academicJournals

Vol. 12(39), pp. 5761-5770, 25 September, 2013
DOI: 10.5897/AJB2013.12412
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Effects of ten years treated wastewater drip irrigation on soil microbiological properties under Mediterranean conditions

Yassine Hidri*, Olfa Fourti, Naceur Jedidi and Abdenaceur Hassen

Water Researches and Technologies Center (CERTE), Borj-Cedria Techno Park, P. O. Box 273, Soliman 8020, Tunisia.

Accepted 5 August, 2013

Water shortage in most countries of the southern Mediterranean basin has led to the reuse of municipal wastewater for irrigation. Despite numerous advantages for soil fertility and crop productivity, recycling wastewater in the soil also has several ecotoxicological and sanitary problems. To evaluate the chronic soil contamination and the cumulative impact of wastewater, we compared two plots, all under orange-grove that had been drip irrigated for 10 years. The first plot was irrigated with treated wastewater; the second one was irrigated with groundwater. No negative effects of treated wastewater drip irrigation treatment were observed on the measured soil parameters (pH, organic matter and cation exchange capacity). A slight increase in the concentration of soil enteric bacteria and soil fungal densities was recorded in the wastewater plot reaching a maximum value in the first soil layer (0 to 20 cm). This result was recorded essentially around the emitters. Groundwater plots and wastewater plots exhibited similar repartitions of soils DNA quantity with depth, with the highest values in the upper layer and a progressive decrease with soil depth. For both soils, DNA quantity was positively affected by soil organic matter content. This work confirms that, under suitable conditions, treated wastewater use in irrigation can have positive effects, not only in the aspects of soil quality, but also in social terms, as it allows the maintenance of irrigated agriculture in areas where groundwater has been polluted by seawater intrusion.

Key words: Treated wastewater, groundwater, drip irrigation, enteric bacteria, soil DNA.

INTRODUCTION

The scarcity of conventional water resources constitutes a social, agricultural and economic problem in most countries of the southern Mediterranean Basin. Water shortage in these countries is a result of a combination of arid climatic conditions and an increase in water requirements due essentially to population growth and the development of tourism. There is thus an urgent need to make alternative water sources available for agriculture to replace the high quality water required for human consumption (Angelakis et al., 1999). In this context, the use of municipal wastewater for irrigation could provide a realistic alternative water supply for agriculture, as has been proved in many countries in the Mediterranean region, such as Israel, Cyprus, Jordan and Tunisia (Angelakis et al., 1999).

With per capita, freshwater of about 450 m^3, Tunisia is one of the most drought-stressed countries in the Middle East and North Africa region. In this country, the reuse of treated wastewater in irrigation is considered as a strategic approach to preserve fresh water resources. This process date back, in fact, to 1965. Treated wastewater currently represents approximately 5% of Tunisia's total

*Corresponding author. E-mail: hidyassine@yahoo.fr. Tel: 00216 79 32 51 22. Fax: 00216 79 32 58 02.

Table 1. Irrigation volume during the years of trials.

Year	Irrigation volume (mm)	
	Site 1	Site 2
1998	248	122
1999	245	125
2000	240	145
2001	250	120
2002	265	140
2003	270	135
2004	300	140
2005	370	130
2006	385	128
2007	380	135

available water; this planned to increase to 11% by 2030 (Shetty, 2004). Treated wastewater can be suitable for a large variety of applications. Among the most common reuse applications are irrigation; residential uses; urban and recreational uses; groundwater recharge; bathing water; aquaculture; industrial cooling water; and drinking water production (Huertas et al., 2008). Water reuse for irrigation has been largely applied to agriculture due to the advantages related to nutrient recovery possibilities, socio-economic implication, decline of fertilizer application and effluent disposal (Candela et al., 2007). However, scientific and technical treated domestic wastewater application for irrigation or aquifer recharge is mainly reduced to countries with a high scientific and technical development and water scarcity (Sheng, 2005).

Water quality criteria being generally applied for agricultural reuse have been mainly based on microbiological aspects, focusing on the existence of potential pathogens (viruses, bacteria and protozoa), which may cause sanitary problems (WHO, 1998), total dissolved solids (TDS) and salinity aspects (Martinez-Beltran, 1999). Haruvy (2006) presents more specific water quality parameters related to water reclamation and reuse. Salinity level of wastewater is generally high, and regular treatment processes do not get rid of salinity unless combined with rather expensive desalination processes and increase of water supply costs (Appelo and Postma, 1993). Research studies have focused on sanitary effects from reused domestic treated wastewater to evaluate the risk of edible crops by sprinkler irrigation (Haas, 1996). The presence of pathogenic microorganisms and NO_2 and CO_2 production for perched aquifers has been mentioned in the literature (Campos, 2008). Possible risk of pesticide leaching on the golf courses application has been evaluated by Cohen et al. (1999). Weber et al. (2006) have evaluated human risk of the organic contaminants in reclaimed wastewater used for irrigation. Candela et al. (2007), Dère et al. (2006) and Rusan et al. (2007) have mentioned the long-term effect of wastewater irrigation on soil and plant quality parameters. In this context, the aim of our study was to evaluate the effects of treated wastewater irrigation, of 10 years' duration, on the abundance soil microbial communities. Experiments were conducted in the "Nabeul-Hammamet" region of northern Tunisia. This is one of the most drought-stressed countries in the Middle Eastern and North African region, with freshwater per capita of around 450 m^3/years. Treated wastewater currently represents approximately 5% of Tunisia's total available water but is expected to increase to 11% by 2030 (Shetty, 2004).

