Deliverable of WG2

Deliverable 8

Crops with highest and lowest potential for contaminants uptake

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Contents

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ACRONYMS

AR	Antibiotic Resistant
ARGs	Antibiotic Resistance Genes
ARB	Antibiotic-Resistant Bacteria
BCF_F	Fruit Bioconcentration Factors
CECs	Contaminants of Emerging Concern
D_{OW}	pH adjusted octanol-water partitioning coefficient ($D_{OW}= K_{OW}/(1+10^{pH-pKa})$)
DOM	Dissolved Organic Matter
d_w	Dry weight
EDCs	Endocrine Disrupting Compounds
ETc	Crop Evapotranspiration
f_n	Fraction of neutral molecules
K_{ow}	Octanol-water partitioning coefficient
NIR	Net Irrigation Requirements
PhACs	Pharmaceuticals
PPCPs	Pharmaceuticals and Personal Care Products Compounds
RCF	Root Concentration Factor
RWW	Reclaimed Wastewater
SCF	Shoot Concentration Factor;
SOM	Soil Organic Matter

Keywords: bioaccumulation; crop evapotranspiration; leafy vegetables; plant physiology; reclaimed wastewater

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Executive summary

The use of reclaimed wastewater (RWW) for irrigation and the use of biosolids and manures as soil amendments constitute significant pathways for the introduction of the contaminants of emerging concern (CECs) to the agricultural environment. Consequently, CECs are routinely detected in RWW-irrigated agricultural soils and runoff from such sites, and in biosolids- and manure-amended soils, as well as in surface and groundwater systems and sediments receiving RWW. During the recent years, crop plants grown in such agricultural environments have been found to uptake and bioaccumulate CECs in their tissues. Therefore, the possible introduction of various CECs in the food chain is presently considered as a high priority issue that needs intensive investigation. The elucidation of the responsible mechanisms for the uptake of CECs by plants, and the understanding of the potential of each crop plant species for CECs uptake, are of outmost importance in the efforts undertaken to reduce the entrance of these contaminants to the food web. Both biotic (plants' genotype and physiological state, soil fauna) and abiotic factors (soil pore water chemistry, physicochemical properties of CECs, environmental perturbations) have been proven to influence the potential for CECs uptake by crop plants. Experimental results revealed that the potential for CECs uptake by crop plants decreased in the order of leafy vegetables > root vegetables > cereals and fodder crops > fruit vegetables; though, the uptake of CECs by important crop plants, such as fruit trees, is not yet evaluated. Overall, more studies must be performed in order to shed light on the uptake of CECs by crop plants under realistic agricultural conditions. Such studies must examine the concentration of CECs in both the edible parts of the plants and in the growing medium aiming at unrevealing the crop plants' potential for CECs uptake and bioaccumulation in the edible tissues.

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1. Introduction

Climate change and global warming effects are widely recognized during the recent decades, and therefore water availability and management issues are of special significance in all arid and semi-arid regions worldwide. Water scarcity already affects every continent, and it is among the main problems to be faced by many societies and the world in the twenty-first century (March et al., 2012). Agriculture is likely to encounter the most serious threats due to water scarcity, as it is the major consumer of water. At the same time, demographic shifts, economic development and lifestyle changes are expected to intensify the competition between agricultural and other uses of water, such as municipal and industrial. Water imbalances in the agricultural sector of most arid and semi-arid countries are expected to be further intensified, as limited and unequally distributed rainfall is following a declining trend, whereas mean temperature is increasing (Milano et al., 2012), having as a result the increase of crop evapotranspiration (ETc) and net irrigation requirements (NIR). As a result, irrigation water needs must be supplied through an adaptation approach where water management is interrelated water scarcity, food security goals, rural development and natural resources management (Iglesias and Garrote, 2015). A great number of integrated water resource management schemes are already being implemented, or are under consideration, with particular focus being given to the exploitation of existing water resources and the utilization of non-conventional water resources, such as reclaimed wastewater (RWW) (Milano et al., 2012). As a result, RWW reuse, mainly for irrigation purposes, is already a well-established practice in almost all arid and semi-arid areas around the world (Bixio et al., 2006). RWW reuse for irrigation represents an advantageous alternative for the mitigation of the ever increasing irrigation water scarcity and demand, since RWW of high quality represents a potentially valuable, nutrient-rich and reliable source of water for the agricultural sector, available all year round (Bixio et al., 2006). Although RWW reuse for irrigation has gained an acceptance as an economic alternate that could substitute nutrient needs and water

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requirement of crop plants, and major advances have been made with respect to producing safe treated effluents for reuse (e.g. successful removal of nutrients, metals, chemical oxygen demand down to low levels), RWW still may contain undesirable chemicals, primarily organic constituents as well as pathogens that pose negative environmental and health impacts (Fatta-Kassinos et al., 2011). Consequently, several important questions concerning the presence of organic microcontaminants, also called contaminants of emerging concern (CECs) (e.g. pharmaceuticals, chemicals from personal care products, endocrine disrupting compounds, plasticizers, flame retardants, surfactants, illicit drugs, transformation products, pesticides, etc.) in treated effluents and their subsequent release to the environment through RWW irrigation are still unanswered and barriers exist regarding the safe/sustainable reuse practices. Available/applied treatment technologies fail to completely remove CECs while no consolidated information exists concerning the fate of CECs within the biomass of activated sludge, in the RWW and in the agricultural environment (i.e. soil, ground/surface waters, plants/crops) in the framework of reuse applications (i.e. irrigation, groundwater replenishment, storage in surface waters for subsequent reuse) (Fatta-Kassinos et al., 2011). CECs, and most specifically pharmaceuticals and personal care products (PPCPs) are introduced into the environment via various human activities, including direct disposal of unused or expired medication, release from pharmaceutical manufacturing plants and hospitals, and veterinary drug use (Grossberger et al., 2014). Moreover, most pharmaceuticals (PhACs) are poorly absorbed and not completely metabolized in human and animal bodies; a high percentage of the intake dosage (30-90%) of most antibiotics is excreted via urine and faeces within hours after application either as the parent compound or as metabolites (Liu et al., 2010; Zhang et al., 2014). As a result, PhACs may directly enter the environment through the application of animal manures to soil and the excretions by grazing livestock (Pan and Chu, 2016). The reuse of RWW for irrigation and the use of biosolids as soil amendments constitute additional significant pathways for the introduction of PhACs in

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the agricultural environment, as conventional wastewater treatment processes are only moderately effective at removing antibiotics from the treated effluent (Michael et al., 2012; Petrie et al., 2015). Consequently, PhACs are routinely detected in RWW and biosolids from the treatment process. Furthermore, PhACs are also detected in RWW-irrigated agricultural soils and runoff from such sites, biosolids- and manure-amended soils, and surface and groundwater systems and sediments receiving RWW (Kolpin et al., 2002; Pedersen et al., 2003; Kinney et al., 2006; Fatta-Kassinos et al., 2011; Gottschall et al., 2012; Luo et al., 2014; Meffe and de Bustamante, 2014).

