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Dynamics of chemical microcontaminants in peri-urban agriculture and evaluation of their potential impact on crops and human health.

Presentada per

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Abstract

Peri-urban agriculture provides environmental, socio-economic functions and ecosystem services in the nearby urban areas. Nonetheless, crops grown in these areas are exposed to organic and inorganic contaminants from industrial emissions and road traffic, as well as the application of biosolids and the use of regenerated waters.

In this regard, in the last years, there has been a growing concern about the presence of chemical contaminants in agricultural crops due to the evidence that plants are able to incorporate, translocate and accumulate them in their edible parts. Although the concentrations detected in food crops are generally low, little is known about the effects of these contaminants on human health. For this reason, field studies are necessary to properly evaluate their incorporation and potential risk to human health. Currently, there is no study that evaluates the exposure and presence of organic and inorganic contaminants simultaneously in agricultural crops in peri-urban areas. The few existing studies at field scale are based on the impact of regenerated water in agricultural crops, considering separately organic and inorganic contaminants.

In this Thesis, it has been assessed the incorporation into plants in real field conditions of some trace elements and organic microcontaminants, chosen by their presence in the environment and their physicochemical properties. For this purpose, 4 agricultural parcels were selected located in the periurban area of Barcelona (NE Spain) and a rural agricultural plot far away from the periurban area, including different irrigation water quality and exposure to urban contamination. In this study, lettuce, tomato, cauliflower and beans were selected as a model plant (leaf, flower and fruit).

The PhD dissertation is divided into six chapters. Chapter I gives an overview of the subject and presents the hypotheses and objectives of the PhD project. Chapter II assesses the occurrence of trace elements and organic microcontaminants in irrigation waters. Irrigation waters from peri-urban areas showed a higher abundance of the selected chemical contaminants than that water from the rural area. Nevertheless, none of the irrigation waters induced phytotoxic effects (seed germination, root elongation) or decrease crop productivity. Chapter III assesses the co-occurrence of these contaminants in soil and lettuce leaves, their bioaccumulation factors and how they affect lipid constituents and leaf sugars. The higher abundance of these contaminants in the irrigation waters and soils from the peri-urban area had no impact on the chlorophyll, carbohydrates and lipid content of lettuce leaves. Chapter IV shows the occurrence of the chemical contaminants in the edible parts of different model crops (lettuce, tomato, cauliflower and broad beans) and estimates the human health risk associated with their consumption. The results obained show that human health risks associated were low and similar among crops grown in peri-urban and rural areas. Chapter V is devoted to the general discussion of three previous chapters, whereas Chapter VI presents the main conclusions of the Thesis.

Resum

L'agricultura periurbana proporciona funcions ambientals, soci-econòmiques i serveis ecosistèmics a les zones urbanes properes. Malgrat això, els cultius conreats en aquestes zones estan exposats a contaminants orgànics i inorgànics procedents d'emissions industrials i del trànsit rodat, així com de l'aplicació de biosòlids i l'ús d'aigües regenerades.

En aquest sentit, en els darrers anys, ha sorgit una preocupació creixent sobre la presència de contaminants químics en els cultius agrícoles degut a l'evidència de que les plantes poden incorporar-los, translocar-los i acumular-los en les seves parts comestibles. Tot i que les concentracions detectades en els cultius alimentaris són generalment baixes, es coneix poc sobre els efectes d'aquests contaminants sobre la salut humana. Per aquest motiu, els estudis de camp són necessaris per avaluar adequadament la seva incorporació i el seu risc potencial per a la salut humana. Actualment no existeix cap estudi que avaluï l'exposició i presència de contaminats orgànics i inorgànics de manera simultània en cultius agrícoles de zones periurbanes. Els pocs estudis existents a escala de camp es basen en l'impacte d'aigües regenerades en cultius agrícoles, considerant de manera separada contaminats orgànics i inorgànics.

En aquesta Tesi, s'ha avaluat la incorporació en les plantes en condicions de camp reals d'alguns elements traça i microcontaminants orgànics, seleccionats en funció de la seva presència en el medi ambient i les seves propietats fisicoquímiques. Amb aquesta finalitat, es varen seleccionar 4 parcel·les agrícoles situades a la zona periurbana de Barcelona (NE Espanya) i una parcel·la agrícola rural llunyana de la zona periurbana, incloent diferents qualitats d'aigua de reg i exposició a la contaminació urbana. Com a cultius objecte d'estudi es van seleccionar l'enciam, el tomàquet, coliflors i faves com a vegetals model (fulla, fruit i flor).

La present Tesi Doctoral es divideix en sis capítols. En el capítol I s'ofereix una visió general de la temàtica de la Tesi i presenta les hipòtesis i objectius del projecte de doctorat. El Capítol II avalua la presència d'elements traça i microcontaminants orgànics en aigües de reg. Les aigües de reg de les zones periurbanes van mostrar una major abundància de contaminants químics seleccionats en relació a l'aigua de la zona rural. Malgrat això, cap de les aigües de reg va induir efectes fitotòxics (germinació de llavors, elongació de l'arrel) o minvar la productivitat del cultiu. El capítol III incideix en la coexistència d'aquests contaminants al sòl i a les fulles d'enciam, els seus factors de bioacumulació i com afecten als constituents lipídics i sucres de les fulles. La major abundància d'aquests contaminants en les aigües de reg i sòls de la zona periurbana no va alterar el contingut en clorofil·les, hidrats de carboni i lípids de fulles d'enciam. El capítol IV mostra la presència dels contaminants químics en la part comestible de diferents cultius model (enciam, tomàquet, coliflor i faves) i avalua el risc potencial per a la salut humana associat al seu consum. Els resultats obtinguts mostren que els riscos associats a la salut humana eren baixos i similars entre els cultius que creixen en les zones periurbana i rural. El capítol V està dedicat a

la discussió general dels tres capítols anteriors, i finalment el capítol VI presenta les principals conclusions de la tesi.

Resumen

La agricultura periurbana proporciona funciones ambientales, socio-económicas y servicios ecosistémicos en las zonas urbanas cercanas. Sin embargo, los cultivos cultivados en estas zonas están expuestos a contaminantes orgánicos e inorgánicos procedentes de emisiones industriales y del tráfico rodado, así como de la aplicación de biosólidos y el uso de aguas regeneradas.

En este sentido, en los últimos años, ha surgido una preocupación creciente sobre la presencia de contaminantes químicos en los cultivos agrícolas debido a la evidencia de que las plantas pueden incorporarlos, traslocarse los y acumularlos en sus partes comestibles. Aunque las concentraciones detectadas en los cultivos alimentarios son generalmente bajas, se conoce poco sobre los efectos de estos contaminantes sobre la salud humana. Por este motivo, los estudios de campo son necesarios para evaluar adecuadamente su incorporación y su riesgo potencial para la salud humana. Actualmente no existe ningún estudio que evalúe la exposición y presencia de contaminantes orgánicos e inorgánicos de manera simultánea en cultivos agrícolas de zonas periurbanas. Los pocos estudios existentes a escala de campo se basan en el impacto de aguas regeneradas en cultivos agrícolas, considerando de manera separada contaminados orgánicos e inorgánicos.

En esta Tesis, se ha evaluado la incorporación en las plantas en condiciones de campo reales de algunos elementos traza y microcontaminantes orgánicos, seleccionados en función de su presencia en el medio ambiente y sus propiedades fisicoquímicas. Con este fin, se seleccionaron 4 parcelas agrícolas situadas en la zona periurbana de Barcelona (NE España) y una parcela agrícola rural alejada de la zona periurbana, incluyendo diferentes calidades de agua de riego y exposición a la contaminación urbana. Como cultivos objeto de estudio se seleccionaron la lechuga, el tomate, coliflores y habas como vegetales modelo (hoja, fruto y flor).

La presente Tesis Doctoral se divide en seis capítulos. En el capítulo I se ofrece una visión general de la temática de la Tesis y presenta las hipótesis y objetivos del proyecto de doctorado. El Capítulo II evalúa la presencia de elementos traza y microcontaminantes orgánicos en aguas de riego. Las aguas de riego de las zonas periurbanas mostraron una mayor abundancia de contaminantes químicos seleccionados en relación al agua de la zona rural. Sin embargo, ninguna de las aguas de riego indujo efectos fitotóxicos (germinación de semillas, elongación de la raíz) o mermó la productividad del cultivo. El capítulo III incide en la coexistencia de estos contaminantes en el suelo y en las hojas de lechuga, sus factores de bioacumulación y cómo afectan a los constituyentes lipídicos y azúcares de las hojas. La mayor abundancia de estos contaminantes en las aguas de riego y suelos de la zona periurbana no alteró el contenido en clorofilas, hidratos de carbono y lípidos de hojas de lechuga. El capítulo IV muestra la presencia de los contaminantes químicos en la parte comestible de diferentes cultivos modelo (lechuga, tomate, coliflor y habas) y evalúa el riesgo potencial para la salud humana asociado a su consumo. Los resultados obtenidos muestran que los riesgos asociados a la salud humana eran bajos y similares entre los cultivos que crecen en las zonas periurbana y rural. El capítulo V está dedicado a la

discusión general de los tres capítulos anteriores, y finalmente el capítulo VI presenta las principales conclusiones de la tesis.

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List of acronyms

2MBT	2- mercaptobenzothiazole
5TTri	5-methyl-2H-benzotriazole
BCF	Bioconcentration factor
BHA	2-*tert*-butyl-4-methoxyphenol
BMDL	Benchmark dose lower confidence limit
BPA	Bisphenol A
BPB	Butylparaben
BPF	Bisphenol F
BT	1,3-benzothiazole
BTri	Benzotriazole
C	Concentration
CBDZN	Carbendazim
CBZ	Carbamazepine
CECs	Contaminants of emerging concern
DEET	N,N-diethyl-meta-toluamide
dw	Dry weight
EC	European Commission
EPB	Ethylparaben
EPOCBZ	10,11-epoxycarbamazepine
EU	European Union
fw	Fresh weight
GRL	Generic residue levels
HQ	Hazard quotient
K_d	Soil-water partition coefficient
K_H	Henry law’s constant
Koa	Octanol-air partition coefficient
Koc	Organic carbon soil - water partition coefficient
Kow	Octanol-water partition coefficient
MPB	Methylparaben
MRL	Maximum residue levels
OHBT	1H-hydroxybenzotriazole
OMCs	Organic microcontaminants
OP	4-*tert*-octylphenol

PAHs	Polycyclic aromatic hydrocarbons
PCA	Principal component analysis
PCBs	Polychlorinated Biphenyls
PCPs	Personal care products
pKa	Constant of acidity
PM	Particulate matter
PPB	Propylparaben
RfD	Reference dose
ROS	reactive oxygen species
SI	Supplementary information
$t_{1/2}$	Half-life
TCEP	(tris(2-chloroethyl) phosphate)
TCPP	Tris(1-chloro-2-propyl) phosphate
TEs	Trace elements
THQ	Total hazard quotient
TTC	Threshold of toxicological concern
TWW	Treated wastewater
VOCs	Volatile organic compounds
WWTPs	Wastewater treatment plants

Motivations and structure of the Thesis

Motivations

In the recent years, a great progress has been achieved in the development of analytical technologies, which has allowed the identification and quantification of microcontaminants (organic and inorganic) in different environmental compartments at ultra-trace concentration levels. Several studies have demonstrated the incorporation of these compounds into crops irrigated with reclaimed waters or grown in polluted soils, as well as their incorporation and translocation into the edible parts of vegetables. Although a great progress has been made in assessing the mechanisms that may affect their incorporation, most of the existing studies on organic microcontaminangts (OMCs) have been performed in the laboratory or in greenhouses, which do not represent the common agricultural practices of commercial agriculture. Furthermore, in any case the simultaneous assessment of trace elements (TEs) and OMCs has been performed. Therefore, field studies are required to properly evaluate their incorporation and elaborate databases to assess the human health risk of the consumption of vegetables exposed to chemical contaminants. This figure could be more pronounced in peri-urban agriculture, where vegetables are exposed to TEs and OMCs through industrial and domestic activities such as the use of wastewater treatment plants (WWTP) effluents for irrigation, soil amending with biosolids, and vehicular emissions, among others (Calderón-Preciado et al., 2011; Singh and Kumar, 2006). Nevertheless, there is no available study in the literature that assesses the effect of that exposure in terms of agricultural productivity and human health implications.

This Thesis assesses for the first time in Spain the exposure and uptake of 50 chemical contaminants (OMCs and TEs) by food crops under real field-scale conditions. Furthermore, this is the first study worldwide that evaluates the implications of the peri-urban agriculture exposition to chemical contaminants on crop productivity and human health. For that propose, 4 farm fields located in the peri-urban area of the city of Barcelona and one rural site, outside the peri-urban area of influence, were selected. Whereas sites in peri-urban area were exposed to atmospheric pollution from the city and irrigated with reclaimed, ground or surface water, the rural site was less affected by urban pollution and irrigated with rainwater/groundwater. Lettuce, tomatoes, cauliflowers and broad beans were selected because of their importance in the agricultural production of the area.

Structure of the Thesis

The PhD dissertation is structured as follows.

Chapter I provides an introductory overview of the concerns regarding plant uptake of contaminants in peri-urban horticulture, describes the relevant contaminants and their main sources as well as their known effects on plants and human health. Moreover, an overview of the selected compounds is detailed. The hypotheses and the objectives of the Thesis are stated

The results obtained along this Thesis as well as their discussion are compiled in chapters III, IV and V.

Chapter II describes the occurrence of the selected contaminants (34 OMCs and 16 TEs), conventional quality parameters and nutrients in the irrigation waters used in the sampling area, which includes four peri-urban and one rural farm plots. Water samples were taken during the growing period of some crops of interest (lettuce and tomato), between February and September of 2016. Moreover, the effects of the presence of contaminants were assessed through a seed germination test (*Lactuca sativa* L.) and the crop productivity (*Lycopersicon esculentum* Mill. cv. Bodar and *Lactuca sativa* L. cv. Batavia). Data analysis, including PCA on the entire dataset that classifies the irrigation waters, is included.

This Chapter is based on the paper Margenat, A., Matamoros, V., Díez, S., Cañameras, N., Comas, J., & Bayona, J. M. (2017). Occurrence of chemical contaminants in peri-urban agricultural irrigation waters and assessment of their phytotoxicity and crop productivity. Science of the Total Environment, 599–600, 1140–1148.

In **Chapter III**, is provided the occurrence of these contaminants in soil and lettuce leaves grown in peri-urban and rural areas, and their bioaccumulation factors. Lettuce crops were sampled in two growing seasons, winter and summer, as they were planted in February-March and June of 2016 and harvested in May and June of 2016.

The effects of the contaminants on the leaf constituents (e.g. chlorophyll, nitrate, lipid and carbohydrate contents) are also detailed and, finally, contains a PCA analysis that sheds light on the most relevant factors in the presence of contaminants in lettuce crops.

This Chapter is based on the paper Margenat, A., Matamoros, V., Díez, S., Cañameras, N., Comas, J., & Bayona, J. M. (2018). Occurrence and bioaccumulation of chemical contaminants in lettuce grown in peri-urban horticulture. Science of the Total Environment, 637–638, 1166–1174.

Chapter IV expands the study of the presence of the contaminants to the edible parts of different vegetables (lettuce, tomato, cauliflower and broad beans) grown in two peri-urban and one rural farm plots, and evaluates the potential risk of their consumption, individual and altogether, to human health. Statistical data analysis on the whole dataset is detailed.

This Chapter is based on the paper under review Margenat, A., Matamoros, V., Díez, S., Cañameras, N., Comas, J., & Bayona, J. M. Occurrence and human health implications of chemical contaminants in vegetables grown in peri-urban agriculture. Environment International.

Finally, a general discussion (**Chapter V**) and conclusions (**Chapter VI**) are included so as to provide an overview of the data obtained along the PhD.

Chapter I: Introduction

1.1 Contamination exposure in peri-urban agriculture

1.1.1 Peri-urban agriculture

Peri-urban agriculture, as well as urban agriculture, emerged due to the urban population expansion (UN-HABITAT, 2006). The movement of people towards cities has accelerated in the past 40 years, particularly in the less-developed regions. While in 1960 just one-third of the world population was living urban areas, half of it was urban in 2008 and it is expected that by 2050, it will reach two-thirds of the total population (FAO, 2011). Therefore, it is necessary to find solutions to guarantee the food supply to this population.

Peri-urban agriculture is undertaken at the surrounding of urban areas. However, there is still no consensus on the definition of urban and peri-urban agriculture. One of the most widely used definition was established by the Organization for Economic Cooperation and Development (OECD) which defines peri-urban agriculture as the one that is being performed in a ratio of 20 km from the urban nucleus with more than 200.000 inhabitants and from 10 km of ratio from the cities between 50.000 and 100.000 inhabitants (OCDE, 1979).

Important productive sectors of urban and peri-urban agriculture include horticulture, livestock, fodder, milk and dairy production, aquaculture and agroforestry; where horticulture is its component and comprises vegetables, fruit crops and tubers, ornamentals, mushrooms and condiments.

There are many advantages of peri-urban agriculture. First of all, the proximity to urban nucleus results in a decrease in transport cost and in the carbon footprint associated with long distance transport. Furthermore, availability of treated wastewater (TWW) of proper quality is guaranteed throughout the year and the availability of biosolids is also viable for use as organic amendments and fertilizers. Nonetheless, competition for resources with other urban sectors, uncertainty of land ownership and presence of diverse hazards derived from its proximity to urban areas (vehicle and industrial emissions, water and soil contamination), constitute some of its major threats (Montasell Dorda and Roda Noya, 2003; Ortolo, 2017). In addition, it is important to notice that urban and peri-urban agriculture is exposed to different contamination sources (contamination originated from air, water, soil, and agricultural practices).

1.1.2 Sources of contaminants

Air

One of the current risks of peri-urban agriculture is the proximity to sources of atmospheric pollution that threaten food production and quality. The air pollutants can be emitted either from anthropogenic or natural sources (primary aerosols) and emitted directly or formed in the atmosphere (secondary aerosols). Concerns have been raised as these pollutants have numerous impacts on the human health and environment (European Environment Agency, 2016). In this regard, Ortolo et al (2017) classified the main urban air chemical pollutants in function of their role in the plant uptake (Figure 1.1).

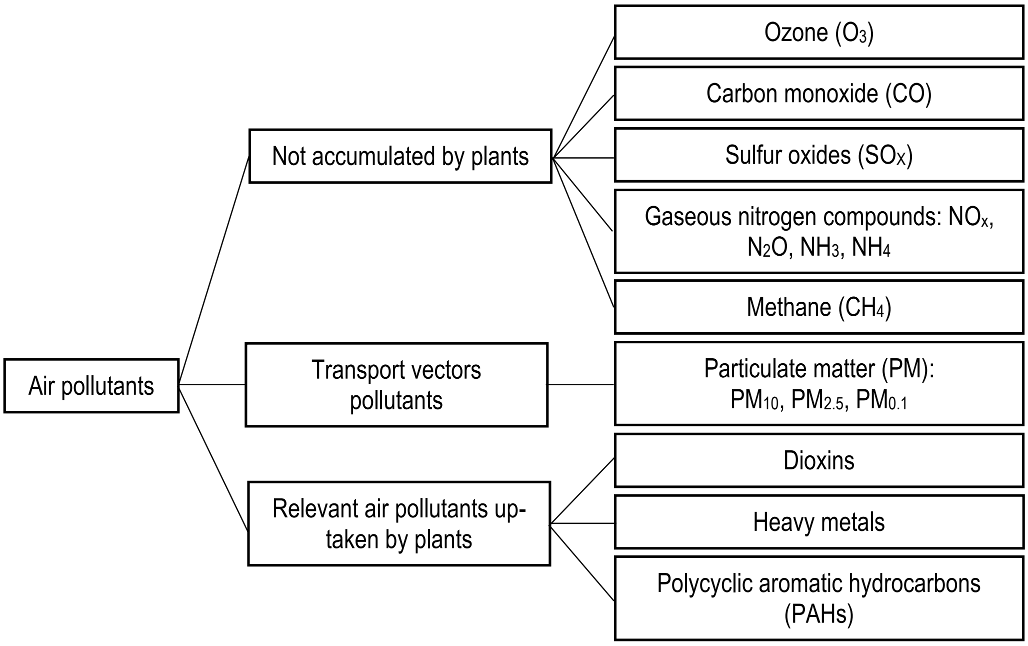


Figure 1.1 Classification of main air pollutants

Not accumulated by plants

Major air pollutants are not accumulated by plants such as O_3 formed by photochemical reactions of precursor pollutants as NO_x and volatile organic compounds (VOC) originated from traffic emissions. CO which is most due to incomplete combustion specially from vehicular exhaust, SO_x emitted by volcanos and industrial processes, gaseous nitrogen compounds from chemical industrial emissions or emissions from fertilizer application and CH_4 produced principally in agriculture (mostly from ruminant animals), followed by waste management and energy production (European Environment Agency, 2016). However, these contaminants can potentially affect food crops altering their plant growth (Pearson and Stewart, 1993), causing foliar chlorosis or necrosis (Markert et al., 2003) or cause the stomata to close and consequently reduction of the transpiration water loss (Barnaby and Ziska, 2012), among others effects.

Transport vector of pollutants

Particulate matter (PM) consists on a mixture of solid particles and liquid droplets suspended in the air made of organic and inorganic substances, which can serve as a transport vector of elements. They are either directly emitted (e.g. dust, fuel burn) or indirectly formed (e.g. when gaseous pollutants previously emitted to air turn into particulate matter) and are classified into three main categories depending on their size: PM_{10} (2.5-10 µm), $PM_{2.5}$ (mainly formed aerosols) and $PM_{0.1}$ (ultra-fine particles). PM can cover plant’s leave and reduce light penetration, blocking the opening of stomata. Also, bounded to organic pollutant can acquire lipophilic nature and, therefore, enter the wax layer that covers the leaves and young twigs (Janhäll, 2015).

Relevant atmospheric pollutants in peri-urban regions

Dioxins and furans

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are by-products produced unintentionally in industrial processes involving chlorine (e.g. waste incineration, chemical and pesticide manufacturing). They can be taken up by the plant's leaves in the vapor phase, via stomata and the wax layer, whilst the particulate phase can be deposited on leaves (Chrostowski and Foster, 1996; Dopico and Gómez, 2015). Dioxins are accumulated in plants, but the risk occurs via meat/dairy products consumption due to magnification along the trophic chain.

Heavy metals

Heavy metals can be found naturally in the environment, in soils, rocks, sediments, waters and microorganisms. Some of the heavy metals are essential nutrients at low concentrations but most of them become toxic at large concentrations. Moreover, heavy metals are persistent in the environment and can be mobilized between different environmental compartments, but they cannot be biodegraded (Ortolo, 2017). The main contaminants being relevant for urban atmospheric pollution risk assessment are cadmium (released through mining and smelting, phosphate fertilizers and various industrial uses and it is also correlated to traffic exposure as added to fuels as preservatives), copper (break wear, automobile emissions, soil and coal via inhalation, fertilizers), lead (the remaining emissions are from the industry sector) and mercury (breakdown of minerals in rocks and soil, human activities such as fertilizers, fungicides and municipal solid waste and industrial wastewater).

Polycyclic aromatic hydrocarbons (PAHs)

PAHs are found in the atmosphere mainly as aerosols, but naphthalene and its derivatives occur depending on temperature in the gas phase. They are persistent and widespread organic pollutants in the environment, whose concern is related to the human health impact since they are carcinogenic, mutagenic and teratogenic. A marginal contribution can be associated to a natural origin such as fires or volcanoes, but the principal sources are anthropogenic and include motor vehicles, stationary power plants and domestic as well as deliberate biomass burning (Holman, 1999).

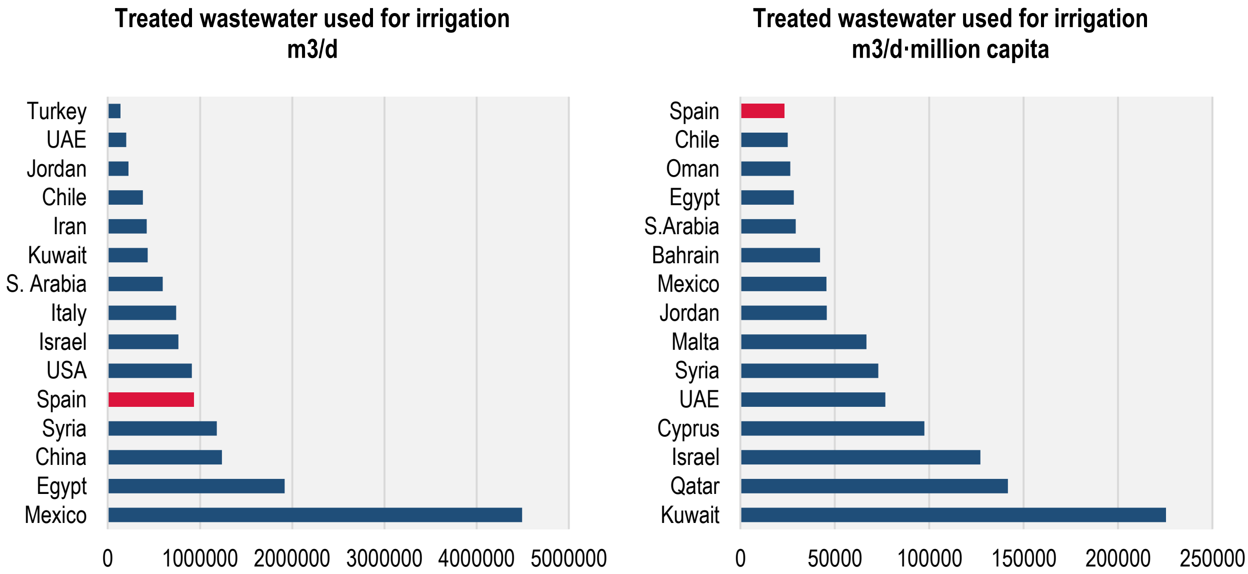
Water

Reclaimed water

Overall, the 70% of global freshwater withdrawals are used for agriculture and this figure can increase up to 90% in underdeveloped countries (UN-WWAP, 2015). The agricultural production in arid and semiarid countries relies on irrigation, especially during the dry season. On the Mediterranean coast, available surface or groundwater resources suitable for irrigation are insufficient and sometimes of low quality (high content of salts) to sustain agriculture demands. Therefore, wastewater reuse has become an effective solution to overcome this issue.

The reclaimed water can be used indirectly if the TWW is released to a waterway (river, canal. stream…) from where it will be taken and used later by farmers; or directly when the TWW is used directly without mixing with other water bodies and distributed by pipeline networks.

There are several countries that actively apply reclaimed water in agricultural irrigation, including Israel, USA, Mexico and Spain (Figure 1.2) (Jiménez and Asano, 2008). Spain is the fifth country to use higher volumes of reclaimed water in irrigation and the fifteenth if the data is given in volume per million capita.


Treated wastewater used for irrigation m3/d

Treated wastewater used for irrigation m3/d·million capita

Figure 1.2. Countries that use major volumes of TWW per in-habitant for agricultural irrigation (Jiménez and Asano, 2008)

Regarding to Catalonia, around 70% of the water resources are used for agriculture. However, in 2015 only 30 hm^3 of the 700 hm^3 of WWTPs releases were reused (ACA, 2016). Moreover, only 7% of this reclaimed water was used for agricultural uses (Figure 1.3).

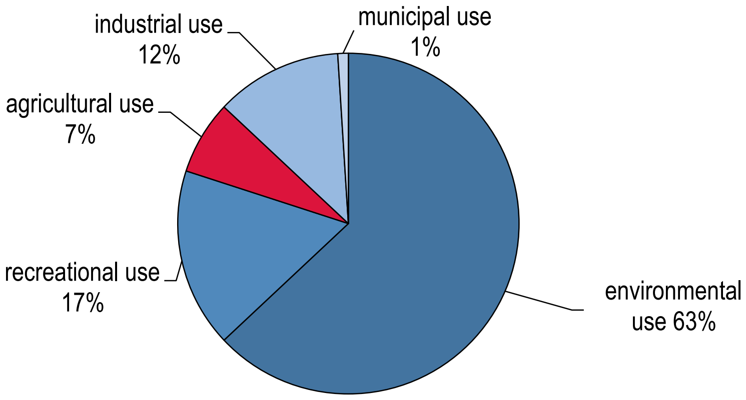


Figure 1.3. Percentages of reuses of regenerated water in Catalonia (ACA, 2016)

Legislation

In Spain, Royal Decree 1620 of 2007 provides the legal framework for the reuse of TWW based on the final use, taking as fundamental criteria the health risk. The five types of uses of regenerated water recognized are: 1) urban (green areas, street cleaning, fires and car wash), 2) agricultural (irrigation and aquaculture), 3) recreational (field irrigation of golf, ponds, ornamental water flow with forbidden access), 4) industrial (process and cleaning waters) and 5) environmental (recharge of aquifers, forestry, maintenance of minimum levels).

Several quality criteria have been established for reclaimed water according to the fate or use, as well as some controls to ensure it does not pose health risks. These are intestinal nematode eggs, *Escherichia coli*, suspended solids and turbidity; for which there is a maximum admissible value according to the final use. In the case of the agricultural use of reclaimed water, the Decree includes the maximum levels (mg L^{-1}) permitted for some metals and metalloids, but although it refers to the organic priority pollutants listed in the Water Framework Directive (2000/60/EC), no maximum level is specified.

Advantages and threats of wastewater reuse

Using TWW for irrigation not only provides crops the necessary water to growth, but also improves soil conditions due to the presence of nutrients, micronutrients, and organic matter that remain in wastewater after treatment (Table 1.1). Nevertheless, one of the major concerns regarding the use of reclaimed water in agriculture is crop exposure to contaminants.

Table 1.1 Advantages and risks associated with the use of reclaimed water (Colon and Toor, 2016)

Benefits	**Risks**
Reliable and economic water source	Presence of salts
Improvement of soil conditions	Presence of biohazards*
Reduced use of fertilizers	Presence of contaminants
	Excess of nutrients

*Bacteria, viruses, helminths, etc...

Reliable and economic water source. Its availability is guaranteed not being subject to the variability of climatic conditions. In addition, the cost of producing regenerated water is lower than that of desalination.

Improvement of soil conditions. They are improved due to the presence of nutrients, micronutrients and organic matter remaining in TWW after treatment (Chen et al., 2013). In addition to the two primary nutrients (nitrogen and phosphorus), reclaimed water also provides micronutrients required by plants such as iron, manganese, zinc, copper, molybdenum, boron, nickel and cobalt (Bedbabis et al., 2014; Lubello et al., 2004; Qian and Mecham, 2005).

Reduced use of fertilizers. Reclaimed water can serve as a reserve of essential nutrients for the growth of plants, which are nitrogen, phosphorus, potassium and some micronutrients. Nevertheless, it can cause many problems, such as nutritional imbalances and groundwater contamination by nitrate (Candela et al., 2007). Total nitrogen concentration in secondary and tertiary effluents from WWTPs are, typically, around 10-20 $mg \cdot L^{-1}$ (Lubello et al., 2004; Pedrero et al., 2010). Unless specific treatment processes (nitrification / denitrification) are used, nitrogenous compounds are not eliminated during the water reclamation treatment. Therefore, reclaimed water can provide a significant portion of the nutrient demand and reduce the need of fertilizers in the production of crops (Chen et al., 2013). In addition, nitrogen excess can cause adverse effects on plants, such as excessive vegetative growth, delays in maturation or decrease in food quality (Chen et al., 2013).

Presence of salts. Salinity in reclaimed water is approximately 1.5-2 times higher than in tap water, so it can affect the quality of the soil and the growth of plants (Chen et al., 2013). It could damage the plant in several ways: 1) it makes difficult the absorption of water and nutrients from the roots (osmotic stress) and 2) the constant accumulation of sodium ions in plant tissues inhibits essential cellular processes (e.g. photosynthesis) (Jorge et al., 2016).

Presence of heavy metals. Conventional wastewater treatment processes effectively eliminate the presence of heavy metals (Qdais and Moussa, 2004). As a result, its presence in reclaimed water is largely insignificant and the concentrations are comparable to the levels found in fresh water. However, heavy metal pollution is significant in peri-urban areas due to industrial activities and the use of fossil fuels (Nabulo et al., 2010). The plant uptake of heavy metals could reduce the productivity of crops by affecting various plant physiological processes, including seed germination, plant growth and photosynthesis (Abbas et al., 2015).

Organic microcontaminants. OMCs can be classified into persistent organic pollutants (POPs) and emerging ones. POPs are toxic chemicals, resistant to degradation, that accumulate in tissues of living beings, can be transported to long-range distance from the source of contamination and generate toxic effects on human health and the environment. According to the Stockholm Convention, they can be divided into intentional (used or produced in an industrial process, e.g. pesticides, perfluorooctane sulfonate (PFOS), polybrominated diphenyl ethers (PBDE), pentachlorobenzene (PECB) and Hexabromobiphenyl (HBB) compounds) and unintentional (originated as a by-product of chemical reactions or processes, e.g. polychlorinated biphenyls (PCBs), PAHs, dioxins and furans).

Emerging organic contaminants (EOCs) can be defined as contaminants that are not included in routine monitoring programs but that may be suggested for future regulations. This is due to the research on its toxicity, potential health effects and monitoring data on its presence in the different environmental compartments. This group includes surfactants, flame-retardants, personal care products (PCPs), gasoline additives and their degradation products, biocides, polar pesticides and their degradation products and other compounds suspected of causing endocrine disruption.

Since conventional wastewater treatments have not been designed specifically to eliminate these chemical compounds, PCPs are widely detected in reclaimed water at concentration levels between ng·L^{-1} and µg·L^{-1} (Calderón-Preciado et al., 2013; Chen et al., 2005). Consequently, the use of reclaimed water for agricultural irrigation results in the crop exposition to EOCs.

Soil

Soil pollution can cause numerous detrimental effects on ecosystems, human, plants and animal health. These harmful effects may come from direct contact with polluted soil or from contact to other resources, such as water or food, which has been grown or been in direct contact with polluted soil. Sources of soil contamination can be differentiated between non-site related causes and site related ones (Figure 1.4).

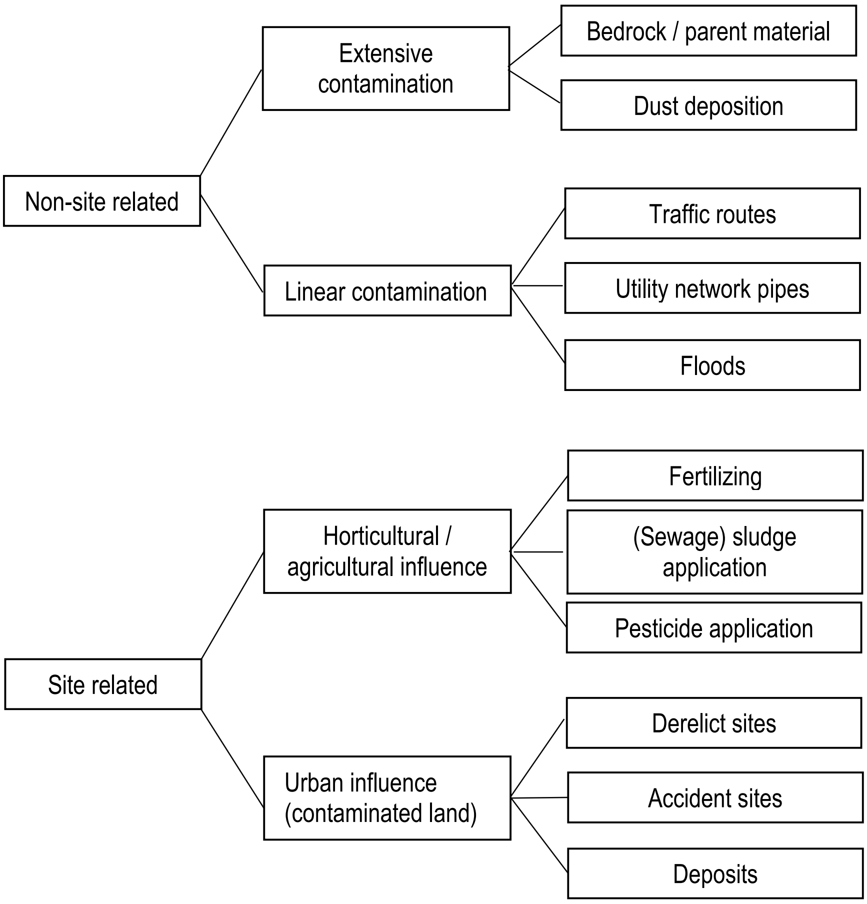


Figure 1.4. Diagram of contaminant sources in the peri-urban environment (Meuser, 2010).

The non-site related causes involve extensive and linear contamination patterns. Extensive contamination can be caused by the presence of metals, metalloids and other TEs in geological materials and from dust deposition, mainly caused by industrial emission. Whereas, linear sources include emission along traffic roads and utility networks pipes as well as flood occurrences in alluvial floodplains.

Nabulo et al. (2006) reported that soils closer to traffic roads contain a greater concentration of heavy metals such as Pb, Zn and Cd (Figure 1.5). Thereby the presence of TEs in peri-urban agriculture due to the traffic emissions needs to be considered as a contamination source.

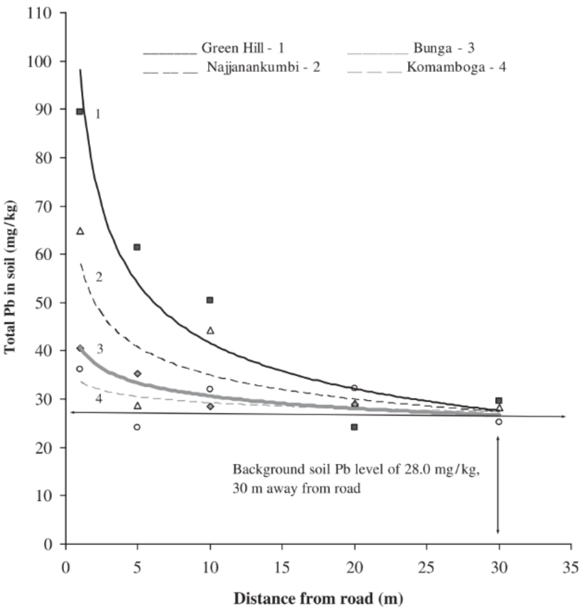


Figure 1.5. Comparison of the total Pb concentrations in soils ranging from high-traffic urban roads to suburban low-traffic places in the city of Kampala (Nabulo et al., 2006)

Numerous contaminant sources are related to traffic. In the past, leaded gasoline emitted Pb and despite the introduction of unleaded gasoline, still trace levels of Pb can be found in soils due to its persistence (Borchers et al., 2010). Residues from tyre and brake wear (Cr, Cu, Ni) can be released to the environment, as well as heavy metals from products of corrosion (Cd, Cu, Zn) and new contaminants based on current car technology (catalytic filter systems) such as platinum and rhodium have arisen (Meuser, 2010).

On the other hand, site related sources of contaminants comprise contaminants that have been used in a specific area (horticultural and agricultural activities) and contaminants generally site-specific such as contaminated derelict sites, heaps, etc. Finally, soils intended for horticultural purposes are potentially exposed to contaminants from fertilizers containing problematical mineral compounds and application of sewage sludge, wastewater and pesticides.

Legislation

In Spain, the Royal Decree 9/2005 (Ministerio de la Presidencia, 2005) establishes generic reference levels (GRL) for certain organic contaminants in soils for human health (industrial, urban or other uses) and ecosystems (agricultural and forest areas or the rest) protection. Additionally, the Regional Decree 5/2017 (Generalitat de Catalunya, 2017) provides the GRL levels for metals and metalloids (Table 1.2).

Table 1.2. GRL values for metals and metalloids established in soils in Catalonia for protection of human health and ecosystems (Regional Decree 5/2017).

	GRL values for protection of human health (mg/kg soil dw)			GRL values for protection of ecosystems (mg/kg soil dw)	
Element	Industrial use	Urban use	Other uses[1]	Agricultural and forest areas[2]	The rest[3]
Antimony (Sb)	30 *	6 **	6 **	6,0	6,0
Arsenic (As)	30 **	30 **	30 **	30	30
Barium (Ba)	1.000 ***	880	500	500	270
Beryllium (Be)	90	40	10	10	4,5
Cadmium (Cd)	55 *	5,5	2,5	2,5	0,6
Cobalt (Co)	90	45	25 **	25	25
Copper (Cu)	1.000 ***	310	90	90	55
Chromium (III)	1.000 ***	1.000 ***	400	400	85
Chromium (VI)	25	10	1	1,0	1,0
Tin (Sn)	1.000 ***	1.000 ***	50	50	7
Mercury (Hg)	30 *	3	2 **	2,0	2,0
Molybdenum (Mo)	70 *	7 *	3,5 **	3,5	3,5
Nickel (Ni)	1.000 ***	470 *	45 **	45	45
Lead (Pb)	550 *	60 **	60 **	60	60
Selenium (Se)	70 *	7 *	0,7	0,7	0,5
Thallium (Tl)	45 *	4,5 *	1,5 **	1,5	1,5
Vanadium (V)	1.000 ***	190	135 **	135	135
Zinc (Zn)	1.000 ***	650 *	170 **	170	110

[1]In the soils where NGRs are applicable to other uses in the protection of human health, the representative surface area of the soil will be that resulting from a homogeneous sample of the first 50 cm, once the natural coverage of the terrain has been removed (the first 5-10 cm); [2]The NGR column defined as agricultural and forestry area will be applicable to all those soil subjected to agricultural fertilization practices. In this case the surface representative sample of the soil will be that resulting from a homogeneous sample of the first 50 cm, once the natural coverage of the ground (5-10 cm) has been removed; [3]Reference levels: upper limit of the confidence interval of Percentile 95 calculated from natural soil samples.

**In application of the criterion of contiguity; **in application of the reference values; ** in application of the reduction criterion*

Agricultural practices

Agricultural practices might have influence in the chemical contamination of food crops. As mentioned previously, the use of TWW for irrigation, use of pesticides and fertilizers and soil amendments together with mulching may constitute an exposure to a wide group of chemicals.

Mulching

The use of field mulching alters the plant microenvironment in order to promote plant growth and thus, increases crop yield. It is useful as a water conservation technique, which increases water infiltration into the soil, reduces soil erosion and surface runoff (Prosdocimi et al., 2016). In addition, plastic mulching suppresses weed growth and reduces competition with weeds for water and nutrients (Abouziena et al., 2008).

The materials applied for mulching can be separated into three main categories: organic (e.g. plant products and animal wastes), inorganic (e.g. plastic and biodegradable plastic film) and special materials (e.g. sand and concrete, which are barely used) (Kader et al., 2017).

The migration of heavy metals from soil to plant, influenced by mulching has been investigated up to a certain point. Li et al. (2010) reported that mulching (semi-transparent plastic film) affects the bioavailability of metals in soil as it slightly increased the bioconcentration factor (BCF) (calculated with the labile portion of metal) for most metals (by 16-58% for Fe, Zn and Cd).

Pesticides

Pesticide application is widespread in agricultural areas in order to provide plant protection. Approximately, from 1 to 2.5 million tons of active pesticides ingredients are applied worldwide every year. They are used to prevent crops from being harmed by disease and infestation and they comprise herbicides, fungicides, insecticides, acaricides, plant growth regulators and repellents. They are divided, based on the chemical constituents, into dithiocarbamates (7.1%), organophosphates (6.7%), phenoxy alkanoic acids (4.7), amides (4.2), bipyridyls (3.2), triazines (2.3), triazoles and diazoles (2%), carbamates (2%), urea derivatives (1.7%) and pyrethoids (1.3%) (Fenner et al., 2013a).

Although their registration for use depends on their non-persistence in the environment after their period of use, their residues are found ubiquitously in the environment from $ng \cdot L^{-1}$ to low $\mu g \cdot L^{-1}$ and not only in ground water, but also in surface waters. Moreover, these substances are often harmful to non-target organisms through consumption of food crops (Li et al., 2010). Hence, the Regulation EC 396/2005 established maximum residue levels (MRL) for residues of pesticides in foodstuff in Europe.

Agricultural soil amendment

Soil amendment includes organic and inorganic substances mixed into the soil in order to improve soil conditions regarding plant productivity. Organic amendments are usually derived from vegetables, by-products from processing plants or mills or waste disposal plants (e.g. processed sewage sludge, compost and biosolids). However, the enhanced physical, chemical and biological properties of soil may promote the migration of heavy metals from soil to crops and thereby constitute a pathway of heavy metal accumulation in crops (Li et al., 2010).

On the other hand, the use of biosolids is one of the major environmental concerns worldwide as they usually contain many toxicants such as heavy metals, pesticides, EOCs, toxic organics, hormone disruptors, detergents and various salts in addition to organic material. Although the presence of nutrients improves the plant growth, a high accumulation of certain heavy metals (such as Cd, Pb and Ni) in seeds has also been reported (Singh and Agrawal, 2010). Therefore, it could serve as a pathway of entering toxic elements into the food chain (Alvarenga et al., 2015; Fijalkowski et al., 2017).

1.2 Plant uptake, translocation and metabolism

1.2.1 Key physical-chemical properties of contaminants

Plant uptake of contaminants is affected by physicochemical properties of contaminants, but also by soil and plant physiology (Dolliver et al., 2007; Khan et al., 2015; Paterson et al., 1990; Trapp and Legind, 2011). The most relevant physical-chemical properties of contaminants and their implication in the plant uptake are detailed below.

- **Half-life ($t_{1/2}$)**

It is defined as the time required by a certain amount of a compound to be reduced by half. Contaminants must be stable in the soil to be incorporated into the plant. Specifically, contaminants with half-life time greater than 14 days are more likely to be incorporated by plants (O'Connor, 1996). On the other hand, compounds with shorter half-lives may suffer degradation during treatment in WWTPs or in water supply networks.

