 ± 2	2.1 ± 0.6	12 ± 3	2.2 ± 0.6	13 ± 3	5.7 ± 0.9	30 ± 5
	D6	7 ± 4	23 ± 13	3 ± 1	19 ± 8	4.2 ± 0.7	25 ± 4	2.89 ± 0.04	15.2 ± 0.2
F0L3	D3	1.9 ± 0.6	6 ± 2	nd	nd	1.8 ± 0.2	10 ± 1	nd	nd
	D4	2.2 ± 0.2	7.4 ± 0.6	2.5 ± 0.1	14.9 ± 0.7	3.0 ± 0.3	17 ± 2	2.1 ± 0.2	11 ± 1
	D5	2.5 ± 0.8	8 ± 3	2.7 ± 0.9	16 ± 6	2.0 ± 0.6	12 ± 3	5.95 ± 0.04	31.2 ± 0.2
	D6	5 ± 1	17 ± 4	2.0 ± 0.7	12 ± 4	7 ± 1	41 ± 9	5 ± 1	28 ± 6
F0L4	D3	2.2 ± 0.9	8 ± 3	nd	nd	5.3 ± 0.9	31 ± 5	6.3 ± 0.8	33 ± 4
	D4	2.2 ± 0.7	7 ± 3	2.5 ± 0.3	15 ± 2	3.0 ± 0.3	17 ± 2	2.1 ± 0.5	11 ± 3
	D5	2.2 ± 0.4	7 ± 1	2.6 ± 0.4	16 ± 2	0.8 ± 0.3	5 ± 2	3.6 ± 0.8	19 ± 4
	D6	3.2 ± 0.9	11 ± 3	3.4 ± 0.5	20 ± 3	3.0 ± 0.8	17 ± 5	5.2 ± 0.4	27 ± 2
F1L1	D3	3.57 ± 0.02	12.08 ± 0.06	0.80 ± 0.02	4.81 ± 0.09	6.6 ± 0.2	38 ± 1	4.76 ± 0.07	24.9 ± 0.4
	D4	4.7 ± 0.6	16 ± 2	3.0 ± 0.3	18 ± 2	12 ± 1	71 ± 7	3.7 ± 0.2	19 ± 1
	D5	4.3 ± 0.5	14 ± 2	5.0 ± 0.4	30 ± 2	14 ± 3	80 ± 16	7.2 ± 0.8	38 ± 4
	D6	4.9 ± 0.8	17 ± 3	8 ± 1	50 ± 9	28 ± 7	160 ± 41	9 ± 1	47 ± 7

Table A7. (Cont.) Average (n=3) detected concentrations of VMSs in plant samples and respective standard deviations (ng/g ww and ng/g dw).

Test Group	Compound	Peas		Pods		Aerial Part		Roots	
		ng/g ww	ng/g dw	ng/g ww	ng/g dw	ng/g ww	ng/g dw	ng/g ww	ng/g dw
F1L2	D3	3.3 ± 0.4	11 ± 1	0.94 ± 0.05	5.6 ± 0.3	5.0 ± 0.1	29.4 ± 0.8	2 ± 2	13 ± 9
	D4	1.3 ± 0.4	4 ± 1	2.9 ± 0.3	17 ± 2	5.62 ± 0.08	32.7 ± 0.5	3.8 ± 0.3	20 ± 1
	D5	5.1 ± 0.1	17.3 ± 0.4	5.0 ± 0.4	30 ± 2	9 ± 2	51 ± 9	7.6 ± 0.8	40 ± 4
	D6	7.9 ± 0.3	26.8 ± 0.9	11 ± 3	67 ± 21	27 ± 4	160 ± 23	13.0 ± 0.3	68 ± 2
F1L3	D3	5.1 ± 0.3	17.3 ± 0.9	3.0 ± 0.4	18 ± 2	3 ± 2	17 ± 9	7.8 ± 0.8	41 ± 4
	D4	2.8 ± 0.4	10 ± 1	3.5 ± 0.9	21 ± 6	3.0 ± 0.6	18 ± 3	5 ± 1	25 ± 6
	D5	8.2 ± 0.6	28 ± 2	5.1 ± 0.6	31 ± 4	4.7 ± 0.7	27 ± 4	8 ± 2	43 ± 12
	D6	15 ± 3	50 ± 10	10 ± 3	60 ± 18	10 ± 4	60 ± 20	14 ± 3	72 ± 15
F1L4	D3	2.270 ± 0.002	7.67 ± 0.01	5 ± 1	30 ± 7	2.1 ± 0.2	12 ± 1	7 ± 1	37 ± 7
	D4	2.3 ± 0.4	8 ± 1	2.3 ± 0.5	14 ± 3	3.3 ± 0.2	19 ± 1	5 ± 1	24 ± 5
	D5	6.72 ± 0.04	22.7 ± 0.1	4 ± 1	27 ± 7	2 ± 1	14 ± 7	6 ± 2	31 ± 8
	D6	12.4 ± 0.8	42 ± 3	12 ± 2	72 ± 15	6 ± 2	34 ± 10	13.2 ± 0.9	69 ± 5
F2L1	D3	2.9 ± 0.3	9.7 ± 0.9	2.0 ± 0.5	12 ± 3	7 ± 1	38 ± 7	6.0 ± 0.7	31 ± 3
	D4	3 ± 1	8 ± 4	5 ± 1	29 ± 8	4.1 ± 0.7	24 ± 4	2.3 ± 0.3	12 ± 2
	D5	1.4 ± 0.5	5 ± 2	0.55 ± 0.04	3.3 ± 0.2	4.8 ± 0.9	28 ± 5	3.2 ± 0.7	17 ± 4
	D6	10 ± 4	34 ± 13	9 ± 3	55 ± 19	10 ± 3	57 ± 15	6 ± 2	33 ± 11
F2L2	D3	11 ± 4	37 ± 15	2.5 ± 0.7	15 ± 4	3.8 ± 0.8	22 ± 5	7 ± 2	37 ± 12
	D4	5 ± 2	16 ± 8	3.2 ± 0.5	19 ± 3	1.4 ± 0.2	8 ± 1	3.4 ± 0.6	18 ± 3
	D5	0.80 ± 0.08	2.7 ± 0.3	4 ± 1	22 ± 8	2.4 ± 0.8	14 ± 4	4.1 ± 0.8	22 ± 4
	D6	8 ± 3	28 ± 9	3.5 ± 0.8	21 ± 5	3.3 ± 1.3	19 ± 7	8 ± 3	42 ± 17
F2L3	D3	0.7 ± 0.5	2 ± 2	9 ± 2	51 ± 11	3.77 ± 0.08	22.0 ± 0.5	6.6 ± 0.9	35 ± 5
	D4	1.8 ± 0.1	6.0 ± 0.4	6.7 ± 0.6	40 ± 3	6 ± 2	34 ± 12	6 ± 3	30 ± 13
	D5	7 ± 1	22 ± 3	23 ± 6	139 ± 35	6 ± 3	37 ± 16	7.6 ± 0.2	40 ± 1
	D6	3 ± 3	11 ± 9	4.4 ± 0.4	26 ± 3	12 ± 3	69 ± 18	20 ± 4	105 ± 22
F2L4	D3	1.6 ± 0.2	5.5 ± 0.8	4.9 ± 0.4	30 ± 3	13.2 ± 0.9	77 ± 5	5.44 ± 0.05	28.5 ± 0.2
	D4	3.6 ± 0.7	12 ± 2	2.5 ± 0.2	15 ± 1	8 ± 3	48 ± 20	2.5 ± 0.2	13.0 ± 0.9
	D5	5 ± 1	18 ± 4	2.9 ± 0.1	17.6 ± 0.6	12.2 ± 0.6	71 ± 3	2.8 ± 0.2	15 ± 1
	D6	6 ± 4	20 ± 13	7 ± 1	42 ± 8	28 ± 5	161 ± 28	14.2 ± 0.8	74 ± 4

