Deliverable of WG2

Deliverable 7

Prioritization of microcontaminants (chemical and biological) and key factors affecting their uptake process

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ACRONYMS

ABs	Antibiotics
ARB	Antibiotic-Resistant Bacteria
ARGs	Antibiotic Resistance Genes
BFR	Brominated Flame Retardant
BT	1,2,3-benzotriazole
CBZ	Carbamazepine
CCL	Contaminant Candidate List
CECs	Contaminants of Emerging Concern
CYP	Ciprofloxacin
DBPs	Disinfection By-Products
DCF	Diclofenac
DDT	Dichlorodiphenylethane
D_{OW}	pH adjusted octanol-water partitioning coefficient ($D_{OW} = K_{OW}/(1+10^{pH-pKa})$)
ECHA	European Chemical Agency
EE2	17α-ethinylestradiol
ENR	Enrofloxacin
FDA	Food and Drug Administration
GABA	Gamma-Aminobutyric Acid
HBDD	Hexabromocyclododecane
HGT	Horizontal Gene Transfer
LMG	Lamotrigine
LCF	Leaf Concentration Factor
MGEs	Mobile Genetic Elements
NDMA	*N*-nitrosodimethylamine
NER	Non-Extractable Residue
PAHs	Polycyclic Aromatic Hydrocarbons
PBDEs	Polybromodiphenyl esters
PFBA	Perfluorobutanoate
PFCA	Perfluorocarboxylic Acid
PFOA	Perfluorooctanoic Acid
PFOS	Perfluorooctane Sulfonate
RWW	Reclaimed Wastewater

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SCF: Seed Concentration Factor

SMX: Sulfamethoxazole

SOM: Soil Organic Matter

TBBPA: Tetrabromobisphenol A

TCC: Threshold Contaminant Concentration

TCP: Tris(2-chloroethyl)phosphate

TCS: Triclosan

TMP: Trimethoprim

TPs: Transformation Products

TSCF: Transpiration Stream Concentration Factor

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Executive summary

Water reclamation and reuse together with soil amendment with biosolids and manure leads to the introduction of a myriad of organic contaminants into the agri-environment. Their fate is very complex and depends on the physical-chemical properties of contaminants but also environmental factors (T, RH, soil properties) including agronomical practices (irrigation technologies, soil management) and plant genotypes (species and cultivars). In this report, we focus on the contaminant physicochemical properties and environmental factors affecting to the crop uptake. A list of contaminants of emerging concern (CECs) based on soil persistence, occurrence in crops and the suspected toxic effects of the CECs is suggested. In addition, a candidate list of ARB and ARGs is also proposed but further research is needed to establish it particularly in endophytic bacteria.

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1. Introduction

The source of the organic contaminants in reclaimed wastewater (RWW) is the partial removal during the different treatment steps (Miege et al., 2009; Murray et al, 2010; Langford and Thomas, 2011). Some of the classes of contaminants found in treated effluents, are the following:

1. Pharmaceuticals
2. Personal care products
3. Illicit drugs
4. Plasticizers
5. Antioxidants
6. Flame retardants
7. Transformation products (TPs) or degradation intermediates

Transformation products (TPs) occurring in RWW can be originated during the wastewater treatment processes but also in case of pharmaceuticals during the human metabolism.

Disinfection by-products (DBPs) have been extensively studied in drinking water (Richardson and Ternes, 2011). They can be generated during the disinfection of RWW by chemical processes, such as chlorination and ozonation, or by physical methods, such as UV mediated processes (photocatalysis) from a specific precursor. The DBPs are process specific and generated from the oxidation or photolysis of organic matter during the disinfection processes (Krasner et al., 2009). Among them, trihalomethanes originated during chlorination are the most widely assessed in reclaimed wastewater (Matamoros et al., 2007). Although they can constitute a class of potential hazardous contaminants in RWW, very few reports exist on their uptake by crops and this aspect will not be covered in this report.

Antibiotics (ABs) used in human health care is one the most relevant class of CECs, since they are commonly detected in RWW (Kümmerer, 2009; Manzetti and Ghisi, 2014), and they might exert a selective pressure to the soil microbiota leading to the formation of antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) (Andersson and Hughes, 2012). The result is that ARB and ARGs may spread to terrestrial and

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aquatic environments and, eventually propagate to other downstream environments (Bondarczuk et al., 2016).

Environmental compartments receiving RWW and biosolids behave as genetic reactors, where bacteria may be mixed and counteract with environmental organisms (Baquero et al., 2008). Thus, the continuous disposal of RWW, biosolids and manure in the environment render soil as the largest environmental reservoir of antibiotic resistance (Nesme et al., 2014). ARGs may persist in the environment, as they can not only multiply in individual hosts but also be transferred to other microbial populations beyond their original hosts (i.e. human pathogens of clinical relevance), through horizontal gene transfer (HGT) of mobile genetic elements (MGEs). Moreover, the impact of AB residues on the aquatic and terrestrial ecosystems cannot be neglected on the basis of ecosystem service concept (Brandt et al., 2015).

Despite the large number of CECs present in RWW, only about one hundred of organic CECs have been assessed in plant uptake studies at different cropping conditions (i.e. *in vitro*, culture, field studies), making thus difficult the comparison of their results (Miller et al., 2016).

Even more, the number of studies reporting on the occurrence of ABGs and ARB in agri-environments is very recent and usually limited to irrigation water and soil, while very few studies investigate plants uptake (Esiobu et al., 2002; Udikovic-Kolic et al., 2014).

