Wastewater reuse in irrigation: A microbiological perspective on implications in soil fertility and human and environmental health

Cristina Becerra-Castro a,b, Ana Rita Lopes a,b, Ivone Vaz-Moreira a,b, Elisabete F. Silva c, Célia M. Manaia a,c, Olga C. Nunes b,c

a CBQF — Centro de Biotecnologia e Química Fina — Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Arquiteto Lobão Vital, 4202-401 Porto, Portugal
b LEPABE, Laboratório de Engenharia de Processos, Ambiente, Biotecnologia e Energia, Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
c Escola Superior de Tecnologia e Gestão, Instituto Politécnico de Viseu, Campus Politécnico de Repeses, 3504-510 Viseu, Portugal

Abstract

The reuse of treated wastewater, in particular for irrigation, is an increasingly common practice, encouraged by governments and official entities worldwide. Irrigation with wastewater may have implications at two different levels: alter the physicochemical and microbiological properties of the soil and/or introduce and contribute to the accumulation of chemical and biological contaminants in soil. The first may affect soil productivity and fertility; the second may pose serious risks to the human and environmental health. The sustainable wastewater reuse in agriculture should prevent both types of effects, requiring a holistic and integrated risk assessment. In this article we critically review possible effects of irrigation with treated wastewater, with special emphasis on soil microbiota. The maintenance of a rich and diversified autochthonous soil microbiota and the use of treated wastewater with minimal levels of potential soil contaminants are proposed as sine qua non conditions to achieve a sustainable wastewater reuse for irrigation.

1. Reasons behind the use of wastewater for irrigation

Water is essential for life, and although covering approximately 70% of the terrestrial crust area, only a small fraction of the water is actually compatible with terrestrial life forms (Shiklomanov, 1993). The small fraction of freshwater, i.e., with low salt concentration (2.5% of the total stock of water in the hydrosphere), is mainly in the form of ice and permanent snow coating in the Antarctic and Arctic regions (68.7%) (Shiklomanov, 1993). One of the first reasons for the observed water scarcity is that the fraction of water available for the human consumption, in rivers and streams, lakes, reservoirs and groundwater aquifers, is not distributed uniformly around the world (Shiklomanov, 1993). As a consequence, 40% of the total land area is dry and includes climate zones classified as arid, semi-arid and dry sub-humid (FAO, 2008). Simultaneously, the increasing need of water resources is a consequence of the demographic growth, the economic
development and the improvement of living standards, climate change and pollution (FAO, 2012). It is estimated that at least in some world regions, water use has been growing more than twice faster than the human population. For instance, the total annual water withdrawal suffered a 6.3-fold increase, rising from less than 600 km³/year, at the beginning of the twentieth century, to more than 3800 km³/year by the beginning of the twenty-first century. The irrigation practices consume up to 70% of this withdrawal (FAO, 2013). Therefore, the use of freshwater has been exceeding the minimum recharge levels, leading to the desiccation of water streams and depletion of groundwater (UNDP, 2006). Water scarcity and droughts are emerging as major issues worldwide, not only in dry lands, but also in world regions where freshwater is abundant (Bixio et al., 2006; EU, 2007; FAO, 2012). For instance, half of the European countries are facing water stress (Bixio et al., 2006) with water scarcity being observed across Europe (EU, 2007). It is estimated that more than 40% of the world’s population will face water stress or scarcity within the next 50 years, a serious incentive to achieve sustainable management options of the water resources (WHO, 2006a). In this context, the reuse of treated wastewater represents a valid option, in some cases urged by the absence of viable alternatives (Niemczynowicz, 1999; WHO, 2006a). Besides the reduction in the use and abstraction of freshwater, wastewater reuse will also contribute to reduce the discharge of effluents into freshwater ecosystems (Bixio et al., 2006; Toze, 2006). This scenario makes wastewater an increasingly valuable resource rather than a waste product. Indeed, irrigation with treated wastewater is already implemented, mainly for agriculture and landscaping, in countries such as France, Italy, Spain, Cyprus, Malta, Israel, Jordan or the USA (Aquarec, 2006; EMWIS, 2007; EPA, 2012; Kalavrouziotis et al., 2013; Ndour et al., 2008; Pedrero et al., 2010). However, despite the aforementioned benefits, the reuse of wastewater involves both health and environmental risks. In this review, we will discuss some implications of the reuse of treated wastewater for irrigation purposes, giving special emphasis to microbiological aspects.

2. Framework of guidelines and policies on wastewater reuse

Wastewater is produced as a result of the multiple human activities, such as domestic, commercial and industrial uses. The quantity and composition of urban wastewater are determined by several factors, including the inhabitant’s lifestyle and standard of living, the proportion of domestic and industrial effluents, or even the design of the sewer and of the treatment systems (Henze and Comeau, 2008; Metcalf and Eddy, 2003). Treated urban wastewater is mainly composed by particulate and dissolved organic matter, and inorganic substances (e.g., N, P, K, Na, Ca, Mg, Cl and B), containing also microorganisms, including pathogens and antibiotic resistant bacteria (Henze and Comeau, 2008; Rizzo et al., 2013; Varela and Manaia, 2013). Additionally, toxic, recalcitrant and/or bioaccumulative chemicals (e.g., trace metals, xenobiotics and natural or semi-synthetic compounds) are normally present, although representing minor components, often designated as micropollutants or microcontaminants (Henze and Comeau, 2008; Metcalf and Eddy, 2003). Given such complexity, the detailed chemical and biological characterisation of treated wastewater is essential to assess its quality, being always difficult to fully predict the effects that may arise from its reuse.

The recommendations on wastewater reuse established by the State of California, the World Health Organisation (WHO) and the US Environmental Protection Agency (EPA) (Aquarec, 2006;
EPA, 2012; WHO, 2006a) constitute the background of most of the legal guidelines proposed in countries such as the USA, Portugal, Spain, Italy, Cyprus, France, Australia, Israel, Jordan, Kuwait, Oman, Saudi Arabia, and China (Table 1). Compliance with these regulatory frameworks requires the analysis of the treated wastewater prior to its reuse. Although the reviewed guidelines and policies cover different applications for the wastewater reuse (e.g. irrigation, groundwater recharge, impoundments and industrial reuse) we will focus our discussion on the use of wastewater for irrigation purposes.

In general, standards are based on the evaluation of physicochemical and microbiological parameters. A summary of the main parameters considered in different guidelines and policies is given in Table 1. Several countries (e.g. the USA, Spain, Jordan) establish different threshold values in function of the type of irrigated crops. For raw-consumed food crops the recommended values are generally stricter than for other crops that will be further processed (processed food) or used as pasture or energy crops. On the other hand, some countries, such as Italy or Israel do not define irrigation categories, and hence the established threshold values should be accomplished regardless the intended use for the irrigated crops (Table 1).

Physicochemical characterisation of wastewater includes the evaluation of several properties such as turbidity [NepheIometric Turbidity Units (NTU) or suspended solids (SS)], acidity (pH), salinity [electrical conductivity (EC), sodium absorption rate (SAR)], organic load [biological oxygen demand (BOD) or chemical oxygen demand (COD)], and nutrients [total N and/or NO₃⁻ and P in form of PO₄³⁻– phosphate] (Table 1). In addition to these parameters, some regulations (e.g. the USA, Italy, Mexico, Oman) require or recommend the determination of potentially toxic agents such as metals or organic contaminants (Table 1). The microbiological characterisation of wastewater is mainly focused on the presence of potential human pathogens and parasites, and is generally based on the enumeration of faecal indicators and nematode eggs (Table 1). In general, the guidelines aim at preventing potential effects on soil productivity and fertility, due to disturbance of physicochemical properties of soil, and possible risks for human health, through the presence of toxic compounds and pathogens (Aquarec, 2006; EPA, 2012; WHO, 2006a). However, the impact of wastewater irrigation on soil ecosystem services, which relies on an adequate equilibrium of diversity and activity of soil microbiota, crucial for soil health (Anderson, 2003; Torsvik and Ovreas, 2002), may be not properly covered by the available guidelines. Another relevant omission refers to the risks of introduction of biological contaminants, such as pathogens and antibiotic resistance genes, through the transmission pathways environment–plants–humans.

