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Published in:
Journal of Environmental Chemical Engineering

Link to article, DOI:
10.1016/j.jece.2018.01.011

Publication date:
2020

Document Version
Peer reviewed version

Accepted Manuscript

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PII: S2213-3437(18)30011-3
DOI: https://doi.org/10.1016/j.jece.2018.01.011
Reference: JECE 2131

To appear in:

Received date: 14-11-2017
Revised date: 2-1-2018
Accepted date: 4-1-2018

Please cite this article as: Benjamin Piña, Josep M.Bayona, Anastasis Christou, Despo Fatta-Kassinos, Emmanuel Guillon, Dimitra Lambropoulou, Costas Michael, Fabio Polesel, Stéphanie Sayen, On the contribution of reclaimed wastewater irrigation to the potential exposure of humans to antibiotics, antibiotic resistant bacteria and antibiotic resistance genes – NEREUS COST Action ES1403 position paper, Journal of Environmental Chemical Engineering https://doi.org/10.1016/j.jece.2018.01.011

On the contribution of reclaimed wastewater irrigation to the potential exposure of humans to antibiotics, antibiotic resistant bacteria and antibiotic resistance genes – NEREUS COST Action ES1403 position paper

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Abstract

Antibiotic resistance (AR) is becoming a worldwide threat due to the increasing occurrence of antibiotic-resistant pathogenic bacterial strains. There is a general consensus about the potential implications of the use of antibiotics in livestock on the onset of antibiotic resistant bacteria (ARB), mainly through meat consumption. However, the ever-increasing use of reclaimed wastewater (RWW) in agriculture may also contribute significantly to the non-accounted exposure to antibiotics, ARB, and antibiotic resistance genes (ARGs). This position paper aims at evaluating the current knowledge concerning the occurrence of antibiotics, ARBs, and ARGs in edible parts of different common crops irrigated with RWW. We will discuss which regulations on the use of RWW may contribute to the minimization of the prevalence of these contaminants in crops, and provide recommendations on how to minimize the impact of these practices.

Keywords

Antibiotics; Reclaimed wastewater; Risk assessment, Plant physiology; Antibiotic resistance; Wastewater treatment and reuse

1. Occurrence of antibiotics (ABs), antibiotic resistant bacteria (ARB), and antibiotic resistance genes (ARGs) in edible plant parts, and their contribution to the general public exposure.

A growing number of reports shows that crops can passively uptake water soluble contaminants through roots [1-3], and that some of these compounds can be translocated and concentrated into the aerial parts of plants, mainly leaves [4]. This effect, intimately related to the plant transpiration, leads to the uptake by plants of any bioavailable contaminant present in the soil, provided its physicochemical properties and persistence to degradation allow it [5]. In case of hydroponic cultures, the uptake of low-volatility contaminants such as ABs is greater than conventional crops in soil. Different reports demonstrated the presence of pharmaceuticals, including ABs, in edible parts of different crops under field experimental conditions, including application of manure or biosolids as fertilizer or RWW irrigation [1, 6-9]. The extent of this uptake is determined by the physicochemical properties of the substance and of the soil, by the plant genotype (species and cultivars), and by the physiological state (transpiration rate) and other stress effects of the plants and climate [3]. Following their uptake, ABs are translocated and reallocated in the various plant tissues including the edible parts, thus entering in the food chain. While leaves or roots are deemed to be the main concentrator of contaminants, trapping mechanisms of ionized ABs in plant transport tissues may lead to preferential translocation to leafy parts or fruits [4, 10]. Commonly detected ABs include sulfonamides (sulfamethoxazole, sulfapyridine), trimethoprim, fluoroquinolones (ciprofloxacin, ofloxacin, enrofloxacin), tetracyclines (tetracycline, oxytetracycline) and macrolides (erythromycin). However, lower accumulation than other organic micropollutants (e.g., carbamazepine) is expected, due to their reduced mobility in soil (fluoroquinolones, tetracyclines [11]), their relatively large molecular size (macrolides) and/or their ionizable nature, resulting in reduced cell permeability [5]. Nevertheless, concentrations up to several μg per kg plant tissue have been observed in edible crops [9]