We compared the microbial quality in two soils that had been irrigated for 10 years, in the same experimental field. The first soil was irrigated with secondary treated wastewater, the second was irrigated with groundwater. The bacterial and fungal abundance in each plot was assessed from counts on synthetic culture media. Enteric bacteria (*Escherichia coli*, fecal coliform and fecal streptococcus), indigenous to the soils irrigated for the same durations, were assessed by using the most probable number (MPN) method.

MATERIALS AND METHODS

Study area and sampling strategy

The valley of Nabeul (North East of Tunisia, 36° 29' N, 10° 42' E) is irrigated since 1980. The climate is temperate and semi arid with mild and rainy winters. The mean mensal temperature range is between 12 and 27°C. Evapo-transpiration (10.3 mm/d) was recorded in July and August, whereas rainfall occurred mainly from September to March. Annual precipitation was around 470 mm. The middle length of sunstroke is between 5.4 and 12.9 h/day. Soils were usually sampled in triplicate at two different sites, all under an orange - grove that had been irrigated for 10 years: Plot 1: soils irrigated with secondary treated wastewater (WWP); plot 2: soils irrigated with groundwater (GWP).

A drip irrigation system was used, with lines running along the citrus tree rows and two droppers situated 50 cm on both sides of each tree; flux per dipper was around 4 l/h. Annual application rates ranged from 240 to 410 mm for the plot 1 and from 120 to 145 mm for the plot 2. Details on the irrigation scheduling are provided in Table 1.

Sampling was carried out using a drill at the end of the dry season extending between the 30 and 31 October, 2007. Each site was divided into three locations or blocks, and composite soil sample from 0-20, 20-40 and 40-60 cm soil depth was taken from each block. Soil samples were collected at three points along a 150 cm transect that was perpendicular to the direction of the drip irrigation system (Figure 1). Soil was sieved (2 mm) in order to remove rocks and root fragments, placed inside plastic vented bags and stored at 5°C for 15 days for later analysis. Physico-chemical soil characteristics were measured at each site for each treatment by the Laboratory of Soil Analysis (INRA-Arras, France, http://www.arras.inra.fr/), using standard methods. The main results are given in Table 2.

Microbial enumeration

Bacteria and fungi were extracted by blending soil samples with 0.8% (w/v) sterile NaCl solution and the homogenous soil suspension was serially diluted tenfold with sterile saline solution. Indirect counting of bacteria was carried out by spreading 100 µl of appropriate dilutions on plate count agar (aerobic plate count agar) (Al-Lahham et al., 2003). Bacterial colonies were counted after 48 h

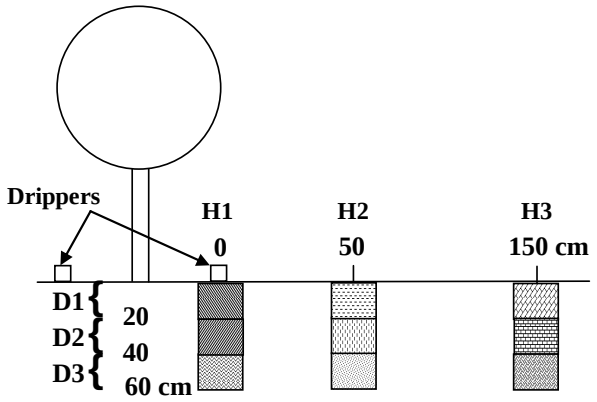

Figure 1. Soil sampling mode. D1: depth 1 (0-20 cm), D2: depth 2 (20-40 cm), D3: depth 3 (40-60 cm), H1: Horizon 1 (0 cm/dripper), H2: Horizon 2 (50 cm/dripper), H3: Horizon 3 (150 cm/dripper).

of incubation at 28°C. Only plates with between 10 and 100 colonies per plate were counted. For fungi, the appropriate soil dilution was spread on malt extract agar (30 g/l malt extract, 3 g/l protease peptone, 1.5% agar, pH 5.6). The number of developing colonies was counted after 7 days of incubation at room temperature and was expressed as the number of colony forming units (CFU) per gram dry weight of soil.