- **Chemical Speciation**

Speciation is an important parameter to take into account during plant uptake of TEs. It refers to the distribution of an element amongst chemical species. It should be highlighted that the total metal contents in soil do not show the biogeochemical behaviour of a metal because its different chemical species have influence (Shahid et al., 2017b). For instance, chromium exhibits contrary effects among its different chemical forms, being Cr (III) and Cr (VI) the most stable and predominant in the nature. However, Cr (VI) is much more mobile in the soil and extremely toxic to organisms compared to Cr (III) as it is highly reactive with other elements (Amin et al., 2013). Meanwhile, Cr (III) is less toxic and mobile due to its precipitation at natural pH values (Shahid et al., 2017b). In addition, both chemical forms are incorporated through different mechanisms (active and passive) and Cr (VI) is known to interfere with the plant uptake of some essential nutrients due their ionic resemblance (e.g. K, Fe, Mn, Mg, Ca and P) (Gardea-Torresdey et al., 2004).

- **Solubility**

Solubility is the ability for a given substance (solute) to dissolve in a solvent and it is measured as the maximum amount of solute dissolved in a solvent at equilibrium. High water solubility compounds tend to have higher soil mobility and, hence, will be less likely to accumulate, bioaccumulate, volatilize and persist in the environment. Generally, the biodegradation and metabolization of these compounds by the microorganisms will be easier with respect to compounds with less solubility.

- **Octanol-water partition coefficient (*K*ow)**

One of the factors that most affects the distribution and bioavailability of a compound is its hydrophobicity, or tendency to dissolve preferably in a lipid phase. Hydrophobicity is measured from the octanol-water partition (K_{ow}) coefficient and represents the distribution of a compound between two immiscible solvents, water (polar solvent) and octanol (relatively non-polar, which represents lipids).

$$K_{OW} = \frac{C_{octanol}}{C_{water}} \tag{1.1}$$

The translocation of organic contaminants in plants takes place with log K_{ow} values between 1 and 4 (Calderón-Preciado et al., 2013; McCutcheon and Schnoor, 2004), with the maximum translocation around log K_{ow} of 1.78 (Briggs et al., 1982). The compounds that have log K_{ow} values in this range, have a higher probability of being incorporated by the plants because they would be sufficiently hydrophobic to mobilize through the lipid bilayer of the cell membranes and would be sufficiently soluble in water to be transported. A high value of log K_{ow} (> 4) represents a high hydrophobicity and would indicate that the compound can be fixed to the organic matter of the soil. Therefore, it would be barely bioaccesible and would rarely be incorporated by the plant via root. On the contrary, if the hydrophilic compound were not set in organic matter, it would have a great mobility to the ground so that it could contaminate the aquifer (Calderón-Preciado et al., 2013).

However, the octanol-water partition coefficient is important only for neutral compounds, since ionic compounds are usually more polar and soluble in water and have been observed to behave differently (Trapp and Legind, 2011). Other mechanisms such as attraction or electrostatic repulsion, and ion trap may affect their accumulation in roots (Wu et al., 2015). Therefore, the incorporation of ionic compounds via root cannot be related to their hydrophobicity, the acidity constants of compounds and variations in the pH of the medium (Trapp, 2004, 2000) are more important.

- **Constant of acidity (pKa)**

As it has mentioned above this value is of great importance since most of the pharmaceutical compounds are ionizable substances (Boxall et al., 2012). Depending on the pH conditions, these compounds can be neutral, cationic, anionic or zwitterionic by having different functionalities in the same molecule and therefore, it will change its possible incorporation and translocation into the plants.

- **Soil-water partition coefficient (*K_d*)**

It is the constant distribution of an organic substance between the soil and the water in equilibrium at a given temperature.

$$K_d = \frac{C_{sorbed\ in\ soil}}{C_{dissolved\ in\ water}}\ (\mathrm{L \cdot kg^{-1}}) \tag{1.2}$$

➢ Organic carbon soil - water partition coefficient (*K_{oc}*)

It represents the capacity of a compound to be adsorbed by the organic matter present in the soil. Therefore, a high value of this parameter would indicate that the compound has a strong affinity to the soil and that a lower proportion of the compound can move through the interstitial water of the ground, so it would be unacceptable to be incorporated by the plants.

$$K_{OC} = \frac{K_d \cdot 100}{\%\ organic\ carbon\ in\ soil} (\mathrm{L \cdot kg^{-1}}) \tag{1.3}$$

➢ Henry constant (*K_H*)

It is the relation between the concentration of a compound in the air respect to its concentration in equilibrium in the water. Therefore, it indicates the volatilization potential of a compound from the water or soil. In addition, the higher the vapour pressure, the greater the potential of volatilization has a compound.

$$K_H = \frac{vapor\ pressure\ in\ liquid}{compound\ solubility} (\mathrm{Pa{\cdot}m^3{\cdot}mol^{-1}\ o\ atm{\cdot}m^3{\cdot}mol^{-1}}) \tag{1.4}$$

$$K_{H'} = \frac{C_{gas\ phase}}{C_{liquid\ phase}} (\mathrm{dimensionless}) \tag{1.5}$$

Substances with $K_{H'} > 10^{-4}$ tend to move in the interstitial spaces of the soil, while with values of $K_H > 10^{-6}$ they move fundamentally in the water. Although the Henry constant is between these two values, the compound will be mobile in the air and in the water so that its potential for incorporation in plants is greater (Linde, 1994).

➢ Octanol-air partition coefficient, K_{oa}

It is a measure of the distribution of a compound between the octanol and the gas phase. Octanol represents the tissue of plants and therefore, this parameter indicates the possible bioaccumulation of the compound in plants from the air.

$$K_{OA} = \frac{C_{octanol}}{C_{air}} \tag{1.6}$$

Aside from the specific factors of the physicochemical properties of the compound, there are also factors that depend on the plant. For example, the incorporation of contaminants may vary between plant species. It has been observed that the incorporation of contaminants from the soil is higher in root vegetables such as carrots than in fruit trees such as apples. However, the incorporation of contaminants from the air is considered to be greater in the opposite case (Trapp and Legind, 2011). Other plant factors are the root system, the shape and size of the leaves and the lipid content. Some studies have shown that plants with a higher lipid content accumulate higher concentrations of contaminants such as PAHs (Simonich and Hites, 1995).

1.2.2 Uptake and translocation

As mentioned above, the greatest concern about the presence of these contaminants in soils is the evidence that they can be incorporated into the plant and accumulated, not only in the roots, but also in the edible parts of plants (Bartha et al., 2010; Khan et al., 2015). Therefore, these contaminants will be incorporated into food chains, which represents a way of exposure for humans. Although the concentrations measured in vegetables are generally low, little is known about the long-term effects of these contaminants on human health (Boxall et al., 2006). The figure 1.6 shows the main plant uptake pathways of contaminants by plants: root uptake from soil solution, dry and wet deposition of particles and gaseous deposition to leaf via cuticle and stomata.

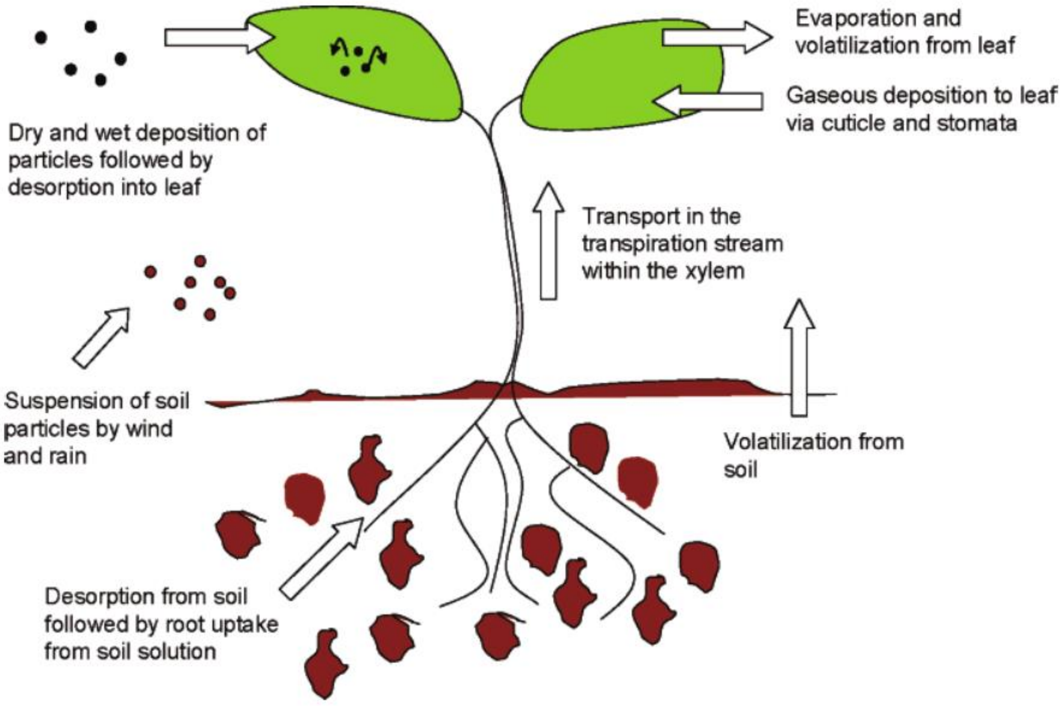


Figure 1.6. Principal plant uptake pathways of chemical contaminants by plants (Collins, 2007)

Root uptake

Although the general uptake pathways are similar, plant uptake mechanisms by roots are very different for organic and inorganic contaminants. This difference remains in the fact that organic contaminants are usually xenobiotic to the plants, so there are no specific transporters for these compounds in the plant membranes (Pilon-Smits, 2005).

In general, TEs such as metal ions are absorbed to soil particles in an insoluble form (e.g. Fe hydroxides in alkaline soil). However, plant roots can influence by releasing protons via membrane H^+-ATPases, which acidify the rhizosphere and create a large membrane potential responsible of cation uptake. While the protons participate in the cation exchange, releasing divalent metal ions that are strongly bounded to soil particles, the acidified rhizosphere can release metals from their hydroxides (Alloway, 2012).

Once heavy metals are incorporated into the roots from the soil solution, they can reach the xylem through same pathways as organic contaminants (symplastic or apoplastic pathways, see figure 1.7). Therefore, although they can migrate into the root apoplastic space, the impermeable Casparian strip in the endodermal cell layer will block this route and then, they have to be actively transported across plasma membrane into the symplast.

In stark contrast to organic contaminants, inorganic transport is mediated by transport proteins in the xylem (Thakur et al., 2016). These transport proteins are naturally found in plants because inorganic elements can be nutrients or chemically similar to them, so they can also be incorporated (e.g. arsenate is incorporated by phosphate transporters, selenate by sulfate transporters). However, transition-metal cations are generally bound by organic ligands upon entry to the symplasm to protect essential cytoplasmic functions (Figure 1.7).

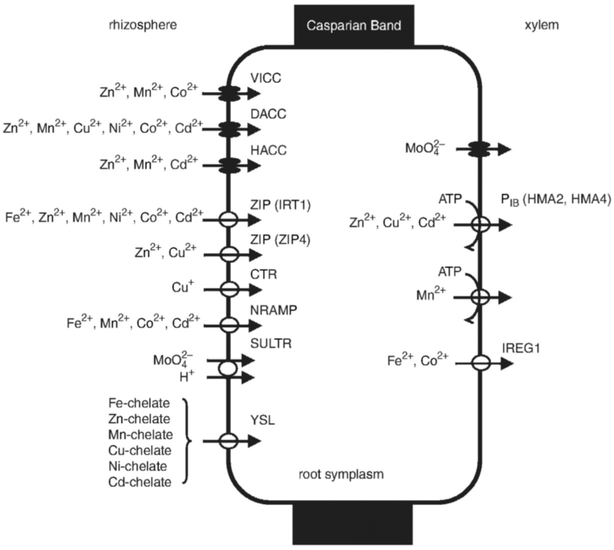


Figure 1.7. Transport proteins in the plasma membranes of root cells implicated in the movement of heavy metals from the rhizosphere to the xylem through the symplasm (White, 2012) VICC: voltage-insensitive cation channels, DACC: depolarization-activated calcium channels, HACC: hyperpolarization activated calcium channels (HACC), ZIP, IRT1: iron-regulated transporter (IRT)-like protein gene family, Z1P4, CTR, NRAMP: natural resistance associated macrophage protein gene family, SULTR: sulphate transporter gene family, YSL: yellow stripe 1 like

Heavy metals can be redistributed from stem and leaf cells through both the xylem and phloem. Selective movement of heavy metals in the phloem allows the delivery of essential elements to developing tissues, tubers, fruits and seeds, whilst toxic elements are retained in older leaves (White, 2012).

Organic contaminants

Plants can incorporate organic contaminants from the soil through the roots (Paterson et al., 1990). The concentration in the soil water of a contaminant is determined by the K_d and as non-ionic organic contaminants are mainly sorbed onto the organic fraction of the soil's solid phase, K_d can be defined in terms of the soil organic carbon content ($K_d=K_{oc}\cdot f_{oc}$, where f_{oc}=fraction organic carbon) (Collins et al., 2007). Correlations between K_{oc} and K_{ow} revealed that soil sorption increases with the Kow, reducing the availability of high K_{ow} compounds for plant uptake (Karickhoff, 1981). Moreover, increases in the f_{oc} diminishes the total amount of contaminant absorbed by vegetation and the optimum K_{ow} for plant uptake.

The uptake of contaminants by plant roots usually is performed by diffusion, which is the simplest passive transport, as it does not require the cell to use energy (Calderón-Preciado et al., 2012; Trapp and Legind, 2011). By contrast, some hormone-like contaminants are incorporated by the active route although it is considered not to be the most important route in the incorporation of organic contaminants as it requires energy to move nutrients and contaminants through the cell membrane (Trapp and Legind, 2011).

Uptake of non-ionic contaminants into plant roots consists of two steps: 1) "equilibration" of the aqueous phase in the plant root with the concentration in the surrounding solution, and 2) "sorption" of the chemical into the lipophilic root solids (lipids in membranes, cell walls, etc.) (Collins et al., 2007). Briggs et al. (1982) reported the linear relationship between the K_{ow} of non-ionic contaminants and the observed root concentration factor (RCF=C in the root/ C in the external solution) in studies about the uptake and translocation of *O*-methylcarbamoyloximes and substituted phenylureas in barley plants. Furthermore, Wild and Jones (1992) published that non-ionic contaminants with $\log K_{ow}>4$ have a high potential for retention in plant roots.

The compounds enter the root with the flow of water and move through the plant through 3 possible pathways (Fig. 1.8): apoplastic (along cell walls through the intercellular space), symplastic (between cells through interconnecting plasmodesmata) and transmembrane route (between cells through cells walls and membranes) (Miller et al., 2016).

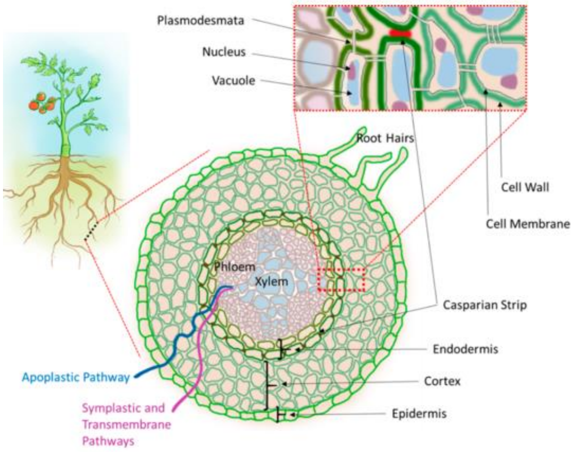


Figure 1.8. Cross-sectional diagram of a root (Miller et al., 2016).

The compound can be mobilized by the apoplast and avoid entering the cells until reaching the endodermis where it has to cross the plasma membranes to enter the symplast and finally reach the xylem from where they can be translocated (Mc Farlane and Trapp, 1994). Nevertheless, for chemicals incorporated in the plant roots must penetrate at least one lipid bilayer to reach the xylem, which are epidermis, cortex, endodermis and pericycle. The pathway used depends on the ability of the compound to cross cell membranes. The Casparian strip acts as hydrophobic barrier between the apoplast and the vascular tissue. Therefore, compounds following the apoplastic route cannot cross the Casparian strip and they must cross at least one lipid bilayer to enter the xylem or phloem. Higher lipophilic contaminants will show more rapid diffusion across lipid bilayers and then, partitioning to lipids and membrane permeability are often estimated from K_{ow}, an approach accurate only for non-ionic organic contaminants because organic ions can be more easily accommodated in lipid bilayers than can n-octanol. Ion trapping occurs when a compound is neutral in the apoplast (pH 4-6) but ionizes inside the cell (pH 7-7.5) leading to accumulation within cells (Trapp, 2004). Once contaminants enter through the roots, they are translocated to the aerial parts through xylem, which is responsible for moving water and nutrients from roots to the upper parts of the plant.

Additionally, Briggs et al. (1982) found the optimal value of log K_{ow} ca. 1.8 (Figure 1.9) for a maximum transpiration stream concentration factor (TSCF) defined as the concentration in xylem respect to the concentration in external solution. Some other authors (Burken and Schnoor, 1998; Hsu et al., 1990) studied this relationship, however, there are significant differences in the TSCF at high (>4) and low log K_{ow} (<1) depending on which model is used (Fig. 1.9).

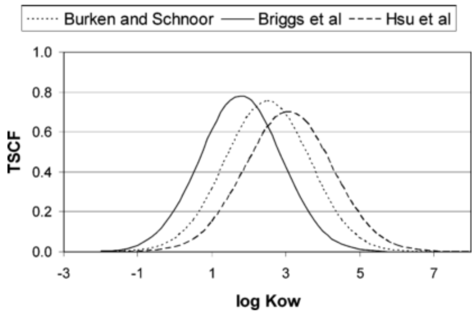


Figure 1.9. Variation in the prediction of the transpiration stream concentration factor (TSCF) with K_{OW} (Collins et al., 2007)

It seems that high polar chemicals are less able to cross hydrophobic lipid membranes, while, lipophilic contaminants also cross the endodermis less efficiently, possibly because they become retained in lipids.

In conclusion, the physicochemical properties of the contaminants are very important to evaluate their potential plant uptake. If the octanol-water partition coefficient is very high, it means that the compound is highly hydrophobic, such as PCBs, PAHs. These compounds would be strongly bounded to the soil and would not dissolve in water, therefore their potential for incorporation would be low (Ryan et al., 1988). It is widely accepted that log values of K_{ow} between 1 and 4 are associated to a greater potential for incorporation (McCutcheon and Schnoor, 2004). With these values the compounds are sufficiently hydrophobic to move through the bilipid layer of cell membranes and sufficiently soluble in water to be transported by cellular fluids (Pilon-Smits, 2005).

Foliar uptake

Inorganic contaminants

As organic contaminants, inorganic can be accumulated in plants leaves due to deposition of atmospheric particles on the leaf surfaces. Unlike root metal uptake, which has been largely studied little is known about foliar metal uptake (Shahid et al., 2017a). The exact mechanism of metal uptake from leaves is not well known, but probably involves gas exchange through the stomata. Heavy metal uptake by leaf surfaces takes place through stoma, cuticular cracks, lenticels, ectodesmata and aqueous pores (Fernández and Brown, 2013). Indeed, the absorption is predominantly through ectodesmata, which consists on non-plasmatic channels positioned mainly in subsidiary and guard cells in the cuticular membrane or epidermal cell wall (Figure 1.10).

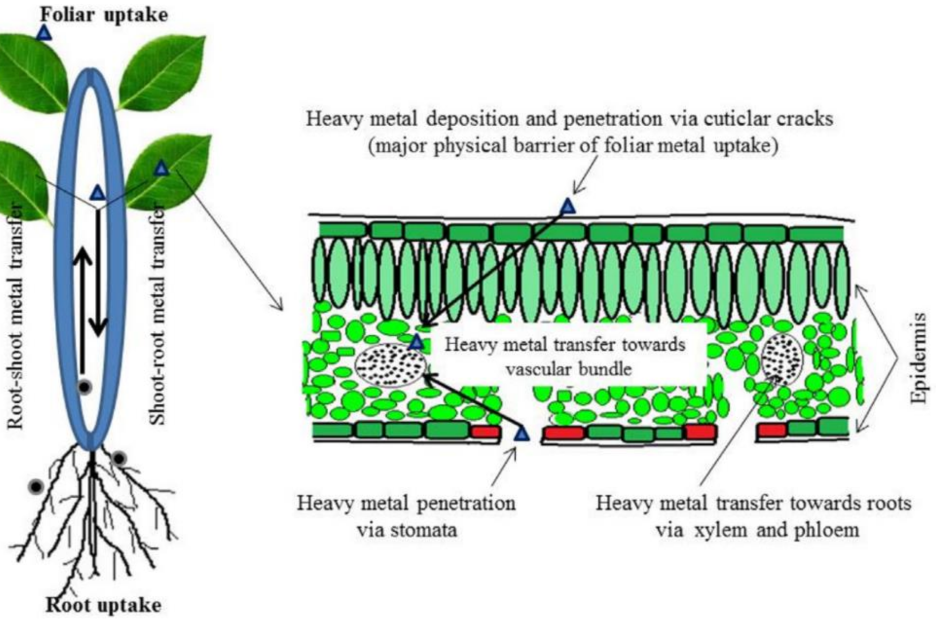


Figure 1.10. Foliar pathways of heavy metal entrance to plants (Shahid et al., 2017a)

Generally, foliar uptake refers to enhanced metal contents in foliar tissues, but it is not easy to differentiate between foliar uptake and transfer inside the plants.

Organic contaminants

Contaminants can be incorporated into the plant via cuticle or stomata by the volatilization of contaminants from soil and deposition on the surface of the plant. This can be an important incorporation pathway for volatile compounds with a Henry's constant exceeding 10^{-4} (Pilon-Smits, 2005). Contaminants in the vapour phase can be taken from the interstitial air of the soil by the roots (O'Connor, 1996), be dissolved in drops of water or adsorbed to particles that are deposited on the surface of the plants and consequently be spread in the plant (Hellström, 2004). Incorporation from the air is simultaneously influenced by other factors such as temperature, humidity, plant species, contaminant concentration and hydrophobicity of the compound.

Dry deposition (free gaseous molecules or dust particles) or wet deposition (contaminants dissolved in water) is the most relevant pathways of foliar exposition to contaminants. The compounds that are deposited on the surface of the plant can be incorporated through the lipid membrane of the cuticle (Riederer, 2002). However, they can also access through the stomata, which are small pores located on the surface of the leaves and which allow the entry of carbon dioxide and other atmospheric gases (Paterson et al., 1990). Once the molecules enter the stomata, they can be translocated by the phloem to other parts of the plant tissue, including the roots (Calderón-Preciado et al., 2012). In this case, the hydrophobicity of the organic contaminant is of great importance, a greater incorporation and translocation has been reported through the stems for compounds with log K_{ow} values between 1 and 3 (Mc Farlane and Trapp, 1994). Unlike the root, the contaminants present in the stems do not have to cross the endodermis, which allows a greater mobility of the polar compounds. The translocation of hydrophobic compounds is limited by their sorption to the cuticle. In addition, they are not easily translocated by the phloem due to their low solubility. In this sense, it has been observed that the permeability of plant cuticles to organic contaminants is linearly related to the K_{ow} of the chemical and inversely related to its molecular volume (Riederer, 2002).

1.2.3 Plant detoxification

Organic contaminants

Awareness that plants can transform xenobiotic compounds, arose in the 1940s, when it was shown that plants had metabolized pesticides (Sandermann, 1994).

Plant detoxification mechanism has been described as the "green liver" model due to its similarities with the processes that occur in liver animals (Sandermann, 1994) (Figure 1.11). According to this model, plants, as autotrophic organisms, do not need organic compounds synthesized by other organisms as a source of carbon, energy and nitrogen. As it is shown in figure 1.11, plants use a three-phase process to convert contaminants, such as herbicides or pharmaceuticals, into intermediate products with less phytotoxicity (phases I, II and III). The ultimate result of these metabolic conversions is often the compartmentation of the contaminant into the vacuole of the plant cell or incorporation into cells walls.

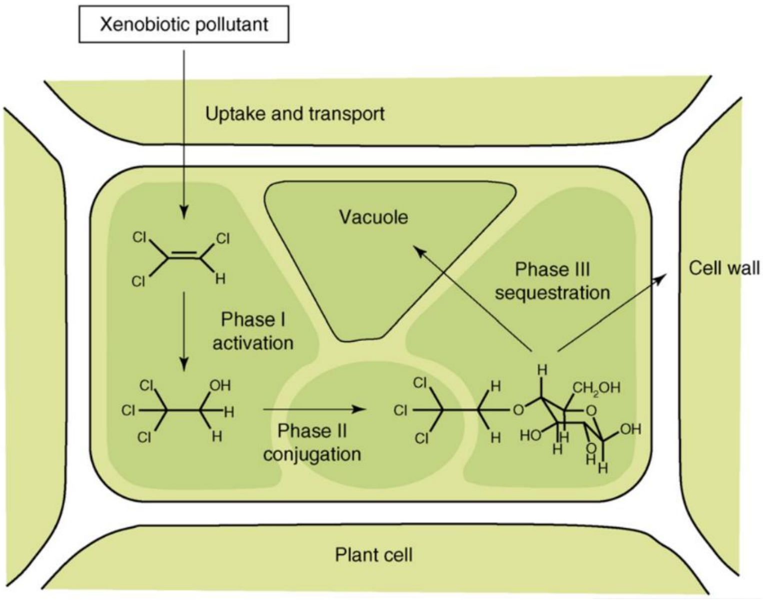


Figure 1.11. Phases of ''green liver'' model (Van Aken, 2008)

Therefore, **phase I** of xenobiotic metabolism consists in the transformation of the compound by oxidation, reduction or hydrolysis. Functional groups are introduced making the molecules more polar, chemically active and more soluble in water (Komives and Gullner, 2005). The first phase is typically an oxygenation carried out by the P-450 cytochrome enzymes. **Phase II** reactions are called conjugation processes and their products are glycosylated, amino acids or peptides conjugates of the xenobiotic compound. The resulting molecule is significantly less toxic than the original molecule or its derivatives of phase I. An enzymatic mediated process by using the glutathione *S*-transferase isoenzyme superfamily is frequently used for the detoxification of a wide-range of toxic chemicals such as herbicides and pharmaceutical compounds (Hu, 2014; Theodoulou et al., 2003). Finally, **phase III** of the xenobiotic metabolism of the plant consists of the storage of soluble conjugates in vacuoles and insoluble conjugates in the cell walls. These molecules resist solubilization in common laboratory solvents and, therefore, are not accessible for the standard waste analysis.

It has been found that metabolized residues from phase I and II may be present in quantities greater than the primary contaminant and, therefore, could represent a source of significant exposure to consumption (Sandermann, 2004). For example, Malchi et al. (2014) found that of the total number of carbamazepine (CBZ) species, only 11 ± 2% in sweet potato leaves and 28 ± 3% in carrot leaves were the parent compound of CBZ, which suggested metabolization of CBZ on the leaves of plants (Malchi et al., 2014) (Fig. 1.12). Similarly, Goldstein et al. (2014) observed the occurrence of these contaminants and metabolites in leaves of tomatoes and cucumbers.

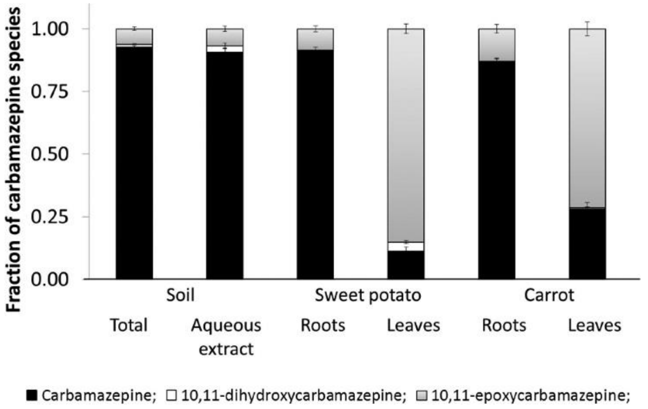


Figure 1.12. Distribution of CBZ and its metabolites, 10,11-epoxycarbamazepine and 10,11-dihydroxycarbamazepine in the bulk, soils, soil aqueous extracts, roots, and leaves of carrots and sweet potatpes (Malchi et al., 2014)

Likewise, LeFevre et al (2015) reported aminoacid conjugates and glycosylated metabolites after benzotriazole plant uptake by *Arabidopsis* spp. (phase II). In addition, in the same study glycosylated benzotriazole conjugates were observed to be excreted by the plants into the hydroponic medium as a detoxification mechanism.

Inorganic contaminants

Baker (1981) defined three plant categories regarding their metal accumulation pattern.

- Excluders, plants that restrict the amount of toxic metal that is transferred to the above-ground biomass
- Accumulators, those plants that tolerate high levels of toxic metals, accumulating them in the above-ground tissues (hyperaccumulators are those that accumulate metals more than 0.1–1 % of the dry weight)
- Indicators, plants whose metal concentration is proportional to the metal concentration in soil

Heavy metals, especially non-essential heavy metals, at higher accumulation generate adverse effects in the functioning of plant cells. Once heavy metals are incorporated in plants, several different types of strategies are employed by the plants to combat the situation. Response of plants to heavy metal depends mainly on efficiency of metal uptake, translocation, and sequestration of heavy metal in specialized tissues or in vacuoles.

The possible mechanisms undertaken by plants in response to heavy metals include exclusion, inclusion and accumulation of heavy metals, binding to the cell wall, reduced transport across the plasma membrane, active efflux, compartmentalization, and chelation (Viehweger, 2014; White, 2012).

Other plant detoxification mechanisms

In addition to plant metabolization, organic and inorganic chemicals can be subject to other detoxification processes diminishing their concentration in the plant system. Indeed, contaminants can undergo transformation processes previously in the rhizosphere, inside the plant and on the plant surface. Once inside the plant, chemicals contaminants may undergo photolytic degradation, phytodegradation (or metabolism), phytoexcretion or phytovolatilization. For the contaminants that are not removed in the aforementioned routes, concentration will increase with chance of causing toxicity, unless growth dilution could counteracts (Limmer and Burken, 2016).

Photolytic degradation on plant surfaces

It may be an important process as the leaves have the tendency to orient themselves to maximize the reception of sunlight (Trapp and Matthies, 1995). The degree of this process on leaf surfaces will influence the aerial deposition and direct contact plant uptake pathways. Trapp and Matthies (1995) discovered that it was the main loss process for 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin in their generic one-compartment model for the uptake of organic chemicals by foliar vegetation.

Phtytoexcretion

There is little information about this process, but may be an important loss mechanism in some plants for hydrophilic and ionized species, where these compounds can be excreted from the leaf through aqueous pores. For instance, Manousaki et al. (2008) found out that *Tamarix smyrnensis* plants grown on contaminated saline soils, excreted Cd via salt glands on the surface of the leaves as a detoxification mechanism, which raised as the soil salinity augmented and afterward this Cd was redeposited on the top soil.

Phytovolatilization

Consists on a process in which plants take up contaminants from soil and release them as volatile form into the atmosphere through transpiration. Organic chemical contaminants with high water solubility and vapour pressure, such as VOCs, can volatilization from foliage to air as plant transpiration flux moves chemicals to sub-stomatal tissues. Moreover, it has been widely detected for inorganic contaminants, as volatile forms of these elements such as Se, As, and Hg (Limmer and Burken, 2016). Jia et al. (2012) reported the As volatilization from rice plants after the uptake of different methylated As species, which was positive related to the trimethylarsine oxide concentration in rice shoots and roots previously exposed to different As concentration levels. However, volatilization from the rice plants accounted just for 0.4 - 3.2% of the total As volatilized from the whole soil-plant system.

Growth dilution

The importance of this process has not been clearly established. There are two scenarios where it can be important: 1) where there is an acute exposure event a growth results in dilution of this peak concentration of contamination, and 2) where the uptake of this contaminants per unit mass is slower than the accumulation of dry matter per unit mass (Collins et al., 2007). Li et al. (2018) reported that the imidacloprid concentration in six leafy vegetable tissues varied significantly according to the plant variety and growth stage (seedling, rapid growth and maturation stages), probably due to growth dilution. It was observed a negative correlation between the daily transpiration, which increases with growth stages, and the log BCF, so it seems that higher daily transpiration values are related to larger shoot biomass, which resulted in lower concentration of imidacloprid in the shoots.

1.3 Effects of environmental contamination in horticulture and human health

Plant uptake of diverse contaminants has become a global concern as contaminants have shown harmful effects to plants and human health directly by their occurrence in plants and/or indirectly, by causing changes in plant metabolism and therefore on nutritional values (Gaweda, 2007; Hurtado et al., 2017; Khan et al., 2015).

This PhD Thesis is focused on the effect of the occurrence of TEs, CECs and pesticides in irrigation water and soil in crop productivity. The occurrence of POPs (PCBs, PAHs, dioxins…) has not been assessed as they are widely legislated, and they are not expected to be present in harmful concentrations.

1.3.1 Effects on plants

Heavy metals, unlike organic substances, are non-biodegradable and hence tend to accumulate in the environment. Then, these elements can be accumulated in living organisms (bioaccumulation) and their concentrations increase as they pass from lower trophic levels to higher trophic levels (biomagnification). Therefore, heavy metals exert different toxicological effects on plants and animals depending on organism and metal (Khan et al., 2015).

Nevertheless, some heavy metals are also essential nutrients for plant growth and development. Plants require 17 nutrients, including water, oxygen, carbon dioxide and 14 mineral elements that can be distinguished in two categories, depending on the relative amount need for plant growth. Macronutrients are generally found in plants at concentrations greater than 0.1% of dry weight (dw) tissue (N, P, K, Ca, S and Mg), while micronutrients or TEs are generally found at concentrations less than 0.01% of tissue dw (Fe, Zn, Mn, Cu, B, Cl, Mo and Ni). The supply of the first three nutrients (C, H and O) is guaranteed by air and water. However, the remaining 14 mineral nutrients should be present in the plant growth medium in a proper concentration (Fageria et al., 2009). These mineral nutrients have numerous functions (Table 1.3) such as being structural components in macromolecules, as cofactors in enzymatic reactions, as osmotic solutes need to maintain proper water potential or as ionized species to provide charge balance in cellular compartments (Grusak, 2001).

Table 1.3. Essential heavy metals for plants (Barker and Pilbeam, 2007; McCauley et al., 2011; Mengel and Kirby, 2004)

Essential nutrients	Chemical symbol	Function	Deficiency
Boron	B	Cell wall component	Chlorosis of young leaves and terminal bud death
Calcium	Ca	Cell wall component	Distorted and dark green leaves, weak stems and poor germination
Chlorine	Cl	Photosynthesis reactions	Chlorotic and necrotic spotting along leaves
Copper	Cu	Chlorophyll production, respiration and protein synthesis	Chlorosis in young leaves, stunted growth, delayed maturity, lodging and in some cases, melanosis (brown discoloration)
Iron	Fe	Chlorophyll synthesis	Interveinal chlorosis, stunted growth
Potassium	K	Activation of enzymes, photosynthesis, protein formation and sugar transport	Reduction of growth rate, chlorosis and necrosis in later stages, older leaves show mottled or chlorotic areas with leaf burn at margins
Magnesium	Mg	Part of chlorophyll and co-factor for ATP production	Interveinal chlorosis and leaf margins becoming yellow or reddish-purple
Manganese	Mn	Activates enzymes, cofactor, chloroplast production	Interveinal chlorosis in young leaves
Molybdenum	Mo	Involved in N fixation and in enzyme activity	Stunted growth and chlorosis
Nitrogen	N	Proteins, nucleic acids (DNA and RNA) and chlorophyll	Chlorosis of lower leaves, stunted growth and necrosis of older leaves
Nickel	Ni	Component of enzymes Required for proper seed germination	Chlorosis and interveinal chlorosis in young leaves that progress to plant tissue necrosis, poor seed germination and decreased crop yield
Phosphorus	P	ATP (energy), sugars and nucleic acids	Stunted growth, dark green plants
Sulfur	S	Amino acids and proteins	Light green, spindly and small plants
Zinc	Zn	Hormone production and important for internode elongation	Interveinal chlorosis, severe stunting

In addition to essential nutrients, more than half of the elements in the periodic table have been detected in some plant tissues. Most of them do not have known benefits to the plant, and many, such as cadmium (Cd) or chromium (Cr), can be detrimental to plant growth (Grusak, 2001). Regarding their role in biological systems, heavy metals and metalloids are classified as essential and non-essential. Heavy metals needed by organisms in tiny quantities for vital physiological and biochemical functions (Fe, Mn, Mn, Cu, Zn, and Ni) are considered as essential, while the ones not needed (Cd, Pb, As, Hg and Cr) are non-essential (Ali et al., 2013). Although small concentrations of some heavy metals are essential for plant growth, high concentrations of them can exert harmful effects on plant growth, hampering the plant germination, growth and production (Ghosh and Sethy, 2013).

The effects on plants shown can be classified in, either seed germination and growth, plant structure or nutritional values.

1.3.2 Seed germination and growth

Seed germination is one of the most important stages in crop development that influences in crop health, better growth and yield at the later growth stages. There are many factors affecting seed germination, such as the light, temperature, germination time, salinity, water availability and mineral composition of soil (Ahmad and Ashraf, 2011; Gray, 1975).

The main effects that heavy metals exert on seeds consist on a decrease in seed germination, reduced root and shoot elongation, dry weight, membrane alteration, altered sugar and protein metabolism and nutrient loss among others, which result in seed toxicity and productivity loss (Ahmad and Ashraf, 2011). For instance, it has been reported that Ni and Cu inhibit the amylase and other enzymes involved in the breakdown of food reserves (e.g. starch and sucrose), thereby retarding seed germination of many crops (Ahmad et al., 2009; Zhang et al., 2009) (Figure 1.13). It must be stated that the assayed concentrations are much higher than most of the agricultural soils.

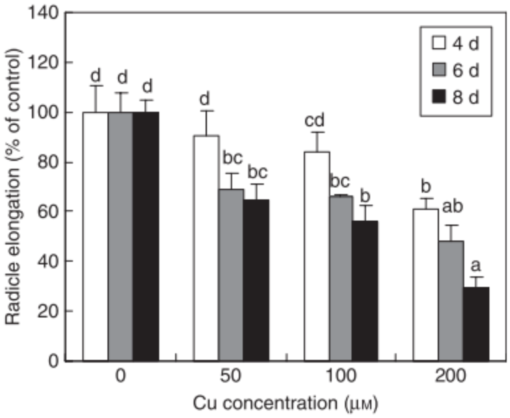


Figure 1.13. Effect of Cu on rice radicle elongation. Rice seeds were treated with the indicated concentrations of Cu for 4, 6 or 8 d. Values shown represent means + s.e. (n = 3) for three different experiments. Means denoted by the same letter did not differ significantly *($p \geq 0.05$ according to Duncan's multiple range test)*

Even though low concentrations of some heavy metals have shown to improve seed growth, high levels are likely to be toxic to plants and inhibit their growth. In addition, high concentrations of some metals may interfere with mineral nutrient uptake. Among the most affected nutrients, the fact that Fe has several resemblances with other heavy metals regarding chemical structure, behaviour, and availability in soils or uptake by plant roots (e.g. Zn, Co, Ni, Cd and Mn) resulted in a Fe plant deficiency, being Zn the most inducing heavy metal (Lešková et al., 2017).

On the other side, effects on seed germination by the occurrence of OMCs have also been studied. Moore and Kröger (2010) studied the effect of three insecticides (diazinon, fipronil, lambda-cyhalothrin) and two herbicides (atrazine, metolachlor) on germination, radicle (root) and coleoptile (shoot) of rice (*Oryza sativa* L.). Although no germination effects of pesticide exposure were observed, significant growth effects were detected between pesticide treatments. Coleoptile growth significantly ($p \leq 0.05$)

diminished in most of the pesticide exposures, compared with controls. On the contrary, radicles of seeds were larger ($p \leq 0.05$) compared to controls.

1.3.3 Plant structure

Bini et al. (2012) showed that the presence of a cocktail of potentially toxic heavy metals (Cu, Fe, Pb, Zn) in soil and plants, is related to micro-morphological changes on the leaf anatomy, such as reduction in leaf thickness, changes in intercellular spaces and in cell structural organization. Balaguer et al. (1998) found that the tomato plant growth was negatively influenced by increasing levels of Ni in the nutrient solution (at 10 and 20 mg·L^{-1}), being strongly altered the fresh weight of stem, branches and leaves but not the water content. Symptoms of Ni toxicity were also reported by Palacios et al. (1998) at nutrient solution containing 15 and 30 mg·L^{-1} of Ni, showing chlorosis, necrosis and stunted growth after 2 weeks of Ni treatments.

In addition, Hurtado et al. (2017) observed that exposure of lettuce to CECs at significant environmental concentrations (0-50 µg·L^{-1}) in irrigation water can cause metabolic alterations in plants as well as the associated morphological changes (height of the leaf and stem width) and variation in the chlorophyll content (Figure 1.14).

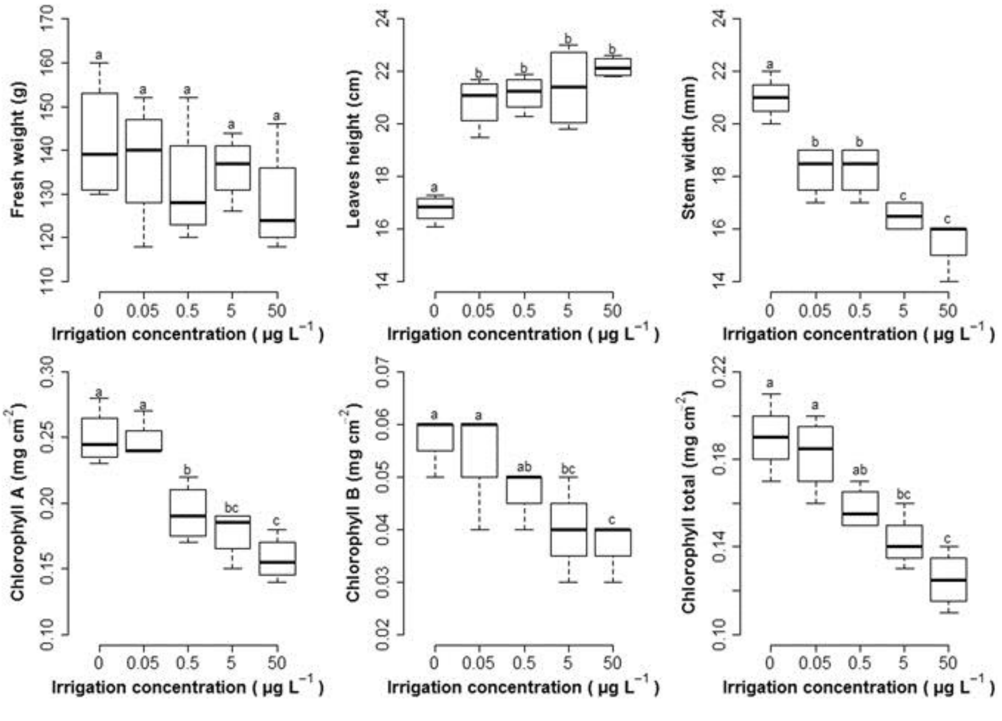


Figure 1.14. Box-plots of different agronomic parameters for different concentrations of pollutants in irrigated water (Hurtado et al., 2017).

Bellino et al. (2018) exposed tomato seeds (*Solanum lycopersicum* L.) to 5 mL of 0, 0.1, 1, 10, 100 and 1000 mg·L^{-1} of a mix of four antibiotics during 10 and 7 days for seed germination and root elongation tests, respectively. Results revealed that the four antibiotics could have phytotoxic effects on tomato root development but not on seed germination, at concentrations from 10 mg·L^{-1} (spectinomycin), 100 mg·L^{-1} (chloramphenicol) to 1000 mg·L^{-1} (spiramycin and vancomycin). This could be due to the reduced permeation of antibiotics through the seed coat, which aims to protect seeds from the noxious effects produced by these molecules (An et al., 2009). Nevertheless, the concentrations tested (ppm) were much higher than the concentrations that are generally found in the environment.

1.3.4 Nutritional values

Heavy metals frequently increase the production of reactive oxygen species (ROS) in plants, resulting in oxidative damages of proteins, lipids, and nucleic acids, which are responsible for several physiological disorders such as growth retardation, nutrient deficiency, reduced transport of nutrients, genotoxicity, and retarded photosynthesis (Khan et al., 2015).

- **Effects on carbohydrates**

Carbohydrate synthesis can be inhibited by an excessive build-up of toxic elements that might destroy the photosynthetic electron transport chain and production of ROS (Sandalio et al., 2001). Gaweda (2007) investigated changes in the carbohydrate content of six vegetable crop species (lettuce, spinach, radish, carrot, red beet and onion) with 0, 250 and 500 $mg \cdot kg^{-1}$ dw of Pb in the substrate; a higher Pb dose, caused a decrease in sucrose content and an increase in starch in the edible part of the plants. Moreover, at high Cd concentration, a general decrease in carbohydrate metabolism occurred (Rodríguez-Celma et al., 2010).

On the other hand, Christou et al. (2019) reported the effects of three pharmaceutical compounds (diclofenac, sulfamethoxazole and trimethoprim), individually and mixed together (10 $\mu g \cdot L^{-1}$), on the quality of tomato fruits. It resulted in no significant alteration of crop productivity, but a significant increase in the soluble solids content and in the transcripts related to the biosynthesis and catabolism of sucrose like and consequently, an increase in the carbohydrate content, which is related to the taste of the fruits.

- **Effects on proteins and amino acids**

Nitrogen and sulphur are the essential nutrients required for synthesis of proteins and amino acids and plant growth. Therefore, the lack of them may affect the metabolic processes (Carfagna et al., 2011). High Cd concentration may impede protein metabolism by modifying physiological functions and synthetic activities (Sandalio et al., 2001) and can also have effects on the decomposition of protein contents (Z. Wu et al., 2014). Likewise, high metal concentrations inhibit protein synthesis by altering the pigment-lipoprotein complex accumulation in photosystems I and II (Wang et al., 2009) and effect ribulose-1,5-bisphosphate carboxylase/oxygenase enzymes (Krantev et al., 2008).