The contamination of the environment, and possibly the food chain with CECs is presently considered as a *serious public health problem* (Rand-Weaver et al., 2013; Malchi et al., 2014; Pan et al., 2014; Prosser and Sibley, 2015). Although the role of aquatic environments as reservoirs and routes for CECs and antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) dissemination is now recognized, knowledge on their fate during RWW reuse in agriculture and on the actual effects in relation to the evolution/release of antibiotic resistance (AR) in the environment is currently not consolidated. Moreover, scientific data are still scarce regarding (1) the potential crop uptake of CECs under actual farming conditions, (2) the fate and adverse effects of the CECs in the environment, and (3) the development of innovative technologies and solutions able to remove such contaminants from RWW and decontaminate the agricultural environment. To avoid negative impacts on the environment and human health due to RWW reuse, regulatory frameworks are required based on validated scientific information. The associated risks are in general unknown, since the vast majority of studies have focused on human pathogen indicators (e.g. coliforms) or soil properties (e.g. heavy metals). Knowledge gaps exist with regard to public health risk assessment as well, since solid data regarding the actual uptake of these CECs by crop plants under actual farming conditions are largely missing. Given the increasing interest in reusing RWW, there is a clear need to include RWW as an additional exposure route for CECs in

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impacted ecosystems, in order to assess the potential risks derived, in both the agricultural ecosystem (soil, water resources, plants, etc.) and the public health.

The uptake and bioaccumulation of CECs in the edible parts of food crops and fodders and their subsequent entry into the food chain have gained prominence over the last decade. Numerous studies, mainly conducted under hydroponic or greenhouse control conditions, as well as in manure- and biosolids-amended soils, highlighted CECs' uptake and bioaccumulation in plants exposed to known concentrations of individual or cocktails of CECs (Tanoue et al., 2012; Wu et al., 2012; Dodgen et al., 2015; Christou et al., 2016). Such studies were proven to be useful in elucidating the mechanisms of CECs' uptake by plants, which was found to be simply driven by the transpiration derived mass flow and largely depended on the chemical properties of the compounds, especially their hydrophobicity and charge. Hydroponic and greenhouse experiments though, even if conducted at environmentally relevant CECs' concentrations, are unable to manifest the complexity of an actual agricultural environment (Malchi et al., 2014).

2. Environmental factors (abiotic stress) that affect the uptake of CECs by plants

Several categories of contaminants of emerging concern (CECs) have been proven to be taken up through roots and translocated to the aerial parts of crop plants grown under hydroponic or greenhouse control conditions, but also in manure- and biosolids-amended and RWW-irrigated soils, in real agricultural systems (Boxall et al., 2012; Tanoue et al., 2012; Goldstein et al., 2014; Wu et al., 2015; Miller et al., 2016; Christou et al., 2017b). However, despite the relatively large number of studies undertaken in order to investigate root uptake of CECs, the mechanistic understanding of CECs uptake by crop plants remains rather limited (Miller et al., 2016). It has been previously shown that the uptake of CECs by crop plants is largely dependent on their bioavailability/bioaccessibility in soil pore water near the rhizosphere (sorption to soil constituents and transformation by soil organisms reduce

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bioavailability), and thus on their physicochemical properties and on the properties of the soil environment (Goldstein et al., 2014). Once taken up, the transport of CECs within the plant vascular translocation system (xylem and phloem) largely depends on their physicochemical properties (i.e. lipophilicity and electrical charge), as well as on the plant's physiology and transpiration rate (Goldstein et al., 2014; Dodgen et al., 2015) and the environmental conditions (i.e. drought stress) (Zhang et al., 2016). CECs have been proven to enter the root through the epidermis of growing root tips and subsequently pass through the cortex and the endodermis to reach the vascular tissues, where they can then be transported via the xylem to aboveground tissues. The movement of CECs from the soil pore water to the vascular tissues of plants may be distinguished to transmembrane, symplastic and apoplastic, depending on the ability of CECs to cross the membranes of plant cells (Miller et al., 2016). The presence of the Casparian strip in the endodermis which acts as a hydrophobic barrier between the apoplast and the vascular tissue, suggests that CECs must at least once follow the symplastic route, which is constituted of selective binding sites and channels (Kong et al., 2007; Tanoue et al., 2012; Malchi et al., 2014). As a result, the lipophilicity and speciation of CECs strongly affects their root uptake by and translocation within the plants. The octanol-water partition coefficient (K_{ow}) has been suggested as a predictor of the uptake behavior of non ionizable organic microcontaminants (Hsu et al., 1990). However, the movement of polar and ionizable microcontaminats (the majority of CECs fall into this category) through plant cell membranes may be impeded by interactions with the negative surface potential of the cytoplasmic membrane (Trapp, 2004), by ion trapping, which is common for sulfonamide antibiotics (Goldstein et al., 2014; Christou et al., 2016) and also by sorption to plant cell walls (Trapp, 2004), thus making K_{ow} an inappropriate indicator for the estimation of ionizable CECs movement within and through plant cells. The pH-dependent speciation of ionic compounds (D_{ow}) is considered to be a more appropriate descriptor for the ability of ionizable CECs to

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cross cell membranes and translocated within the plant than K_{ow} (Wu et al., 2013b; Hyland et al., 2015).