Pathogenic contamination

Soil examination

The mass of 10 g of soil was dispersed in 90 ml of sterile distilled water. They were then submitted to a mechanical shaking for 2 h (Edmond Buhler, type KI-2), in order to remove bacteria from their organo-mineral substrates. Finally, soil suspensions were used for *E. coli*, fecal coliform and fecal streptococcus determination using the most probable number (MPN) method and following the 3 replications × 5 dilution scheme (APHA, 1998).

Water examination

All the glassware used was cleaned with hot water and a suitable detergent, rinsed with hot water to remove all traces of the detergent used, and finally rinsed with distilled water. The sampling glass bottles WEre sterilized in an autoclave at 121°C for 15 min (APHA, 1998).

Samples of treated wastewater and conventional water are collected in sterilized glass bottles from the wastewater treatment plant and from the Nabeul groundwater. 10% of sodium thiosulfate ($Na_2S_2O_3$) was added to the samples that have been treated with chlorine, as a de-chlorinating agent to neutralize any residual chlorine and to prevent the continuation of its action on bacteria thereafter. Solution of $Na_2S_2O_3$ at the rate of 0.1 ml of 10% and 0.1 ml of 3% to 100 ml sample bottles are added to the two water samples. Wastewater samples were collected and transported directly to the laboratory at +4°C and kept in the refrigerator for later analysis. The samples were examined within 24 h for the presence of coliforms and streptococci bacteria group, and the total bacterial count was done in accordance with APHA (1998).

Soil DNA extraction

Microbial DNA was extracted from independent triplicates of soils sampled at each site, according to the method described by Ranjard et al. (2003). Briefly, 1 g from each soil sample was mixed with 4 ml of a solution containing 100 mm Tris (pH 8.0), 100 mm EDTA (pH 8.0), 100 mM NaCl and 2% (wt/Vol) sodium dodecyl sulfate. 2 g of 106 µm-diameter glass beads and 8 glass beads of 2-mm diameter were added to the mixture in a bead-beater-tube. The samples were then homogenized for 30 s at 1600 RPM in a mini bead-beater cell disruptor (Mikro-dismembrator S. B. Braun Biotech International). The samples were incubated for 20 min at 70°C, then centrifuged at 14 000 *g* for 1 min at 4°C. The collected supernatants were incubated for 10 min on ice with 1/10 volume of 3 M potassium acetate (pH 5.5) and centrifuged at 14 000 *g* for 5 min. After precipitation with one volume of ice-cold isopropanol, the nucleic acids were washed with 70% ethanol. DNA was separated from the residual impurities, particularly humic substances, by centrifuging through two types of minicolumn. Aliquots (100 µl) of crude DNA extract were loaded onto polyvinyl polypyrrolydone minicolumns (BIORAD, Marne-la-Coquette, France) and centrifuged at 1000 *g* for 2 min at 10°C. The collected eluate was then purified with the Geneclean turbo kit (Q-Biogene, Illkirch, France). Purified DNA was quantified by spectrophotometry (Bio-Rad Smart Spec^{TM} Plus, France) (Leckie et al., 2004).

Statistical analysis

The effect of irrigation on soil physicochemical properties and microbial abundance was tested by the SPSS statistical program (SPSS 10.05 for Windows; SPSS Inc., Chicago, IL, USA) and differences between means were tested with the Student-Newman-Keuls test.

RESULTS AND DISCUSSION

Water characteristics

Treated wastewater

This study was conducted at the SE4 Wastewater Treatment Plant (Nabeul -north east Tunisia-) which was set up in May 1979. The SE_4 wastewater treatment plant is an activated sludge-extended aeration system that involves a mechanical screen, grit removal tanks, primary sedimentation tanks, extended aeration tanks and finally sedimentation tanks. The characteristics of the waste water used for irrigation varied both within and between the years of application. The wastewater was, on average, alkaline with a basic pH value of 7.8 and had a moderate level of total dissolved solids (TDS) of 1556 mg/l. It contained considerable amounts of nitrate (31 mg/l), ammonia (53 mg/l) phosphate (17.8 mg/l) and potassium (53.3 mg/l). It presented an electrical conductivity of 3.27 (mmhos cm^{-1}), a chemical and biochemical oxygen demand of 95 and 17.4 mg/l, respectively.

On the other hand, the concentrations of micronutrients and heavy metals in the wastewater were relatively low with 0.0007 mg L^{-1} of Cd, 0.02 of Co, 0.01 of Cu, 0.05 of Mn, 0.19 of Fe, 0.05 of Ni, 0.03 of Pb, 0.03 of Zn and 0.03 of Cr.

Microbiological contamination and organic matter in the wastewater can produce detrimental effects on human health (Al-Shammiri et al., 2003). A recent WHO report concluded that crop irrigation with untreated wastewater

Table 2. Physico-chemical soil characteristics.