Much attention has been given to the potential of different wastewater treatment technologies to overcome the emerging challenges, as the removal of novel classes of contaminants or the sustainable reuse of wastewater for irrigation. In particular, pharmaceutical products, pesticides and disinfectants are foresighted in most of the discussions around wastewater treatment and quality (Michael et al., 2013; Pal et al., 2014; Rivera-Utrilla et al., 2013). A recent literature review by Norton-Brandão et al. (2013) offers a comprehensive overview of the technologies commonly used to treat wastewater reused for irrigation, making a comparison based on the parameter’s salinity, pathogens, nutrients and heavy metals. The use of technologies based on sedimentation, filtration or disinfection processes, such as chlorine dioxide, UV, ozone or TiO₂ seems adequate for wastewater treatment for irrigation, depending on the
raw wastewater quality and application demands (Norton-Brandão et al., 2013). Regarding the removal of microorganisms, membrane bioreactor (MBR) systems show high efficiency, meeting also other relevant objectives such as the removal of heavy metals. Other processes such as constructed wetlands, ponds and disinfection oxidants may also offer good removal rates of microorganisms, although may be not so efficient on the achievement of adequate levels of other parameters, in particular salinity (Norton-Brandão et al., 2013). The choice of the best methods for the wastewater treatments must represent a compromise between cost effectivity and the production of water with adequate quality for irrigation. In this respect, nowadays, binding quality criteria should include also the absence of emerging contaminants as pharmaceuticals or antibiotic resistant bacteria. Since the implementation and maintenance costs and the environmental impacts cannot be ignored, some times it may be challenging to achieve an ideal compromise.

3. Influence of wastewater irrigation on soil microbial communities

Soil microbial communities are established on basis of a complex net- work of interrelations between abiotic (physical and chemical soil properties) and biotic factors (macro- and microbiological soil components). The impact of wastewater on soil microbial communities is supposed to depend on direct inputs of exogenous microbiota, which, in an improbable worst case scenario, would lead to the elimination of autochthonous microorganisms by competition. In addition, and probably not less relevant, are the indirect effects produced by wastewater, which may contribute to change the physicochemical soil properties and, there- fore, induce microbial community disturbances. Both types of impact are almost uncharacterized, constituting deep gaps of knowledge. To the best of our knowledge, up to now few studies have applied commonly used methodological approaches to assess microbial community structure and activity on the evaluation of effects of wastewater irrigation on the soil microbiota (Table 2). However, nowadays, some methodological limitations are becoming easier to overcome thanks to the multiple applications of the next generation sequencing methods. In the next sections the direct and indirect effects that wastewater irrigation may have on soil microbiota and, therefore, on its properties will be discussed.

3.1. Physicochemical soil properties versus soil microbiota

Several physicochemical parameters, such as pH or organic matter content, have been shown to influence both abiotic and biotic soil parameters (Table 3). Because wastewater irrigation has the potential to cause variation in those physicochemical parameters, possible effects on soil properties and microbiota will be discussed in the next sections.
3.1.1. pH

Soil pH influences the availability of nutrients and metals, the cation exchange capacity, as well as the mineralisation of organic matter (Table 3). The increase of soil pH was observed after long-term irrigation with wastewater (4 to 60 years), in soils with distinct management regimens (arable or in grazed pastoral soils) and irrigated with different types of wastewater (secondary-treated municipal wastewater and dairy wastewater) (Table 4). In opposition, the decrease of soil pH was reported in agricultural soils dedicated to lettuce, livestock fodder and orange production irrigated with wastewater for more than 15, 20 and 40 years, respectively (Table 4). Although the effects on soil microbiota due to the abovementioned pH variations were not explored in those studies, this parameter seems to be an important determinant of the richness (number of different species) and diversity (variety of organisms) of soil bacterial communities (Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2010). In a comprehensive study comparing soil bacterial communities from distinct ecosystems it was observed that communities of sites with identical pH values share similar indices of bacterial diversity and richness, irrespective of other factors such as climate conditions or edaphic properties (Fierer and Jackson, 2006). In general, soil habitats with pH values in the neutrality range tend to present a higher bacterial diversity than those more acidic or alkaline (Fierer and Jackson, 2006; Lauber et al., 2009). In contrast, fungal communities may be not so vulnerable to pH variations (Rousk et al., 2010). Variations on pH can also influence the solubility of different soil components, such as metals (Bloom, 2000; Sparks, 2003) (Table 3). The increase of free metals in soil irrigated with wastewater was related to a decrease of soil pH (Rattan et al., 2005). In turn, the concentration and availability of metals have the potential to affect the microbial communities (Bååth, 1989; Brookes, 1995; Chander and Brookes, 1991; Müller et al., 2002).

3.1.2. Organic matter

Soil organic matter is essential as nutrient reservoir and in soil structure. Through the formation and stabilisation of aggregates, organic matter content contributes for the capacity of soil to retain water, affecting the drainage properties and resistance to compaction. Organic matter is also a reservoir of nutrients (such as N, P and S) important for plant growth, providing also a substantial part of the soil’s cation exchange capacity (CEC) (Baldock and Nelson, 2000; Powlson et al., 2013; Sparks, 2003) (Table 3). In this way, organic matter content is of vital importance for soil fertility.

The organic matter-related pools are amongst the soil properties most affected by wastewater irrigation, as has been suggested by different studies reporting an increase of total organic C and/or N in irrigated soil (Table 4). Depending on the type of organic matter inputs, an increase in the organic matter availability may promote the selection of specific bacterial populations (Sun et al., 2014; Torsvik and Ovreas, 2002). For instance, the supplying of easily metabolisable nutrients may contribute to select bacterial groups with high growth rates, such as members of the classes Alpha- and Beta-Proteobacteria (Smit et al., 2001). Effects on soil aggregate stability and water retention capacity due to inputs of organic matter from irrigation with wastewater has been described (Levy et al., 2013; Vogeler, 2008). However, these effects may depend on the concentration and composition of organic matter and on
soil texture. While in loamy sand soils wastewater irrigation led to an increase in the aggregate stability, in sandy clay and clay soils resulted in a decrease (Levy et al., 2012). The characteristics of the soil aggregates are very important, since 40-70% of the soil bacteria are associated with stable microaggregates and clay particles with sizes lower than 20 μm (Ranjard and Richaume, 2001). Therefore, the interference on the formation of soil aggregates due to wastewater irrigation is expected to modify soil microhabitats and, hence, influence the soil microbial communities (Torsvik and Ovreas, 2002). In general, the increase of soil organic matter was associated with a positive effect on soil structure and water retention (Baldock and Nelson, 2000; Sparks, 2003). However, this is not a general rule, and the opposite effect may be observed. The coating of soil particles with organic matter or even microbial biofilms can, in some circumstances, promote the hydrophobicity of the soil and therefore the water repellence (Doerr et al., 2000; Nadav et al., 2013; Tarchitzky et al., 2007). The complexity of implications of wastewater irrigation limits the establishment of possible cause-effect relationships, and the supplying of organic matter is of this a good example. Although most of the studies report an increase on organic matter due to wastewater irrigation, some did not detect significant variations but still reported alterations on microbiological and biochemical parameters (Table 4). The influence of organic matter supply on the microbiota is suggested by the fact that wastewater irrigation was sometimes associated with an increase in soil microbial biomass and in soil enzyme activities (Table 4). Examples are the activities of enzymes such as dehydrogenase, laccase, cellulase, protease and urease (Chevremont et al., 2013; Friedel et al., 2000; Liu and Haynes, 2011; Morugán-Coronado et al., 2013). These effects are probably due to the stimulation of microbial growth and activity, boosted by the organic matter and nutrients supplied by wastewater (Adrover et al., 2012; Chevremont et al., 2013; Friedel et al., 2000; Liu and Haynes, 2011; Morugán-Coronado et al., 2013; Tam, 1998).

### 3.1.3. Nitrogen, phosphorus and other plant nutrients

The availability of plant nutrients such as N, P or K is essential for plant growth (Haygarth et al., 2013; White and Greenwood, 2013). One of the advantages of wastewater irrigation is the supplying of nutrients that may be sufficient to replace, or at least to reduce, the use of synthetic fertilizers in agriculture (Adrover et al., 2012; Fatta-Kassinos et al., 2011; Toze, 2006). The fertilizing potential of the wastewater (assuming an application rate of 5000 m³/ha year) can reach annual values as high as 250 kg/ha for N, 50 kg/ha for P and 150 kg/ha for K (Pescod, 1992). In addition, wastewater can also be a source of other macronutrients and micronutrients such as Ca, Mg, B, Mg, Fe, Mn or Zn (Henze and Comeau, 2008). Indeed, irrigation with wastewater has been shown to significantly increase the yields of non-edible crops such as lemon grass or rosebushes (Lal et al., 2013; Marinho et al., 2013), and to have a positive impact on oil’s quality recovered from sunflower and castor crops (Tsoutous et al., 2013).

The increase of different N forms, i.e. NO₃-N, NH₄-N or total N, has been observed
after wastewater irrigation of orchards (citrus or hazel), sugarcane, corn, forage crops and mangrove for less than 1 year up to 20 years (Table 4). Identically, long term wastewater irrigation contributes to increase the available P (Table 4). Both N and P contents may impact soil microbial communities, in particular the activity associated with the cycling of these elements. Tam (1998) observed a concomitant increase of inorganic forms of N (NO$_3^-$-N and NH$_4^+$-N) and of ammonia and nitrite-oxidizing bacterial counts in wastewater irrigated soils. Similar findings were reported by Habteselassie et al. (2013) when N sources, from different origins including dairy wastes, were added to agricultural soils. N availability is also reported as able to interfere, either increasing or decreasing the bacterial richness. Gelsomino et al. (2006) reported that the increase of total N observed in soils flooded with wastewater was concomitant with a decrease in the genetic diversity of ammonia-oxidizing bacteria when compared to controls. However, the addition of ammonium nitrate to soils was shown to increase the bacterial richness (Turlapati et al., 2013). Friedel et al. (2000) studying a soil irrigated with wastewater for more than 18 years demonstrated that denitrifying activity was stimulated comparing to those under rainfed agriculture. This difference was probably due to the availability of NO$_3^-$ released as product of the activity ammonia and nitrite-oxidizing bacterial groups.