Contaminants can be uptaken by plants from soil by **roots** and atmosphere (gas phase and suspended particles) and by **shoots** and **leaves** (Collins and Finnegan, 2010). The uptake from soil is basically from contaminants dissolved in pore water, in equilibrium usually with soil particles and colloids. Volatile contaminants in soil can be uptaken by leaves by volatilization usually through cuticle and the smallest molecules through stomata and then, translocated by the plant vascular system (Fig. 1). All of these processes can be parameterized and taken into account in some of the predictive models described in the Deliverable 2.4.

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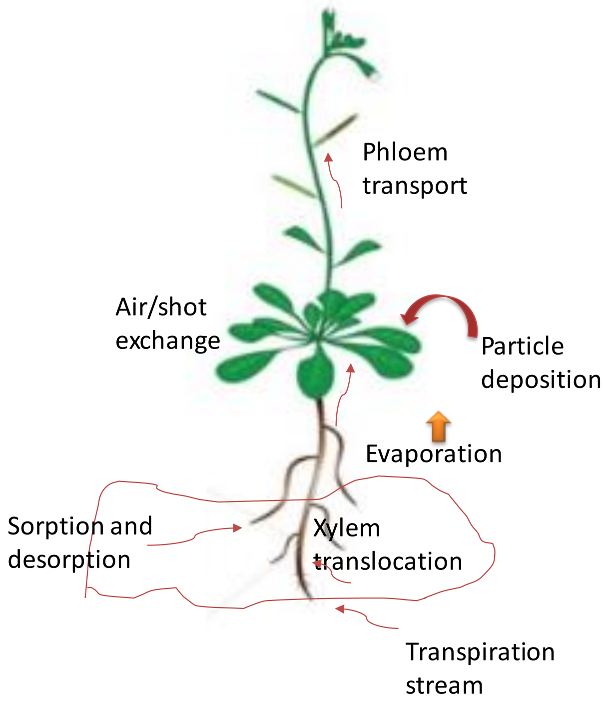

Figure 1 - Plant uptake pathways

Endophytic bacteria play a significant role in the degradation of uptaken contaminants by plants (Afzal et al., 2014), but their pathways are not well understood and they appear to be mobilized through xylem (Yadeta and Thomma, 2013).

Biosolids and **manure**, widely used as soil amendment, can be sources of CECs including veterinary antibiotics (Hu et al., 2010; Walters et al., 2010; Clarke and Smith, 2011), biocides, pharmaceuticals and personal care products (Hu et al., 2010; Walters et al., 2010; Clarke and Smith, 2011).

2. Key factors affecting the uptake processes of organic microcontaminants

The uptake of organic microcontaminants by plants is primarily controlled by their bioavailability in the soil-root system. In the next section the key factors are briefly described (Figure 2).

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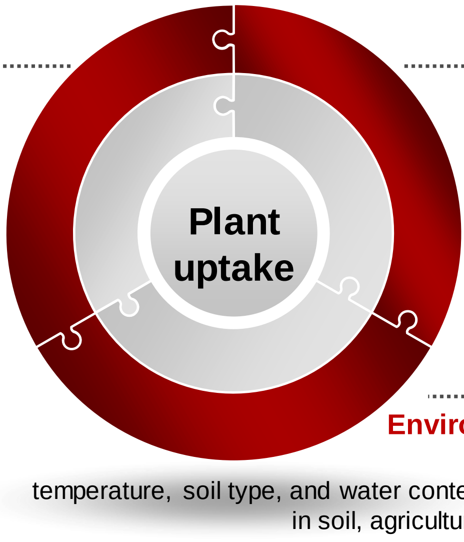

Figure 2 - Key factors affecting their uptake process

2.1. Growth conditions

The growing conditions can substantially affect the contaminant uptake by crops. **Greenhouse** cropping is widely used in the intensive agriculture and it is expected that it provides the highest exposure to crops (no rainfall dilution or wind drift) together with higher plant transpiration.

Organic agriculture is not using synthetic agrochemicals, while soil fertilization is carried out with manure amendment. Since **manure** can contain veterinary drugs, it is not surprising that some crops grown under organic agriculture can harbour a higher abundance of ARGs than conventional agriculture (Zhu et al., 2017).

Agronomical practices, such as soil surface coverage with **plastic films** or **soil mulching** with biomass are commonly used in herbaceous crop production (i.e. lettuce, strawberry), in order to avoid the weed proliferation and to prevent soil moisture depletion. Although the effect of these agronomical practices in terms of contaminant uptake by crops has not been experimentally assessed, it can be inferred that these practices may enhance the potential for contaminants uptake by plants. Such a trend may be attributed to the increased exposure of contaminants to plants as a result of the significant reduction of the contaminant dissipation processes, such as volatilization, photooxidation and biodegradation from the topsoil. Therefore, this practice could increase the root exposure to contaminants and minimize the uptake from the aerial parts

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of the plant. Accordingly, it could enhance the bioaccumulation of contaminants uptaken by roots. However, further research is necessary to ascertain this hypothesis.