Sample	Clay (%)	Silt (%)	Sand (%)	pH	Tot org. C (mg. g^{-1})	N tot. (mg. g^{-1})	Org. M. (mg.g^{-1})	CEC (Cmol^{+}. kg^{-1})
WWH1D1	14	8.7	77.3	7.07^{a} (±0.85)	18.07^{a} (±3. 47)	1.88^{c} (±0.36)	29.80^{a} (±5.78)	9.57^{gh} (±1.42)
WWH1D2	10.4	6.1	83.5	7.97^{cd} (±0.38)	5.6^{bc} (±1.79)	0.65^{ab} (±0.20)	9.70^{bc} (±3.12)	5.82^{abcd} (±0.76)
WWH1D3	11.07	7.03	81.9	8.25^{cd} (±0.14)	3.01^{c} (±0.50)	0.37^{a} (±0.04)	5.21^{c} (±0.87)	4.91^{abcd} (±0.14)
WWH2D1	12.53	8.07	79.4	7.69^{b} (±0.38)	15.03^{a} (±3.87)	1.66^{c} (±0.41)	26.07^{a} (±6.69)	8.38^{efg} (±1.34)
WWH2D2	8.8	6.3	84.9	8.24^{cd} (±0.30)	4.77^{c} (±0.99)	0.53^{a} (±0.12)	8.24^{c} (±1.70)	4.63^{abc} (±0.17)
WWH2D3	7.83	4.17	88	8.40^{cd} (±0.30)	2.15^{c} (±0.50)	0.27^{a} (±0.06)	3.73^{c} (±0.88)	3.73^{a} (±0.70)
WWH3D1	10.87	6.2	82.93	7.99^{cd} (±0.18)	10.06^{b} (±3.11)	1.10^{b} (±0.36)	17.43^{b} (±5.40)	5.83^{abcd} (±0.84)
WWH3D2	8.77	4.87	8637	8.50^{d} (±0.14)	2.70^{c} (±0.37)	0.33^{a} (±0.03)	4.68^{c} (±0.63)	3.88^{ab} (±0.21)
WWH3D3	9.3	4.67	86.03	8.55^{d} (±0.16)	2.58^{c} (±1.08)	0.31^{a} (±0.09)	4.47^{c} (±1.87)	4.37^{abc} (±0.72)
GWH1D1	22.83	18.5	58.7	8.61^{d} (±0.09)	14.2^{a} (±1.93)	1.53^{c} (±0.22)	24.57^{a} (±3.36)	8.97^{fgh} (±1.15)
GWH1D2	20.03	16.4	63.33	8.77^{d} (±0.03)	7.10^{bc} (±1.19)	0.68^{ab} (±0.12)	12.29^{bc} (±2.08)	6.94^{efg} (±0.65)
GWH1D3	23.43	17.7	58.8	8.79^{d} (±0.02)	4.77^{c} (±1.35)	0.48^{a} (±0.07)	8.25^{c} (±2.34)	6.25^{cde} (±0.26)
GWH2D1	22.63	21.5	55.87	8.25^{cd} (±0.08)	18.17^{a} (±4.10)	1.91^{c} (±0.39)	31.43^{a} (±7.10)	10.48^{h} (±1.18)
GWH2D2	19.23	16.9	63.83	8.67^{d} (±0.05)	6.49^{bc} (±0.36)	0.71^{ab} (±0.02)	11.2^{bc} (±0.53)	7.05^{efg} (±0.78)
GWH2D3	20.60	15.7	63.7	8.71^{d} (±0.15)	4.82^{c} (±0.69)	0.48^{a} (±0.04)	8.33^{c} (±1.19)	6.29^{cde} (±1.23)
GWH3D1	18.60	19.2	62.2	8.31^{cd} (±0.14)	16.17^{a} (±1.12)	1.69^{c} (±0.08)	27.97^{a} (±1.94)	8.55^{fg} (±0.05)
GWH3D2	19.53	17.6	62.87	8.54^{d} (±0.14)	6.15^{bc} (±0.87)	0.68^{ab} (±0.02)	10.64^{bc} (±1.49)	7.05^{efg} (±1.05)
GWH3D3	20.23	15.6	64.10	8.61^{d} (±0.05)	4.19^{c} (±0.55)	0.499^{a} (±0.08)	7.24^{c} (±0.961)	6.09^{bcd} (±0.92)

(a, b, c, ...): for each property, means followed by the same letter are not significantly different according to the Student-Newman-Keuls test at $P < 0.05$. WW, wastewater; GW, groundwater, horizontal transect (H1: 0 cm/drip, H2: 50 cm/drip and H3: 150 cm/drip), vertical transect (D1: 0-20 cm, D2: 20-40 cm and D3: 40-60 cm). Each value is the mean of three replicates. Tot org. C, total organic carbon; N tot, total nitrogen; Org. M., organic manure; CEC, cation exchange capacity.

