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Review

Co-contaminant risks in water reuse and biosolids application for agriculture☆

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ABSTRACT

Agriculture made the shift toward resource reuse years ago, incorporating materials such as treated wastewater and biosolids. Since then, research has documented the widespread presence of contaminants of emerging concern in agricultural systems. Chemicals such as pesticides, pharmaceuticals and poly- and -perfluoroalkyl substances (PFASs); particulate matter such as nanomaterials and microplastics; and biological agents such as antibiotic resistance genes (ARGs) and bacteria (ARB) are inadvertently introduced into arable soils where they can be taken up by crops and introduced to the food-web. Thus, concern about the presence of contaminants in agricultural environments has grown in recent years with evidence emerging linking agricultural exposure and accumulation in crops to ecosystem and human health effects. Our current assessment of risk is siloed by working within disciplines (i.e., chemistry and microbiology) and mostly focused on individual chemical classes. By not acknowledging the fact that contaminants are mostly introduced as a mixture, with the potential for interactions, with each other and with environmental factors, we are limiting our current approach to evaluate the real potential for ecosystem and human health effects. By uniting expertise across disciplines to integrate recent understanding regarding the risks posed by a range of chemically diverse contaminants in resources destined for reuse, this review provides a holistic perspective on the current regulatory challenges to ensure safe and sustainable reuse of wastewater and biosolids to support a sanitation-agriculture circular economy.

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1. Introduction

Global food security is a complex issue determined by the interactions of numerous driving forces operating on both the demand and supply sides. To meet future global food demand, estimated to increase by 56 % in 2050 (van Dijk et al., 2021), the pressures on the primary resources of energy, land, water, and biomass are expected to increase significantly. For example, crop irrigation accounts for 70 % of water use worldwide (FAO, 2020) and despite water scarcity already being an issue on every continent, recent estimates suggest that it is expected to increase in more than 80 % of global croplands by 2050, stemming from a decrease in fresh water availability and increased water demand (Liu et al., 2022a). Concurrently at a time of increased demand for agricultural produce, agricultural systems are coming under intense pressure from both policy and commercial drivers for improved sustainability (e.g. UN Sustainable Development Goals (SDGs)), and the need for companies to source ingredients that meet consumer expectations for environmentally acceptable farming (Melović et al., 2020).

These pressures are opening the door to solutions embedded in ancient practices which minimise waste and promote circular approaches to agriculture (Sands et al., 2023). One such possibility is the reuse of organic waste streams from livestock and sanitation, with the resulting procurement of water, organic matter (C) and macro-nutrients (N, P, K) to help reduce our reliance on energy-intensive manufacturing of mineral fertilizer, mining phosphate and potash rock and freshwater irrigation (Ofori et al., 2020; Walsh et al., 2023). Organic waste streams for example include by-products from wastewater treatment such as treated sludges and treated wastewater (TWW) as well as livestock manures (Carter et al., 2019; Zucker et al., 2015). Reuse therefore enables the recovery of resources and adds economic value to what is conventionally perceived as waste whilst reducing demand pressures on primary resources. However, as agriculture has started to shift towards increased resource reuse, research has documented the inadvertent release of contaminants into agricultural systems, which are present in treated sludges and wastewater (Carter et al., 2024). Concerns about the presence of contaminants have grown in recent years following studies, that have linked exposure via the agricultural food chain to ecosystem and potential human health effects (Ben Mordechay et al., 2022b; Topaz et al., 2024).

Contaminants in the environment can be defined as chemicals, materials or biological agents of natural or anthropogenic origin that are present in environmental matrices including soils, sediments, water bodies and the biota that inhabit them. Whilst anthropogenic contaminants are artificially introduced into the environment, naturally occurring chemicals materials or biological agents are considered contaminants when they are detected in locations they are not normally found, or in concentrations that do not occur naturally. The term "contaminant" therefore covers a wide variety of substances including legacy contaminants such as heavy metals and persistent organic pollutants (Boshir et al., 2017), as well as emerging contaminants (ECs; e.g. poly- and -perfluoroalkyl substances (PFAS), pharmaceuticals, microplastics, and biological contaminants). The term "emerging" describes both the contaminant and the emerging concern the contaminant poses. In this way, ECs are often called 'contaminants of emerging concern' or 'chemicals of emerging concern' (Sauvé and Desrosiers, 2014). ECs can therefore include well-known pollutants such as dichlorodiphenyltrichloroethane (DDT), which has been at the forefront of research and regulatory agendas for many years, when evidence emerges which suggests it can result in a newly discovered harmful impact or mode of action (Burgos-Aceves et al., 2021) as well as newly discovered contaminants where technology advances have enabled lower concentrations to be detected. It is important to acknowledge that the presence of a contaminant does not always result in a toxicological concern. For example, a certain concentration (or threshold) for a chemical contaminant (e.g. pharmaceutical) must be reached in order to elicit an effect and thus pose a risk. Whereas, for biological contaminants (e.g. ARGs), the risk of developing resistance in a plant, animal, or human only occurs if the ARG is expressed within a known pathogen, and this excludes many of the ARGs we are now detecting in the environment (Martínez et al., 2015).

To date, efforts to remove contaminants during wastewater treatment have largely focused on the need to reduce the organic load in general rather than advanced treatment options to remove ECs owing to the fact that many ECs were not included in existing wastewater treatment legislation (e.g. EU; Directive, 2000/60/EC, Directive, 2008/56/EC, Directive, 2013/39/EU). ECs are therefore widely detected in sewage sludges and effluents worldwide, due to their incomplete removal during wastewater treatment processes (Petrie et al., 2015; Rede et al., 2024). Chemical contaminants for example are challenging to remove owing to their capabilities of making complexes, toxic derivatives, by-product formation, and dynamic partitioning. ECs are also typically present in wastewater at trace ppt levels and such low concentrations often render the available technologies (e.g. bioremediation or adsorption to activated carbon) insufficient (Boshir et al., 2017). When chemical contaminants are removed from the wastewater this is commonly attributed to biological degradation or sorption to sludge (Cheng et al., 2021); sludge can therefore be considered as a sink or a reservoir of ECs, in particular hydrophobic pollutants which are more likely to partition to the organic solid fraction (Hyland et al., 2012).

Alongside the presence of chemical contaminants, monitoring studies frequently detect the widespread co-occurrence of ARGs and ARB in sludges and TWW owing to the continual input of sewage that contains human and animal faeces as well as wastewater from food processing which can contain microbes from infected livestock and poultry. Despite the documented removal of ARGs via physical treatment processes such as membrane filtration, and advanced oxidation processes (Gao et al., 2022), WWTPs are known antimicrobial resistance (AMR) reservoirs, where chemicals (e.g. antimicrobials) and AMR genes and microbes meet, resulting in the further selection of resistant genes (Sims et al., 2023). During wastewater treatment 'stress-inducing' conditions such as chemical exposure can stimulate ARG development and dissemination by exerting a pressure on the exposed microorganism thus inducing resistance to itself, and/or stimulating the transfer of mobile genetic elements (MGEs) responsible for the dissemination of resistance determinants (Nguyen et al., 2021). For example, exposure to the antibiotic trimethoprim has been shown to increase the rate of horizontal gene transfer (HGT) in an activated sludge bacterial community (Li et al., 2019) and even at low concentrations of tetracycline, the HGT of ARG determinants in WWTP activated sludge and effluent was still stimulated (Kim et al., 2014).

In the context of a sanitation-agricultural circular economy, which promotes reuse, ECs can therefore enter the environment through two primary routes: (i) the use of TWW as a source of irrigation; and (ii) through the use treated sewage sludge (biosolids) (Fig. 1). Application of biosolids to land as a fertilizer is common practice in many countries such as Australia where 85 % of biosolids was beneficially used in 2023 (Australian & New Zealand Biosolids Partnership, 2025). Data from the EU estimates that sludge use in agriculture is between about 30 and 50 % of the total amount of sludge produced. However, this percentage masks the large differences in recovery to land across EU countries (Gianico et al., 2021). For example, in the Netherlands, the spreading of treated sludge on farmland is banned (European Commission, 2023).

The use of TWW is seen as a necessary step toward meeting global crop irrigation demands, especially in arid and semi-arid countries, where access to freshwater is limited (Carter et al., 2019; Ungureanu et al., 2020). Israel and other Mediterranean countries also have a long history of irrigating crops with TWW, and several publications have demonstrated the widespread presence of ECs in TWW used for irrigation and the corresponding fields (Ben Mordechay et al., 2022b, 2021; Murrell et al., 2021; Picó et al., 2019a; Riemenschneider et al., 2016). However, wastewater is not always treated prior to reuse. Raw or partially TWW has been used for decades in settings with limited sewage connectivity or poorly functioning treatment systems (Contreras et al., 2017), for sustainability and economic reasons (Adegoke et al., 2018), where it is expected that concentrations of ECs will be higher (Igbinosa et al., 2011).