Table A7. (Cont.) Average (n=3) detected concentrations of VMSs in plant samples and respective standard deviations (ng/g ww and ng/g dw).

Test Group	Compound	Peas		Pods		Aerial Part		Roots	
		ng/g ww	ng/g dw	ng/g ww	ng/g dw	ng/g ww	ng/g dw	ng/g ww	ng/g dw
F3L1	D3	9.3 ± 0.6	31 ± 2	1.9 ± 0.8	11 ± 5	5 ± 1	30 ± 8	4 ± 1	20 ± 5
	D4	7 ± 2	22 ± 8	2.0 ± 0.4	12 ± 2	3.0 ± 0.5	17 ± 3	2.1 ± 0.6	11 ± 3
	D5	7 ± 2	25 ± 8	3.6 ± 0.9	22 ± 6	4 ± 1	21 ± 6	2.0 ± 0.2	11 ± 1
	D6	9 ± 1	29 ± 4	8.21 ± 0.09	49.2 ± 0.5	16 ± 2	90 ± 10	11 ± 2	58 ± 9
F3L2	D3	2.6 ± 0.9	9 ± 3	2.9 ± 0.8	18 ± 5	6 ± 3	35 ± 20	2.0 ± 0.7	10 ± 4
	D4	3.3 ± 0.4	11 ± 2	1.9 ± 0.4	12 ± 2	2.2 ± 0.3	13 ± 2	2.0 ± 0.2	10.7 ± 0.9
	D5	3.37 ± 0.05	11.4 ± 0.2	2.5 ± 0.6	15 ± 3	3 ± 1	19 ± 8	2.9 ± 0.2	15.1 ± 0.9
	D6	11.6 ± 0.3	39.3 ± 0.9	6 ± 1	33 ± 8	8 ± 5	47 ± 28	15 ± 6	79 ± 33
F3L3	D3	6.4 ± 0.7	21 ± 2	5 ± 1	28 ± 9	5 ± 1	29 ± 6	4.2 ± 0.9	22 ± 5
	D4	2.3 ± 0.4	8 ± 1	3.1 ± 0.6	19 ± 4	2.5 ± 0.6	15 ± 3	1.9 ± 0.2	9.9 ± 0.9
	D5	2.4 ± 0.3	8 ± 1	2.8 ± 0.1	17.1 ± 0.6	1.9 ± 0.4	11 ± 2	3.9 ± 0.7	21 ± 4
	D6	4.4 ± 0.8	15 ± 3	4 ± 1	25 ± 7	4 ± 2	24 ± 13	15.7 ± 0.2	82 ± 1
F3L4	D3	3 ± 1	12 ± 4	1.4 ± 0.4	8 ± 2	3.5 ± 0.3	21 ± 2	2.7 ± 0.8	14 ± 4
	D4	3.7 ± 0.3	13 ± 1	3.1 ± 0.6	19 ± 4	3.1 ± 0.7	18 ± 4	2.7 ± 0.4	14 ± 2
	D5	3.9 ± 0.5	13 ± 2	2.5 ± 0.2	15 ± 1	2.8 ± 0.4	16 ± 2	7 ± 5	35 ± 25
	D6	6 ± 1	20 ± 4	3.5 ± 0.3	21 ± 2	6 ± 2	33 ± 9	21 ± 11	108 ± 56

Annex 7 CECs Mass Balances in Soil-Plant System

In Tables A8 through A16 the mass balances for some of the target CECs are presented. The compounds included were all detected in at least one of the analyzed matrix types (including both sown and unsown pots).

Table A8. Mass Balance of D3.