Despite the possible benefits for crop productivity, the excessive provision of nutrients in soil may have adverse effects. Nutrients such as available P and nitrate may be leached into the surface and groundwater, causing eutrophication or toxicity in other habitats (Candela et al., 2007; Knobloch et al., 2000; Wu, 1999) (Table 3). The excess of nutrients can also disturb the autochthonous soil microbial communities. For example, the accumulation of inorganic-N in soils may affect the microbial catabolic activity, in particular the biodegradation of recalcitrant carbon compounds present in soil (DeForest et al., 2004; Ramírez et al., 2012).

3.1.4. Salinity

Wastewater contains higher concentrations of dissolved inorganic substances, including soluble salts, than freshwater. Therefore, wastewater irrigation may promote soil salinisation (increase of soluble salts concentration) or sodification (increase of sodium ions relative to other cations). In turn, salinisation and sodification are also associated with the increase of electrical conductivity. These are the most commonly reported negative effects caused by wastewater irrigation (EPA, 2012; Pescod, 1992; WHO, 2006a) (Table 4). Excessive soil salinity imposes hyperosmotic, oxidative stress and ion toxicity, constituting a limiting factor for plant growth, development and productivity (Levy and Tai, 2013; Ngara et al., 2012). On the other hand, sodification affects negatively the stability of soil aggregates and soil structure, leading to an increase of soil compaction, loss of soil permeability and reduction of hydraulic conductivity (Sparks, 2003) (Table 3). These factors will interfere not only with plant growth but also with soil microbiota. Effects on microbial communities are mainly related with the alteration of the soil structure and with the decrease of osmotic potential (Chowdhury et al., 2011; Sarig et al., 1993; Torsvik and Ovreas, 2002; Wong et al., 2008). Increase in soil salinity has been shown to reduce fungal and bacterial counts (Omar et al., 1994; Pankhurst et al., 2001), as well as, to reduce microbial diversity and microbial biomass (Ke et al., 2013; Tripathi et al., 2006). Rietz and Haynes
(2003) related salinity and sodicity with increased levels of microbial stress and a reduction on the metabolical efficiency of the microbial community. Indeed, soil salinity seems to influence the C and N mineralization, and the retardation of nitrification (Azam and Ifzal, 2006; Sarig et al., 1993).

3.1.5 Contaminants

Wastewater transports different types of contaminants (e.g., metals, organic micropollutants), which through irrigation may accumulate in soil. These contaminants may diffuse or propagate to the surrounding environment and in soil may hinder its fertility and/or disturb the microbial communities (Table 3). The incapacity of the soil ecosystem to eliminate contaminants supplied by biosolids or water has been designated terraccumulation (Rooklidge, 2004) (Table 3). Metals such as Fe, Cr, Zn, Pb, Ni, Cd and Cu, abundant in wastewater, are on the top list of potential contaminants accumulating due to wastewater irrigation (Table 4). Besides the potential phytotoxicity and consequent effects on plant growth and/or contamination, metals may also disturb the au- tochthonous microbial communities (e.g. reduction of microbial bio- mass or alteration of the community structure, Table 3). In addition, microbial functions impaired by metal contamination, include C and N mineralization, soil enzyme activity and litter decomposition (Bååth, 1989; Brookes, 1995; Chander and Brookes, 1991; Müller et al., 2002) (Table3). Some studies reporting metal accumulation in soil after irrigation with wastewater related this accumulation with the reduction of alkaline phosphatase activity and soil ATP content, the suppression of the sporulation and diversity alteration of arbuscular mycorrhizal fungi and the emergence of metal resistance in bacteria (Faryal et al., 2007; Ortega-Larroceaeal., 2001; Yim and Tam, 1999). Leaching is an- other possible consequence of metal accumulation in soils, mainly after long periods (~20 years) of irrigation with wastewater (Xu et al., 2010). Moreover, accumulated metals may interact synergistically with other contaminants, such as antibiotics, exacerbating their potential effects (Kong et al., 2006; Peltier et al., 2010). Boron is a semi-metallic element and also a pollutant commonly present in wastewater (EPA, 2012; WHO, 2006a). Besides its toxicity to human and plants (Nable et al., 1997; Parks and Edwards, 2005), B may also affect microbial communities, although the reported effects due to its excess in soils are scarce in literature and commonly associated with effects of soil salinity. Nevertheless, B has been shown to reduce bacterial diversity and dehydrogenase activity of soils (Ibekwe et al., 2010; Khan et al., 2012; Nelson and Mele, 2007).

Organic contaminants transported by wastewater, such as phenolic compounds, surfactants, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and pharmaceutical products may also accumulate in soils due to long-term irrigation with wastewater. These compounds may increase soil toxicity and represent a potential ecological risk in the soil ecosystem (Fatta-Kassinos et al., 2011; Song et al., 2006b). Potential adverse effects include also the disturbance of soil microbiota (Table 3). Phenolic compounds may promote changes in the soil microbial community structure and function, for instance through the stimulation of primary phenol degraders and trophically related populations (DeRito et al., 2005; Sinsabaugh, 2010). Surfactants, such as linear alkylbenzene sulfonate (LAS), are other common waste- water contaminants, which accumulation in soil may have adverse effects. Although these effects are not always observed and depend on the surfactant type (Abu-Zreig et al., 2003; Sklarz et al., 2013), their accumulation may generate water-repellent soils, with effects in hydraulic properties and crop
productivity (Doerr et al., 2000; Wiel-Shafran et al., 2006). In addition, changes in the structure of soil bacterial communities due to surfactant contamination have been reported (Sánchez-Peinado et al., 2010). Also the exposure to PCBs is capable of diminishing soil bacterial abundance (Correa et al., 2010), and phenanthrene, a model compound for PAHs, has negative effects on several bacterial groups, reducing soil richness and evenness (Ding et al., 2012).

Endocrine-disruptors and a myriad of pharmaceutical products (e.g. antimicrobials, personal care products, lipid regulator agents, anti-inflammatory drugs, betablockers, cancer therapeutics, contraceptives and other hormones) are contaminants of emerging concern (Chen et al., 2011; Fatta-Kassinos et al., 2011; Kinney et al., 2006; Muñoz et al., 2009; Shi et al., 2012; Thiele-Bruhn, 2003; Toze, 2006). The fate and effect of these compounds in soils will depend on several key factors, which go far behind the chemical properties of each one or of the soil characteristics. Compounds with low mobility, such as the antimicrobials ciprofloxacin, sulfamethoxazole, and carbamazepine, were shown to accumulate in soils with the irrigation period (0–100 years), in contrast with others highly mobile (diclofenac, naproxen, bezafibrate) (Chefetz et al., 2008; Dalkmann et al., 2012; Gibson et al., 2010; Kinney et al., 2006).

Antimicrobial compounds in soils may disturb the structure and activity of microbial communities (Ding and He, 2010; Kong et al., 2006; Liu et al., 2012; Müller et al., 2002) (Table 3). Indeed, disturbances on N transformation, methanogenesis, and sulphate reduction were reported (Ding and He, 2010). Oxytetracycline and sulfamethoxazole have been shown to decrease the functional diversity of soil microbial community (Kong et al., 2006; Liu et al., 2012), and tylosin, to alter the bacterial structure of soil (Müller et al., 2002). Such effects may be more pronounced for antimicrobials with high tendency to adsorb to soil particles, since adsorption promotes the concentration of the compound without loss of activity (Chander et al., 2005). Some antimicrobial compounds are known to suffer degradation in soils (Lin and Gan, 2011; Monteiro and Boxall, 2009; Xu et al., 2009; Zhang et al., 2012). However, highly mobile contaminants and/or degradation products thereof are not of less concern, since due to leaching processes can contaminate groundwater. For instance, amoxicillin degradation products were observed to contaminate groundwater in an agricultural field irrigated with wastewater (Gozlan et al., 2013).