causes significant excess intestinal nematode infection in crop consumers and field workers, while irrigation with adequately treated wastewater does not (Al-Shammiri et al., 2003). And there is no actual health risk from using wastewater to irrigate crops (Takashi, 1994). WHO recommended that treated wastewater intended for crop irrigation should contain less than 1 viable intestinal nematode egg per liter and less than 10^3 fecal coliform bacteria (FCB) per 100 ml. In the effluent from preliminary treatment, the number of fecal coliforms, of fecal streptococci bacteria and of *E. coli* are respectively 1.6×10^5, 5.4×10^4 and 9.1×10^4 MPN/100 ml. The secondary treatment reduced concentrations of fecal coliform bacteria by about 1 log units and concentrations of *E. coli* and fecal *streptococci* bacteria by about 2 log units. In the pond, the average concentrations of fecal coliforms, streptococci bacteria and *E. coli* is respectively 1.1×10^3, 9.0×10^2 and 9.1×10^2 MPN/100 ml.

According to World Health Organization (1998), wastewater reclaimed by the SE_4 Wastewater Treatment Plant could be used for fruit tree irrigation. Irrigation should be stopped 2 weeks before harvest and no fruit should be picked off the ground.

Groundwater

The groundwater was, on average, alkaline with a basic pH value of 7.5 and had an electrical conductivity of 3.42 (mmhos/cm). It contained considerable amounts of nitrate (24 mg/l), ammonia (53 mg/l), phosphate (1.58 mg/l) and potassium (55.6 mg/l) which are considered essential nutrients for improving plant growth together with soil fertility and productivity levels. It presented a chemical and biochemical oxygen demand of 14.8 and 11.4 mg/l, respectively.

The concentrations of micronutrients and heavy metals in the groundwater were relatively low with 0.005 mg/l of Cd, 0.02 of Co, 0.005 of Cu, 0.019 of Mn, 0.05 of Fe, 0.036 of Ni, 0.039 of Pb, 0.028 of Zn and 0.03 of Cr. It is evident from the data that the heavy metal content was greater in the treated wastewater than groundwater. However, the level of all the heavy metals was under the Tunisian standards for wastewater reuse irrigation (N. A. W. M., 2001).

In the groundwater, the number of fecal coliform, fecal streptococci bacteria and of *E. coli* is respectively 4.3×10^2, 2.5×10^2 and 3.1×10^2 MPN/100 ml. The presence of enteric bacteria in the groundwater could be attributed to the anthropogenic influence.

On the whole, chemical proprieties of the water used in this study satisfy the standards for wastewater reuse irrigation of Tunisian (N. A. W. M., 2002). These kinds of waters could thus be considered of good quality.

Soil characteristics

On the whole, soil texture was sandy clayey for the GWP and only sandy for the WWP.

Impact of water quality on soil physicochemical properties

Soil pH

Soil pH ranged from 7.07 to 8.55 pH units for the field irrigated with treated wastewater for 10 years and from 8.25 to 8.77 pH units for the field irrigated with groundwater (Table 1). In the two studied fields, pH value exhibits the same pattern all over vertical and horizontal transects. Student-Newman-Keuls statistical test ($p \leq 0.05$) reveals a significant decrease in soil pH value due to the treated wastewater irrigation. This result agrees with those reported by Mohamed and Mazahreh (2003) who found a decrease in the pH value as a result of the wastewater irrigation. This fact is due to the oxidation of organic compounds and nitrification of ammonium. Tarchouna et al. (2010) found that soil pH increased as the result of successive several years of wastewater irrigation and they attributed this pH increase to the chemistry and high content of alkaline cations such as Na, Ca and Mg in the wastewater used for a long period of irrigation. Bicarbonate and carbonate ions combined with calcium or magnesium will precipitate as calcium carbonate ($CaCO_3$) or magnesium carbonate ($MgCO_3$). This will cause an alkalizing effect and will increase slightly the pH level. Therefore when a water analysis indicates high pH level, it may be a sign of a high content of carbonate and bicarbonate ions.

Soil organic matter (SOM) and cation exchange capacity (CEC)

SOM contents as affected by the type of water used for irrigation along the studied transects are shown in Table 2.

In the WWP, SOM values along the studied transect varied from 1.73 to 2.98% for the superficial first soil layer (0 to 20 cm), from 0.47 to 0.97% for the second soil layer (20 to 40 cm) and from 0.45 to 0.52% for the third soil layer (40 to 60 cm). The highest values were recorded at the beginning point of the studied transect (0 cm/dripper). These results show the positive effect of treated wastewater content on soil organic matter content (Rusan et al., 2007). On the whole, SOM was lower in the treated wastewater irrigated soil as compared to the groundwater irrigated one. Such a diminution, despite the organic matter supplied by the TWW, has been observed elsewhere (Gloaguen et al., 2007) and it is likely related to an intensification of microbial activity due to labile C and N supplied by the TWW (Tarchouna et al., 2010). In this case, this result may be due to soil texture that was sandy clayey for the GWP and only sandy for the WWP.

Along the studied transects, SOC and soil total nitrogen (STN) exhibited the same variations as compared to soil organic matter values.