Test Group	CEC mass in soil t_0 (ng dw)	Average CEC Mass per Pot (ng dw)						Partition %							
		Sown pots					Plant-free pots	Sown pots						Plant-free pots	
		Peas	Pods	Aerial Part	Roots	Soil t_f	Soil t_f	Peas	Pods	Aerial Part	Roots	Soil t_f	Losses	Soil t_f	Losses
Controls	6012	3 ± 2	0	0	0	0	2988 ± 113	0.06	0.00	0.00	0.00	0.00	99.94	49.69	50.31
F0L1	6052	8 ± 5	0	15 ± 6	0	0	1809 ± 134	0.13	0.00	0.25	0.00	0.00	99.62	29.89	70.11
F0L2	6096	20 ± 3	0	5 ± 2	0	3679 ± 1240	3156 ± 2546	0.33	0.00	0.09	0.00	60.35	39.23	51.76	48.24
F0L3	6180	6 ± 3	0	8.7 ± 0.8	0	2685 ± 1637	3402 ± 1223	0.10	0.00	0.14	0.00	43.44	56.32	55.04	44.96
F0L4	6289	9 ± 4	0	36 ± 3	59 ± 6	2578 ± 486	4094 ± 1304	0.15	0.00	0.58	0.95	40.99	57.34	65.09	34.91
F1L1	6052	9 ± 3	4 ± 2	35 ± 12	17 ± 12	0	2545 ± 707	0.15	0.06	0.58	0.28	0.00	98.93	42.04	57.96
F1L2	6080	11 ± 2	3.2 ± 0.5	22 ± 4	14 ± 6	0	4195 ± 476	0.17	0.05	0.36	0.22	0.00	99.19	69.00	31.00
F1L3	6148	16 ± 8	13 ± 3	18 ± 10	45 ± 19	0	3995 ± 2193	0.26	0.21	0.29	0.73	0.00	98.51	64.98	35.02
F1L4	6236	9.0 ± 0.4	20 ± 1	13.8 ± 0.4	57 ± 2	0	5657 ± 1497	0.14	0.32	0.22	0.92	0.00	98.40	90.71	9.29
F2L1	6052	8 ± 4	5 ± 1	33 ± 27	24 ± 9	3801 ± 1411	3369 ± 673	0.13	0.08	0.55	0.40	62.79	36.05	55.65	44.35
F2L2	6097	18 ± 5	4 ± 2	9 ± 6	36 ± 29	2893 ± 188	4958 ± 1105	0.29	0.07	0.15	0.58	47.45	51.45	81.32	18.68
F2L3	6181	0	19 ± 4	13 ± 4	26 ± 9	1952 ± 769	4039 ± 1817	0.00	0.31	0.21	0.42	31.58	67.49	65.33	34.67
F2L4	6292	4 ± 1	16 ± 1	52 ± 12	22 ± 6	4615 ± 1953	5143 ± 1724	0.07	0.26	0.82	0.36	73.34	25.15	81.74	18.26
F3L1	6051	14 ± 7	4 ± 2	12 ± 4	15 ± 8	0	1470 ± 25	0.23	0.07	0.20	0.24	0.00	99.25	24.29	75.71
F3L2	6093	5 ± 2	7 ± 4	18 ± 8	10 ± 5	2165 ± 946	1626 ± 1016	0.08	0.12	0.29	0.16	35.52	63.83	26.69	73.31
F3L3	6174	14 ± 5	11 ± 4	20 ± 13	19 ± 8	2655 ± 243	1751 ± 321	0.23	0.18	0.33	0.30	43.00	55.95	28.35	71.65
F3L4	6279	12 ± 9	4 ± 1	19 ± 5	14 ± 4	0	2250 ± 427	0.19	0.07	0.31	0.22	0.00	99.21	35.83	64.17

dw – dry weight; t_0 – initial time (day 0); t_f – final time (pot collection day).

Table A9. Mass Balance of D4.

Test Group	CEC mass in soil t_0 (ng dw)	Average CEC Mass per Pot (ng dw)						Partition %							
		Sown pots					Plant-free pots	Sown pots						Plant-free pots	
		Peas	Pods	Aerial Part	Roots	Soil t_f	Soil t_f	Peas	Pods	Aerial Part	Roots	Soil t_f	Losses	Soil t_f	Losses
Controls	2699	0	0	15.7 ± 12	22 ± 24	2946 ± 0	2043 ± 346	0.00	0.00	0.58	0.80	109.12	-10.50	75.70	24.30
F0L1	3241	6 ± 2	1.3 ± 0.5	9 ± 5	3 ± 2	510 ± 305	1040 ± 254	0.19	0.04	0.26	0.08	15.72	83.70	32.10	67.90
F0L2	3832	7 ± 4	3 ± 3	8 ± 5	9 ± 5	2506 ± 2157	1196 ± 12	0.17	0.08	0.20	0.22	65.39	33.94	31.21	68.79
F0L3	4965	2 ± 0.9	8 ± 4	16 ± 3	10 ± 3	1033 ± 648	1582 ± 802	0.03	0.16	0.32	0.20	20.80	78.49	31.86	68.14
F0L4	6442	9 ± 3	13 ± 1	21 ± 3	19 ± 4	2472 ± 261	6400 ± 388	0.13	0.20	0.32	0.30	38.37	60.68	99.34	0.66
F1L1	3239	15 ± 0.8	9 ± 2	53 ± 4	17 ± 8	901 ± 506	5177 ± 1265	0.45	0.29	1.63	0.51	27.81	69.30	159.86	-59.86
F1L2	33070	4 ± 1	9 ± 0.5	25 ± 5	22 ± 3	3291 ± 3247	10702 ± 936	0.01	0.03	0.07	0.07	9.95	89.87	32.36	67.64
F1L3	63441	10 ± 2	15 ± 6	19 ± 5	29 ± 17	1321 ± 75	13800 ± 7399	0.02	0.02	0.03	0.05	2.08	97.80	21.75	78.25
F1L4	103054	9 ± 1	9 ± 3	22 ± 4	39 ± 17	13568 ± 19172	50021 ± 5817	0.01	0.01	0.02	0.04	13.17	86.76	48.54	51.46
F2L1	22540	6 ± 3	13 ± 5	18 ± 10	11 ± 3	1723 ± 539	11152 ± 1925	0.03	0.06	0.08	0.05	7.64	92.14	49.48	50.52
F2L2	44185	11 ± 12	9 ± 4	4 ± 3	18 ± 15	1699 ± 639	18524 ± 4645	0.03	0.02	0.01	0.04	3.85	96.06	41.92	58.08
F2L3	85670	3 ± 0.3	15 ± 3	19 ± 4	21 ± 8	1369 ± 402	39238 ± 10965	0.00	0.02	0.02	0.02	1.60	98.33	45.80	54.20
F2L4	139781	9 ± 2	11 ± 5	34 ± 22	10 ± 3	2877 ± 1129	69702 ± 31362	0.01	0.01	0.02	0.01	2.06	97.90	49.87	50.13
F3L1	34564	9 ± 1	4 ± 1	7 ± 1	8 ± 5	1302 ± 769	11052 ± 9096	0.03	0.01	0.02	0.02	3.77	96.15	31.97	68.03
F3L2	69324	10 ± 1	6 ± 3	9 ± 4	10 ± 5	1933 ± 653	42884 ± 28078	0.01	0.01	0.01	0.01	2.79	97.16	61.86	38.14
F3L3	135949	5 ± 2	8 ± 5	10 ± 4	8 ± 3	2754 ± 1127	63315 ± 5989	0.00	0.01	0.01	0.01	2.03	97.95	46.57	53.43
F3L4	222852	12 ± 6	11 ± 4	17 ± 5	14 ± 4	5489 ± 4227	129889 ± 6259	0.01	0.00	0.01	0.01	2.46	97.51	58.29	41.71