The discussion about the effects of environmental contaminants is often oversimplified since most of the times a single compound and target function or parameter are assessed. However, contaminants seldom occur alone in wastewater and, thus, the combination of different compounds may make the prediction of possible effects, even more complex. It is arguable that in soil, antimicrobial compounds may interact with other contaminants. Indeed, it is documented that antibiotics may favour the soil adsorption of metals such as Cu and Cd, reducing their toxicity to plants (Jia et al., 2008; Wan et al., 2010). In turn, metals can also enhance the effects of antibiotics accumulation in soils (Kong et al., 2006; Peltier et al., 2010). In comparison with the individual contaminants, the combination of oxytetracycline and Cu was observed to significantly decrease the bacterial diversity, evenness and soil microbial community function (Kong et al., 2006). Last but not least, the possibility that these pollutants contribute for antibiotic resistance selection cannot be ignored (Chee-Sanford et al., 2009; Wang et al., 2014).
3.2. Effects on microbial abundance and activity

The increase of soil microbial biomass due to wastewater irrigation was observed in several studies (Table 4). As described above, this effect may be due to the provision of organic carbon, as is suggested by the simultaneous increase in the activity of dehydrogenase, a parameter generally indicative of biological oxidation of organic compounds (Alguacil et al., 2012; Elifantz et al., 2011; Frenk et al., 2014; Friedel et al., 2000) (Table 2). Through the supplying of organic matter and nutrients, soil irrigation with wastewater is expected to stimulate different organisms and metabolic pathways. The increase in the activity of some enzymes (e.g. hydrolytic, proteolytic, laccases, cellulases, phosphatase) has been reported in soils irrigated with treated wastewater (Adrover et al., 2012; Alguacil et al., 2012; Chevremont et al., 2013; Elifantz et al., 2011; Frenk et al., 2014; Friedel et al., 2000; Meli et al., 2002; Morugán-Coronado et al., 2013). It is, thus, suggested that wastewater irrigation may stimulate the activity of microorganisms involved in the biochemical balance of elements such as C, N and P. In addition, organic matter may stabilize the enzymes, which remain active in the extracellular medium, independently of the soil microbial (Trasar-Cepeda et al., 2008a). However, these modifications are not necessarily beneficial and the stimulation of the soil microbial abundance and activity may have negative impacts on soil properties. For instance, Magesan et al. (1999) observed that the bacterial growth stimulated by irrigation with wastewater led to the formation of biofilms, with the concomitant clogging of the pore spaces between particles, with implications in the soil hydraulic conductivity. Again, the complexity of the cause–effect relationships associated with wastewater irrigation is emphasized. In the literature available it is not possible to find a general agreement about the promotion of microbial abundance and activity in soils irrigated with wastewater. Indeed, either a decrease or no noticeable effects on the microbial biomass or enzymatic activity were reported by several authors (Table 4). Tam (1998) did not observe changes in phosphatase or dehydrogenase activities in mangrove soils irrigated with wastewater. Kayikcioglu (2012) reported a decrease of the activities of enzymes aryl sulfatase, dehydrogenase, urease, alkaline phosphatase and β-glucosidase in wastewater irrigated agricultural soil. The inhibition of microbial growth or activity after wastewater irrigation is probably explained by the fact that besides nutrients also contaminants, such as heavy metals, are being supplied (Kayikcioglu, 2012; Yim and Tam, 1999).

4. Autochthonous versus exogenous microbiota

4.1. Soil and wastewater bacterial diversity

Soil and wastewater have quite different characteristics, but both are inhabited by a wide diversity of bacteria. A first glance to evaluate the likelihood of disturbances in soil microbial communities due to the introduction of exogenous bacteria through irrigation with wastewater can be given by a comparative diversity assessment (Fig. 1). Most of the recent studies on the bacterial diversity of these habitats are based on culture independent methods (16S rRNA gene-PCR-DGGE, clone libraries and high-throughput sequencing methods). Although these approaches may offer a reliable overview of the bacterial community, microbial identification is only possible above the genus level, a fact that hinders a thorough comparison of the microbiota of soil and waste-water.
A summary of studies reporting the bacterial diversity of agricultural soils and treated wastewaters is shown in Fig. 1 and Table S1. Due to the lack of a consensual soil classification, the data herein reported for agricultural soils is independent of soil texture, edaphic characteristics and type of crops. The data of treated wastewater include those treated in lagoons and/or in wastewater treatment plants, treating municipal, industrial, including pharmaceutical, and/or animal wastewater.

At the phylum level, Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, Acidobacteria and Verrucomicrobia are amongst the most abundant bacterial groups thriving in both types of habitat (Fig. 1, Table S1). In contrast, some bacterial groups commonly found in treated wastewater are seldom, if ever, detected in agricultural soil. This was the case for the phyla Deinococcus-Thermus, Thermotogae, Synergistetes or for members of the orders Methylophilales, Neisseriales, Alteromonadales, Methylococcales, Thiotrichales, Vibrionales, Desulfobacterales, Erysipelotrichales, Selenomonadales, Holophagales, Opitutales, Puniceicoccales, Phycisphaerales, Planctomycetales, and Spirochaetales (Fig. 1, Table S1). Not surprisingly, some of these bacteria comprise members of the human microbiome, i.e. are commensal or pathogenic bacteria, and were detected in the human gastrointestinal or urogenital tracts (Vibrionales, Erysipelotrichales, Selenomonadales, Spirochaetales) (NIH Human Microbiome Project catalog, http://www.hmpdcc.org/catalog/). It is thus suggested that, if not outcompeted, bacteria of human origin may become part of the soil microbiome through the use of treated wastewater for irrigation. Although some bacteria inhabiting wastewater are ubiquitous and may occur in different habitats, their passage through the human body may increase the chances of acquisition of exogenous genetic material. In this respect, mobile genetic elements, antibiotic resistance and virulence genes are of special concern. For instance, members of the orders Aeromonadales, Alteromonadales, Enterobacteriales, Legionellales, Pseudomonadales, Vibrionales, Xanthomonadales, Actinomycetales, Bacillales, Clostridiales, Lactobacillales and Bacteroidales are common members of the human microbiome (NIH Human Microbiome Project catalog, http://www.hmpdcc.org/catalog/) and described as carriers of multiple determinants of antibiotic resistance (Vaz-Moreira et al., 2014).

On the other hand, the use of wastewater for irrigation may be a source of beneficial bacteria for the soils. For example, the introduction of bacteria involved in the N cycling or other with the ability to remediate soil contaminants (e.g. pesticides, heavy metals or antibiotics) may contribute for the improvement of the soil quality (Hanjra et al., 2012; Oved et al., 2001). Oved et al. (2001) reported that soils irrigated with treated wastewater were enriched with different Nitrosomonas like species. Although bacterial groups such as the ammonia and nitrite oxidizing bacteria (e.g. Nitrosomonas and Nitrobacter or Nitrospira, respectively) are not frequently detected in bacterial diversity studies of treated wastewaters (Table S1, Fig. 1), they may eventually be enriched in soils explaining the results of Oved et al. (2001).

One of the challenges concerning the microbiological impacts of wastewater irrigation is to understand if and how the introduced micro-organisms will affect the soil microbial community. These effects may be related not only with the interference that exogenous populations may have on the soil microbial community, but also with the capacity of the exogenous organisms to survive in soil and constitute a health risk to humans and livestock. To the best of our knowledge,
there are no studies reporting the fate of wastewater microorganisms introduced in soil through irrigation, neither describing their influence on the soil native microbial communities. Because plant and human health risks posed by wastewater irrigation are relevant issues, some inferences based on the literature available are the subject of the next subsections.

4.2 Risk of dissemination of pathogens through wastewater reuse

The occurrence of pathogens in treated domestic wastewater is well documented (Okoh et al., 2007; Varela and Manaia, 2013) and their transmission to humans by direct contact or through the food chain is of concern (Hussain et al., 2002; Solomon et al., 2002; Steele and Odumeru, 2004; Wachtel et al., 2002). In addition, indirect transmission pathways include the air, due to the formation of aerosols, or water, due to runoff or leaching (Hussain et al., 2002). The risks posed to humans by pathogens transmitted through wastewater irrigation are difficult to estimate, but will depend, amongst other factors, on the survival of pathogens in the environment, the infective dose, and the host immunity (Hussain et al., 2002; Shuval and Fattal, 2003). In the environment, in particular in soil, the survival of exogenous microorganisms, including pathogens, depends on factors such as the antagonism with native microbiota, moisture content, organic matter, pH, and temperature (Brandl, 2006; Shuval and Fattal, 2003). The survival periods in soil or crops for some pathogenic bacteria and parasites may vary from only few days (e.g. *Campylobacter* spp.) up to one year for the highly resistant eggs of the helminth *Ascaris* (Shuval and Fattal, 2003), or much longer periods for fungi spores (e.g. *Hemileia vastatrix*, *Puccinia melanocephala*, *Puccinia striiformis* f. sp. *tritici*) or highly resistant bacterial endospores (e.g. *Bacillus cereus*, *Clostridium botulinum* or *Clostridium perfringens*) produced by some phyto- and human pathogens, respectively (Brown and Hovmoller, 2002; Brown, 2000; Harris et al., 2003).