The uptake and translocation of CECs within RWW-irrigated crop plants grown in real agricultural systems, where a cocktail of CECs is present in real RWW and the complexity of soil-plant-environment interactions are also taken into account, has not been extensively studied. Few studies followed experimental setups where real RWW was applied for the irrigation of crop plants in the field, or where biosolids and manure was applied as soil amendment in agricultural fields, representing actual farming practices, or genuine soil, or ecological conditions typical for commercial agriculture farming (Calderón-Preciado et al., 2011a; Calderón-Preciado et al., 2011b; Shenker et al., 2011; Pannu et al., 2012; Goldstein et al., 2014; Malchi et al., 2014; Marsoni et al., 2014; Pan et al., 2014; Prosser et al., 2014; Wu et al., 2014; Riemenschneider et al., 2016; Christou et al., 2017b; Calderón-Preciado et al., 2013), simultaneously allowing for the assessment of the actual potential uptake of CECs by crops and its integration into a database for risk assessment (Malchi et al., 2014; Prosser and Sibley, 2015).

These studies have enabled researchers to better understand the effects of the agricultural environment on the uptake and bioaccumulation of CECs in the edible parts of crop plants. Results showed that the properties of the agricultural soil and the soil environment in general, as well as the environment of the agro-ecosystems, greatly shape and determine the uptake of CECs by crop plants. More precisely, accumulating evidence show that crops growing in agricultural soils with low levels of organic matter, or sandy soils, or soils that have low proportion of clay compared to silt and sand may have higher potential for uptake of CECs compared to clay soils or soils rich in organic matter (Goldstein et al., 2014; Malchi et al., 2014). Malchi et al. (2014) studied the uptake of PhACs compounds by carrots and sweet potatoes grown in lysimeters filled with three different soil types (sandy clay, loamy sand and sandy loam) and found that PhACs were in higher concentrations in the roots and leaves of

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those plants grown is sandy soils compared with those grown in loamy soils. Similarly, Goldstein et al. (2014) reported that nonionic pharmaceuticals were taken up and accumulated at higher levels in the leaves and fruits of tomato and cucumber plants grown in soils of lower organic matter and clay content. Moreover, Zhang et al. (2016) studied the uptake of three antibiotics (sulfamethoxazole, lincomycin, and oxytetracycline) by lettuce plants grown in three soils with variability in texture (loam, sandy loam, and sand) and found that only sulfamethoxazole was detected in lettuce leafs, with increasing uptake corresponding to increasing sand content in soil. These studies, highlight the role of soil texture and soil environment on the potential for CECs uptake by plants, as these variables greatly determine the fate of CECs in the soil, thus their bioavailability and bioaccessibility for microbial degradation and uptake by plants (Fig. 1). It is now well documented that once introduced into soil, CECs are subjected to sorption/desorption and transformation processes (both biotic and abiotic), which influence the concentrations available for biodegradation, transport into soil (runoff and leaching) and plant uptake, ultimately specifying the potential of accumulation of antibiotics in soil and plants (Dalkmann et al., 2012; Grossberger et al., 2014). The chemical properties of CECs that significantly impact and shape their environmental fate are polarity, hydrophobicity and water solubility. Polar and ionizable CECs engage in interactions with the soil organic matter (SOM), the mineral surfaces and the dissolved organic matter (DOM), which include hydrophobic partitioning, electron donor-acceptor interactions (e.g., hydrogen bonding), cation-anion exchange, protonation, water binding, cation binding and surface complexation (Thiele-Bruhn et al., 2004; Vasudevan et al., 2009). Therefore, the physicochemical properties of CECs, the chemistry of soil pore-water (i.e. pH, mineral concentration, cation exchange capacity, dissolved organic matter), and the soil structure (i.e. clay composition) and soil organic matter (SOM) content are critical factors controlling the retention of antibiotics in soil, and therefore their bioavailability and potential for plant uptake (Vasudevan et al., 2009; Wu et al., 2013a; Miller et al., 2016; Park and Huwe, 2016).

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Moreover, well aerated soils may facilitate the uptake of CECs by plants compared with partially- or not-aerated soils, such as compacted and waterlogged soils, as the aeration of soil may facilitate the respiration and functionality of roots in the rhizosphere, as well as the water and nutrient uptake, and plant growth (Niu et al., 2012), and therefore the uptake of CECs. Soils with basic pH values (e.g. soil pH>pKa) results in the predominant presence of the anionic form of ionizable acidic contaminants in soil (repulsion of CECs from the negatively charged soil surfaces enhance leaching potential), thus affecting their uptake by plants due to repulsion forces (roots epidermis is negatively charged) (Goldstein et al., 2014; Miller et al., 2016). In contrary, soils with acidic pH values (soil pH<compound pKa) may result in the presence of contaminants in their neutral form (high fraction of neutral molecules (f_n) values) thus facilitating their uptake (Fig. 1); Trimethoprim antibiotic was reported to be predominantly presence in its neutral form is soils with pH values higher than 8, thus up taken by tomato plants (Christou et al., 2017b).

Other environmental factors that may affect the uptake of CECs by crop plants are the ambient temperature, the wind speed and air humidity, as these factors greatly shape the crop evapotranspiration (water and nutrient uptake by plants) (Allen et al., 1998). It is well accepted that high temperature, increased wind speed and low air humidity result in increased evapotranspiration rates of plants. Plants grown in such conditions show increased water and nutrient uptake (if available in the soil), thus the potential for CECs uptake is also increased (Fig. 1). Overall, it can be assumed that crop plants grown in hot and dry agricultural sites (e.g. Mediterranean region) may show increased rates of contaminants uptake compared with plants grown in cold regions. Drought stress, implying dry climatic conditions and limited water availability, has recently been reported to affect the uptake of CECs. Zhang et al. (2016) showed that increased drought stress resulted in increased uptake of lincomycin and decreased uptake of oxytetracycline and sulfamethoxazole in wastewater irrigated lettuce plants. Dodgen et al. (2015) showed that plant transpiration may play a significant role in the