Repeated application of biosolids and wastewater therefore provides a pathway by which a range of biological, chemical and particulate pollutants can enter and accumulate in agricultural soils. However, the fate and exposure of ECs in soil-plant systems are largely considered on a contaminant-specific basis and do not fully account for the chemical and biological complexity that exists in the natural environment. This review first presents the foundation for understanding the current challenges associated with the presence of chemical, particulate and biological ECs in soil-plant systems which have been grouped into six categories: 1) plastic materials and natural fibres, 2) PFAS, 3) pesticides, 4) engineered nanomaterials, 5) pharmaceuticals, and 6) biological contaminants. Selected ECs include a large variety of substance families and physico-chemical properties; and were prioritized based on the following criteria: (i) occurrence in resources destined for reuse; (ii) recently identified potential risks to ecosystems and human health; (iii) the availability of the analytical data; and (iv) limited existing minimum quality standards for safe reuse.

We then highlight the potential interactivity between contaminants to provide a novel perspective on how the risks to ecosystems and human health may change in response to another contaminant stressor. The concluding section presents suggestions for the path forward, emphasizing the importance of improving links between researchers and policymakers, and better outreach to the public, water industry, and agricultural practitioners. We use this platform as a call for action, to form a global harmonized approach on our current understanding of the risks posed by contaminants destined for reuse that will centralize scientific findings across various contaminant classes. Insights from joined-up thinking such as this will help to mitigate risks and facilitate more sustainable use of resources for future agricultural production.

2. ECs in the environment

2.1. Particulate contaminants

1) Synthetic polymer particles and natural fibres

Plastics are a large group of materials which are generally composed of various types of natural or synthetic polymeric chains forming solid polymers (Geyer et al., 2017). These often also contain additives that improve plastic stability and provide some functionality such as colour, material flexibility, and UV resistance. Under environmental conditions, larger plastic items tend to degrade into smaller fragments or particles in the micro- (broadly defined as being <5 mm in size) and nano-range (<100 nm) particles, often named microplastics and nanoplastics, respectively (Hartmann et al., 2019), as well as releasing chemical additives in the process (Pfohl et al., 2022).

Microplastics have been reported in soils at concentrations up to 5047 particles per kg (Yoon et al., 2024), however the true extent of material exposure in agricultural systems is unknown and largely stems from the limited development of methodologies to isolate environmentally relevant particles from complex matrices such as soils, in particular nano-sized particles. Nevertheless, biosolids application to land has been identified as a major source of microplastics (Frehland et al., 2020), as microplastics are largely removed from the wastewater by precipitation and collection in the sludge (>98 % (Horton et al., 2021)). Nizzetto et al. (2016) estimated that in Europe up to 430,000 tonnes of microplastic enter agroecosystems annually through biosolids alone, while in North America estimates range from 44,000 to 300,000 tonnes of microplastics annually. Biosolids application has also been linked to the presence of plastic additives in soil, increasing the chemical burden. Aging and fragmentation of plastics can result in the release of additives and their degradation products from plastics (Hofmann et al., 2023), although evidence to date is mostly limited to phthalates. Plasticizers such as di(2-diethylhexyl) phthalate (DEHP) and Di-n-butyl phthalate (DnBP) are the most frequently detected plasticizers in biosolids but are typically reported at relatively low concentrations in biosolid-amended soils, linked with degradation during the wastewater treatment process (Armstrong et al., 2018; Estoppey et al., 2024). Nevertheless, in the environment the release of hydrophobic phthalates such as DEHP can extend over centuries due to slow diffusional aqueous boundary mass transfer (Henkel et al., 2022).

Research has also revealed the presence of microplastics in soils irrigated with recycled wastewater (Pérez-Reverón et al., 2022) as well as additional sources such as littering, composts and mulches and neighbouring land use (Bläsing and Amelung, 2018) and atmospheric deposition (Stefano and Pleissner, 2022). Furthermore, natural fibres of cellulosic (e.g. cotton) and animal (e.g. wool) origin are ubiquitous in wastewater resources and have emerged as a common, and often dominant, anthropogenic particle in microplastic research (Stanton et al., 2019). Natural fibre concentrations in the environment regularly

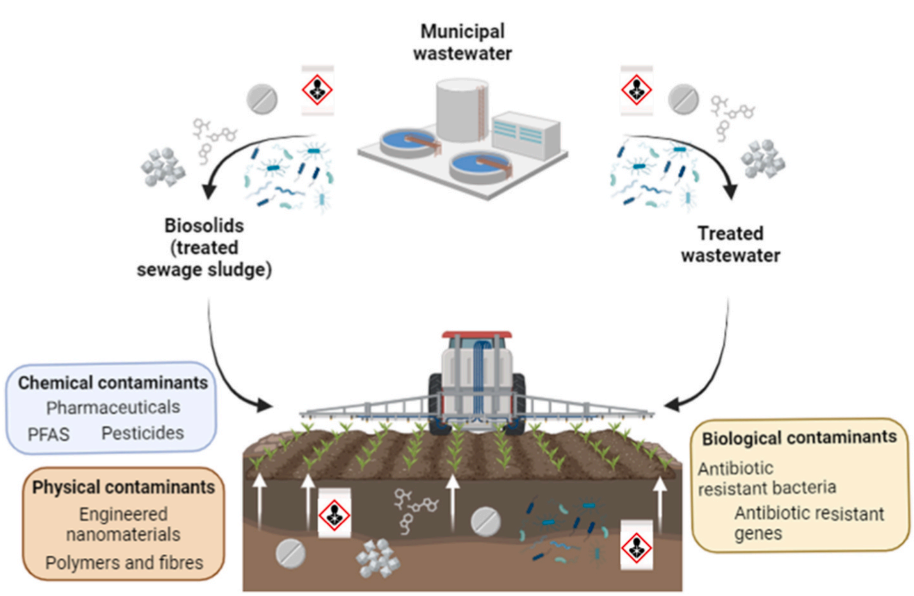

Fig. 1. Pathways of emerging contaminants in agricultural systems following treated wastewater and biosolids reuse.

outnumber that of plastic fibres where both are quantified, which has been shown in multiple environmental matrices including air (Liu et al., 2023), marine water (Suaria et al., 2020), and biota (Le Guen et al., 2020), worldwide, with further evidence from archaeological studies which has shown that individual natural fibres persist in the environment for decadal, and even centennial time scales (Kirkinen et al., 2023). Knowledge gaps persist in the natural textile fibre research space, however, their persistence and early investigations which have confirmed these natural textile fibres can lead to ecotoxicological harm (Siddiqui et al., 2023) provides evidence that the agricultural setting represents a key pathway for natural fibre introduction to, and accumulation in, the environment.

Plastic material plant uptake and effects

The presence of microplastics has been shown to effect soil bulk density, water holding capacity and alter soil structure with particle morphology such as fibres influencing this further (De Souza MacHado et al., 2018; Guo et al., 2020). Additionally, microplastic particles in soils have affected microbial activity in soils; while polystyrene and polyacrylic fibres decreased the microbial activity of the soil, low-density polyethylene significantly modified the microbial composition of the rhizosphere of wheat plants (Qi et al., 2020). However, due to non-monotonicity of the reported responses, it is difficult to predict how increased plastic particle concentrations will affect soil properties and ultimately plant responses.

Nevertheless, the presence of microplastics in soil has been observed to impact soil health which has the potential to lead to further impacts on food production and biotic health (Khalid et al., 2020). Effects such as these can influence the function and structure of soils which can indirectly impact on plant growth and development (Mateos-Cárdenas et al., 2021). For example, as microplastic particles have been found to accumulate in the topsoil (Cohen and Radian, 2022), they are likely to accumulate on plant roots and germinating seed surfaces, obstructing water and nutrient uptake or rupturing cell-to-cell connection (Roy et al., 2023).

Microplastics can also directly affect plant health following their uptake and accumulation (Ullah et al., 2021). For example, the accumulation of particles in leaves has been linked to oxidative stress, thus reducing leaf growth and photosynthesis (Colzi et al., 2022; Lian et al., 2021). However, as highlighted in Table 1, limited data exists which demonstrates in-field accumulation of plastic materials into plants following direct use of biosolids and TWW. A majority of our understanding stems from hydroponics studies which have demonstrated that nano- and micro-sized plastic particles could be absorbed by plant roots and translocated to aerial parts such as leaves (Wang et al., 2022). Apoplastic transport has been suggested to be the main pathway for the uptake and translocation of plastic particles to above ground plant organs. Further research has shown that nanoplastics have greater potential to enter plant cell walls as compared to microplastic particles (Azeem et al., 2021). Nevertheless, both 80 nm and 1 μm polystyrene microspheres were observed to accumulate in the vascular systems of rice plant tissues, especially the root stele, stem vascular bundles and in leaf veins, and were found to mostly aggregate on cell walls and in the intercellular regions (Liu et al., 2022b). However it is important to highlight that, hydroponic exposures to micro- and nanoplastics will not fully reflect exposure when in soils where interactions between solid mass and water phases will change availability to plants as well as possible transformation processes (e.g. eco-corona formation (Zettler et al., 2013)) which won't occur in the same way in hydroponic systems. Typically, hydroponic exposure has been considered a worst-case scenario for exposure.

Table 1
Demonstrated in plant accumulation of synthetic polymer particles.