When considering transmission of human pathogens through the food chain, one important parameter to be considered is the type of plants produced by the irrigated soil and the method employed for irrigation. For fruit trees or vegetables cultivated on vines and not in direct contact with irrigation water, the risks of transmission may be lower than for vegetables which grow in direct contact with the soil and irrigation wastewater (Cirelli et al., 2012; Melloul et al., 2001). In addition, methods that increase the probability of direct contact between wastewater and edible part of plants (e.g., furrow irrigation) seem to promote greater contamination of plants than subsurface drip irrigation (Song et al., 2006a). However, Christou et al. (2014) reused a tertiary treated effluent for irrigation of tomato crops and did not find evidences of microbiological contamination of the edible parts.

Rhizosphere is a habitat for plant-beneficial bacteria, however it may also host potential human pathogens (Berg et al., 2005; Holden et al., 2013). Bacterial genera such as *Burkholderia*, *Enterobacter*, *Herbaspirillum*, *Ochrobactrum*, *Pseudomonas*, *Ralstonia*, *Staphylococcus* and *Stenotrophomonas* are inhabitants of plant roots also able to colonize humans (Berg et al., 2005). Besides colonizing the vegetables surface, some bacteria may live inside the plants (endophytes). A positive correlation between plant contamination and irrigation with wastewater has been described, suggesting that wastewater can be an important source of bacteria that will colonize plants (Armon et al., 1994; Howard and Hutcheson, 2003; Ibenyassine et al., 2006; Tyler and Triplett,
2008). Part of these bacteria can be transmitted to the consumers, mainly through uncooked vegetables. Opportunistic human pathogens described as endophytic bacteria include members of the genus *Staphylococcus* and family *Enterobacteriaceae* (*e.g.*, *Hafnia, Yersinia, Pantoea, Salmonella, Enterobacter, Citrobacter* and *Klebsiella*) (Markova et al., 2005; Opelt et al., 2007; Rosenblueth and Martínez-Romero, 2006; Sturz et al., 2000; Wang et al., 2006). Although some *Enterobacteriaceae* have the ability to promote plant growth (Rosenblueth and Martínez-Romero, 2006; Tyler and Triplett, 2008), risks for human health cannot be ignored. Species comprising opportunistic pathogens such as *Escherichia coli, Enterobacter cloacae* and *Klebsiella pneumoniae* were detected in vegetables after irrigation with wastewater (Al-Lahham et al., 2003; Ibenyassine et al., 2007). A good example is the enterohemorrhagic strain *E. coli* O157:H7 that can be internalized into lettuce when exposed to contaminated irrigation water or soil (Solomon et al., 2002; Wachtel et al., 2002). Moreover, because plants are privileged hosts for these bacteria, if supplied in irrigation water, they can proliferate and survive for longer periods when plants are present than in their absence (Gagliardi and Karns, 2002; Ibekwe et al., 2004; Tyler and Triplett, 2008). Another risk is that bacteria transmitted by this pathway may be inherited through plant seeds, as has been described for some endophytic bacteria (Burnett et al., 2000; Cooley et al., 2003; Guo et al., 2001; Rosenblueth and Martínez-Romero, 2006; Tyler and Triplett, 2008; Wang et al., 2006). The risks associated with the dissemination of human pathogens due to the use of treated wastewater for irrigation can be considered low (Bichai et al., 2012; Shuval and Fattal, 2003). However, a pathway of transmission, comprising treated wastewater, soil, vegetables and human consumers, can be defined and should be considered when the implications of wastewater irrigation are discussed.

Another possible adverse effect of wastewater irrigation is the introduction of phytopathogens in the soils. Although the risks of propagation of phytopathogens to soils through wastewater are scarcely addressed in literature, it is recognised that several plant pathogens, either bacteria, fungi, viruses, parasitic nematodes or oomycetes (*e.g.* *Phytophthora* and *Pythium*) are waterborne (Bush et al., 2003; Hong and Moorman, 2005). Evidences of possible risks are supported by reports involving the use of recycled freshwater in plant nurseries and greenhouses, concluding that this can be identified as an important source and vehicle for the spread of plant pathogens (Stewart-Wade, 2011). Also the cross checking of potential phytopathogens in the list of microorganisms normally occurring in treated wastewater, suggests some candidates. Bacterial phytopathogens found in irrigation systems include *Corynebacterium flaccumfaciens, Erwinia* spp., *Pseudomonas syringae, Ralstonia solanacearum* and *Xanthomonas* spp. (Hong and Moorman, 2005). Some members of these genera (*e.g.* *Pseudomonas* and *Xanthomonas*) and other plant pathogens, such as members of the genera *Acidovorax* and *Herbaspirillum* and plant viruses (families *Tobamoviridae, Geminiviridae, Nanoviridae* and the genus *Tobamovirus*) (Alhamlan et al., 2013; Fegan, 2006; Rosario et al., 2009) are also common inhabitants of treated wastewater. The scant information on the presence of phytopathogens in treated wastewater hampers decisive conclusions on the risks posed by wastewater irrigation. However, the introduction of plant pathogens through irrigation with wastewater cannot be ruled out until further studies are conducted focusing this issue.
4.3. Risks and precautions associated with antibiotic resistance

Treated wastewater contains high loads of bacteria. Most of these are of environmental origin and a non-negligible fraction (up to $10^3$ colony forming units per mL) is derived from human and animal guts (Novo and Manaia, 2010; Varela and Manaia, 2013). Inevitably, these bacteria harbour antibiotic resistance genes, frequently associated with mobile genetic elements, which means that these genes have a high potential to be propagated amongst the bacterial community (Rizzo et al., 2013; Vaz-Moreira et al., 2014). In part this situation can be attributed to the fact that although conventional secondary waste-water treatment processes can reduce the cultivable bacterial loads around 2 logarithmic cycles, it fails to reduce the prevalence of antibiotic resistance and, sometimes, can even contribute for its increase (Ferreira da Silva et al., 2006; Luczkiewicz et al., 2010; Manaia et al., 2010; Novo et al., 2013; Rizzo et al., 2013; Zhang et al., 2009).

This means that treated wastewater will contain high doses of antibiotic resistant bacteria. An estimate made for ciprofloxacin resistant coliforms, suggests that, per minute, a treatment plant will discharge $10^9$ resistant bacteria (Vaz-Moreira et al., 2014). Over the years, antibiotic resistance has accumulated in the environment, humans and other animals. In particular old antibiotics such as aminopenicillins, sulfonamides, tetracyclines or erythromycin are nowadays inactive against bacterial groups formerly susceptible to those drugs. The prevalence of resistance to these old antibiotics can reach more that 50% of some bacterial populations discharged in the final effluents of wastewater treatment plants with conventional treatment (Manaia et al., 2012; Rizzo et al., 2013). The time elapsed between the emergence of a new resistance gene in clinical settings and its detection in municipal wastewater is frighteningly short (Szczepanowski et al., 2009). For instance, bacteria resistant to last resort antibiotics mainly or exclusively used in hospitals, such as carbapenems resistant Gram-negative bacteria, vancomycin resistant enterococci (VRE), or methicillin resistant Staphylococcus aureus (MRSA) are nowadays detected in municipal wastewater worldwide (Manaia et al., 2012; Rizzo et al., 2013; Baker-Austin et al., 2006; Fluit and Schmitz, 2004; Hernández et al., 1998; Miyahara et al., 2011). The wide contamination of the environment and the food chain with these genetic determinants may represent a public health calamity. Even if no emerging resistance types occur in wastewater, the dose of bacteria resistant to currently used and old antibiotics that will be discharged in an irrigated field is very high, and the possibility that these organisms can proliferate in soils and/or plants cannot be over ruled. The fate of antibiotic resistant bacteria and resistance genes in soils after wastewater irrigation is still poorly understood. Even though some wastewater bacteria will be outcompeted in soil, two types of negative consequences can be anticipated: i) some antibiotic resistant populations can proliferate in soil or plants, behaving as invasive species; and ii) some antibiotic resistance genes can be horizontally transferred (by conjugation, transduction or transformation) from wastewater bacteria to soil or plant bacteria. Hypothetically, both can take place simultaneously. However, the studies available do not strongly support those effects. Instead, the few publications available on the subject, show some controversial findings. While Negreanu et al. (2012) and Gatica and Cytryn (2013) failed to find any evidences that antibiotic resistant bacteria from treated wastewater could compete or survive in soils after for 6–8 years of irrigation, Wang et al. (2014) reported opposite results (Table 4). Wang et al. (2014) observed higher diversity and abundance of antibiotic resistance genes in soils irrigated with wastewater.
than in a pristine soil. Furthermore, soils irrigated with wastewater presented higher abundance of the gene encoding a class 1 integrase (intI1). Confirming the hypothesis that the abundance of the gene intI1 could indicate a high potential for horizontal gene transfer in soils, Wang et al. (2014) observed significant positive correlations between that gene and genes encoding resistance for tetracycline and sulfonamide (tetG, sul1, and sul2). One of the concerns associated with the continuous discharges of antibiotic resistant bacteria in soils is the high stability that some resistance genotypes may have. Although it might be generally assumed that antibiotic resistant bacteria or their genes can only persist if they have a competitive advantage, being selected by the presence of antibiotics, it has been demonstrated that this is not the case. Indeed, even in the absence of selective pressures or at concentrations found in many natural environments antibiotic resistant bacteria can be maintained or even enriched (Andersson and Hughes, 2010; Gullberg et al., 2011; Heuer et al., 2008). In addition, antibiotic resistance genes may occur in the environment also as naked DNA. It is known that extracellular DNA easily adsorbs to the sediment matrix and organic matter, prevalent in soils, and when adsorbed, DNA may be more resistant against DNase I degradation than the free naked DNA (Crecchio and Stotzky, 1998; Lorenz and Wackernagel, 1994). Hence, the persistence of antibiotic resistance genes in the soils irrigated with wastewater will depend mainly on the ability of the host bacteria to survive and proliferate in the receiving habitat or on the ability of the native microbiota to acquire the naked DNA fragments and proliferate after the acquisition. Although it is still very difficult to predict what may be the effect of the naked DNA in the soil, it was possible to demonstrate that antibiotic resistance genes (from both intra- and extracellular DNA) could be detected in soil in high doses by quantitative PCR after 16 months of environmental exposure (Hong et al., 2013; Selvam et al., 2012). These arguments show that even if immediate impacts are not observed, long term effects of accumulation of resistant bacteria, resistance genes and antibiotic residues should not be ignored (Dalkmann et al., 2012).