2.2. Soil properties

Soil properties play a big role in the crop uptake of CECs affecting their bioavailability. Sandy soils with low soil organic matter (SOM) content are the ones with the lowest interaction with organic microcontaminants and the more prone to their accumulation by crops (Goldstein et al., 2014). Cation exchange capacity is also an important parameter, relevant in case of ionic or ionized contaminants (Domínguez et al., 2014). The soil pH plays a significant role in the chemical speciation of the compound, and the neutral forms are the ones with the highest root bioconcentration factors (Miller et al., 2016). Alkaline ions (Ca^{2+}) in calcareous soils and metallic cations (Cu^{2+}) can form stable chelates or complexes with the anionic organic microcontaminants, decreasing thus their solubility in pore water and then their bioavailability by plants (Graouer-Bacart et al., 2016).

High-energy interactions between contaminants and soil or physical trapping in the layered clays can lead also to a strong decrease in the contaminant bioavailability (Pan et al., 2009). Usually this fraction of contaminant is referred as non extractable residue (NER) but changes in the soil redox status can release a NER fraction becoming bioavailable by plants (Jechalke et al., 2014). At present, no consensus exist if this fraction should be considered or not in the risk assessment evaluations.

2.3. Plant physiology

Transpiration rate and plant metabolism related to plant genotype can play a significant role in the uptake and accumulation of organic contaminants. They are related to the plant genotype and can play a significant role in the uptake, metabolization and accumulation of persistent organic contaminants (dichlorodiphenylethane (DDTs), polycyclic aromatic hydrocarbons (PAHs) and chlordane) in cucurbitaceae (Mattina et al., 2006). In addition, significant differences in metformin bioconcentration factors were found between different carrot cultivars from spiked soil (Eggen and Lillo et al., 2012).

Foliar and root lipid content can be of particular relevance, since crops characterised by a high lipid content can give rise to a faster sorption kinetics of hydrophobic contaminants due to equilibrium partitioning processes (Yang et al., 2016).

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2.4 Chemical specific properties

Molecular weight is a relevant property in the uptake of contaminants in the rizosphere. In fact, water and small molecules (<500 D) can enter through the epidermis of growing root tips, including root hairs (Miller et al., 2016). However, higher molecular weight compounds, such as the deca-brominated flame retardant BDE-209 (Mw = 959 g mol^{-1}) have been detected in roots and shoots of different plant species (i.e. ryegrass, alfalfa, pumpkin, summer squash, maize, radish). However, the uptake is not well understood for such large molecules (Huang et al., 2010).

Hydrophobicity and ionization extent are key properties affecting the uptake of CECs by crops. For neutral molecules, hydrophobicity is measured as the octanol-water partition coefficient K_{OW}. However, for the ionizable contaminants, in spite of K_{OW}, the D_{OW}, which is the pH of the pore water adjusted octanol-water partitioning coefficient, is used. Neutral contaminants at the soil pH with moderate hydrophobicity (log K_{OW} = 1-3), are the ones, which exhibit the highest bioconcentration factors and can be translocated to different plant organs (Trapp 2004). Hydrophobic contaminants (log K_{OW} >4) can be adsorbed on the plant roots leading to a high root bioconcentration factors. For instance, the antimicrobial triclosan and triclocarban can be adsorbed on the root lipid membranes in hydroponic systems but they are not prone to be translocated to other plant organs (Matthews et al. 2014).

Acidic contaminants in neutral or basic soils occur as **negatively** charged and as a consequence, they exhibit electrostatic repulsion with the negatively charged membranes of the root surface (Trapp 2004). As a consequence, they are not prone to be accumulated by plants through roots (Calderón-Preciado et al., 2011). Nevertheless, in long-term studies of crops irrigated with RWW, diclofenac has been reported in tomato fruit following several years of irrigation with reclaimed water (Christou et al., 2017).

Basic compounds at the soil pH can be ionized in the acidic pH of the apoplast and can be accumulated in cells by the so-called **ion trap** mechanism but not translocated (Trapp 2004). For instance, lamotrigine, which occurs as neutral at soil pH but can be protonated in the apoplast exhibited high concentrations in carrot leaves (Malchi et al., 2014).

Complex molecules, such as ABs might contain multiple functional groups in the molecule. As a consequence, many chemical species might coexist depending of soil pH and the same molecule can contain positive and negative charges simultaneously

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(**zwitterion**). Accordingly, their behaviour is compound and soil specific (Brett-Sallach et al., 2015).

Finally, chemical contaminants with **structure** similar to the other **biogenic molecules** can be counter-gradient transported by protein membranes due to the low molecular selectivity with plant energy expenses (Miller et al., 2016). For instance, the corrosion inhibitor benzotriazole has been hypothesized to be taken up into *Arabidopsis* by nitrogen transporters due to the structural similarity to tryptophan aminoacid (Lefevre et al., 2015).

2.5. Exposure medium and environmental factors

Hydroponic culture is a growing technology particularly in high-tech agriculture. Under hydroponics as growing medium, the highest bioconcentration factors due to the lack of soil partitioning have been detected. **Sand-perlite** growing medium shows the smallest interaction with contaminants and in this respect, the experimental bioconcentration factors can approach those found in hydroponics (Hurtado et al., 2016b). ***In vitro* culture** under sterilized conditions has also been used to evaluate the contaminants' uptake by crops usually with agar-agar growing medium (Calderón-Preciado et al., 2012). Under these experimental conditions, photooxidation can be a significant removal pathway of contaminants from the growing medium.

