Irrigation with treated wastewater: Potential impacts on microbial function and diversity in agricultural soils

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List of abbreviations

COD	Chemical oxygen demand
BOD	Biological oxygen demand
TSS	Total suspended solids
PAHs	Polycyclic aromatic hydrocarbons
PCBs	Polychlorinated biphenyls
UI	Treated wastewater used in unrestricted irrigation
RI	Treated wastewater used restricted irrigation
WW	Wastewater
n.a.	Not available
CFUs	Colony forming units
USDA	United States Department of Agriculture
WRB	World Reference Base for soil resources
CST	Chinese Soil Taxonomy
ASC	Australian Soil Classification
SiBCS	Brazilian Soil Classification
U	Urban
I	Industrial
st	Secondary treated
tt	Tertiary treated
NR	Not reported

Abstract

The reuse of treated wastewater could be a promising measure to attenuate the water scarcity burden. In agriculture, irrigation with wastewater may contribute to improve production yields, reduce the ecological footprint and promote socioeconomic benefits. However, it cannot be considered exempt of adverse consequences in environmental and human health. Apart from the introduction of some biological and chemical hazardous agents, the disturbance of the indigenous soil microbial communities and, thus, of vital soil functions impacting soil fertility may occur. The consequences of these disturbances are still poorly understood.

This chapter summarizes physicochemical and microbiological alterations in soil resultant from irrigation with treated wastewater that are described in scientific literature. These alterations, which involve a high complexity of variables (soil, wastewater, climate, vegetal cover) may have impacts on soil quality and productivity. In addition, possible health risks may arise, in particular through the direct or indirect contamination of the food chain with micropollutants, pathogens or antibiotic resistance determinants. The current state of art suggests that irrigation with treated wastewater may have a multitude of long-term implications on soil productivity and public health. Although further research is needed, it seems evident that the analysis of risks associated with irrigation with treated wastewater must take into account not only the quality of water, but other aspects as diverse as soil microbiota, soil type or the cultivated plant species.

Keywords

Microbial communities, Environmental contamination, Public health, Sustainable reuse

1. Introduction

The high demand of freshwater for anthropogenic activities sometimes exceeding the minimum recharge levels has been leading to the desiccation of water streams and depletion of groundwater [1]. The water stress index, defined as the ratio between total freshwater abstraction and the total annual renewal of water (volume), is a useful indicator to seek an adequate balance between available water resources and water uses. The reuse of treated wastewater has increasingly been regarded as an important measure to attenuate the water scarcity burden, promoting an adequate balance between water resources and water uses [2, 3]. This is observed for some countries with severe water stress indexes (e.g. Spain and Israel) that already have mature wastewater reuse practices [4]. Among the activities requiring freshwater resources worldwide, irrigation consumes the highest fraction (~70 %) [5]. For this reason, wastewater reuse in agriculture and landscaping has been implemented in countries such as the USA, Israel, Malta, Cyprus, France, Italy, Jordan or Spain [4, 6-8]. The reuse of treated wastewater in agriculture may contribute to improve production yields, reduce the ecological footprint and have beneficial socioeconomic implications. In the socioeconomic domain, this practice can contribute to human wellbeing through environmental protection and economic sustainability, supporting increased production with reduced costs, and fixing populations and employment in areas at risk of desertification [6, 9-11]. Additionally, it can contribute to reduce the discharges of effluents in the environment, minimizing the deterioration of freshwater ecosystems through eutrophication and algal blooms [11].

The arguments presented above make the reuse of treated wastewater inevitable, at least in some world regions. However, the associated environmental and human health risks cannot be ignored. Since wastewater results from human activities, the occurrence of chemical compounds and microorganisms that can persist even after conventional and advanced wastewater treatment may be incompatible with a reuse. For instance, the occurrence of pathogens in treated domestic wastewater is well documented [12, 13]. With different ability to survive in the environment, some of these pathogens can persist and spread after treated wastewater discharge, with the possibility of infecting new hosts by direct contact or entering the food chain [14-17]. Wastewater contains also numerous recalcitrant chemical compounds, some of which are potentially toxic, teratogenic or even carcinogenic. Many of these are not completely removed during wastewater treatment and are released with the final effluent [10, 18-24]. The awareness of the risks associated with these biological and chemical hazards has motivated the introduction of guidelines and legislation concerning the safe use of treated wastewater for irrigation and other purposes (Table 1). However, it should be noted that some adverse effects of treated wastewater reuse cannot be evaluated based on those legal recommendations.