dw – dry weight; t_0 – initial time (day 0); t_f – final time (pot collection day).

Table A10. Mass Balance of L4.

Test Group	CEC mass in soil t_0 (ng dw)	Average CEC Mass per Pot (ng dw)					Partition %								
		Sown pots					Plant-free pots	Sown pots						Plant-free pots	
		Peas	Pods	Aerial Part	Roots	Soil t_f	Soil t_f	Peas	Pods	Aerial Part	Roots	Soil t_f	Losses	Soil t_f	Losses
Controls	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F0L1	150	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F0L2	315	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F0L3	629	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F0L4	1040	0	0	0	0	0	710 ± 176	0.00	0.00	0.00	0.00	0.00	100.00	68.34	31.66
F1L1	150	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F1L2	183	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F1L3	365	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F1L4	604	0	0	0	0	0	777 ± 73	0.00	0.00	0.00	0.00	0.00	100.00	128.74	-28.74
F2L1	81	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F2L2	170	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F2L3	339	0	0	0	0	0	536 ± 247	0.00	0.00	0.00	0.00	0.00	100.00	158.04	-58.04
F2L4	561	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F3L1	99	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F3L2	208	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F3L3	415	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F3L4	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-

dw – dry weight; t_0 – initial time (day 0); t_f – final time (pot collection day).

Table A11. Mass Balance of D5.

Test Group	CEC mass in soil t_0 (ng dw)	Average CEC Mass per Pot (ng dw)						Partition %							
		Sown pots					Plant-free pots	Sown pots						Plant-free pots	
		Peas	Pods	Aerial Part	Roots	Soil t_f	Soil t_f	Peas	Pods	Aerial Part	Roots	Soil t_f	Losses	Soil t_f	Losses
Controls	2454	0	0	41 ± 18	10 ± 5	1303 ± 115	2941 ± 758	0.00	0.00	1.69	0.39	53.11	44.81	119.84	-19.84
F0L1	25879	7 ± 1	1.31 ± 0.01	9 ± 5	2 ± 1	1797 ± 470	24249 ± 5865	0.03	0.01	0.03	0.01	6.94	92.98	93.70	6.30
F0L2	51433	6 ± 5	3 ± 3	10 ± 6	41.24 ± 1	13760 ± 19049	36478 ± 11268	0.01	0.01	0.02	0.08	26.75	73.13	70.92	29.08
F0L3	100412	7.5 ± 0.2	9 ± 7	10 ± 2	42.0 ± 0.2	3991 ± 614	81968 ± 21357	0.01	0.01	0.01	0.04	3.97	95.96	81.63	18.37
F0L4	164298	9 ± 2	14 ± 1	6 ± 2	34 ± 4	6254 ± 1226	230496 ± 23172	0.01	0.01	0.00	0.02	3.81	96.16	140.29	-40.29
F1L1	25776	20 ± 11	21 ± 7	69 ± 9	38 ± 15	3165 ± 789	35845 ± 7565	0.08	0.08	0.27	0.15	12.28	87.14	139.06	-39.06
F1L2	90960	16 ± 1	17.0 ± 0.4	37.4 ± 0.3	45 ± 15	21069 ± 30352	82467 ± 10603	0.02	0.02	0.04	0.05	23.16	76.71	90.66	9.34
F1L3	179467	30 ± 2	21 ± 4	29 ± 4	39 ± 18	6388 ± 2441	136558 ± 51791	0.02	0.01	0.02	0.02	3.56	96.37	76.09	23.91
F1L4	294910	27 ± 1	18 ± 8	17 ± 11	48 ± 2	80088 ± 125128	335312 ± 60095	0.01	0.01	0.01	0.02	27.16	72.81	113.70	-13.70
F2L1	50039	3.4 ± 0.8	1.0 ± 0.9	23 ± 16	16 ± 6	8164 ± 7016	47181 ± 1595	0.01	0.00	0.05	0.03	16.31	83.60	94.29	5.71
F2L2	101950	0.87 ± 0	5 ± 2	8.3 ± 0.9	20 ± 13	8813 ± 7984	101262 ± 26330	0.00	0.00	0.01	0.02	8.64	91.32	99.33	0.67
F2L3	201445	11.1 ± 0.4	50 ± 2	20 ± 2	31 ± 20	11173 ± 6411	187439 ± 36119	0.01	0.02	0.01	0.02	5.55	94.40	93.05	6.95
F2L4	331222	14 ± 3	9.6 ± 0.6	48 ± 13	12 ± 4	15077 ± 11075	339550 ± 55614	0.00	0.00	0.01	0.00	4.55	95.42	102.51	-2.51
F3L1	67978	10 ± 1	7.2 ± 0.4	9 ± 4	6.2 ± 0.2	3608 ± 1101	55173.39 ± 38085	0.01	0.01	0.01	0.01	5.31	94.64	81.16	18.84
F3L2	139459	9 ± 2	7 ± 6	18 ± 9	15 ± 9	8365 ± 7448	174372 ± 90955	0.01	0.01	0.01	0.01	6.00	93.97	125.03	-25.03
F3L3	276463	5 ± 2	8 ± 5	8 ± 6	17 ± 8	26285 ± 28794	292818 ± 36224	0.00	0.00	0.00	0.01	9.51	90.48	105.92	-5.92
F3L4	455165	13 ± 6	9 ± 3	16 ± 7	36 ± 31	69140 ± 90184	548197 ± 55036	0.00	0.00	0.00	0.01	15.19	84.79	120.44	-20.44