- **Effects on lipids**

Little knowledge is available on heavy metal impact on lipid content (Upchurch, 2008). Khanna-Chopra (2012) determined that heavy-metal-induced oxidative stress results in chloroplast degradation and lipid peroxidation affecting the nutritional status of the contaminated plant.

- **Effects on vitamins**

Although leafy vegetables are considered as a good source of nutrients (Gupta and Bains, 2006), they can be affected by the presence of heavy metals. Environment has a strong influence on vitamin contents and in extreme conditions with high heavy metal concentrations, temperature, and pH, the vitamin contents are significantly reduced (Ipek et al. 2005). Moreover, lipid peroxidation can also reduce vitamin content (Seven et al., 2012). Indeed, there is a negative correlation between heavy metals and vitamins (Widowati, 2012), as a Cd increase resulted in a decrease of 61.7% of vitamin A and a decline of 74.7% in vitamin C in three aquatic vegetables.

1.4 Effects on human health

Entrance of TEs into human body is possible by different pathways such as consumption of contaminated food, drinking water and/or air. It has been highlighted the contribution of vegetables to the total metal intake in human diet, accounting for around 90%, while the other 10% is due to dermal contact and inhalation of dust contamination (Martorell et al., 2011). Moreover, these elements may accumulate in vital body organs such as liver, heart, kidney, and brain disturbing normal biological functioning. Some of these elements (e.g. Zn, Cu, Mg, Co…) as occurred for plants are essential for human body but ingested at higher concentrations may be toxic. By contrast, some other heavy metals (Pb, Hg…) do not have known favourable effects on human health and they become toxic once they are accumulated in the body (Rehman et al., 2018). Toxic heavy metals can cause different health problems depending on the heavy metal concerned, its concentration and oxidation state, etc. Table 1.4 shows some examples of harmful effects of selected heavy metals on human health.

Moreover, OMCs constitute a broad family of compounds, but in this work only CECs and pesticides have been selected. The assessment of CECs in vegetables is important because the risk they might pose to human health is not fully understood. To date, CECs have not been included yet guidelines and regulations. However, information about human health effects resulting from the use of pesticides has been widely reported.

The type of pesticide, the duration and route of exposure, and the individual health status are relevant to assess the possible health effect, also pesticides may be metabolized, excreted, stored, or bioaccumulated in the body fat (Pirsaheb et al., 2015). Furthermore, it should be noted that washing and peeling vegetables and fruits cannot completely remove pesticide residues (Reiler et al., 2015). The numerous of negative health effects (Table 1.4) that have been associated with chemical pesticides include, among other effects, dermatological, gastrointestinal, neurological, carcinogenic, respiratory, reproductive, and endocrine effects.

Table 1.4.Harmful effects of the chemicals studied on human health with oral exposure according to different sources (Ali et al., 2013; ATSDR, 2018; EPA, 2018; Fenner et al., 2013b; Pereira et al., 2015; World Health Organization, 2003)

Chemical	**Harmful effects**
As	As (V) (as arsenate) is an analogue of phosphate and thus interferes with metabolic processes such as ATP synthesis and oxidative phosphorylation Hyperpigmentation, keratosis and possible vascular complications
B	Most ingested boron is absorbed and leaves the body within 4 days. Decreased fetal weight (developmental)
Ba	Barium is a competitive potassium channel antagonist that block the passive efflux of intracellular potassium, results in a decrease of K in the blood plasma. Hypokalemia, which can result in ventricular tachycardia, hypertension and/or hypotension, muscle weakness, and paralysis.
Cd	Carcinogenic, mutagenic, and teratogenic; endocrine disruptor; interferes with calcium regulation in biological systems; causes renal failure and chronic anemia
Co	Has both beneficial and harmful effects on human health. It is a part of the vitamin B12, has been used for the treatment of anemia because it causes red blood cells.
Cr	Chromium is a human carcinogen mainly by inhalation exposure in occupational sceneries. Hair loss
Cu	Elevated levels have been found to cause brain and kidney damage, liver cirrhosis and chronic anemia, stomach and intestinal irritation
Hg	Anxiety, autoimmune diseases, depression, difficulty with balance, drowsiness, fatigue, hair loss, insomnia, irritability, memory loss, recurrent infections, restlessness, vision disturbances, tremors, temper outbursts, ulcers and damage to brain, kidney and lungs
Li	A single large dose may result in vomiting and diarrhea.
Mn	Central nervous system effects
Mo	Increases uric acid levels
Ni	Allergic dermatitis known as nickel itch; inhalation can cause cancer of the lungs, nose, and sinuses; cancers of the throat and stomach have also been attributed to its inhalation; hepatotoxic, immunotoxin, neurotoxic, genotoxic, reproductive toxic, pulmonary toxic, nephrotoxic, and hepatotoxic; causes hair loss
Pb	Its poisoning causes problems in children such as impaired development, reduced intelligence, loss of short-term memory, learning disabilities and coordination problems; causes renal failure; increased risk for development of cardiovascular disease.
Sb	Affects longevity, blood glucose, and cholesterol
Zn	Over dosage can cause dizziness and fatigue.
Amide pesticides	Their symptoms include abdominal cramps, anemia, ataxia, dark urine, cyanosis, hypothermia, collapse, convulsions, diarrhea, etc.

Bipyridyl herbicides	The main effects are dehydration (resulted from vomiting), their high oxidative stress causes necrosis in the gastrointestinal tract, kidney tubules, liver, and lung; in the latter case, respiratory failure and pulmonary fibrosis may take place.
Carbamate pesticides	They poorly penetrate the blood-brain barrier. Their main symptoms of carbamates intoxication are miosis, salivation, sweating, tearing, rhinorrhoea, behavioural change, abdominal pain, vomiting, diarrhea, urinary incontinence, bronchospasm, dyspnea, and so on.
Dithiocarbamate pesticides	Low acute oral and dermal toxicity due to their slow absorption. The metabolite that derives from dithiocarbamates biotransformation is ethylenethiourea, which induces thyroid cancer and modifies thyroid hormones.
Organophosphate pesticides	The skin, conjunctiva, gastrointestinal tract, and lungs rapidly absorb most these compounds and their metabolites arise 12 to 48 h. The main symptoms are the muscarinic syndrome, nauseas, vomiting, and diarrhea; and provokes urinary incontinence, bronchospasm, miosis, and bradycardia.
Phenoxy alkanoic acids herbicides	They are mostly absorbed by the gastrointestinal tract rather than by the lungs or skin, and they are not stored in the fat. The main symptoms are nausea, dizziness, vomiting, burning in the mouth, constipation, abdominal pain, numbness, diarrhea, gastrointestinal bleeding, among others.
Pyrethroid pesticides	After their absorption, fast distribution occurs in the organism, where they undergo biotransformation via two mechanisms. Some of their injuring symptoms are tremors, spasms, incoordination, drooling, convulsions, and hypersensitivity to stimuli.
Triazine herbicides	Human exposure has been associated with carcinogenicity and endocrine disruption, but these effects are still debatable.
Triazole, diazole pesticides	Propiconazole was classified as a possible human carcinogen by EPA and its ingestion of can irritate the gastric mucosa.
Urea derivative pesticides	For example, isoproturon has been in commercial use for a short period and no cases of human poisoning have been reported.

Regarding to the occurrence of CECs in edible parts of vegetables, although the effect they pose to human health risk is not fully understood, there are some studies that consider that the consumption of some vegetables could represent a risk to human health, principally due to the presence of genotoxic compounds. Malchi et al. (2014) observed, in a field study watered with TWW, higher concentrations of pharmaceuticals compounds in leaves rather than in roots and CBZ metabolites, mainly EPOCBZ (10,11-epoxycarbamazepine, genotoxic compound), rather than the parent compound. Although for CBZ and caffeine, hundreds of kilograms of carrots or sweet potatoes should be ingested by an adult to reach the threshold of toxicological concern (TTC), for lamotrigine and EPOCBZ the TTC could be surpassed easily. A child (25 kg) and an adult (70 kg) could reach the threshold of toxicological concern (TTC) by consuming half carrot (~60 $g \cdot day^{-1}$) and two carrots a day (~180 $g \cdot day^{-1}$), respectively. Thus, indicating that specific toxicity analysis of these contaminants is needed. Riemenschneider et al. (2016) reported the uptake of 28 microcontaminants and CBZ metabolites in 10 field-grown vegetable species irrigated with TWW and evaluated the human health risk associated to the consumption of these crops.

For most of the compounds assessed, no risk is shown according to the TTC approach as at least 9 kg of vegetable is allowable. However, for the genotoxic ciprofloxacin and EPOCBZ, further toxicological data are required. The TTC value for EPOCBZ and ciprofloxacin could be surpassed by an adult (70 kg) by consuming only one potato (~100 $g \cdot day^{-1}$) or half an eggplant (~177 $g \cdot day^{-1}$). Another field study (Christou et al., 2017b), reported that the estimated TTC and hazard quotient (HQ) values of tomatoes watered with TWW in three consecutive years, represents a *de minimis* risk to human health as low values (≤ 0.015) of HQ were obtained and the daily consumption of tomato by an adult (70 kg) or a toddler (12 kg) to reach the TTC at least is 9.04 and 1.55 $kg \cdot day^{-1}$, respectively. Therefore, in order to conduct a human health risk assessment on the occurrence of TEs, pesticides and OMCs in vegetables, hazard quotient (HQ) and TTC approaches can be used. For further details on HQ and TCC approaches, see Chapter 4 section 4.2.6.

1.5 Overview of the selected contaminants

In this Thesis, chemical contaminants were selected based on their occurrence in the in peri-urban agriculture due to its proximity to relevant contaminant sources (e.g. WWTP effluents, industrial runoff and road networks), and their potential to be incorporated and accumulated into edible parts of plants. Nevertheless, pesticides applied by farmers to control pests, including weeds, were also included in the study, as well as TEs listed in the Spanish Royal Decree 1620/2007 for water reuse. In fact, consumption of vegetables has shown to be the main source of human exposure to heavy metals (Martorell et al., 2011). In addition, the selected OMCs comprise pesticides used in the area of study and CECs with a high plant uptake potential, presence in irrigation waters, persistence in the environment and potential harmful effects for human health.

1.5.1 TEs

Many different sources contribute to the release of TEs into the environment. Some of the most significant natural sources consist on weathering of minerals, erosion and volcanic activity; while anthropogenic sources include mining, smelting, pesticides, fertilizers, sewage sludge, atmospheric deposition, among others (Ansari et al., 2016). From 58 elements analysed in this Thesis, only 16 were finally studied being the most frequent and relevant elements in irrigation waters (Table 1.5).

Table 1.5. Anthropogenic sources of selected inorganic contaminants in the environment

Element	Sources	Reference
As	Pesticides, veterinary pharmaceuticals and wood preservatives	9
B	Production of glass, ceramics, surfactants, fire retardants, pesticides, cosmetics, photographic materials and high energy fuels	10
Ba	Petroleum and steel industry, production of semiconductors and medicinal uses	5
Cd	Cd-Ni battery production, paints, pigments for plastics and enamels, fumicides, phosphate fertilizers and electroplating and metal coatings	7, 9
Co	Steel and alloy production, paint and varnish drying agent and pigment and glass manufacturing	9
Cr	Tanneries, steel industries, fly ash, chromium plating and allows in motor vehicles are considered to be a more probable source	4
Cu	Pesticides, fertilizers, industry and sewage sludge, textile mills, cosmetic manufacturing and hardboard production sludge	4, 9
Hg	Electrical apparatus manufacture, electrolytic production of Cl and caustic soda, pharmaceuticals, paints, plastics, paper products, Hg batteries, pesticides and burning of coal and oil	9
Li	Lithium batteries	1
Mn	Fertilizers, sewage sludge and ferrous smelters	4
Mo	Super alloys, nickel base alloys, lubricants, chemicals, glass workings, ink, pigments and electronics	2
Ni	Production of stainless steel, alloys, automobiles batteries, storage batteries, spark plugs, magnets and machinery	8, 9
Pb	Emission from combustion of leaded gasoline in the past, battery manufacture, herbicides and insecticides	9, 11
Rb	Used in electronics, special glass and in the production of semi-conductors and photocells.	3
Sb	Plastics, pigments of paints, liners of automobile brakes, red rubber production, ceramics, fire retardants, electronics, and glass industries	6
Zn	Mining, smelting and industrial processing of ores and metals, coal combustion, batteries, accumulators, plastics and paints	11

1. Aral and Vecchio-Sadus (2008); 2. Halmi and Ahmad (2014); 3. Kabata-Pendias and Mukherjee (2007); 4. Khan et al. (2007); 5. Kravchenko et al. (2014); 6. Mubarak et al. (2015); 7. Pulford and Watson (2003); 8 Tariq et al. (2006); 9. Thangavel and Subbhuraam (2004); 10. USEPA (2008); 11. Wuana and Okieimen (2011)

1.5.2 OMCs

The OMCs selected in this study can be classified in the following categories (Table 1.6):

Table 1.6. Classification of organic analytes

Category	Analytes
Anticonvulsants and antidepressants and related metabolites	primidone, lamotrigine, diazepam, lorazepam, oxazepam, carbamazepine, 10,11-epoxycarbamazepine
Benzothiazoles	benzothiazol, 2-mercaptobenzothiazole, 2-hydroxybenzothiazole
Benzotriazoles	benzotriazole, 5-methyl-1H-benzotriazole
Chlorinated flame retardants	tris (1-chloro-2propyl) phosphate, tris (2-chloroethyl) phosphate
Parabens	methylparaben, ethylparaben, propylparaben, butylparaben
Pesticides	atrazine, simazine, diazinon, carbendazim, DEET, azoxystrobin, dimethomorph, pyrclostrobin, chlorpyrifos, indoxacarb, pymetrozin
Plasticizers	bisphenol A, bisphenol F
Surfactants and derivatives	surfynol 104, octylphenol
Tert-butylphenols	2-tert-butyl-4mehtoxyphenol

Anticonvulsants and antidepressants

Benzodiazepines such as diazepam, lorazepam and oxazepam are usually prescribed against anxiety and related emotional disorders; as muscle relaxants and inducing agents in anaesthesiology. They have been detected in effluents from WWTPs and rivers. Diazepam and oxazepam concentrations in the Llobregat River were 3 and 20 $ng \cdot L^{-1}$, respectively (Huerta-Fontela et al., 2011). Carbamazepine, lamotrigine and primidone are used as anticonvulsant drugs that have shown potential to be translocated in plants (Goldstein et al., 2014; Wu et al., 2013). Although only up to 30% of CBZ is excreted unmodified from the human body, its inefficiency removal in WWTPs allow it to reach the environment and it is detected in surface water as well in groundwater (Riemenschneider et al., 2017). A plant metabolite from CBZ, EPOCBZ, has also been included in this study. Ben Mordechay et al. (2018) observed higher concentrations of CBZ and its metabolites in soils irrigated with TWW rather than in soils amended in biosolids, being the irrigation water a major contribution of uptake of contaminants. Moreover, it has been found in the leaves of root vegetables in a higher concentration than the parent compound (Malchi et al., 2014).

Benzothiazoles

Benzothiazoles are a class of high production volume chemicals with various applications in industry, but mostly used as vulcanization accelerators (Kloepfer et al., 2005). For instance, 2-mercaptobenzothiazole (2MBT) is a high-volume production product due to its use as a vulcanization accelerator in rubber manufacture, including vehicular tires (LeFevre et al., 2016). It is also used as a corrosion inhibitor in antifreeze coolant, industrial fungicides, and in paper production as corrosion inhibitor (Kloepfer et al., 2005). They are continuously released through WWTP effluents and exhibit a significant lifetime in surface waters.

Benzotriazoles

Benzotriazoles are a broadly used corrosion inhibitors produced in a high volume and applied in dishwashing detergents, cooling and freezing fluids, metal processing, polymer stabilization, in the production of dyes, pharmaceutical, fungicides, as antifogging component in photographic applications and as ultraviolet stabilizers (Huntscha et al., 2014). Moreover, it is an ubiquitous compound in aquatic environment, found at concentrations up to10 $\mu g \cdot L^{-1}$: In fact, it has been described as one of the most abundant contaminants in the aquatic environment (Giger et al., 2006; Huntscha et al., 2014).

Chlorinated flame retardants

Flame retardants are added to many products such as plastics, electronics, textiles, foams and furniture, automobile interiors and many others. The most extensively used were polybrominated diphenyl ethers (PBDEs) until their commercial mixtures were forbidden, and then alternative chemicals such as organophosphate flame retardants (OPEs) became available as substitutes. OPEs, such as TCPP (tris (chloroisopropyl phosphate) and TCEP (tris(2-chloroethyl) phosphate) have been reported in river water, seawater and sediment as early as 1970s (Kurt-Karakus et al., 2018). In addition, they show high bioconcentration factors (>1) for leaf and seed with a disparity between crop species (Eggen et al., 2013).

Parabens

Parabens are ester of *p*-hydroxybenzoic acid and they include methylparaben (MPB), ethylparaben (EPB), propylparaben (PPB) and butylparaben (BPB), among others. They are mostly used as preservatives in cosmetics (like lotions and sunscreens), pharmaceuticals or in a limited range in foodstuffs. Because of their low economic costs and high effectiveness, these products are widely used and are currently ubiquitous in the environment (Czarczyńska-Goślińska et al., 2017; Nowak et al., 2018). Although some of them are synthetized by bacteria or plants (Baardseth and Russwurm, 1978; Peng et al., 2006), all the parabens available are synthetically produced and the major source of contamination are WWTPs. Although the removal percentage in WWTPs is really high (96-99%), their high presence in the raw wastewater (up to about 80 000 $ng \cdot L^{-1}$ for MPB) make them detectable in the effluents (around 4000 $ng \cdot L^{-1}$ the most concentrated) (Błędzka et al., 2014) .

Pesticides

In the last decades, the widespread use of pesticides residues caused significant contamination of aquatic and terrestrial ecosystems. However, their occurrence and behavior in WWTPs has been barely reported due to the fact that agricultural applications represents the main source rather than urban use (Köck-Schulmeyer et al., 2013). In this Thesis, 11 pesticides have been selected, including herbicides, fungicides and insecticides. Atrazine and simazine are triazine herbicides, which are commonly used for the control of broadleaf in agricultural and roadway applications (Hodgson, 2012; L. Li et al., 2018). Azoxystrobin, carbendazim, dimethomorph and pyraclostrobin are used as fungicides; while chlorpyrifos, diazinon, DEET, indoxacarb and pymetrozin are insecticides. Pesticides were selected due to their occurrence in irrigation waters from the area (González et al., 2012; Köck-Schulmeyer et al., 2012; Masiá et al., 2015) and due to their use by local farmers (information provided by Baix Llobregat Agrarian Park).

Plasticizers

BPA (bisphenol A) is the monomer in the production of polycarbonates and epoxy resins commonly applied in food containers, electronics (CDs and DVDs), electronic equipment, medical devices, dental fillings, paper products (e.g., thermal receipts, water pipes and toys, etc.) (Česen et al., 2018). Moreover, BPF (bisphenol F) is one of the main substitutes of BPA and has many applications such as lacquers, varnishes, liners, adhesives plastics, and water pipes, as well as in dental sealants, oral prosthetic devices, tissue substitutes and coatings for food packaging (Chen et al., 2016). Despite the high removal efficiency of bisphenols in WWTPs (≥96.2%), they can still be detected in the WWTP effluents (Česen et al., 2018). Moreover, they have shown to cause endocrine disruptive effects (Czarczyńska-Goślińska et al., 2017).

Surfactants and derivatives

Surfynol 104 is the commercial name for 2,4,7,9-tetramethyl-5-decyne-4,7-diol (TMDD), a non-ionic surfactant used as a wetting and defoamer agent in many waterborne applications such as coatings, paints, inks, adhesives, pigment manufacture, overprint varnishes and paper coatings, agricultural chemicals, among others (Ash and Ash, 2004). It has been found to be detrimental to fish and aquatic invertebrates only at high concentrations (Guedez and Püttmann, 2014). In addition, effluents from WWTPs must be the major source of Surfynol 104 in aquatic environment, as its removal in WWTPs is incomplete (33-68%) (Guedez and Püttmann, 2011).

Alkylphenols (APs) are used for surfactants and antioxidants production (Siddique et al., 2016). OP (4-*tert*-octylphenol) is mainly used in the production of octylphenol ethoxylates, and in a minor proportion for the obtainment of phenolic resins, for tyres and ink production (Miyagawa et al., 2016). It is an endocrine disrupter and its released to the environment as a degradation product of its ethoxylated derivatives in WWTPs effluents (Höhne and Püttmann, 2008).

Tert-butylphenols

Synthetic phenolic antioxidants (SPAs) are widely used as an antioxidant and preservative in food, cosmetics, plastics, rubber and pharmaceuticals; where 2-tert-butyl-4-methoxyphenol (BHA) is one of the most commonly used SPAs, but recent studies have associated its occurrence with adverse health effects in animals (Wang and Kannan, 2018).

Physical-chemical properties of the selected organic contaminants in the environment

A summary of the physical-chemical properties of each class of OMCs is provided in Table 1.7 (see Chapter 2, Table S2.1 for further information).

Table 1.7. Summary of the physical-chemical properties of the selected organic contaminants

Category	Molecular weight		Solubility (mg/L)		Log K_{OW}		Log D (pH=7.4)		Log K_{OA}		Log K_{AW}	
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Benzothiazoles	135	167	120	4300	2.2	2.9	2.09	2.21	6.8	8.2	-5.8	-4.8
Benzotriazoles	119	147	914	22580	0.1	1.7	0.07	1.69	1.5E-05	6.9	-5.2	-5.1
Chlorinated flame retardants	285	328	1200	7000	1.6	2.9	1.42	2.32	5.3	8.2	-5.6	-3.9
Parabens	152	180	207	2500	2.0	3.6	2.09	3.12	8.8	10	-6.8	-6.5
Pesticides	191	528	0.1	666	0.9	5.5	1.61	4.78	8.3	19	-13	-3.9
Anticonvulsants, antidepressants and its related metabolites	218	321	21	500	0.7	3.3	0.61	2.92	9.0	12	-11	-8.1
Plasticizers	200	228	120	543	3.1	3.6	2.90	3.63	12.6	12.7	-9.7	-9.4
Surfactant and derivatives	206	226	3.1	26	3.6	5.5	2.94	5.47	8.6	9.2	-5.0	-3.7
tert-butylphenols	180		213		3.5		3.14		9.0		-6.5	

The probable uptake pathways of these contaminants by plants can be discussed from the physical-chemical properties point of view. At first glance, these compounds possess low values of log Kow (<4), which could mean that they will not be mainly absorbed in the organic matter of the soil and therefore, they will be available for root uptake.

There is a wide disparity in the solubility of each category of compounds due to disparate chemical structures within the same category. The parameters log K_{OA} and log K_{AW} (dimensionless Henry's law constant) are indicator of whether these compounds will be incorporated from the atmosphere or from the soil. All the studied OMCs show negative log K_{AW} values (dimensionless Henry's law constant), which indicates that are non-volatile compounds. Moreover, most of the log K_{OA} values are below 11, therefore confirms that gaseous uptake probably will not be the main uptake pathway.

1.5.3. Occurrence of the selected organic contaminants in the environment

Table 1.8 shows the concentrations of the studied OMCs reported in the aquatic environment. As it is shown, their concentration decline from raw wastewater to surface water due to the WWTP treatment, but for some compounds such as carbamazepine or lamotrigine, these concentrations are similar, which indicates that they are not eliminated by WWTP processes.

Table 1.8. Occurrence of the selected OMCs in the aquatic environment

Compound	WWTP influent (ng·L^{-1})	WWTP effluent (ng·L^{-1})	Surface water (ng·L^{-1})	Reference
Anticonvulsants, antidepressants and its related metabolites				
Carbamazepine	nd-1680	0.03-2100	5-11581	25, 32, 34
10,11-epoxycarbamazepine	nd-69	nd-117	nd-282	1, 11, 29
Diazepam	nd-8.0	nd-72	nd-75.5	2, 8, 32
Lamotrigine	144-1270	353-1726	455	3, 13, 31
Lorazepam	nd-334	nd-227	1.1-5.7	32, 34
Oxazepam	nd-77	nd-94	1.2-5.5	14, 32, 34
Primidone	180	430-710	nd-180	19
Benzothiazoles				
Benzothiazole	500-1050	70[a]-2530	nd-200	20, 23
2-hydroxybenzothiazole	160[a]-660	60[a]-2200	nd-300	20, 23
2-mercaptobenzothiazole	20[a]-260	nd-50[a]	nd	20, 23
Benzotriazoles				
Benzotriazole	500-210000	nd-100000	7000	20
5-methyl-1H-benzotriazole	nd-8000	20-17000	nd-2160000	20
Chlorinated flame retardants				
TCEP	0.06-0.50	0.06-2.40	nd-79.5	21, 25
TCPP	0.18-4	0.10-21	3.30-214	25
Parabens				
Methylparaben	nd-79500	nd-3830	nd-27500	9, 18
Ethylparaben	nd-10500	nd-290	nd-30500	9, 18
Propylparaben	nd-40000	nd-230	nd-52100	9, 18
Butylparaben	nd-5600	nd-1000	nd-19900	9, 18
Pesticides				
Atrazine	0.02-28	0.004-0.73	nd-1400	6, 10, 25
Azoxystrobin	-	-	nd-59.8	6, 26
Carbendazim	14-78	60-80	nd-45	6, 15, 28
Chlorpyrifos			1.01-65.0	6, 26
DEET	2560-3190	6.4-15800	2.0-69	15, 22, 25
Diazinon	4.0	0.7-4160	0.12-20.39	24, 25
Dimethomorph	-	-	nd	5, 26
Indoxacarb	-	-	-	
Pymetrozin	-	-	-	
Pyraclostrobin	-	-	-	
Simazine	7.27	169	nd-47.95	6, 24
Plasticizers				
Bisphenol A	nd-11800	nd-4090	nd-1950	9, 12
Bisphenol F	nd-16.4	nd	nd-2850	7, 33
Surfactants and derivatives				
Surfynol 104	572-1877	335-950	514	16, 17
4-*tert*-octylphenol	Nd-8.7	0.004-1.3	1-641	25
***Tert*-butylphenols**				

BHA	nd-48.7	nd-75	134-256	4, 27, 30

1. Bahlmann et al.(2014); 2. Baker and Kasprzyk-Hordern (2013); 3. Bollmann et al. (2016); 4. Calderón-Preciado et al. (2011); 5. Casado et al. (2018); 6. Ccanccapa et al. (2016); 7. Česen et al. (2018); 8. Clara et al. (2005); 9. Czarczyńska-Goślińska et al. (2017); 10. De Gerónimo et al. (2014); 11. de Jongh et al. (2012); 12. Deblonde et al. (2011); 13. Ferrer and Thurman (2012); 14. Golovko et al. (2014); 15. Gonzalez-Rey et al. (2015); 16. Guedez and Püttmann (2011); 17. Guedez et al. (2010); 18. Haman et al. (2015); 19. Hass et al. (2012); 20. Herrero et al. (2014); 21. Kim and Kannan (2018); 22.Kim et al. (2017); 23. Kloepfer et al. (2005); 24.Köck-Schulmeyer et al. (2013); 25. Luo et al. (2014); 26. Reilly et al. (2012); 27. Rodil et al. (2010); 28. Singer et al. (2010); 29. Valcárcel et al. (2011); 30. Wang and Kannan (2018); 31. Writer et al. (2013); 32. Wu et al. (2015); 33. Yamazaki et al. (2015); 34. Yuan et al. (2013)

[a] *Values between LOD and LOQ; - not reported, nd non-detected*

1.5.4. Occurrence of the studied contaminants in edible parts of vegetables in field-scale studies

Although there is no much information available on the occurrence of OMCs in edible vegetables at real field scale, the Table 1.9 provides a compilation of existing studies for TEs and OMCs. This table shows that pesticides, parabens, chlorinated flame retardants and benzotriazole compounds are the most abundant OMCs, whereas Zn and Mn are the most abundant TEs in vegetables.

Table 1.9. Occurrence of the selected contaminants in vegetable tissues

Compound	Crop	Concentration	Reference
Anticonvulsants, antidepressants and its related metabolites (ng·g^{-1} fw)			
Carbamazepine	Lettuce	0.001-0.002[b]	19
	Tomato	nd	19
Primidone	Lettuce	nd	19
Benzotriazole (ng·g^{-1} fw)			
Benzotriazole	Lettuce	0.46-3.99[b]	9
	Lettuce	0.27-6.89[b]	
Chlorinated flame retardants (ng·g^{-1} fw)			
TCEP	Lettuce	0.7-2.3	2
TCPP	Tomato	2.8-4.7	2
	Lettuce	1.7-2.5	2
Parabens (ng·g^{-1} dw)			
Methylparaben	Bean products	nd	13
	Fruits	150	13
	Vegetables	50	13
	Lettuce	nd-1500	3
Ethylparaben	Bean products	980	13
	Fruits	960	13
	Vegetables	70	13
Propylparaben	Bean products	nd	13
	Fruits	960	13
	Vegetables	20	13
Butylparaben	Bean products	60	13
	Fruits	nd	13
	Vegetables	nd	13
Pesticides (ng·g^{-1} fw)			
Azoxystrobin	Lettuce	nd-290	10, 12

Carbendazim	Lettuce	210	4
Chlorpyrifos	Cauliflower	5-440	1, 6, 8, 11, 18
	Lettuce	5.2-1524	4, 6, 12
	Tomato	3.8-295	1, 4,6, 8, 12, 18, 21
Diazinon	Tomato	0.3-29.5	21
Dimethomorph	Lettuce	120	10
Indoxacarb	Tomato	12-8	4
Pymetrozin	Lettuce	80	10
	Tomato	45-380	4, 10
Pyraclostrobin	Lettuce	80	10
	Tomato	10	10
	Plasticizers (ng·g^{-1} fw)		
Bisphenol A	Tomato	1.6-8.4	2
	Lettuce	3.3-8.4	2
4-*tert*-octylphenol	Tomato	nd	20
	Lettuce	nd	20
	TEs (mg·kg^{-1} fw) [b]		
As	Cauliflower	0.01	15
As	Lettuce	0.01-0.03	5, 22
As	Tomato	0.002-0.003	22
Ba	Vegetables	0.49[a]	7
Cd	Cauliflower	nd-0.09	15
Cd	Lettuce	nd-0.03	5, 15, 22
Cd	Tomato	0.004-0.039	14, 22
Cr	Vegetables	0.16[a]	7
Cr	Cauliflower	0.04	15
Cr	Lettuce	0.002-0.056	5, 15
Cu	Vegetables	0.94[a]	7
Cu	Cauliflower	nd-1.47	16,17
Cu	Lettuce	0.03-0.04	22
Cu	Tomato	0.01-0.10	14, 22
Hg	Cauliflower	0.001	15
Hg	Lettuce	0.002	15
Mn	Vegetables	2.24[a]	7
Mo	Vegetables	0.16[a]	7
Ni	Vegetables	0.36[a]	7
Ni	Cauliflower	0.063	15
Ni	Lettuce	0.05	15
Pb	Cauliflower	nd-0.02	15, 16
Pb	Lettuce	nd-0.08	5,15, 22
Pb	Tomato	0.01-1.06	14, 22
Sb	Vegetables	0.01[a]	7
Zn	Vegetables	5.69[a]	7
Zn	Cauliflower	1.85-6.28	16,17
Zn	Tomato	0.24-0.73	14, 22

nd non-detected; [a] Median value is given; [b] data has been transformed to fw using as lettuce's moisture 95.5%, tomato's moisture 80.7% and 91.4% from cauliflower.

1. Alamgir et al. (2013); 2. Albero et al. (2017); 3. Aparicio et al. (2018); 4. Bakirci et al. (2014); 5. Chang et al. (2014); 6. Chen et al. (2005); 7. Generalitat de Catalunya (2015); 8. Latif et al. (2011); 9. LeFevre et al. (2017); 10. Lemos et al.(2016); 11. Lozowicka (2015); 12. Mac Loughlin et al. (2018); 13. Maher et al. (2018); 14. Mohod (2015); 15. Pan et al. (2016); 16.Singh and Singh (2014); 17.Singh and Kumar (2006); 18. Sinha et al. (2012); 19. Wu et al. (2014); 20. Yang and Ding (2005); 21. Yu et al. (2016); 22. Zhou et al. (2016)

1.6 Hypothesis and objectives

Taking into consideration the evidence on the absorption of chemical contaminants by plants reported in the literature, the following hypothesis were proposed:

1. Peri-urban agriculture is exposed to a greater concentration of chemical contaminants through water irrigation, air or soil may end up with vegetables, which have high concentration of chemical contaminants.
2. Vegetables from peri-urban agriculture exposed to a higher concentration of contaminants may have negative yield and human health implications compared to rural farming.

To address these hypotheses, the following objectives were established.

The overall aim of this Thesis is the evaluation of the occurrence of several chemical contaminants in peri-urban agriculture (irrigation water, soil and vegetables) as well as their effect on crop productivity and human health, compared with rural agriculture.

Therefore, in order to accomplish the general objective, this Thesis comprises the following specific objectives:

1. Evaluate the presence of the selected chemical contaminants (OMCs and TEs) in the irrigation waters used in the four peri-urban and one rural farm plots located in the Baix Llobregat Agrarian Park (Barcelona, Spain),
2. Assess the effect of the above-mentioned irrigation waters on seed germination and crop productivity.
3. Evaluate the effect of seasonality on the presence of the contaminants in soil and lettuce leaves and estimate their bioconcentration factors.
4. Assess the effects of the studied chemical contaminants in lettuce leave components (chlorophyll, nitrate, lipid and carbohydrate content).
5. Evaluate the concentration of contaminants (OMCs and TEs) in different food crops (i.e. lettuce, tomato, cauliflower and broad beans) grown under peri-urban and rural agriculture.
6. Evaluate the potential human health risk of the consumption of vegetables grown under peri-urban agriculture in comparison to those grown under rural agriculture by applying HQ and TTC approaches.

Chapter II: Occurrence of chemical contaminants in peri-urban agricultural irrigation waters and assessment of their phytotoxicity and crop productivity

This chapter is based on the article:

Margenat, A., Matamoros, V., Díez, S., Cañameras, N., Comas, J., & Bayona, J. M. (2017). Occurrence of chemical contaminants in peri-urban agricultural irrigation waters and assessment of their phytotoxicity and crop productivity. Science of the Total Environment, 599–600, 1140–1148.

Water scarcity and water pollution have increased the pressure on water resources worldwide. This pressure is particularly important in highly populated areas where water demand exceeds the available natural resources. In this regard, water reuse has emerged as an excellent water source alternative for peri-urban agriculture. Nevertheless, it must cope with the occurrence of chemical contaminants, ranging from TE to OMCs. In this study, chemical contaminants (i.e., 15 TEs, 34 OMCs), bulk parameters, and nutrients from irrigation waters and crop productivity (*Lycopersicon esculentum* Mill. cv. Bodar and *Lactuca sativa* L. cv. Batavia) were seasonally surveyed in 4 farm plots in the peri-urban area of the city of Barcelona. A pristine site, where rain-groundwater is used for irrigation, was selected for background concentrations. The average concentration levels of TEs and OMCs in the irrigation water impacted by TWW were 3 ($35 \pm 75\ \mu g \cdot L^{-1}$) and 13 ($553 \pm 1050\ ng \cdot L^{-1}$) times higher than at the pristine site respectively. Principal component analysis was used to classify the irrigation waters by chemical composition. To assess the impact of the occurrence of these contaminants on agriculture, a seed germination assay (*Lactuca sativa* L.) and real field-scale study of crop productivity (i.e., lettuce and tomato) were used. Although irrigation waters from the peri-urban area exhibited a higher frequency of detection and concentration of the assessed chemical contaminants than those of the pristine site (P1), no significant differences were found in seed phytotoxicity or crop productivity. In fact, the crops impacted by TWW showed higher productivity than the other farm plots studied, which was associated with the higher nutrient availability for plants.

2.1 Introduction

Water scarcity is increasing with global changes and already affects almost every continent and >40% of the world's population (UN-WATER 2016). Agriculture accounts for 70% of global water withdrawals, a figure that rises to 80% in arid and semiarid regions. In this context, the direct or indirect reuse of TWW for crop irrigation can be considered a reliable and strategic water supply, quite independent from seasonal drought and weather variability and able to cope with peaks in water demand. This can be very beneficial to farming activities that rely on a continuous water supply during the irrigation period, reducing the risk of crop failure and income losses (EC 2016). Appropriate nutrient appraisal in TWW could also reduce fertilization needs, resulting in environmental benefits and a reduction in production costs (Haruvy, 1997). This is particularly important in peri-urban agriculture, which is characterized by a high water demand and proximity to TWW (Kurian et al., 2013).

However, TWW may contain pollutants and pathogens, which can constitute a threat to human health when the TWW is used for agricultural irrigation (Becerra-Castro et al., 2015; Prosser and Sibley, 2015). Those contaminants include so-called CECs, chemicals of a synthetic origin or deriving from a natural source that have recently been found to have possible harmful effects on environmental and public health, although the extent of the risk has yet to be determined (Naidu et al., 2016). Moreover, the use of TWW also raises the levels of metals, such as Cu, Zn, Fe, Pb, and Ni, in the receiving soils and, as a consequence, in the medium term can affect agricultural productivity and human health if they are uptaken by crops (Rattan et al., 2005). Therefore, although the occurrence of some metals in crops is already regulated in different countries due to their human health implications (Khan et al., 2015, 2013) CECs remain unregulated.

Recent reports have shown that the occurrence of OMCs in irrigation waters is highly dependent on the source of water used. For instance, Calderón-Preciado et al. (2013) observed that irrigation water from a secondary TWW contains a higher concentration of OMCs such as pharmaceuticals and personal care products (772 ng L^{-1} on average) than groundwater (31 ng L^{-1} on average). Recent laboratory and greenhouse studies have shown that OMCs can produce phytotoxic, morphological and physiological changes in crop plants (Carter et al., 2015; Carvalho et al., 2014; Christou et al., 2016; Marsoni et al., 2014; Shahid et al., 2015), but until now there is no evidence of their effect on crop productivity at real field scale. Rattan et al. (2005) observed that irrigation with TWW for 20 years resulted in a significant build-up of extractable TEs such as Zn (208%), Cu (170%), Fe (170%), Ni (63%), and Pb (29%) compared to adjacent soil irrigated with tube-well water. According to the FAO, the threshold levels of TEs for crop production depend on the crop and the element (FAO 1985). For instance, As toxicity to plants ranges from 12 mg L^{-1} for Sudan grass to <0.05 mg L^{-1} for rice. Co is phytotoxic to tomato plants at 0.1 mg L^{-1} in nutrient solution, and Cu is phytotoxic at 0.1 to 1.0 mg L^{-1}. Zn is phytotoxic to many plants at widely varying concentrations, while Ni is phytotoxic to a number of plants at 0.5 to 1.0 mg L^{-1}; in both cases, toxicity is reduced at neutral or alkaline soil pH. Finally, while 0.2 mg L^{-1} of B in water is essential for some crops, at concentrations of 1 to 2 mg L^{-1} it becomes phytotoxic. Recent findings suggest that the presence of macronutrients (e.g. N, P, K, Ca, Mg) and micronutrients (e.g. Cu, Fe, Zn, Mn, etc.) in the TWW leads to an increase in crop productivity (Li et al., 2015; Urbano et al., 2017). Notwithstanding these findings, there is no information about the co-occurrence of OMCs and TEs in irrigation waters impacted by TWW effluents and their potential effect on crop productivity in real field conditions.

This study aimed to assess the occurrence of chemical pollutants (15 TEs, 34 OMCs, and nutrients) in irrigation waters, as well as their effect on crop phytotoxicity (i.e., seed germination) and productivity (i.e., lettuce and tomato) in 4 farm plots located in the peri-urban area of the city of Barcelona (NE Spain). The results were also compared with background concentrations from a farm plot located far away from the peri-urban area.

2.2 Material and methods

2.2.1 Description of the study area

The area of study was located in the delta and low valley of the Llobregat River (NE Spain). This traditionally rich farmland, also known as the Baix Llobregat Agrarian Park (BLAP), is a protected farmland precinct spanning 3300 ha in the metropolitan area of Barcelona (Paül and McKenzie, 2013). In this region, farmland, the river, and natural or semi-natural sites exist side by side with urban sprawl, with the concomitant population pressure and environmental impact. The peri-urban area of the BLAP is characterized by a gradient of atmospheric and irrigation water pollution originating from industrial, urban, and agricultural activities. In this study, 5 plots were selected based on their irrigation water source. The plots included 4 farm plots located inside the peri-urban area (P2-P5, <50 m asl) and 1 pristine site (P1) located 400 m asl on a Karstic massif in the west of the BLAP (Fig. 2.1).

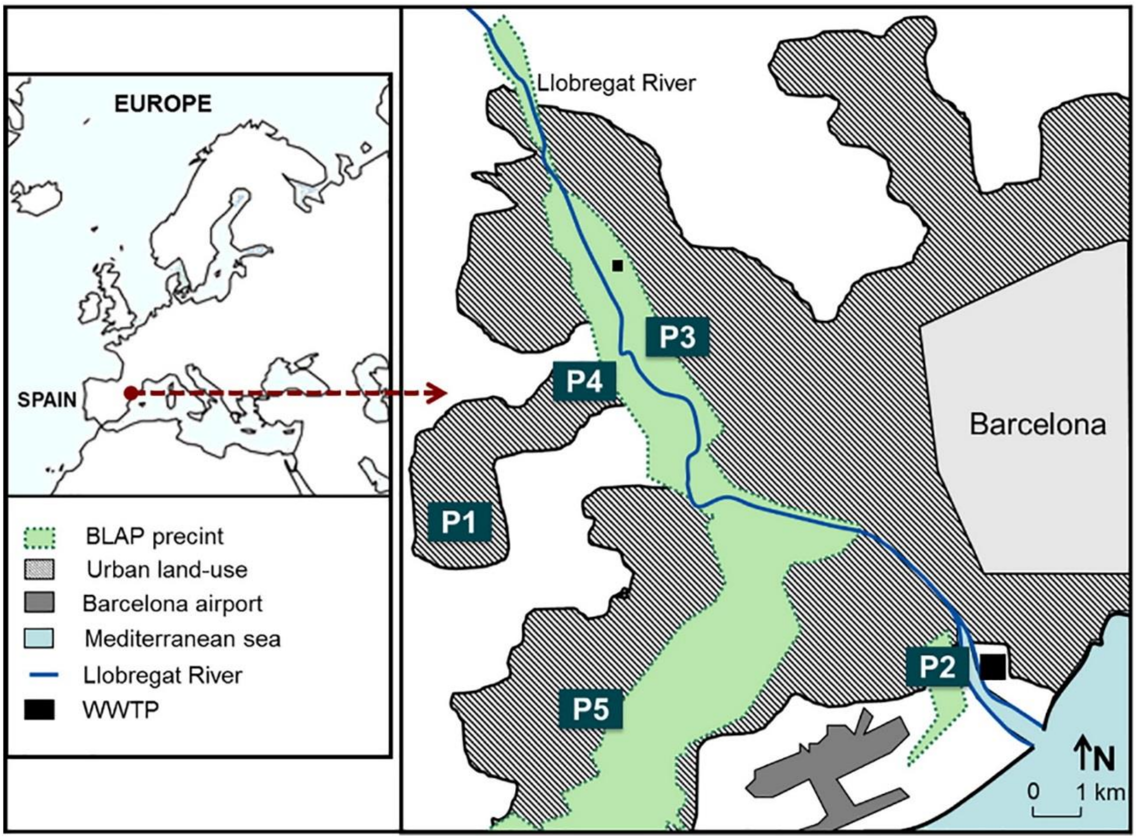


Figure 2.1 Map of the sampling area. P1. Begues; P2. Prat de Llobregat P3. Sant Joan Despí; P4. Sant Boi de Llobregat; P5. Viladecans.

Each farm plot had a surface area of over 0.1 ha planted with different seasonal vegetables (i.e., lettuce, tomato, onion, and cauliflower). The soil in the peri-urban sites is formed by rich sediments deposited over the years by the river from which this area takes its name. All places have coastal Mediterranean climate. The soil analysis performed prior to lettuce cultivation indicated for the 5 sites an adequate level of fertility to grow vegetables (N-NO_3 > 2 mg L^{-1}; P > 15 mg L^{-1}; K^+ > 180 mg L^{-1}) and similar electrical conductivity (2.2 - 2.8 dS m^{-1}) and pH (7.6 -7.8). The 5 plots presented very permeable soils, P1, P3 and P4 soil's texture was sandy loam and P2 and P5 sandy.

Most of the BLAP area (1240 ha) is watered with irrigation water from the Llobregat River. The river's average flow rate is 137 hm^3 $year^{-1}$, and it drains an area of 4948 km^2. The Llobregat River and its two main tributaries, the Cardener River and Anoia Stream, receive discharges from 80 urban and industrial WWTPs. Furthermore, the central area of the basin receives brine leachates from natural salt formations and mining operations, which have caused an increase in water salinity downstream. The river water in the BLAP area flows through interconnected open-air concrete distribution channels (P2 and P4), but additional water sources such as well water are also used (P5). The irrigation water for site P3 originates in the Infanta Channel, which is mostly made up of TWW from 10 WWTP effluents (Rubí Creek). Therefore, whereas irrigation waters from P2 and 4 are a clear example of unplanned indirect water reuse, P3 is of planned indirect water reuse. Most of the WWTPs impacting irrigation waters (P2-P4) consisted of conventional activated sludge treatments without any additional polishing system. The sampling site located in Viladecans (P5) uses well water impacted by industrial and road runoff.

Additionally, a reference site (P1) was selected for the purposes of comparison in a pristine area in the Littoral Mountains where ground-rainwater is used for irrigation.

Drip irrigation system was used in the reference plot (P1), while furrow irrigation was applied in the other farm plots (P2 - P5). The volume of irrigation water supplied to the cultures was similar among sites P2 - P5 (lettuce: 150 - 170 mm irrigation + 120 mm precipitation; tomato: 570 mm irrigation + 80 mm precipitation) and P1 (lettuce: 80 mm irrigation + 160 mm precipitation; tomato: 520 mm irrigation + 70 mm precipitation).