The microbiological risks associated with the use of treated wastewater in soil irrigation include three major lines: i) the disturbance of indigenous microbial communities of soil, jeopardizing their activity and, in turn, affecting soil health and long-term fertility; ii) the introduction of phytopathogens that may cause a reduction on either the yields or the quality of the crops or other cultivated plants; iii) the introduction of human or animal pathogens or antimicrobial resistant microorganisms which can be hosted by plants, contaminating the environment and/or the food chain, with implications in environmental and human health (Figure 1). This holistic perspective of the implications of wastewater reuse involves different thematic areas such as soil microbial ecology, plant-microbe interactions and environmental-clinical microbiology. This review presents a summary of the possible direct or indirect effects of wastewater reuse on the soil microbial communities, based on studies that assessed possible alterations in soil properties after irrigation with treated wastewater. Major uncertainties, gaps of knowledge and risks associated with wastewater irrigation are discussed. The impacts of irrigation with wastewater will depend strongly on the plasticity of soil microbial communities and on the composition of wastewater. Both microbial habitats, soil and wastewater, are briefly described in the two following sections.

2. Wastewater composition

Urban raw wastewater usually comprises domestic, industrial, and sometimes storm water. Wastewater composition is normally characterized based on few standard parameters. The chemical oxygen demand (COD), biological oxygen demand (BOD), and total suspended solids (TSS) are used to express the content in organic matter. Other parameters, such as the content in different forms of N and P and electrical conductivity are commonly used to assess the availability of nutrients (NH_4-N, NO_3-N and P) and salinity, respectively. Avoiding the impossible task of surveying specific pathogens and parasites, the enumeration of indicators of faecal contamination, such as total and/or faecal coliforms and nematodes eggs, are the standard methods to assess the microbiological quality of water. A general overview of the raw wastewater composition is given in Table 1. A typical secondary treatment of urban wastewater is expected to significantly reduce the initial parameters such as BOD, TSS, total N and P contents. Nevertheless, the extent of removal depends on several factors, such as the composition of the raw wastewater and the treatment configuration and efficiency, which thus have an important influence on the characteristics of the final effluent. There is a general agreement about some standards with which treated wastewater discharge must comply and they are widely recommended with the aim of minimizing environmental and public health negative impacts. These quality criteria are the basis for the legal standards or guidelines of treated wastewater to be discharged to surface water as well as for irrigation (Table 1). However, in the majority of the countries, routine monitoring of wastewater does not include potentially harmful agents. Although at low densities, they are inevitably present in treated effluents and may have undesirable effects on environment and human health This is the reason why countries such as the USA, Mexico, Israel, Jordan, Oman or Italy, require the determination of some trace metals and/or organic contaminants [e.g., As, Pb, Mg, Cr, Cd, phenols, polycyclic aromatic hydrocarbons (PAHs), phthalates] before the discharge of treated wastewater into the environment [6, 25-28]. However, other potentially harmful agents, such as pesticides, personal care and pharmaceutical products, including antibiotic residues, are not routinely monitored. Furthermore, treated wastewater contains antibiotic resistance genes and bacteria with potential adverse effects on human heath [22, 29-38]. Although treatment reduces the microbial load, treated wastewater still contains a considerable diversity and number of chemicals and microorganisms (up to 10^6-10^7 CFU/100 mL) [39-41]. Among these, though not considered pathogenic, antibiotic resistant bacteria can also negatively impact the microbiological quality of wastewater. Moreover, given the abundance of nutrients and close contact between bacteria, the occurrence of antibiotic resistance acquisition, mainly via horizontal gene transfer, may occur either in the municipal collector or during wastewater treatment [33, 40, 42]. As an example, based on experimental data from different wastewater treatment plants, it was estimated that, irrespective of treatment type and efficiency, plant size or world region, a domestic wastewater treatment may release up to 10^9 ciprofloxacin resistant coliforms per minute to the environment, depending on the volume and flow of water [36]. Thus, even if it can be considered with adequate quality, treated wastewater contains chemical and microbiota components which may negatively impact soil quality and characteristics.