The apparent controversial results on the detection of contaminant antibiotic resistance in soil due to wastewater irrigation can be attributed to several factors, which obviously include the quality of the waste- water and of the soil. However, it is important to note that the detection of soil contamination with antibiotic resistance genes and/or bacteria in soil is technically challenging. In first place, since antibiotic resistance is a natural property of bacteria that can be observed in natural communities, it is critical to define in each case the resistance back- ground naturally existing in soil in order to assess the level of contamination coming from wastewater (Cytryn, 2013; Knapp et al., 2010). On the other hand, contamination by antibiotic resistant bacteria or genes may occur at very low levels, which in spite of the potential biological impact (e.g. facilitating horizontal gene transfer) may be below the quantification limits of the techniques commonly used (e.g. qPCR of DNA extracts produced from less than one gram of soil).

Antibiotic resistance is increasingly recognised as a global threat for human health and efforts to contain the dissemination of resistance genes and resistant bacteria are urgently needed (WHO, 2014). Wastewater reuse for irrigation poses a serious risk of contamination of soils, ground- and surface water, the wild life and the food-chain. Therefore, the current state of knowledge alerts for the need to apply the precautionary principle, i.e. avoid the irrigation with wastewater containing high levels of antibiotic resistance, as those normally resultant from most conventional wastewater treatment processes. In this respect, it seems advisable to assume that advanced treatment processes may be required for a safe wastewater reuse in irrigation.
5. Conclusions

The use of wastewater for irrigation is regarded as a way to address the imbalance between water demand and water supply. However, the literature shows that irrigation with treated wastewater is not exempt of implications, some of them adverse. Alterations, such as the increase of organic matter related pools, salinity and soil accumulation of contaminants are the most commonly reported effects, with several examples being found in literature. Also, the responses of soil microbiota to wastewater irrigation are varied and include the increase of microbial biomass and activity and different types of alterations on the microbial community structure. However, the most evident outcome of the literature search is that the effects on soil microbiota are neglected in the majority of studies on irrigation with wastewater. This is a major gap in the knowledge, given the importance of soil microbiota on soil health and fertility. A critical review of the current knowledge on soil and wastewater microbiology gives unequivocal indications that wastewater irrigation, in spite of the unquestionable benefits, may have adverse impacts on both physicochemical and microbiological properties of the soil. These will influence soil fertility and productivity, raising important concerns about the sustainability of continued reuse of treated wastewater in agriculture. Studies on the effects on soil of wastewater irrigation are challenging, since direct cause–effect relationships can hardly be found. It is suggested that multi-parametric analyses and holistic studies may bring additional and reliable insights into this problem.

The risks posed for human health represent another question at the heart of any discussion on wastewater reuse. These risks cannot be accurately estimated at the moment, but cannot be ignored. The evidences reported in the literature, as well as the critical analyses on the limitations of some experimental approaches highlight the importance of the accumulation and propagation of biological contaminants in soils due to wastewater irrigation. Human and animal pathogens, phytopathogens and antibiotic resistant bacteria and their genes are important biological contaminants that can be transported by wastewater and/or be enriched in soil. Also numerous chemical contaminants, included in categories such as xenobiotics, pharmaceuticals and metals, can threaten the environmental and human health. The mixture of these contaminants may have unpredictable consequences in both environmental and human health.

For a sustainable and safe wastewater reuse, parameters affecting both soil health and safety should be included in the quality standards, avoiding the disturbance of soil properties and the dissemination of emerging chemical and biological contaminants. Simultaneously, affordable technological solutions with minimal environmental impacts must be developed in order to assure wastewater treatment processes compatible with sustainable uses.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2014.11.001.

Acknowledgements

This work was supported by the National Funds from FCT – Fundação para a Ciência e a Tecnologia through projects PEst-OE/EQB/ LA0016/2013, PEst-C/EQB/UI0511/2013 and NORTE-07-0124-FEDER-000025 and CBC grant SFRH/BPD/87152/2012, IVM grant SFRH/BPD/87360/2012, and
ARL grant SFRH/BPD/92894/2013. This Review was produced under the scope of the COST Action ES1403 - New and emerging challenges and opportunities in wastewater reuse (NEREUS).

References


Baldrian, P., Kolarik, M., Stursova, M., Kopecky, J., Valaskova, V., Vetrovsky, T., et al., 2012. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. ISME J. 6, 248–258.


European Union (EU), Brussels (COM/2007/0414).


Kuramae, E., Gamper, H., van Veen, J., Kowalchuk, G., 2011. Soil and plant factors driving the community of soil-borne microorganisms across


and enzymatic activities in the rhizosphere of *Trachypogon plumosus* Ness. in three acid sandy soils. Agric. Ecosyst. Environ. 103, 405-411.


Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: a review. Water Res. 47, 957-995.


Monteiro, S.C., Boxall, A.B.A., 2009. Factors affecting the degradation of


Real Decreto 1620/2007 de 7 de diciembre, por el que se establece el régimen jurídico de la reutilización de las aguas depuradas. Ministerio de la Presidencia, España.


Song, Y.F., Wilke, B.M., Song, X.Y., Gong, P., Zhou, Q.X., Yang, G.F., 2006b. Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and heavy metals (HMs) as well as their genotoxicity in soil after long-term wastewater irrigation. Chemosphere 65, 1859-1868.


Xu, J., Wu, L., Chang, A.C., 2009. Degradation and adsorption of selected...
pharmaceuticals and personal care products (PPCPs) in agricultural soils. Chemosphere 77, 1299-1305.


Fig. 1. Dendrogram representation of the bacterial diversity observed in treated wastewater and agricultural soil. The dendrogram was constructed with the iTOL – interactive tree of life (Letunic and Bork, 2007, 2011), based on the taxon ID codes, corresponding to the identifications provided in each of the publications cited (see Table S1).
**Table 1**  
Guidelines on wastewater reuse for irrigation in different world regions.