The irrigation technology can also affect the uptake of organic microcontaminants. Usually, drip irrigation can provide the lowest contaminant intake to crops due to the smallest volume of water used. On the other hand, sprinkling irrigation with reclaimed water can lead to a direct contact of the dissolved contaminants in RWW with the edible parts of crops. Accordingly, contaminants that cannot be translocated can be in contact with the edible part of vegetables (Calderón-Preciado et al., 2013).

Dissipation time usually measured as DT_{50} (time necessary to degrade the 50% of the original contaminant concentration) is also relevant in the crop uptake of CECs (Table 1). The DT_{50} values span in several orders of magnitude depending on the contaminant and soil conditions (Hurtado et al., 2016a). In this regard, the DT_{50s} in the rizosphere where specific microbiome occur with a high specific activity enhanced by root exudates, these values can be smaller than non cropped soil (Zhuang et al., 2007; Lundberg et al., 2012).

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3. Priorization Criteria

3.1 Contaminants of emerging concern (CECs)

The following criteria have been established for priorization. Usually, a single CEC has been chosen as a marker or representative of a broader family of contaminants with similar properties.

1. High frequency of detection in treated effluents. It is related to high patterns of use and recalcitrance during the wastewater treatment process.
2. Environmental, agricultural or health concern. At least one of the following criteria should be met by the target CECs:
    a. DT50 in soil > 14 d (O'Connor, 1996)
    b. Phytotoxicity at environmental relevant concentrations
    c. Promote a selective pressure to soil microbiota
    d. Potential human health effects according to threshold contaminant concentration (TCC) criteria.
3. Significant uptake rate by crops. Usually, bioconcentration factors (RCF=[root]/[growing medium]; LCF=[leaf]/[growing medium]; FCF=[fruit]/[growing medium]) higher than 1.

3.2 Antibiotic Resistance Genes and Antibiotic-Resistant Bacteria

- Prevalence in RWW
- Prevalence in soils irrigated with RWW
- Potential for internalization in plant tissues (Ye et al., 2016; Zhang et al., 2016)

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4. List of Priority CECs

According to the criteria described in the Section 2.1, the following CECs have been selected.

Table 1 - List of prioritized CECs, relevant properties and metabolites and toxicity

Compound	CAS Nr	Class	p*Ka* values*	log K_{OW}	DT_{50} (d)	Relevant metabolites	Toxicity effects & related genes
Carbamazepine (CBZ)	298-46-4	Anticonvulsant	----	2.23	60-693	10,11-epoxide, 10,11-diol dihydro	Genotoxic metabolites
Diclofenac** (DCF)	15307-86-5	NSAID	4.15	4.51	0.5	Glycoside and glutathione conjugates	Renal failure vulture
Enrofloxacin (ENR)	93106-60-6	Quinolone antibiotic	6.17; 8.8	0.7	>152	Ciprofloxacin	ARG: *qnrS*
Sulfamethoxazole (SMZ)	723-46-6	Sulfonamide antibiotic	+/0: 1.6 0/-: 6.2	0.7	2-33	5-methylhydroxy; N4-acetyl-5-methyl-hydroxy-; sulfamethoxazole-N1 glucuronide	ARG: *sul*1
17α-ethinyl estradiol** (EE2)	57-63-6	Contraceptive	---	3.67	NA	Conjugates	Estrogenicty
Lamotrigine (LMG)	84057-84-1	Epileptic seizure and bipolar disorders	5.7	0.99	NA	N2 glucuronide Conjugates	Genotoxicity
Trimethoprim (TMP)	738-70-5	inhibitor of dihydrofolate reductase	7.2	0.91	>152	hydroxy; 4-desmethyl-	ARGs: *tetM, tetW*

*(0) depicts the neutral chemical species, (-) anionic and (+) cationic forms

**Included in the EU Watch List 2008/105/EC.

According to the Cramer's TOXTREE, all the former contaminants belong to the Class III, except from the 17α-ethinyl estradiol (EE2), which is classified as Class II (http://tinyurl.com/kv62kfy). However, EE2 exhibits a strong estrogenicty close to the endogenous estradiol at low concentrations (Bhandari et al., 2015).

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Carbamazepine (CBZ). It is used primarily in the treatment of epilepsy and neuropathic pain. It is used in schizophrenia along with other medications and as a second line agent in bipolar disorder. The main CBZ metabolites are 10,11-epoxide, which can be found at higher concentration than CBZ in leaves of sweet potato and carrot (Malchi et al., 2014).

The reported DT_{50} CBZ values in soil range from 60 to 693 days. In the soil-root system occurs in the neutral form and according to optimal log K_{OW} exhibits one of the highest transpiration stream concentration factor (TSCF). In fact, its leaf BCFs in soybean, radish, cucumber, tomato and sweet potato ranges from 0.6 to 425 kg kg^{-1} dw. The root BCFs in soybean, radish, cucumber, tomato, sweet potato ranges from 0.1 to 8.3 kg kg^{-1} dw and the fruit BCFs in cucumber and tomato from 0.4 to 27 kg kg^{-1} dw. Accordingly, the highest accumulation pattern of CBZ is in leaves and the reported BCF values (up to BCF=425 kg kg^{-1} dw) are very high, which is consistent with uptake and translocation through phloem facilitated by the neutral charge of the molecule and its moderate hydrophobicity. From the risk assessment point of view, the major metabolites should be measured since their concentration can exceed to the parent compound.

Diclofenac (DCF). It is a non-steroidal anti-inflammatory drug used for the treatment of pain and inflammation associated with arthritis. It has been included in the Watch List of the Water Framework Directive from the EU (2008/105/EC).