2.2.2 Sampling plan

Irrigation water

The sampling was carried out between February and September of 2016 during the growing period of the different crops of interest (lettuce and tomato). Fig.2.1 shows the location of both the sampling points in the irrigation network from the peri-urban area of the BLAP (P2 - P5) and the reference site (P1). In each farm plot, between four and ten irrigation water samples were analysed (Table 2.1). All water samples were collected directly from the irrigation canals, except in the P5 were water samples were collected in the irrigation pipeline, after the ferti-irrigation system. Water samples for TE determination were collected in acid-washed (2% HNO_3) 125 mL fluorinated ethylene propylene (FEP) bottles (Thermo Scientific Nalgene, Rochester, NY, USA). After each field campaign, < 2h after their collection and once in the laboratory, the water samples were filtered (< 0.45 µm) with nylon membranes using a syringe filtration unit acidified with nitric acid (pH < 2). The samples were then stored in the fridge in a pre-cleaned bottle until analysis by ICP-MS and ICP-OES. Water samples for the determination of OMCs and conventional water quality parameters were collected in pre-cleaned 2.5 L amber glass bottles. All samples were kept refrigerated during transport to the laboratory, where they were stored at 4 °C until they were analyzed.

Crops

Lettuce (*Lactuca sativa* L. cv. Batavia) and tomatoes (*Lycopersicon esculentum* Mill. cv. Bodar) were harvested when they reached commercial size. The lettuce seedlings were planted in March 2016, and the plants were harvested in May 2016 (P1 - P5), whereas the tomato seedlings were planted in May - June and harvested in September 2016 (P1, P3, and P5). In each farm plot, 50 lettuces and 50 tomatoes fruits were randomly harvested, weighted over an area of 0.1 ha (P1 - P5). The same integrated management plan (fertilization and pesticide application) was used in all farm plots from the BLAP.

2.2.3 Analytical procedures

Water quality parameters

Conventional water quality parameters, including ammonium nitrogen (NH_4^+-N), NO_3-N, total phosphorous (TP), and total suspended solids (TSS), were determined in all the water samples. The nutrients were measured with Hach Lange NH_4^+-N, NO_3-N, and TP cell tests (LCK 303, 304, 339, and

349) on a spectrophotometer (Hach Lange DR 1900 Portable Spectrophotometer). Measurements of water pH, conductivity, temperature, and dissolved oxygen (DO) were taken using Hach Lange sensors.

Trace elements

The TEs were selected due to their inclusion in the Spanish Royal Decree 1620/2007 for water reuse, and since their accumulation by food crops, particularly vegetables, is of increasing concern because of the potential human health risks to the consumers (Khan et al., 2008). An inductively coupled plasma optical emission spectrometer (Thermo Scientific, iCAP 6500 ICP-OES) and an inductively coupled plasma mass spectrometer (Thermo Scientific, XSeries 2 ICP-MS) were used for the determination of TEs in the water samples. The most frequently found elements (i.e., 13 out of the 58 elements analyzed) were divided into three categories: major TEs (Ba, B, Mn, and Li), most common TEs (Mo, Pb, Zn, Cu, Ni, Co, and Cr), and other non-common TEs (Rb and Sb). Reagent water was used as a blank matrix, and laboratory reagent blank (LRB) was treated exactly the same as a sample, including exposure to all glassware, equipment, solvents, and reagents used with the other samples. A limit of detection (LOD) of 0.2 μg L^{-1} was determined from three times the standard deviation obtained from the analysis of ten runs of blank samples on the same day as the determinations. Similarly, the limit of quantification (LOQ) was calculated by multiplying the standard deviation by ten (0.67 μg L^{-1}).

Organic microcontaminants (OMCs)

In this survey study, the prioritization of OMCs was based on the compounds' potential plant uptake, log Kow < 4.0 (Table S2.1), occurrence in irrigation waters, persistence, and potential harmful effects for human health (Banjac et al., 2015; Ginebreda et al., 2010; Prosser and Sibley, 2015). The determination of OMCs was performed as described by Matamoros and Bayona (2006). Briefly, 250 mL of filtered water samples was spiked with 100 ng of a surrogate standard mixture (see section 2.5.2).

The samples were then percolated through a conditioned 200 mg STRATA X solid-phase extraction cartridge (Phenomenex, Torrance, USA). Elution was performed with 15 mL of ethyl acetate. After that, the eluted extract was evaporated under a gentle nitrogen stream until ca. 250 μL and 100 ng of triphenylamine was added. Derivatized and non-derivatized aliquots of the sample extracts were analyzed with an EI-GC–MS/MS Bruker 450-GC gas chromatograph coupled to a Bruker 320-MS triple-stage quadrupole mass spectrometer (Bruker Daltonics Inc., Billerica, MA, USA).

The derivatization of samples was carried out by methylation of the acidic hydroxyl groups in a programmed temperature vaporizing (PTV) injector of the gas chromatograph by adding 10 μL TMSH to a 50 μL sample aliquot before injection. A volume of 5 μL was injected into a Bruker 450-GC gas chromatograph coupled to a Bruker 320-MS triple quadrupole mass spectrometer (Bruker Daltonics, Billerica, MA, USA) fitted with a 20 m × 0.18 mm ID, 0.18 μm film thickness Sapiens X5-MS capillary column coated with 5% diphenyl 95% dimethyl polysiloxane from Teknokroma (Sant Cugat del Vallès, Spain). The PTV injector was set at 60 °C for 0.5 min and then rapidly heated up to 300 °C at 200 °C min^{-1} and held for 10 min. It was then cooled to the initial 60 °C at 200 °C min^{-1}. The gas flow rate was set at 0.6 mL min^{-1}. The ion source temperature and transfer line were both held at 250 °C. A solvent delay of 7 min was applied. Argon gas was used for CID at a pressure of 1.8 mTorr, and the optimum collision energy (CE) was selected for each transition.

Qualitative and quantitative analyses were performed based on retention time and the selection reaction monitoring (SRM) mode of two product ions, and the ratio between the product ions was used for confirmation. The LOD and LOQ were defined as the mean background noise in a blank triplicate plus three and ten times, respectively, the standard deviation of the background noise from three blanks. The LOD ranged from 0.1 to 50 ng L^{-1}, and the LOQ from 0.3 to 80 ng L^{-1}, except for benzotriazole and derivatives, which exhibited an order of magnitude higher. The monitoring ions, CEs, LODs, LOQs, and recoveries can be found in the supporting information (Tables S2.2–2.5).

2.2.4 Seed germination bioassay and crop productivity

The seed germination assay was performed as previously described by (Marsoni et al., 2014). Briefly, lettuce seeds (Vilmorin Jardin, St Quentin Fallavier Cedex, France) were sterilized with 2.5% sodium hypochlorite for 15 min and thoroughly washed with distilled water. Hydrated seeds were transferred to Petri dishes (100 mm diameter) containing a GF/F filter in the presence of 5 mL of distilled water (control) or irrigation waters. For each irrigation water, 10 dishes containing 10 seeds each (n = 100 per irrigation water type) were prepared and incubated in the dark at 25 °C. After 72 h, the germinated seeds were counted. Seeds were considered germinated when root elongation was >3 mm; a minimum of 80% germinated seeds in the control dishes was required.

Lettuce and tomato yields were determined at commercial size in 0.1 ha plots in commercial fields. The time needed for them to reach commercial size was also recorded. Crop productivity for lettuce was calculated by multiplying the measured fresh weight per a survival factor of 0.8 by the number of crops per square meter (6.5 plants m^{-2}). 50 tomatoes fruits (10 fruits × 5 sections × plot) were sampled to determine their average weight.

2.2.5 Data analysis

The experimental results were statistically evaluated using the SPSS v. 22 package (Chicago, IL, US). All data sets were checked for normal distribution using the Kolmogorov–Smirnov test to ensure that parametric statistics were applicable. The comparison of means of the occurrence of chemical pollutants between farm plots was performed with a two-paired (Wilcoxon) signed-rank test (the concentration of each compound was compared between farm plots). A Mann-Whitney U test was used for the comparison of conventional quality parameters, and a one-way ANOVA was used for the productivity studies (n =50-100). Principal component analysis (PCA) was conducted on the concentration levels of TEs, OMCs, and nutrients. Once the data matrix had been completed, it was autoscaled to have zero mean and unit variance (correlation matrix). Statistical significance was defined as $p \leq 0.05$.

2.3 Results and discussion

2.3.1 Conventional water quality parameters

Table 2.1 shows the conventional water quality parameter values for the 5 irrigation plots studied. Irrigation waters from the peri-urban area of Barcelona had higher electrical conductivity than irrigation waters from the pristine zone (site P1). This can be attributed mainly to two factors: 1) the high impact of TWW on irrigation sites P2, P3, and P4) the impact of salt mining on the Llobregat River Basin (Momblanch et al., 2015). Additionally, the high electrical conductivity observed in the groundwater from the Llobregat Delta (site P5) may be due to seawater intrusion and agricultural activities (Miracle, 1989; Otero and Soler, 2002). Similarly, the concentration of TSS was higher at sites P2, P3, and P4 than at sites P1 and P5 ($p < 0.05$) and ammonium was lower at sites P1, P2, and P4 than at sites P3 and P5 ($p < 0.05$). This is in keeping with the fact that the irrigation waters of the former (i.e., P2, P3, and P4) originated from surface water bodies (i.e., Llobregat River and TWW effluents), whereas the irrigation water source of sites P1 and P5 was groundwater. The high concentration of TP, ammonium, and nitrates at site P5 was accounted for by the fact that the water samples were collected in the irrigation pipeline, after the ferti-irrigation system. When the P5 site was excluded, P3 showed the highest levels of conductivity and nutrients ($p < 0.05$). This is consistent with the fact that the water from the Infanta Channel is fed by Rubí Creek, a stream that is mainly composed of worse-quality TWW than that of the Llobregat River (González et al., 2012). Nevertheless, the irrigation water quality complied with the Spanish guidelines for water reuse in accordance with the general quality parameters assessed in this study (Royal Decree 1620/2007). In addition to these parameters, Spanish Royal decree also includes intestinal parasites, *Salmonella* sp., *Escherichia coli* (not included in this study), and the TEs listed in Table 2.2.

Table 2.1 Minimum, maximum and average levels of general quality parameters in the studied irrigation waters. Levels below the LOD were replaced by ½ LOD.

	Plot 1 (n = 5)	Plot 2 (n = 4)	Plot 3 (n = 10)	Plot 4 (n = 8)	Plot 5 (n = 4)[a]
Conductivity (µS cm^{-1})	(968–1211) 1049	(1519–1645) 1584	(1490–2148) 1944	(1255–1707) 1482	(1272–2370) 1663
NH_4^+–N (mg L^{-1})	(0.002–0.167) 0.05	(0.1–0.7) 0.3	(3–47) 14	(0.1–0.6) 0.2	(0.1–12.8) 4.2
Nitrates (mg L^{-1})	(2.8–4.6) 3.9	(1.8–2.7) 2.1	(3.4–7.4) 5.4	(1.5–2.5) 2.0	(4–175) 55
TP (mg L^{-1})	(0.03–3.0) 0.6	(0.2–2.4) 0.8	(0.6–2.5) 1.5	(0.2–0.7) 0.3	(0.6–6.2) 2.5
TSS (mg L^{-1})	(10–55) 21	(13–84) 33	(14–94) 46	(13–90) 63	(2–40) 18
pH	(7.5–8.6) 8.1	(8.1–8.6) 8.4	(7.7–8.1) 7.9	(6.7–8.6) 8.1	(6.8–7.8) 7.4

TP: total phosphorous; TSS: total suspended solids.

[a] Water samples collected from the irrigation pipeline (may contain chemical fertilizers due to ferti-irrigation).

2.3.2 Occurrence of trace elements (TEs)

Table 2.2 shows that 13 out of the 58 TEs studied were detected above the LOQ in all irrigation waters. The TEs detected at the highest concentrations in all irrigation waters were B and Ba. This high abundance is consistent with their predominant geogenic origin (Kabata-Pendias and Mukherjee, 2007), although B can also be anthropogenically derived and can be distinguished by stable isotopic fractionation (Bassett et al., 1995). The total average concentration of TEs per site was as follows: 164 µg L^{-1} (P1), 259 µg L^{-1} (P2), 269 µg L^{-1} (P4), 330 µg L^{-1} (P5), and 550 µg L^{-1} (P3). A two-paired test showed that irrigation water from the pristine site (P1) was less polluted by TEs than the irrigation waters from the peri-urban area of Barcelona ($p < 0.05$) and that site P3 was the most polluted. Sites P3 and P5 showed the highest concentration of Zn (from 34 to 68 µg L^{-1} on average) and Mn (23 to 63 µg L^{-1} on average). Zn is phytotoxic, but at much higher concentrations than those found in the irrigation waters in this study. Bouain et al. (2014) observed that a concentration higher than 23 mg L^{-1} of Zn was required to reduce shoot and root dry weight in hydroponic lettuce cultures. Mn is an essential micronutrient that participates in the structure of photosynthetic proteins and enzymes (Millaleo et al., 2010), but it is also used in pesticide formulations as an active ingredient (e.g., mancozeb). This is in keeping with the presence of high levels of industrial, urban, and agricultural activity in the peri-urban area of Barcelona.

The detected TE concentration levels were consistent with those previously detected in the Llobregat River, TWWs, and groundwater (Cabeza et al., 2012). It should be noted that Cabeza et al. (2012) only monitored the occurrence of 4 TEs (Cd, Ni, Hg, and Pb), and only Ni and Pb were found at the concentration range of µg L^{-1} in all types of waters with a frequency of detection (FOD) higher than 25%. The results of this study were slightly lower than those reported for Pb, but with a similar order of magnitude to those reported for Ni, especially in groundwater. The content of the TEs analyzed in all the irrigation water samples complied with both standards, i.e., Spanish Royal Decree 1620/2007 (SRD, 2007) for the agriculture use of TWW and the UN Food and Agriculture Organization guidelines (FAO, 1985), as shown in Table 2.2.

Table 2.2 Frequency of detection (FOD), minimum, maximum and average concentration of metals and metalloids in the water irrigation from the different studied plots. The threshold levels of trace elements for crop production of the Spanish Royal (SRD, 2007) and the Food and Agriculture Organization (FAO, 1985) are shown. Levels below the LOD were replaced by ½ LOD.

	Plot 1		Plot 2		Plot 3		Plot 4		Plot 5		SRD (mg L^{-1})	FAO (mg L^{-1})
	FOD (%)	Concentration (µg L^{-1})	FOD (%)	Concentration (µg L^{-1})	FOD (%)	Concentration (µg L^{-1})	FOD (%)	Concentration (µg L^{-1})	FOD (%)	Concentration (µg L^{-1})		
As	100	(2.9–3.3) 3.1	100	(1.6–2.6) 2.3	100	(4.6–7.0) 5.6	100	(2.5–3.1) 2.9	100	(2.9–17) 8.5	0.1	0.1
B	100	(98–131) 112	100	(47–84) 62	100	(276–334) 298	100	(85–182) 123	100	(113 − 123) 119	0.5	n/a
Ba	100	(57–66) 60	100	(37–79) 49	100	(30–42) 36	100	(51–64) 58	100	(37–59) 49	n/a	n/a
Cd	nd	< 0.2	nd	< 0.2	nd	< 0.2	nd	< 0.2	nd	< 0.2	0.01	0.01
Co	100	(0.6–1.1) 0.8	100	(1.3–2.5) 1.7	100	(2.2–4.8) 3.1	100	(0.7–1.2) 0.9	100	(0.5–1.2) 0.8	0.05	0.05
Cr	100	(1.4–3.4) 2.2	100	(0.2–0.7) 0.4	100	(2.3–7) 4.8	100	(0.6–1.9) 1.4	100	(0.3–0.6) 0.5	0.1	0.1
Cu	100	(1.2–2.8) 2	100	(2.2–7.3) 3.4	100	(2.8–4.7) 3.7	100	(0.9–2) 1.5	100	(3.7–17.6) 8.4	0.2	0.2
Li	100	(18.8–21) 20	100	(22–29) 26	100	(22.6–46.9) 35.6	100	(12.8–19.8) 16.5	100	(11–15.1) 13.8	n/a	2.5
Mn	100	(7.5–23.8) 16	100	(0.3–2.5) 1.5	100	(27.8–96.1) 63.3	100	(5.7–43.4) 21.6	100	(8.4–37.5) 22.8	0.2	0.2
Mo	100	(2.3–3.5) 3	100	(2–3) 2.3	100	(6.1–16.2) 9.7	100	(2.1–6.5) 3.3	100	(3–7.2) 4.4	0.01	0.01
Ni	100	(11.6–17.5) 14.5	100	(2.4–3.9) 2.9	100	(11–17.5) 13.5	100	(10.4–20.4) 16.8	100	(2.7–47.4) 17.9	0.2	0.2
Pb	100	(0.2–0.2) 0.2	nd	< 0.1	86	(0.1–0.4) 0.3	nd	< 0.1	67	(0.1–0.6) 0.4	n/a	5
Rb	100	(11.4–11.9) 11.7	100	(2.5–3.2) 2.7	100	(13.9–20) 16.5	100	(8.9–11.8) 10.4	100	(6.7–21.4) 16.5	n/a	n/a

	Plot 1		Plot 2		Plot 3		Plot 4		Plot 5		SRD (mg L^{-1})	FAO (mg L^{-1})
	FOD (%)	Concentration (μg L^{-1})	FOD (%)	Concentration (μg L^{-1})	FOD (%)	Concentration (μg L^{-1})	FOD (%)	Concentration (μg L^{-1})	FOD (%)	Concentration (μg L^{-1})		
Sb	100	(0.7–1.3) 1	100	(0.7–1) 0.8	100	(2.2–5.2) 3.2	100	(0.8–1.2) 1	nd	< 0.2	n/a	n/a
Zn	100	(11–14) 12	100	(3–14) 8	100	(29–38) 34	100	(9–17) 12	100	(26–110) 68	n/a	2

n/a: no data available; nd: not detected

2.3.3 Occurrence of organic microcontaminants (OMCs)

Table 2.3 shows the FOD and concentration of OMCs in the 5 irrigation plots. As can be seen, 3 of the 34 OMCs were below the LOD in all the irrigation waters (i.e., EPB, indoxacarb, and TCCP). The FOD ranged from 20% at the pristine site (P1) to 100% at all sites. The other sites exhibited intermediate values (59% at P2, 55% at P4, and 43% at P5). The concentration of OMCs ranged from <1 to 12,745 ng L^{-1} for 5-TTri. Surfynol 104 was the most abundant contaminant in all the irrigation waters of the BLAP area (>1000 ng L^{-1}), with values ranging from 55 to 4933 ng L^{-1} (average 2095 ng L^{-1}). This is in keeping with previously reported studies that found this compound to be one of the most abundant contaminants in surface waters (Guedez et al., 2010). Indeed, higher Surfynol 104 concentrations have been reported in rivers (up to 63.5 μg L^{-1}) and in TWW effluents (up to 310 μg L^{-1}) affected by industrial effluents (paper recycling industry and factories producing paint and printing ink) in Germany (Guedez and Püttmann, 2014). In fact, Surfynol 104 is a non-ionic surfactant used as an industrial defoaming agent that has various benefits in coatings, wood finishes, varnishes, cements, and metalworking fluids. A two-paired test showed that the concentration of OMCs in irrigation waters collected from groundwater (i.e., sites P1 and P5) was much lower than in those collected from surface waters (i.e., sites P2, P3, and P4) ($p < 0.05$). This is consistent with the previously reported low occurrence of OMCs in groundwater compared to surface waters (Sui et al., 2015). Site P3 presented the highest concentration of OMCs. In addition to Surfynol 104, 2-MBT, 5-TTri, BTri, and EPOCBZ were the most abundant (>1000 ng L^{-1}). A high concentration of 2-MBT was found in irrigation water from site P3 originating from TWW, which is consistent with previous studies that reported high levels of this compound in treated water (Matamoros et al., 2010). Benzotriazole compounds (5-TTri, BTri), which are used as anticorrosive agents in industry, have also been found at high concentration levels in TWW effluents and Llobregat River water samples (Asimakopoulos et al., 2013; Banjac et al., 2015; Matamoros et al., 2010). This result is consistent with the high concentration of these compounds in raw wastewater, as well as their moderate removal in WWTPs (Matamoros et al., 2010). Finally, results for EPOCBZ, which is the biotic transformation product of carbamazepine (Martínez-Hernández et al., 2016), show that the ratio between this compound and the parental counterpart (carbamazepine) was almost constant in all irrigation waters (3.1 ± 1.0). This result is consistent with the ubiquity and recalcitrance of carbamazepine in the aquatic environment and the fact that EPOCBZ is mainly formed by the detoxification phase I (oxidation), which occurs after human intake or crop uptake of carbamazepine (Malchi et al., 2014; Paltiel et al., 2016). These results suggest that both compounds are recalcitrant in the studied irrigation water bodies and should be targeted in crop uptake studies.

Table 2.3 Frequency of detection (FOD), minimum, maximum and average concentration of CECs in the studied irrigation waters. Levels below the LOD were replaced by ½ LOD for the calculation of the average concentration.

	Plot 1		Plot 2		Plot 3		Plot 4		Plot 5	
	FOD (%)	Concentration (ng L^{-1})	FOD (%)	Concentration (ng L^{-1})	FOD (%)	Concentration (ng L^{-1})	FOD (%)	Concentration (ng L^{-1})	FOD (%)	Concentration (ng L^{-1})
Atrazine	nd	< 2	50	(< 2–7) 4	100	(13–43) 22	13	(< 2–8) 3	nd	< 2
Azoxystrobin	nd	< 4	nd	< 4	50	(< 4–57) 11	nd	< 4	25	(< 4–9) 4
2-tert-Butyl-4-methoxyphenol (BHA)	nd	< 3	nd	< 3	100	(4–44) 20	nd	< 3	25	(< 3–18) 6
Chlorpyrifos	nd	< 10	nd	< 10	30	(< 10–75) 24	13	(< 10–16) 6	100	(75–149) 119
DEET	20	(< 17–33) 14	100	(33–48) 39	100	(86–813) 395	88	(< 17–62) 45	100	(48–52) 51
Diazepam	nd	< 1	100	(3 − 10) 5	100	(28–1019) 163	88	(< 1–5) 3	50	(< 1–2) 1
Diazinon	nd	< 1	100	(8–12) 11	100	(20–115) 49	100	(3–27) 15	100	(4–37) 13
Dymethomorph	40	(< 1–9) 3	75	(< 1–2) 1	10	(1–106) 21	75	(< 1–3) 2	75	(< 1–2) 1
Indoxacarb	nd	< 18	nd	< 18	nd	< 18	nd	< 18	nd	< 18
Simazine	nd	< 4	nd	< 4	20	(< 4–24) 5	nd	< 4	nd	< 4
Surfynol 104	100	(55–683) 343	100	(2234–3374) 2860	100	(607–4564) 2393	100	(711–4933) 2332	100	(58–157) 113
TCEP	40	(< 5–7) 5	100	(27–51) 38	100	(56–171) 100	100	(10–56) 26	100	(7–8) 8
2-Mercaptobenzothiazole (2-MBT)	40	(< 18–61) 23	100	(78–157) 114	100	(618–8844) 2391	100	(28–515) 144	50	(< 18–172) 54
5-Methyl-2H-benzotriazole (5-TTri)	20	(< 12 − 30) 12	100	(267–507) 356	100	(1070–12,745) 4190	100	(95–412) 263	75	(< 12–92) 67
Bisphenol A (BPA)	100	(9–210) 62	100	(66–74) 69	100	(70–274) 165	100	(26–109) 55	100	(6–42) 16
Bisphenol B (BPB)	nd	< 1	nd	< 1	10	(< 1–7) 1	nd	< 1	nd	< 1
Bisphenol F (BPF)	60	(< 14–291) 79	100	(26–302) 167	90	(7–390) 97	63	(7–361) 93	100	(52–189) 111
1,3-Benzothiazole (BT)	60	(< 32–173) 66	100	(50–115) 85	100	(48–154) 93	100	(22–166) 71	100	(37–111) 61
Benzotriazole (BTri)	nd	< 670	nd	< 670	100	(1064–5849) 2617	nd	< 670	nd	< 670
Carbamazepine	nd	< 10	100	(149–261) 186	100	(373–1280) 891	100	(47–191) 133	50	(< 10 − 13) 10
Carbendazim	20	(< 2–3) 2	100	(28–110) 57	100	(53–2411)599	100	(8–132) 47	100	(9–24) 20
Ethyl paraben (EPB)	nd	< 26	nd	< 26	nd	< 26	nd	< 26	nd	< 26
Lorazepam	nd	< 3	100	(28–48) 36	100	(42–420) 207	100	(8–42) 27	nd	< 3
Methyl paraben (MPB)	60	(< 56–191) 75	100	(57–90) 79	80	(< 56–142) 86	38	(< 56–119) 57	50	(28–120) 66
1-Hydroxybenzotriazole (OHBT)	60	(< 10–35) 14	100	(32–64) 48	100	(299–900) 530	100	(17–159) 56	100	(21 − 113) 50
Octylphenol (OP)	nd	< 10	nd	< 10	100	(17–38) 25	nd	< 10	nd	< 10
Oxazepam	nd	< 2	100	(29–50) 36	100	(37–307) 178	100	(11–49) 25	nd	< 2
Propyl paraben (PPB)	60	(< 2–25) 8	75	(< 2–5) 3	nd	< 2	75	(< 2–6) 3	50	(< 2–3) 2
Primidone	nd	< 4	100	(18–35) 26	100	(88–215) 145	100	(12–28) 21	25	(< 4–22) 7
Lamotrigine	nd	< 6	nd	< 6	20	(< 6–46) 12	nd	< 6	nd	< 6
Pymetrozin	nd	< 2	nd	< 26	40	(< 2–7) 3	20	(< 2–4) 2	nd	< 2
Pyraclostrobin	100	(2–2) 2	nd	< 1	40	(< 1–29) 6	nd	< 1	nd	< 1
Carbamazepine-10,11-epoxide (EPOCBZ)	nd	< 48	100	(447–767) 573	100	(1176–3304) 2447	100	(163–706) 490	nd	< 48
Tris(1-chloro-2-propyl) phosphate (TCPP)	nd	< 197	nd	< 197	nd	< 197	nd	< 197	nd	< 197

2.3.4 Irrigation water sample classification

PCA was performed on the whole data set to gain further insight into their sources and the distribution behaviour of the different assessed parameters in the irrigation waters (Table 2.4). The PCA reduced the 43 measured variables to 7 principal components with eigenvalues >1, which explains the 99% of the total variability observed. Components explaining small data variance (i.e., <5%) were not retained and were assumed to be mostly due to background and noise contributions. Therefore, only the first three principal components, which accounted for 64% of total variability, were studied.

The first principal component (PC1), which accounted for 47% of the variance, had high positive loading values (>0.8) for electrical conductivity, ammonium, B, Cr, Mn, Co, Mo, Sb, atrazine, TCEP, 5-TTri, BTri, carbamazepine, lorazepam, OHBT, OP, oxazepam, primidone, and EPOCBZ, indicating a common source for these compounds. The positive loadings of these compounds correlated with a high TWW impact; the highest contribution was for irrigation waters from site P3. This result indicated that PC1 was associated with TWW discharges.

PC2 accounted for 11% of the variance, with high positive loadings (<0.7) for TP, Cu, Zn, As, Pb, and chlorpyrifos. The positive loadings of this component explained the groundwater pollution by TEs and chlorpyrifos at site P5. Finally, the third principal component (PC3) accounted for 6% of the variance. It had a high positive loading (>0.5) for Surfynol 104, but negative loadings for nitrates and 2-MBT. This negative correlation between parameters indicates that whereas nitrates and 2-MBT were ubiquitously distributed in all the irrigation waters (except for site P3), Surfynol 104 was only detected at higher concentrations in the surface irrigation waters. The higher hydrophobicity of Surfynol 104 (log Kow=3.6) and its lower groundwater mobility compared to nitrates and 2-MBT (log Kow = 2.7) could explain this different behaviour. The positive loadings for Surfynol 104 were associated with surface irrigation waters.

Table 2.4 Variance explained and loadings for the two PCAs.

	All irrigation water samples			**Excluding irrigation water from site 3**		
	PC1	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
Percent of variance	47	11	6	29	20	9
Cumulative percentage	47	58	64	29	49	58
Conductivity	**0.820**	0.088	0.215	0.763	0.315	0.390
NH_4^+–N	**0.818**	0.058	− 0.186	0.091	0.695	0.125
Nitrates	0.630	0.154	**− 0.523**	− 0.684	0.030	–0.133
TP	0.309	**0.805**	0.219	− 0.067	**0.884**	–0.043
TSS	0.129	− 0.159	− 0.267	0.380	− 0.108	0.245
Li	0.759	− 0.214	0.031	− 0.372	− 0.560	–0.223
B	**0.886**	0.108	− 0.190	0.492	0.387	0.603
Cr	**0.817**	− 0.106	− 0.032	0.762	− 0.204	0.025
Mn	**0.862**	0.065	0.041	0.538	0.513	− 0.120
Co	**0.827**	− 0.111	0.029	− 0.494	− 0.400	0.225
Ni	0.123	0.196	0.328	0.457	0.340	–0,019
Cu	0.056	**0.819**	− 0.024	− 0.275	0.728	0.001

	All irrigation water samples			**Excluding irrigation water from site 3**		
	PC1	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
Zn	0.307	**0.779**	0.086	– 0.077	**0.860**	– 0.070
As	0.279	**0.777**	– 0.008	– 0.068	0.760	0.124
Rb	0.571	0.628	0.104	0.473	**0.839**	0.042
Mo	**0.849**	0.159	0.175	0.272	0.600	0.099
Sb	**0.820**	– 0.148	– 0.124	0.387	– 0.761	0.088
Ba	– 0.521	– 0.035	0.441	0.387	0.044	0.668
Pb	0.473	**0.753**	0.219	0.023	**0.901**	– 0.017
Atrazine	**0.958**	– 0.104	0.085	0.435	– 0.094	-0.045
BHA	0.712	– 0.047	0.233	– 0.092	0.497	– 0.172
Chlorpyriphos	– 0.030	**0.841**	– 0.125	– 0.277	**0.847**	0.041
DEET	0.733	0.075	0.011	0.511	0.534	– 0.243
Diazepam	0.409	0.012	– 0.318	0.748	– 0.153	– 0.108
Diazinon	0.791	0.019	0.116	0.442	0.410	– 0.172
Dimethomorph	0.255	– 0.066	– 0.499	– 0.184	– 0.239	– 0.198
Surfynol 104	0.435	– 0.373	**0.525**	**0.819**	– 0.212	– 0.138
TCEP	**0.864**	– 0.179	0.172	**0.904**	– 0.148	-0.073
2-MBT	0.517	– 0.020	**– 0.689**	0.349	– 0.152	0.682
5-TTri	**0.819**	– 0.122	0.306	**0.930**	– 0.076	-0.097
BPA	0.706	– 0.291	0.107	0.234	– 0.343	-0.420
BPF	0.092	– 0.072	0.180	0.281	– 0.055	-0.414
BT	0.286	– 0.133	0.067	0.338	– 0.069	– 0.302
BTri	**0.889**	– 0.077	0.051	– 0.245	– 0.186	**0.878**
CBZ	**0.945**	– 0.138	0.073	**0.924**	– 0.195	– 0.080
CBDZN	0.751	– 0.161	0.329	0.708	0.020	– 0.110
Lorazepam	**0.888**	– 0.110	– 0.066	**0.916**	– 0.219	0.024
MPB	0.275	– 0.108	0.225	– 0.056	– 0.053	–0.031
OHBT	**0.899**	– 0.008	– 0.191	0.462	0.024	0.005
OP	**0.879**	0.029	– 0.288	0.245	0.186	**0.878**
Oxazepam	**0.946**	– 0.158	0.082	**0.898**	– 0.200	0.024
Primidone	**0.928**	– 0.075	– 0.159	**0.868**	0.068	0.170
EPOCBZ	**0.928**	– 0.110	– 0.007	**0.856**	– 0.200	– 0.036

In bold the variables with the highest loading values.

Fig. 2.2a shows the score plot for PC1 vs. PC2, since it was the only plot that grouped samples. The plot separated the irrigation water samples into three distinct groups. Group I includes the irrigation waters with polluted groundwater (site P5), Group II includes the samples highly impacted by TWW (site P3), and Group III includes the irrigation waters less impacted by the discharge of TWW effluents and groundwater pollution (sites P1, P2, and P4).

To gain further insight into the sources of the remaining samples, the irrigation water samples from site P3 were excluded, and a new PCA was performed on water samples from sites P1, P2, P4, and P5 (Fig. 2.2b). The loadings are reported in Table 2.4. Two principal components with eigenvalues >1 were extracted, and the PCA method led to a reduction of the initial size of the data set to three components explaining 58% of the data variation. PC1 accounted for 29% of the variance and had positive loadings

(>0.8) for Surfynol 104, TCEP, 5-TTri, carbamazepine, lorazepam, oxazepam, and primidone. These OMCs are highly recalcitrant compounds that have already been detected in the Llobregat River at high concentration levels (Banjac et al., 2015). Thus, the positive loadings for these components were associated with the Llobregat River irrigation water. In contrast, PC2 (20%) had positive loadings for TP, Zn, Rb, Pb, and chlorpyrifos.

These results are consistent with the presence of Pb and chlorpyrifos in previous studies carried out with Llobregat Delta groundwater (Cabeza et al., 2012). Therefore, this component was associated with groundwater pollution. Fig. 2.2b shows the score plot for PC1 vs. PC2. Interestingly, the samples formed three different groups: Group I includes the irrigation water from the Llobregat Delta groundwater (site P5), while Group II includes the irrigation water from the Llobregat River (sites P2 and P4). Group III clusters the irrigation water samples from the pristine site (i.e., site P1, the least polluted location). These results are consistent with a similar water quality for sites P2 and P4 characterized by a mixture of river water and TWW effluents.

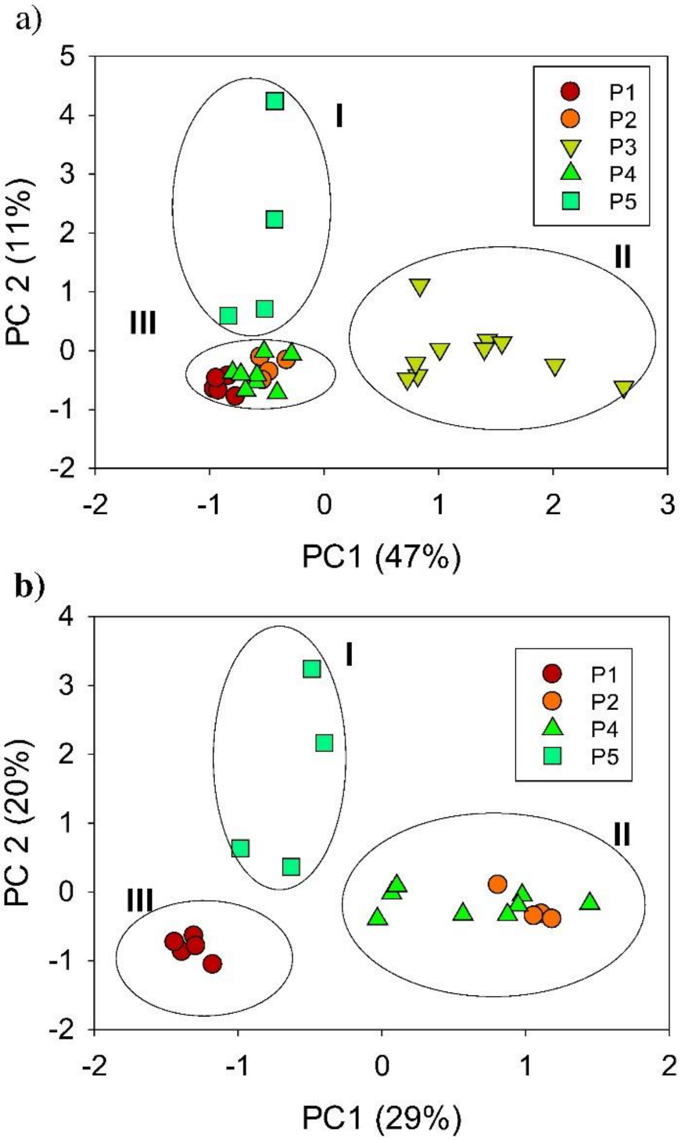


Figure 2.2 Principal Component Analysis (PCA) results. (a) PC 1 vs. PC2 score plot with all irrigation water samples (P1–P5), (b) PC1 vs. PC2 score plot excluding irrigation water samples from site P3.

2.3.5 Phytotoxicity studies (lettuce seed germination)

Table 2.5 shows that germination was higher in seeds watered with irrigation waters than with distilled water (88 vs. 98-99%). This may be explained by the higher concentration of macro- and micronutrients such as nitrates and salts in the irrigation waters that would help break dormancy to facilitate seed germination (Hilton, 1985; Rezvani et al., 2014). The root elongation was higher at the sampling site P4 than in the distilled water ($p = 0.01$), but no differences were found among the irrigation waters from sites P1, P3, and P4 ($p > 0.05$). Likewise, no differences were observed between distilled water and the irrigation water for sites P1 and P3 ($p > 0.05$). Therefore, the higher FOD and concentration of OMCs, TEs, and nutrients in the irrigation water from site P3 did not affect seed germination. Similar results were reported by Marsoni et al. (2014), who observed that pharmaceutical compounds only affected seed germination at concentrations higher than 10 mg L^{-1}.

Table 2.5 Effect of irrigation waters on in vitro seed germination of lettuce (n=100).

	Control*	Plot 1	Plot 3	Plot 4
Germination (%)	88	98	98	99
Root elongation (mm)	17.2 ± 0.9^{d}	19.4 ± 0.8	19.5 ± 0.8	20.8 ± 0.9^{a}

*Seeds were grown in distilled water; Significant differences between plots are shown (control = ^{a}, P1 = ^{b}, P3 = ^{c}, and P4 = ^{d}), statistical differences at $p = 0.05$.

2.3.6 Crop productivity: a field study

Many authors reported the importance of the pedological conditions related to agricultural and horticultural production systems (Andrews et al., 2002; Armenise et al., 2013; Bouma and Droogers, 1998; Mukherjee and Lal, 2014; Vasu et al., 2016), but in our study soil composition was similar; therefore, differences may be related to the water irrigation quality. Unfortunately, crop productivity can also be affected by other environmental conditions. For instance, the pristine site (P1) was located at 400 m asl, whereas the other plots were at sea level. Table 2.6 shows the crop productivity for lettuce and tomato fruits in the 5 farm plots (P1-P5). The productivity values for tomatoes fruits and lettuce were in keeping with those found in other studies. For instance, Serna et al. (2012) reported a lettuce plant yield of 34,000 ± 1000 kg ha^{-1}. The results from farm plot P3 should be approached with caution, since they were harvested after 77 cropping days (yield of 96,428 Kg ha^{-1}), whereas the other plots were harvested after 61- 69 days (yield of 40,178–52,187 Kg ha^{-1}). Casals et al. (2010) observed an average tomato fruit weight of 138 g, which is in the range of the yield observed at the pristine site (P1), whereas the average tomato weight observed in the peri-urban area was slightly higher (189 to 208 g per fruit on average). Therefore, the results showed that lettuce and tomato productivity was significantly higher in the farm plot in which indirect water reuse with TWW prevails (P3) ($p < 0.05$), probably due to the higher nutrient content (Table 2.1). Suspended, colloidal, and dissolved solids present in TWW contain macronutrients (e.g. N, P, K, Ca, Mg) and micronutrients (e.g. Cu, Fe, Zn, Mn, etc.) required by many crops (Abu-Zeid., 1998). This is in keeping with the results found by Urbano et al. (2017) who observed that the concentration of some soil nutrients (e.g. K, Ca, Al, and S) increased after irrigation with TWW and that lettuce production (in terms of fresh weight) was higher in lettuce cultivated in TWW than in those cultivated by conventional fertilization. Similarly, Li et al. (2015b) observed that irrigation with TWW increased tomato biomass and yield by 9%. Furthermore, the productivity results are consistent with the fact that the concentration levels of TEs in irrigation waters were between 10 and 100 times lower than the values observed to produce phytotoxicity (see Table 2.2).

Table 2.6 Fresh weight per unit, crop growing time and productivity for lettuce and tomatoes in the 5 farm plots studied.

	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5
Lettuce (g, fresh weight)	$773 \pm 38^{c,d}$	$795 \pm 37^{c,d}$	$1854 \pm 71^{a,b,d,e}$	$1004 \pm 44^{a,b,c,e}$	$805 \pm 40^{c,d}$
Lettuce (growing time, days)[f]	61	62	77^{c}	69	66
Lettuce yield (kg ha^{-1}, fresh weight)[g]	40,178 ± 6342	41,332 ± 9640	96,428 ± 13,264	52,187 ± 6753	41,877 ± 12,332
Tomatoes (g, fresh weight per unit)	$157 \pm 41^{c,d}$	–	207 ± 39^{a}	189 ± 46^{a}	–

Statistical differences at $p = 0.05$; super index letters show significant differences between plots (P1 = ^{a}, P2 = ^{b}, P3 = ^{c}, P4 = ^{d}, and P5 = ^{e}); [f] time required to reach commercial size. [g] fresh weight. Statistical assessment for lettuce yield is the same than for lettuce.

These results and the non-effect of the presence of chemical contaminants on lettuce seed germination and crop productivity suggest that the use of water impacted by TWW effluents for crop irrigation is beneficial due to the reduction of fertilization costs and water availability over the production cycle.

2.4 Conclusions

The results of this study show that the occurrence of OMCs, TEs, and nutrients in irrigation waters depends on the water source used (surface water vs. groundwater). Nevertheless, irrigation waters from peri-urban areas are more likely to contain chemical contaminants than those from pristine areas. The following key conclusions can be drawn:

- The irrigation waters from the peri-urban area of the BLAP showed higher conductivity and nutrient levels than the pristine site.
- Ba and B were the TEs with the highest concentration in all irrigation waters, whereas the irrigation water from the peri-urban area of the BLAP had the highest concentration levels of Zn and Mn.
- Irrigation waters originating from surface water bodies had a higher FOD and concentration of OMCs than irrigation water originating from groundwater, and Surfynol 104 was the most abundant compound.
- The irrigation water from site P3 (Infanta Channel) was the most impacted by nutrients, TEs, and OMCs since it is mainly made up of TWW.
- The higher occurrence of TEs and OMCs in the peri-urban irrigation waters did not affect seed germination, root elongation, or crop productivity.

Although our study shows that peri-urban agriculture is exposed to a higher concentration of TEs and OMCs and this did not affect crop productivity, further research is needed to exclude possible adverse human health effects or nutritional crop changes associated with the use of irrigation waters containing these substances.

2.5 Supporting Information

2.5.1 Materials and Reagents

Flame retardants (i.e, TCEP and TCPP), benzotriazoles and benzothiazoles (i.e., 1,3-benzothiazole, 2-mercaptobenzothiazole, benzotriazole, 5-methyl-2H-benzotriazole and 1-hydroxybenzotriazole), parabens (methylparaben, etylparaben,butylparaben and propylparaben), antioxidant (i.e., butylated hydroxyanisole (BHA)), plastifiers (bisphenol A, bisphenol F and 4-tert-octylphenol), tensioactive (2,4,7,9-tetramethyl-5-decyne-4,7-diol (surfynol 104)), some pharmaceuticals (i.e., carbamazepine, diazepam, lamotrigine, lorazepam, primidone, oxazepam) and some pesticides (i.e., azoxystrobin, dymethomorph, pyraclostrobin, chlorpyrifos, diazinon, pymetrozin, indoxacarb, DEET) were purchased from Sigma-Aldrich (Bornem, Belgium).

Other pesticides (i.e., carbamazepine-10,11-epoxide, carbendazim, atrazine and simazine) were supplied by Fluka (Buchs, Switzerland).

Surrogates used were bisphenol A-d_{16}, carbamazepine-^{13}C6, diazepam-d_5, 5,6-dimethyl-1H-benzotriazole (XbTri), ethylparaben-^{13}C and lamotrigine-$^{13}C_{15}N_4$ purchased from Sigma-Aldrich (Bornem, Belgium) and caffeine-$^{13}C_3$ obtained from Fluka (Buchs, Switzerland). Internal standard triphenylamine (TPhA, 98%) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and trimethylsulfonium hydroxide (TMSH) was obtained from Fluka (Buchs, Switzerland). Suprasolv® grade methanol, hexane, ethyl acetate were purchased from Merck (Darmstadt, Germany). Reagent water was deionized using the ultrapure water system Arium 611 from Sartorius (Aubagne, France).

Strata-X solid phase extraction (SPE) cartridges (200mg / 6 mL) were purchased from Phenomenex (Torrance, CA, USA) and 0.70µm of glass filters 47mm in diameter were obtained from Whatman (Maidstone, UK).

2.5.2 Sample extraction

CECs in wastewater and interstitial water samples were analyzed following a previously described methodology (Matamoros and Bayona, 2006).

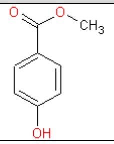
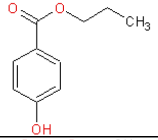
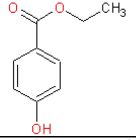
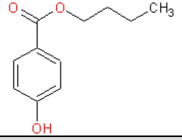
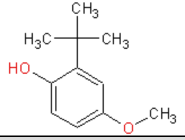
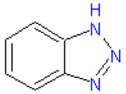
A sample volume of 250 mL was spiked at 0.25 ppb of a surrogate standard mix. The spiked sample was percolated through a polymeric solid-phase extraction cartridge, 200 mg Strata X from Phenomenex (Torrance, CA). Cartridges were conditioned with 6 mL of n-hexane, 6 mL of ethyl acetate, 10 mL of methanol and 10 mL of distilled water (pH=7). The spiked samples were percolated through the cartridges under vacuum, were allowed to dry for 30 min and eluted with 15 mL ethyl acetate. Then, the extract was evaporated until ca. 250 µL under a gentle nitrogen stream, and 25µL of triphenylamine (TPhA) as internal standard was added.