<table>
<thead>
<tr>
<th>Country or organism (year)</th>
<th>Irrigation categories</th>
<th>pH (uS/cm)</th>
<th>EC (µS/cm)</th>
<th>SAR (mg/L)</th>
<th>NTU (mg/L)</th>
<th>SS (mg/L)</th>
<th>BOD (mg/L)</th>
<th>COD (mg/L)</th>
<th>DO (mg/L)</th>
<th>TNK or TN (mg/L)</th>
<th>N-N03 (mg/L)</th>
<th>P (mg/L)</th>
<th>Salphate (mg/L)</th>
<th>Total coliform (CFU/100 mL)</th>
<th>Fecal coliform (CFU/100 mL)</th>
<th>E. coli (CFU/100 mL)</th>
<th>Nematode eggs (no/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UN-EPF (2012)</strong></td>
<td>R</td>
<td>6-9</td>
<td>2</td>
<td>30</td>
<td>10</td>
<td>Absent</td>
<td>-</td>
<td>2-10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>WHO (2009a)</strong></td>
<td>R</td>
<td>6-9</td>
<td>2</td>
<td>Present</td>
<td>-</td>
<td>-</td>
<td>2-10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>California (1978)</td>
<td>ND</td>
<td>6-9</td>
<td>2</td>
<td>-</td>
<td>Present</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Italy (2003)</strong></td>
<td>ND</td>
<td>6-9</td>
<td>2</td>
<td>2-10</td>
<td>-</td>
<td>2</td>
<td>500</td>
<td>-</td>
<td>2-10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>France (2010)</strong></td>
<td>R</td>
<td>6-9</td>
<td>2</td>
<td>Present</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>22-10</td>
<td>10^3</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Spain (2007)</strong></td>
<td>R</td>
<td>6-9</td>
<td>2</td>
<td>Present</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2-10</td>
<td>10^2</td>
<td>2-10^3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Portugal (2006)</strong></td>
<td>R</td>
<td>6-9</td>
<td>2</td>
<td>Present</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2-10</td>
<td>10^3</td>
<td>2-10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Australia (2000)</strong></td>
<td>R</td>
<td>6-9</td>
<td>2</td>
<td>Present</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2-10</td>
<td>10^3</td>
<td>2-10^3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Israel (1990)</strong></td>
<td>R</td>
<td>6-9</td>
<td>2</td>
<td>Present</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2-10</td>
<td>10^3</td>
<td>2-10^3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Tunisia (1989)</strong></td>
<td>R</td>
<td>6-9</td>
<td>2</td>
<td>Present</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2-10</td>
<td>10^3</td>
<td>2-10^3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Jordan (2002)</strong></td>
<td>R</td>
<td>6-9</td>
<td>2</td>
<td>Present</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2-10</td>
<td>10^3</td>
<td>2-10^3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Kuwait (2001)</strong></td>
<td>R</td>
<td>6-9</td>
<td>2</td>
<td>Present</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2-10</td>
<td>10^3</td>
<td>2-10^3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Oman (1993)</strong></td>
<td>R</td>
<td>6-9</td>
<td>2</td>
<td>Present</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2-10</td>
<td>10^3</td>
<td>2-10^3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Saudi Arabia (2000)</strong></td>
<td>R</td>
<td>6-9</td>
<td>2</td>
<td>Present</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2-10</td>
<td>10^3</td>
<td>2-10^3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>China (2007)</strong></td>
<td>R</td>
<td>6-9</td>
<td>2</td>
<td>Present</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2-10</td>
<td>10^3</td>
<td>2-10^3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Mexico (1987)</strong></td>
<td>R</td>
<td>6-9</td>
<td>2</td>
<td>Present</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2-10</td>
<td>10^3</td>
<td>2-10^3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

UR, unrestricted irrigation; R, restricted irrigation; EC, electrical conductivity; SAR, sodium absorption rate; NTU, Nephelometric Turbidity Unit; TSS, total suspended solids; BOD, biological oxygen demand; COD, chemical oxygen demand; DO, dissolved oxygen; TNK, total nitrogen (Kjeldahl); TN, total nitrogen; N-N03, nitrate nitrogen; P, phosphate; log reduction value; MPN/100 mL; n.d., no determination.

Table 2
Overview of methodological approaches used to assess soil microbial community composition, structure and activity.

<table>
<thead>
<tr>
<th>Main goal</th>
<th>Approach</th>
<th>Specific goal</th>
<th>Outcome</th>
<th>Methods</th>
<th>Eg References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Community activity and function</td>
<td>Biochemical assays</td>
<td>Detection or quantification of specific activities</td>
<td>Respiratory activity</td>
<td>Quantification of CO₂ evolution</td>
<td>[1]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Activity of enzymes involved in biogeochemical cycles (e.g., dehydrogenase, laccase, urease)</td>
<td>Enzymatic assays</td>
<td>[2–5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Detection of compounds produced during specific microbiological transformations (e.g., nitrifiers, ammonia for ammonifiers)</td>
<td>Colorimetric assays; chromatography</td>
<td>[6]</td>
</tr>
<tr>
<td>Culture dependent</td>
<td>Quantification of specific activities</td>
<td></td>
<td>Enumeration of total cultivable metabolic groups of organisms (e.g., ammonifiers, denitrifiers, diazotrophs, autotrophs)</td>
<td>MPN, plating or membrane filtration techniques</td>
<td>[3, 7, 8]</td>
</tr>
<tr>
<td></td>
<td>Profiling</td>
<td></td>
<td>Enumeration of total metabolic active cells (e.g., community level physiological profiling)</td>
<td>MPN, (e.g., eco-BIOLOG)</td>
<td>[7, 9]</td>
</tr>
<tr>
<td>Nucleic acid dependent</td>
<td>Quantification of specific activities (targeted)</td>
<td></td>
<td>Detection or quantification of genes involved in particular reactions (e.g., CO₂ N cycle)</td>
<td>FISH-Confocal/epifluorescence microscopy; qPCR; clone libraries</td>
<td>[10–13]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Functional gene microarrays (e.g., GeoChip)</td>
<td>[14]</td>
</tr>
<tr>
<td></td>
<td>Quantification and identification of total potential activities (non-targeted)</td>
<td></td>
<td>Metagenomics</td>
<td>Total DNA sequencing</td>
<td>[12, 15–18]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Metatranscriptomics</td>
<td>Total mRNA (cDNA) sequencing</td>
<td>[19, 20]</td>
</tr>
<tr>
<td>Culture dependent</td>
<td></td>
<td></td>
<td>Enumeration of total cultivable organisms or groups of organisms (e.g., total heterotrophic aerobic/anerobic bacteria, fungi, actinomycetes)</td>
<td>MPN, plating or membrane filtration techniques</td>
<td>[3, 7, 8]</td>
</tr>
<tr>
<td>Community size, composition and structure</td>
<td>Estimation of size</td>
<td></td>
<td>Enumeration of total cells</td>
<td>Nucleic acid staining (e.g., DAP), Confocal/epifluorescence microscopy Chloroform fumigation extraction</td>
<td>[3]</td>
</tr>
<tr>
<td>Culture independent</td>
<td></td>
<td></td>
<td></td>
<td>DCGE, IRFL, microarray (e.g., PhyloChip)</td>
<td>[2, 9, 18, 21]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Quantification of microbial biomass (C and/or N)</td>
<td>PELA extraction, chromatography</td>
<td>[18, 23–25]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Small subunit rRNA gene sequence based (16S or 18S rRNA genes)</td>
<td>Clone libraries; high throughput technologies</td>
<td>[12, 15–18]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phospholipid-derived fatty acids based</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Assessment of taxonomic diversity and abundance of each taxa</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Identification of community members involved in the degradation of specific substrates</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Small subunit rRNA gene sequence based (16S or 18S rRNA genes)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Isotope labeled small subunit rRNA gene sequence based (16S or 18S rRNA genes)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3
Examples of soil physicochemical, biochemical and microbiological properties influenced by the variation of selected parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Associated effects in the soil (and environment)</th>
<th>Microbiological properties</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Availability of nutrients and trace metals&lt;br&gt; Mineralisation of organic matter&lt;br&gt; Cation exchange capacity (CEC)</td>
<td>Community richness and diversity</td>
<td>[1-6]</td>
</tr>
<tr>
<td>Organic matter</td>
<td>Aggregate formation and stabilisation of soil structure&lt;br&gt; Water retention&lt;br&gt; Content of nutrients (N, P and S)&lt;br&gt; Cation exchange capacity&lt;br&gt; Enzymatic activity&lt;br&gt; Availability of organic and inorganic contaminants</td>
<td>Selection of specific populations&lt;br&gt; Soil microhabitats</td>
<td>[2, 7-11]</td>
</tr>
<tr>
<td>Nutrients (N, P, K)</td>
<td>Improvement of soil fertility&lt;br&gt; Increase of soil organic matter&lt;br&gt; Water retention&lt;br&gt; Elements cycling&lt;br&gt; Leaching to groundwater and risk of eutrophication of aquatic environments</td>
<td>Disturbance of soil microbial communities&lt;br&gt; Microbial catalytic activity</td>
<td>[12–20]</td>
</tr>
<tr>
<td>Salinity</td>
<td>Soil salinisation or sodification&lt;br&gt; Decrease of aggregate stability and soil structure&lt;br&gt; Soil permeability and water retention&lt;br&gt; Increase of soil compaction&lt;br&gt; Soil pH&lt;br&gt; Negative impact on soil fertility&lt;br&gt; Dynamics of organic and inorganic compounds&lt;br&gt; Leaching of heavy metals</td>
<td>Soil microhabitats&lt;br&gt; Community diversity and activity</td>
<td>[2, 9, 21–25]</td>
</tr>
<tr>
<td>Contaminants</td>
<td>Soil toxicity, terracumulation, leaching&lt;br&gt; Potential direct or indirect contamination of the food chain&lt;br&gt; Examples of specific effects: Metals:&lt;br&gt; - Enhance effects of antibiotics&lt;br&gt; Boron:&lt;br&gt; - Plant toxicity&lt;br&gt; Surfactants:&lt;br&gt; - Water repellency&lt;br&gt; Antibiotics:&lt;br&gt; - Contribute for antibiotic resistance&lt;br&gt; Endocrine-disrupting products and other pharmaceutical products:&lt;br&gt; - Interfere with normal functioning of hormone systems in wildlife&lt;br&gt; - Influence plants development</td>
<td>Community structure and diversity&lt;br&gt; Increase of microbial tolerance to contaminants&lt;br&gt; and/or biodegradation&lt;br&gt; Spread of antibiotic resistance</td>
<td>[26–35]</td>
</tr>
</tbody>
</table>

Table 4

Observed effects of wastewater (WW) irrigation on soil properties.