Crop species	Location/study type	In plant accumulation	Reference
Pear *(P. communis)*, broccoli *(B. oleracea italic)*, lettuce *(L. sativa)*, carrot *(D. carota)*	Commercial produce from local markets in Catania, Italy	MPs <10 μm ranged by 52,050 to 233,000 particles/g depending on vegetable samples* demonstrates microplastics are prevalent in agricultural produce but the study does not link to specific pathways (i.e. biosolids use)	Oliveri Conti et al. (2020)

Although uptake of micro- and nanoplastics into plants has been observed, and recent work has shown they have to potential to cause negative effects and plants (Maity and Pramanick, 2020; Schultz et al., 2021), the long-term consequences of exposure for crops need to be elucidated. Approaches to estimate the human health risks associated with consuming produce also need to be extended to consider dietary uptake of plastics from contaminated crops specifically (Senathirajah et al., 2021). However it is crucial that when examining their fate, and potential toxicity that microparticles of high environmental relevancy are used, in terms of physicochemical characteristics and concentrations (Rubin et al., 2021). For example, plastic particles reaching agricultural soils are expected to have oxidised and shredded surfaces and may also be coated with organic and inorganic substances or "eco-corona" (Zettler et al., 2013) which need to be accounted for to enable an accurate assessment of risk to be made. Thus, future research efforts need to focus on an improved understanding of the fate of micro- and nanoplastics in soil-plant systems to inform the potential for adverse human health effects following the consumption of contaminated produce. So far research has revealed that exposure of aquatic embryos to micro- and nanoplastics led to their growth delay and developmental toxicity (Bashirova et al., 2023; Bhagat et al., 2020; Brun et al., 2019; Kögel et al., 2020). Similar adverse effects of nanoplastics were recently documented in embryos of terrestrial species (i.e. mice, chick) (Nie et al., 2015; Wang et al., 2023). More studies are therefore needed to assess the accurate risks to human health following inadvertent EC exposure.

2) Engineered nanomaterials (ENMs)

During the lifecycle of nano-enabled products in applications including medicine, agriculture and consumer products, engineered nanomaterials (ENMs) (between 1 and 100 nm in at least one dimension) are released, particularly during the use phase. In the case of consumer products such as textiles and personal care products, material flow analysis indicates that their use will result in releases to WWTPs through washing or other down the drain releases. However quantification of ENMs in these systems is very limited by the challenges around detection of ENMs in complex matrices. Nevertheless, pilot scale studies have found for example, that >90 % of ENMs such as titanium dioxide (TiO_2), silver (Ag) and zinc oxide (ZnO) are in sludge at the end of treatment process (Ma et al., 2014). As such, biosolids application to agricultural land is an important release pathway for ENMs to soils. ENMs have also been shown to undergo different transformation processes either during use or release from nano-enabled products. These can include chemical transformations, such as redox reactions, dissolution, coating degradation, and organic matter, protein, and macromolecule binding, and physical transformations including homo or heteroagglomeration (Spurgeon et al., 2020; Svendsen et al., 2020). These transformations will change the physicochemical properties of the ENMs. For example, ageing processes in waste streams can result in dissolution and chemical transformations such as sulfidation or phosphatisation of ENMs such as ZnO and Ag (Kaegi et al., 2013; Ma et al., 2014). Thus, release of ENMs to the environment will likely not be in their pristine manufactured form (Surette et al., 2019) and an understanding these transformations is important in elucidating the hazard and risk of ENMs released to the environment (Spurgeon et al., 2020).

Table 2
Demonstrated in plant accumulation of engineered nanonmaterials (ENMs) following wastewater reuse or biosolid application to soil.

Crop species	Location/study type	In plant accumulation	Reference
Tomato *(Solanum lycopersicum)*	Biosolid amended soil in a laboratory study	Bioavailability of Ag from AgNPs and Ag2S-NPs was low and no greater than that of ionic Ag+	Judy et al. (2015)
Spinach (*Spinacia oleracea)*	Microcosm study using irrigation water spiked with NPs	Bioaccumulation of Zn and Cu in roots and shoots. Low translocation from roots to shoots. Accumulation not greater that ionic Zn or Cu	Singh and Kumar (2020)
Lettuce *(Lactuca sativa)*	Biosolid amended soil in a laboratory study	Bioavailability of Ag from AgNPs and Ag2S-NPs was low and no greater than that of ionic Ag+. Plant uptake generally low (0.02 %). Low translocation of Ag from the roots to the shoots in lettuce	Doolette et al. (2015)
Rice seedlings *(Oryza sativa)*	Microcosm greenhouse study using treated wastewater	Demonstrated plant uptake. Roots accumulated a larger amount of Cu (37.0–553.7 mg/kg) compared to shoots (17.0–63.0 mg/kg) or grains (4.9–7.9 mg/kg)	Phung et al. (2022)

ENM plant uptake and effects

As demonstrated in Table 2, ENMs can be taken up by plants following TWW irrigation and biosolids application to land (Judy et al., 2015; Singh and Kumar, 2020). The bioavailability of ENMs in soil is driven by both the physicochemical characteristics of the nanomaterial and soil characteristics such as soil pH and organic matter content (Khodaparast et al., 2022; Lahive et al., 2023) with uptake potential also a factor of the plant species in question. Research has shown that both primary ENMs and the dissolved ions (from metallic ENMs) can be directly taken up by plants, including ENMs with diameters larger than the size exclusion limits of plant roots (e.g. less than 20 nm) (Ma and Yan, 2018). ENMs can penetrate the cell wall and cell membrane of root epidermis via a range of pathways (Avellan et al., 2021) leading to the accumulation of ENMs in the plant roots.

ENMs can induce the formation of nanoscale membrane holes in the root cells that allow their accumulation in the roots (Zhu et al., 2012) or pass through the crack-entry mode where the endodermal cells are not yet mature in the discontinuous regions in the Casparian band of the root tip as well as at sites of secondary root initiation (Li et al., 2020; Murazzi et al., 2022). Additional research has established that surface charge influences the interaction between nanoparticles and plant cell surfaces, affecting their uptake and transport through processes such as electrostatic interactions and alterations to membrane permeability (Ma and Quah, 2016; Zhu et al., 2012) Following uptake, nanoparticle bioaccumulation is often highest in plant roots although transfer to shoots and grains have been found in some cases (Khodaparast et al., 2022).

The uptake and accumulation of ENMs can negatively affect plant growth and seed germination at high concentrations (100–400 ppm (Siddiqi and Husen, 2016)). However, these often exceed concentrations that are measured or predicted in the environment which range from pg/kg to μg/kg (Giese et al., 2018). ENMs have also been observed to increase plant growth at these lower nanoparticle concentrations (1 μg/mL (Feichtmeier et al., 2015)). Interestingly, effects of ENMs applied in biosolids have been found to be less compared with their application to soil as pristine nanomaterials or bulk metal forms (Oleszczuk et al., 2019; Schlich et al., 2018), although not in every case (Lahive et al., 2017). In more recent years, the impact of transformation processes on the bioavailability and toxicity of ENMs has also received greater attention, where more realistic exposure levels and release forms are used to assess impacts on soil environments (Spurgeon et al., 2020). For example, low (environmentally relevant) concentrations TiO_2 nanoparticles applied in irrigation water were not toxic to the soil microcosm and instead appeared to mitigate the harmful impacts of aged Ag nanoparticles (Liu et al., 2019a). Findings such as this highlight the importance of considering contaminant combinations in the environment as in some circumstances they could result in beneficial effects.

2.2. Chemical contaminants

3) Poly- and perfluoroalkyl substances (PFAS)

PFAS are a family of amphiphilic compounds containing hydrophobic perfluoroalkyl moieties (C_nF_{2n+1}) and a soluble negatively charged head group (i.e., carboxylic acids, sulfonic acids, phosphonic acids). The physicochemical properties of these compounds make them desirable in many industrial applications, but also render them highly persistent, pervasive and mobile in the environment. Moreover, exposure to some types of PFAS is of particular concern as there have been multiple health risks linked to human exposure including carcinogenic, metabolic, hepatotoxic, immune, and neurodevelopmental effects (Bell et al., 2021; Brown et al., 2020; Sunderland et al., 2019).

The continuous and widespread use of PFAS in applications such as non-stick packaging or cookware and firefighting foams, has caused them to accumulate in many environmental compartments, including soils and the wider agricultural environment (Lenka et al., 2021; Wang et al., 2017a). A major point source for PFAS in agricultural soils is irrigation using wastewater and biosolids application as fertilisers (Ghisi et al., 2019). For example, wastewater is suggested to account for 85 % of perfluorooctane sulfonic acid (PFOS) releases on a global scale (Sunderland et al., 2019). Chain length and functional groups impact the pathway of exposure and occurrence in the environment; short-chained PFAS (<5C) are more soluble and volatile and are therefore more mobile, however, the concentrations of PFAS detected are often comparable between long and short chains due to the historic widespread use of long-chain PFAS and their persistence in the environment (Pike et al., 2021). Still, overtime, as regulations are tightened, we would expect the concentrations of the new generation compounds in the environment to surpass those of older generation PFAS due to their more polar nature.