The potential uptake of ARB and ARGs by RWW-irrigated crops is currently largely unexplored [9]. Recent developments on DNA sequencing allowed for the characterization of complex microbiomes not only associated to roots and to the adjacent soil (rhizosphere), but also to the skin of the aerial parts, and inside the plant itself [12]. It is unclear what portion of this microbiome reflects the composition of the soil microbiome, but it is known that bacteria can enter into the plant vascular system and can be translocated to the aerial parts [13]. While it is unknown which fraction, if any, of these bacterial community qualifies as ARB, recent studies detected ARGs in this endophytic or periphytic microbiome [14, 15]. For example, plants grown in sulfonamide-contaminated soil showed internalization of ARB and ARGs in leaves [16]. The ability of these genes and bacteria to colonize the gut microbiome of human and animals exposed to these crops is to date largely unknown.

2. Potential contribution of water reuse strategies to the contamination of agricultural soils and crops

Similarly to other biologically active compounds, like pharmaceuticals or ingredients of personal care products, some ABs are notoriously recalcitrant to conventional biological treatment (e.g., biological activated sludge) in urban wastewater treatment plants

(WWTPs), and are therefore continuously released into agroecosystems receiving RWW [17, 18]. Sulfonamides and macrolides seem to deserve special attention when considering the potential emissions via RWW reuse. Sulfonamides typically undergo incomplete removal in WWTPs, with additional formation during biological treatment through deconjugation of excreted human metabolites [19, 20]. Macrolides have been also considered as recalcitrant [21] and included in the latest version of the EU Watch List of Chemicals from the Water Framework Directive [22]. Among other ABs, fluoroquinolones and tetracyclines are mostly released to soils through fertilization with biosolids or manure, due to significant accumulation in sewage sludge [23, 24] and widespread use for animal therapy.

Given their continuous release into the environment, these contaminants are commonly considered as pseudo-persistent contaminants of emerging concern, or CEC [25]. CECs may enter agroecosystems through wastewater irrigation and soil amendment with animal manures or biosolids. Once in the soil, these contaminants can interact with other pollutants such as metals or alkaline earth cations (Fe, Cu, Al, Ca), forming complexes or chelates, which dramatically modify their fate. Limited information exists on how these cations influence the effect and the fate of pollutants [26-29], and, hence, the ability of plants to uptake them.

Antibiotics are routinely detected in RWW-irrigated agricultural soils runoff and management practices associated with concentrated animal feeding operations (CAFOs) often involve the application of highly contaminated wash and runoff water to agricultural lands, with AB concentrations ranging from low μg L^{-1} to low mg L^{-1} [9, 30-32]. The dissemination of ABs in agricultural soils can disrupt essential soil functions (e.g., nitrification processes and iron reduction), thus leading to a loss of crop productivity [33, 34]. There are many reports demonstrating that irrigation by RWW alters soil microbiome, reduces complexity and favors the appearance of ARB and ARGs due to the introduction of CECs in soil [35, 36]. While the direct connection between these observations and the use of RWW for irrigation is not yet clear, the presence of antibiotics in RWW and the receiving agricultural soils is a potential hazard that needs to be addressed. Although not so solidly tested, ARB and ARGs generated by the treatment of animals and humans with antibiotics could conceivably reach soils through irrigation with RWW. In any case, their capacity to compete with the resident soil microbiome is still to be determined.

3. Effects of AB, ARB and ARGs pollution on crop growth.

While the linkage between the presence of ARB/ARGs in edible plants and the onset of pathogenic ARB in human and animals is still to be assessed, there are some other hazards that should be explored for the risk assessment of the use of RWW for irrigation [37]. The effects of antibiotics on plant morphology and physiology have been described for different plant species, including both hormetic (positive and negative effects occurring at low exposure concentrations) and phytotoxic (at high exposure concentration) effects [7, 38, 39]. Plants exposed to environmentally relevant or higher concentrations of ABs (isolated or in combination) show lower rates of germination, inhibition of growth, tissues deformation, reduced photosynthetic rate and chlorophyll