Weak but significant variations in CEC values were observed between the two studied sites. This result was true for the horizontal and for the vertical transects (Table 2). In the WWP, a good correlation was found ($R^2 = 0.99$) for the first (0-20 cm) soil layer between the CEC values and the soil horizons.

Impact of water quality on soil microbial properties

Soil microbial abundance

The microbial density in the different soils of the studied site was evaluated by counting the bacteria and fungi on culture media (Table 3). The average numbers of bacteria in the WWP ranged from 29.72 (± 0.57) x 10^4 to 31.35 (± 2.64) x 10^5 CFU g^{-1} soil and from 3.53 (± 0.30) x 10^4 to 9.16 (± 0.25) x 10^4 in the GWP (Table 3). Similar significant differences were recorded for fungi, both between the different soils and between the studied transects. Altogether, these data revealed that irrigation with wastewater induced a significant increase in soil microbial abundance. This growth of microorganisms might be explained by the ready source of easily degradable compounds in the oligotrotrophic soil environment brought about by wastewater irrigation (Ramirez-Fuentes et al., 2002).

Soil DNA content

Soil DNA content as affected by the type of the water used for irrigation along the studied transects is shown in Figures 2 and 3.

In the WWP, soil DNA content values along the studied transect varied from 2.03 to 3.77 $\mu g.g^{-1}$ dry weight for the first soil layer (0-20 cm), from 0.89 to 2.63 µg/g dry weight for the second soil layer (20-40 cm) and from 0.92 to 1.65 µg/g dry weight for the third soil layer (40-60 cm). The highest values were recorded at the beginning point of the studied transect (0 cm/dripper). The weak significance of the positive relationship ($r = 0.55$, $p = 0.05$) between the increase in C_{org} contents and soil DNA content could be partly explained by the sandy texture of the soils, that would promote greater and a more rapid organic matter mineralization. This fact would lead to a transitory increase in C with a significant resiliency due to the rapid decrease in the stock of fresh organic matter (Lejon et al., 2007).

In the GWP, soil DNA content values along the studied transect varied from 5.24 to 5.50 µg/g dry weight for the first (0-20 cm), from 2.68 to 4.28 µg/g dry weight for the second soil layer (20-40 cm) and from 0.90 to 1.90 µg/g dry weight for the third soil layer (40-60 cm). The highest values were recorded at the upper soil layer (0-20 cm). The observed stratification of soil DNA content with soil depth generally corresponded to the decrease in C_{org} and N_{org} contents, as classically observed by Lejon et al. (2005) and Lejon et al. (2007).

Altogether, our results show that there is more DNA in soil irrigated with ground water as compared to soil irriga-

Table 3. Bacterial and fungal counts in soils from the studied sites.

Sample	Heterotrophic bacteria (cfu.g^{-1} soil)	Filamentous fungi (cfu.g^{-1} soil)
WWH1D1	31.35^{a} (± 2.64) x 10^{5}	3.42^{a} (± 0.43) x 10^{5}
WWH1D2	91.18^{c} (± 2) x 10^{4}	9.44^{b} (± 0.11) x 10^{4}
WWH1D3	32.99^{e} (± 2.08) x 10^{4}	3.49^{c} (± 0.25) x 10^{4}
WWH2D1	29.45^{b} (± 1) x 10^{5}	9.08^{b} (± 0.35) x 10^{4}
WWH2D2	65.69^{d} (± 4) x 10^{4}	7.98^{b} (± 0.51) x 10^{4}
WWH2D3	29.72^{e} (± 0.57) x 10^{4}	3.07^{cd} (± 0.05) x 10^{4}
WWH3D1	87.4^{c} (± 4) x 10^{4}	8.04^{b} (± 0.30) x 10^{4}
WWH3D2	57.23^{d} (± 1) x 10^{4}	7.82^{b} (± 0.61) x 10^{4}
WWH3D3	92.77^{f} (± 3.05) x 10^{4}	3.00^{cd} (± 0.11) x 10^{4}
GWH1D1	9.16^{f} (± 0.25) x 10^{4}	8.96^{de} (± 0.30) x 10^{3}
GWH1D2	6.13^{f} (± 0.30) x 10^{4}	6.52^{de} (± 0.20) x 10^{3}
GWH1D3	4.16^{f} (± 0.35) x 10^{4}	2.02^{e} (± 1.79) x 10^{3}
GWH2D1	8.40^{f} (± 0.5) x 10^{4}	8.49^{de} (± 0.15) x 10^{3}
GWH2D2	6.46^{f} (± 0.83) x 10^{4}	6.14^{de} (± 0.40) x 10^{3}
GWH2D3	3.96^{f} (± 0.20) x 10^{4}	1.96^{e} (± 1.73) x 10^{3}
GWH3D1	6.8^{f} (± 0.20) x 10^{4}	8.27^{de} (± 0.20) x 10^{3}
GWH3D2	4.53^{f} (± 1) x 10^{4}	5.82^{de} (± 0.30) x 10^{3}
GWH3D3	3.53^{f} (± 0.30) x 10^{4}	ND

(a, b, c, ...): For each count, means followed by the same letter are not significantly different according to the Student-Newman-Keuls test at P = 0.05. WW, wastewater; GW, groundwater; H1: 0 cm/drip, H2: 50 cm/drip and H3: 150 cm/drip, horizontal transect; D1: 0-20 cm, D2: 20-40 cm and D3: 40-60 cm; vertical transect. Each value is the mean of 3 replicates; ND = non-detectable.