PFAS plant uptake and effects

The main pathway for PFAS uptake into plants has been identified as passive uptake from the soil pore water to the plant root. PFAS need to overcome selective membrane barriers to be taken up and transported within plants leading to higher bioaccumulation in vegetative compartments than in reproductive and storage organs (Ghisi et al., 2019; Lesmeister et al., 2021; Sunderland et al., 2019). A review of literature reveals that PFAS bioaccumulation decreases with increasing chain length, mainly due to sorption of the longer PFAS chains to the soil organic matter and reduced. However, the exact reasons for this remain unknown (Ghisi et al., 2019). Previous findings suggest that the selectivity towards uptake of shorter PFAS stems from solubility and availability in the soil solution, and the ability to transport via selective proteins within the plant. However, some hydroponic experiments have shown that the uptake is not correlated to chain length, suggesting the availability in the soil solution (and respective adsorption to the soil particles) may be the reason for higher uptake of shorter PFAS in soil (Ghisi et al., 2019; Lesmeister et al., 2021). It has also been suggested that the functional head group may also impact PFAS uptake with the carboxylic group being more prone to bioaccumulate, however, this is not as significant as the chain length of the compound. Soil physicochemical properties can also affect the uptake rates of PFAS in plants (Adu et al., 2023). Mainly the composition and concentration of soil organic matter was found to alter uptake patterns, where bioaccumulation of PFAS decreased with increasing soil organic matter concentration and age (fresh compost vs aged humic substances) (Qi et al., 2022). This also suggests that reduced bioavailability of the more hydrophobic PFAS hinders their uptake by plants.

Table 3
Demonstrated in plant accumulation of poly- and -perfluoroalkyl substances (PFAS) following wastewater reuse or biosolid application to soil.

Crop species	Location/study type	In plant accumulation	Reference
Yam (*Dioscorea* spp.), maize (*Zea mays*) and sugarcane (*Saccharum officinarum*)	Crop plants irrigated with treated wastewater in agricultural areas in Kampala, Uganda	PFHpA, PFOA, PFNA, PFBS and FOSA were detected in plant samples. Yam root and sugarcane stem had similarly high ΣPFAS concentrations (360 ± 170 and 350 ± 64 pg/g dw, respectively), while those in maize cobs were lower (200 ± 64 pg/g dw)	Dalahmeh et al. (2018)
Lettuce (*L. sativa*) and tomato (*Lycopersicon lycopersicum*)	Greenhouse trial using industrially impacted biosolids-amended soil, a municipal biosolids-amended soil (biosolids:soil dry weight ratio of 1:10)	Lettuce uptake <266 and 236 ng/g (dw) for perfluorobutanoic acid (PFBA) and perfluoropentanoic acid (PFPeA), respectively, and reached 56 and 211 ng/g (dw) for PFBA and PFPeA in tomato, respectively. PFBA had the highest bioaccumulation factor in lettuce (56.8) and PFPeA the highest in tomato (17.1)	Blaine et al. (2013)
Alfalfa (*Medicago sativa*), mint (*Mentha spicata*) and lettuce (*L. sativa*)	Wastewater irrigated soil in Jordan	PFAS detected in soils but no PFAS detected in alfalfa and mint plants. Lettuce not analysed due to technical difficulties	Shigei et al. (2020)
Corn silage and tall fescue (for animal feed)	Irrigated crops at a mixed-use agricultural and forested site (US)	The majority (>84 %) of the PFAS present in the crops were short-chain compounds, including PFBA, PFPeA, and PFHxA. There was slight seasonality in the number of PFAS compounds in the Fescue haylage with increased variability in the fall, compared to spring	Mroczko et al. (2022)

Overall PFAS compounds have been detected in all plant organs and compartments and in a plethora of plant species (Table 3). Out of the thousands of PFAS in use, monitoring efforts have only focused on a small fraction, namely the most common legacy PFAS, PFOS and perfluorooctanoic acid (PFOA). This presents an incomplete picture of risk associated with uptake into food crops as different compounds accumulate in different plant compartments when plants are exposed to a mixture of PFAS (Lesmeister et al., 2021). For example, PFOA and PFOS accumulated to a low extent in potatoes and cereal, while short-chain compounds accumulated at higher levels in leafy vegetables and fruits. Still, studies have shown that many fluorinated compounds such as fluorotelomer alcohols, fluorotelomer polymers, perfluoroalkane sulfonamides, and sulfonamidoethanols, degrade in the environment to PFOA and PFOS (Dinglasan et al., 2004; Rhoads et al., 2008; Washington et al., 2009). These findings are therefore valuable for risk assessment.

PFAS uptake via crop consumption has been identified as a major exposure route to humans (Ghisi et al., 2019). The importance of this was highlighted by Brown et al. (2020) who showed that consumption of vegetables irrigated with PFAS-impacted water, which meets the current EPA lifetime health advisory limits, may not be protective of dietary exposures to PFAS contaminants specifically. Elevated PFAS concentrations in meat and dairy products have also been reported, suggesting contaminated crops are a source of dietary exposure for farm animals as well (Sunderland et al., 2019). However, there is a need to better quantify, monitor and evaluate PFAS toxicity especially shorter chain and more mobile compounds.

4) Pesticides

Pesticides are intrinsically toxic and deliberately spread in agricultural and forestry environments to control pests and therefore their presence in soils is to be expected. In addition to direct application, studies have also reported the widespread presence of pesticides in TWW (Firouzsalari et al., 2019; Xie et al., 2021) originating from use in grass management, rodent/mosquito control, and the use of algicides in paints and horticulture. Wastewater-derived pesticides such as DDT and hexa-chlorocyclohexanes have therefore been observed to accumulate in the soil subsurface following irrigation events (Haddaoui et al., 2016). Interestingly, DDT was the predominant organochlorinated pesticide in Tunisian soils irrigated with treated wastewater (94 % of the total organochlorinated pesticide) despite a ban on DDT use in many countries since 1970s. Current use pesticides are more biodegradable in nature and are considered to be less toxic and persistent as compared to previously used organochlorinated pesticides. However, monitoring studies have detected a suite of pesticides in TWW, demonstrating the potential for these chemicals to re-enter agricultural systems including pyrethroids, carbaryl and imidacloprid at levels exceeding US EPA aquatic life benchmarks for chronic exposure to invertebrates (Sutton et al., 2019). Furthermore, a survey of ECs in biosolids destined for land application also revealed the presence of pesticides such as 1,4-dichlorobenzene alongside widely reported ECs such as polycyclic aromatic hydrocarbons (PAHs) and pharmaceuticals (Kinney et al., 2006). The use of agro-wastewater for irrigation, containing residues of pesticides from the clean-up of equipment after application (Aliste et al., 2022) also presents another pathway for pesticides to inadvertently enter agricultural soils.

Pesticide plant uptake and effects

The fate of soil-applied pesticides in terms of plant uptake involves complex interactions between the pesticide's chemical properties, soil characteristics, environmental conditions, and plant physiology. The adsorption capacity of pesticides modifies their environmental behaviour and is key factor linked to pesticide leaching, (bio)degradation, volatilization, bioavailability and ultimately plant uptake. Whilst organic amendments including biosolids and animal wastes have been shown to increase the adsorption of pesticides by soils reducing the fraction pesticide available for plant uptake (García-Delgado et al., 2020), a fraction of soil-applied pesticides reach the root surface by bulk transport in soil water.

Pesticides can then move into plants primarily through root uptake which is mainly recognised as a passive diffusion process (Sicbaldi et al., 1997). Ultimately, the extent of root uptake is influenced by the pesticide's water solubility, the presence of transport proteins, and the plant's metabolic activity. However, some studies also found that active uptake (e.g. protein-mediated energy-dependent uptake) might also be involved in the root uptake of pesticides and might coexist with the passive uptake process (Fu et al., 2016). Following uptake, pesticides can translocate to higher parts of the plant such as fruits and buds or remain in the root system (Liu et al., 2019b; Zhang and Yang, 2021). Transpiration and movement via the xylem and phloem facilitates the uptake and bioaccumulation of pesticides with moderate lipophilicity to the leaves and fruit. Specifically, pesticides with a log octanol water partition coefficient (log K_{OW}) ~ 2 exhibit moderate hydrophobicity, making them sufficiently soluble in both water and lipid environments. This characteristic enhances their solubility in phloem sap, which is primarily aqueous but contains organic solutes, facilitating their movement within the plant (Hsu and Kleier, 1996).

Pesticide uptake by plants has been extensively studied (Liu et al., 2024). However, the accumulation of pesticides in plants has been largely attributed to the direct use of pesticides from a pest management perspective, despite the identified presence of pesticides in TWW and biosolids (Table 4). Pesticides are well known environmental toxicants, to the extent that the World Health Organisation recommends the classification of pesticides by hazard, and maximum residue limits are defined for food products (European Commission, 2005). For example,

Table 4
Demonstrated in plant accumulation of pesticides following wastewater reuse or biosolid application to soil.