3. Soil holds rich and diverse microbial communities

Soil is considered the most complex and heterogeneous biomaterial on earth [43], holding structurally and metabolically diverse microbial communities [44, 45]. Due to such metabolic diversity, microbial communities are responsible for cycling abundant elements such as C and N (e.g., [46-53]), and less abundant, although essential, elements such as S and Fe (e.g., [46, 54-56]). Therefore, while each metabolic type of microorganisms has a key role in the recycling of elements, a well-balanced microbial community is essential for an adequate biogeochemical equilibrium of the soil. Microorganisms are also essential to the maintenance of soil structure, in particular soil aggregation [57-59]. Moreover, rhizosphere soil microorganisms play a key role in plants' development and health. Through the interaction with roots, microorganisms promote processes that are crucial for plant nutrition and growth (e.g., N_2 fixation, P solubilisation; siderophore production) and confer protection against phytopathogens [60-64]. Therefore, from both perspectives of soil quality and plant protection, the maintenance of the physiological and metabolic diversity of microorganisms can be considered as one of the most important determinants of soil fertility.

Other important functions are attributed to soil microorganisms. A good example is the biodegradation of several micropollutants which contribute to attenuate the negative impacts of xenobiotics or other noxious compounds discharged in soil (e.g., pesticides, organochlorides, PAHs, antibiotics, birth control and natural hormones) (e.g., [65-74]). Hence, due to biodegradation activity, soil microorganisms contribute to avoid the dissemination of micropollutants to the surrounding environment through surface run-off and leaching into aquifers. However, soil microbial communities may have a limited capacity to regenerate soils submitted to frequent discharges of xenobiotics (not naturally produced) or natural exogenous substances that will act as pollutants [75-78]. In general, it can be hypothesised that the long-term wastewater reuse, mainly if the minimal quality standards are not met, will have implications either on the turnover of some chemical components or on the adequate balance of microbial populations in soils. Both have adverse impacts on soil health and agriculture production.

4. Possible effects of irrigation with treated wastewater on the soil microbial communities

Structure and function of soil microbial communities are greatly influenced by a wide variety of abiotic and biotic factors, such as soil texture, pH, organic matter content, N and P inputs, presence of different types of micropollutants, land use history, agricultural management, vegetal cover, introduction of exogenous organisms, among others (e.g., [79-87]). Considering the complex composition of treated wastewater and the myriad of factors capable of affecting soil microbial communities, it is likely that irrigation with treated wastewater disturbs the soil microbiome. Such effects may be direct, through the introduction of exogenous microorganisms, or indirect through the alteration of soil physicochemical properties resulting in a change of the microbial activities and populations. Some of these effects are illustrated by case studies assessing the effect of the reuse of treated wastewater on physicochemical and microbiological soil properties (Table 2). The studies analysed are representative of different regions (e.g., Spain, India, Mexico, France, Pakistan, Italy, China, Greece, Turkey, Brazil, Australia, Senegal, Israel, USA), type of treated wastewater reused (urban, industrial or synthetic), type of soil use (e.g. golf course, land near to a wastewater treatment plan, orchard land, agricultural, horticultural, grazed pastoral soils), and history of wastewater irrigation (from 4 months to 90 years). Most of these studies aimed to evaluate the effect of treated wastewater irrigation on the soil productivity and physicochemical quality (e.g., [88-91]). Other studies assessed the potential environmental impacts of metals and antibiotics introduced in soil through wastewater irrigation (e.g., [92-96]). The approach used in the majority of the studies involved the comparison of soil characteristics when irrigated with treated wastewater and with natural freshwater. The analysed edaphic parameters were soil pH, organic matter content, exchangeable cations, Na concentration, electrical conductivity, total available P and total N content, metal and micropollutants' concentrations, including antibiotics (Table 2). The microbiological parameters included the soil biomass content, enzymatic activity, and the abundance of specific microbial groups, such as the total aerobic bacteria or fungi. Few studies focused on the diversity of specific bacterial groups, such as the ammonia oxidizing bacteria, or antibiotic resistant bacteria and their genetic determinants.