dw – dry weight; t_0 – initial time (day 0); t_f – final time (pot collection day).

Table A12. Mass Balance of L5.

Test Group	CEC mass in soil t_0 (ng dw)	Average CEC Mass per Pot (ng dw)						Partition %							
		Sown pots					Plant-free pots	Sown pots						Plant-free pots	
		Peas	Pods	Aerial Part	Roots	Soil t_f	Soil t_f	Peas	Pods	Aerial Part	Roots	Soil t_f	Losses	Soil t_f	Losses
Controls	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-
F0L1	820	0	0	0	0	0	938 ± 281	0.00	0.00	0.00	0.00	0.00	100.00	114.32	-14.32
F0L2	1715	0	0	0	0	0	1601 ± 351	0.00	0.00	0.00	0.00	0.00	100.00	93.39	6.61
F0L3	3430	0	0	0	0	0	3339 ± 803	0.00	0.00	0.00	0.00	0.00	100.00	97.36	2.64
F0L4	5666	0	0	0	0	910 ± 250	9120 ± 599	0.00	0.00	0.00	0.00	16.05	83.95	160.96	-60.96
F1L1	817	0	0.0	0	0	0	1062 ± 181	0.00	0.00	0.00	0.00	0.00	100.00	130.03	-30.03
F1L2	1890	0	0	0	0	0	2423 ± 254	0.00	0.00	0.00	0.00	0.00	100.00	128.18	-28.18
F1L3	3780	0	0	0	0	851 ± 150	4466 ± 1409	0.00	0.00	0.00	0.00	22.51	77.49	118.15	-18.15
F1L4	6245	0	0	0	0	2671 ± 3066	8355 ± 1484	0.00	0.00	0.00	0.00	42.76	57.24	133.79	-33.79
F2L1	883	0	0	0	0	0	986 ± 94	0.00	0.00	0.00	0.00	0.00	100.00	111.67	-11.67
F2L2	1847	0	0	0	0	0	1970 ± 725	0.00	0.00	0.00	0.00	0.00	100.00	106.67	-6.67
F2L3	3693	0	0	0	0	1035 ± 52	3975 ± 322	0.00	0.00	0.00	0.00	28.02	71.98	107.64	-7.64
F2L4	6102	0	0	0	0	1153 ± 465	5908 ± 973	0.00	0.00	0.00	0.00	18.90	81.10	96.81	3.19
F3L1	1050	0	0	0	0	0	986 ± 162	0.00	0.00	0.00	0.00	0.00	100.00	93.88	6.12
F3L2	2196	0	0	0	0	0	2174 ± 961	0.00	0.00	0.00	0.00	0.00	100.00	99.00	1.00
F3L3	4392	0	0	0	0	1172 ± 713	4031 ± 366	0.00	0.00	0.00	0.00	26.68	73.32	91.77	8.23
F3L4	7256	0	0	0	0	2413 ± 1895	7356 ± 844	0.00	0.00	0.00	0.00	33.25	66.75	101.37	-1.37

dw – dry weight; t_0 – initial time (day 0); t_f – final time (pot collection day).

Table A13. Mass Balance of D6.