Crop species	Location/study type	In plant accumulation	Reference
Barley, cabbage, green beans, chili, tomatoes and zucchini	Agricultural wastewater irrigation in the Al Hayer area (Saudi Arabia)	Seven pesticides detected. Diazinon detected in irrigation waters and subsequently in barley root, eggplant, tomato and green beans. Imidacloprid and malathion also detected in wastewaters used for irrigation and in cabbage and chilli plants	Picó et al. (2019b)
Cucumber, pepper, and melon	Controlled conditions greenhouse with irrigated with TWW and with TWW spiked with chemicals	17 pesticides found in the crops roots, with 6 common to all crop types (average of 276 μg/kg f.w in melon root, 30.8 μg/kg f.w in cucumber root and 9.7 μg/kg f.w in pepper root). Propamocarb and atrazine were the pesticides that showed the highest accumulation rates in the non-edible parts of each crop. Only cucumber showed pesticides accumulation in the fruits	García-Valverde et al. (2024)

many current use pesticides such as glyphosate and malathion are probable or possible carcinogens, and there have been reports of endocrine disruption in humans and other organisms (Cressey, 2015). It is therefore important that the contribution of the 'urban wastewater' pathway (i.e. biosolids or wastewater irrigation) is accounted for in current human and environmental pesticide risk assessments (e.g. Biocidal Products, Regulation (EU) 528/2012).

5) Pharmaceuticals

Pharmaceuticals comprise a large and diverse group of biologically active chemicals, including both prescription and over-the-counter substances intended for the treatment of livestock and humans. After utilization, pharmaceuticals are excreted or washed from the body existing either in their original parent form or as metabolites and subsequently enter the sewage system (Besse et al., 2009; Fu et al., 2019). Owing to the partial removal rates of pharmaceuticals in WWTPs (Yang et al., 2011; Zonja et al., 2016), irrigation using TWW exposes the soil and crops to ECs, in their most bioavailable form (i.e., soluble). Numerous studies have documented the presence of pharmaceuticals such as carbamazepine, lamotrigine and venlafaxine, in soils receiving TWW at concentrations typically in the range of 0.02–10,000 ng/g (Ben Mordechay et al., 2023; García-Valverde et al., 2023). Land application of biosolids, also provides a pathway by which pharmaceuticals can enter agricultural environments with antimicrobials, stimulants, and antidepressants some of the most commonly detected pharmaceuticals in treated sludges, with reported concentrations up to high mg/kg levels (Martin and Hart, 2023; Sellier et al., 2022; Verlicchi and Zambello, 2015). However, for biosolid-associated pharmaceuticals to become bioavailable for plant uptake, they must first be desorbed into the soil solution (Ben Mordechay et al., 2018; Bourdat-Deschamps et al., 2017).

Pharmaceutical plant uptake and effects

The uptake of pharmaceuticals into plants is governed by the physicochemical properties of the chemical including its charged state, lipophilicity (log K_{OW} >3.5), molecular weight in combination with soil properties which affect their available concentration in soil solution, and their bioavailability (Ben Mordechay et al., 2022a; Trapp, 2004; Xu et al., 2021; Briggs et al., 1983; Carter et al., 2014). Positively charged pharmaceuticals are associated with higher rates of sorption to soil solids reducing their bioavailability, whilst negatively charged compounds tend to repel from the root membrane and soil smectites (Trapp, 2000).

Once taken up, non-ionic pharmaceuticals exhibiting moderate hydrophobicity (log K_{OW} between 1 and 3) can cross the Casparian Strip and be translocated with the water flux in the xylem towards the leaves and accumulate there, whereas more hydrophobic compounds typically remain in the roots (Limmer and Burken, 2014). Therefore, leaves and roots tend to exhibit high concentrations of pharmaceuticals and other ECs as compared to other plant organs such as fruits, grains, and bulbs, which receive most of their solutes through the phloem sap. Non-ionized pharmaceutical accumulation in the higher parts of crop is governed via lipophilicity, sorption to proteins, and the air-water partition coefficient (Trapp et al., 2023) whereas for ionizable pharmaceuticals, barriers restricting in-plant transportation exist. For example, for acids (pKa > pH 5.5) translocation via the phloem will be inhibited, and preferential fate is via the xylem (pH 5.5), whereas weak bases (pKa > pH 8) will be translocated via the phloem and are subject to both the upward and downward flow of the phloem. Due to the range of factors with potential to influence plant uptake and accumulation, considerable variability in plant concentrations has been reported (Table 5) following TWW irrigation or biosolids applications (Ben Mordechay et al., 2022b; Bourdat-Deschamps et al., 2017).

Accumulation of ECs such as pharmaceuticals in edible portions of crops presents toxicological concerns for the agroecosystem as well as

Table 5
Demonstrated in plant accumulation of pharmaceuticals following wastewater reuse or biosolid application to soil.

Crop species	Location/study type	In plant accumulation	Reference
8 crop types including leafy greens (lettuce (*L. sativa)* carrot *(D. carota),* orange *(Citrus X sinensis),* tangerine *(C. reticulata),* tomato *(S. lycopersicum),* potato *(S. tuberosum),* avocado *(P. americana),* banana *(Musa)*	Reclaimed wastewater used in agricultural fields in Israel (data from 445 commercial fields)	Anticonvulsants (carbamazepine, lamotrigine, and gabapentin) were the most dominant therapeutic group found in the reclaimed wastewater-soil-plant continuum (<1000 ng/g). Antimicrobials were detected in ~85 % of water/soil samples but low detection frequencies and concentrations in crops. Leafy greens exhibited the highest concentration of pharmaceuticals	Ben Mordechay et al. (2021)
Tomato (*S. lycopersicum* L)	Commercial greenhouses irrigated with treated wastewater in Spain	Sulfonamides detected in tomato were sulfadiazine, sulfapyridine, sulfamethazole, sulfamethoxazole and sulfadimethoxine. Sulfamethoxazole was the antibiotic with highest concentration in tomato fruit, exceeding 30 μg/kg	Camacho-Arévalo et al. (2021)
Tomatoes (variety H9909), potatoes (*Solanum tuberosum*), carrots (*D. carota*) and sweet corn (*Z. mays*)	Field experiment using soil fertilized with municipal biosolids in Ontario, Canada	Eight of the 141 analytes were detected in one or two crop replicates at concentrations ranging from 0.33 to 6.25 ng/g dry weight, but no analytes were consistently detected above the detection limit in all triplicate treated plots. Detections included atenolol, trimethoprim, sulfamethazine in tomatoes	Sabourin et al. (2012)

human health following the consumption of wastewater-derived contaminated produce. Early ecotoxicological studies suggested that pharmaceutical uptake could negatively affect plant growth at high concentrations (Gworek et al., 2021). Links have been made between pharmaceutical mode and action and conserved receptors in plants (Garduño-Jiménez and Carter, 2023) which is supported by emerging research which has shown that at low concentrations, pharmaceuticals can effect main plant physiological processes such as phytohormone concentrations (Carter et al., 2015), with unknown implications for crop productivity and yield in the long term. Furthermore, the potential risk to human health following the consumption of pharmaceutical-contaminated produce is evidenced by biomonitoring data which has detected carbamazepine and its metabolites in urine collected from healthy individuals (including pregnant women), confirming unintentional and persistent exposure to this drug in the population which can be attributed to exposure via the food chain (Paltiel et al., 2016; Schapira et al., 2020). A recent paper assessing exposure to pharmaceuticals in various scenarios highlighted that several pharmaceuticals may exceed acceptable daily intake values in extreme cases of high consumption of highly contaminated leafy vegetables (Ben Mordechay et al., 2022b). Nevertheless, a recent meta-analysis revealed that in plant concentration data exists for a small number of pharmaceuticals approved for use (Sleight et al., 2023). This lack of data, in addition to the considerable knowledge gaps concerning in-plant metabolism (Carter et al., 2018; Malchi et al., 2014), adds further uncertainty regarding previous conclusions that minimal health effects are expected following the consumption of pharmaceutical contaminated produce.

2.3. Biological contaminants

6) Antibiotic resistance genes (ARGs) and microorganisms

Where advanced treatment methods (tertiary treatment) are in operation at WWTPs such as filtration, chlorination, ultraviolet (UV) radiation, or ozonation, these processes have been shown to effectively reduce microbial loads, including many organisms considered to be AMR facilitating elements. However, substantial loads of biological contaminants remain in TWW, including pathogenic bacteria (Rizzo et al., 2013; Ye and Zhang, 2011), fungi and their spores (Assress et al., 2019b; Ezeonuegbu et al., 2022), parasites (Ramo et al., 2017), protozoa (Rusiñol et al., 2020) and viruses (López-Gálvez et al., 2016), along with toxins produced by these organisms (Zhang et al., 2019) and their genes e.g. ARGs (Rizzo et al., 2013). Furthermore, biosolids can also contain a variety of microbial contaminants, including bacteria, viruses, and parasites (Al-Gheethi et al., 2018; Sidhu and Toze, 2009) where there is the potential for resistant determinants to grow back, especially if biosolids are stored for considerable time on the side of the field before spreading (Pepper et al., 2006; Zaleski et al., 2005). Log removal of *E. coli* (or fecal coliforms) is the "gold standard" for estimating the capacity of a specific water treatment technology to mitigate pathogens. However, in certain treatment schemes it may not accurately predict the removal capacity of other bacterial and viral pathogen indicators (Wang et al., 2021) suggesting that when possible other indicators should be evaluated in addition to *E. coli*. The tricycle protocol (WHO, 2021) measures ESBL-producing (cefotaxime resistant) *E. coli* concomitant to total *E. coli*, providing a platform that can easily be adopted by stakeholders that is highly informative regarding the antibiotic resistance status of wastewater and irrigated environments (Davidovich et al., 2025).