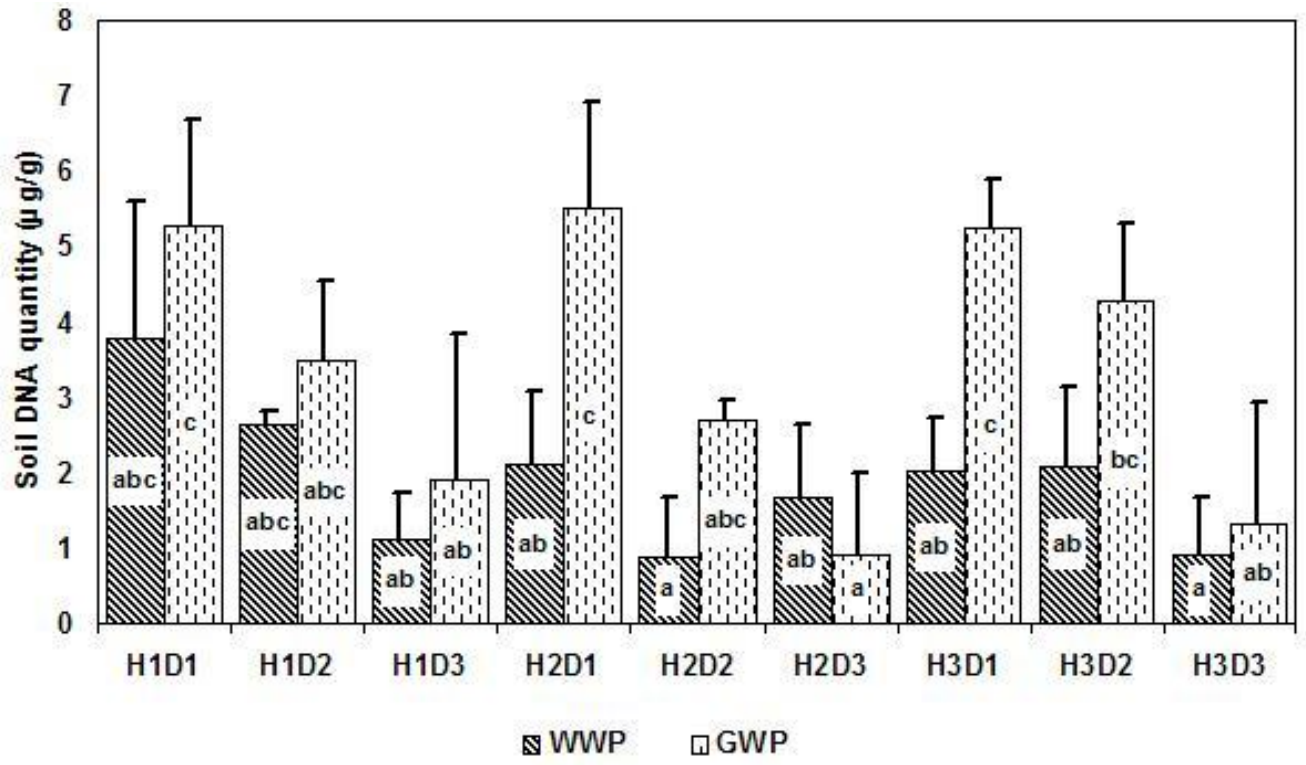

Figure 2. Soil DNA content in the wastewater plot as affected by the vertical (D1: 0-20 cm, D2: 20-40 cm and D3: 40-60 cm) and the horizontal (H1: 0 cm/drip, H2: 50 cm/drip and H3: 150 cm/drip) soils transects, WWP: wastewater plot, GWP: groundwater plot. Vertical bars represent standard deviation (*n* = 3 replicates for soil sample), (a, b, c ...): For each count, means followed by the same letter are not significantly different according to the Student-Newman-Keuls test at *P < 0.05*, WW: wastewater.