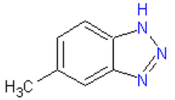
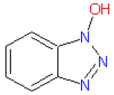
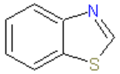
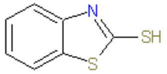
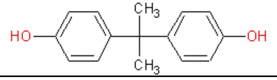
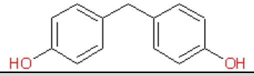
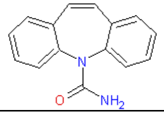
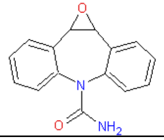
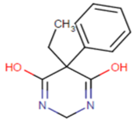
2.5.3 GC-MS/MS determination

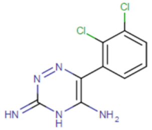
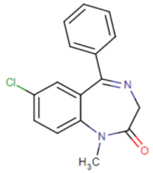
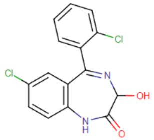
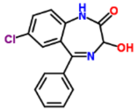
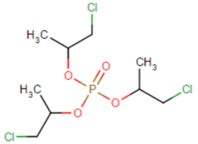
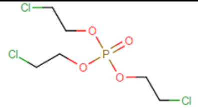
Aliquots of the sample extracts were analyzed with an EI-GC-MS/MS Bruker 450-GC gas chromatograph coupled to a Bruker 320-MS triple-stage quadrupole mass spectrometer (Bruker

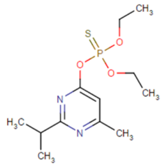
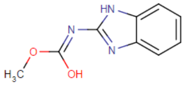
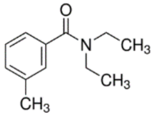
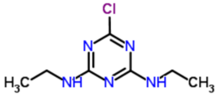
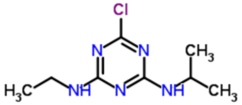
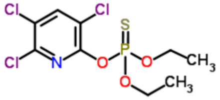
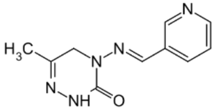
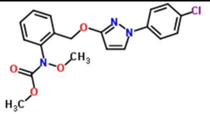
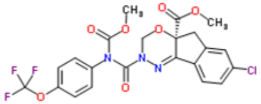
Daltonics Inc., Billerica, MA, USA). The linearity range was from 0.80 to 500 µg·L^{-1}. The correlation coefficients (R^2) of the calibration curves were always higher than 0.99. Physicochemical properties of the CECs of study (Table S2.1), monitoring ions (Table S2.2 and Table S2.3), LODs and LOQs (Table S2.4) and recoveries of the surrogates used (Table S2.5) can be found in the supplementary information.

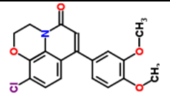
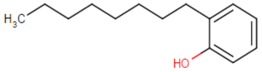
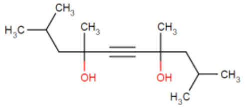
Table S2.1 Physicochemical properties of the CECs of study

Name	Molecular structure	CAS Number	Molecular Weight	Molecular formula	pKa[1]	Solubility (mg L^{-1})	Log K_{OW}[2]	Log D_{OW} (pH=7.4)	Log K_{OA}[2]	Henry LC (atm-m^3/mole)	Log K_{AW}[2]
Parabens											
Methyl paraben		99-76-3	152.15	$C_8H_8O_3$	8.50[0/-]	2500(a)	2.00	2.09	8.791	3.61E-009	-6.831
Propylparaben		94-13-3	180.21	$C_{10}H_{12}O_3$	8.50[0/-]	500(a)	2.98	2.81	9.624	6.37E-009	-6.584
Ethylparaben		120-47-8	166.18	$C_9H_{10}O_3$	8.50[0/-]	885(a)	2.49	2.48	9.178	4.79E-009	-6.708
Butylparaben		94-26-8	194.23	$C_{11}H_{14}O_3$	8.47[0/-]	207(a)	3.57	3.12	10.032	8.45E-009	-6.462
***Tert*-butylphenols**											
2-tert-Butyl-4-methoxyphenol (BHA)		121-00-6	180.25	$C_{11}H_{16}O_2$	10.57[0/-]	212.8	3.50	3.14	8.956	8.56E-008	-5.456
Benzotriazoles											
Benzotriazole (BTri)		95-14-7	119.12	$C_6H_5N_3$	8.37[0/-]	5957	1.44	-	6.661	1.47E-007	-5.221

5-Methyl-2H-benzotriazole (5-TTri)		136-85-6	133.15	$C_7H_7N_3$	0.77[+/0] 8.85[0/-]	1769	1.71	1.69	6.889	1.62E-007	-5.179
1-Hydroxybenzotriazole (OHBT)		2592-95-2	135.13	$C_6H_5N_3O$	6.88[0/-]	2.258E+004	0.11	0.07	-	-	-
Benzothiazoles											
1,3-Benzothiazole (BT)		95-16-9	135.18	C_7H_5NS	2.28[+/0]	4300(a)	2.17	2.09	6.826	3.74E-007	-4.816
2-Mercaptobenzothiazole (2MBT)		149-30-4	167.24	$C_7H_5NS_2$	10.90[0/-]	120(c)	2.86	2.21	8.249	3.63E-008	-5.829
Plasticizers											
Bisphenol A		80-05-7	228.29	$C_{15}H_{16}O_2$	9.78[0/-] 10.39[-/2-]	120(d)	3.64	3.63	12.747	9.16E-012	-9.427
Bisphenol F		620-92-8	200.24	$C_{13}H_{12}O_2$	9.84[0/-] 10.45[-/2-]	542.8	3.06	2.90	12.582	5.2E-012	-9.672
Anticonvulsants, antidepressants and its related metabolites											
Carbamazepine		298-46-4	236.28	$C_{15}H_{12}N_2O$	13.9[0/-]	112(e)	2.25	2.28	10.805	1.08E-010	-8.355
Carbamazepine-10,11-epoxide		36507-30-9	252.27	$C_{15}H_{12}N_2O_2$	15.96 [0/-]	276.8	0.95	1.31	11.503	6.84E-013	-10.553
Primidone		125-33-7	218.26	$C_{12}H_{14}N_2O_2$	2.36[+/0] 3.94[0/-] 5.42[-/2-]	500(c)	0.73	0.61	9.011	1.94E-010	-8.101

Lamotrigine		84057-84-1	256.10	$C_9H_7Cl_2N_5$	8.53[+/0] 9.21[0/-]	139.7	0.99	1.68	11.612	2.22E-011	-9.042
Diazepam		439-14-5	284.75	$C_{16}H_{13}ClN_2O$	2.92[+/0]	50(c)	2.70	2.92	9.647	3.64E-009	-6.827
Lorazepam		846-49-1	321.16	$C_{15}H_{10}Cl_2N_2O_2$	10.61[0/-] 12.46[-/2-]	80(g)	2.41	2.49	10.166	4.1E-010	-7.776
Oxazepam		604-75-1	286.71	$C_{15}H_{11}ClN_2O_2$	1.55[+/0] 10.9[0/-]	20.71	3.34	2.06	10.101	4.24E-009	-6.761
Chlorinated flame retardants											
Tris(1-chloro-2-propyl) phosphate (TCPP)		13674-84-5	327.57	$C_9H_{18}Cl_3O_4P$	-9.8[0]	1200(b)	2.89	2.32	8.203	5.96E-008	-5.613
Tris(2-Chloroethyl) Phosphate (TCEP)		115-96-8	285.49	$C_6H_{12}Cl_3O_4P$	-9.06[0]	7000(h)	1.63	1.42	5.311	3.29E-006	-3.871
Pesticides											

Diazinon		333-41-5	304.35	$C_{12}H_{21}N_2O_3PS$	4.19[+/0]	40(i)	3.86	3.80	9.145	1.13E-007	-5.335
Carbendazim		10605-21-7	191.19	$C_9H_9N_3O_2$	1.12[+/0] 2.76[0/-] 12.64[-/2-]	29(j)	1.55	1.61	10.582	2.12E-011	-9.062
N,N-Diethyl-meta-toluamide (DEET)		134-62-3	191.27	$C_{12}H_{17}NO$	-	666	2.18	2.24	8.250	2.08E-008	-6.070
Simazine		122-34-9	201.66	$C_7H_{12}ClN_5$	1.62[+/0]	6.2	2.18	2.30	9.594	3.37E-009	-7.414
Atrazine		1912-24-9	215.68	$C_8H_{14}ClN_5$	1.60 [+/0]	34.7	2.61	2.66	9.626	4.47E-009	-7.016
Chlorpyrifos		2921-88-2	350.59	$C_9H_{11}Cl_3NO_3PS$	-	1.12(k)	4.96	4.78	8.882	2.52E-006	-3.922
Pymetrozin		123312-89-0	217.23	$C_{10}H_{11}N_5O$	4.37 [+/0] 11.4 [0/-]	290(j)	0.89	-	11.729	3.54E-013	-10.839
Pyraclostrobin		175013-18-0	387.82	$C_{19}H_{18}ClN_3O_4$	0.44[0/-]	0.08	5.45	4.07	18.778	1.15E-015	-13.328
Indoxacarb		144171-61-9	527.83	$C_{22}H_{17}ClF_3N_3O_7$	-			3.69			

Dimethomorph		110488-70-5	387.86	$C_{21}H_{22}ClNO_4$	-	18.72	2.68	3.31	16.064	1.01E-015	-13.384
Azoxystrobin		131860-33-8	403.39	$C_{22}H_{17}N_3O_5$	0.94 [+/0]	10[j]	2.50	3.54	14.049	8.01E-014	-11.549
Surfactants and derivatives											
Octylphenol (OP)		27193-28-8	206.33	$C_{14}H_{22}O$	10.30[0/-]	3.114	5.50	5.47	9.235	4.5E-006	-3.735
Surfynol 104		126-86-3	226.36	$C_{14}H_{26}O_2$	13.15[0/-] 13.83[-/2-]	26.35	3.61	2.94	8.611	2.44E-007	-5.001

[1] Dissociation reaction, [0]: neutral; [+]: cationic; [-]: anionic.

[2] Log K_{OW}, Log K_{OA}, Henry LC and Log K_{AW} from database provided by Episuite v4.11 (http://www.epa.gov/opptintr/exposure/pubs/episuite.htm)

Note: Log KOW, Log KOA, Henry LC and Log KAW are estimate values.

(a) Yalkowsky, S.H. (2003): Handbook of Aqueous Solubility Data. CRC Press.

(b) Chemicals Inspection and Testing Institute (1992): Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan. Japan Chemical Industry Ecology - Toxicology and Information Center.

(c) Yalkowsky, S.H., Dannenfelser, R.M. (1992): Aquasol database of aqueous solubility. Coll. Pharmacy, Univ. Arizona, Tucson, AZ.

(d) Dorn PB et al. (1987): Chemosphere 16: 1501-7

(e) Ferrari, B., Paxéus, N., Giudice, R. Lo, Pollio, A., Garric, J. (2003): Ecotoxicological impact of pharmaceuticals found in treated wastewaters: study of carbamazepine, clofibric acid, and diclofenac. Ecotoxicol. Environ. Saf. 55, 359–370

(g) Budavari, S. (1996): The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. Merck

(h) Muir DCG (1984): Handbook of Environmental Chemistry. Germany: Springer-Verlag 3: 41-66

(i) Sharom MS et al. (1980): Water Res 14: 1095-100

(j) Tomlin, C.D.S. (1997): The Pesticide Manual, British Crop Protection Council, 11th ed., Surrey, UK.

(k) Yalkowsky, S.H., He, Y., Jain, P. (2016): Handbook of Aqueous Solubility Data, Second Edition. CRC Press.

Table S2.2 Monitoring ions in GC-MS/MS of the underivatized contaminants

Segment	Compound	RT (min)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)
1	Surfynol 104	9.34	151	109	9
			109*	67	11
2	BHA	10.23	180	165	14
			165*	137	12
3	DEET	11.36	190	145	21
			119*	91	12
4	Simazine	13.39	201*	173	10
			201	138	15
4	Atrazine	13.46	215*	200	15
			215	173	15
4	TCEP	13.55	249*	125	15
			249	99	30
5	TCPP	13.95	125	99	16
			99*	81	21
5	Diazinon	13.91	304*	179	15
			179	137	24
6	Caffeine-$^{13}C_3$	14.74	197*	111	18
			111	57	14
6	Tonalide-d_3	14.79	261	246	12
			246*	190	10
7	Chlorpyrifos	16.22	313	258	17
			97*	79	16
8	TPhA	16.91	245*	167	30
			245	141	21
9	Carbamazepine-10,11-epoxide	17.49	178	150	23
			207*	178	26
10	Pymetrozin	18.85	113*	98	11
			98	70	14
11	Lamotrigine-$^{13}C_{15}N_4$	22.16	262	187	22
			185*	114	22
11	Lamotrigine	22.08	255*	185	16
			185	123	18
11	Diazepam-d_5	21.84	289	261	20
			261*	226	17
11	Diazepam	21.85	284	256	16
			256*	221	13
12	Pyraclostrobin	26.23	164*	132	15
			132	77	21
13	Indoxacarb	26.97	203*	134	17
			203	106	25
14	Dimethomorph	27.77	387	301	19
			301*	165	16
14	Azoxystrobin	27.53	388	360	14
			344*	329	17

* Transition used for quantification

Table S2.3 Monitoring ions for derivatized compounds in GC-MS/MS

Segment	Compound	RT (min)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)
1	1,3-Benzothiazole (BT)	7.85	135*	108	20
			108	82	14
2	Benzotriazole (Btri)	9.09	133*	105	12
			105	90	19
2	Methylparaben (MPB)	9.12	166	135	13
			135*	77	18
3	Etylparaben (EPB)	9.89	180	152	11
			135*	77	18
3	Etylparaben-13C (EPB 13C)	9.91	186	158	12
			141*	82	20
4	5-methyl-2H-benzotriazole (5TTri)	10.40	147	118	14
			118*	77	17
5	Propylparaben (PPB)	11.05	194	152	12
			135*	77	18
5	4-*tert*-octylphenol (OP)	11.00	220	149	12
			149*	121	15
6	1-hydroxybenzotriazole (OHBT)	11.55	165*	136	17
			136	109	17
6	2-mercaptobenzothiazole (2MBT)	11.89	181*	148	16
			148	104	14
7	5,6-dimethyl-1H-benzotriazole (XbTri)	12.30	161	132	16
			132*	91	16
7	Butylparaben (BPB)	12.34	208	152	12
			152*	135	14
8	Carbendazim	14.68	219*	160	15
			160	132	16
9	Carbamazepine	16.51	193*	191	23
			193	167	18
9	Carbamazepine-$^{13}C_6$	16.53	199	197	29
			199	171	22
9	Bisphenol F (BPF)	16.21	228*	197	15
			197	165	17
10	Primidone	16.62	218	175	17
			146*	117	15
10	Triphenylamine (TPhA)	16.90	245*	167	30
			245	141	21
11	Bisphenol A (BPA)	17.32	256	241	13
			241*	133	15
11	Bisphenol A-d_{16} (BPA d_{16})	17.23	270	252	14
			252*	139	20
12	Oxazepam	24.43	314	209	21
			228*	193	17
13	Lorazepam	25.58	348*	209	20
			209	165	35

* Transition used for quantification

Table S2.4 Limits of detection (LOD) and quantification (LOQ) of the selected ECs

Compound	LOD ($ng \cdot L^{-1}$)	LOQ ($ng \cdot L^{-1}$)
1,3-Benzothiazole (BT)	32.3	46.6
1-hydroxybenzotriazole (OHBT)	2.03	3.12
2-mercaptobenzothiazole (2MBT)	17.6	32.2
5-methyl-2H-benzotriazole (5TTri)	12.5	15.9
Atrazine	2.40	2.63
Azoxystrobin	4.04	4.12
Benzotriazole (Btri)	672	755
Butylhydroxyanisol (BHA)	3.35	3.42
Bisphenol A (BPA)	3.91	5.62
Bisphenol F (BPF)	13.5	20.7
Butylparaben (BPB)	0.91	1.04
Carbamazepine	10.3	12.2
Carbamazepine-10,11-epoxide	47.5	57.4
Carbendazim	1.63	2.31
Chlorpyrifos	9.78	13.0
DEET	17.0	27.9
Diazepam	1.00	1.30
Diazinon	0.64	0.87
Dimethomorph	1.19	1.52
Etylparaben (EPB)	26.7	29.1
Indoxacarb	17.7	18.9
Lamotrigine	5.90	5.91
Lorazepam	3.19	3.72
Methylparaben (MPB)	55.9	74.5
4-*tert*-octylphenol (OP)	10.7	13.7
Oxazepam	2.92	4.01
Primidone	4.00	6.00
Propylparaben (PPB)	2.24	3.39
Pymetrozin	2.42	2.51
Pyraclostrobin	0.14	0.30
Simazine	4.40	5.11
Surfynol 104	16.3	20.4
TCEP	4.92	8.03
TCPP	197	310

Table S2.5 Recoveries of the surrogates

Compound	Recovery (%)
Bisphenol A-d_{16}	75±9
Caffeine-$^{13}C_3$	106±8
Carbamazepine-$^{13}C_6$	102±10
Diazepam-d_5	95±8
5,6-dimethyl-1H-benzotriazole (XbTri)	104±9
Etylparaben-^{13}C	93±9
Lamotrigine-$^{13}C_{15}N_4$	52±12

Chapter III: Occurrence and bioaccumulation of chemical contaminants in lettuce grown in peri-urban horticulture

This chapter is based on the article:

Margenat, A., Matamoros, V., Díez, S., Cañameras, N., Comas, J., & Bayona, J. M. (2018). Occurrence and bioaccumulation of chemical contaminants in lettuce grown in peri-urban horticulture. Science of the Total Environment, 637–638, 1166–1174.

Peri-urban horticulture performs environmental and socio-economic functions and provides ecological services to nearby urban areas. Nevertheless, industrialization and water pollution have led to an increase in the exposure of peri-urban vegetables to contaminants such as TEs and OMCs. In this study, the occurrence of chemical contaminants (i.e., 16 TEs, 33 OMCs) in soil and lettuce leaves from 4 farm fields in the peri-urban area of the city of Barcelona was assessed. A rural site, outside the peri-urban area of influence, was selected for comparison. The concentration of TEs and OMCs ranged from non-detectable to 803 mg kg^{-1} dw and from non-detectable to 397 µg kg^{-1} dw respectively in the peri-urban soil, and from $6\cdot10^{-5}$ to 4.91 mg/kg fw and from non-detectable to 193 µg kg^{-1} fw respectively in lettuce leaves. Although the concentration of Mo, Ni, Pb, and As in the soil of the peri-urban area exceeded the environmental quality guidelines, their occurrence in lettuce complied with human food standards (except for Pb). The many fungicides (carbendazim, dimethomorph, and methylparaben) and chemicals released by plastic pipelines (tris(1-chloro-2-propyl)phosphate, bisphenol F, and 2-mercaptobenzothiazole) used in agriculture were prevalent in the soil and the edible parts of the lettuce. The occurrence of these chemical pollutants in the peri-urban area did not affect the chlorophyll, lipid, or carbohydrate content of the lettuce leaves. PCA showed that soil pollution, fungicide application, and irrigation water quality are the most relevant factors determining the presence of contaminants in crops.

3.1 Introduction

Peri-urban horticulture performs environmental and socioeconomic functions and provides ecological services to nearby urban areas. These include fresh vegetables with a low carbon footprint, as well as the provision of recreational, landscape structure, and other ecological services (Veenhuizen, 2007). Nevertheless, peri-urban agriculture is exposed to atmospheric and water pollution. For instance, air pollution associated with transportation infrastructure (airports, harbours, highways) or the use of reclaimed water containing OMCs and TEs for irrigation lead to their accumulation in soil and potentially in plants (Colon and Toor, 2016; Liacos et al., 2012). In particular, the application of manure and biosolids for soil amendment and of reclaimed water for irrigation have been reported as the main sources of OMCs in agriculture (Eggen and Lillo, 2012).

On the other hand, as water scarcity is increasing due to climate change and population growth, the application of reclaimed water for crop irrigation in peri-urban agriculture is becoming a reliable alternative water supply, especially in arid and semi-arid regions (WHO, 1989). Despite the widespread occurrence of OMCs such as pharmaceuticals or personal care products in reclaimed water, their concentrations are generally low, ranging from ng L^{-1} to µg L^{-1}; their continual release into the environment coupled with their transformation products makes them behave in a "pseudo-persistent" way (Daughton and Ternes, 1999). Reclaimed water irrigation is also one of the main sources of TEs in agriculture, along with biosolid and manure amendments and atmospheric deposition (Liacos et al., 2012; Lough et al., 2005; Nabulo et al., 2006). As a result, TEs have a significant impact on peri-urban agriculture (Singh and Kumar, 2006).

Bioaccumulation of OMCs have been frequently observed in crops grown under field conditions irrigated with treated wastewater (X. Wu et al., 2014). The highest concentrations have been detected in the edible parts of leafy vegetables (carbamazepine and its metabolites at 347 ng g^{-1} dw lettuce) rather than in root or fruit-bearing vegetables (Riemenschneider et al., 2016). Knowledge about the bioaccumulation of OMCs in vegetables irrigated with treated wastewater under field conditions is scarce (Calderón-Preciado et al., 2011). One long-term study (3 consecutive years) revealed that a longer duration of treated wastewater irrigation may lead to a significant uptake and bioaccumulation of some OMCs (Christou et al., 2017b). Moreover, TEs such as Pb, Cd, and Zn have been found at greater concentrations in crops grown on the roadside in peri-urban areas than in those from rural sites (Nabulo et al., 2010, 2006). Therefore, concerns regarding human exposure to OMCs and TEs have arisen as they have been detected in the edible parts of plants (Khan et al., 2008; X. Wu et al., 2014). However, only field studies can fully assess the incorporation of these compounds in real scenarios (Colon and Toor, 2016), and to date few such studies exist (Malchi et al., 2014; X. Wu et al., 2014). Another important adverse effect of the use of reclaimed water in agriculture is its high nitrate content. Nitrate content in vegetables is the major human dietary source of nitrate, and 5% of ingested nitrate is transformed into the toxic form nitrite (Santamaria, 2006). In this regard, nitrate fertilization and the reuse of treated wastewater contribute to nitrate uptake by food crops (Castro et al., 2009).

The occurrence of TEs and OMCs in agricultural soils can also lead to morphological and physiological changes in the exposed plants. For instance, Hurtado et al. (2017) observed that the occurrence of OMCs in irrigation waters at environmentally relevant concentrations resulted in a decrease in the chlorophyll content, morphological changes, and alterations in the metabolic profile of lettuces. Carter et al. (2015) observed a reduction in biomass productivity and changes in hormone and nutrient content in zucchini due to the presence of carbamazepine and verapamil at environmentally relevant concentrations (0.005–10 mg L^{-1} in soil). The occurrence of heavy metals such as Pb in plants can inhibit chlorophyll biosynthesis and decrease vegetable carbohydrate content (Gaweda, 2007; Peralta-Videa et al., 2009), while the occurrence of Cd can cause lipid peroxidation (Monteiro et al., 2007; Rodríguez-Serrano et al., 2006). However, there is no available information about the impact of the co-occurrence of OMCs and TEs in crops in real-life scenarios.

Furthermore, although the occurrence of TEs and pesticide residues in food products is regulated in the EU (EFSA), USA (FDA), China, and Australia-New Zealand, and the FAO has published international guidelines (Codex Alimentarius) (FAO, 2016), no regulations exist for the other detected OMCs.

In a previous study, several chemical pollutants were detected in irrigation waters from the peri-urban horticultural area of the city of Barcelona (Margenat et al., 2017). The present study aims to assess the occurrence of these pollutants (16 TEs and 33 OMCs) in lettuce cultivation (soils and plants), as well as bioaccumulation factors and their potential impacts on leaf constituents (i.e. chlorophyll, nitrates, lipids, and carbohydrate content). The study was conducted in 4 farm fields located in the peri-urban area of Barcelona (NE Spain) and a remote organic farming plot located in a rural area.

3.2 Material and methods

3.2.1 Sampling site description

Five locations in the Llobregat River delta and its lower valley (NE Spain) were sampled. Further information is described in section 2.2.1.

3.2.2 Sampling strategy

Soil

Soil was sampled in May of 2016 when the lettuce was harvested. A composite soil sample from a horizon of 0 to 20 cm was obtained from five subsamples in each farm plot. Soil samples were sieved through a 2.0 mm mesh and stored at -20 °C. According to the USDA (1987) classification, soil samples from farm plots P1, P3, and P4 were loamy sand, while the soils from farm plots P2 and P5 were sandy. The soil samples were also characterized by UNE-EN ISO/IEC 17025:2005 accredited laboratories.

Table S3.1 provides information about the physicochemical properties of each of the studied soils.

Lettuce

Lettuce was selected for being one of the most cultivated vegetables in the peri-urban agricultural area of Barcelona. The lettuces (*Lactuca sativa* L. cv. Batavia) were harvested when they reached their commercial size. The lettuce seedlings were planted in February-March and June 2016, and the plants were harvested in May 2016 (P1-P5) and June 2016 (P3-4) for the winter and summer seasons respectively (Fig. 2.1). Each farm field was divided in 5 sections, and 10 lettuces were collected per section. For each section, a quarter of each lettuce was mixed and comminuted together using liquid nitrogen and a porcelain mortar. These samples were then stored at -20 °C until they were analyzed. Thus, 5 samples were obtained per plot.

3.2.3 Analytical procedures

The chemicals and reagents used for the analytical methodologies described below are listed in section 3.5.1.

Nitrate content in lettuce

Extraction was performed according to the procedure recommended by the AOAC (AOAC, 1997; Guadagnin et al., 2005). Briefly, 40 mL of Milli-Q water were added to a tube containing 5 g of fresh weight (fw) lettuce leaves, and it was heated to 70 °C for 15 min. Once the extract was at room temperature, Milli-Q water was added to a total volume of 100 mL. It was then filtered through a filter paper (Whatman No. 4) prior to spectrophotometric measurement. Nitrate content was measured with a Hach-Lange spectrophotometer (DR 1900 Portable Spectrophotometer) at a wavelength of 460 nm as nitrate nitrogen (NO_3-N).

Lipids and carbohydrate extraction in lettuce leaves

The method was adapted from that described by (Yang et al., 2016). Extraction was carried out by adding 15 mL of ethanol/hexane (1:1, v/v) to a glass tube with 3 g of fw sample. The sample was sonicated for 15 min and centrifuged at 2500 rpm for 15 min. It was then filtered through a 0.22 µm nylon filter (Scharlab, Barcelona, Spain) into a preweighed glass tube. After removing the solvent by purging and drying with nitrogen gas, the tube and filter were weighed. The sample remaining in the tube was operationally defined as lipid content, whereas the sample on the filter was operationally defined as carbohydrates.

Chlorophyll content in lettuce

Chlorophyll content in lettuce was measured with a chlorophyll meter (CCM200Plus, Opti-Sciences) (Hudson, NH, USA) in triplicate based on the outer and inner leaf absorbance of each head of lettuce. A calibration curve was obtained to relate the chlorophyll content to the absorbance previously measured with the chlorophyll content meter. To this end, rounded samples of leaves (4 cm diameter) were extracted with 5 mL of N,N-dimethylformamide (DMF) and kept in the dark at 4 °C for 48 h before the spectrophotometric determination. The extracts were measured at two wavelengths, 647 and 664.5 nm, so that chlorophylls (a, b, and total) could be calculated using Inskeep and Bloom's coefficients (Inskeep and Bloom, 1985; Porra, 2002).

Trace element (TE) extraction

- ***Soil***

A slightly modified pseudo-total digestion method (Lee et al., 2006) using a strong acid (HNO_3-$HClO_4$) was used. A portion of 0.1 g of homogenized, dried sample was sieved through 2 mm mesh (CISA, Spain) and placed in a polyethylene tube. Then, 10 mL of 65% HNO_3 and 10 mL of concentrated $HClO_4$ were added, and the mix was heated up to 135 °C for 16 h. The digested samples were then evaporated, resuspended with 3mL of HNO_3, and then heated to ensure dissolution. A 1 mL aliquot was diluted with 24 mL of Milli-Q water, and the sample was filtered (0.2 µm) prior to analysis. An inductively coupled plasma optical emission spectrometer (Thermo Scientific, iCAP 6500 ICP-OES) and an inductively coupled plasma mass spectrometer (Thermo Scientific, XSeries 2 ICP-MS) were used for the determination of major and minor TEs respectively in both the soil and lettuce samples. The content of Hg was determined using an advanced mercury analyser (AMA-254, Altec, Prague, Czech Republic).

- ***Lettuce***

A portion of 1 g of plant leaf tissues, dried and sieved, was digested with 4 mL (1:1) HNO_3 and 10 mL of (1:4) HCl in a closed Teflon vessel using a six-position EvapoClean heating block (EvapoClean, Deltalabo, France) at 95 °C for at least 3 hosed. Afterwards, samples were transferred to a 100-mL volumetric flask and centrifuged; then, a 10 mL aliquot was diluted with 40 mL Milli-Q water prior to analysis. The determination method was the same as for the soil, but values are expressed in fw basis. The applied methodology was validated by NIST 1570a (Gaithersburg, USA), with certified values for As, B, Cd, Co, Cu, Mn, Hg, Ni, Zn in lettuce. For accuracy, excellent extraction efficiencies were noted for these elements (92-107%). Further information is provided in section 3.5.2.

Organic microcontaminant (OMC) extraction

Physicochemical properties (molecular weight, pKa, solubility, and log K_{OW},) of the studied OMCs are provided in Table S2.1.

- ***Soil***

Soil extraction was adapted from a previously reported method (Xu et al., 2008). Briefly, 5 g of soil (fw), homogenized and sieved through 2.0mm mesh, were placed in a glass tube. It was fortified with 31.25 ng of a mixture of 6 surrogates and left to equilibrate for 30 min. The extraction was performed by sonication for 15 min three times with 5 mL of acetone/ethyl acetate (1:1, v/v). The extract was then centrifuged at 3100 rpm for 10 min and the supernatants were combined and evaporated to ca. 0.5 mL under a gentle stream of nitrogen.

Next, 2 mL of methanol were added to the final extract, which was reconstituted with 250 mL of deionized water prior to percolation through previously conditioned SPE cartridges (STRATA X, 100 mg, 6 mL). The cartridges were dried under vacuum and eluted with 10 mL of ethyl acetate. The extracts were concentrated to ca. 250 µL under a stream of nitrogen and 37.25 ng of triphenylamine (TPhA) were added as an internal standard. Finally, a 50 µL aliquot was analyzed by GC-MS/MS without derivatization, and another 50 µL aliquot was analyzed derivatized with 10 µL of TMSH. The analytical quality parameters (LOD, LOQ, and recoveries) are provided in Tables S3.2-3.4 in the SI section.

- ***Lettuce***

The extraction of OMCs from plant leaf tissues was performed according to Calderón-Preciado et al. (2009). Briefly, the extraction of samples (0.5 g fw) was performed with a matrix solid-phase dispersion method previously spiked with 12.5 ng of a mixture of surrogates and equilibrated for 30 min. Neutral-basic and acid fractions were obtained by solvent partitioning at neutral and acid pH, respectively.

After clean-up, fractions were reduced to ca. 80 μL and 37.25 ng of TPhA were added. A 50 μL aliquot and another one derivatized with 10 μL of TMSH were analyzed in GC-MS/MS. The extraction of CBZ and EPOCBZ from lettuce samples was carried out by sonication followed by liquid chromatography tandem mass spectrometry (LC-MS/MS). To this end, 0.5 g of fw lettuce was spiked with 50 ng of carbamazepine-13C and left to stand for 30 min.

Samples were sonicated with 10 mL of MeOH for 15 min and centrifuged 15 min at 3000 rpm. The extraction was performed twice, and the extracts were combined and reduced to ca. 1 mL with nitrogen gas and reconstituted with 10 mL of LiChrosolv water. The samples were then percolated through SPE cartridges (STRATA X, 100 mg·6 mL), previously conditioned with 1 mL of MeOH and water, respectively. The cartridges were washed with water/methanol (95:5, v/v) and eluted with 2 mL of a mixture of MeOH/ethyl acetate (1:1, v/v). The final extracts were reduced almost to dryness, resuspended in 1 mL of water, and filtrated (0.22 μm) prior to LC-MS/MS analysis. The LODs and LOQs were calculated for each analyte as three and ten times the signal from the baseline noise (S/N ratio), respectively. Analytical quality parameters (LOD, LOQ, and recoveries) are provided in Tables S3.5-7 in the SI section. Further details on the GC–MS/MS and LC-MS/MS are provided elsewhere (section 3.5.2).

3.2.4 Data analysis

Data values for the soil and plants are presented in dw and fw respectively. This is in agreement with legislated units for each of the studied matrices. The bioconcentration factor (BCF) was calculated for TEs and OMCs as the ratio between the concentrations (mg·kg^{-1} dw) in the edible parts of lettuce plants and soil content. The units used were mg/kg dw for TEs and μg/kg dw for OMCs.

$$BCF = \frac{C\ in\ edible\ part\ of\ the\ plant}{C\ soil} \tag{3.1}$$

The experimental results were statistically evaluated using the SPSS v. 22 package (Chicago, IL, US). All data sets were checked for normal distribution using the Kolmogorov–Smirnov test to ensure that parametric statistics were applicable. The comparison of means of the occurrence of chemical pollutants between farm plots was performed with a two-paired (Wilcoxon) signed-rank test (the concentration of each compound was compared between farm plots). Principal Component Analysis (PCA) was conducted on the concentration levels of TEs, OMCs, lettuce constituents (chlorophyll, nitrates, lipids, and carbohydrates), and soil properties. Once the data matrix had been completed, it was autoscaled to have zero mean and unit variance (correlation matrix). Statistical significance was defined as $p \leq 0.05$.

3.3 Results and discussion

3.3.1 Occurrence of trace elements (TEs)

Soil

Table 3.1 shows the concentration of 16 TEs in the soil samples and their corresponding maximum values established for agricultural use by Catalan Law 5/2017 in accordance with Spanish Royal Decree 9/ 2005. The TE concentrations ranged from non-detectable (Cd in all plots except P3) to 802 mg/kg dw (Mn in P5). The most abundant TEs were Mn, Ba, Cr, Pb, Zn, Cu, and B in all the sampling sites. The total median concentration of TEs per site was as follows: 824 mg/kg dw (P1), 1353 mg/kg dw (P2), 1518 mg/kg dw (P3), 1623 mg/kg dw (P4), and 1896 mg/kg dw (P5). Based on these results, P1 was thus the least polluted site, and P5 the most polluted. A two-paired test showed that soil from the rural site (P1) was less polluted by TEs than any of the soils from the peri-urban area of Barcelona (P2-P5, $p< 0.05$). In fact, the concentration of Mo, Ni, Pb, Zn, and As in the soils of the peri-urban area (P2-P5) exceeded the maximum soil concentration limit established for agricultural use in the regional decree (Generalitat de Catalunya, 2017). These results are consistent with the abundance of Mn (103–13,584 mg/kg dw), Zn(23–214mg/kg dw), Cr (12–57 mg/kg dw), Ni (9–111 mg/kg dw), Cu (4–170 mg/kg dw), Cd (0.1–130 mg/kg dw), and Pb (20–86 mg/kg dw) reported in soil from the Baix Llobregat area (Zimakowska-Gnoińska et al., 2000). Finally, the high concentration of As in the peri-urban soil was in agreement with the fact that phosphatic fertilizers generally contain the highest concentrations of most heavy metal(loid)s including As, Cd, U, Th and Zn (Alloway, 2012), whereas in the rural site organic amending was used for soil fertilization.

Table 3.1 Concentration of TEs (mg/kg dw) in the agricultural soil from the different studied plots. The generic reference levels (GRL, mg/kg dw) of these elements for contaminated soils in Catalonia are shown.

	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	GRL agricultural use
B	92	132	94	81	58	–
Ba	86	257	226	305	412	500
Cd	<0.56	<0.56	0.79	<0.50	<0.60	2.5
Co	5.7	11	9.7	10	17	25
Cr	26	42	45	44	62	400
Cu	33	68	89	178	89	–
Li	10	27	26	28	43	–
Mn	361	439	520	494	803	–
Mo	2.0	3.7	3.6	2.0	2.9	3.5
Ni	17	31	57	33	49	45
Pb	15	83	216	164	77	60
Rb	28	35	37	39	58	–
Sb	<0.56	<0.56	<0.54	<0.50	<0.60	6.0
Zn	132	195	148	208	198	170
As	16	27	45	38	27	30
Hg	0.01	0.30	0.37	0.37	0.19	2

The high concentration levels of Pb are consistent with the fact that, in the past, Pb particles were widely released into the environment through vehicle emissions from leaded gasoline engines (half-life in soil of about 53,000 years). Industrial emissions and paints can also contribute to the release of Pb into the environment (Nabulo et al., 2006). Ba and Mn come mainly from natural sources in both urban and rural areas (Davis et al., 2009), while the source of B can be either geogenic or anthropogenic (fertilizers, households detergents, discharges from industrial plants, etc.) (Pedrero et al., 2010). Finally, Zn is naturally present in all soils in concentrations typically ranging from 10 mg/kg to 100 mg/kg; and human activities have enriched them through atmospheric deposition, fertilizers, and sewage sludge (Alloway, 2012). The proximity of road networks can also lead to considerable exposure to Zn through brake and tire wear, tailpipe emissions of motor oil, and anti-wear additives (Lough et al., 2005).

Lettuce

Table 3.2 shows the minimum, maximum, and median concentrations (mg/kg fw) of TEs in lettuce samples. B, Ba, Mn, and Zn were the most abundant TEs, in keeping with the occurrence of these elements in soil samples (Table 3.1). The detected levels were in the same range as those published for garden-grown vegetables (leafy greens, herbs, roots, and fruits) (McBride et al., 2014) for Ba (3.7 mg/kg fw) and Cd (0.028 mg/kg fw), but slightly higher for Pb (0.099 mg/kg fw). The values of Cd were compliant with Commission Regulation (EC) No 1881/2006 of 19 December 2006, which sets maximum levels for certain contaminants in food stuffs. In contrast, Pb in P3 in the summer season slightly exceeded the maximum legislated concentration. Since the winter levels were below the regulated concentrations, further studies are needed to establish the significance of these data.

Table 3.2 Minimum, maximum and median concentration (mg/kg fw) of TEs in lettuce samples. Only Plots 3 and 4 were planted during the summer season.

	Plot 1 winter	Plot 2 winter	Plot 3 winter	Plot 3 summer	Plot 4 winter	Plot 4 summer	Plot 5 winter	Maximum value legislated
B	(1.50–2.12) 1.76	(1.43–1.89) 1.73	(1.54–2.44) 1.98	(1.62–2.02) 1.86	(1.56–2.43) 1.92	(1.58–2.42) 2.00	(1.30–1.97) 1.58	–
Ba	(0.62–1.16) 0.83	(0.56–0.66) 0.59	(0.38–0.66) 0.48	(0.55–0.80) 0.62	(0.44–0.71) 0.60	(0.48–0.85) 0.61	(0.40–0.58) 0.47	–
Cd	(0.004–0.008) 0.006	(0.02–0.04) 0.03	(0.02–0.03) 0.02	(0.03–0.03) 0.03	(0.01–0.02) 0.02	(0.01–0.02) 0.01	(0.01–0.02) 0.01	0.20
Co	(0.02–0.03) 0.02	(0.01–0.02) 0.01	(0.00–0.01) 0.01	(0.01–0.02) 0.01	(0.01–0.02) 0.01	(0.01–0.02) 0.01	(0.01–0.02) 0.02	–
Cr	(0.12–0.34) 0.20	(0.10–0.20) 0.14	(0.08–0.26) 0.15	(0.08–0.78) 0.25	(0.09–0.54) 0.20	(0.20–0.57) 0.33	(0.11–0.17) 0.13	–
Cu	(0.40–0.66) 0.49	(0.57–0.78) 0.67	(0.46–0.69) 0.59	(0.48–0.76) 0.65	(0.64–0.89) 0.78	(0.60–0.96) 0.78	(0.45–0.81) 0.57	–
Li	(0.04–0.07) 0.05	(0.05–0.05) 0.05	(0.02–0.04) 0.03	(0.04–0.08) 0.06	(0.03–0.05) 0.04	(0.03–0.05) 0.04	(0.04–0.07) 0.06	–
Mn	(2.77–4.91) 3.60	(1.29–1.77) 1.47	(2.65–4.20) 3.32	(3.29–4.28) 3.59	(1.50–2.46) 1.89	(1.73–2.79) 2.16	(1.89–2.70) 2.33	–
Mo	(0.02–0.03) 0.03	(0.02–0.03) 0.02	(0.02–0.04) 0.03	(0.02–0.06) 0.03	(0.02–0.05) 0.03	(0.03–0.06) 0.04	(0.02–0.03) 0.02	–
Ni	(0.05–0.16) 0.09	(0.05–0.09) 0.07	(0.04–0.10) 0.06	(0.08–0.61) 0.21	(0.05–0.41) 0.13	(0.19–0.32) 0.25	(0.05–0.12) 0.09	–
Pb	(0.03–0.08) 0.05	(0.09–0.15) 0.11	(0.08–0.19) 0.13	(0.13–0.45) 0.28	(0.13–0.21) 0.16	(0.13–0.25) 0.20	(0.13–0.22) 0.19	0.30
Rb	(0.32–0.50) 0.37	(0.41–0.49) 0.45	(0.28–0.53) 0.40	(0.30–0.42) 0.35	(0.37–0.59) 0.50	(0.39–0.60) 0.50	(0.33–0.56) 0.44	–
Sb	(0.02–0.07) 0.03	(0.02–0.02) 0.02	(0.01–0.01) 0.01	(0.02–0.03) 0.02	(0.01–0.01) 0.01	(0.02–0.02) 0.02	(0.01–0.02) 0.01	–
Zn	(1.00–1.52) 1.23	(2.36–3.41) 2.78	(1.73–2.92) 2.28	(1.30–1.96) 1.71	(2.15–3.12) 2.53	(2.01–2.95) 2.50	(1.47–2.69) 1.91	–
As	($5.75\cdot10^{-5}$–$6.51\cdot10^{-4}$) $4.10\cdot10^{-4}$	($5.10\cdot10^{-4}$–$8.55\cdot10^{-4}$) $6.77\cdot10^{-4}$	($2.16\cdot10^{-4}$–$9.36\cdot10^{-4}$) $6.01\cdot10^{-4}$	($5.67\cdot10^{-4}$–$2.30\cdot10^{-3}$) $1.54\cdot10^{-3}$	($4.45\cdot10^{-4}$–$1.17\cdot10^{-3}$) $8.35\cdot10^{-4}$	($4.19\cdot10^{-4}$–$1.10\cdot10^{-3}$) $6.88\cdot10^{-4}$	($2.67\cdot10^{-4}$–$1.40\cdot10^{-3}$) $8.43\cdot10^{-4}$	–
Hg	($3.01\cdot10^{-4}$–$4.59\cdot10^{-4}$) $3.65\cdot10^{-4}$	($3.65\cdot10^{-4}$–$1.67\cdot10^{-3}$) $1.06\cdot10^{-3}$	($5.20\cdot10^{-4}$–$1.12\cdot10^{-3}$) $8.37\cdot10^{-4}$	($6.95\cdot10^{-4}$–$1.45\cdot10^{-3}$) $1.07\cdot10^{-3}$	($6.90\cdot10^{-4}$–$1.56\cdot10^{-3}$) $9.74\cdot10^{-4}$	($5.26\cdot10^{-4}$–$8.56\cdot10^{-4}$) $7.45\cdot10^{-4}$	($6.36\cdot10^{-4}$–$1.66\cdot10^{-3}$) $1.01\cdot10^{-3}$	–

A two-paired test was used to compare differences between sampling points regarding the TEs present in lettuce samples. No statistical differences ($p > 0.05$) were obtained for Cr, Mn, or Ba content at the study sampling sites. In contrast, they were obtained among sites for the rest of the TEs. The most polluted crops were found in site P3 (total concentration of 192 mg/kg fw in winter and 170 mg/kg fw in summer), whereas crops least polluted by TEs (total concentration 140 mg/kg fw) were grown in the rural farm plot. Nevertheless, no statistical differences were found in the total metal concentration measured in vegetable crops from each farm plot ($p > 0.05$).

3.3.2 Bioconcentration factor for TEs

Table S3.8 shows the BCFs of selected TEs in lettuce for each of the studied farm plots. The BCFs ranged from 0.0002 (As in P5) to 1.78 (Cd in P2). Sb and Cd were the TEs with the highest BCFs. Ratios > 1 denote a positive accumulation of metals in plant organs. Only Cd (P2 and P4) and Sb (P1, P2, P3, and P5) exhibited values above 1. Although most of the values obtained were <1, it must be recalled that only the edible part of the plant was analyzed. The BCF of a TE depends on the plant cultivated and the soil properties, such as pH, OMC, and the distribution of metals in different soil fractions (Kos et al., 2003). These results are in keeping with other studies in which Cd is the TE most likely to accumulate in leafy vegetables, with BCFs ranging from 0.01 to 3.10 (Chang et al., 2014).