4.1 pH

Soil pH variation, either increase or decrease, may result from irrigation with treated wastewater (Table 2). Although the analysed studies did not assess alterations in the microbial communities, both increase and decrease of pH are known to have a strong influence on the soil microbial richness (number of different species) and diversity (variety of organisms) [80, 97, 98] depending on the buffer capacity of the soil. In addition, pH variation can influence the solubility of different compounds, in particular metals and ionisable organic compounds and, therefore, affect the soil chemical composition [94, 99].

4.2 Organic matter

In some studies, soil organic matter-related pools increased due to irrigation with treated wastewater (Table 2). However, through the comparison of the different studies it is suggested that the influence of wastewater irrigation on soil properties may depend on the concentration and composition of organic matter in water as well as on the soil texture [100, 101]. In either case, variations on organic matter content and the type of organic inputs will influence the indigenous microbial communities of soil [45, 86]. Indeed, in most of the case studies in which variation in the organic matter content were reported, it was also observed fluctuation in one or more microbial parameters (Table 2).

4.3 Salinization

The increase of soil electrical conductivity/salinity (i.e., water in soil) was observed in the majority of the reviewed studies (Table 2). Soil salinity may strongly affect soil structure and it is described as having negative impacts on soil microbial diversity, microbial biomass and activity. The hindering of functions related with C and N mineralization had also been described [102-106]. For these reasons, salinity may reduce soil fertility and productivity.

4.4 Nutrients and macro-elements

Wastewater has high contents of total N and P, and exchangeable cations (e.g., K, Na, Mg, Ca) [107] (Table 1). This is one of the potential beneficial aspects of irrigation with wastewater, since it may supply nutrients and macro-elements, substituting synthetic fertilization [9, 11, 108]. However, it should be noted that adverse effects can also result from the leaching of excess of available P and NO_3-N into natural waters, causing contamination [109] and eutrophication of these habitats [110]. Indeed, biological P- and N-removal technologies have been developed as a measure to reduce the impact of the introduction of these nutrients in the environment [111]. The increase of total available P content in wastewater irrigated soils was consistently reported [9, 88, 91, 93, 112, 113], with a single exception , where the reference soil is an uncultivated land with high P content [99]. In some studies, irrigation with wastewater did not affect the soil total N content [89, 114], but in others, it led to an increase [113, 115]. Simultaneously, N-related pools were also influenced by wastewater irrigation, with the increase in NO_3-N, NH_4-N or organic N reported in different studies [89, 114, 116]. Such variation on the impact of wastewater irrigation on the soil N may be due to presence of different N-forms and concentration both in water and soils. The increase in the content of total available P and NO_3-N, and the simultaneous accumulation of macro-elements in soils, may contribute to change the diversity and catabolic activity of microbial communities [83, 117-120]. Whether these variations have positive or negative impacts on soil microbiota and productivity was not clear from the analysed studies. Probably because ammonia oxidizing bacteria populations do vary in response to N inputs [118], increase in the abundance of ammonia and nitrite oxidizing bacteria was observed in soils irrigated with synthetic wastewater [114]. This is a clear example of how chemical inputs from wastewater may lead to alterations in the soil microbiota.

Wastewater irrigation influenced the abundance of exchangeable cations. However, no general trend was observed, since the abundance of exchangeable cations either decreased or increased after irrigation [91, 99, 112, 121, 122]. These observations suggest that many factors in soil and other external conditions may influence the fate of nutrients and macro-elements supplied in wastewater.