<table>
<thead>
<tr>
<th>WW</th>
<th>Soil description* / culture / period of irrigation (years / country)</th>
<th>Effects on physicochemical parameters</th>
<th>Effects on microbiological parameters</th>
<th>Reported implications for microflora and/or plant production</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>U, la</td>
<td>Vertic Xerochrept (USDA); citrus orchard (15 / Italy)</td>
<td>Organic matter pools pH Salinity</td>
<td>Metals Others</td>
<td>Increment of available nutrients improves metabolic efficiency of soil microflora.</td>
<td>[1]</td>
</tr>
<tr>
<td>U, st</td>
<td>Calciosoils (WWB) / alfalfa, maize, barley, oats (&gt; 20) / Spain</td>
<td>OC</td>
<td>ApP</td>
<td>Increment of available P and water-soluble organic carbon is related to the increases of soil microbial biomass and activity.</td>
<td>[2]</td>
</tr>
<tr>
<td>U</td>
<td>Vertisols (WWB) / cereals and vegetables / 80 / Mexico</td>
<td>OC</td>
<td>Pb, Cd, Cu, Zn</td>
<td>Denitrification activity</td>
<td>[3]</td>
</tr>
<tr>
<td>U</td>
<td>Typic Hapludult (USDA) / hazel orchard (20 / Italy)</td>
<td>OC, TN</td>
<td>DH</td>
<td>Increase of available decomposable organic material increases soil microbial biomass and activity.</td>
<td>[3]</td>
</tr>
<tr>
<td>U, st</td>
<td>Xerochrept (USDA); orange-tree orchard (43 / Spain)</td>
<td>OC</td>
<td>ApP</td>
<td>Diversity of the anaerobic-oxidizing bacteria</td>
<td>[4]</td>
</tr>
<tr>
<td>U</td>
<td>NR/cereals, millers, vegetable and fodder crops / India</td>
<td>OC</td>
<td>Pp</td>
<td>Wastewater flooding leads to the functional uniformization of soil microflora.</td>
<td>[4]</td>
</tr>
<tr>
<td>U</td>
<td>NR/cereals, millers, vegetable and fodder crops / 30 / India</td>
<td>OC, TN</td>
<td>Zn, Fe, Ni, Pb</td>
<td>Reduction of the arbuscular mycorrhizal fungi diversity but crop viability and productivity is not affected.</td>
<td>[5]</td>
</tr>
<tr>
<td>U</td>
<td>NR/cereals, millers, vegetable and fodder crops / 20 / India</td>
<td>OC</td>
<td>Zn, Cu, Fe, Ni, Pb</td>
<td>Metals may be accumulated by plants.</td>
<td>[6]</td>
</tr>
<tr>
<td>U, st</td>
<td>Loamy fine sand texture / alfalfa hay, sudan grass</td>
<td>OC, TN</td>
<td>Cr, Cu, Ni, Zn</td>
<td>Metals may be accumulated by plants and may also be leached.</td>
<td>[7]</td>
</tr>
<tr>
<td>Site Description</td>
<td>Geochemistry</td>
<td>Microbiome</td>
<td>Impact</td>
<td>Literature</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>--------------</td>
<td>------------</td>
<td>--------</td>
<td>------------</td>
<td></td>
</tr>
<tr>
<td>Fine texture/forage crops, Jordan</td>
<td>Cu, PO, Cd</td>
<td>AvP, K</td>
<td>Monitorisation of soil and plant is needed for a safe use of wastewater.</td>
<td>[9]</td>
<td></td>
</tr>
<tr>
<td>NIR persimmon orchard, Israel</td>
<td>OC</td>
<td>NO₂⁻N</td>
<td>Soil bacterial composition and function are affected by wastewater irrigation.</td>
<td>[10,11]</td>
<td></td>
</tr>
<tr>
<td>NIR orange grove, Tunisia</td>
<td>OC</td>
<td>Ni, Zn, Co</td>
<td>Microbial abundance</td>
<td>Modification of the genetic structure of soil bacterial and fungal communities.</td>
<td>[12]</td>
</tr>
<tr>
<td>Xerorthent (USDA)/grape crop, Spain</td>
<td>OC</td>
<td>CEC, WR, AvP</td>
<td>AP, UR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xerofluvent (USDA)/&quot;green filter&quot;, Spain</td>
<td>OC</td>
<td>CEC, Ag, AvP</td>
<td>AP, BC, UR</td>
<td>Modification of soil quality indices</td>
<td>[13]</td>
</tr>
<tr>
<td>Xerorthent (USDA)/orange-tree orchard, Spain</td>
<td>OC</td>
<td>AvP</td>
<td>AP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xerofluvent (USDA)/grape crop, Spain</td>
<td>OC</td>
<td>CEC, WR, AgSt</td>
<td>AP, BC, UR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sandy loam texture/Mangrove swamp, China</td>
<td>TN</td>
<td>Cu, Zn, Cd, Mn</td>
<td>NH₄⁺, NO₃⁻, AvP</td>
<td>Aerobic and anaerobic bacteria Ammonia- and nitrite-oxidizing bacteria</td>
<td>Increment of available nutrients stimulates microbial growth and activity.</td>
</tr>
<tr>
<td>U</td>
<td>Leptosols (WRB)/cereals and vegetables (&lt; 80°C) Mexico</td>
<td>OC</td>
<td>Pb, Cd, Cu, Zn</td>
<td>DM</td>
<td>Denitrification activity</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>I</td>
<td>Chromosols and Teneosols (ASC)/grazed pastoral/60/ Australia</td>
<td>OC, TN</td>
<td>Ca, Mg, Fe, K</td>
<td>C:N ratio</td>
<td>AvP, Ex Na, K</td>
</tr>
<tr>
<td>U, 25</td>
<td>Horticultural soil/NR/1/Canada</td>
<td>OM</td>
<td></td>
<td></td>
<td>Lacase, cellulase, PL, UR</td>
</tr>
<tr>
<td>U</td>
<td>Typic Hapludust (USDA)/sugar cane/1/Brazil</td>
<td>OC, TN</td>
<td>NO₃-N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>Eutric Arenosol (WRB)/kaolinite/15/Senegal</td>
<td>OC, TN</td>
<td>NH₃-N, NO₃-N, N</td>
<td>HD</td>
<td>Bacterial community structure</td>
</tr>
<tr>
<td>I</td>
<td>Loamy texture/Fodder, cereals/NR/Pakistan</td>
<td>OM</td>
<td>Zn, Cu, Ni, Cr</td>
<td>Ex SO₄, NO₃-N, AvP</td>
<td>Heavy metal resistant bacteria</td>
</tr>
<tr>
<td>U, 25</td>
<td>Argosols and Cambosols (CST)/cereals and vegetables/40/China</td>
<td>OC</td>
<td>Cd, Cr, Cu, Ni, Pb, Zn</td>
<td>HA</td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>NR/barley, corn, cotton, alfalfa, sorghum/80/USA</td>
<td>Zn</td>
<td>SoilChem</td>
<td>AvP</td>
<td></td>
</tr>
<tr>
<td>Soil Type</td>
<td>Microbial Abundance (total aerobic bacteria)</td>
<td>Low Risk of Microbial Aquifer Contamination</td>
<td>Heavy Metals Reduce Plant Production and Decrease Alkaline AP Activity</td>
<td>Microbial Abundance and Diversity</td>
<td>Effects of Wastewater on Enzyme Activities are Dependent of Plant Type</td>
</tr>
<tr>
<td>-----------</td>
<td>--------------------------------------------</td>
<td>---------------------------------------------</td>
<td>---------------------------------------------------------------------</td>
<td>---------------------------------</td>
<td>-------------------------------------------------------------------</td>
</tr>
<tr>
<td>Silty sand texture/perennial ryegrass</td>
<td>BC, Na</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartzarenic Neosol (Silic/S) eucalyptus</td>
<td>Cd, Cr, Cu, Ni, Zn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR/mangrove</td>
<td>Fe, Cr, Zn, Pb, Ni, Cd, Cu</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhizosphere soil/wheat</td>
<td>Cr, Cu, Ni, Zn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mollic Leptosol and Eutric Vertisol (WRB)/maize/S. 98/Mexico</td>
<td>Cr, Cu, Ni, Zn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR/agricultural</td>
<td>Fe, Ni, Zn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EURIC Histosol (WRB)/different grasses</td>
<td>WC, Eh</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EURIC Histosol (WRB)/salix</td>
<td>WC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR/Parks</td>
<td>Ab, Dp</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silty clay loam texture</td>
<td>ED, Ph</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>