3.3.3 Occurrence of organic microcontaminants (OMCs)

Soil

Table 3.3 shows that only 25 of the 33 OMCs studied in the sampled agricultural soils were detected over the LOQs in at least one site. Unexpectedly, the concentrations of OMCs ranged from non-detectable to 397 ng/g dw for TCPP (P1). The highest concentration values were recorded for TCPP and bisphenol F (Plots 1 and 5). The high values of TCPP and BPF could be due to the use of plastic tubing for drip and sprinkler irrigation. In fact, TCPP is used as a raw material in the manufacture of polyester, plastic foam, binder, and resins, while bisphenol F and other bisphenol analogues have gradually emerged as substitutes for bisphenol A in various applications, such as the plastic and canning industries, due to their similar physicochemical properties (Regueiro and Wenzl, 2015). The concentrations of OMCs detected in the soils of the area of study were in the same range as those published in the peri-urban horticultural area of La Plata (Buenos Aires, Argentina) for azoxystrobin (0.8–153 ng/g dw) and lower than those for chlorpyrifos (79–2258 ng/g dw) (Mac Loughlin et al., 2017). The average concentration of OMCs in soil was higher in P1 and P5 (>14 ng/g dw) than in P2- P4 (<4 ng/g dw). This is probably due to the use of plastic tubing for water irrigation in these two farm plots. Paired t-tests showed statistical differences ($p < 0.05$) in all the sites.

Table 3.3 Concentration of OMCs (ng/g dw) in soil samples during the summer campaign.

	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5
Azoxystrobin	nd	nd	nd	3.82	nd
Chlorpyrifos	nd	nd	nd	6.68	nd
N,N-Diethyl-meta-toluamide (DEET)	1.4	<0.22[a]	<0.22	0.48	0.56
Diazinon	nd	nd	nd	nd	3.24
Dimethomorph	nd	nd	8.96	nd	16.9
Surynol 104	<0.10	<0.10	<0.10	<0.10	<0.10
Tris(2-chloroethyl) phosphate (TCEP)	1.1	nd	0.23	nd	2.3
2-Mercaptobenzothiazole (2MBT)	<4.3	<4.3	5.4	<4.3	<4.3
5-Methyl-2H-benzotriazole (5TTri)	<0.12	<0.12	<0.12	<0.12	<0.12
Bisphenol A (BPA)	<4.2	<4.2	<4.2	<4.2	<4.2
Butylparaben	<0.15	nd	nd	nd	0.28
Bisphenol F (BPF)	199	<9	10	<9	106
Benzothiazole	<4.1	<4.1	7.9	<4.1	18
Benzotriazole	<0.41	<0.41	6.8	<0.41	13
Carbamazepine	<0.12	<0.12	0.62	0.14	0.34
Carbendazim	nd	nd	nd	nd	1.27
Lorazepam	<5.91	nd	<5.91	nd	<5.91
Metylparaben (MPB)	<6.2	30	<6.2	<6.2	18
1-Hydroxybenzotriazole (OHBT)	5.6	5.5	5.8	5.8	6.8
Octylphenol	<0.63	<0.63	<0.63	<0.63	<0.63
Propylparaben	<0.19	<0.19	<0.19	<0.19	0.48
Pymetrozin	2.0	1.4	2.4	1.3	2.1
Pyraclostrobin	nd	nd	0.23	nd	2.7
Carbamazepine-10,11-epoxide	nd	<0.21	<0.21	<0.21	<0.21
Tris (chloroisopropyl) phosphate (*TCPP*)	397	<21	<21	<21	114

nd = not detected, concentration values have been corrected by the recoveries.

[a] Values between LOQ and LOQ are shown.

Lettuce

Table 3.4 shows that 8 of the 25 compounds detected in soil samples were above the LOQs in the lettuce samples. Similarly, as in the agricultural soil, methylparaben (106-193 ng/g fw in P1), 2-mercaptobenzothiazole (2.39-40.5 ng/g fw in P1) and bisphenol F (17.8-104 ng/g fw in P3) were among the ones detected at highest concentrations. Whereas the occurrence of bisphenol F and 2MBT can be attributed to the presence of plastic materials in the agricultural fields (pipelines or plastic mulch or film), the high abundance of MPB may be due to its use as a fungicide in agriculture, although it can also be biosynthesized by some plants (Calvo-Flores et al., 2018). MPB is also released into water bodies via domestic and industrial wastewater (Becerra-Herrera et al., 2018). Fungicides, dimethomorph, and carbendazim were only detected in winter in P3 and P5, at concentrations up to 39 ng/g fw (Table 3.4). This is consistent with the need for an early application of fungicide in winter to reduce downy mildew and lettuce drop disease. Hence, direct foliar application of these fungicides could be the main source of their presence in lettuce crops. These results are higher than the concentration levels at which carbendazim has been detected in lettuce (2-7 ng/g fw) sold at farmers' markets from January to March in Hatay (Turkey) (Esturk et al., 2014). In addition, carbamazepine, one of the most widely reported pharmaceuticals in crops due to its high plant uptake, and its transformation product (epoxy-carbamazepine) were detected in almost all the farm plots. This is in keeping with the results reported Malchi et al. (2014), where CBZ and EPOCBZ were detected in carrots and sweet potatoes irrigated with secondary treated wastewater under field conditions.

Table 3.4 Minimum, maximum and median concentration (ng/g fw) of the OMCs detected in lettuce samples during summer and winter campaigns. Only Plots 3 and 4 were planted during the summer season. Concentration values have been corrected by recoveries.

	Plot 1 winter	Plot 2 winter	Plot 3 winter	Plot 3 summer	Plot 4 winter	Plot 4 summer	Plot 5 winter
Dimetomorph	nd	nd	(15.3–20.5) 19.5	nd	nd	nd	(0.42–0.62) 0.59
Surfynol 104	<4.07	<4.07	(<4.07–8.10) 7.57	<4.07	<4.07	<4.07	(<4.07–6.77)
2MBT	(2.39–40.5) 15.3	<0.66	<0.66	<0.66	(<0.66–0.74)	<0.66	(<0.66–1.74) 1.51
BPF	(1.11–1.91) 1.61	(1.58–3.92) 3.01	(17.8–104) 62.0	(1.34–3.41) 2.28	(15.9–31.2) 22.2	(13.3–49.6) 15.5	(0.59–2.32) 2.16
Carbamazepine	(0.18–0.23) 0.20	(0.11–0.13) 0.12	(0.13–0.32) 0.23	(0.33–0.56) 0.36	(0.08–0.18) 0.11	(0.10–0.51) 0.16	(0.13–0.20) 0.14
Carbendazim	nd	nd	(<0.24–2.61) 2.24	<0.24	nd	nd	(<0.24–38.9) 19.9
MPB	(106–193)136	(45.2–47.2) 46.4	(24.9–31.9) 28.7	(23.6–31.5) 25.0	(25.2–47.9) 31.6	(25.8–41.4) 34.9	(48.3–83.3) 64.3
Carbamazepine epoxide	(0.19–0.24) 0.21	nd	(0.05–0.07) 0.06	(0.08–0.10) 0.09	(0.08–0.25) 0.16	0.09	(0.08–0.13) 0.11

nd= not detected

In summary, although previous studies have reported the occurrence of 33 OMCs in the irrigation waters (Margenat et al., 2017), only two were taken up by plants from the water (i.e. carbamazepine and surfynol 104). Therefore, this study shows that the occurrence of OMCs in irrigation waters is not the main source of OMCs in crops, as other pollutants released by the irrigation system or pesticide applications (carbendazim, dimethomorph, 2MBT, BPF, and MPB) were detected in higher concentrations. Based on the two-paired test, it was concluded that the concentrations of OMCs in groundwater irrigated vegetables (P1 and P5) are statistically different ($p < 0.05$) from those observed in surface water irrigated crops (P2 to P4). Higher occurrence of 2MBT in groundwater irrigated crops (P1) can be attributed to the use of drip and sprinkle irrigation, whereas the higher occurrence of MPB is of unknown origin. Conversely, although BPF appeared only in soil samples from drip (P1) and sprinkle irrigation fields (P5), it was detected in the crops of all farm plots, which demonstrates its ubiquity. The values of the detected fungicides - carbendazim and dimethomorph (P3 and P5) - were compliant with the levels established for lettuce by Regulation (EC) No 396/2005 on maximum residue levels of pesticides in food (EC, 2005), as the legislated values are about 15 mg/kg fw for dimethomorph and 0.1 mg/kg fw for carbendazim.

3.3.4 Bioconcentration factors for OMCs

The BCFs for detected compounds ranged from to 1 to 375 (Table S3.9). The compounds to show the highest BCFs were carbendazim, due to its direct application as a fungicide (375), and bisphenol F, which is released by the drip irrigation system (200). Except for these two compounds, all other BCF values were in the range of those observed in plants grown in soil but lower than those observed in plants grown under hydroponic conditions (Wu et al., 2015). For instance, in a real field-scale study, Wu et al. (2015) found that carbamazepine had BCF values up to 20 as it is easily transferred from soil to plant, which is in keeping with the values found in the present study (6 to 53). BCFs from crops harvested in summer were lower than those from winter season. This can be explained by the higher lipidic content in summer (Table 3.5).

Table 3.5 Minimum, maximum and average levels of different lettuce quality parameters studied (*n* = 5).

	Plot 1 winter	Plot 2 winter	Plot 3 winter	Plot 3 summer	Plot 4 winter	Plot 4 summer	Plot 5 winter
Water content (%)	95.0	95.3	96.1	95.2	95.7	95.2	95.8
Chl_T (mg/cm^2)	(0.40–0.80) 0.58	(0.4–0.7) 0.6	(0.4–0.7) 0.6	(1.1–1.2) 1.2	(0.5–0.6) 0.6	(1.1–1.2) 1.1	(0.2–0.6) 0.4
Nitrates (mg/kg)	(1113–1543) 1331	(729–862) 793	(1467–1854) 1648	(1264–1411) 1316	(739–1039) 843	(628–835) 736	(1202–1827) 1427
Lipids (%)	(0.13–0.17) 0.15	(0.10–0.23) 0.16	(0.10–0.16) 0.13	(0.22–0.23) 0.23	(0.13–0.23) 0.20	(0.17–0.27) 0.22	(0.17–0.33) 0.24
Carbohydrates (%)	(4.03–4.46) 4.20	(3.78–5.53) 4.37	(3.36–4.23) 3.66	(2.76–5.23) 4.20	(4.58–5.94) 5.15	(5.20–6.96) 5.71	(4.64–5.43) 5.03

3.3.5 Effects of the occurrence of chemical pollutants in lettuce

The chlorophyll, nitrate, lipid, and carbohydrate content of the sampled lettuces were analyzed to evaluate the effects of chemical pollutants in fresh lettuces (Table 3.5). Lettuces harvested in summer showed statistically higher chlorophyll concentration levels ($p < 0.05$) than those harvested in winter, which can be accounted for by the longer exposure to sunlight during the summer season (Gent, 2014). The concentration of nitrates ranged from 628 to 1854 mg/kg. Although nitrate concentrations in vegetables are considered to be of low toxicity, nitrate is easily reduced to nitrite, which can pose a risk to human health (Guadagnin et al., 2005). The European Union established maximum values for nitrate content in lettuce produced in open fields of 2500 mg/kg in summer and 4000 mg/kg in winter (Regulation (EC) No 1881/2006) (EC, 2006). None of the harvested lettuce showed values above the maximum legislated levels. Since the nitrate concentration in vegetables depends on the harvesting period, agricultural system, maturation stage, and plant part, almost all the sampling sites had a statically different ($p < 0.05$) nitrate content from the other sites. Furthermore, unlike other (Guadagnin et al., 2005; Woese et al., 1997), the organic farming plot (P1) was not found to have lower nitrate concentrations. This could support the idea that nitrate content is subject to many factors. For instance, in this study nitrate content in irrigation waters (Margenat et al., 2017) seemed to be a relevant factor since it was higher in P1, P3, and P5. Fig. S3.1 shows a strong relationship between nitrate content in lettuce and irrigation water (Pearson correlation coefficient of 0.897 and *p*-value < 0.05), which means that the main source of nitrates in crops is plant uptake from irrigation water.

Finally, the lipid and carbohydrate content were analyzed, as the lipid portion has been shown to be a major reservoir for the storage of OMCs (Yang et al., 2016). The concentration of lipids and carbohydrates was shown to be strongly dependent on both the farm plot and the season. Specifically, P4 and P5 had a higher carbohydrate content than the other farm plots studied, and lettuce harvested in summer had higher lipid and carbohydrate content than lettuce harvested in winter (P3). This is consistent with the fact that greater sunlight intensity in summer promotes higher sugar accumulation in plants (Gent, 2014). Furthermore, the results show a negative relationship between nitrate content and sugar content, as has been previously reported for the stabilization of osmotic potential in plant tissues (Blom-Zandstra and Lampe, 1985).

3.3.6 Correlation analysis (PCA)

PCA with Varimax normalized rotation was performed, reducing the 76 measured variables to six principal components with eigenvalues >1 explaining 95.7% of the total variance observed.

The first principal component (PC1), which explains 31.4% of the variance in the dataset, showed strong positive loadings (>0.8, Table S3.10) for some OMCs in soil (PPB, carbendazim, surfynol 104, BPB, BT, TCEP, dimethomorph), other OMCs in lettuce (carbendazim), TEs in soil (Mn, Rb, Mg, Co, Cr, Li, and K), a few TEs in lettuce (Li), soil moisture, and soil cation-exchange capacity. The positive loadings of these compounds correlated with a high chemical pollution in the soil as well as fungicide application in lettuce (carbendazim); the highest contribution was for the P5 site. This finding indicates that PC1 was associated with pollution.

Another 25.0% of the variance is explained by the second principal component (PC2), which has high positive loading values (Table S3.10) for TEs in soil (Hg, Ca, Na), TEs in lettuce (Hg, Zn), EPOCBZ in soil, and total organic carbon (TOC) in soil. This correlated with sampling sites collated in the peri-urban area, where Na and TOC values are high. Negative loading values were found for MPB in lettuce and TCPP and BPF in soil, which correlates with the control site.

Finally, the third principal component (PC3) explains 19.9% of the variance in the dataset and has high loadings for soil electrical conductivity, some OMCs in soil (2MBT, carbamazepine, pymetrozin), Mn and carbamazepine in lettuce, and nitrate content in lettuce. This correlates with the impact of reclaimed water (P3), as has previously been determined in the characterization of the irrigation waters (Margenat et al., 2017), a finding that is consistent with the higher soil conductivity and carbamazepine concentration observed in this farm plot.

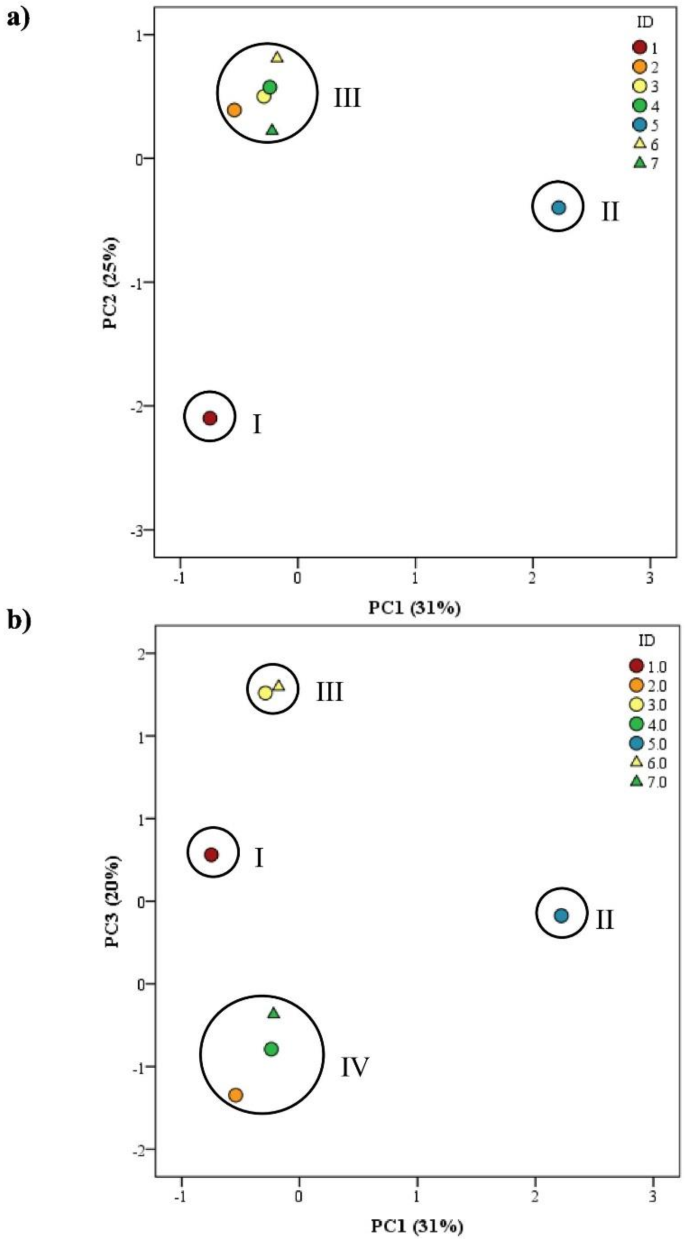


Figure 3.1 Principal Component Analysis (PCA) results. a) Scores plot PC1 vs PC2 (ID1=Plot1 winter, ID2=Plot2 winter, ID3=Plot3winter, ID4=Plot4 winter, ID5=Plot5 winter, ID6=Plot3 summer, ID7=Plot4 summer), b) Scores plot PC1 vs PC3

Fig. 3.1a shows the three main groups differentiated with regard to the first two PCs. The three main groups are the rural plot (I), P5 (II), and P2–P4 (III). As can be seen, the first differential characteristic depends on the proximity of the sampling site to the urban area. P2–P5 are located in the peri-urban area, whereas P1 is located in a rural area unexposed to urban pollution. However, P5 is impacted by industrial effluents and other soil pollution due to the use of urban biosolids and pesticides. This makes this farm plot the most polluted site. Fig. 3.1b shows the score plots of PC1 vs PC3. As can be seen, the farm plots from the peri-urban area can be grouped into 3 clusters: the plots irrigated with river water (group IV, P2 and P4), the farm plot irrigated with reclaimed water (group III, P3), and the farm plot irrigated with groundwater (group II, P5). Therefore, the main differences observed in this second score plot (Fig. 3.1b) with regard to pollution (organic and inorganic) in soil and lettuce are due to the quality of the irrigation water.

3.4 Conclusions

The results of this study demonstrate that peri-urban pollution increases the occurrence of pollutants but does not affect lipid and carbohydrate content.

- The concentrations of TEs and OMCs ranged from 0.790 to 803 mg/kg dw and from <0.1 to 397 ng/g dw, respectively, in the peri-urban soil, whereas they ranged from $6{\cdot}10^{-5}$ to 5 mg/kg fw and from b0.1 to 193 ng/g fw, respectively, in the lettuce crops.

- The concentrations of metals in the soil from the rural area were always below the Catalonian guidelines, but limits were exceeded for Mo, Ni, Pb, and As in the soils of the peri-urban area. However, their occurrence in lettuce complied with human food safety standards (except for Pb in peri-urban area).

- The many fungicides (carbendazim, dimethomorph, and MPB) and chemicals released by plastic pipelines (TCPP, BPF, and 2-MBT) used in agriculture were prevalent in the soil or the edible parts of the lettuce. In contrast, chemicals from irrigation waters (carbamazepine, surfynol 104) were not.

- The BCFs of TEs ranged from 0.0002 to 2, whereas for OMCs such as pesticides and plastic-related compounds it depended on whether or not they came into direct or indirect (use of plastic pipelines) contact with the lettuce leaf surface.

- Chlorophyll, lipid, and carbohydrate content in crops grown in the peri-urban area were not affected by soil or irrigation water pollution, whereas nitrate content depended on the irrigation water quality.

- PCA showed that peri-urban pollution and water irrigation quality could explain a large share of the variance in the dataset.

Although the present study showed that lettuce exposure to peri-urban pollution did not affect lipid and carbohydrate content, further studies are necessary to assess changes in agri-food quality associated with peri-urban pollution. Similarly, further work is needed on the potential effects of chemical pollutants released by plastic irrigation pipes on crops.

3.5 Supporting Information

3.5.1 Materials and reagents

Most of the reagents, OMCs and surrogates are described in section 2.5.1.

N,N-dimethylformamide was obtained from Merck and and 0.70 µm of glass-fiber filters 47 mm in diameter were obtained from Whatman (Maidstone, UK).

3.5.2 Analytical determination of chemical pollutants in soil and crop samples

ICP-MS and ICP-OES determination

An inductively coupled plasma optical emission spectrometer (Thermo Scientific, iCAP 6500 ICP-OES) and an inductively coupled plasma mass spectrometer (Thermo Scientific, XSeries 2 ICP-MS) were used for the determination of TEs.

Major elements were determined by ICP-OES (Ba and Mn), while the rest of TEs were determined by ICP-MS.

Reagent water was used as a blank matrix, and laboratory reagent blank was treated exactly the same as a sample. A limit of detection (LOD) of 0.2 µg/L in the solution analyzed was determined from three times the standard deviation obtained from the analysis of ten runs of blank samples on the same day as the determinations.

GC-MS/MS determination (SI)

GC-MS/MS determination is described in section 2.2.3.
LODs, LOQs, recoveries of the surrogates and recoveries of the targeted compounds are reported in Tables S3.2-3.4 and Tables S3.5-3.7.

LC-MS/MS determination

Samples extracted by sonication, were analyzed with a Waters Acquity Ultra-Performance liquid chromatography system coupled to a Waters TQ-Detector (Manchester, UK). Autosampler was set at 15ºC and a volume of 10 µL of sample was injected to the liquid chromatography system fitted with an Ascentis Express RP-Amide column (5 cm x 2.1mm, 2.7 µm particle size, Supelco, Bellefonte, USA) and with a guard column (0.5 cm x 2.1 mm) containing the same packing material.

The flow rate was 0.35 mL/min and the gradient conditions of mobile phase A (acetonitrile 0.1% formic acid) and mobile phase B (water + 0.1% formic acid) were set as follows: 0-1 min 3% of A, 1-7 min 3-25% of A, 7-10 min 25-95% of A, 10-15 min 95% of A, 15-17% min, 15-17 min 95-3%, 17-23 min 3% of A. Column oven was set at 25ºC. Ions were generated with an electrospray in positive mode (ESI+). Source and desolvation temperature were set to 80ºC and 350ºC, respectively. Qualitative analysis was performed as in GC-MS/MS, quantitative analysis was performed through matrix-matched calibration.

Table S3.1. General parameters of soil samples

	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Methodology
Humidity at 105ºC (%)	<1	<1	<1	<1	<1	Gravimetry
Nitrogen-nitric (mg/Kg)	9	19	10	2	12	Colorimetry
Phosphorous (mg/Kg)	64	15.6	67	35	102	Spectrophotometry UV-VIS
Potassium (mg/Kg)	375	183	309	346	512	Spectrophotometry ICP-OES
Calcium (mg/Kg)	2984	6422	6498	6561	6598	Spectrophotometry ICP-OES
Magnesium (mg/Kg)	379	330	390	360	510	Spectrophotometry ICP-OES
Sodium (mg/Kg)	32	152	161	136	109	Spectrophotometry ICP-OES
Cation exchange capacity (mS·cm^{-1})	7.5	8.0	9.3	9.7	11.5	Volumetric titration
pH	7.6	7.8	7.6	7.7	7.8	Potentiometry
Electrical conductivity (mS·cm^{-1})	2.3	2.3	2.8	2.2	2.3	Conductimetry
Texture	Sandy loam	Sand	Sandy loam	Sandy loam	Sand	Granulometry

Table S3.2 Limits of detection (LOD) and quantification (LOQ) of soil samples

Analyte	LOD (ng/g dw)	LOQ (ng/g dw)
Atrazine	0.44	0.46
Azoxystrobin	0.36	0.37
2-tert-Butyl-4-methoxyphenol (BHA)	0.03	0.04
Chlorpyrifos	0.04	0.06
N.N-Diethyl-meta-toluamide (DEET)	0.19	0.22
Diazepam	0.11	0.12
Diazinon	0.36	0.37
Dimethomorph	0.29	0.30
Indoxacarb	0.29	0.30
Simazine	0.37	0.38
Surfynol 104	0.96	1.01
Tris(2-Chloroethyl) Phosphate (TCEP)	0.17	0.18
2-Mercaptobenzothiazole (2-MBT)	4.33	4.51
5-Methyl-2H-benzotriazole (5-TTri)	0.12	0.13
Bisphenol A (BPA)	4.22	4.24
Butylparaben (BPB)	0.14	0.16
Bisphenol F (BPF)	9.57	10.06
1,3-Benzothiazole (BT)	4.04	4.09
Benzotriazole (Btri)	0.39	0.40
Carbamazepine (CBZ)	0.46	0.47
Carbendazim	0.22	0.23
Ethyl paraben (EPB)	3.09	3.63
Lorazepam	5.91	5.92
Methyl paraben (MPB)	6.18	6.92
1-Hydroxybenzotriazole (OHBT)	10.8	11.0
Octylphenol (OP)	0.63	0.64
Oxazepam	0.51	0.52
Propyl paraben (PPB)	0.20	0.21
Primidone	0.17	0.18
Pymetrozin	0.88	0.89
Pyraclostrobin	0.04	0.06
Carbamazepine-10,11-epoxide (EPOCBZ)	0.21	0.40
Tris(1-chloro-2-propyl) phosphate (TCPP)	20.9	21.4

Table S3.3 Recoveries (%) of surrogates in soil samples

Surrogate	R(%) at 7 ng/g dw
Bisphenol A-d_{16}	88±5
Caffeine-$^{13}C_3$	10±0.9
Carbamazepine-$^{13}C_6$	65±5
Diazepam-d_5	68±8
5,6-dimethyl-1H-benzotriazole (XbTri)	50±6
Etylparaben-^{13}C	67±3

Table S3.4 Absolute recoveries (%) of analytes in soil samples

Compound	R(%) at 7 ng/g dw
Atrazine	39±5
Azoxystrobin	71±0.3
Chlorpyrifos	32±4
N.N-Diethyl-meta-toluamide (DEET)	49±5
Diazepam	38±5
Diazinon	33±4
Dimethomorph	61±9
Indoxacarb	46±1
Simazine	62±1
Surfynol 104	44±5
Tris(2-Chloroethyl) Phosphate (TCEP)	50±0.8
5-Methyl-2H-benzotriazole (5-TTri)	54±5
Bisphenol A (BPA)	72±9
Butylparaben (BPB)	42±0.6
Bisphenol F (BPF)	66±21
1.3-Benzothiazole (BT)	76±16
Benzotriazole (Btri)	10±0.8
Carbamazepine (CBZ)	58±3
Carbendazim	75±7
Ethyl paraben (EPB)	54±4
Lorazepam	68±6
Methyl paraben (MPB)	120±22
1-Hydroxybenzotriazole (OHBT)	85±13
Octylphenol (OP)	46±2
Oxazepam	56±3
Propyl paraben (PPB)	53±2
Carbamazepine-10,11-epoxide (EPOCBZ)	84±6
Tris(1-chloro-2-propyl) phosphate (TCPP)	87±13

nd: non detected

Table S3.5 Limits of detection (LOD) and quantification (LOQ) of lettuce's samples

Compound	LOD (mg/kg fw)	LOQ(mg/kg fw)
Atrazine	0.11	0.18
Azoxystrobin	0.22	0.35
2-tert-Butyl-4-methoxyphenol (BHA)	0.025	0.028
Chlorpyrifos	0.56	0.94
N.N-Diethyl-meta-toluamide (DEET)	0.32	0.7
Diazepam	0.03	0.05
Diazinon	0.35	0.63
Dimethomorph	0.03	0.06
Indoxacarb	0.49	0.83
Simazine	0.19	0.3
Surfynol 104	4.1	6.5
Tris(2-Chloroethyl) Phosphate (TCEP)	0.82	1.5
2-Mercaptobenzothiazole (2-MBT)	0.66	1
5-Methyl-2H-benzotriazole (5-TTri)	0.26	0.43
Bisphenol A (BPA)	0.24	0.39
Butylparaben (BPB)	0.16	0.22
Bisphenol F (BPF)	0.51	0.77
1.3-Benzothiazole (BT)	1.2	1.7
Benzotriazole (Btri)	0.96	1.6
Carbamazepine (CBZ)	0.05	0.15
Carbendazim	0.24	0.33
Ethyl paraben (EPB)	0.39	0.66
Lorazepam	0.82	1.3
Methyl paraben (MPB)	0.81	0.99
1-Hydroxybenzotriazole (OHBT)	0.38	0.6
Octylphenol (OP)	0.11	0.18
Oxazepam	0.38	0.63
Propyl paraben (PPB)	15	24
Primidone	0.26	0.33
Pymetrozin	3.2	5.1
Pyraclostrobin	3.7	6.6
Carbamazepine-10,11-epoxide (EPOCBZ)	0.1	0.3
Tris(1-chloro-2-propyl) phosphate (TCPP)	16	23

Table S3.6 Recoveries (%) of surrogates in lettuce's samples

Surrogate	R(%) at 10 ng/g fw
Bisphenol A-d_{16}	38±3
Caffeine-$^{13}C_3$	46±4
Carbamazepine-$^{13}C_6$	111±14
Diazepam-d_5	15±2
5.6-dimethyl-1H-benzotriazole (XbTri)	73±7
Etylparaben-^{13}C	74±8

Table S3.7 Absolute recoveries (%) of analytes in lettuce's samples

Compounds	R(%) at 10 ng/g fw
1-hydroxybenzotriazole (OHBT)	76±5
2-mercaptobenzothiazole (2MBT)	85±6
2-tert-Butyl-4-methoxyphenol (BHA)	74±4
4-tert-octylphenol (OP)	48±3
5-methyl-2H-benzotriazole (5TTri)	71±1
Azoxystrobin	35±8
Benzothiazole	66±1
Benzotriazole	86±3
Bisphenol A	34±2
Bisphenol F	20±2
Butylparaben	39±4
Carbamazepine	152±6
Carbamazepine-10.11-epoxide (EPOCBZ)	95±14
Carbendazim	40±2
DEET	46±3
Dimethomorph	35±3
Etylparaben	60±4
Indoxacarb	n.a.
Lamotrigine	n.a.
Lorazepam	n.a.
Methylparaben	58±8
Oxazepam	96±2
Primidone	52±5
Propylparaben	72±3
Pymetrozin	70±6
Pyraclostrobin	n.a.
Simazine	41±1
Surfynol 104	89±13
Tris(1-chloro-2-propyl) phosphate (TCPP)	44±6
Tris(2-chloroethyl) phosphate (TCEP)	37±4

n.a. not evaluated

Table S3.8 BCF for the TEs selected in this study

	Plot 1 winter	**Plot 2 winter**	**Plot 3 winter**	**Plot 3 summer**	**Plot 4 winter**	**Plot 4 summer**	**Plot 5 winter**
B	0.36	0.28	0.50	0.41	0.54	0.47	0.62
Ba	0.19	0.05	0.05	0.06	0.05	0.04	0.03
Cd	-*	-	0.69	0.79	-	-	-
Co	0.07	0.03	0.02	0.02	0.02	0.02	0.03
Cr	0.12	0.06	0.07	0.06	0.06	0.15	0.05
Cu	0.28	0.20	0.15	0.16	0.10	0.08	0.13
Li	0.09	0.04	0.03	0.04	0.03	0.03	0.03
Mn	0.19	0.07	0.15	0.14	0.09	0.08	0.07
Mo	0.22	0.14	0.16	0.13	0.28	0.46	0.18
Ni	0.07	0.04	0.03	0.04	0.05	0.17	0.05
Pb	0.06	0.03	0.01	0.03	0.02	0.02	0.06
Rb	0.24	0.27	0.25	0.19	0.29	0.24	0.17
Sb	-*	-	-	-	-	-	-
Zn	0.17	0.29	0.35	0.25	0.28	0.23	0.22
As	0.00045	0.0005	0.0003	0.0008	0.0006	0.0004	0.0002
Hg	0.64	0.07	0.06	0.07	0.05	0.04	0.12

**soil concentration below LOD*

Table S3.9 BCF of the CECs selected in this study

	Plot 1 winter	Plot 2 winter	Plot 3 winter	Plot 3 summer	Plot 4 winter	Plot 4 summer	Plot 5 winter
Dimetomorph	*	*	64	*	*	*	1
2-MBT	*	*	*	30	*	*	*
BPF	1	*	200	6	*	*	2
Carbamazepine	*	*	10	6	53	24	8
Carbendazim	*	*	*	*	*	*	375
MPB	*	30	*	*	*	*	85

**soil concentration below LOD*

Table S3.10 Loadings for PCA.

	Component					
	1	2	3	4	5	6
PPB (ng/g) soil	.980	-.175	-.040	-.080	.016	-.037
Carbendazim (ng/g) soil	.978	-.176	-.039	-.092	.015	-.040
Carbendazim (ng/g) lettuce	.976	-.152	.026	-.111	.105	-.021
Mn (ng/g) soil	.974	.206	.081	.013	.048	.028
Moisture (%) soil	.972	-.154	-.058	.164	.031	.029
Rb (ng/g) soil	.969	.184	-.144	.069	-.020	.034
BPB (ng/g) soil	.945	.296	-.090	-.099	.041	.020
Mg (mg/Kg) soil	.928	-.209	.301	.046	.028	-.029
BT (ng/g) soil	.918	.071	.273	-.024	-.030	.278
Co (ng/g) soil	.901	.334	-.214	-.170	.035	.014
CAP.Interc.Cat. (meq/100g) soil	.868	.349	.025	.322	.070	.128
Cr (ng/g) soil	.866	.489	-.040	-.068	.054	.049
Li (ng/g) lettuce	.847	-.050	.052	-.418	-.220	-.234
Li (ng/g) soil	.843	.488	-.205	-.061	.049	.061
TCEP (ng/g) soil	.830	-.545	.042	-.072	-.009	-.087
Dimethomorph (ng/g) soil	.829	.119	.522	-.150	.039	-.051
Btri (ng/g) soil	.814	.173	.386	.001	-.048	.396
K (mg/Kg) soil	.770	-.407	.154	.461	.031	.060
Ba (ng/g) soil	.770	.524	-.298	.081	.142	.131
P (mg/Kg) soil	.712	-.310	.621	.087	.025	-.051
B (ng/g) soil	-.696	.168	-.160	-.663	-.055	-.141
MPB (ng/g) lettuce	.030	**-.987**	.011	-.113	-.011	-.107
TCPP (ng/g) soil	-.070	**-.981**	.115	.031	-.058	-.123
BPF (ng/g) soil	.167	**-.971**	.112	.010	-.054	-.116
Hg (ng/g) soil	-.113	.967	-.006	.149	.064	.160
Na (mg/Kg) soil	-.050	.939	.290	-.157	.054	.061
TOC(%) soil	.258	.926	.206	-.157	.062	.066
Ca (mg/Kg) soil	.357	.916	-.125	-.022	.064	.119
Hg (ng/g) lettuce	.357	.829	.376	-.194	.018	-.081
2MBT (ng/g) lettuce	-.277	**-.821**	.320	.076	-.314	-.205
As (ng/g) soil	.004	.799	.056	.393	-.449	.052
Zn (ng/g) lettuce	.037	.786	-.458	-.042	.412	-.016
Cu (ng/g) lettuce	-.127	.783	-.343	.495	.059	.068
Cd (ng/g) lettuce	-.190	.774	.415	-.336	-.034	-.279
Pb (ng/g) soil	-.053	.768	.064	.422	-.471	.049
B (ng/g) lettuce	-.216	.713	.235	.123	.609	-.061
Carbamazepine epoxide (ng/g) lettuce	-.050	-.680	-.091	.620	.063	-.373
Co (ng/g) lettuce	.644	-.676	-.308	-.158	-.034	-.087
Zn (ng/g) soil	.306	.568	-.541	.089	.470	.249
As (ng/g) lettuce	.411	.504	.428	.158	-.474	-.381
Mn (ng/g) lettuce	.090	-.166	**.968**	.087	.131	-.044
Carbamazepine (ng/g) lettuce	-.127	.182	**.952**	-.036	-.209	-.001
Pymetrozin (ng/g) soil	.234	-.071	**.951**	-.159	.018	-.104
Conductivity (mS) soil	-.107	.291	**.931**	-.179	.028	-.063
Lorazepam (ng/g) soil	-.181	.360	**-.900**	.108	.000	.125

2-MBT (ng/g) soil	.061	.429	**.897**	-.074	.047	-.017
Nitrates(mg/Kg) lettuce	.187	-.232	**.879**	-.091	.328	-.153
Carbamazepine (ng/g) soil	.212	.459	**.860**	-.033	.055	.000
pH soil	.404	.251	**-.849**	-.227	-.006	.024
Ni (ng/g) soil	.411	.632	.645	-.104	.063	.017
N-NO_3 (mg/Kg) soil	.113	-.044	.046	-.957	-.054	-.253
MPB (ng/g) soil	.304	.067	-.508	-.785	-.045	-.162
Mo (ng/g) soil	.066	.478	.447	-.738	.008	-.152
Carbohydrates(%) lettuce	.264	.216	-.527	.697	-.341	.060
Cu (ng/g) soil	.038	.549	-.403	.678	.065	.266
Sb (ng/g) soil	.598	-.329	.302	-.632	.104	-.181
Moisture(%) lettuce	.384	.226	.222	.043	.854	-.143
BPF (ng/g) lettuce	-.199	.383	.273	.225	.819	.133
Dimethomorph (ng/g) lettuce	-.102	.217	.557	-.155	.762	.165
Cd (ng/g) soil	-.024	.327	.600	-.032	-.699	-.207
Surfynol 104 (ng/g) lettuce	.539	.075	.456	-.193	.668	.117
Sb (ng/g) lettuce	-.483	-.380	.415	-.155	-.649	.071
Pb (ng/g) lettuce	.405	.555	.328	.256	-.577	.143
Chl_T(mg/cm2) lettuce	-.376	.443	.276	.304	-.571	.409
Rb (ng/g) lettuce	.163	.504	-.525	.343	.559	.116
Lipids(%) lettuce	.406	.307	-.247	.524	-.530	.354
Cr (ng/g) lettuce	-.043	-.002	-.190	.274	.077	.939
Mo (ng/g) lettuce	.040	.290	-.199	.261	.174	.881
Ni (ng/g) lettuce	.189	.193	-.149	.307	-.277	.857
Ba (ng/g) lettuce	-.351	-.463	-.140	.409	-.137	-.676

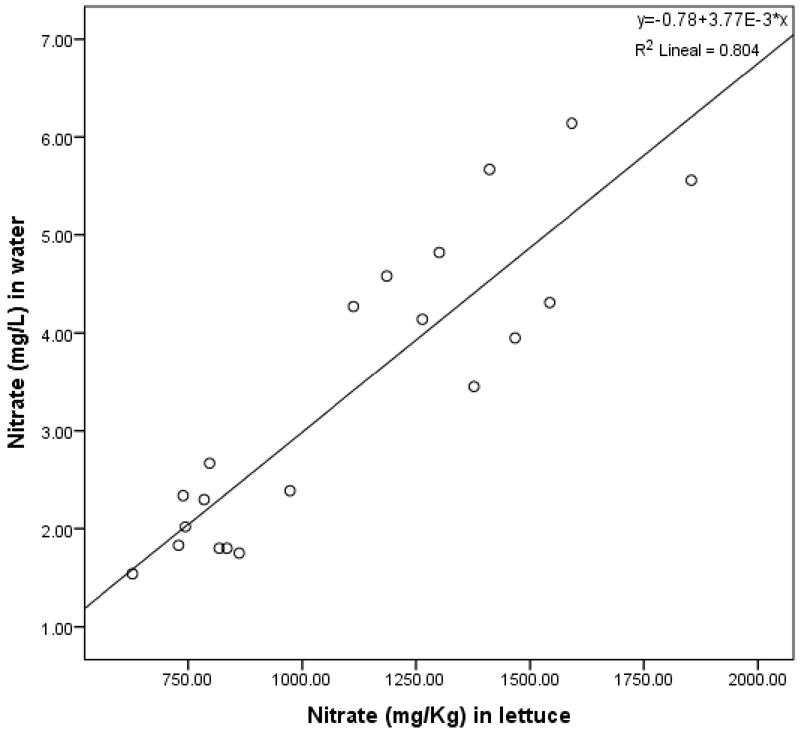


Figure S3.1 Dispersion diagram and linear correlation between nitrate content in water and in lettuce

Chapter IV: Occurrence and human health implications of chemical contaminants in vegetables grown in peri-urban agriculture

This chapter is based on the article under review:

Margenat, A., Matamoros, V., Díez, S., Cañameras, N., Comas, J., & Bayona, J. M. Occurrence and human health implications of chemical contaminants in vegetables grown in peri-urban agriculture. Environment International.

Recent studies have proven that vegetables cultivated in peri-urban areas are exposed to a greater concentration of OMCs and TEs than those grown in rural areas. In this study, the occurrence and human health risk of chemical contaminants (16 TEs and 33 OMCs) in edible parts of lettuce, tomato, cauliflower, and broad beans from two farm fields in the peri-urban area of the city of Barcelona and one rural site outside the peri-urban area were assessed. The concentration of TEs and OMCs ranged from non-detectable to 17.4 mg kg^{-1} and from non-detectable to 256 µg kg-^{1}, respectively. Tomato fruits showed the highest concentration of TEs and OMCs. Principal component analysis indicated that the occurrence of chemical contaminants in crops depended on the commodity rather than the location (peri-urban vs rural). Risk assessment using hazardous quotient (HQ) and threshold of toxicological concern (TTC) approaches showed that the risk for the consumption of target vegetables in the peri-urban area was low and similar to that observed for the rural site. Total HQ values for TEs were always below 1, and a minimum consumption of 150 g day^{-1} for children and 380 g day^{-1} for adults is required to reach the TTC due to the presence of pesticides. Further studies are needed to estimate the combined effect of TEs and OMCs on human health.

4.1 Introduction

The consumption of vegetables grown with peri-urban agriculture has grown exponentially due to the increase in urban demand (Olsson et al., 2016). Nevertheless, recent studies have highlighted that vegetables cultivated in peri-urban areas are generally exposed to a higher concentration of chemical contaminants than those grown in rural areas (Christou et al., 2017b; Säumel et al., 2012).

Many studies have demonstrated that industrial activities, dense traffic flows, and the reuse of treated wastewater for irrigation are important sources of contamination of food crops (Christou et al., 2017a; Kalavrouziotis et al., 2008; Khan et al., 2008; Nabulo et al., 2006). Therefore, concerns about the chemical contamination of vegetables grown on peri-urban farms have risen due to the potential human health risk implications of consuming contaminated vegetables (Augustsson et al., 2018; Huang et al., 2018). The main health risks associated with peri-urban horticulture include the contamination of vegetables with agrochemical residues and TEs such as heavy metals (Birley and Lock, 1999). Nevertheless, it is important to notice that soil amending may result in a higher vegetable exposure to heavy metals (Antisari et al., 2015). Conversely, the use of reclaimed water for crop irrigation might end with a reduction of heavy metals uptake in crops caused by antagonist effects (Kalavrouziotis and Koukoulakis, 2010), but it can also increase crop exposition to OMCs.

The detection of OMCs in the edible parts of plants has recently raised concerns regarding human exposure to these contaminants (Kalavrouziotis et al., 2012; Khan et al., 2008; Pan et al., 2014). In this regard, it has been reported that the presence of OMCs such as lamotrigine and 10,11-epoxycarbamazepine in sweet potatoes and carrots could have adverse effects on human health (Malchi et al., 2014; Prosser and Sibley, 2015). In the case of TEs such as heavy metals, although the potential risk associated with their presence in peri-urban vegetables is minimal, it may exceed the target hazard quotient for vulnerable populations such as children or pregnant woman (Hough et al., 2004; Nabulo et al., 2010). Nevertheless, few field studies exist on the occurrence of OMCs in vegetables (Malchi et al., 2014; X. Wu et al., 2014), and no risk assessment study has been done on the co-occurrence of metals, pesticides, and OMCs in peri-urban horticulture.

Estimation of the dietary exposure to chemical contaminants requires data on food consumption and the occurrence of each contaminant, which must then be compared with the pertinent health-based guidance value for the chemical of concern (WHO, 2008). Risk assessment can be based on either deterministic or probabilistic approaches. Deterministic approaches use a single value, such as a mean or percentile, to describe model variables. Their main drawback is thus the lack of insight they offer into the range of possible exposures and the proportion of the population that remains at risk. In contrast, probabilistic approaches, which are increasingly widely used (Quijano et al., 2017), account for the variability in food consumption and consumer body weight (BW), as well as the variability in the occurrence of the contaminant. Thereby, they address uncertainty and variability (Mondal and Polya, 2008), taking all possible scenarios into account (EFSA, 2012; FAO, 2009).

Although risk assessment methodologies have mainly focused on individual chemicals and a single exposure route (oral, inhalational, or dermal), many of these chemicals act on the same target receptor by similar pathways. Therefore, the cocktail effect has been deemed an important factor in assessing the interaction between chemicals (WHO, 2017). This effect can take the form of an independent action, a dose addition (whereby one compound in the mixture is replaced by another) or an interaction (either synergistic or antagonistic compared to the predicted values based on dose addition) (Borchers et al., 2010). A conservative risk assessment approach consists of the sum of the hazard quotients (HQs), assuming that the effect of exposure to the individual chemical contaminants is additive and there are no synergistic or antagonistic effects (Prosser and Sibley, 2015).

In previous studies, we found that vegetables cultivated in peri-urban areas are exposed to a higher concentration of chemical contaminants than those from rural sites, but the occurrence of these contaminants in lettuce vegetables was similar (Margenat et al., 2018). The present study aims to expand on those results and assess the occurrence of these contaminants (16 TEs and 33 OMCs) in several vegetables (lettuce, tomato, cauliflower, and broad beans), as well as to assess the potential risk to human health associated with the consumption of vegetables grown on peri-urban farms. This work was carried out on two farm plots located in the peri-urban area of Barcelona (NE Spain) and a rural plot located in a pristine area. The hypothesis was that the higher contamination exposure in peri-urban agriculture could result in a greater occurrence of contaminants in eatable vegetables and, consequently, in a greater human health risk.

4.2 Material and methods

4.2.1 Sampling site description

The study was carried out in the Llobregat River delta and its low valley (NE Spain) (Fig.2.1). Only farm plots P1, P3 and P4 were sampled. More details are provided in section 2.2.1

4.2.2 Sampling regime

Four types of vegetables (lettuce, tomato, cauliflower and broad beans) were sampled, as they are some of the most consumed vegetables in the metropolitan area of Barcelona city. Vegetables were collected when they reached its commercial size and only the edible part was used for analysis.