content, and other stress-related phenomena, classical phytotoxic effects [40]. Although not definitely demonstrated, it is likely that complex mixtures of ABs and/or other CECs may exert synergistic or additive effects in plants, as demonstrated for aquatic and terrestrial organisms [41]. Moreover, increasing evidences show that the growth and production of crops is influenced by their interaction with the soil microbiome, being the nitrogen-fixing bacteria only an extreme case [42]. Following the same line of thought, endophytic bacteria appear as important elements on plant physiology, not only as parasites, but also by reinforcing different metabolic functions of the plant. Related to this, there is increasing information showing that irrigating crop plants with water polluted with different CEC alters the plant metabolism and, in some cases, reduces its growth [43]. While it is not clear that the quality of the final edible product may decrease due to this, it seems obvious that at least some of its organoleptic properties may also vary. If this relationship between CEC pollution and crop outcome is confirmed, it will add uncertainties to the cost/benefit balance of RWW irrigation.

4. **Public health risks associated with the unintended intake of CEC due to the consumption of RWW-irrigated agricultural products**.

Antibiotic resistance is becoming a worldwide concern due to the occurrence of pathogenic bacterial strains increasingly recalcitrant to treatment with the existing antibiotic medications. One of the most notorious cases is the methicillin-resistant *Staphylococcus aureus* (MRSA), one of the most prominent pathogens linked to hospital- and livestock-associated infections, which constitute a virtual pandemy capable to affect not only hospitalized patients but also healthy individuals in the community [44]. As the origin and maintenance of ARBs ultimately depends on the presence of sublethal concentrations of ABs in the microbiome environment [45, 46], it is of paramount importance to evaluate the contribution of ABs present in RWW-irrigated vegetables to the total AB burden in the human(and animal) gut.

A recent seminal study demonstrated that the antiepileptic drug carbamazepine and its metabolites can be detected at considerable concentrations in the urine of people consuming RWW-irrigated vegetables [47]. This proof of concept study indicates that consumers may be inadvertently exposed to ABs, in addition to other pharmaceuticals, due to the consumption of RWW-irrigated agricultural products. Therefore, the unintended intake of a certain antibiotic and its transformation products (TPs) at trace or higher levels and its presence in blood and urine may elicit physiological, and potentially unfavorable responses, which may vary according to the exposure concentration and the potential simultaneous exposure to a cocktail of pharmaceuticals or other classes of pollutants.

Different tools have been developed for the assessment of the antibiotics-mediated risks to human health, with the most important being the estimation of the daily or annual exposure of humans to antibiotics and its conversion to medical dose equivalent [8, 48, 49], the threshold of toxicological concern (TTC) [50], and the hazard quotient approach [51]. Most of these studies considered negligible the risk of consuming RWW-irrigated vegetables to human health [9, 48, 51]. However, the current parameters in ABs intake risk assessments may have to be readjusted to incorporate new knowledge on ABs toxicity and potential hazards. For example, special attention should be drawn to antibiotics with structural alert for potential genotoxicity and carcinogenicity (i.e. sulfapyridine, sulfamethoxazole and ciprofloxacin), for which the toxicological

thresholds are extremely low (2.5 ng kg body weight^{-1} day^{-1}) [50]. Moreover, risk assessment tools underestimate the hazard associate to AB transformation products (TPs) produced in the human and animal gut, during the WWTP process, and in the plants themselves. TPs are present in all environmental matrices, including crops, and they may accumulate in greater concentrations and exert higher toxic effects that the parent compounds [9].

When focusing on final consumers, risk assessment studies need to consider the cocktail effects of many different ABs and other CECs, and of their TPs, which are present in RWW-irrigated products. This complex mixture may produce additive or synergistic effects, and it can interact in unknown ways with medications already prescribed for the treatment of pre-existing infections [51]. Finally, food processing and/or cooking can increase the complexity of the total CEC intake.