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uptake and translocation of PPCP/EDCs, which may have a pronounced effect in arid and hot climates where irrigation with RWW is common. Therefore, the evapotranspiration rate of crop plants, determined by climatic and plant specific values (Kc, crop coefficient) may be a good indicator of the potential uptake of CECs by plants (Table 1). Plants with high evapotranspiration rate and NIR values are expected to show higher potential for CECs uptake (Fig. 1). Moreover, plants with high NIR values, grown in hot and dry conditions (thus irrigated in short intervals), have the highest potential for CECs uptake. Such crop plants are bananas, citrus, fruit trees, walnut (fruit trees), cucumber, eggplant, green beans, melons, pepper, tomatoes (vegetable plants), peanuts and alfalfa (arable crops). In addition, seasonal crops that are growing during the summer period (i.e. vegetables) and crops grown in greenhouses (RWW irrigation is needed; not rainfed crops), as well as perennial crops for which RWW irrigation is practiced all year round for a prolonged period (i.e. fruit trees) may be categorized as plants with high potential for CECs uptake. On the other hand, crops grown during the autumn and winter period, where irrigation is irregularly practiced because of the precipitation events, as well as crops with relatively small root system and succulent plants, may well be categorized as crop plants with the lowest potential for CECs uptake. Importantly, leafy vegetables (i.e. lettuce, spinach, cabbage, broccoli, celery, etc.) may bioaccumulate greater concentrations of CECs in their edible tissues, since all the above-ground parts of these plants are edible. This implies that there are no barriers between the transpiration flow system of these plants, i.e. xylem/phloem, or leaves, stems and fruits), and that all CECs up taken by the roots of leafy vegetable may easily translocated and bioaccumulated in their above-ground edible parts, thus allowing higher concentrations of CECs in their edible parts.

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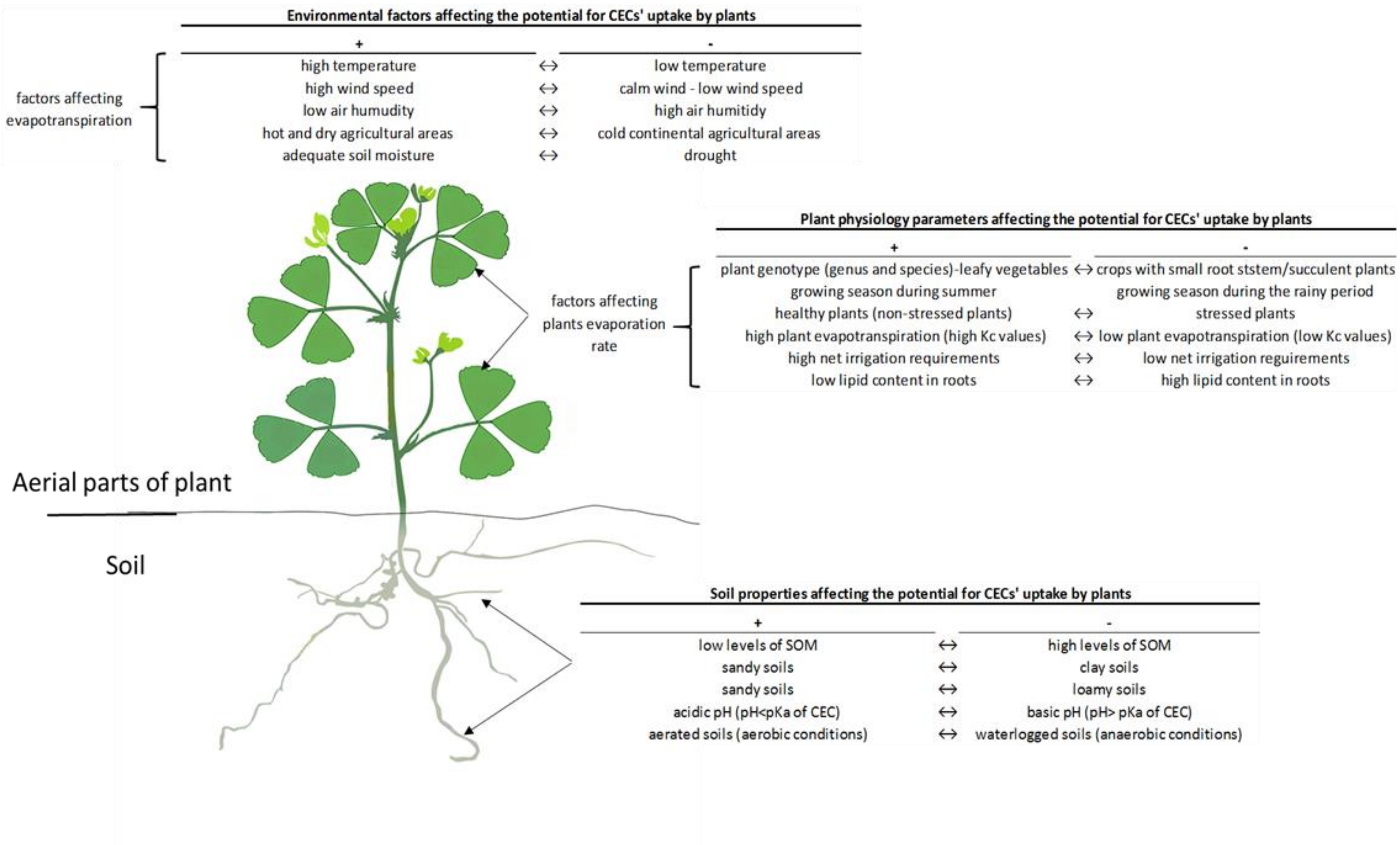

Fig. 1. The potential for uptake by and bioaccumulation of CECs within the edible parts of crop plants is affected by a plethora of parameters, including plant physiology parameters, soil properties, environmental conditions and perturbations. + and - indicates the factors that increase or decrease the potential for CECs uptake by crop plants, respectively. Figure is modified from Christou et al. (2016).

3. Plant physiology factors affecting to the uptake of CECs

The physiology of plants greatly determines the potential for CECs uptake. Plants exposed to environmental perturbations respond by inducing various adaptive mechanisms, such as the antioxidant defense mechanism, the closure of stomata for controlling respiration, the osmoregulation by accumulating osmolytes and other macromolecules, as well as ion sequestration and exclusion, hormone regulation, etc. Therefore, plants that are exposed to adverse environmental conditions may exert different patterns of CECs uptake compared with

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plant grown under optimum environmental conditions. It can be assumed that non-stressed plants have higher potential for CECs uptake than stressed ones, as the rate of plant growth and development (water and nutrient uptake and photosynthetic efficiency) of plants under non stress conditions is significantly higher compared to that in stressed plants (Fig. 1). Goldstein et al. (2014) reported that the concentration of CECs in cucumber and tomato leaves of plants grown in three different soils in pots were of similar order, whereas their concentrations in the tomato fruit were much lower than in the cucumber fruit, attributing this to differences in fruit physiology. This may be due to the fact that cucumber fruits exert physiological responses and functions similar to those of leaves, as the chlorophyll content of the exocarp and the efficiency of PSII of fruits is similar to that of leaves (the photosynthesis by cucumber fruits, through direct fixation of atmospheric CO_2 and recapture of respired CO_2, makes an important contribution to fruit growth) (Sui et al., 2017). The lipid content of plant tissues may also affect the uptake of CECs, as higher uptake of metformin was found in carrot cultivars with low-lipid content in roots compared with cultivars with high-lipid content in roots (Eggen et al., 2011).