Lettuce (*Lactuca sativa* L. cv. Batavia) was planted in March 2016 and harvested in May 2016. More information of lettuce sampling is given in section 3.2.2. Tomatoes seedlings (*Lycopersicon esculentum*) were cultivated in summer 2016 and picked them up in September 2016. Cauliflower

(*Brassica oleracea* L. cv Skywalker) and broad beans were planted in winter and harvested in March 2017. All vegetables were planted in all studied sites, except broad beans which were not planted in P1.

Each farm plot was divided in 5 sections and from 5 to 15 specimens of vegetables or fruits were collected per section. A quarter of every lettuce and cauliflower were mixed and comminuted together with the aid of liquid nitrogen and a porcelain mortar, while tomatoes and broad beans were entirely used. Finally, they were stored at -20ºC until they were analyzed.

4.2.3 Analytical procedures

Chemicals and reagents used for the analytical methodologies are described in the supporting information section 4.5.1.

Trace element (TEs) extraction

A 1 g of vegetable tissue, previously dried, was extracted using HNO_3/HCl_4 digestion at 95ºC for 3h (EPA, 1994). The samples were then transferred to a 100-mL volumetric flask and centrifuged. A 10 mL aliquot was then diluted with 40 mL Milli-Q water prior to analysis in ICP-MS and ICP-OES. Further information is provided in section 4.5.2. of the SI.

OMCs extraction

Briefly, 2 g of fresh sample (cauliflowers, tomato and broad beans), previously spiked with 12.5 ng of a surrogate mix (section 4.5.1. SI), were transferred to a glass centrifuge tube containing 4 g Na_2SO_4 and 1 g of NaCl. Then, 10 mL of ACN were added to the tubes, and they were sonicated 15 min and centrifuged at 3000 rpm. The supernatant was collected and transferred to another tube, and 75 mg of C18, 75 mg of PSA, and 1350 mg of Na_2SO_4 were added. The tube was sonicated for 15 min and centrifuged as above.

Finally, the supernatant was evaporated under a gentle stream of nitrogen to approximately 1 mL, and 50 mL of water were added. The samples were percolated through SPE cartridges (100 mg/6mL), preconditioned with 2mL methanol and 2mL of MilliQ water, and eluted with ethyl acetate. The final extracts were evaporated to ca. 100 µL and spiked with 37 ng of TPhA. A 50 µL aliquot was directly analyzed by GC-MS/MS, and another 50 µL aliquot was derivatized by adding 10 µL of TMSH in the chromatograph injector port.

Extraction of carbamazepine and 10,11-epoxycarbamazepine from vegetable samples was carried out by sonication followed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) (Tadić et al., n.d.). For this purpose, 0.5 g of fresh-weight vegetable was spiked with 50 ng of carbamazepine-$^{13}C_6$ and left to equilibrate for 30 min. The samples were then sonicated with 10 mL of MeOH for 15 min and centrifuged 15 min at 3000 rpm. The extraction was performed twice, and the extracts were combined and reduced to ca. 1 mL with nitrogen gas and reconstituted with 10 mL of LiChrosolv water. The samples were subsequently percolated through SPE cartridges (STRATA X, 100 mg/6 mL), previously conditioned with 1 mL of MeOH and water, respectively. The cartridges were washed with water/methanol (95:5, v/v) and eluted with 2 mL of a mixture of MeOH/ethyl acetate (1:1, v/v). The final extracts were reduced almost to dryness, resuspended in 1 mL of water, and filtered (0.22 µm) prior to LC-MS/MS analysis.

LODs and LOQs were calculated for each analyte as three and ten times the signal from the baseline noise (S/N ratio) of 3 blank samples, respectively. Further details on the GC-MS/MS and LC-MS/MS and analytical quality parameters (LOD, LOQ, and recoveries) are provided elsewhere (section 4.5.2 and tables S4.1-4.3).

It is important to notice that the extraction method used for the determination of OMCs in lettuce, as well as the analytical quality parameters are likewise reported in section 3.2.3.

4.2.4 Human health risk assessment

TEs- Hazard Quotient (HQ)

The potential risk to human health resulting from consumption of TEs in vegetables was conducted through the hazard quotient (HQ) approach, which was calculated as follows:

$$\mathrm{HQ}=\frac{\mathrm{EDI}}{\mathrm{RfD}} \tag{4.1}$$

where RfD (reference dose) is the maximum tolerable daily intake (µg/kg bw/day) of a specific metal (EPA, 2015; WHO, n.d.) that does not result in any harmful health effect, and EDI is the estimated daily intake (µg/kg bw/day). The EDI was calculated as follows:

$$\mathrm{EDI}=\frac{\mathrm{DI}\times \mathrm{C_M}}{\mathrm{BW}} \tag{4.2}$$

where DI denotes the daily intake of edible parts of vegetables in g per day (the DI for each of the vegetables and population classes is given in Table S.4.4), C_M is the 95th percentile value for the concentration of each TE in the vegetable tissue (µg/g fresh weight (fw)), and BW is the body weight (kg) of the target individual. The DI of fresh vegetables in Spain used in the calculations was taken from the EFSA’s Comprehensive Food Consumption Database. For these data, all varieties of lettuce were considered, as well as tomatoes and tomato by-products, cauliflower, and all types of beans. An EDI value for a specific TE in excess of the corresponding RfD (i.e., HQ>1) implies a potential risk to consumers. RfD values are obtained from chronic oral exposure studies (IRIS, n.d.). Finally, the total hazard quotient (THQ) of each sampling site was calculated as the sum of all the HQs for all the chemicals to which an individual might be exposed. The values for selected metals are shown in Table 4.3. The RfD value for Cr was calculated considering the Cr^{3+} form, as it is the main chromium species present in lettuce (Asfaw et al., 2017).

OMCs - Threshold of toxicological concern (TTC)

The human health risk associated with the intake of the selected OMCs through the consumption of the vegetable crops was assessed with the threshold of toxicological concern (TTC) approach using Toxtree software (Toxtree V2.6.13), which is based on the decision trees of Cramer and Kroes (Cramer et al., 1976; Kroes et al., 2000). This method is suitable for evaluating chemical compounds found at low concentrations in food products without toxicity data (Kroes et al., 2000). As a result, the OMCs were classified into three categories (I, II, III), corresponding to increasing toxicity or their potential genotoxicity and carcinogenicity. Class I contains substances with simple chemical structures with efficient modes of metabolism, suggesting a low order of oral toxicity. Class II consists of intermediate compounds and substances. Class III includes those that allow for no strong initial presumption of safety or may even suggest significant toxicity.

The selected TTC values were 30, 9, and 1.5 μg/kg bw/day for Classes I, II, and III, respectively (Munro et al., 1996). For the genotoxic substances, a TTC value of 0.0025 mg/kg bw/day was selected (Kroes et al., 2004). TTC values were calculated based on analysis of the chronic toxicity data of chemicals in three structural classes identified according to the Cramer decision tree (Cramer et al., 1976). The intake of OMCs above TTC values could pose a potential risk of exposure and requires a specific toxicity analysis of the targeted OMCs. In this study, the average body weights of a Catalan male adult (20-65 years) and child (4-9 years) were used, i.e. 70 and 24 kg, respectively (Generalitat de Catalunya, 2015). The daily consumption (DC, kg/day) by an adult or child to reach the TTC was calculated as follows:

$$DC = \frac{TTC \times BW}{C_{OMC}} \tag{4.3}$$

where C_{OMC} is the 95th percentile value for the concentration of each OMCs in the vegetable tissue (μg/kg fw).

4.2.5 Data analysis

The experimental results were statistically evaluated using the SPSS v. 22 package (Chicago, IL, US). All data sets were checked for normal distribution using the Kolmogorov–Smirnov test to ensure that parametric statistics were applicable. The overall comparison of the occurrence of chemical contaminants between farm plots (rural vs peri-urban) was performed with a paired-sample t-test (dependent samples), whereas the comparison of the concentration of each chemical contaminant between plots was analyzed by independent samples t-test. Statistical significance was defined as $p \leq 0.05$. Principal component analysis (PCA) was performed on the concentration of TEs and OMCs in vegetables by using a correlation matrix.

4.3 Results and discussion

4.3.1 Occurrence of trace elements (TEs)

Table 4.1 shows the occurrence of TEs in vegetable crops from different sampling sites. Different vegetable species and cultivars differ in their ability to uptake, accumulate, and tolerate heavy metals. The concentration of TEs in vegetables ranged from non-detectable to 17 mg/kg fw. Zn, B, and Mn were the most abundant, each of them with a concentration higher than 1 mg/kg fw. No statistical differences were generally observed between the rural and peri-urban sites (paired t-test taking into account all compound differences between sites, p-value>0.05). Nevertheless, the concentrations of As, Cd, Pb, Mo, and Hg were greater in vegetables harvested in the peri-urban area (p-value <0.05; broad beans were not included due to the lack of data for the rural site), except for Ba, Co, Mn and Sb which showed higher abundance in vegetables from rural agriculture. These findings are consistent with a previous study conducted in the same area for lettuce, in which Cd and Pb showed greater concentrations in the peri-urban site than in the rural one (Margenat et al., 2018). They were also similar to those of other studies carried out in urban gardens (0.014 mg/kg fw Cd in fruit and 0.028 mg/kg fw Cd in leafy vegetables) (McBride et al., 2014).

The total concentration of TEs per vegetable was as follows (calculated as the average of the total concentration of TEs in the 3 plots): 26 mg/kg fw (broad beans), 19 mg/kg fw (tomato fruits), 9.0 mg/kg fw (lettuce), and 8.9 mg/kg fw (cauliflower). Zn, Cu, B, and Mn, were the most abundant TEs in broad beans (14 mg/kg fw for Zn, 4.0 mg/kg fw for Cu, 2.9 mg/kg fw for B, and 3.0 mg/kg fw for Mn, on average) and tomato fruits (8.0 mg/kg fw for Zn, 3.1 mg/kg fw for Cu, 4.1 mg/kg fw for B, and 2.5 mg/kg fw for Mn, on average), whereas Cu and B were lower than 0.7 and 2.0 mg/kg fw respectively in lettuce and cauliflower. Mn concentration was similar in all of the studied vegetables. These results are in keeping with those of other studies conducted in the Basque Country (Spain) (Trebolazabala et al., 2017), which showed that Zn and Cu were detected in tomato fruits in a similar range of concentrations (12.43-26.7 mg/kg fw for Zn and 3.563-16 mg/kg fw for Cu). Similarly, in a study carried out in Turkey (Bagdatlioglu et al., 2010), Cu was detected at a higher concentration in tomato fruits than in lettuce. Furthermore, in the same study broad beans showed maximum levels for both Cu and Zn. Another study conducted in Bangladesh observed that the concentration of Cu was higher in tomato fruits than in the rest of vegetables studied (brinjal, bean, carrot, green chilli, onion, and potato) (Shaheen et al., 2016).

Finally, it should be noted that Li, Cd, Sb, and As were only detected in lettuce, due to their higher accumulation in the edible parts of this crop compared to root and fruit vegetables (Singh, 2012). The values of Cd and Pb were compliant with the maximum levels for these TEs in foodstuffs set out in Directive 1881/2006/EC of 19 December 2006 (0.2 mg/kg and 0.05 mg/kg fw for Cd in lettuce and the other vegetables, respectively, and 0.3 mg/kg and 0.10 mg/kg fw for Pb in lettuce and cauliflower and in tomatoes and broad beans, respectively).

Table 4.1 Average and 95th percentile concentration values of selected TEs in vegetables (mg/kg fw). Average and standard deviation of TEs in vegetables grown in rural and peri-urban agriculture.

	Lettuce			Tomato			Cauliflower			Broad beans		Rural*	Peri-urban*
	Plot 1	Plot 3	Plot 4	Plot 1	Plot 3	Plot 4	Plot 1	Plot 3	Plot 4	Plot 3	Plot 4		
As	$3.38\cdot10^{-4}$ $(6.28\cdot10^{-4})$	$5.94\cdot10^{-4}$ $(9.17\cdot10^{-4})$	$8.41\cdot10^{-4}$ $(1.14\cdot10^{-3})$	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$	$<5\cdot10^{-5}$	$<5\cdot10^{-5}$	$<5\cdot10^{-5}$	$<10^{-4}$	$<10^{-4}$	$1.38\cdot10^{-4}\pm2.08\cdot10^{-4}$	$2.73\cdot10^{-4}\pm3.68\cdot10^{-4}$**
B	1.77 (2.11)	2.00 (2.38)	1.91 (2.33)	3.98 (4.14)	3.77 (4.85)	4.60 (5.18)	2.35 (2.74)	1.69 (1.84)	1.99 (2.13)	3.12 (3.99)	2.62 (2.94)	2.70±1.00	2.66±1.21
Ba	0.83 (1.09)	0.50 (0.63)	0.60 (0.70)	0.19 (0.21)	0.16 (0.20)	0.21 (0.33)	0.21 (0.23)	0.19 (0.23)	0.21 (0.23)	0.28 (0.30)	0.17 (0.19)	0.41±0.33	0.31±0.19**
Cd	0.01 (0.01)	0.02 (0.03)	0.02 (0.02)	<0.008	<0.008	<0.008	<0.004	<0.004	<0.004	<0.008	<0.008	0.004±0.002	0.009±0.009**
Co	0.02 (0.03)	0.01 (0.01)	0.01 (0.02)	<0.008	<0.008	<0.008	<0.004	<0.004	<0.004	<0.008	<0.008	0.009±0.010	0.005±0.003**
Cr	0.20 (0.32)	0.15 (0.24)	0.18 (0.46)	0.18 (0.19)	0.14 (0.16)	0.17 (0.18)	0.15 (0.20)	0.13 (0.14)	0.12 (0.13)	0.27 (0.28)	0.28 (0.29)	0.17±0.06	0.15±0.08
Cu	0.49 (0.62)	0.61 (0.69)	0.76 (0.88)	3.28 (4.77)	2.83 (3.54)	2.87 (3.55)	0.61 (0.75)	0.44 (0.48)	0.46 (0.54)	3.89 (4.09)	4.01 (4.21)	1.57±1.57	1.33±1.13
Hg	$3.66\cdot10^{-4}$ $(4.43\cdot10^{-4})$	$8.34\cdot10^{-4}$ $(1.09\cdot10^{-3})$	$9.76\cdot10^{-4}$ $(1.47\cdot10^{-3})$	$3.15\cdot10^{-4}$ $(4.36\cdot10^{-4})$	$5.33\cdot10^{-4}$ $(5.82\cdot10^{-4})$	$2.80\cdot10^{-4}$ $(3.31\cdot10^{-4})$	$3.55\cdot10^{-4}$ $(6.12\cdot10^{-4})$	$1.74\cdot10^{-4}$ (2.02E-04)	$1.54\cdot10^{-4}$ (2.44E-04)	$1.67\cdot10^{-4}$ $(1.86\cdot10^{-4})$	$2.10\cdot10^{-4}$ $(3.50\cdot10^{-4})$	$3.50\cdot10^{-4}\pm1.36\cdot10^{-4}$	$4.92\cdot10^{-4}\pm3.63\cdot10^{-4}$**
Li	0.04 (0.06)	0.03 (0.04)	0.04 (0.05)	<0.008	<0.008	<0.008	<0.004	<0.004	<0.004	<0.008	<0.008	0.013±0.018	0.014±0.016
Mn	3.59 (4.73)	3.33 (4.05)	1.89 (2.38)	3.00 (3.61)	2.12 (2.40)	2.38 (2.99)	1.56 (1.87)	2.13 (2.30)	1.64 (1.74)	3.31 (3.61)	2.67 (2.81)	2.76±1.05	2.25±0.64**
Mo	0.03 (0.03)	0.03 (0.04)	0.03 (0.05)	<0.008	0.07 (0.12)	0.12 (0.16)	<0.004	<0.004	<0.004	1.18 (1.46)	1.04 (1.17)	0.01±0.01	0.04±0.05**
Ni	0.09 (0.15)	0.06 (0.09)	0.11 (0.34)	<0.008	0.07 (0.12)	0.12 (0.19)	<0.004	<0.004	<0.004	0.38 (0.44)	0.29 (0.32)	0.03±0.05	0.06±0.09
Pb	0.05 (0.07)	0.14 (0.18)	0.16 (0.20)	<0.008	<0.008	0.15 (0.19)	<0.004	<0.004	<0.004	<0.008	<0.008	0.02±0.03	0.08±0.08**
Rb	0.37 (0.47)	0.40 (0.51)	0.50 (0.59)	0.95 (1.33)	1.17 (1.27)	0.60 (0.70)	0.32 (0.39)	0.29 (0.33)	0.29 (0.31)	0.40 (0.44)	0.24 (0.29)	0.57±0.37	0.54±0.31
Sb	0.02 (0.03)	0.01 (0.01)	0.01 (0.01)	<0.008	<0.008	<0.008	<0.004	<0.004	<0.004	<0.008	<0.008	0.009±0.010	0.005±0.003**
Zn	1.23 (1.51)	2.29 (2.85)	2.50 (3.01)	7.45 (9.61)	6.98 (7.36)	9.13 (11.3)	3.79 (4.17)	3.56 (3.82)	4.60 (4.85)	14.03 (14.70)	14.09 (16.60)	4.16±2.59	4.85±2.6

** broad beans were not included for the statistical analysis, when values were <LOD, LOD/2 value has been considered ** p-value<0.005.*

4.3.2 Occurrence of OMCs

Only 10 of the 33 OMCs assessed in the vegetables were detected in at least one site. Overall, carbamazepine was the most frequently detected OMC (75%) (Table 4.2). This is consistent with the demonstrated ubiquity of this compound in surface water samples, as well as its high plant uptake in greenhouse and field-grown experiments. Due to its neutral molecular form in a wide range of pH values and its log Kow (2.45), meaning it is rapidly uptaken by plants and accumulated at higher concentrations (Franklin et al., 2016; Goldstein et al., 2014; Riemenschneider et al., 2016). The concentration of OMCs in vegetables ranged from non-detectable to 256 µg/kg fw (dimethomorph). Dimethomorph (110-256 µg/kg fw for tomato fruits in the peri-urban site P3), methyl paraben (106-193 µg/kg fw for lettuce in P1), bisphenol F (32-92 µg/kg for tomato fruits in P4), and TCEP (97-124 µg/kg fw for tomato fruits in P4) were among the OMCs detected at the highest concentrations. This is consistent with the fact that direct application of fungicides, WWTP effluents (methylparaben), and chemicals released by plastic pipelines (bisphenol F) have been demonstrated to be the main sources of pollution in lettuce (Margenat et al., 2018). Similarly, dimethomorph has already been detected in tomato fruits due to its direct application in agriculture (Walorczyk, 2013). Although the paired t-test did not reveal statistical differences between the concentrations of OMCs in vegetables from the rural and peri-urban areas (p-value>0.05), individual analyses showed that the concentrations of dimethomorph, TCEP, and MPB were different between the two areas (p-value<0.05). The higher concentrations of dimethomorph and TCEP in the peri-urban area can be explained due to the application of fungicide and the wet/dry deposition of the fire-retardant compound on the vegetable surface, respectively. The occurrence of fire-retardant compounds in the atmosphere in areas close to cities has already been demonstrated (Ren et al., 2016), mostly in the particle phase (gas phase <5%), and it has been found that rainfall can lead to a scavenging effect, promoting the wet deposition of these compounds.

The total concentration of OMCs per vegetable was as follows (calculated as the average of the total concentration of OMCs in the 3 plots): tomato fruits (163 µg/kg fw), lettuce (104 µg/kg fw), broad beans (19 µg/kg fw), and cauliflower (5 µg/kg fw). The highest average concentrations were found in tomato fruits for TCEP (64 µg/kg fw) and bisphenol F (33 µg/kg fw). Finally, it is important to note that the concentration of pesticides (dimethomorph, carbendazim, and indoxacarb) in vegetables complied with the maximum residue levels of pesticides in or on food and feed of plant and animal origin under Council Directive 91/414/EC (EC, 2006) (Table S4.5).

Table 4.2 . Average and 95th percentile concentration values of selected OMCs in vegetables (µg/kg fw). Average and standard deviation of TEs in vegetables grown in rural and peri-urban agriculture. Minimum, maximum and median concentrations of TEs (ng g^{-1} fw) in vegetable samples

	Lettuce			Tomato			Cauliflower			Broad beans	
	Plot 1	Plot 3	Plot 4	Plot 1	Plot 3	Plot 4	Plot 1	Plot 3	Plot 4	Plot 3	Plot 4
Dimethomorph	<0.29	18.7 (20.4)	<0.29	12.9 (17.7)	172 (247)	0.58 (0.98)	<0.03	<0.03	<0.03	2.04 (2.91)	<0.03
Surfynol 104	<4.07	7.57 (8.04)	<4.07	<4.1	<4.1	<4.1	<4.1	<4.1	<4.1	<4.1	<4.1
2-mercaptobenzothiazole	18.3 (37.2)	<0.66	<0.66	<4.80	<4.80	<4.80	<4.80	<4.80	<4.80	<4.80	<4.80
Bisphenol F	1.56 (1.88)	61.6 (101)	22.83(30.35)	55.6 (55.7)	50.5 (62.2)	65.2 (88.9)	<0.51	<0.51	<0.51	<0.51	<0.51
Carbamazepine	0.20 (0.23)	0.23 (0.31)	0.12 (0.17)	0.16 (0.19)	0.12 (0.14)	0.13 (0.21)	<0.05	<0.05	<0.05	0.15 (0.22)	0.18 (0.30)
Carbendazim	<0.22	1.00(2.46)	<0.22	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24
Methylparaben	150 (192)	28.5 (31.7)	34.1 (46.2)	23.3 (30.2)	12.5 (17.2)	15.4 (22.5)	<0.81	<0.81	<0.81	<0.81	28.7 (34.9)
Carbamazepine epoxide	0.21 (0.23)	0.06 (0.07)	0.16 (0.24)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
TCEP	<0.17	<0.17	<0.17	<0.82	64.3 (78.8)	<0.82	<0.82	<0.82	<0.82	<0.82	<0.82
Indoxacarb	<0.29	<0.29	<0.29	<0.49	<0.49	<0.49	0.80 (0.89)	3.63 (6.11)	<0.49	<0.49	2.15 (2.92)

** FOD: frequency of detection*

4.3.3 Sources and Distribution of chemical pollutants (PCA)

Principal component analysis (PCA) was performed on the whole data set to gain further insight into the sources and distribution behavior of the various parameters assessed in the irrigation waters (Table S4.6). The PCA reduced the 23 measured variables to 6 principal components with eigenvalues > 1, which explained 75% of the total variability observed. Components explaining small data variance (i.e., < 10%) were not retained and were assumed to be mostly due to background and noise contributions. Therefore, only the first three principal components, accounting for 55% of the total variability, were studied. The first principal component (PC1), which accounted for 23% of the variance, had high positive loading values (> 0.6) for B, Cu, and Mo, but negative loadings for Co, Ba, and MPB. This negative correlation between parameters indicates that while the positive variables were not accumulated in plants, the negative ones were. This fit perfectly with the lettuce plants. The second component explained 18% of the variance and had positive loadings (>0.6) for Cr, Mn, Co, Ni, Mo, carbamazepine, and MPB. This component correlated with the chemicals that were highly abundant in lettuce and broad beans. The third component accounted for 13% of the variance and had positive loadings (>0.6) for B, Rb, TCEP, and BPF. It correlated with the chemicals found to be most abundant in tomato fruits.

Fig. 4.1 shows the score plots for PC1 vs PC2 (PC1 vs PC3 is provided in Fig. S4.1). Both plots grouped samples in 4 groups depending on the vegetable (lettuce, tomato, cauliflower, or broad bean). Hence, the abundance of chemicals in the various samples collected depended on the vegetable rather than the location.

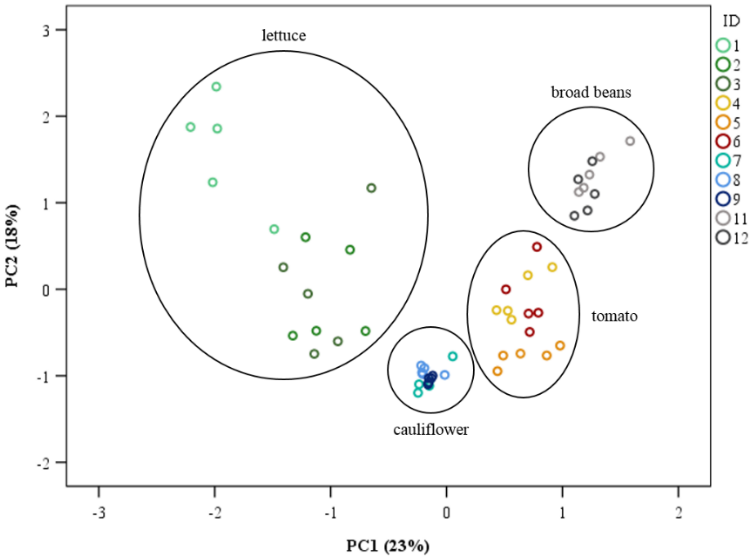


Figure 4.1 Principal Component Analysis (PCA) results. Scores plot PC1 vs PC2 (ID1= Plot 1 lettuce, ID2= Plot 3 lettuce, ID3= Plot 4 lettuce, ID4= Plot 1 tomato, ID5= Plot 3 tomato, ID6= Plot 4 tomato, ID7= Plot 1 cauliflower, ID8= Plot 3 cauliflower, ID9= Plot 4 cauliflower, ID11=Plot 3 broad beans and ID12= Plot 4 broad beans).

4.3.4 Potential human health risk associated with the consumption of vegetables

Risk assessment for TEs exposure

The estimated daily intake (EDI) of TEs was compound and vegetable dependent. On average, Zn was the compound with the highest intake (9.0×10^{-3} and 1.0×10^{-2} mg/day for adults and children, respectively), whereas tomatoes had the highest concentration of TEs on average per farm plot (4.3 - 7.2 mg/kg fw). Oral reference doses (RfDs) were used to assess the human health risk (Table 3), except for Co, Hg, Li, and Rb, for which the oral RfD was not available. Therefore, the risk assessment was evaluated for 12 TEs. HQs ranged from 4.8×10^{-8} to 0.2 for As in broad beans and Pb in tomato fruits (P3), respectively. Of these 12 TEs, on average, Pb posed the greatest health risk to adults and children, followed by Zn, As, Mn, B, Mo, Cd, Cu, Ni, Ba, As, and Cr. These findings are consistent with those of other studies carried out in China and Ethiopia (Chang et al., 2014; Dziubanek et al., 2017; Woldetsadik et al., 2017), where food crops irrigated with either river water or treated wastewater in rural and urban sites did not pose risks to human health.

Pb was also assessed with three Benchmark Dose Lower Confidence Limits (BMDLs) to evaluate the effects of lead exposure in humans: $BMDL_{10}$ for chronic kidney disease development at 0.63 μg/kg bw/day, $BMDL_{01}$ for systolic blood pressure effects at 1.5 μg/kg bw/day, and $BMDL_{01}$ for developmental neurotoxicity (which would apply to fetuses and infants) at 0.5 μg/kg bw/day, as determined by the WHO (1986) and EFSA (2010). The EDI values found for Pb in the present study due to the ingestion of these vegetables (Table S4.7) were in the range of $5.2 \cdot 10^{-5}$ to $5.2 \cdot 10^{-1}$ μg/kg bw/day. These values were lower than all three of the aforementioned BMDLs, except for tomato fruits in Plot 3. This is in agreement with the high lead content found in the soil from this plot (164 mg/Kg dw) in a previous study (Margenat et al., 2018).

The THQs obtained for all age groups were less than 1 for all vegetables and sites studied. Tomato fruits showed the highest THQ on average (0.40 and 0.57 on average for adults and children, respectively), followed by lettuce (0.23 and 0.30 for adults and children, respectively), cauliflower (0.02 and 0.003 for adults and children, respectively), and broad beans (0.0001 and 0.005 for adults and children, respectively). The fact that the highest THQ was observed for tomatoes is probably due to the high consumption of this fruit in Spain compared to the other studied vegetables (see the Material and Methods section). Nevertheless, no statistical differences were observed in the individual and total HQs between vegetables grown in the peri-urban area and rural site (paired t-test, $p > 0.05$). Although for some TEs such as Pb in tomatoes and Zn in lettuce the HQs were greater in the peri-urban agriculture, statistical analysis, comparing rural and peri-urban vegetables (including all vegetables), resulted in no statistical differences between both areas for any of the studied TEs. The total sums of the THQs (for adults) for each farm plot, taking into consideration all TEs and the consumption of all the studied vegetables, were as follows: 0.82 (P4), 0.59 (P3), and 0.54 (P1). Farm plots irrigated with unplanned reclaimed water (*de facto* reuse) showed the highest risk (P3-4), although it was still less than 1 for adults; hence no risk was assumed (Zeng et al., 2018). These findings are consistent with previous studies, which have found that farm plots irrigated with treated wastewater result in a higher risk due to the occurrence of TEs than control plots irrigated with groundwater (Khan et al., 2008).

Although the THQs obtained were lower than 1 in all sites, it should be taken into account that the assessment included only the consumption of the analyzed vegetables. The values could be higher if other vegetables are considered. Therefore, future studies should evaluate the risk associated with other vegetables consumed per person and day in a real-life scenario.

Table 4.3 HQ and THQ of TEs in vegetable samples for an adult (70 kg) and a child (24 kg).

		Lettuce			Tomato			Cauliflower			Broad beans		RfD (µg/kg bw/day)
		Plot 1	**Plot2**	**Plot 3**	**Plot 1**	**Plot2**	**Plot 3**	**Plot 1**	**Plot2**	**Plot 3**	**Plot2**	**Plot 3**	
As	Adult	$2.59\cdot10^{-3}$	$3.79\cdot10^{-3}$	$4.69\cdot10^{-3}$	$4.66\cdot10^{-4}$*	$4.66\cdot10^{-4}$*	$4.66\cdot10^{-4}$*	$5.80\cdot10^{-5}$*	$5.80\cdot10^{-5}$*	$5.80\cdot10^{-5}$*	$4.78\cdot10^{-8}$*	$4.78\cdot10^{-8}$*	0.3**
	Child	$3.31\cdot10^{-3}$	$4.83\cdot10^{-3}$	$5.99\cdot10^{-3}$	$6.55\cdot10^{-4}$*	$6.55\cdot10^{-4}$*	$6.55\cdot10^{-4}$*	$8.33\cdot10^{-6}$*	$8.33\cdot10^{-6}$*	$8.33\cdot10^{-6}$*	$2.15\cdot10^{-6}$*	$2.15\cdot10^{-6}$*	
B	Adult	$1.31\cdot10^{-2}$	$1.47\cdot10^{-2}$	$1.44\cdot10^{-2}$	$5.79\cdot10^{-2}$	$6.77\cdot10^{-2}$	$7.24\cdot10^{-2}$	$4.77\cdot10^{-3}$	$3.20\cdot10^{-3}$	$3.72\cdot10^{-3}$	$5.73\cdot10^{-6}$	$4.22\cdot10^{-6}$	200**
	Child	$1.67\cdot10^{-2}$	$1.88\cdot10^{-2}$	$1.84\cdot10^{-2}$	$8.14\cdot10^{-2}$	$9.52\cdot10^{-2}$	$1.02\cdot10^{-1}$	$6.85\cdot10^{-4}$	$4.59\cdot10^{-4}$	$5.34\cdot10^{-4}$	$2.58\cdot10^{-4}$	$1.90\cdot10^{-4}$	
Ba	Adult	$6.77\cdot10^{-3}$	$3.93\cdot10^{-3}$	$4.34\cdot10^{-3}$	$2.94\cdot10^{-3}$	$2.76\cdot10^{-3}$	$4.68\cdot10^{-3}$	$4.06\cdot10^{-4}$	$4.04\cdot10^{-4}$	$4.08\cdot10^{-4}$	$4.36\cdot10^{-7}$	$2.70\cdot10^{-7}$	200**
	Child	$8.65\cdot10^{-3}$	$5.02\cdot10^{-3}$	$5.54\cdot10^{-3}$	$4.10\cdot10^{-3}$	$3.87\cdot10^{-3}$	$6.58\cdot10^{-3}$	$5.83\cdot10^{-5}$	$5.80\cdot10^{-5}$	$5.85\cdot10^{-5}$	$1.96\cdot10^{-5}$	$1.21\cdot10^{-5}$	
Cd	Adult	$9.74\cdot10^{-3}$	$3.74\cdot10^{-2}$	$2.25\cdot10^{-2}$	$1.12\cdot10^{-2}$*	$1.12\cdot10^{-2}$*	$1.12\cdot10^{-2}$*	$6.96\cdot10^{-4}$*	$6.96\cdot10^{-4}$*	$6.96\cdot10^{-4}$*	$1.15\cdot10^{-6}$*	$1.15\cdot10^{-6}$*	1**
	Child	$1.24\cdot10^{-2}$	$4.78\cdot10^{-2}$	$2.87\cdot10^{-2}$	$1.57\cdot10^{-2}$*	$1.57\cdot10^{-2}$*	$1.57\cdot10^{-2}$*	$1.00\cdot10^{-4}$*	$1.00\cdot10^{-4}$*	$1.00\cdot10^{-4}$*	$5.16\cdot10^{-5}$*	$5.16\cdot10^{-5}$*	
Cr	Adult	$2.61\cdot10^{-4}$	$2.00\cdot10^{-4}$	$3.81\cdot10^{-4}$	$3.46\cdot10^{-4}$	$2.89\cdot10^{-4}$	$3.38\cdot10^{-4}$	$4.58\cdot10^{-5}$	$3.17\cdot10^{-5}$	$2.92\cdot10^{-5}$	$5.40\cdot10^{-8}$	$5.61\cdot10^{-8}$	1500**
	Child	$3.33\cdot10^{-4}$	$2.55\cdot10^{-4}$	$4.87\cdot10^{-4}$	$4.87\cdot10^{-4}$	$4.07\cdot10^{-4}$	$4.75\cdot10^{-4}$	$6.57\cdot10^{-6}$	$4.55\cdot10^{-6}$	$4.20\cdot10^{-6}$	$2.43\cdot10^{-6}$	$2.52\cdot10^{-6}$	
Cu	Adult	$1.93\cdot10^{-3}$	$2.13\cdot10^{-3}$	$2.72\cdot10^{-3}$	$3.33\cdot10^{-2}$	$2.47\cdot10^{-2}$	$2.48\cdot10^{-2}$	$6.56\cdot10^{-4}$	$4.22\cdot10^{-4}$	$4.72\cdot10^{-4}$	$2.93\cdot10^{-6}$	$3.02\cdot10^{-6}$	400***
	Child	$2.46\cdot10^{-3}$	$2.72\cdot10^{-3}$	$3.47\cdot10^{-3}$	$4.68\cdot10^{-2}$	$3.48\cdot10^{-2}$	$3.49\cdot10^{-2}$	$9.41\cdot10^{-5}$	$6.06\cdot10^{-5}$	$6.78\cdot10^{-5}$	$1.32\cdot10^{-4}$	$1.36\cdot10^{-4}$	
Mn	Adult	$4.18\cdot10^{-2}$	$3.58\cdot10^{-2}$	$2.11\cdot10^{-2}$	$7.21\cdot10^{-2}$	$4.79\cdot10^{-2}$	$5.97\cdot10^{-2}$	$4.65\cdot10^{-3}$	$5.71\cdot10^{-3}$	$4.34\cdot10^{-3}$	$7.40\cdot10^{-6}$	$5.76\cdot10^{-6}$	140**
	Child	$5.34\cdot10^{-2}$	$4.57\cdot10^{-2}$	$2.69\cdot10^{-2}$	$1.01\cdot10^{-1}$	$6.74\cdot10^{-2}$	$8.40\cdot10^{-2}$	$6.68\cdot10^{-4}$	$8.20\cdot10^{-4}$	$6.22\cdot10^{-4}$	$3.33\cdot10^{-4}$	$2.59\cdot10^{-4}$	
Mo	Adult	$8.13\cdot10^{-3}$	$9.02\cdot10^{-3}$	$1.19\cdot10^{-2}$	$2.24\cdot10^{-3}$*	$6.77\cdot10^{-2}$	$9.06\cdot10^{-2}$	$1.39\cdot10^{-4}$*	$1.39\cdot10^{-4}$*	$1.39\cdot10^{-4}$*	$8.38\cdot10^{-5}$	$6.69\cdot10^{-5}$	5**
	Child	$1.04\cdot10^{-2}$	$1.15\cdot10^{-2}$	$1.52\cdot10^{-2}$	$3.14\cdot10^{-3}$*	$9.52\cdot10^{-2}$	$1.27\cdot10^{-1}$	$2.00\cdot10^{-5}$*	$2.00\cdot10^{-5}$*	$2.00\cdot10^{-5}$*	$3.77\cdot10^{-3}$	$3.01\cdot10^{-3}$	
Ni	Adult	$9.10\cdot10^{-3}$	$5.78\cdot10^{-3}$	$2.12\cdot10^{-2}$	$5.59\cdot10^{-4}$*	$1.71\cdot10^{-2}$	$2.67\cdot10^{-2}$	$3.48\cdot10^{-5}$*	$3.48\cdot10^{-5}$*	$3.48\cdot10^{-5}$*	$6.27\cdot10^{-6}$	$4.66\cdot10^{-6}$	20**
	Child	$1.16\cdot10^{-2}$	$7.38\cdot10^{-3}$	$2.70\cdot10^{-2}$	$7.86\cdot10^{-4}$*	$2.41\cdot10^{-2}$	$3.75\cdot10^{-2}$	$5.00\cdot10^{-6}$*	$5.00\cdot10^{-6}$*	$5.00\cdot10^{-6}$*	$2.82\cdot10^{-4}$	$2.10\cdot10^{-4}$	
Pb	Adult	$2.64\cdot10^{-2}$	$6.22\cdot10^{-2}$	$7.18\cdot10^{-2}$	$3.19\cdot10^{-3}$*	$3.19\cdot10^{-3}$*	$1.49\cdot10^{-1}$	$1.99\cdot10^{-4}$*	$1.99\cdot10^{-4}$*	$1.99\cdot10^{-4}$*	$3.28\cdot10^{-5}$*	$3.28\cdot10^{-7}$*	3.5
	Child	$3.37\cdot10^{-2}$	$7.94\cdot10^{-2}$	$9.17\cdot10^{-2}$	$4.49\cdot10^{-3}$*	$4.49\cdot10^{-3}$*	$2.10\cdot10^{-1}$	$2.86\cdot10^{-5}$*	$2.86\cdot10^{-5}$*	$2.86\cdot10^{-5}$*	$1.47\cdot10^{-5}$*	$1.47\cdot10^{-5}$*	
Sb	Adult	$9.29\cdot10^{-2}$	$4.49\cdot10^{-2}$	$4.07\cdot10^{-2}$	$2.79\cdot10^{-2}$*	$2.79\cdot10^{-2}$*	$2.79\cdot10^{-2}$*	$1.74\cdot10^{-3}$*	$1.74\cdot10^{-3}$*	$1.74\cdot10^{-3}$*	$2.87\cdot10^{-6}$*	$2.87\cdot10^{-6}$*	0.4**
	Child	$1.19\cdot10^{-1}$	$5.73\cdot10^{-2}$	$5.20\cdot10^{-2}$	$3.93\cdot10^{-2}$*	$3.93\cdot10^{-2}$*	$3.93\cdot10^{-2}$*	$2.50\cdot10^{-4}$*	$2.50\cdot10^{-4}$*	$2.50\cdot10^{-4}$*	$1.29\cdot10^{-4}$*	$1.29\cdot10^{-4}$*	
Zn	Adult	$6.22\cdot10^{-3}$	$1.18\cdot10^{-2}$	$1.24\cdot10^{-2}$	$8.95\cdot10^{-1}$	$6.86\cdot10^{-2}$	$1.05\cdot10^{-1}$	$4.84\cdot10^{-3}$	$4.43\cdot10^{-3}$	$5.63\cdot10^{-3}$	$1.41\cdot10^{-5}$	$1.59\cdot10^{-5}$	300**
	Child	$7.95\cdot10^{-3}$	$1.50\cdot10^{-2}$	$1.59\cdot10^{-2}$	$1.26\cdot10^{-1}$	$9.64\cdot10^{-2}$	$1.48\cdot10^{-1}$	$6.95\cdot10^{-4}$	$6.36\cdot10^{-4}$	$8.09\cdot10^{-4}$	$6.32\cdot10^{-4}$	$7.14\cdot10^{-4}$	
THQ	Adult	$2.19\cdot10^{-1}$	$2.32\cdot10^{-1}$	$2.28\cdot10^{-1}$	$3.02\cdot10^{-1}$	$3.40\cdot10^{-1}$	$5.73\cdot10^{-1}$	$1.82\cdot10^{-2}$	$1.71\cdot10^{-2}$	$1.75\cdot10^{-2}$	$1.25\cdot10^{-4}$	$1.05\cdot10^{-4}$	
	Child	$2.80\cdot10^{-1}$	$2.96\cdot10^{-1}$	$2.91\cdot10^{-1}$	$4.24\cdot10^{-1}$	$4.78\cdot10^{-1}$	$8.06\cdot10^{-1}$	$2.77\cdot10^{-3}$	$2.45\cdot10^{-3}$	$2.51\cdot10^{-3}$	$6.01\cdot10^{-3}$	$4.73\cdot10^{-3}$	

Calculated from the LOD/2 **(EPA, 2015) *(WHO, n.d.)*

Risk assessment for OMCs exposure

The health risk associated with the occurrence of OMCs in the different studied vegetables was assessed using the TTC approach. Only compounds identified above the LOQ were considered for the risk assessment study (10 of the 33 monitored compounds). The OMCs were classified as Class III (dimethomorph, surfynol 104, 2-MBT, BPF, CBZ, carbendazim, TCEP, and indoxacarb), Class I (MPB), and genotoxic (10,11-epoxycarbamazepine). Table 4 shows the DC required to reach TTC levels for each of the studied OMCs. The DC ranged from 0.38 kg/day (dimetomorph in tomatoes, P3) to 7000 kg/day (dimethomorph in cauliflowers and broad beans, except for P3) for adults, and from 0.15 kg/day to 2400kg/day for children. Hence, in the worst-case scenario, the daily consumption of tomato fruits grown in P3 required for a child or an adult to reach the TCC level is 150 g/day and 380 g/day, respectively, which would not be a typical DI for tomato fruits and by-products (94.3 g/day and 196 g/day for children and adults, respectively, as shown in Table 4-SM). Similarly, the daily consumption of lettuce, tomato, and broad beans did not reach the TCC for any of the studied OMCs and therefore did not pose a risk to human health. Nevertheless, exposure to other compounds, such as 10,11-epoxycarbamazepine, should be taken into consideration in lettuce as the TTC can be reached with a consumption of 700 g/day or 300 g/day of lettuce for adults and children, respectively. These findings are consistent with those of Riemenschneider et al. (2016), who studied 28 OMCs (including 9 CBZ metabolites) in 10 vegetable species, including lettuce and tomato fruits irrigated with TWW. In that study, the daily consumption of lettuce required to reach the TCC was higher than 9 Kg for all the studied OMCs, except 10,11-epoxycarbamazepine, for which it was 40 g for lettuce for adults; in contrast, it was higher than 300 kg for tomato fruits.

Since pesticides were the most important compounds in the risk assessment obtained with the TTC approach, and because they are already regulated through maximum residue levels (MRLs), the risk (HQ) for consumers was also assessed. This was done using the quotient between the EDI and the ADI, where the ADI was obtained from the MRL established by EFSA for pesticides in each vegetable and the daily intake (EC, 2005). Table 8-SM shows the HQs for pesticides in the different vegetables. As indicated by the TTC approach, dimethomorph posed the highest risk in tomatoes (HQ=0.56), whereas the other pesticides showed HQs less than 0.04. The risk for pesticides is considered unacceptable when the HQ exceeds 1; thus, the risk was not noticeable. These findings are consistent with other studies carried out in pesticides for several vegetables and fruits with HQ values always below 1 (Lemos et al., 2016; F. Li et al., 2017; Lozowicka, 2015). In general, the risk associated with pesticide application was found to be more relevant than that associated with other OMCs that can originate from irrigation or soil pollution.

Table 4.4 The daily consumption (kg/day) required to reach TTC levels for the selected OMCs in an adult (70 kg) and in a child (24 kg).