Hazards linked to the presence of ARB and ARGs in food and their relationship with RWW-irrigation practices are poorly understood. The current knowledge cannot exclude the possibility that ARB thriving in the environment can be transmitted to humans [52, 53], even at very low abundance. This may result in an asymptomatic long-term colonization, noticed only when for some reason the general health condition is compromised [52]. Several limitations prevent the establishment of adequate recommendations about maximum admissible threshold values or to define critical control points or critical sources for ARB dissemination. It is obvious from the above that further field studies need to be performed in order to obtain more solid information on the safety of RWW use for irrigation.

5. Risk mitigation strategies

Risk mitigation strategies should be implemented at various levels, including the administrative-legislative level, the policy makers, the scientific community, the farmers, and the general public. The obvious first strategy to minimize the contamination of soils and crops by partially depurated RWW is to limit the amount of contaminants in the source. Consumption of antibiotics is difficult to tackle in human populations, but there are many good reasons for limiting it at maximum -- if not, to prevent the onset of ARBs in human guts. Sensitive facilities (hospitals, AB-producing factories) should have separated specific water treatment systems, to prevent massive contamination of the public sewerage [54, 55]. While being extremely significant at the local scale, AB loads from hospitals are globally insignificant compared to emissions from households, as a result of common therapy practices or short hospitalization periods [56]. The reduction of the overuse of antibiotics in human medicine practice along with encouraging local authorities to set up and implement collection systems and inactivation of the unused and expired medicines, are expected to significantly control the release of antibiotics through RWW irrigation. Finally, the use of ABs in animal farming has to be reduced and controlled, in line with the modern tendencies in different countries, with the regulation of antibiotics usage in the farmers' level, the reduction of meat consumption, and the adoption of users fees to be the most important [57]. Management decisions should also consider the benefits of the use of antibiotics in animals, such as improved animal welfare, reduction in losses due to morbidity and mortality, and any production efficiencies or food safety benefits that may arise from the use of antibiotics.

At the legislative level, the implementation of a European legislative tool on the use of RWW, which could determine the wastewater treatment levels and quality criteria, the reuse practices and other agricultural practices when RWW is applied for irrigation, would facilitate the mitigation of all evolving risks, mediated from the release of insufficiently treated RWW and the implementation of improper agricultural practices. Preventing the use of RWW for the irrigation of leafy vegetables would mean that consumers will be exposed to lower AB levels, as leafy vegetables seem to be the crops with the higher for the uptake and bioaccumulation of CECs with high translocation potential [58]. The regulated use of RWW through modern irrigation systems, which promote water use efficiency and the application of the precise irrigation water at the right place and the right time, would mitigate the input of RWW-applied antibiotics to the agroecosystems and therefore their presence in RWW-irrigated agricultural products.

Finally, the use of RWW for irrigation in agricultural soil rich in organic matter or clay content, rather than sandy soils, could also mitigate the uptake of CEC by crop plants, as the bioavailability of CEC for plant uptake is proven to be lower in soils rich in organic matter and clay content. To this end, the use of sorbent materials, such as biochar, compost or zeolite, in RWW-irrigated soils may also minimize the bioavailability of CECs for plant uptake, by their sequestration and inactivation in the sorbents' surfaces [59].

6. Conclusions

Our position is that the use of RWWs for irrigation of edible crops poses hazards associated to their content in ABs, ARBs and ARGs, the magnitude of which needs to be evaluated. We propose first to limit contamination in the sources, promoting a rational use of ABs and of their disposal both for human and veterinarian treatments. A second preventing strategy would be to reduce the use of RWWs in crops that are consumed raw, particularly edible leaves (e.g., lettuce) and sweet fruits. Finally, advanced water treatment methodologies able to remove CECs from wastewaters would be advisable if reclaimed waters are to become a general commodity to irrigate edible crops. The contribution of the scientific community towards the implementation of this task is crucial, as treatment technologies that would sufficiently eliminate these contaminants from effluents at economically-feasible prices, while simultaneously promote the circular economy, are still to be developed.

Acknowledgement

The authors would like to acknowledge the COST Action ES1403 NEREUS "New and emerging challenges and opportunities in wastewater reuse", supported by COST (European Cooperation in Science and Technology, www.cost.eu) for enabling the collaboration among the authors of the paper.

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