ARGs including genes conferring resistance to tetracyclines and beta-lactams have been shown to accumulate in soils irrigated with TWW, with ARG abundance in soil observed to positively correlate with irrigation intensity (Kampouris et al., 2021; Slobodiuk et al., 2021). However, whilst TWW irrigation was found to promote the spread of ARGs and the class 1 integron integrase gene, *intI1* in soil at greater levels compared to freshwater irrigation, it had a negligible effect on 16S rRNA absolute abundance and the soil microbial community composition (Kampouris et al., 2021). Biosolids-amended soils also have higher levels of ARGs and ARB, including various pathogenic bacteria. For example, a long term field experiment detected 130 unique ARGs and 5 mobile genetic elements (MGEs) following application of sewage sludge and manure, and observed a significantly increased abundance and diversity of ARGs in the soil (Chen et al., 2016). Genes conferring resistance to beta-lactams, tetracyclines, and multiple other drugs are typically dominant in biosolid amended soils (Chen et al., 2016; Qin et al., 2022). Despite the widespread presence of ARGs in biosolid amended soils, contrasting results exist with respect to the role that biosolids application plays in increasing the percentage of antibiotic-resistant culturable bacteria above background soil levels (Brooks et al., 2007; Mays et al., 2021).

Biological contaminant plant uptake and effects

Several studies have reported high counts of total coliforms and fecal coliforms in crops irrigated with TWW (Akponikpè et al., 2011; Mutengu et al., 2007; Sacks and Bernstein, 2011), while others have detected bacterial pathogens such as *Salmonella*, *Streptococci*, *Clostridium*, *Shigella*, and *Vibrio* spp. (Mañas et al., 2009) (Table 6). Bacteria (e.g. *Salmonella*) are known to attach to parts of the crop, particularly the stomata or areas where damage has occurred to the plant (Wiedemann et al., 2014). Once attached, some bacteria may colonise crop surfaces, resulting in the potential formation of biofilms on the edible parts of the plant (Alegbeleye et al., 2018). In addition to accumulation on crops, pathogens have been shown to internalise into plant tissues, although studies

Table 6
Demonstrated in plant accumulation of biological contaminants following wastewater reuse or biosolid application to soil.

Crop species	Location/study type	In plant accumulation	Reference
Tomato crops (*Lycopersicon esculentum* Mill. cv. Bodar)	Two commercial tomato fields irrigated with using water from a channel impacted by treated wastewater in Spain	ARGs and intl1 sequences were found in leaves and fruits at levels representing from 1 to 10 % of those found in roots or soil. High levels of *tetM*, *mecA*, and bla_{OXA-5}. Correlation between the prevalence of *Pseudomonadaceae* and the levels of different ARGs, particularly in fruits and leaves	Cerqueira et al. (2019b)
Legume plant *Vicia faba* (broad beans)	Samples collected from a peri urban plot in Spain irrigated with reclaimed wastewater	Most of the studied genetic elements (*intl1*, ARGs) were only found in significant amounts in soil and root endophytes but bla_{TEM} was detected in 100 % of samples. Bacterial abundance in both roots and leaves was around 1–10 % of the levels found in soils	Cerqueira et al. (2019a)
Strawberry plants (*Fragaria ananassa* cv Camarosa)	Greenhouse trial using treated wastewater, surface water from a dam and blended water (wastewater and dam water)	*Overall, the bacterial population on the leaves was greater than that detected on the fruits. E. coli was the dominant bacterium detected on strawberry leaves and fruits independent of water type. E. coli* population increased from 1.8×10^2 in DW-irrigated fruits to 2.8×10^3 colony forming unit per gram in TWW-irrigated fruits	Al-Karablieh et al. (2024)
Corn, potatoes, cabbage, parsley, carrots	Several different fields and lysimeter experiments irrigated with different water sources	Levels of several the targeted ARGs were high in several irrigation water samples, but below limits of detection on irrigated plants, and in general, there was almost no correlation between ARG abundance in irrigation water and irrigated soil or crops	Marano et al. (2019)

have produced varied results due to differences in experimental design, systems tested and pathogens and crops used (Alegbeleye et al., 2018; Hirneisen et al., 2012). Bacterial and viral pathogens (e.g. *E. coli*, Hepatitis A) may internalise via the roots, however the evidence supports this happening in hydroponic growth conditions rather than plants grown in soils which often show little to no internalisation (Hirneisen et al., 2012). Interestingly, contrasting evidence indicates that ARB and ARGs persist poorly in soil and on plants irrigated with TWW, suggesting that "ecological boundaries" associated with biotic and abiotic factors restrict spread of antibiotic resistance elements from TWW to irrigated plants (Marano et al., 2019). In addition to pathogens, ARGs have also been detected in agricultural crops following irrigation with treated wastewater. For example, ARGs between (−0.9)—5.6 log copies/g of soil have been detected in tomato (*Lycopersicon esculentum)*, beans (*Vicia faba* L) and lettuce (*Lactuca sativa*) with higher concentrations of *sul1, tet*M, *mec*A, *qnr*S *and* bla_{TEM} typically in the crop roots in comparison to the leaves or fruit (Leiva et al., 2021).

One of the major human health concerns relating to resource reuse is exposure to pathogenic organisms (Adegoke et al., 2018).

AMR is disseminated by direct contamination of produce by resistant pathogens in the irrigation water, or by indirect contamination from non-pathogenic bacteria that harbour mobile ARGs, that can be transferred to pathogens present in soil, plant or human microbiomes. Treated and untreated wastewater can harbour microbial pathogens and AMR genes that can potentially be transferred to humans through ingestion of irrigated produce (Piña et al., 2020). Whilst the vast majority of data collected to date suggests minimal risks of pathogen and AMR-associated contamination of produce in regions that irrigate with high quality (secondary and tertiary) wastewater, a recent study highlighted the presence of "under the radar" elements that are only identified following enrichment of treated wastewater irrigated soils in pathogen-inducing media (Marano et al., 2021).

Future research should characterize the abundance and associated risks of non-bacterial, potentially resistant pathogens, such as viral, fungal and parasitic microorganisms that may also be transmitted via TWW through the agro-ecosystem. For example, antifungal resistant yeasts and moulds have been isolated from TWW alongside high concentrations of azoles (Assress et al., 2021, 2019a), which may further enrich antifungal resistant pathogens in wastewater or in the field where this treated waste may be applied. However, it is unclear whether these concentrations are selective given the limited research into minimal selective concentrations of antifungals (Stevenson et al., 2022). Specific focus should also be placed on newly emerging parasitic protists (Durigan et al., 2024) and viruses (Corpuz et al., 2020), have also been found in TWW but their impacts and risks following contamination of agroecosystems following wastewater irrigation have received little research attention to date.

In water-stressed low income regions raw (untreated or minimally treated) sewage is commonly used for irrigation, and it is estimated that globally this practice exceeds TWW irrigation by 30-fold (Drechsel et al., 2022). Loads and risks of the above-mentioned pathogens in irrigated crops are significantly higher than for TWW irrigation, yet they are minimally addressed in the literature (Heyde et al., 2025). Developing practical measures to control these pathogens in the water cycle, as well as in irrigated crops and soil, is crucial for safeguarding public health on both local and global scales. This effort must adopt a one-health perspective, taking into account the interconnected movement of people and produce worldwide.

3. Co-contaminant exposure in the environment: observed accumulation and toxicity

Many contaminants co-occur in municipal wastewater as WWTPs serve to collect and treat wastes that contain a suite of biological and chemical contaminants. The complexity of this chemical mixture is exemplified by the fact that sanitation systems do not source segregate waste streams so contaminants are discharged into sewers from domestic areas as well as industries, and hospitals creating a complex chemical soup. The utilization of wastewater treatment products (biosolids and TWW) for agricultural purposes therefore presents a scenario where ECs co-exist and are likely to interact with each other which has the potential to exacerbate their individual effects. To date our understanding of co-contaminant mixtures in agricultural systems largely stems from studies that have investigated interactions with contaminants from the same contaminant class. For example, exposure of lettuce seeds (*Lactuca sativa)* to individual pharmaceuticals (paracetamol, ibuprofen and amoxicillin) revealed adverse effects following short-term exposure (i.e. acute toxicity) but exposure to mixtures of the same chemicals did not result in the same inhibitory effect, suggesting antagonistic effects (Rede et al., 2024). Comparatively, mixtures of pesticides including azole fungicides and cholinesterase inhibiting pesticides can act synergistically whereby they enhance the effect of other chemicals, so that they jointly exert a larger effect than predicted (Cedergreen, 2014). Even though synergistic effects for pesticides typically only comprise a small number of tested mixture combinations (approximately 5 %) (Boobis et al., 2011; Cedergreen et al., 2008), if these 5 % are combinations often co-occur in humans and the environment, they might nonetheless be of quantitative importance.