		Lettuce			Tomato			Cauliflower			Broad beans		
		Plot 1	**Plot 3**	**Plot 4**	**Plot 1**	**Plot 3**	**Plot 4**	**Plot 1**	**Plot 3**	**Plot 4**	**Plot 1**	**Plot 3**	**Plot 4**
Dimethomorph	Adult	724*	5.2	724*	5.9	0.4	107	7000*	7000*	7000*	7000*	32	7000*
	Child	248*	1.8	248*	2.0	0.1	37	2400*	2400*	2400*	2400*	12	2400*
Surfynol 104	Adult	52*	13	52*	51*	51*	51*	51*	51*	51*	51*	51*	51*
	Child	18*	4.5	18*	18*	18*	18*	18*	18*	18*	18*	18*	18*
2-mercaptobenzothiazole	Adult	2.8	318*	318*	318*	318*	318*	318*	318*	318*	318*	318*	318*
	Child	1.0	109*	109*	109*	109*	109*	109*	109*	109*	109*	109*	109*
Bisphenol F	Adult	56	1.0	3.5	1.9	1.7	1.2	412*	412*	412*	412*	412*	412*
	Child	19	0.4	1.2	0.6	0.6	0.4	141*	141*	141*	141*	141*	141*
Carbamazepine	Adult	466	338	619	553	763	500	4200*	4200*	4200*	64	426	313
	Child	160	116	212	189	262	171	1440*	1440*	1440*	24	164	120
Carbendazim	Adult	955*	43	955*	875*	875*	875*	875*	875*	875*	875*	875*	875*
	Child	327*	15	327*	300*	300*	300*	300*	300*	300*	300*	300*	300*
Methylparaben	Adult	11	66	45	70	122	93	5185*	5185*	5185*	73	63	54
	Child	3.7	23	16	24	42	32	1778*	1778*	1778*	28	24	21
Carbamazepine epoxide	Adult	0.8	2.4	0.7	3.5*	3.5*	3.5*	3.5*	3.5*	3.5*	3.5*	3.5*	3.5*
	Child	0.3	0.8	0.3	1.2*	1.2*	1.2*	1.2*	1.2*	1.2*	1.2*	1.2*	1.2*
TCEP	Adult	1235*	1235*	1235*	256*	1.3	256*	256*	256*	256*	256*	256*	256*
	Child	424*	424*	424*	88*	0.5	88*	88*	88*	88*	88*	88*	88*
Indoxacarb	Adult	724*	724*	724*	429*	429*	429*	105	15	429*	429*	58	32
	Child	248*	248*	248*	147*	147*	147*	20	3	147*	147*	11	6

**Calculated from the LOD/2*

4.4 Conclusions

Although the occurrence of some chemical contaminants, such as Pb or dimethomorph, was greater in vegetables grown in the peri-urban area than the rural one, the abundance of chemical contaminants depended on the vegetables evaluated (cauliflowers, tomatoes and lettuce). The following key conclusions can be drawn:

- The concentrations of TEs ranged from non-detectable to 4.9 mg/kg fw in lettuce, from 2.3×10^{-4} to 12 mg/kg fw in tomato fruits, from 1.1×10^{-4} to 4.9 mg/kg fw in cauliflower, and from 1.5×10^{-4} to 17 mg/kg fw in broad beans. In contrast, the concentration of OMCs ranged from non-detectable to 193 µg/kg fw in lettuce, from non-detectable to 256 µg/kg fw in tomato fruits, from non-detectable to 156 µg/kg fw in cauliflower, and from non-detectable to 206 µg/kg fw in broad beans.

- The concentrations of TEs and pesticides in vegetables were compliant with EC Directives 1881/2006/EC and 396/2005/EC, respectively.

- PCA showed that the abundance of chemicals in the different plot sites depended on the vegetable rather than the farm location.

- THQs for TEs were below 1 in all studied vegetables and ranged from 0.0001 to 0.40 on average in adults and from 0.0005 to 0.57 in children.

- The daily consumption of food crops required to reach the TTC values for OMCs was 150 g/day for children and 380 g/day for an adult. As the actual daily consumption of these vegetables is below these values, no risk due to the intake of these food crops is foreseen, although more toxicological data is needed.

The present study showed that the occurrence of target chemical contaminants in peri-urban agriculture did not affect the human health risk. However, further research is required, especially on the potential human health risk of the combined effects of OMCs and TEs.

4.5 Supporting Information

4.5.1 Materials and reagents

Most of the reagents, CECs and surrogates are detailed in sections 2.5.1 and 3.6.1.

Sodium chloride was purchased from Sigma-Aldrich (Bornem, Belgium), sodium phosphate from Merck (Darmstadt, Germany), C18 from Millipore (Bedford, MA, USA) and PSA was bought from Agilent Technologies (Santa Clara, CA, USA).

4.5.2 Analytical determination of chemical pollutants in soil and crop samples

ICP-MS and ICP-OES determination

Total TEs concentrations were determined by an inductively coupled plasma optical emission spectrometer (Thermo Scientific, iCAP 6500 ICP-OES) and an inductively coupled plasma mass spectrometer (Thermo Scientific, XSeries 2 ICP-MS). Major elements were determined by ICP-OES (Ba and Mn), while the rest of TEs were determined by ICP-MS.

The Hg concentration was determined using an advanced mercury analyzer (AMA-254, Altec, Prague, Czech Republic).

Method validation was carried out by NIST 1570a (Gaithersburg, USA), with certified values for As, B, Cd, Co, Cu, Mn, Hg, Ni, Zn. For accuracy, excellent extraction efficiencies were noted for these elements (92–107%).

Reagent water was used as a blank matrix, and laboratory reagent blank was treated exactly the same as a sample. A limit of detection (LOD) of 0.2 μg/L in the solution analyzed was determined from three times the standard deviation obtained from the analysis of ten runs of blank samples on the same day as the determinations.

GC-MS/MS determination

Description of the GC-MS/MS determination and quantification is provided in section 2.3.3. LODs, LOQs, recoveries of the surrogates and recoveries of the targeted compounds are reported in Tables S4.1-4.3.

LC-MS/MS determination

Details on the LC-MS/MS determination and quantification of CBZ and EPOCBZ is given in section 3.5.2. LODs, LOQs, recoveries of the surrogates and recoveries of the targeted compounds are detailed in Tables S4.1-4.3.

Table S4.1 LODs and LOQs of the studied OMCs in vegetables (tomatoes, cauliflowers and broad beans).

Compound	LOD (µg/kg fw)	LOQ (µg/kg fw)
Atrazine	0.26	0.46
Azoxystrobin	0.19	0.31
2-tert-Butyl-4-methoxyphenol (BHA)	0.22	0.29
Chlorpyrifos	21	38
N.N-Diethyl-meta-toluamide (DEET)	0.45	0.8
Diazepam	0.05	0.06
Diazinon	0.26	0.61
Dymethomorph	0.013	0.015
Indoxacarb	0.15	0.18
Simazine	1.47	2.28
Surfynol 104	11.2	11.5
Tris(2-Chloroethyl) Phosphate (TCEP)	0.28	0.55
2-Mercaptobenzothiazole (2-MBT)	4.80	10.8
5-Methyl-2H-benzotriazole (5-TTri)	0.19	0.29
Bisphenol A (BPA)	2.89	5.36
Butylparaben (BPB)	0.27	0.71
Bisphenol F (BPF)	13.8	31.6
1.3-Benzothiazole (BT)	1.2	1.7
Benzotriazole (Btri)	2.53	5.72
Carbamazepine	0.03	0.10
Carbendazim	0.39	0.45
Ethyl paraben (EPB)	0.75	0.98
Lorazepam	3.17	4.92
Methyl paraben (MPB)	7.44	7.66
1-Hydroxybenzotriazole (OHBT)	5.72	12.0
Octylphenol (OP)	0.40	0.72
Oxazepam	2.0	2.7
Propyl paraben (PPB)	0.24	0.48
Primidone	0.38	0.59
Pymetrozin	4.45	6.71
Pyraclostrobin	0.31	0.45
Carbamazepine-10.11-epoxide (EPOCBZ)	0.05	0.16
Tris(1-chloro-2-propyl) phosphate (TCPP)	38.7	55.6

Table S4.2 Recoveries of the surrogates in vegetables

Surrogate	R (%) tomato	R (%) cauliflower	R (%) broad beans
Bisphenol A-d_{16}	52±4	54±1	48±6
Caffeine-$^{13}C_3$	53±8	60±1	52±9
Carbamazepine-$^{13}C_6$	72±12	64±7	83±14
Diazepam-d_5	52±8	78±2	69±7
5.6-dimethyl-1H-benzotriazole (XbTri)	62±10	55±5	71±3
Etylparaben-^{13}C	57±7	58±8	52±7

Table S4.3 Recoveries of the studied OMCs in vegetables

Compounds	R (%) tomato	R (%) cauliflower	R (%) broad beans
1-hydroxybenzotriazole (OHBT)	43±2	66±5	53±3
2-mercaptobenzothiazole (2MBT)	89±5	81±8	85±1
2-tert-Butyl-4-methoxyphenol (BHA)	91±16	7.7±10	89±9
4-tert-octylphenol (OP)	84±2	55±9	55±2
5-methyl-2H-benzotriazole (5TTri)	54±3	42±10	47±2
Atrazine	71±5	54±12	78±4
Azoxystrobin	90±8	47±5	98±1
Benzothiazole	54±14	38±13	40±3
Benzotriazole	43±6	58±6	38±3
Bisphenol A	51±8	59±10	66±9
Bisphenol F	44±6	57±11	55±7
Butylparaben	51±2	58±2	76±2
Carbamazepine	92±26	152±24	52±10
Carbamazepine-10.11-epoxide (EPOCBZ)	134±31	95±14	93±4
Carbendazim	110±10	88±5	84±3
Chlorpyrifos	n.d.	n.d.	41±10
DEET	83±7	86±5	73±5
Diazepam	53±8	81±5	66±1
Diazinon	96±9	79±8	87±8
Dimethomorph	94±1	87±5	89±7
Etylparaben	73±4	84±10	75±4
Indoxacarb	69±5	65±3	70±7
Lorazepam	n.d.	35±5	61±1
Methylparaben	92±11	81±7	86±4
Oxazepam	70±11	60±4	78±4
Primidone	49±3	51±3	52±6
Propylparaben	54±3	52±4	58±1

12.967	5.967	48
13.714	6.714	54
13.536	6.536	52

Pymetrozin	n.d.	n.d.	n.d.
Pyraclostrobin	n.d.	n.d.	47±7
Simazine	66±5	54±12	77±2
Surfynol 104	86±6	97±9	82±3
Tris(1-chloro-2-propyl) phosphate (TCPP)	98±9	103±2	99±2
Tris(2-chloroethyl) phosphate (TCEP)	73±1	64±10	77±5

Table S4.4 Daily consumption in Spain for each of the studied vegetables and population class

Vegetable	Population class	Dietary survey	Nº subjects	Nº consumers	Value	Consumption (g/day)
Lettuce	Adult	AESAN-FIAB	981	662	P95	86.7
	Other children	Food patterns of Spanish school children and adolescent	156	45	P95	38.0
Tomato	Adult	AESAN	410	370	P95	196
	Other children	Food patterns of Spanish schoolchildren and adolescent	156	92	P95	94.3
Cauliflower	Adult	AESAN-FIAB	981	88	P95	24.4
	Other children	Nutrition survey2005	399	7	Mean	1.20
Broad beans	Adult	AESAN-FIAB	981	567	P95	0.02
	Other children	Nutrition survey2005	399	256	P95	0.31

Table S4.5 Maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending council directive 91/414/EEC

Vegetable	Carbendazim and benomyl (sum of benomyl and carbendazim expressed as carbendazim)	Indoxacarb (sum of indoxacarb and its R diastereomer)	Dimethomorph (sum of isomers)
Tomato	0.3	0.5	1
Cauliflower	0.1	0.3	0.6
Lettuce	0.1	3	15
Beans (without pods)	0.1	0.02	0.04

Table S4.6 Loadings for PCA

	Component					
	1	**2**	**3**	**4**	**5**	**6**
B	**0.6**	0.0	**0.6**	-0.2	-0.1	-0.2
Cr	0.3	**0.7**	-0.1	0.1	-0.2	0.2
Mn	-0.1	**0.8**	0.2	0.0	0.3	-0.2
Co	**-0.7**	**0.6**	0.2	-0.2	0.0	0.1
Ni	0.4	**0.7**	-0.1	0.3	-0.2	0.2
Cu	**0.8**	0.4	0.3	0.0	0.1	0.0
Zn	**0.9**	0.4	0.0	0.1	0.0	0.0
Rb	0.3	-0.1	**0.8**	-0.3	0.3	0.2
Mo	**0.6**	**0.6**	-0.3	0.2	0.0	0.1
Ba	**-0.8**	0.5	0.1	0.0	-0.1	0.1
Pb	-0.4	0.1	0.5	0.4	-0.5	0.0
Hg	-0.4	-0.2	0.3	**0.5**	0.0	0.5
Indoxacarb	0.2	0.0	-0.4	0.0	0.0	0.0
Dimetomorph	0.2	-0.2	0.5	-0.2	0.4	0.4
TCEP	0.3	-0.1	**0.6**	0.0	-0.5	-0.4
Surfynol	-0.2	-0.1	0.0	0.6	0.4	-0.3
MBT	-0.5	0.4	0.0	-0.4	0.1	-0.2
BPF	0.0	-0.1	**0.6**	0.2	-0.3	0.0
Carbamazepine	0.0	**0.6**	0.4	0.2	0.2	0.0
EpoxyCBZ	-0.5	0.3	0.1	-0.2	-0.1	0.2
Carbendazim	-0.2	0.0	0.1	**0.6**	0.4	-0.3
MPB	**-0.6**	**0.6**	0.1	-0.3	0.1	-0.1

Table S4.7 EDI (mg/kg bw/day) of TEs in vegetables for an adult (70 kg) and a child (24 kg). *Calculated from the LOD/2

		Lettuce			**Tomato**			**Cauliflower**			**Broad beans**	
		Plot 1	**Plot 3**	**Plot 4**	**Plot 1**	**Plot3**	**Plot 4**	**Plot 1**	**Plot 3**	**Plot 4**	**Plot 3**	**Plot 4**
As	Adult	$7.77\cdot10^{-7}$	$1.14\cdot10^{-6}$	$1.41\cdot10^{-6}$	$1.40\cdot10^{-7}$*	$1.40\cdot10^{-7}$*	$1.40\cdot10^{-7}$*	$1.74\cdot10^{-8}$*	$1.74\cdot10^{-8}$*	$1.74\cdot10^{-8}$*	$1.43\cdot10^{-11}$*	$1.43\cdot10^{-11}$*
	Child	$9.94\cdot10^{-7}$	$1.45\cdot10^{-6}$	$1.80\cdot10^{-6}$	$1.96\cdot10^{-7}$*	$1.96\cdot10^{-7}$*	$1.96\cdot10^{-7}$*	$2.50\cdot10^{-9}$*	$2.50\cdot10^{-9}$*	$2.50\cdot10^{-9}$*	$6.46\cdot10^{-10}$*	$6.46\cdot10^{-10}$*
B	Adult	$2.62\cdot10^{-3}$	$2.94\cdot10^{-3}$	$2.88\cdot10^{-3}$	$1.16\cdot10^{-2}$	$1.35\cdot10^{-2}$	$1.45\cdot10^{-2}$	$9.55\cdot10^{-4}$	$6.40\cdot10^{-4}$	$7.43\cdot10^{-4}$	$1.15\cdot10^{-6}$	$8.45\cdot10^{-7}$
	Child	$3.35\cdot10^{-3}$	$3.76\cdot10^{-3}$	$3.68\cdot10^{-3}$	$1.63\cdot10^{-2}$	$1.90\cdot10^{-2}$	$2.03\cdot10^{-2}$	$1.37\cdot10^{-4}$	$9.18\cdot10^{-5}$	$1.07\cdot10^{-4}$	$5.16\cdot10^{-5}$	$3.80\cdot10^{-5}$
Ba	Adult	$1.35\cdot10^{-3}$	$7.86\cdot10^{-4}$	$8.67\cdot10^{-4}$	$5.83\cdot10^{-4}$	$5.51\cdot10^{-4}$	$9.36\cdot10^{-4}$	$8.12\cdot10^{-5}$	$8.07\cdot10^{-5}$	$8.15\cdot10^{-5}$	$8.72\cdot10^{-8}$	$5.40\cdot10^{-8}$
	Child	$1.73\cdot10^{-3}$	$1.01\cdot10^{-3}$	$1.11\cdot10^{-3}$	$8.19\cdot10^{-4}$	$7.75\cdot10^{-4}$	$1.32\cdot10^{-3}$	$1.17\cdot10^{-5}$	$1.16\cdot10^{-5}$	$1.17\cdot10^{-5}$	$3.93\cdot10^{-6}$	$2.43\cdot10^{-6}$
Cd	Adult	$9.74\cdot10^{-6}$	$3.74\cdot10^{-5}$	$2.25\cdot10^{-5}$	$1.12\cdot10^{-5}$*	$1.12\cdot10^{-5}$*	$1.12\cdot10^{-5}$*	$6.96\cdot10^{-7}$*	$6.96\cdot10^{-7}$*	$6.96\cdot10^{-7}$*	$1.15\cdot10^{-9}$*	$1.15\cdot10^{-9}$*
	Child	$1.25\cdot10^{-5}$	$4.78\cdot10^{-5}$	$2.88\cdot10^{-5}$	$1.57\cdot10^{-5}$*	$1.57\cdot10^{-5}$*	$1.57\cdot10^{-5}$*	$1.00\cdot10^{-7}$*	$1.00\cdot10^{-7}$*	$1.00\cdot10^{-7}$*	$5.17\cdot10^{-8}$*	$5.17\cdot10^{-8}$*
Cr	Adult	$3.91\cdot10^{-4}$	$3.00\cdot10^{-4}$	$5.72\cdot10^{-4}$	$5.19\cdot10^{-4}$	$4.34\cdot10^{-4}$	$5.07\cdot10^{-4}$	$6.86\cdot10^{-5}$	$4.75\cdot10^{-5}$	$4.39\cdot10^{-5}$	$8.10\cdot10^{-8}$	$8.41\cdot10^{-8}$
	Child	$5.00\cdot10^{-4}$	$3.83\cdot10^{-4}$	$7.31\cdot10^{-4}$	$7.30\cdot10^{-4}$	$6.10\cdot10^{-4}$	$7.13\cdot10^{-4}$	$9.86\cdot10^{-6}$	$6.82\cdot10^{-6}$	$6.30\cdot10^{-6}$	$3.65\cdot10^{-6}$	$3.79\cdot10^{-6}$
Cu	Adult	$7.70\cdot10^{-4}$	$8.51\cdot10^{-4}$	$1.09\cdot10^{-3}$	$1.33\cdot10^{-2}$	$9.90\cdot10^{-3}$	$9.92\cdot10^{-3}$	$2.62\cdot10^{-4}$	$1.69\cdot10^{-4}$	$1.89\cdot10^{-4}$	$1.17\cdot10^{-6}$	$1.21\cdot10^{-6}$
	Child	$9.84\cdot10^{-4}$	$1.09\cdot10^{-3}$	$1.39\cdot10^{-3}$	$1.87\cdot10^{-2}$	$1.39\cdot10^{-2}$	$1.39\cdot10^{-2}$	$3.77\cdot10^{-5}$	$2.42\cdot10^{-5}$	$2.71\cdot10^{-5}$	$5.28\cdot10^{-5}$	$5.44\cdot10^{-5}$
Mn	Adult	$5.86\cdot10^{-3}$	$5.01\cdot10^{-3}$	$2.95\cdot10^{-3}$	$1.01\cdot10^{-2}$	$6.71\cdot10^{-3}$	$8.36\cdot10^{-3}$	$6.51\cdot10^{-4}$	$8.00\cdot10^{-4}$	$6.07\cdot10^{-4}$	$1.04\cdot10^{-6}$	$8.06\cdot10^{-7}$
	Child	$7.49\cdot10^{-3}$	$6.41\cdot10^{-3}$	$3.77\cdot10^{-3}$	$1.42\cdot10^{-2}$	$9.43\cdot10^{-3}$	$1.18\cdot10^{-2}$	$9.35\cdot10^{-5}$	$1.15\cdot10^{-4}$	$8.71\cdot10^{-5}$	$4.66\cdot10^{-5}$	$3.63\cdot10^{-5}$
Mo	Adult	$4.07\cdot10^{-5}$	$4.51\cdot10^{-5}$	$5.95\cdot10^{-5}$	$1.12\cdot10^{-5}$*	$3.39\cdot10^{-4}$	$4.53\cdot10^{-4}$	$6.96\cdot10^{-7}$*	$6.96\cdot10^{-7}$*	$6.96\cdot10^{-7}$*	$4.19\cdot10^{-7}$	$3.34\cdot10^{-7}$
	Child	$5.20\cdot10^{-5}$	$5.76\cdot10^{-5}$	$7.61\cdot10^{-5}$	$1.57\cdot10^{-5}$*	$4.76\cdot10^{-4}$	$6.37\cdot10^{-4}$	$1.00\cdot10^{-7}$*	$1.00\cdot10^{-7}$*	$1.00\cdot10^{-7}$*	$1.89\cdot10^{-5}$	$1.51\cdot10^{-5}$
Ni	Adult	$1.82\cdot10^{-4}$	$1.16\cdot10^{-4}$	$4.23\cdot10^{-4}$	$1.12\cdot10^{-5}$*	$3.42\cdot10^{-4}$	$5.33\cdot10^{-4}$	$6.96\cdot10^{-7}$*	$6.96\cdot10^{-7}$*	$6.96\cdot10^{-7}$*	$1.25\cdot10^{-7}$	$9.32\cdot10^{-8}$
	Child	$2.33\cdot10^{-4}$	$1.48\cdot10^{-4}$	$5.41\cdot10^{-4}$	$1.57\cdot10^{-5}$*	$4.82\cdot10^{-4}$	$7.50\cdot10^{-4}$	$1.00\cdot10^{-7}$*	$1.00\cdot10^{-7}$*	$1.00\cdot10^{-7}$*	$5.64\cdot10^{-6}$	$4.20\cdot10^{-6}$
Pb	Adult	$9.23\cdot10^{-5}$	$2.18\cdot10^{-4}$	$2.51\cdot10^{-4}$	$1.12\cdot10^{-5}$*	$1.12\cdot10^{-5}$*	$5.22\cdot10^{-4}$	$6.96\cdot10^{-7}$*	$6.96\cdot10^{-7}$*	$6.96\cdot10^{-7}$*	$1.15\cdot10^{-9}$*	$1.15\cdot10^{-9}$*
	Child	$1.18\cdot10^{-4}$	$2.78\cdot10^{-4}$	$3.21\cdot10^{-4}$	$1.57\cdot10^{-5}$*	$1.57\cdot10^{-5}$*	$7.34\cdot10^{-4}$	$1.00\cdot10^{-7}$*	$1.00\cdot10^{-7}$*	$1.00\cdot10^{-7}$*	$5.17\cdot10^{-8}$*	$5.17\cdot10^{-8}$*
Sb	Adult	$3.72\cdot10^{-5}$	$1.79\cdot10^{-5}$	$1.63\cdot10^{-5}$	$1.12\cdot10^{-5}$*	$1.12\cdot10^{-5}$*	$1.12\cdot10^{-5}$*	$6.96\cdot10^{-7}$*	$6.96\cdot10^{-7}$*	$6.96\cdot10^{-7}$*	$1.15\cdot10^{-9}$*	$1.15\cdot10^{-9}$*
	Child	$4.75\cdot10^{-5}$	$2.29\cdot10^{-5}$	$2.08\cdot10^{-5}$	$1.57\cdot10^{-5}$*	$1.57\cdot10^{-5}$*	$1.57\cdot10^{-5}$*	$1.00\cdot10^{-7}$*	$1.00\cdot10^{-7}$*	$1.00\cdot10^{-7}$*	$5.17\cdot10^{-8}$*	$5.17\cdot10^{-8}$*
Zn	Adult	$1.87\cdot10^{-3}$	$3.53\cdot10^{-3}$	$3.73\cdot10^{-3}$	$2.68\cdot10^{-2}$	$2.06\cdot10^{-2}$	$3.15\cdot10^{-2}$	$1.45\cdot10^{-3}$	$1.33\cdot10^{-3}$	$1.69\cdot10^{-3}$	$4.22\cdot10^{-6}$	$4.76\cdot10^{-6}$
	Child	$2.39\cdot10^{-3}$	$4.52\cdot10^{-3}$	$4.77\cdot10^{-3}$	$3.77\cdot10^{-2}$	$2.89\cdot10^{-2}$	$4.43\cdot10^{-2}$	$2.09\cdot10^{-4}$	$1.91\cdot10^{-4}$	$2.43\cdot10^{-4}$	$1.90\cdot10^{-4}$	$2.14\cdot10^{-4}$

**Calculated from the LOD/2*

Table S4.8 HQ of the studied OMCs in the vegetable samples

	Lettuce			Tomato			Cauliflower			Broad beans	
	Plot 1	Plot 3	Plot 4	Plot 1	Plot 3	Plot 4	Plot 1	Plot 3	Plot 4	Plot 3	Plot 4
Dimethomorph	$9.7\cdot10^{-6}$*	$1.4\cdot10^{-3}$	$9.7\cdot10^{-6}$*	0.04	0.56	$2.2\cdot10^{-3}$	$7.1\cdot10^{-6}$*	$7.1\cdot10^{-6}$*	$7.1\cdot10^{-6}$*	$1.7\cdot10^{-4}$	$8.7\cdot10^{-7}$*
Carbendazim	$1.1\cdot10^{-3}$*	0.02	$1.1\cdot10^{-3}$*	$9.0\cdot10^{-4}$*	$9.0\cdot10^{-4}$*	$9.0\cdot10^{-4}$*	$3.4\cdot10^{-4}$*	$3.4\cdot10^{-4}$*	$3.4\cdot10^{-4}$*	$2.8\cdot10^{-6}$*	$2.8\cdot10^{-6}$*
Indoxacarb	$4.8\cdot10^{-5}$*	$4.8\cdot10^{-5}$*	$4.8\cdot10^{-5}$*	$1.1\cdot10^{-3}$	$1.1\cdot10^{-3}$	$1.1\cdot10^{-3}$	$8.4\cdot10^{-4}$	0.01	$2.3\cdot10^{-4}$*	$1.9\cdot10^{-4}$	$3.4\cdot10^{-4}$

**Calculated by using the LOD/2 value*

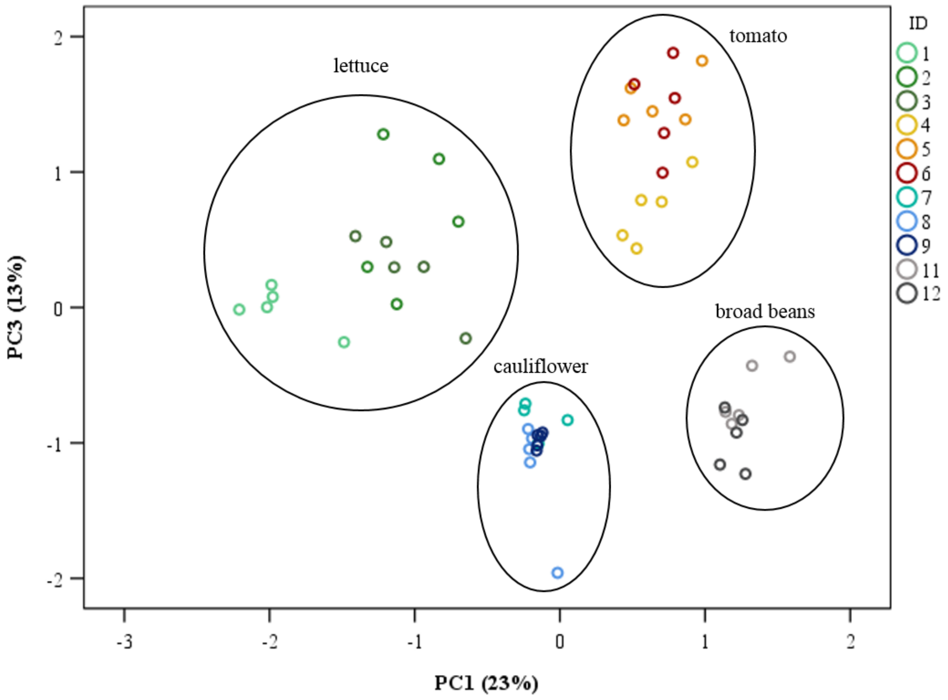


Figure S4.1 Principal Component Analysis (PCA) results. Scores plot PC1 vs PC3 (ID1= Plot 1 lettuce, ID2= Plot 3 lettuce, ID3= Plot 4 lettuce, ID4= Plot 1 tomato, ID5= Plot 3 tomato, ID6= Plot 4 tomato, ID7= Plot 1 cauliflower, ID8= Plot 3 cauliflower, ID9= Plot 4 cauliflower, ID11=Plot 3 broad beans and ID12= Plot 4 broad beans).

Chapter V: General Discussion

Peri-urban agriculture performs environmental, social and economic functions and services to the nearby urban areas (FAO, 2011). Nevertheless, industrialization and irrigation with TWW have led to an increase of the peri-urban agricultural exposure to chemical contaminants. For example, heavy metal accumulation in soil caused by industrial run-off, airborne pollution by traffic or industrial emissions, or the use of reclaimed water with a high content of CECs (Nabulo et al., 2010; X. Wu et al., 2014).

Concerns regarding human exposure to chemical contaminants have arisen since they have been detected in the edible parts of food crops at detectable levels (Pan et al., 2014), but the risk that accumulated residues may pose to humans via crop consumption is still not well documented. Furthermore, most of the current studies available in the literature are conducted in hydroponic cultures, or greenhouse settings, but real scale conditions have barely been studied for OMCs (Riemenschneider et al., 2016). Currently, there are only few field studies done at real scale, none of them in Spain, which demonstrate the uptake of micropollutants, needless to say the co-occurrence of organic and inorganic contaminants, in vegetables irrigated with reclaimed water (Christou et al., 2017b). Therefore, this Thesis aimed to evaluate the presence of OMCs and TEs in irrigation water and later in the soil-plant system in different farming conditions and evaluates their possible plant and human health risk effects. This study has been conducted in four farm plots located in the peri-urban area of Barcelona (NE Spain) and a pristine farm plot far away from the peri-urban area for comparison. A summary of the principal distinctive of each farm plot is provided in Chapter II. Many studies reported the presence of OMCs in TWW due to an incomplete removal of these chemicals in WWTPs as well as their posterior plant uptake (Calderón-Preciado et al., 2011; de Jongh et al., 2012; Gonzalez-Rey et al., 2015; Masiá et al., 2015). In this regard, Chapter II conducted an assessment of the occurrence of the selected chemical contaminants in irrigation waters (TEs and OMCs) from peri-urban area of Barcelona. As it was expected, Plot 3, located in the peri-urban area, showed the highest concentration levels for TEs and OMCs (Tables 2.2 and 2.3) due to their irrigation water impacted by WWTP effluents and the proximity to a traffic network. On the contrary, Plot 1, outside the peri-urban area, showed lower concentration levels of TEs and FOD of OMCs. Additionally, PCA analysis provided an insight into irrigation water nutrients and contaminants and classified these waters in four main groups: ground water (P1), surface water (P2 and P4), surface water impacted by WTTP effluents (P3) and groundwater impacted by industrial run-off (P5). However, the irrigation waters analyzed did not affect either seed germination, root elongation and crop productivity, indicating the suitability of using TWW for agricultural irrigation. Gvozdenac et al., (2016) assessed the phytotoxicity of the water from the Stara Tisa meander (Serbia), which contained low TEs and pesticides levels (<2 µg L^{-1}) in 6 vegetable seedlings and observed that while some species (sunflower, cucumber, maize and white mustard) are sensitive to abiotic stress, others (cabbage and radish) are tolerant showing little alterations. D'Abrosca et al. (2008) also observed different sensitivity among plant species in phytotoxicity assays probably due to different plant physiology. Therefore, in view of these studies, other seed species should be tested to assess the phytotoxicity of irrigation waters on seed germination.

Once chemical contaminants reach agricultural soil, their fate and behaviour depend on several factors. OMCs can undertake chemical changes or degrade into products with different toxicity than the parent compound. Whereas TEs cannot break down, their characteristics can be altered and thereby, their ability to be taken up by plants. Chapter III provides co-occurrence of TEs and OMCs in soil and lettuce irrigated with the irrigation waters reported in Chapter II. Two-thirds of the farm plots (P1, P3 and P4) sampled contained sandy loam soils with a smaller proportion of clay particles. The soils in the sampling area had the following TOC content: 0.77% for P1, 2.86% for P2, 2.65% for P3, 3.53% for P4 and 1% for P5. Examining table S3.8 about TEs' BCF, it would seem that generally food crops grown in soils with lower TOC content (P1 and P5) bioaccumulate TEs more than soils with a lower TOC content (P2-P4). This is consistent with the fact TEs present in the soil phase are able to undergo precipitation and decomposition, ionic exchange and adsorption and desorption; depending on the pH and the presence of clay minerals, humic substances, iron oxides and hydroxides, and manganese found in the soil (Petruzzelli et al., 2010). The importance of clay minerals and soil texture in the distribution of TEs between soil solid and liquid phases, affects the TEs bioavailability of plants. In sandy soils, TEs are more soluble and available for plant uptake than in clay soils. However, the organic fraction has a great influence on metal mobility and bioavailability due to the tendency of metals to bind with humic compounds in both the solid and solution phases in soil. Similarly, the mobility of OMCs in the soil compartment will depend on compounds and soil characteristics and basically consists on adsorption or desorption processes on solid soil phase. Generally, compounds with high K_{OW} values and low solubility will be mostly retained in the soil and consequently, less available for plant uptake. Indeed, compounds with $\log K_{OW} > 4.0$ hardly mobilize. On the contrary, organic contaminants with $\log K_{OW}<1$ will not be retained in soil, so they could be mobile and finally found in aquifers. In general, BCF for OMCs were lower in summer than in winter cultivation campaigns, this might be due to the rapid growth of summer crops (1 month) compared to winter crops (4-5 months) which could have cause growth dilution. PCA analysis determined the most relevant factors determining the presence of OMCs and TEs in lettuce crops, which were soil pollution, fungicide application, and irrigation water quality.

Finally, in Chapter IV, the occurrence of chemical contaminants in different vegetables crops and the human health risk by their consumption were examined. As it has been reported in the literature, vegetable species differ widely in their ability to take up and accumulate TEs, even among cultivars and varieties within the same species (Zhou et al., 2016). For example, Säumel et al. (2012) reported that Zn concentrations in green beans, tomato, potato, kohlrabi, and carrots were significantly lower than the concentrations in leafy vegetables. Moreover, Singh (2012) reported the pattern of some TEs (Zn, Cu, Pb and Ni) distribution in different vegetable types and observed a major concentration of these elements in roots (Cu, Cd and Ni) or leaves (Pb) being the minor translocation into fruit crops. However, other processes such as element interactions may alter the uptake patterns. The presence of heavy metals in TWW irrigation waters could become worse by the adverse effects of element interactions, that is continuously occurring among nutrients, heavy metals, soil physicochemical and biological properties within the TWW, soil and plants (Kalavrouziotis, 2017). The mechanism of synergistic interactions is still not well documented. On the contrary, the antagonist effects of the elemental interactions consist on the competition between essential nutrients and heavy metals occurring in the plants and soil. During the transfer of ions from the soil solution into the cytoplasm were there is a binding at transport sites in the plasma membrane, is where competition between ions with the same electrical charge may occur (Marschner, 1995) .

OMCs uptake and translocation also varied between types of food crops. For instance, uptake of contaminants from soil is likely to be higher in root vegetables (e.g., carrots) than tree fruits (e.g., apples) because root crops are in close contact to soil, whereas tree fruits are not. By contrast, uptake of contaminants directly from air is expected to be higher for tree fruits than root crops (Trapp and Legind, 2011). Many other plant parameters may influence in the assimilation of contaminants including the root system, transpiration rate, shape and size of leaves, and lipid content. Collins et al. (2007) published a summary of the principal crop nutrients in function of the principals crop classes (Table 5.1):

Table 5.1 Details of water, lipid, carbohydrate, and fiber content of crops relevant to risk assessment

Crop class	% water	% lipids	% carbohydrates	% fiber
Leafy crops	94.1	0.16	3.5	1.5
Bulbs, stems, tubers	83.6	0.16	13.9	1.8
Roots crops	83.9	0.27	13.7	3.9
Fruit crops	88.4	0.36	7.9	2.0

It can be noticed from the table above that leafy crops contain the major value of water content and the minor value of carbohydrate percentage, whereas root and fruit crops are the ones with highest values regarding lipid composition. There are few researches that point out that the lipid content is of significance in plant uptake, while others required also the fiber and carbohydrate content (Zhan et al., 2013). However, other plant constituents such as waxes, lignin and suberin may also absorb OMCs (Q. Li et al., 2017).

In this section, PCA analysis indicated that the occurrence of chemical contaminants in crops depended on the commodity rather than the location (peri-urban vs rural).

Furthermore, it should be remarked that chemical contaminants assimilated by plants could be metabolized. In some cases, these metabolites present a similar bioactivity than the parent compound. Malchi et al. (2014) observed that CBZ was rapidly metabolized in carrots and sweet potatoes, being EPOCBZ the metabolite that a demonstrated a greater toxic effect than the parent compound (Tomson et al., 1990).

Metabolism likely controls the final accumulation of PPCPs in plants but is in general poorly understood for CECs (Fu et al., 2017). Chuang et al. (2018) published that after 6 days of the experiment, the 50% of caffeine was dissipated in lettuce grown in a hydroponic culture, 20% due to demethylation and 8 metabolites (phase I) were found. Likewise, Hurtado et al. (2018) reported that the conjugated fraction (phase II metabolites) of several CECs accounted from 27 to 83% of the parent compound and generally increased with the hydrophobicity and concentration. Therefore, current research should focus on the identification of the metabolites generated after the uptake of chemical contaminants in order to perform a proper health risk assessment.

Hypothesis validation

At the beginning of this study, a pair of hypotheses were raised. Firstly, it was raised the question on whether crops watered with irrigation waters of different physicochemical properties would provide vegetable with differential quality properties ($p<0.05$) and secondly, whether peri-urban agriculture may result in vegetables of low food quality and subsequent human health risk associated compared to rural farming.

Nevertheless, both of two hypotheses have been rejected along the experiments comprised in this Thesis as no statistically differences ($p>0.05$) in crop constituents (e.g. chlorophyll, nitrate, carbohydrate and lipid content) were obtained in lettuce samples irrigated with different water qualities (e.g. ground and surface waters, some impacted by industrial run-off and WWTP effluents) or cultivated under different farming conditions (peri-urban and rural horticulture).

The fact that some researchers reported significant differences in crop constituents at closely environmental concentrations of contaminants (10 and 0.5 µg L^{-1}) (Christou et al., 2019; Hurtado et al., 2017) strengths the conclusion that neither irrigation water quality nor farming practices covered in this study, were sufficient to significantly impact on crop composition.

5.1 Future research needs

Metabolomic response

From the present study it has been revealed that chemical contaminants, from TWW irrigation water and other urban anthropogenic sources, can be incorporated and translocated into edible parts of the plants in detectable concentrations; but the crop components (chlorophyll, nitrate, carbohydrate and lipid content) tested were not significantly different (p>0.05) comparing peri-urban and rural sites and seasonality. However, it is widely reported in the literature that plant metabolism is involved in physiological regulation and defense response to biotic and abiotic environmental stress factors, such as drought, salt, low oxygen due to waterlogging or flooding of the soil, temperature, light, and oxidative stress (Jorge et al., 2016).

Recent progress in metabolomics techniques has allowed the analysis of plant metabolic changes. Plant metabolomics aims to study the plant system at the molecular level to provide a non-biased characterization of the total metabolite pool (metabolome) of a plant's tissue in response to its environment (Jorge et al., 2016). It comprises the analysis of a wide range of chemical compounds from ionic inorganic compounds to biochemically derived hydrophilic carbohydrates, organic and amino acids, and a range of hydrophobic lipid-related compounds; an estimated 200,000 different metabolites in the plant kingdom (Fiehn, 2001), with a wide range of physicochemical properties and thereby, powerful analytical tools are requiring for the separation, characterization and quantification of this vast compound diversity present in plant matrix. Consequently, no single analytical technology can cover the whole metabolome due to metabolite's chemical diversity and broad dynamic range in cellular abundance. Thus, different extraction techniques and combinations of analytical methods are often employed to achieve adequate metabolite coverage. Analytical methodologies based on gas chromatography-mass spectrometry (GC-MS), liquid chromatography (LC)-MS, capillary electrophoresis (CE)-MS and nuclear magnetic resonance spectroscopy (NMR) are commonly used in plant metabolomics research. Nevertheless, NMR has poor sensitivity and poor dynamic range compared to MS, so it is only able to properly evaluate the most abundant metabolites and it is used as a fingerprinting technique, in other words, a forerunner to metabolic profiling (Jorge et al., 2016).

Few plant metabolomic research has been done for metals (e.g. Pb and Cd) and some pesticide application (e.g. $Cu(OH)_2$ nanoparticles) (Wang et al., 2015; Zhao et al., 2018, 2016). For example, Wang et al. (2015) reported greater accumulation of glucose, galactose and fructose under Pb treatment and a decrease of maltose, turanose, α-D-glucopyranoside and β-D-glucopyranose in radish roots, suggesting that photoassimilates were stored as hexoses in radish after Pb exposure. However, metabolomic studies on plant response to CECs exposure have been barely performed up to date.

Recently, Hurtado et al. (2017) demonstrated that plant exposure to relevant environmental concentrations of CECs (from 0.05 to 50 µg L^{-1}) could affect plant morphology and plant physiology. Changes related to metabolic variations in plants (carbohydrate metabolism, the citric acid cycle, pentose phosphate pathway and glutathione pathway) were observed in leaf high, stem width and chlorophyll A and B content of lettuce at the low concentration of 0.05µg L^{-1}.

Aside from evaluating the effects of chemical contaminants in the metabolome, the use of metabolomics for food identification purposes (e.g. geographical origin, farming practices, vegetable varieties, etc.) has gained interest (Yang et al., 2018). Llano et al. (2017) explored the effect of organic and conventional conditions on specific chemicals (carotenoids, ascorbic acid and pesticides) using targeted metabolomics and examined overall phytochemicals by untargeted metabolomics approach. Even though metabolome level of the golden berry fruits tested was influenced by farming practices, no significant differences were found regarding carotenoids and ascorbic acid content. Despite the fact that some studies reported changes in metabolome due to organic and conventional farming (Novotná et al., 2012; Vallverdú-Queralt and Lamuela-Raventós, 2016) the limited number of available reports makes impossible to achieve an accurate conclusion.

In this regard, obtaining conclusions from a metabolomic study of vegetables grown in the different farm plots studied in this PhD is difficult, since in addition to the effect of the presence of the studied chemical contaminants, other constituents from water, soil or air may play an important role. Therefore, it would be better to study factors separately. This implies to study irrigation water, soil, air pollution individually under controlled conditions, preferably in a greenhouse facility.

Risk assessment approaches and other compounds of concern

The co-occurrence of contaminants in edible parts of food crops have been scarcely considered in field studies. Nevertheless, it is convenient to take an overview of the chemical contaminants present in foodstuff for a reliable risk assessment. In this context, the study of antibiotics, antibiotic resistant bacteria and antibiotic resistant genes is of great interest due to their spread by the application of manure and biosolids amendment, and irrigation with TWW (Christou et al., 2017a). Metabolites of chemical contaminants in plant tissues ought to be monitored, they may surpass the concentration of the parent compounds and show more acute toxicity in some cases (Christou et al., 2017b; Prosser and Sibley, 2015). Similarly, the nonexistence of risk assessment approaches regarding co-occurrence of organic and inorganic contaminants and the lack of information about the toxicity of CECs and metabolites remain a current limitation. As toxicological profile data is not available for CECs, the TTC approach appears a suitable option to estimate their potential risk associated by comparing the chemical structures of these contaminants with other ones from which toxicity data are accessible. Hence, there is the need of obtaining the toxicological data of these compounds.

In addition, the major drawbacks of field experiments are their less controllable and reproducible conditions, that could originate variances among individual plant samples (Riemenschneider et al., 2016). Although in this study, no significant differential (p>0.05) effects on plant composition were found among different irrigation systems and water qualities; it remains unclear the possible release of plasticizers and vulcanization agents from the irrigation tubing used in drip and sprinkle irrigation. Therefore, it should be recommended to deepen the effects of the irrigation system used in the release of contaminants into agricultural fields. In the same way, more research studies are needed to assess other agricultural practices such as mulching. The presence of microplastics in the soil could change soil properties and microplastics may be transported by soil organisms or act as vectors for other soil pollutants. Li et al. (2010) reported the influence of plastic mulching on the translocation of some elements, particularly of Fe in broad beans). Besides, Du et al. (2009) investigated the uptake of di-(2-ethylhexyl)phthalate from plastic mulch film by 10 vegetable plants; where wax gourd, cucumber and pumpkin crops appeared to be a potential source of this contaminant in human diet. Consequently, further studies are needed to appropriately estimate the contribution of agricultural practices to food contamination and quality.

Chapter VI: Conclusions

The general main conclusions extracted from the research conducted in this Thesis are summarized as follows:

- Crops irrigated with surface waters in peri-urban areas are exposed to a higher FOD and concentration of TEs and OMCs than those irrigated with groundwater from the rural areas.
- B and Ba were the TEs with the highest concentration in all irrigation waters, while Zn (34-68 μg L^{-1} on average) and Mn (23-63 μg L^{-1} on average) were the most abundant in peri-urban irrigation waters. Surfynol 104 (113-2860 ng L^{-1} on average) was the most abundant OMCs in all irrigation waters.
- The high concentration levels of TEs, OMCs and nutrients found in surface irrigation waters, especially in those impacted by WWTP effluents (P3), did not affect seed germination, root elongation (*Lactuca sativa* L. cv. Batavia), or crop productivity (*Lactuca sativa* L. cv. Batavia and *Lycopersicon esculentum* Mill. cv. Bodar).
- Seasonal cultivation of lettuce (winter *vs.* summer) did not result in a significant difference in the total TE concentration. However, fungicides such as dimethomorph and carbendazim were only detected in the winter season.
- BCF for TEs ranged from 0.0002 to 1.79 but only Cd and Sb exhibit BCF above 1 in several farm plots. BCF for OMCs varied between 1 and 375 highlighting the predominance of some OMCs that may be introduced directly from pesticide application or the irrigation system (use of plastic pipelines).
- The higher abundance of chemical compounds (TEs and OMCs) in peri-urban agriculture did not impact chlorophyll, carbohydrate and lipid content in lettuce leaves. Nevertheless, chlorophyll content varied because of seasonality and nitrate content depended on the irrigation water quality.
- Concentration levels of TEs and OMCs varied between different types of vegetables, having tomato fruits the highest concentration for OMCs and together with broad beans for TEs.
- The risk associated with the consumption of the selected food crops (lettuce, tomato, cauliflower and broad beans) grow in the area of study was estimated to be low and similar between peri-urban and rural sites. THQ for TEs were below 1 and a minimum consumption of 150 g/day for children and 380 g/day for adults is required to surpass the TTC due to the presence of pesticides. Nevertheless, the combined effect of TEs and OMCs could not be assessed.
- Statistical analysis revealed that the variance of the concentration of chemical contaminants from different vegetable crops depended on the crop rather than the farm location (peri-urban and rural agriculture). In addition, the variance in a single vegetable type was principally explained by the irrigation water, which aided to classify its origin.

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