4.5 Trace metals

Given the frequent occurrence of trace metals in wastewater (Table 1), irrigation may lead to the increase of their content in soil [92-94, 96, 99, 114, 123-126]. Some of these metals, such as Fe, Zn and Cu, have a beneficial role in the functioning of biological systems when present at low concentrations [127, 128]. Others, such as Pb, Cr or Cd, may be toxic to microbes and plants, even at low concentrations. The adverse effects of metals may be aggravated by the fact that they may bioaccumulate in plants and enter the food chain [92, 94, 96, 99, 126, 129]. In soil, metals accumulation may induce changes in the soil functional activity and in the abundance and diversity of fungi and bacteria [93, 99, 123]. Some trace metals have bacteriostatic properties and may cause cross resistance against antibiotics [130]. The selective effect of metals can be inferred from the fact that higher density of metal-resistant organisms was observed in soils with increased concentration of metals due to irrigation with wastewater than in control soils [99, 123]. The phytotoxicity of some metals and the risk of metal leaching after long periods (~20 years) of soil irrigation with wastewater [96, 126] are also important negative impacts that may result from wastewater irrigation.

4.6 Organic micropollutants

The introduction of personal care and pharmaceutical products, including endocrine-disrupting chemicals (e.g. antibiotics, lipid regulator agents, anti-inflammatory drugs, cancer therapeutics, beta-blockers, contraceptives and other hormones) in the environment via wastewater irrigation is also a well described problem [10, 11, 22, 31, 36, 131-134]. Depending on the mobility of the micropollutants, different risks are posed. Highly mobile micropollutants can leach into and contaminate groundwater, while those strongly adsorbing to soil particles, such as tetracycline, can accumulate in the top soil layer [135]. The contamination of the food chain, via the uptake of some pharmaceuticals, including antibiotics, by plants is another possible consequence of wastewater irrigation [134, 136-143]. For antibiotics, the role of these pollutants in resistance acquisition and selection cannot be ignored [95, 135]. The current state of the art shows that treated wastewater is a reservoir of antibiotic-resistant bacteria, resistance genes and mobile genetic elements [13, 33, 40, 95, 144-146] and contributions in this book. Therefore, the hypothesis that irrigation of soils with treated wastewater is a route for resistance dissemination cannot be discarded. This is not a clear issue, since some contradictory results were found. While the discharge of treated wastewater in freshwater receiving environments is known to expand the levels of antibiotic resistant bacteria and resistance genes, it is not clear if irrigation with treated wastewater contributes to raise antibiotic resistance levels in the soil microbiome [95, 147, 148]. The possibility of occurrence of horizontal gene transfer between the exogenous bacteria (derived from wastewater) and the established soil or plant microbiota is, thus, a reason of concern.

Other organic micropollutants, such as surfactants, PAHs or polychlorinated biphenyls (PCBs), among others, may also accumulate in the soil due to long-term irrigation with wastewater. Although it is known that some micropollutants have the potential to disturb soil microbial communities [149, 150], to the best of our knowledge, studies assessing such effects due to irrigation with wastewater are not available. This is a gap of knowledge that needs to be filled.

4.7 Microbiological parameters

Most of the analysed case studies concluded that irrigation with treated wastewater, either of urban or of industrial origin, may lead to an increase of the soil microbial biomass (Table 2) [9, 112, 115, 124, 151]. When an increase in the microbial biomass was observed, it may have been due to the supplying of additional organic C and other nutrients by wastewater [9, 124, 152]. The observed increase in the activity of different enzymes involved in the biochemical turnover of elements such as C, N and P, such as dehydrogenase, laccase, cellulase, beta-glucosidase, as well as alkaline phosphatase, hydrolase, protease and urease, corroborates this [88, 124, 153-155]. The input of organic matter due to irrigation with treated wastewater may be beneficial for soil, stimulating not only the catabolism of labile compounds but also of complex substrates. However, some adverse effects of excessive microbial growth can also be observed, for instance when biofilms cause the clogging of soil particles, affecting the hydraulic conductivity [156].

The biogeochemical activity of microbiota is considered the most important aspect of soil quality, with implications in soil fertility and quality of plants. One of the concerns related with irrigation with treated wastewater is the disturbance of the soil microbiota, which may hinder the extent and rate of biogeochemical transformations. These aspects were not clearly explored in the analysed studies, although some evidences of functional redundancy were reported. Functional redundancy means that, despite the alterations on the microbial populations, the same reactions will be undertaken, involving alternative microbial groups [53, 157]. For instance, this explains why ammonia and nitrite oxidation in soils are not affected by irrigation with wastewater [115, 157]. Nevertheless, although maintaining the normal activity, functional redundancy processes may lead to a decrease in the genetic diversity. This effect was observed for ammonia oxidizing populations after a long-term (20 years) irrigation with wastewater [115]. In general, the decrease of genetic diversity may be considered an impoverishment of soil and, thus, an undesirable effect.