Whilst limited data currently exists, results also reveal that interactions of contaminants from different chemical classes can also result in effects in non-target species different to those observed for individual contaminants (Table 7). A recent review of synergistic interactions of environmentally relevant chemical toxicants from distinct chemical classes revealed that synergy occurred in 7 %, 3 % and 26 % of the 194, 21 and 136 binary pesticide, metal and antifoulant mixtures (Cedergreen, 2014). Furthermore, findings from co-contaminant mixture effects in aquatic systems (Spurgeon et al., 2022) and terrestrial species such as bees and enchytraeids (Bart et al., 2022; Robinson et al., 2017) demonstrate the need consider mixture effects across contaminant classes.

Increasing research has also evidenced the role of microparticles as a vector to transport other contaminants in the environment since many

Table 7
Existing evidence of co-contaminant interactions for per- and polyfluoroalkyl substances (PFAS), plastics and fibres, engineered nanomaterials (ENM), pesticides, pharmaceuticals and antibiotic resistant genes (ARG) and bacteria (ARB) + limited data to support potential for contaminant interactions ++ existing data demonstrates contaminant interactions in the environment+++ existing data demonstrates contaminant interactions and implications for human health.

			Contaminant					
			Particulate		Chemical			Biological
			Plastics/fibres	ENMs	PFAS	Pesticides	Pharmac-euticals	ARB/ARG
Contaminant	Particulate	Plastics/Fibres			++	++	++	++
		ENMs		++				
	Chemical	PFAS			++			
		Pesticides				++	+	+++
		Pharmaceuticals					++	+++
	Biological	ARB/ARG						+++

polymers (PET, PS, PP, and PE) are naturally hydrophobic materials, which facilitates interactions with organic contaminants such as pharmaceuticals and personal care products (Atugoda et al., 2021) and PFAS (Llorca et al., 2018). The presence of microplastics has been shown to result in higher PFAS bioconcentration factors but lower bioconcentration factors for pesticides terbuthylazine and chlorpyrifos when co-exposed with microplastics (Álvarez-Ruiz et al., 2021). Further research by Rubin and Zucker (2022) demonstrated that triclosan-sorbed microplastics had an order of magnitude higher toxicity toward Caco-2 cells (used as a model of the intestinal epithelial barrier) than pristine microplastics and that more environmentally realistic microparticles (e.g. 'weathered') have an increased adsorption capacity for organic chemicals leading to even more pronounced *in vitro* toxic effects for oxidised microplastics compared to their pristine counterparts. Ultimately, it is critical that any assessment of risk also accounts for realistic environmental exposure conditions, including sorption interactions in soil systems which are directly linked factors such as pH, the presence of organic matter, and ionic strength and alter the bioavailable fraction of the chemical available for uptake into plants.

Emerging research has started to link chemical pollutants commonly detected in TWW and biosolids such as antibiotics (Wang et al., 2020), non-antibiotic pharmaceuticals (Jiang et al., 2022), pesticides (Qiu et al., 2022) and microplastics (Perveen et al., 2023; Pham et al., 2021) with ARB and ARGs. Although research has demonstrated that these chemicals in isolation can affect resistant bacteria and ARGs, far less is known about the implications of these chemicals in combination. Microplastics can be colonised by microbial communities distinct to surrounding free-living communities (Bryant et al., 2016), which may harbour antibiotic resistance (Liu et al., 2021) and/or pathogens (Quilliam et al., 2023), though not always at greater abundances (Liu et al., 2021). Whether this is due to potentially unique properties of microplastics compared to other substrates, such as the ability antibiotics (e.g. Ji et al. (2024)) or metals (e.g. Gao et al. (2021)) to sorb to microplastic surfaces, remains unresolved. A holistic approach which aims to effectively capture the interactions between microplastics (physical), antimicrobials (chemical) and antibiotic resistance (biological) is yet to be described, and its development will be limited until significantly more data are generated on individual effects and interactions across different types of contaminants.

Current approaches to assess human and environmental health risk typically focus on ECs in isolation and assessment does not typically consider the potential for co-contaminant interaction. More work is urgently needed to understand if interactions between contaminant classes and resulting adverse effects in aquatic systems and other terrestrial species are mirrored in soil-plant systems and how they ultimately influence any potential risk to ecosystem and human health. It is also important that as a research community we do not lose sight of legacy pollutants that remain ubiquitous in the environment and could be interacting with ECs; the potential for contaminant interaction goes beyond the contaminant classes discussed in this review.

4. Call to action: identified future research needs across all contaminants

Co-contaminant effects and synergistic interactions of chemicals in mixtures pose a great concern to the environment and public health and need to be addressed. Concern stems from 1) the uncertainty as to whether we are monitoring and regulating the most harmful contaminants in the first place; and 2) whether contaminants, regulated on an individual basis and deemed "safe", present a new, uncharacterized risk when present in mixtures. By not considering combined exposure to co-contaminant mixtures, risks could be over- or underestimated. Research is urgently needed to confirm several underlying assumptions and to increase available data to make better, more informed assessments. Key priority areas are highlighted and discussed below.

1) Improved monitoring and surveillance

An improved understanding of exposure is an essential step to ensure risk is appropriately, and proportionately assessed, and accounts for unknown contaminants and the inadvertent release of chemical and biological agents. Without an accurate assessment of contaminant exposure in the agricultural environment, both top-down (whole mixture) and bottom-up (prioritized individual contaminants) approaches to co-contaminant risk assessment will fail. Existing monitoring campaigns for ECs in the agricultural environment is very geographically biased, with most studies carried out in China, Europe and North America (Khalid et al., 2018; Wang et al., 2017b) and very little published data for the rest of the world. Studies in sub-Saharan Africa and Southeast Asia largely focused on microbiological contaminants, as chemical contaminants have generally been considered to be lower priority health risks in low-income countries (Bos et al., 2009; Dickin et al., 2016). It is imperative that we have better, globally relevant exposure data to ensure the risks associated with the use of wastewater products are relevant to the scenario considered. Use of wastewater as a source of irrigation in regions with minimal or no wastewater treatment, requires accurate exposure data to develop customized technologies, policies and regulations that minimise risks, while being achievable within the context of the local infrastructure (Carter et al., 2024).

While improved monitoring of contaminants in the environment enables and enhances effective regulation, it is also considered an unscalable and costly task. Research needs to capitalize on rapidly developing capability in technologies such as High-Resolution Mass Spectrometry (HRMS) for a broader, more comprehensive, assessment of chemicals including transformation products in resources destined for reuse. The first untargeted EC study in the Tula Valley in Mexico recently detected over one hundred pollutants never previously measured in the area, including diclofenac, carbamazepine metabolites, and the herbicide fomesafen (Garduño-Jiménez et al., 2023). Comparatively 160 PFAS from 42 classes were detected from target screening and homologue-based nontarget screening in organic waste products destined for land application in France. Approaches such as these show that vast numbers of contaminants are present in these waste products which need to be considered in the context of co-contaminant mixtures. Methodologies developed for sensing ECs present in environmental samples such as fluorescence spectroscopy (a non-contact method) which has been proven for the detection of pharmaceuticals, pesticides, and urban contaminants (e.g. polycyclic aromatic hydrocarbons) (Wasswa et al., 2019) also offer promise to increase surveillance capacity by overcoming existing issues associated with the submersion of sensors in environmental media. However, further advancements with sensing technologies are required to increase sensitivity and selectivity of measurements, to make them applicable to trace analysis in complex environmental matrices. Linked to this is the use of artificial intelligence (AI) to facilitate routine monitoring of parameters such as electrical conductivity, pH, turbidity, temperature and oxidation, which can be used as proxies for other contaminants not easily measurable in the field (e.g. organic loads, metals) (Nallakaruppan et al., 2024; Rana et al., 2023; Shirkoohi et al., 2022). The geographical extent of such research must not only reflect the availability of these technologies. The application of these methods must be global and low cost, and their application to parts of the world with fewer research resources must integrate local knowledge to ensure sampling campaigns are representative of, and sensitive to, local circumstances. Furthermore, an alternative approach to reducing the cost and resource burden of monitoring is to be smarter in measuring only what we need to measure and identifying the risk drivers (see *Prioritization* below).

2) Prioritization

Due to the immense number and diversity of ECs in the agricultural environment there is a need to rank them in accordance with their perceived risk or based on available monitoring (i.e. occurrence) and toxicity data. Numerous attempts have been made to do this in relation to the aquatic environment but more comprehensive prioritization efforts are needed for agricultural systems (Burns et al., 2018; Zhou et al., 2019). In any case, since not all ECs can be monitored due to their enormous number, selecting representative ECs based on their occurrence, toxicity, and representativeness is crucial. Further work is needed to understand if all co-contaminants in a mixture contribute to a combined effect, to prioritize contaminants of most concern. For example as previously highlighted, interactions between microplastics, chemicals and AMR entities exist and efforts are needed to prioritize future actions by establishing if the greater risk is from chemicals, plastics, and/or resistant pathogens, or from their interaction (in which case does removing one of them mitigate the risk). Existing research offers promise in this respect by successfully identifying risk drivers in contaminant mixtures to help aid prioritization efforts. For example, in 95 % of 69 described cases where synergism was observed for pesticide mixtures, these included cholinesterase inhibitors or azole fungicides; and Spurgeon et al. (2022) established that the most toxic chemical contributed ≥20 % of the calculated mixture effect in >99 % of all measured groundwater and surface water samples.