Table 1. Average annual crop evapotranspiration (ETc) and net irrigation values (NIR) of the main cultivated crop species in the Mediterranean region, estimated during the 1990-2014 period (Christou et al., 2017a).

Crops	**ETc**	**NIR**
	Total ETc (m^3 water/ ha/ year)	**Total NIR (m^3 water/ ha/ year)**
Tree crops		
Almonds	3445	3364
Bananas	12184	11340
Citrus & Avocado	8237	7615
Fruit trees (Lowlands / plains)	7973	7836
Pistachio	3443	3362
Table grapes	3023	2845
Table olives	4186	3867
Walnut (Pecan)	9676	9358
Vegetables		
Artichokes	4390	3715
Cabbage	2972	2559

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Carrots	4180	2998
Celery	4216	3576
Cucumber (greenhouse)	5713	5713
Cucumber	4649	4456
Eggplants	5766	5448
Green beans (greenhouse)	4414	4414
Green beans	5992	5696
Lettuce	3265	2268
Marrows	4976	4772
Melons	5069	4865
Okra	6616	6289
Onions (dried)	3571	3186
Peas	1926	1594
Pepper	5399	5173
Potatoes (spring)	2994	2641
Potatoes (mid season)	1360	759
Potatoes (late season)	4760	4300
Radish	4051	3410
Spinach	3535	3101
Taro (Kolokasi)	23355	22510
Tomatoes (greenhouse)	7342	7342
Tomatoes	6363	6136
Watermelons	4976	4772
Arable crops		
Haricot beans	4440	4300
Peanuts (Monkey nuts)	5153	4927
Effective rainfall	2543	
	2540	

4. Plant species and genotype

Plant species within the same genus have been reported to have different patterns of CECs uptake. For example, different species of the *Brassica* genus have been shown to exert distinct uptake patterns for carbamazepine, salbutamol, sulfamethoxazole, and trimethoprim. More precisely, Cabbage (*Brassica rapa* var. pekinensis) and Wisconsin Fast Plants (*Brassica rapa*) grown hydroponically in nutrient solution spiked with the above four pharmaceuticals displayed different concentrations in all tissues tested, i.e. roots, leaves, stems, and seedpods (Herklotz et al., 2010). Moreover, varieties of the same species may also exert different uptake pattern for CECs. Eggen et al. (2011) reported that four carrot cultivars grown in soil spiked with metformin, in pots in greenhouse, displayed significantly different uptake patterns, with the bioconcentration factor for leafs in low-lipid-content carrot roots being significantly higher

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(in the order of 2- to 4-fold) compared with that in high-lipid carrot roots. Worth noting, cultivated crop species may be available to farmers in dozens or even more varieties, which are adapted to different climatic conditions, or have different productivity and fruit characteristics (taste, resistant to pest and diseases, resistance to salinity or other stress factors, etc.). A number of different cultivars may be even cultivated in a specific region due to farmers or consumers'preferences, highlighting the difficulties and challenges in defining the potential for CECs uptake by a single cultivated specie (e.g. carrot, tomato, etc.) (Fig. 1).

5. Studies reporting CECs uptake by crop plants in real agricultural environments receiving reclaimed WW irrigation or amended with manure of biosolids

Pan et al. (2014) evaluated the accumulation of antibiotics (tetracycline, sulfamethazine, norfloxacin, erythromycin, chloramphenicol) in vegetable crops irrigated with sewage for a prolonged period (20 years) in Southern China and found that the concentration of antibiotics in the different edible parts of various crops followed specific trends. Norfloxacin was consistently found at the highest concentrations (4.6-23.6 μg kg^{-1} dw) in crop tissues, followed by chloramphenicol (2.6-22.4 μg kg^{-1} dw) and tetracycline (4.0-10.1 μg kg^{-1} dw), whereas sulfamethazine and erythromycin were not detected in most of the vegetable crops tested (Chinese cabbage, water spinach, Chinese radish, corn and rice). Wu et al. (2014) evaluated the uptake of 16 pharmaceuticals (acetaminophen, caffeine, meprobamate, atenolol, trimethoprim, carbamazepine, diazepam, gemfibrozil, primidone, sulfamethoxazole, dilantin, diclofenac, naproxen, ibuprofen, atorvastatin and fluoxetine) and 3 personal care products (PCPs) (N,N-diethyl-metatoluamide; DEET), triclosan, and triclocarban) in eight common vegetables (carrot, celery, lettuce, spinach, cabbage, cucumber, bell pepper, and tomato) grown in field and irrigated with spiked (250 ng L^{-1} of each PCP) and non-spiked tertiary treated wastewater. The analysis of the edible tissues of these plants showed that the studied

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CECs where detected in the 64% and 91% of the samples collected from the treated wastewater and the fortified wastewater treatments, respectively. Thus, it was shown that the concentration of the CECs in the irrigation water is a key factor in the uptake process. The edible samples from the two treatments contained the same PPCPs, including caffeine, meprobamate, primidone, DEET, carbamazepine, dilantin, naproxen, and triclosan. Worth noting, leafy vegetables (celery, lettuce, spinach, cabbage) and root vegetables (carrot) systemically displayed higher CECs concentrations in their edible parts compared with fruit vegetables (cucumber, bell pepper, and tomato). The total concentrations of PPCPs detected in edible tissues from the treated wastewater and fortified wastewater irrigation treatments were in the range of 0.01–3.87 and 0.15–7.3 μg kg^{-1} dw, respectively, while the annual exposure of PPCPs from the consumption of mature vegetables irrigated with the fortified wastewater was estimated to be only 3.69 μg per capita.