Test Group	CEC mass in soil t_0 (ng dw)	Average CEC Mass per Pot (ng dw)						Partition %							
		Sown pots					Plant-free pots	Sown pots						Plant-free pots	
		Peas	Pods	Aerial Part	Roots	Soil t_f	Soil t_f	Peas	Pods	Aerial Part	Roots	Soil t_f	Losses	Soil t_f	Losses
Controls	0	0	0	45 ± 36	15 ± 20	2765 ± 902	4518 ± 809	-	-	-	-	-	-	-	-
F0L1	5039	31 ± 6	15 ± 9	45 ± 10	9 ± 1	4978 ± 1395	6906 ± 967	0.61	0.30	0.90	0.17	98.80	-0.79	137.05	-37.05
F0L2	10535	8 ± 4	17 ± 7	16 ± 12	16 ± 11	9971 ± 7454	9959 ± 1968	0.07	0.16	0.15	0.15	94.65	4.82	94.53	5.47
F0L3	21070	13 ± 2	5 ± 2	34 ± 6	34 ± 14	10084 ± 1273	23783 ± 3748	0.06	0.02	0.16	0.16	47.86	51.74	112.88	-12.88
F0L4	34812	12 ± 2	18 ± 3	20 ± 5	50 ± 7	15339 ± 1024	62956 ± 4740	0.04	0.05	0.06	0.14	44.06	55.65	180.84	-80.84
F1L1	5016	15 ± 2	28 ± 3	138 ± 8	45 ± 32	5790 ± 1156	18058 ± 5385	0.31	0.55	2.75	0.90	115.43	-19.94	359.97	-259.97
F1L2	46342	25 ± 1	37 ± 8	119 ± 6	76 ± 15	15264 ± 7661	46779 ± 13582	0.05	0.08	0.26	0.16	32.94	66.51	100.94	-0.94
F1L3	92683	53 ± 9	42 ± 14	65 ± 25	65 ± 26	20254 ± 7297	63239 ± 33585	0.06	0.05	0.07	0.07	21.85	77.90	68.23	31.77
F1L4	153129	49 ± 5	47 ± 2	41 ± 17	111 ± 32	57842 ± 39690	183868 ± 31741	0.03	0.03	0.03	0.07	37.77	62.06	120.07	-20.07
F2L1	29152	26 ± 12	24 ± 8	50 ± 43	31 ± 13	13613 ± 2405	31803 ± 1119	0.09	0.08	0.17	0.11	46.70	52.85	109.09	-9.09
F2L2	60955	10 ± 2	8 ± 2	9 ± 4	52 ± 60	21830 ± 8067	68920 ± 13750	0.02	0.01	0.02	0.09	35.81	64.06	113.07	-13.07
F2L3	121910	6 ± 4	10 ± 0	39 ± 13	81 ± 40	46743 ± 10206	115755 ± 29688	0.00	0.01	0.03	0.07	38.34	61.55	94.95	5.05
F2L4	201417	36 ± 24	23 ± 3	110 ± 43	58 ± 16	69968 ± 26578	243144 ± 16599	0.02	0.01	0.05	0.03	34.74	65.15	120.72	-20.72
F3L1	49862	19 ± 6	21 ± 4	34 ± 16	44 ± 24	18476 ± 5611	51962 ± 20962	0.04	0.04	0.07	0.09	37.05	62.71	104.21	-4.21
F3L2	104256	31 ± 9	22 ± 7	54 ± 37	82 ± 74	33738 ± 9771	138860 ± 67640	0.03	0.02	0.05	0.08	32.36	67.46	133.19	-33.19
F3L3	208512	10 ± 4	12 ± 11	16 ± 8	43 ± 5	83678 ± 29485	225899 ± 34893	0.00	0.01	0.01	0.02	40.13	59.83	108.34	-8.34
F3L4	344499	19 ± 10	12 ± 4	30 ± 5	109 ± 71	180467 ± 97586	400821 ± 43459	0.01	0.00	0.01	0.03	52.39	47.57	116.35	-16.35

dw – dry weight; t_0 – initial time (day 0); t_f – final time (pot collection day).

Table A14. Mass Balance of ADBI.

Test Group	CEC mass in soil t_0 (ng dw)	Average CEC Mass per Pot (ng dw)						Partition %							
		Sown pots					Plant-free pots	Sown pots						Plant-free pots	
		Peas	Pods	Aerial Part	Roots	Soil t_f	Soil t_f	Peas	Pods	Aerial Part	Roots	Soil t_f	Losses	Soil t_f	Losses
Controls	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F0L1	489	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F0L2	1023	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F0L3	2045	0	0	0	0	0	819 ± 362	0.00	0.00	0.00	0.00	0.00	100.00	40.05	59.95
F0L4	3379	0	0	0	0	0	1953 ± 290	0.00	0.00	0.00	0.00	0.00	100.00	57.81	42.19
F1L1	487	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F1L2	1394	0	0	0	0	0	716 ± 6	0.00	0.00	0.00	0.00	0.00	100.00	51.34	48.66
F1L3	2788	0	0	0	0	0	964 ± 222	0.00	0.00	0.00	0.00	0.00	100.00	34.58	65.42
F1L4	4606	0	0	0	0	1059 ± 873	2082 ± 641	0.00	0.00	0.00	0.00	23.00	77.00	45.19	54.81
F2L1	783	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F2L2	1637	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F2L3	3274	0	0	0	0	0	842 ± 199	0.00	0.00	0.00	0.00	0.00	100.00	25.71	74.29
F2L4	5409	0	0	0	0	0	2163 ± 757	0.00	0.00	0.00	0.00	0.00	100.00	39.98	60.02
F3L1	1217	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F3L2	2545	0	0	0	0	0	544 ± 246	0.00	0.00	0.00	0.00	0.00	100.00	21.39	78.61
F3L3	5090	0	0	0	0	0	722 ± 20	0.00	0.00	0.00	0.00	0.00	100.00	14.18	85.82
F3L4	8410	0	0	0	0	1144 ± 155	2067 ± 459	0.00	0.00	0.00	0.00	13.60	86.40	24.58	75.42

dw – dry weight; t_0 – initial time (day 0); t_f – final time (pot collection day).

Table A15. Mass Balance of HHCB.