In the neutral-basic soils, DCF occurs predominantly in the anionic. Its DT_{50} in common soils is not reported experimentally but in soils subjected to long-term wastewater irrigation shows a short DT_{50} values (<0.1-1.4 d) but these values maybe not representative of soil subjected to RWW irrigation (Dalkman et al., 2014). Radish leaf and bulb exhibited BCFs of 11.53 and 5.39 kg kg^{-1} dw respectively and ryegrass 6.82 kg kg^{-1} dw (Carter et al., 2014). The major DCF metabolites in *Typha latifolia* L. are the glycoside and the glutathione conjugates (Bartha et al., 2014).

Enrofloxacin (ENR). It is a bactericidal agent belonging to the flouroquinolone class used in veterinary applications. Enrofloxacin has demonstrated a significant post-antibiotic effect for both Gram-negative and Gram-positive bacteria and is active in both stationary and growth phases of bacterial replication. It is stable to hydrolysis but photolabile. Its antibiotic potency depends on the fluorine at the C-6 position (Thiele-Bruhn 2003). Enrofloxacin is partially deethylated by CYP450 into the active metabolite ciprofloxacin (CYP), which is also a fluoroquinolone antibiotic, used preferentially in humans.

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ENR and CYP are frequently detected in RWs and exhibit a high stability in soil (Table 1). Depending on the soil pH, it presented both as ionized carboxylic acid and zwiterionic species (Figure 3). ENR has been detected in carrot (BCF=11 referred to pore water) but the concentration of ENR was found to be below the LOD in leafy vegetables (lettuce) (Boxall et al. 2006).

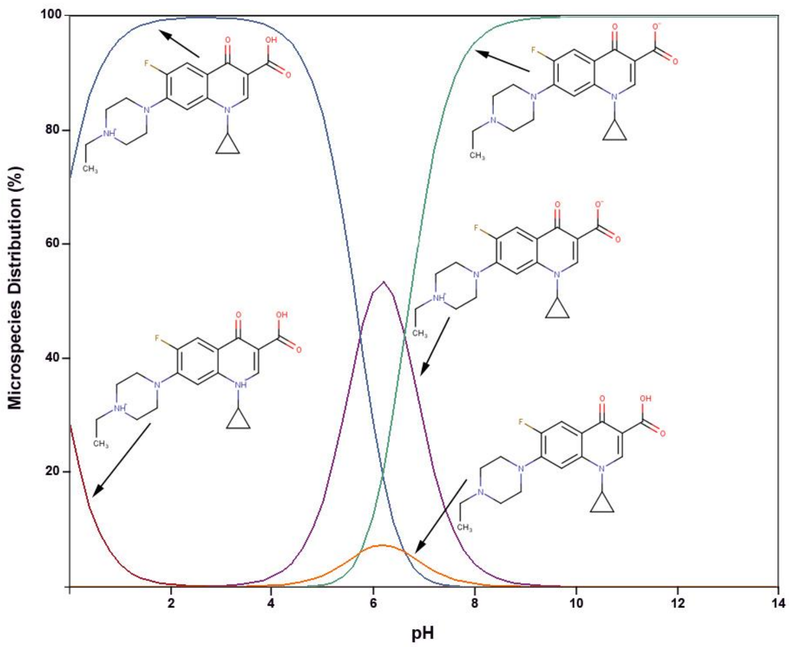

Figure 2 - Distribution of ENR species according to the soil pH obtained from MarvinSketch v. 17.6 software.

Sulfamethoxazole (SMX). SMX is the most prominent short-acting representative of sulfonamide antibiotics used in high amounts in human and veterinary applications to treat and prevent bacterial infections of both Gram-positive and -negative species. The bacteriostatic effect of sulfonamides on cell reproduction originates from the sulfanilamide toxicophore and is based on competitive enzyme inhibition and metabolic interference due to its similarity in molecular structure to *p*-aminobenzoic acid, an essential carboxylic acid involved in the natural intracellular folic acid synthesis of bacteria. In neutral soil pH, both negatively charged and neutral species coexist (Figure 3). Its reported DT_{50} is quite high (Table 1).

Concentrations of SMX in lettuce grown under different soil textures (sandy, sandy-loam and loam) ranged from 54 to 107 $\mu g\ kg^{-1}$ fw (Zhang et al. 2016) and from 88 125 $\mu g\ kg^{-1}$ fw when wastewater was used for irrigation (Brett Sallach et al., 2015). In another work, SMX was detected in grass at concentrations from 7 and 21 $\mu g\ kg^{-1}$ and watercress 4 to

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7.5 μg kg^{-1} when soil was spiked at concentrations from 5 to 10 mg kg fw (Chitescu et al., 2013). Ahmed et al (2015) evaluated the uptake of SMX in cucumber, tomato and lettuce when soil was spiked at different concentrations (5, 10 20 mg kg^{-1}). The highest concentrations were detected in roots (10-35 mg kg $^{-1}$) followed by lettuce leaves (3 mg kg^{-1}). Hu et al (2010) in a field study at the north of China evaluated the SMX occurrence in radish, rape, celery, and coriander found the highest concentrations in radish (0.9-2.7 μg kg^{-1}). Holling et al (2012) evaluated the uptake of SMX in Chinese cabbage (*Brassica campestris*) in fortified organic matter rich soil and biosolids at environmental relevant concentrations (low μg kg^{-1}). Mean root and aerial concentrations were 260.3 and 179.2 μg kg^{-1} respectively in high SOM but it was not detected in biosolid grown plants. Reported BCFs refereed to soil in lettuce ranged from 0.0045 to 0.0055 (Ye et al., 2016), 0.223 in cabbage shoot (Holling et al., 2012), 0.4 to 0.833 in carrot root and 1.25 to 5 in sweet potato root (Malchi et al., 2014), 0.47 to 5.362 in tomato fruit (Christou et al., 2017).