5. Conclusions

Microbial communities are extremely important to assure soil quality and productivity. Both wastewater microbiological and chemical composition may have impacts on soil physicochemical properties, microbial abundance, diversity and biogeochemical activity. Although often reporting contradictory trends, the analysed case studies demonstrated changes in chemical and microbiological soil parameters due to wastewater irrigation. However, the comparison of the different studies indicates clearly that many variables influence the impact of irrigation with treated wastewater on soil. Whereas no clear predictions are possible at the moment, it seems clear that soil quality and productivity may be affected by long-term use of treated wastewater for irrigation. The factors conditioning the possible impacts may vary among different ecosystems, and there is always a degree of uncertainty regarding the preferential target populations/functional activities or the interplay between different variables. Multidisciplinary studies involving the characterization of the system wastewater-soil-plant as a whole are necessary, supporting a deeper understanding of the impacts of irrigation with wastewater. If these studies are not possible, at least in the short term, then the precautionary principle should be applied.

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Table 1. Overview of physicochemical and biological properties of urban raw wastewater and the legal standards or guidelines for treated wastewater used in unrestricted (UI) and restricted irrigation (RI) (units, mg/L, unless indicated).

	Parameter	**Raw WW**[a]	**Treated WW (UI)**[c]	**Treated WW (RI)**[c]
Physicochemical	Chemical oxygen demand (COD)	500-1200	10-200	60-500
	Biological oxygen demand (BOD)	230-560	10-200	10-300
	Total N	30-100	5-45	10-70
	NH_4- N	20-75	n.a.	n.a.
	Organic N	10-25	n.a.	n.a.
	NO_3-N + NO_2-N	0.1-0.5	n.a.	n.a.
	Total Kjeldahl N	30-100	n.a.	n.a.
	Total P	6-25	2-30	30
	Ortho-P	4-15	n.a.	n.a.
	Organic P	2-10	n.a.	n.a.
	Total suspended solids (TSS)	250-600	10-60	30-150
	pH	7-8	4.5-9.5	5.5-9
	Electrical conductivity (mS/m)	70-120	100-300	270
	Na adsorption ratio	n.a.	8-10	9-10
	As	n.a.	0.02-0.10	0.02-0.10
	Cl	200-600	250-350	250-350
	Cd	1-4	0.005-0.010	0.005-0.010
	Cr	10-40	0.1-0.2	0.1-0.2
	Cu	30-100	1.0-0.2	1.0-0.2
	Pb	25-80	0.1-5.0	0.1-5.0
	Mg	1-3	0.001-0.002	0.001-0.002
	Ni	10-40	0.2	0.2
	Zn	100-300	0.5-5.0	0.5-5.0
	Phenol	0.02-0.10	0.10	0.10
	PAHs	0.5-2.5	n.a.	n.a.
	Phthalates	0.1-0.3	n.a.	n.a.
Biological	Faecal coliforms (CFU/100 mL)	10^6 [b]	$0 - 2x10^4$	$2x10^2 - 4x10^4$
	Nematode eggs (no./L)	n.a.	0.1-1	0.1-1

n.a. not available; CFUs, colony forming units

The values are from: [a] [107]; [b] [39]; [c] Values of legal standards from: [6, 25-28, 158-165].

Table 2. Case studies of potential impacts of irrigation with treated wastewater.

Main alterations in physicochemical or microbiological soil parameters when irrigation with treated wastewater was compared with freshwater irrigation.