The uptake of nonionic pharmaceuticals (carbamazepine, caffeine, and lamotrigine) and ionic pharmaceuticals (metoprolol, bezafibrate, clofibric acid, diclofenac, gemfibrozil, ibuprofen, ketoprofen, naproxen, sulfamethoxazole, and sildenafil) by RWW-irrigated root crops (carrots and sweet potatoes) grown in lysimeters was monitored, in a recent field study (Malchi et al., 2014). In both crops, the nonionic pharmaceuticals were detected at significantly higher concentrations than ionic pharmaceuticals, whereas pharmaceuticals in leaves were found at higher concentrations than in the roots.

Goldstein et al. (2014) studied the uptake and translocation of several pharmaceuticals (carbamazepine, caffeine, lamotrigine, metoprolol, bezafibrate, clofibric acid, gemfibrozil, ibuprofen, ketoprofen, naproxen, sulfamethoxazole, sildenafil and sulfapyridine) in cucumber and tomato plants with the aim to elucidate the effects of the physicochemical properties of pharmaceuticals, the soil type, and the irrigation-water quality on the uptake and translocation of pharmaceuticals by plants. Nonionic pharmaceuticals were taken up and accumulated at higher levels in plants grown in soils of lower organic matter and clay content. While the

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concentration of most pharmaceuticals in cucumber and tomato leaves were of similar order, their concentrations in the tomato fruit were much lower than in the cucumber fruit. Lamotrigine, carbamazepine and caffeine were found in higher concentrations in cucumber fruits, whereas, ibuprofen, clofibric acid, caffeine, sildenafil and carbamazepine were found in higher concentrations in tomato fruit, compared with the other studied pharmaceuticals.

Riemenschneider et al. (2016) studied the uptake of 28 micropollutants and carbamazepine metabolites in 10 different field-grown vegetable species (among them carrot, lettuce, potato, and zucchini) irrigated with water of the Zarqa River, consisting of RWW as the main component and spring and runoff water, in Jordan. Results revealed that a total of 12 micropollutants and six carbamazepine metabolites, could be detected in all of the samples in concentrations ranging from 1.7 to 216 μg kg^{-1} dw. In edible tissues, the total concentration of micropollutants decreased in the order of leafy (247–533) > root (73–126) > fruit-bearing (5–76 μg kg^{-1} dw) vegetables.

By conducting a field study, Christou et al. (2017b) explored the long-term (three years) effects of two distinctly tertiary treated wastewater effluents applied for the irrigation of tomato plants under commercial agricultural farming on the fate of diclofenac, sulfamethoxazole and trimethoprim in soil and their uptake and bioaccumulation in tomato fruits. The concentration of these pharmaceuticals was determined in fruits harvested at the end of the harvesting period (last harvest) for the first two years of the study, while at the third year of the study pharmaceuticals' concentrations were determined at fruits harvested at the beginning (first harvest), middle (fourth harvest) and the end of the harvesting period (seventh harvest) (seven to eight harvests took place in each year of the study). The pharmaceutical with the highest soil concentration throughout the studied period was sulfamethoxazole (0.98 μg kg^{-1} dw), followed by trimethoprim (0.62 μg kg^{-1}dw) and diclofenac (0.35 μg kg^{-1} dw). Diclofenac was not found in tomato fruits harvested from RWW-irrigated plants during the first year of the study. However, diclofenac displayed the highest fruit concentration (11.63 μg kg^{-1} dw)

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throughout the study (as a result of prolonged RWW irrigation), followed by sulfamethoxazole (5.26 μg kg^{-1} dw) and trimethoprim (3.40 μg kg^{-1} dw). The calculated fruit bioconcentration factors (BCF_F) were extremely high for diclofenac in the 2^{nd} (108) and 3^{rd} year (132) of the experimental period, with the respective values for sulfamethoxazole (0.5-5.4) and trimethoprim (0.2-6.4) being significantly lower. (Christou et al., 2017b).

Wu et al. (2010) studied the uptake of three pharmaceuticals (carbamazepine, diphenhydramine, and fluoxetine) and two chemicals from personal care products (triclosan and triclocarban) by soybean (*Glycine max* (L.) Merr.) plants grown in soil, in pots in greenhouse, and either amended with biosolids or irrigated with RWW. Results showed that carbamazepine, triclosan, and triclocarban were concentrated in root tissues and translocated into above ground parts including beans, whereas accumulation and translocation for diphenhydramine and fluoxetine was limited. The uptake of the selected compounds differed by treatment, with biosolids application resulting in higher plant concentrations, probable due to higher loading. However, compounds introduced by irrigation appeared to be more available for uptake and translocation. In another study, Wu et al. (2012) found that carbamazepine, diphenhydramine, and triclocarban were taken up and bioaccumulated in lettuce, radish, tomato, pepper and collard grown in biosolids-treated soils. Root concentration factor (RCF) and shoot concentration factor (SCF) were found highest for carbamazepine followed by triclocarban and diphenhydramine. Positive correlation between RCF and root lipid content was observed for carbamazepine but not for diphenhydramine and triclocarban.

Moreover, Holling et al. (2012) studied the uptake of carbamazepine, sulfamethoxazole, salbutamol and trimethoprim by Chinese cabbage (*Brassica campestris*) plants grown in fortified soil, as well as the uptake of these PhACs and triclosan from plants grown in biosolid amended soil, under greenhouse conditions. All PhACs were detected in the roots and the aerial parts of cabbage grown in the fortified soil with median concentrations of 255.4 μg kg^{-1} in the aerial parts and 272.9 μg kg^{-1} in roots for carbamazepine; 222.8 μg kg^{-1} in the aerial

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parts and 260.3 μg kg^{-1} in roots for sulfamethoxazole; 108.3 μg kg^{-1} in the aerial parts and 140.6 μg kg^{-1} in roots for salbutamol; and 20.6 μg kg^{-1} in the aerial parts and 53.7 μg kg^{-1} in roots for trimethoprim (all in dry weight). Although all studied compounds were present in the biosolids-amended planting soil, only carbamazepine (317.6 μg kg^{-1} in the aerial parts and 416.2 μg kg^{-1} in roots), salbutamol (21.2 μg kg^{-1} in the aerial parts and 187.6 μg kg^{-1} in roots), and triclosan (22.9 μg kg^{-1} in the aerial parts and 1220.1 μg kg^{-1} in roots) were detected in the aerial parts of the cabbage. Results revealed the differences in the bioavailability and bioaccessibility of PhACs for uptake by plants in fortified and biosolids-amended soils, probably due to the effects of the increased SOM in the biosolids-amended soils.