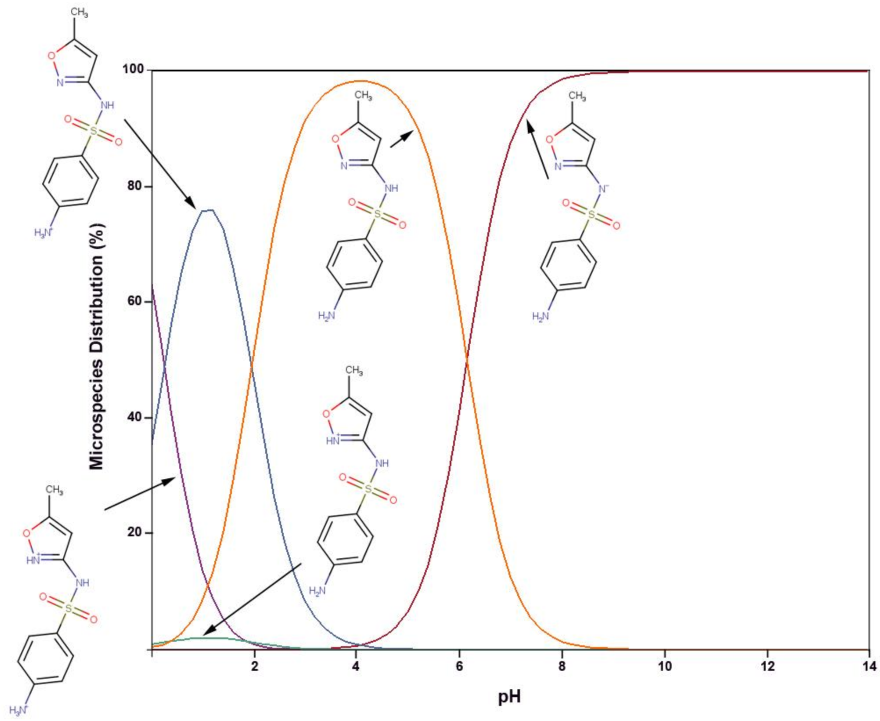

Figure 3 - Distribution of SMX species according to the soil-pore water pH obtained from MarvinSketch v. 17.6 software.

17α-ethynylestradiol (EE2). It is a synthetic, steroidal estrogen and a derivative of estradiol, the major endogenous estrogen in humans used as contraceptive. It has been included in the Watch List of the Water Framework Directive (2008/105/EC) from the EU.

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It is a neutral and moderately hydrophobic compound. Its uptake has been evaluated in spiked sand and soil and the uptake has been evaluated in bean plants (Karnjanapiboonwong et al., 2011). The highest bioconcentration values were obtained for root in sand and increased with the exposure time (1 week RCF=31; 4 week RCF=1218 dry wt.) whereas in soil it decreased with time (1 week RCF=154; 4 week RCF=32 dry wt.). No evidence of translocation exists for this compound.

Lamotrigine (LMG). It is a synthetic phenyltriazine with anti-epileptic and analgesic properties. Lamotrigine enhances the action of gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter, which may result in a reduction of pain-related transmission of signals along nerve fibers. This agent may also inhibit voltage-gated sodium channels, suppress glutamate release, and inhibit serotonin reuptake. It is generally accepted to be a member of the sodium channel blocking class of antiepileptic drugs, but it could have additional actions since it has a broader spectrum of action than other sodium channel antiepileptic drugs such as phenytoin and carbamazepine and is effective in the treatment of the depressed phase of bipolar disorder, whereas other sodium channel blocking antiepileptic drugs are not, possibly on account of its sigma receptor activity. In soil, the non-ionized form is predominant. Malchi et al (2014) reported from 5 to 20 μg kg^{-1} of LMG in carrot leaves and from 2 to 10 in carrot's root.

Trimethoprim (TMP). It is primarily used in the treatment of urinary tract infections, although it may be used against any susceptible aerobic bacterial species. It may also be used to treat and prevent *Pneumocystis jiroveci pneumonia*. It is generally not recommended for the treatment of anaerobic infections such as *Clostridium difficile colitis* (the leading cause of antibiotic-induced diarrhea). Resistance to trimethoprim is increasing, but it is still a first line antibiotic in many countries. It's a hydrophilic compound and occurs as neutral compound in basic or positively charged in neutral soils (Figure 4). It is characterized by a high DT_{50} value. Holling et al (2012) evaluated the uptake of TMP in Chinese cabbage (*Brassica campestris*) in fortified organic matter rich soil and biosolids at environmental relevant concentrations (low μg kg^{-1}). It occurs as cationic forms in soils. Mean root and aerial concentrations were 232.2 and 14.5 respectively. TMP was not detected in crops grown on fortified biosolids. The BCFs (whole plant/soil) in lettuce and carrot were 0.06 and 0.08, respectively (Boxall et al., 2006).