Wastewater origin*	**Soil description* / culture / period of irrigation (years) / country**	**Physicochemical changes**	**Microbiological changes**	**Reference**
U, st	Calcisols WRB / alfalfa, maize, barley, oats / >20 / Spain	↑ pH, water-soluble organic C, total available P	↑ microbial biomass, activity of beta-glucosidase, alkaline phosphatase	[9]
I (dairy)	Chromasols and Tenosols ASC/ grazed pastoral / > 60 / Australia	↑ pH; ≡ total organic C, total N; ↓ C/N ratio; ↑ total available P, exchangeable Na, K, electrical conductivity	↑ microbial biomass C and N, soil basal respiration; ≡ metabolic quotient	[112]
U (flooding)	Typic Haplustand USDA / hazel orchard/ 20 / Italy	↑ pH, total organic C, active soil C resources, total N	↑ microbial biomass C, basal and substrate-induced respiration; ↓ genetic diversity of the ammonia-oxidizing bacteria	[115]
U	Xerofluvent USDA / grape crop / 2 / Spain	↑ pH; ≡ total organic C; ↑ total available P, electrical conductivity; ≡ cation exchange capacity, water holding capacity, aggregate stability	≡ activity of phosphatase, urease, beta-glucosidase	[91]
	Xerorthent USDA / grape crop / 20 / Spain	↓ pH; ↑ total organic C, total available P, cation exchange capacity, water holding capacity; ≡ electrical conductivity; ↓ aggregate stability	↑ activity of phosphatase, urease; ↓ activity of beta-glucosidase	
	Xerofluvent USDA / "green filter" / 20 / Spain	↓ pH; ↑ total organic C, total available P, cation exchange capacity, electrical conductivity, aggregate stability; ≡ water holding capacity	↑ activity of phosphatase, urease, beta-glucosidase	
	Xerorthent USDA / orange-tree orchard / 40 / Spain	↓ pH; ↑ total organic C, electrical conductivity, total available P; ≡ cation exchange capacity, water holding capacity, aggregate stability	↑ activity of phosphatase; ≡ activity of urease, beta−glucosidase	
U, st	Loamy fine sand texture / alfalfa hay, sudan grass, and winter grains / 3, 8, 20 / USA	↓ pH; ↑ organic matter content, electrical conductivity, salinity, metals (Cr, Cu, Ni and Zn)	NR	[126]
U, st	Argosols and Cambosols CST / cereals and vegetables / > 40 / China	≡ pH; ↑ humic acids, metals (Cd, Cr, Cu, Ni Pb, Zn)	NR	[92]
U, st	Fine texture / forage crops / 2, 5, 10 / Jordan	≡ pH; ↑ organic matter content, total N, total available P, K, salinity; ≡ metals (Cu, Pb, Cd)	NR	[166]
U	NR / barley, corn, cotton, alfalfa, sorghum / 80 /USA	≡ pH; ↑ soil compaction, ↓ Mg; ≡ total available P, electrical conductivity, metals (Zn)	NR	[122]
U, st	Fine clay and silt loam texture / corn / NR / China	↑ total organic C, total N, total available P	NR	[113]

Table 2. (Cont.)