The above studies have reported the concentration of CECs in both the soil (either irrigated with non-spiked or spiked RWW or amended with manure or biosolids), as well as in the edible parts of crop plants, thus allowing the estimation of the bioconcentration factor (BCF). The BCFs, reported in these studies for carbamazepine, triclosan and sulfamethoxazole in the various crop plant species are summarized in Table 2, allowing for the identification of the crops with the highest and lowest potential for CECs uptake.

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Table 2. Bioconcentration factors, as calculated in published studies.

PPCP	Plant species	Type of application	soil type	Concentration in soil (ng g^{-1} dw)	Plant tissue	Concentration in plant tissue (ng g^{-1} dw)	Bioconcentration factor	Reference
Carbamazepine	cucumber	reclaimed WW	alluvial	8.5	fruit	10	1.176	Goldstein et al., 2014
			aeolian	1.2	fruit	13	10.833	
			sand	1.2	fruit	11	9.167	
		spiked reclaimed WW	alluvial	17	fruit	10	0.588	
			aeolian	1.5	fruit	45	30.000	
			sand	2.5	fruit	27	10.800	
	tomato	reclaimed WW	alluvial	15	fruit	0.9	0.060	
			aeolian	2	fruit	1	0.500	
			sand	2	fruit	1.2	0.600	
		spiked reclaimed WW	alluvial	18.5	fruit	0.85	0.046	
			aeolian	5	fruit	1.4	0.280	
			sand	4.5	fruit	1.5	0.333	
	carrot	spiked reclaimed WW	sandy clay	4	leaves	12.5	3.125	Malchi et al., 2014
				4	root	8	2.000	
			loamy sand	3	leaves	22	7.333	
				3	root	9.5	3.167	
			sandy loam	7	leaves	17	2.429	
				7	root	7	1.000	
	sweet potato	spiked reclaimed WW	sandy clay	4.5	leaves	5	1.111	
				4.5	root	1	0.222	
			loamy sand	1.7	leaves	3.5	2.059	
				1.7	root	0.5	0.294	
			sandy loam	6	leaves	3.5	0.583	
				6	root	0.5	0.083	
	soybean	biosolids-amended soil		44.2	leaves	110	2.489	Wu et al., 2010

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				44.2	bean	0.17	0.004	
	cabbage	biosolids-amended soil		93.1	shoot	658	7.068	Holling et al., 2012
	lettuce	biosolids-amended soil		98.3	shoot	600	6.104	Wu et al., 2012
	radish	biosolids-amended soil		90.3	shoot	350	3.876	
	tomato	biosolids-amended soil		96.8	shoot	400	4.132	
Sulfamethoxazole	cucumber	reclaimed WW	alluvial	0.2	fruit	0	0	Goldstein et al., 2014
			aeolian	0.2	fruit	0	0	
			sand	0.2	fruit	0	0	
		spiked reclaimed WW	alluvial	0.2	fruit	0	0	
			aeolian	0.2	fruit	0	0	
			sand	0.2	fruit	0	0	
	tomato	reclaimed WW	alluvial	2	fruit	0	0	
			aeolian	1	fruit	0	0	
			sand	1	fruit	0	0	
		spiked reclaimed WW	alluvial	2	fruit	0	0	
			aeolian	2	fruit	0	0	
			sand	2	fruit	0	0	
	carrot	spiked reclaimed WW	sandy clay	0.15	leaves	0	0	Malchi et al., 2014
				0.15	root	0.1	0.667	
			loamy sand	0.25	leaves	0	0	
				0.25	root	0.1	0.400	
			sandy loam	0.3	leaves	0	0	
				0.3	root	0.25	0.833	
	sweet potato	spiked reclaimed WW	sandy clay	0.1	leaves	0	0	
				0.1	root	0.2	2.000	
			loamy sand	0.1	leaves	0	0	
				0.01	root	0.05	5.000	

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			sandy loam	0.2	leaves	0	0	
				0.2	root	0.25	1.250	
	tomato	reclaimed WW	sandy clay loam	0.56	fruit	0.263	0.470	Christou et al., 2017
				0.55	fruit	0.397	0.722	
				0.64	fruit	0.406	0.634	
				0.43	fruit	0.547	1.272	
				0.38	fruit	1.014	2.668	
				0.98	fruit	5.255	5.362	
	cabbage	biosolids-amended soil		67.4	shoot	15	0.223	Holling et al., 2012
Triclosan	soybean	biosolids-amended soil		13.2	leaves	120	9.091	Wu et al., 2010
				13.2	bean	12.6	0.955	
	cabbage	biosolids-amended soil		433	shoot	62	0.143	Holling et al., 2012
	lettuce	biosolids-amended soil		1000	shoot	10	0.010	Pannu et al., 2012
		biosolids-amended soil		4600	shoot	120	0.026	
		biosolids-amended soil		10000	shoot	900	0.090	
	radish	biosolids-amended soil		990	root	100	0.101	
		biosolids-amended soil		4500	root	1200	0.267	
		biosolids-amended soil		9900	root	9200	0.929	
	soybean	biosolids-amended soil		55	fruit	17	0.309	
	radish	biosolids-amended soil		10.3	root	24.800	2.408	Prosser et al., 2014
	carrot	biosolids-amended soil		1	root	49.300	49.300	

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	soybean	biosolids-amended soil		6.2	fruit	2.8	0.452	
	cucumber	biosolids-amended soil		29.2	fruit	4	0.137	
	tomato	biosolids-amended soil		29.2	fruit	2.8	0.096	
	pepper	biosolids-amended soil		29.2	fruit	2.8	0.096	