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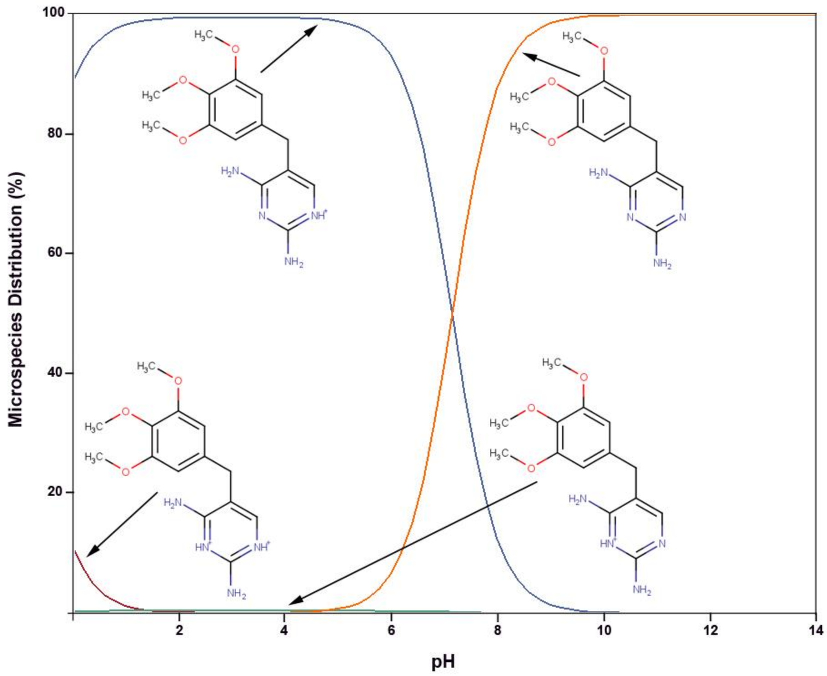

Figure 4 - Distribution of ENR species according to the soil-pore water pH obtained from MarvinSketch v. 17.6 software.

5. Candidate lists of contaminants

These lists contain contaminants (chemical and microbiological), which occur in RWW and are prone to be uptaken by plants or there are limited experimental evidences, which suggest that they can be uptaken for some crops. They can be categorized in chemical contaminants and biological contaminants, which includes antibiotic-resistant bacteria and antibiotic resistant genes.

5.1 Candidate list of chemical contaminants

Compound	CAS Nr	Class	pKa	neutral log Kow	DT_{50} soil (d)	Toxicity effects
Triclosan (TCS)	3380-34-5	biocide	8.8	5.21	18-187	endocrine disruptor
Tris(2-chloroethyl) phosphate (TCP)	115-96-8	flame retardant	NA	1.72-2.59	167	reproductive toxicity

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1,2,3-Benzotriazole (BT)	95-14-7	antioxidant, UV stabilizer	8.2	1.44	NA	anti-androgenic activity
N-nitrosodimethyl amine (NDMA)	62-75-9	by-product waste water treatment	3.52	0.57	21	probable carcinogen to human (2A)

Triclosan (TCS). Despite the fact that it is still registered as a pesticide, it has been used in many personal care products (soaps, deodorants or toothpastes) as biocide. TCS is fat-soluble compound that can penetrate the skin and enter to the bloodstream (Moss et al., 2000). Laboratory studies suggested that TCS has reproductive endocrine-disrupting effects (Wang and Tian, 2015). Therefore, in 2015 the European Chemicals Agency (ECHA) restricted the use of TCS and in 2016, the US Food and Drug Administration (FDA) also banned from soaps, although it can still be used in toothpaste and other personal care products with a maximum concentration of 0.3% w/w (FDA, 2016).

Several studies have evaluated the uptake of TCS in different crops but large differences in BCF have been reported ranging from 0.010 in lettuce (shoot) to 49,300 in carrot (root) (Wu et al., 2010; Holling et al., 2012; Pannu et al., 2012; Prosser et al 2012; Hurtado et al, 2016). These results suggest that root crops are prone to uptake TCS but with negligible translocation to aerial parts of plant. However, for the same crop (radish) and in biosolid amended soils, the reported RCFs range from negligible values (RCF=0.101) (Pannu et al., 2012) up to extremely high values (RCF=2,408) (Proser et al., 2018)

Tris(2-chloroethyl) phosphate (TCP). Besides as a flame retardant, it can be used also as plasticizer and viscosity regulator in different polymers. TCP has been found in many environmental matrices and due to its suspected reproductive toxicity, it was listed by the European Chemical Agency (ECHA) as a substance of very high concern (European Comission, ED/6/2009).

The TCP uptake has been evaluated in several crops (i.e. barley, wheat, oilseed rape, meadow fescue and four cultivars of carrot) (Eggen et al., 2013). The leaf concentration factor (LCF) was 3.9 in meadow and 42.3 in carrot. The RCF from carrot cultivars ranged from 1.7 to 4.6.

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1,2,3-Benzotriazole (BT). It is classified as a high production volume chemical by the U.S. EPA (EPA, 2000). They have been extensively used as corrosion inhibitors, partially water soluble, non-biodegradable, and UV-resistant. Accordingly, they are widespread in the aquatic environment as consequence of their persistence an high usage (surface water, groundwater, RWs) (Matamoros et al., 2010). Limited results in hydroponics suggest that their uptake is kinetically limited and not affected by the solution pH but strong influence in the temperature (Castro et al., 2004).