3) Improved understanding of the 'environmental' component of exposure

As outlined in the contaminant-specific examples above, uptake and accumulation of contaminants by plants are influenced by a combination of contaminant physico-chemical properties, soil characteristics, crop parameters and biosolid/irrigation application methods. Therefore, an assessment of risk needs to be made using an environmentally relevant and realistic exposure scenario accounting for species abundance and type, as well as mixtures, transformation products and the form of the contaminant (i.e. not pristine). For example, it is expected that subsurface irrigation is a strategy that reduces the risk of microbial contamination to crops irrigated with TWW as well as contaminants such as macroparticles (Gurtler and Gibson, 2022; Helmecke et al., 2020). Consideration of source attribution will also enable an assessment of exposure that accounts for the fact that all resource types are unlikely to contribute equally for all contaminant types. An understanding of whether simultaneous exposure to all ECs occurs at the same time, or which combinations are most likely to be present at higher concentrations is also needed to elucidate when co-contaminant exposure is likely to occur, therefore increasing the likelihood of an effect. Herein, it is important to highlight that the environmental-food route is one of many potential exposure pathways that present a potential risk to human health. We need to account for the complexities of the environmental exposure scenario alongside all potential exposure routes (e.g. air/inhalation) to deliver a risk assessment that is fit for purpose.

4) Shared protocols and synthesized databases

A globally harmonized approach to monitoring, and an assessment of the risk of co-contaminants in agricultural systems is needed to avoid a duplication of efforts and to ensure consistency in adopted regulatory approaches. Specifically, an agreement on how to assess risk, i.e. which endpoints to consider and for which species would also support joined up thinking and regulation in this space, noting that exposure will be largely defined by sewer connectivity which is known to vary on a geographical basis. Initiatives such as this could be supported by the Intergovernmental Panel on Chemical Pollution (IPCC) which was formed to provide a scientifically sound, and balanced view of major chemical pollution issues, including an evaluation of different options for chemicals management. However, as highlighted in this review, for contaminant risk assessment to be truly protective, biological risks need to be integrated into initiatives such as IPCC given the links between chemical and biological entities (e.g. antibiotic use, enrichment of ARB and ARGs following antibiotic exposure). Consensus among researchers, industry and Governments on extraction protocols and analytical methodologies (including quality control and assurance) would also support such a harmonized approach to addressing co-contaminant risks and allow for easier comparability of datasets between regions and exposure scenarios. Protocols need to be open source and aligned to a synthesized database for contaminant monitoring and exposure in the agricultural environment. Shared resources such as this will better support joined up policy thinking and regulation.

5) Holistic approach to risk assessment

In response to projected increases in future resource reuse, policies are being developed to regulate ECs exposure. For example, in June 2023, new requirements for the safe reuse of TWW for agricultural irrigation were introduced across most of the European Union (Water Reuse Regulation (EU) 2020/741). However, despite a focussed effort to set minimum quality requirements, these policies still neglect to account for the presence of many known ECs discussed in this review. Policies therefore need to be revised to account for emerging hazards and risks following the use of biosolids and TWW, where there is also no consistent approach to managing ECs. However, it is important to highlight that changes or implementation of regulation need to be considered in the broader context of risks associated with not using TWW or biosolids (e.g. unsustainable extraction of freshwater resources, disposal via landfill or incineration) and be underpinned by clear evidence to support suspected adverse health effects; as presence does not necessarily result in adverse impact. We need to better link cause and potential effect and develop dose-response relationships for a wider suite of ECs in co-contaminant exposures. Risk characterisation also needs to account for scenario specific parameters, for example crop characteristics (peel on or without peel), proximity to TWW or biosolids, whether produce is cooked or eaten raw, and the method of application (e.g. surface application or drip vs. flood irrigation), which have all been shown to be important determinants of risk. An understanding of these parameters will ensure they are incorporated into assessment criteria used by industry and regulators to reduce the risks of ECs wastewater irrigated or biosolid amended products to an acceptable level.

6) Improved understanding of exposure and effects

For many ECs included in the review, permissible thresholds or safe levels of exposure do not exist. Acceptable Daily Intake (ADI) is an example of a health-based guidance value based on hazard identification and characterisation. Combined with estimates of exposure ADI can be used to assess risk. However to be truly protective, it is imperative that when an ADI is used the worst case scenario is first considered (e.g. teratogenicity) and that it accounts for individual susceptibility (e.g. fetuses, children, elderly) through the use of uncertainty factors as well as chronic exposure over a lifetime where the risk may be greater. It is widely acknowledged that a new experimental methodology to characterize chemical hazards to human health, not only to reduce the amount of animal testing, but also to assess the hazards at lower and more realistic doses. As such, a systems approach has been recommended to characterize hazards that utilizes novel tools to predict biological responses such as *in vitro* methods, -omics technologies, quantitative structure–activity relationships (QSARs), read-across, toxicokinetic (TK) modelling, thresholds of toxicological concern (TTC) approaches, adverse outcome pathways (AOPs), or integrated approach to testing and assessment (IATA). In this regard, capitalizing on advances in toxicological science, molecular biology, and computational methodologies has the potential to generate new and meaningful data for a wider range of contaminants, including data on the mechanisms underlying mixture effects that enable a better understanding of toxicity pathways and the prediction of combined adverse effects (Bopp et al., 2019).

To this end, animal embryos are extremely sensitive to adverse conditions in their surrounding environment, which can influence their growth, development and health during gestation, with consequences throughout their lifetime (Bambino and Chu, 2017; Hamdoun and Epel, 2007; Hamlin and Guillette, 2011). Embryos have therefore been used to investigate the effects of contaminants in aquatic systems (Galus et al., 2013; Huang et al., 2024; Yang et al., 2023), but this raises the question of whether effects are limited to teleost embryos, which lack an amniotic sac and are in a direct contact with the pollutant. As the manner of exposure of terrestrial embryos (reptiles, avian and mammalian), to pollutants differs greatly from that of teleost embryos, experimental settings and evaluation of effects are more challenging, leading to fewer studies compared to aquatic species. Studies have reported that contaminants can reach the hen, or the laid egg, and can impair avian embryo development (Kohl et al., 2019; Nordén et al., 2016; Wang et al., 2023). Combined with evidence concerning the susceptibility of mammalian embryos to contaminants which were transferred via maternal exposure (Bai et al., 2024; Kaushik et al., 2016; Meyyazhagan et al., 2023) this urgently warrants further investigation.

Future exposure and effects analysis needs to better integrate 'the environment' into an assessment of the potential to develop antibiotic resistance by considering the role of non-human sources. A recent modelling paper suggested that although human-human transmission was the most common route, non-human sources of antibiotic resistance (such as via the food chain) also play a role in the community carriage of antibiotic resistant *E. coli* (Mughini-Gras et al., 2019). There is some evidence that exposure to antibiotic resistance sources, particularly consumption of raw plants, can result in increased colonisation and infection in humans (Stanton et al., 2022). However, if and by how much these risks may be elevated by resource reuse in agricultural settings, and the relative risk of each (e.g. application biosolids vs TWW) has yet to be researched. Importantly, these findings are also limited to studies on antibiotic resistance in bacteria, whilst it has been highlighted above in this review that other antimicrobial resistant organisms also warrant further study.

5. Conclusion

The widespread occurrence, high diversity, and persistence of ECs in agricultural systems following TWW irrigation and biosolid application necessitate a holistic approach to consider the associated ecosystem and human health risks and enable any potential adverse effects to be managed appropriately. Improved visibility of the scale of human exposure and ecological impact will support much-needed changes in global efforts for policy-making and regulation. For both individual ECs and co-contaminant mixtures, efforts need to first define what is considered "safe" from ecosystem, agricultural and human health perspectives. It is crucial that this understanding is supported by an awareness that the systems we are focused on (i.e. health and environment) are inherently complex and dynamic as this can ultimately alter exposure and the potential for effects. An assessment of risk therefore needs to be made using an environmentally relevant and realistic exposure scenario and in the absence of data and scientific uncertainty, assessments of risk need to include appropriate uncertainty factors.

Improved monitoring and surveillance, underpinned by prioritization, are needed to identify pollutant hotspots, support the implementation of regulation and assess the effectiveness of potential mitigation measures to minimise contaminant-induced risks. Based on our fragmented understanding to date, ecosystem and human health impacts will be quite diverse, and underpinned by the presence of co-contaminants in the environment. To ensure that irrigation and nutrient demands are met in a safe and sustainable manner it is important that these new challenges are accounted for in existing policy and frameworks to avoid unnecessary duplication of efforts. A holistic and transdisciplinary approach is needed linking expertise in (amongst others) environmental chemistry, ecotoxicology microbiology, human health, plant biology, water and soil science and supported by improved global collaboration and sharing of expertise.

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Declaration of competing interest

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No data was used for the research described in the article.

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