Test Group	CEC mass in soil t_0 (ng dw)	Average CEC Mass per Pot (ng dw)						Partition %							
		Sown pots					Plant-free pots	Sown pots						Plant-free pots	
		Peas	Pods	Aerial Part	Roots	Soil t_f	Soil t_f	Peas	Pods	Aerial Part	Roots	Soil t_f	Losses	Soil t_f	Losses
Controls	1472	0	0	2.2 ± 0.4	6 ± 6	0	0	0.00	0.00	0.15	0.42	0.00	99.43	0.00	100.00
F0L1	145173	0	0	2.4 ± 0.9	10 ± 4	8436 ± 1407	31270 ± 5956	0.00	0.00	0.00	0.01	5.81	94.18	21.54	78.46
F0L2	301938	0	0	2 ± 2	145 ± 42	50841 ± 26953	93899 ± 41898	0.00	0.00	0.00	0.05	16.84	83.11	31.10	68.90
F0L3	602403	0	0	2.7 ± 0.2	466 ± 13	115467 ± 17281	232841 ± 75392	0.00	0.00	0.00	0.08	19.17	80.75	38.65	61.35
F0L4	994315	0	0	4 ± 2	397 ± 116	193058 ± 54344	635637 ± 113396	0.00	0.00	0.00	0.04	19.42	80.54	63.93	36.07
F1L1	144543	0	0	5.2 ± 0.7	27 ± 15	13460 ± 2427	51367 ± 23335	0.00	0.00	0.00	0.02	9.31	90.67	35.54	64.46
F1L2	312769	0	0	13 ± 11	106 ± 51	56317 ± 40408	137850 ± 56352	0.00	0.00	0.00	0.03	18.01	81.96	44.07	55.93
F1L3	624066	5 ± 2	0	0	125 ± 58	92803 ± 2307	268253 ± 71875	0.00	0.00	0.00	0.02	14.87	85.11	42.98	57.02
F1L4	1030106	4.4 ± 0.2	0	0	322 ± 34	316202 ± 210502	617248 ± 152128	0.00	0.00	0.00	0.03	30.70	69.27	59.92	40.08
F2L1	162712	2 ± 1	3 ± 2	0	18 ± 6	14343 ± 4775	48404 ± 23198	0.00	0.00	0.00	0.01	8.82	91.17	29.75	70.25
F2L2	338609	2 ± 1	4 ± 4	1.4 ± 0.2	30 ± 18	37840 ± 1194	81084 ± 23054	0.00	0.00	0.00	0.01	11.18	88.81	23.95	76.05
F2L3	675747	1.0 ± 0.1	0	6 ± 1	110 ± 60	102438 ± 21761	26643 ± 80044	0.00	0.00	0.00	0.02	15.16	84.82	39.43	60.57
F2L4	1115491	0	0	3.6 ± 0.8	170 ± 79	209678 ± 18921	573522 ± 181556	0.00	0.00	0.00	0.02	18.80	81.19	51.41	48.59
F3L1	199845	0	0	3 ± 1	7.7 ± 0.4	14461 ± 1248	45724 ± 15694	0.00	0.00	0.00	0.00	7.24	92.76	22.88	77.12
F3L2	416251	2 ± 1	0	2.7 ± 0.4	45 ± 23	39030 ± 15572	128328 ± 59950	0.00	0.00	0.00	0.01	9.38	90.61	30.83	69.17
F3L3	831029	0	0	0	77 ± 36	95429 ± 20136	238412 ± 45352	0.00	0.00	0.00	0.01	11.48	88.51	28.69	71.31
F3L4	1372044	9 ± 6	0	4 ± 0	158 ± 44	307179 ± 138189	635010 ± 79867	0.00	0.00	0.00	0.01	22.39	77.60	46.28	53.72

dw – dry weight; t_0 – initial time (day 0); t_f – final time (pot collection day).

Table A16. Mass Balance of AHTN.

Test Group	CEC mass in soil t_0 (ng dw)	Average CEC Mass per Pot (ng dw)						Partition %							
		Sown pots					Plant-free pots	Sown pots						Plant-free pots	
		Peas	Pods	Aerial Part	Roots	Soil t_f	Soil t_f	Peas	Pods	Aerial Part	Roots	Soil t_f	Losses	Soil t_f	Losses
Controls	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F0L1	20383	0	0	0	0	10350 ± 229	7196 ± 1237	0.00	0.00	0.00	0.00	50.78	49.22	35.30	64.70
F0L2	42618	0	0	0	53 ± 21	28147 ± 10112	18918 ± 4114	0.00	0.00	0.00	0.12	66.05	33.83	44.39	55.61
F0L3	85236	0	0	0	160 ± 3	49299 ± 5382	47373 ± 9907	0.00	0.00	0.00	0.19	57.84	41.97	55.58	44.42
F0L4	140825	0	0	0	251 ± 70	80401 ± 10038	147384 ± 21855	0.00	0.00	0.00	0.18	57.09	42.73	104.66	-4.66
F1L1	20293	0	0	0	26 ± 10	11659 ± 2888	15694 ± 4160	0.00	0.00	0.00	0.13	57.45	42.42	77.34	22.66
F1L2	83573	0	0	0	68 ± 13	32894 ± 852	48640 ± 10391	0.00	0.00	0.00	0.08	39.36	60.56	58.20	41.80
F1L3	167145	0	0	0	92 ± 43	61439 ± 11397	89075 ± 23980	0.00	0.00	0.00	0.05	36.76	63.19	53.29	46.71
F1L4	276153	0	0	0	214 ± 40	130423 ± 29050	255184 ± 85363	0.00	0.00	0.00	0.08	47.23	52.69	92.41	7.59
F2L1	50674	0	0	0	27 ± 9	19269 ± 3669	25607 ± 2545	0.00	0.00	0.00	0.05	38.03	61.92	50.53	49.47
F2L2	105954	0	0	0	48 ± 27	51032 ± 10338	68264 ± 12673	0.00	0.00	0.00	0.05	48.16	51.79	64.43	35.57
F2L3	211908	0	0	0	156 ± 47	125670 ± 6952	129412 ± 46101	0.00	0.00	0.00	0.07	59.30	40.62	61.07	38.93
F2L4	350109	0	0	0	224 ± 86	211154 ± 53814	312016 ± 52677	0.00	0.00	0.00	0.06	60.31	39.62	89.12	10.88
F3L1	78911	0	0	0	58 ± 29	40866 ± 14058	50034 ± 4514	0.00	0.00	0.00	0.07	51.79	48.14	63.41	36.59
F3L2	164996	0	0	0	114 ± 62	78988 ± 10714	122294 ± 50618	0.00	0.00	0.00	0.07	47.87	52.06	74.12	25.88
F3L3	329993	0	0	0	166 ± 52	165110 ± 18084	226021 ± 49256	0.00	0.00	0.00	0.05	50.03	49.92	68.49	31.51
F3L4	545206	0	0	0	241 ± 123	294783 ± 70307	441137 ± 76797	0.00	0.00	0.00	0.04	54.07	45.89	80.91	19.09

dw – dry weight; t_0 – initial time (day 0); t_f – final time (pot collection day).