***N*-Nitrosodimethylamine (NDMA).** It is included in the Contaminant Candidate List (CCL) 3 of the US EPA and classified as probable carcinogen to human (2A) according to the IARC. They are formed during chlorination and chloramination of drinking water potabilization and many unintentional industrial processes (rubber manufacturing and processing, leather tanning, metal casting and food processing) have identified in irrigation waters and biosolids (Calderón-Preciado et al., 2011; Venkatesan et al., 2014). Only a very old report exists on the NDMA uptake by lettuce and spinach (Dean-Raymond and Alexander, 1976) but no further studies have been recently carried out.

Perfluorosulfonic and perfluorocarboxylic acids (PFCAs). The perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been used in many applications (coatings, fire-fighting foams, adhesives, antistatic agents). They are hardly biologically degradable once released in the environment. During the wastewater treatment, they are adsorbed to the sewage sludge and can cause contamination of agricultural soil if biosolids are applied as fertilizer. Since they behave as strong acids, in the environment, they occur in negative charged forms. Accordingly, they exhibit low bioconcentration factors ($[plant_{fw}]/[soil_{dw}]<0.88$) in several crops (cucumber, potato, carrot) (Lechner and Knapp, 2011). In another study, the uptake of perfluoroalkyl acids of different alkyl chain length (C4-C8) was evaluated in radish, tomato, and sugar snap pea grown in different soils amended with municipal or industrial biosolids (Blaine et al., 2014). The highest BCFs in edible portions were obtained by the perfluorobutanoate (PFBA) in celery shoot and in pea fruit and perfluoroocatoante in radish root. The authors suggest a decrease of 0.54-0.58 log in the seed concentration factor (SCF) according with a CF_2 decrease in the perfluoroalkyl chain. Further research is needed to ascertain this trend and the uptake pathway.

Brominated flame retardants (BFRs). The depletion of polybromodiphenyl ethers (PBDEs) from tri- through deca- in soil have been evaluated in different crops (ryegrass, pumpkin, maize) in a greenhouse study (Huang et al., 2011). No concentrations have

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been measured in crops but they found a higher depletion of the lower bromine-substituted congeners. BDE-209 has also been evaluated in the soil-plant system (Huang et al., 2010) in six plant species (ryegrass, alfalfa, pumpkin, summer squash, maize and radish). The root uptake was positively correlated with root lipid content and the translocation factor (C_{shoot}/C_{root}) was inversely related to its concentration in root.

The uptake of tetrabromobisphenol A (TBBPA) and hexabromocyclododecane (HBDD) diastereomers by plants (cabbage and radish) has been investigated (Li et al., 2011). They found from 3.5 to 10.0 fold higher HBCD than TBBPA in all plant tissues. In case of HBDD the root uptake is diastereomer specific. From the mechanistic point of view, it is not clear why the BFRs are uptaken by crops since they are hydrophobic with a high soil interaction and then of limited bioavailability by root uptake.

5.2 Candidate list of ARB and ARGs

Lettuce and endive grown on soil-amended manure showed higher abundance of ARGs (*sul*1, *tet*G, *tet*C, *tet*A and *tet*M) and *intI*1 including endophytes and and phyllosphere organisms (Wang et al., 2015).

In fact, only *sul* 1 and 2 genes have been reported in lettuce where the soil amendment with biochar decreased the richness of SA-resistant endophytes and the abundance of *sul* genes in lettuce tissues (Ye et al., 2016).

Bacterial groups and genetic determinants suggested as possible indicators in environmental setting are detailed below (Berendonk et al., 2015) but further information is needed in the case of agri-ecosystems.

ARB
Escherichia coli
Klebsiella pneumoniae
Aeromonas spp.
Pseudomonas aeruginosa
Enterococcus faecalis
Enterococcus faecium
Staphylococcus
Salmonella

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Genetic determinants and the proteins they encode
qnrS (quinolone pentapeptide repeat family)
vanA (vancomycin resistance operon gene)
mecA (penicillin binding protein)
tetM (ribosomal protection protein, associated with tetracycline resistance)
aph (aminoglycoside phosphotransferase)
bla_{TEM} and $bla_{CTX\text{-}m}$ (β-lactamases frequently identified in Enterobacteriaceae)
bla_{OXA}
bla_{KPC} (Klebsiella pneumonia carbapenemase)
sul1, sul2 (sulfonamide-resistant dihydropteroate synthase)
ermB and *ermF* (rRNA adenine N-6-methyltransferase, associated with macrolide resistance)
intI1 (integrase gene of class 1 integrons)
tetW (tetracycline resistant protein)

However, a definitive list of priority ARGs is still to be issued, as data on their distribution and on the relevance of the encompassed resistance is still lacking. For example, bla-TEM, may not be considered relevant anymore due to their widespread distribution.

6. Research needs

- Assessment of toxic intermediate formation and accumulation. Identification of dead-end metabolites.
- Risk assessment including toxic metabolites
- Further insight in the uptake mechanisms for ionized, ionic and zwiterionic molecules
- The proposed priority list is preliminary and needs to be updated periodically once consolidated knowledge is acquired.
- In-depth understanding of the translocation of organic microcontaminants into edible parts of food crops at detectable levels
- Fate of ARGs and ARB in agri-ecosystems

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- Threshold concentrations of ARB to exert selective pressure on bacteria communities.

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