Wastewater origin*	**Soil description* / culture / period of irrigation (years) / country**	**Physicochemical changes**	**Microbiological changes**	**Reference**
U, st	Xerorthent USDA / orange-tree orchard / 43 / Spain	↑ total organic C, total available P	↑ activity of alkaline phosphatase, urease, dehydrogenase, protease, beta-glucosidase; ↓ arbuscular mycorrhizal fungi diversity	[88]
U	Vertisols WRB / cereals and vegetables / < 80 / Mexico	↑ total organic C, salinization, metals (Pb, Cd, Cu, Zn)	↑ microbial biomass, activity of dehydrogenase, denitrification activity; ↓ adenylate energy charge ratios	[124]
	Leptosols WRB / cereals and vegetables / < 80 / Mexico	≡ total organic C; ↑ salinization, metals (Pb, Cd, Cu, Zn)		
U	NR / cereals, millets, vegetable and fodder crops / 5 / India	↑ total organic C, metals (Fe)	NR	[94]
	NR / cereals, millets, vegetable and fodder crops / 10 / India	↑ total organic C, metals (Zn, Fe, Ni, Pb)		
	NR / cereals, millets, vegetable and fodder crops / 20 / India	↑ total organic C, metals (Zn, Cu, Fe, Ni, Pb; ↓ Mn)		
U, st	Vertic Xerofluvent USDA / maize / 0.25 / Turkey	≡ total organic C	↑ ratio C_{mic}/C_{org}; ↓ activity of dehydrogenase, urease, alkaline phosphatase, aryl sulphatase	[151]
U	Typic Haplustox USDA / sugarcane / >1 / Brazil	≡ total organic C, total N; ↑ NO_3-N	NR	[89]
U, tt	Horticultural soil / NR / 1 / France	≡ organic matter content	↑ activity of laccase, cellulase, protease, urease; ≡ functional diversity of soil microorganisms CLPP	[152]
I (textile)	Loamy texture / fodder, cereals / NR / Pakistan	↓ organic matter content, total available P, exchangeable cations; electric conductivity, total soluble salts, SO_4, NO_3-N; ↑metals (Zn, Cu, Ni, Cr)	↑ population of bacteria, vesicular arbuscular mycorrhizae, heavy metal resistant bacterial strains	[99]
U (lagoon)	Vertic Xerocrept USDA / citrus orchard / 15 / Italy	↑ organic N ,NH_4-N, NO_3-N	≡ microbial biomass C and N; ↑ activity of hydrolase, phosphatase	[116]
Synthetic wastewater with 0 or 1.5% salinity	Sandy loam texture / mangrove swamp / 0.25 /China	≡ total N; ↑ NH_4-N, NO_3-N, total available P, metals (Cu, Zn, Cd, Mn)	↑ aerobic and anaerobic bacteria, ammonia- and nitrite-oxidizing bacteria; ≡ activity of dehydrogenase, phosphatase	[114]
U	Mollic Leptosol and Eutric Vertisol WRB / maize / 5 and 90 / Mexico	↑ total available P, metals (Cr, Cu, Ni, Zn, Pb)	↓ arbuscular mycorrhizal fungi free spores irrigation 90 years	[93]
U, tt	Silty sand texture / perennial ryegrass / 3 / Spain	↑ Ca, Mg, salinization	≡ microbial abundance total aerobic bacteria	[121]
U (lagoon)	Quartzarenic Neosol SiBCS/ eucalyptus / 5 / Brazil	↑ Na, Na adsorption ratio, exchangeable Na	NR	[90]
I (factories)	Rhizosphere soil / wheat / ~10 / India	↑ metals (Fe, Cr, Zn, Pb, Ni, Cd, Cu)	↑ abundance of metal resistant *Azotobacter chroococcum* isolates	[123]

Table 2. (Cont.)

Wastewater origin*	Soil description* / culture / period of irrigation (years) / country	Physicochemical changes	Microbiological changes	Reference
I oil refinery	NR / agricultural / 12 /India	↑ metals (Fe, Ni, Zn)	≡ microbial dynamics viable counts of aerobic heterotrophs, actinomycetes, fungi and potentially asymbiotic diazotrophs	[125]
Synthetic industrial wastewater	NR / Mangrove / 0.5 / China	↑ metals (Cd, Cr, Cu, Ni, Zn)	↓ activity of alkaline phosphatase	[96]
U and I	Silty clay loam texture / crops / 50 / China	↑ endocrine-disrupting chemicals e.g. triclocarban and pharmaceuticals e.g. oxytetracycline, tetracycline	NR	[131]
U, st	Dune quartz sand / citrus orchard lysimeter / 12 / Israel	NR	≡ enumeration of antibiotic resistant bacteria, antibiotic resistance genes	[148]
	Vertisol 60% clay / avocado orchard / 12 / Israel			
	Loam 20% clay / cotton, wheat / 15 / Israel			
	Vertisol 52% clay / olive trees / 6 / Israel			
U	NR / parks / NR / China	↑ antibiotics and degradation products	↑ diversity and abundance of antibiotic resistance and integrase genes	[95]

* According to the information reported in the reference. Soil classification was used when available and indicated in parenthesis USDA, United States Department of Agriculture; WRB, World Reference Base for soil resources; CST, Chinese Soil Taxonomy; ASC, Australian Soil Classification, SiBCS, Brazilian Soil Classification

U, urban; I, industrial; st, secondary treated; tt, tertiary treated; NR, not reported; ↑ increase; ↓ decrease; ≡ no variation

Figure 1. Wastewater treatment and reuse for irrigation in agriculture: possible effects and human and environmental health implications.