Annex 8 BCF Values of CECs across Plant Compartments

In Tables A17 and A18 the calculated values of the Bioconcentration Factor for each plant compartment and test group, considering the estimated initial pollutant concentrations in the pots, are presented. In cells containing a dash the calculation was not done because the initial concentration of the respective pollutant in the pot was zero and therefore the BCF would mathematically tend to infinity despite no accumulation of soil pollutants having taken place.

Table A17. Calculated Bioconcentration Factors of VMSs according to plant compartment and test group.

Test Groups	Bioconcentration Factors															
	D3				D4				D5				D6			
	Peas	Pods	Aerial Part	Roots	Peas	Pods	Aerial Part	Roots	Peas	Pods	Aerial Part	Roots	Peas	Pods	Aerial Part	Roots
Controls	1.20	0.00	0.00	0.00	0.00	0.00	24.56	7.06	0.00	0.00	51.36	2.00	-	-	-	-
F0L1	1.02	0.00	4.05	0.00	2.44	1.11	5.36	0.48	0.36	0.14	0.69	0.10	7.79	8.56	14.97	1.65
F0L2	3.79	0.00	1.22	0.00	2.64	2.78	4.61	2.45	0.19	0.30	0.31	0.72	2.70	2.27	2.88	1.77
F0L3	1.27	0.00	2.05	0.00	0.43	3.71	4.57	2.04	0.10	0.20	0.14	0.38	1.01	0.72	2.39	1.62
F0L4	1.49	0.00	6.12	6.50	1.43	2.88	3.38	2.10	0.06	0.12	0.04	0.14	0.39	0.73	0.62	0.98
F1L1	2.45	0.98	7.81	5.07	6.04	6.88	27.10	7.30	0.69	1.44	3.80	1.79	4.08	12.32	39.31	11.44
F1L2	2.27	1.15	5.96	2.63	0.16	0.65	1.22	0.73	0.23	0.41	0.68	0.54	0.71	1.79	4.24	1.81
F1L3	3.48	3.58	3.41	8.22	0.19	0.41	0.35	0.48	0.19	0.21	0.19	0.30	0.66	0.80	0.80	0.97
F1L4	1.53	5.98	2.39	7.31	0.09	0.17	0.23	0.29	0.10	0.11	0.06	0.13	0.34	0.58	0.28	0.56
F2L1	1.97	2.42	7.82	6.39	0.46	1.60	1.29	0.65	0.11	0.08	0.68	0.42	1.45	2.34	2.40	1.38
F2L2	7.52	3.01	4.51	7.49	0.45	0.53	0.22	0.49	0.03	0.26	0.17	0.26	0.56	0.42	0.39	0.84
F2L3	0.49	10.24	4.40	6.96	0.09	0.57	0.50	0.44	0.14	0.85	0.23	0.24	0.11	0.26	0.70	1.06
F2L4	1.09	5.83	15.24	5.64	0.11	0.13	0.43	0.12	0.07	0.07	0.27	0.05	0.12	0.26	0.99	0.46
F3L1	6.40	2.30	6.09	4.05	0.79	0.43	0.61	0.39	0.45	0.39	0.39	0.19	0.72	1.21	2.23	1.44
F3L2	1.75	3.54	7.01	2.12	0.20	0.21	0.23	0.19	0.10	0.13	0.17	0.13	0.46	0.39	0.55	0.93
F3L3	4.30	5.61	5.79	4.41	0.07	0.17	0.13	0.09	0.04	0.08	0.05	0.09	0.09	0.15	0.14	0.49
F3L4	2.29	1.60	4.09	2.82	0.07	0.10	0.10	0.08	0.04	0.04	0.04	0.10	0.07	0.08	0.12	0.39

Table A18. Calculated Bioconcentration Factors of SMs according to plant compartment and test group.

Test Groups	Bioconcentration Factors							
	HHCB				AHTN			
	Peas	Pods	Aerial Part	Roots	Peas	Pods	Aerial Part	Roots
Controls	0	0	0.534	4.650	0	0	0	-
F0L1	0	0	0.005	0.064	0	0	0	0.000
F0L2	0	0	0.005	0.422	0	0	0	1.425
F0L3	0	0	0.001	0.710	0	0	0	1.723
F0L4	0	0	0.001	0.267	0	0	0	1.195
F1L1	0	0	0.011	0.225	0	0	0	1.525
F1L2	0	0	0.013	0.361	0	0	0	0.899
F1L3	0.009	0	0	0.224	0	0	0	0.606
F1L4	0.004	0	0	0.247	0	0	0	0.607
F2L1	0.020	0.009	0.004	0.197	0	0	0	0.674
F2L2	0.010	0.002	0.003	0.187	0	0	0	0.626
F2L3	0.004	0	0.003	0.282	0	0	0	1.510
F2L4	0	0	0.001	0.236	0	0	0	1.007
F3L1	0	0	0.008	0.082	0	0	0	1.234
F3L2	0.007	0	0.003	0.142	0	0	0	0.891
F3L3	0	0	0.0006	0.141	0	0	0	0.760
F3L4	0	0	0.0007	0.151	0	0	0	0.386