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6. General discussion

Based on the bioconcentration factors of carbamazepine, sulfamethoxazole and triclosan in various plant species (as shown in Table 2), as well as on studies reporting the uptake of CECs by several plant species and their bioaccumulation in their edible parts (Kang et al., 2013; Pan et al., 2014; Wu et al., 2014), it can be concluded that leafy vegetables (i.e. lettuce, spinach, cabbage, arugula) constitute the crop plants with the highest potential for CECs uptake. This may be attributed to the fact that all the aerial parts of these plants are edible; this means that no barriers exist (i.e. stems, leaves) for the translocation of CECs upon their uptake by roots, leading to their maximum bioaccumulation in the edible parts of these plants. Following leafy vegetables, root vegetables, such as carrot, radish, sweet potato and potatoes, display high potential for CECs uptake. Fruit vegetables (cucumber, tomato, pepper, bean) and cereal crops (corn, rice, wheat) have been shown to exert lower potential for CECs uptake compared with leafy and root vegetables. It is worth noting that cucumber show higher potential for CECs uptake compared with tomato, due to fruit physiology effects (Sui et al., 2017). Fruit trees are assumed to have similar potential for CECs uptake with fruit vegetables; though, more studies are required in order to better elucidate the potential for CECs uptake by fruit trees, since no studies have been performed so far for the determination of CECs concentration in fruit trees irrigated with RWW, or grown in biosolids- and manure-amended soils.

Moreover, by taking into account the plant physiology-related parameters that affect the potential for CECs uptake by plants, such as the growing period, and the ETc and NIR values, the main crop species may be sorted based on their potential for CECs uptake. Leafy vegetables are identified as the crops with the highest potential for CECs uptake. Though, based on their physiological parameters, the tentative classification of the potential for CECs uptake of leafy vegetables plants may decrease in the order of celery > spinach > lettuce > cabbage, whereas that of root vegetables may decrease in the order of sweet potato > carrots

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> radish > late-season potatoes (grown during July to early December) > spring potatoes (grown during January to May) > mid-season potatoes (grown during October to early March) (Fig. 2). Moreover, the tentative classification of the potential of fruit vegetables for CECs uptake may decrease in the order of cucumber > green beans > okra > marrows > tomato > watermelons> melons > pepper > eggplants. Cereal and arable crops may be sorted in the order of maize > alfalfa > sorghum > peanuts > haricot beans > wheat > barley, while fruit trees may be sorted in the way of bananas > walnut (Pecan) > citrus and avocado > fruit trees in lowland and plains > fruit trees in mountains > pistachio > table olives > almonds > table grapes (Fig. 2).

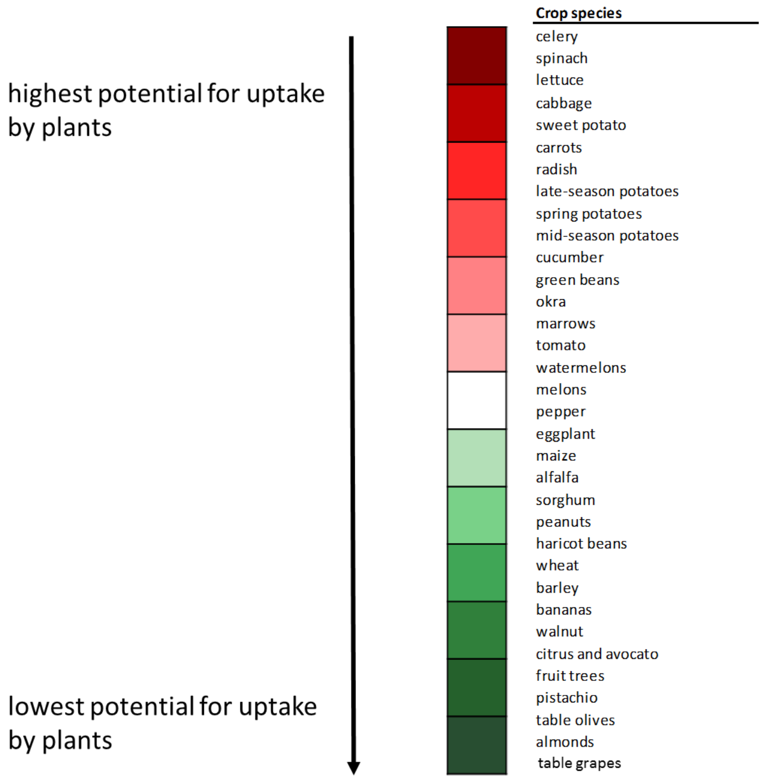

Fig. 2. Heat map showing the tentative classification of the potential of the main crop species for CECs uptake. This ranking was based on the plant physiology related parameters (growing period, ETC and NIR values) and the calculated bioconcentration factors reported in Table 2. The highest potential for uptake is indicated with dark red; the lowest potential with dark green.

7. Concluding remarks and research needs

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Accumulating evidence shows that the uptake of CECs by plants may be influenced by a variety of factors, both biotic and abiotic. The main biotic factors that may influence the uptake of CECs by plants are the plant itself (encompassing the species, the variety and cultivar, the genotype, and the physiological state of the plant), and the soil fauna, which constitute the main cause for the biodegradation and biotransformation of CECs within the soil. Climatic conditions and other environmental perturbations (such as temperature, wind speed, UV radiation, salinity, drought, environmental pollution, etc.) constitute the main abiotic factors that influence the potential for CECs uptake by crop plants. The majority of studies with regard to CECs uptake, either conducted in controlled laboratory or greenhouse conditions or under field or simulated conditions, employed mostly (a) vegetables (leafy vegetables such as lettuce and cabbage, fruit vegetables such as tomato and cucumber, and root vegetables such as carrot and radish) and (b) cereals and fodder crops (i.e. maize, wheat, alfalfa). Experimental results revealed that the potential for CECs uptake by crop plants decreased in the order of leafy vegetables > root vegetables > cereals and fodder crops > fruit vegetables. Though, the uptake of CECs by important crop plants, such as fruit trees, is not yet evaluated. Fruit trees, such as citrus, bananas, apple and other fruit bearing trees, have high net irrigation requirements and evapotranspiration rates, which may render them as plants with moderate to high potential for CECs uptake (similar to that of fruit vegetables). In addition, field studies, where a wider range of plant species will be employed (fruit trees included), must be performed in order to shed light on the uptake of CECs by crop plants under realistic agricultural conditions. Moreover, the quantification of the examined CECs in both the edible parts of the examined plants and in the growing medium (RWW-irrigated or biosolids- or manure-amended soil, substrates, etc.) is of high importance, as it will allow for the calculation of the BCF and the better understanding of crop plants' potential for CECs uptake.

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