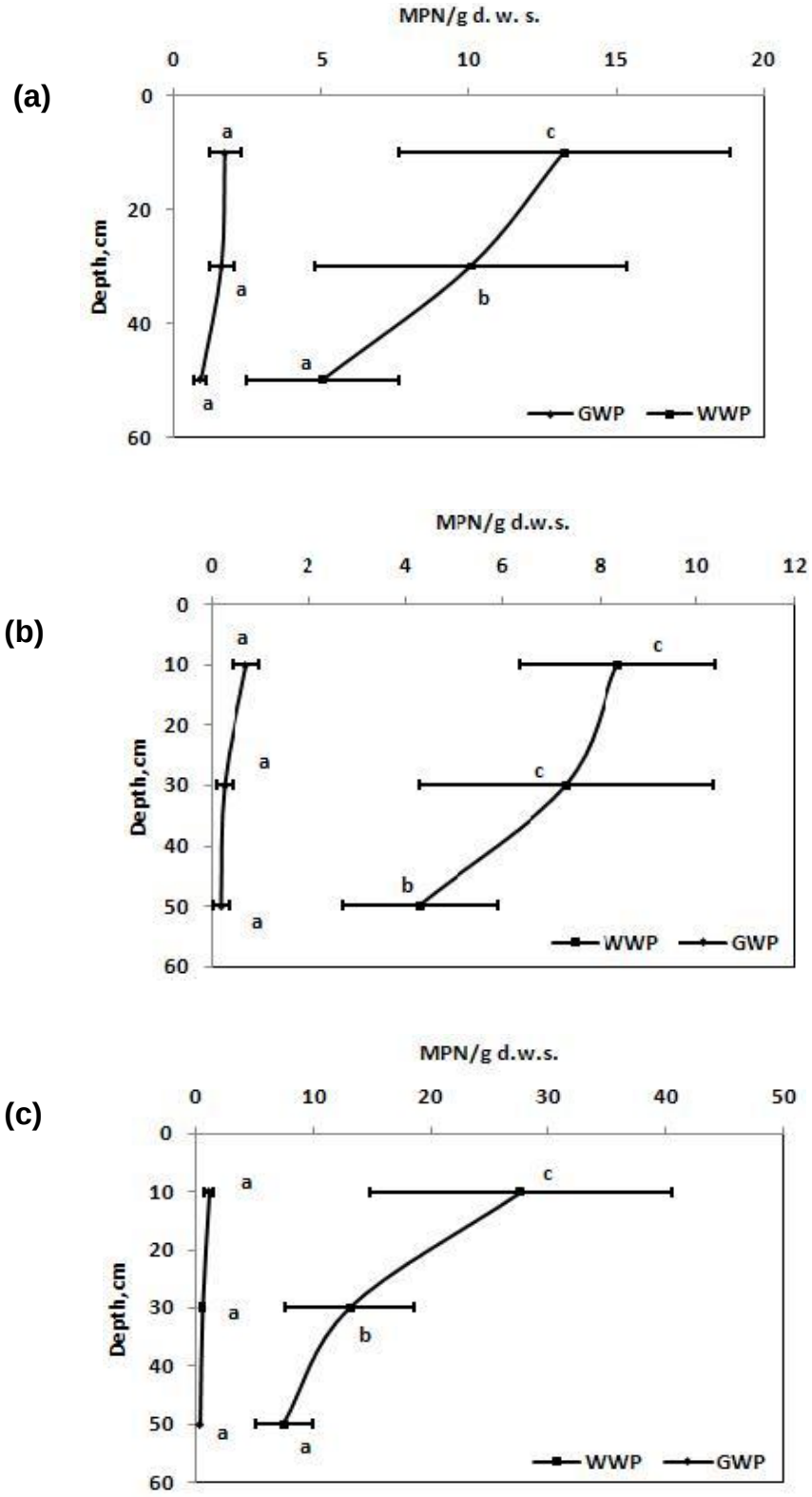

Figure 3. Fecal coliforms (a), *Escherichia coli* (b) and fecal Streptococci (c) concentrations as affected by the vertical (D1: 0-20 cm, D2: 20-40 cm and D3: 40-60 cm) and the horizontal (H1: 0 cm/drip, H2: 50 cm/drip and H3: 150 cm/drip) soils transects. Horizontal bars represent the standard deviation (n = 3 replicates for soil sample), (a, b, c ...): For each count, means followed by the same letter are not significantly different according to the Student-Newman-Keuls test at $P < 0.05$, WWP: wastewater pilot, GWP: groundwater plot.

ted with wastewater for the same period. This unexpected result could be partly explained, in the WWP, by the soil's sandy texture, which would lead to weak accumulation of organic matter in the topsoil due to the significance of mineralization process. Results published by Hidri et al. (2010) show that long-term (26 years) irrigation with wastewater significantly stimulated microbial growth by providing a nutrient source.

Soil microbiological examination for fecal coliforms, Escherichia coli and fecal streptococci

Soil microbial contamination was assessed by measuring the number of fecal coliforms, of *E. coli*, and of fecal *Streptococci* on samples taken at depths of 0-20, 20-40 and 40-60 cm, after seasonal irrigation. Results show that WWP soil was significantly ($p \leq 0.05$) more microbial polluted than GWP soil (Table 4).

A slight increase in the concentration of fecal coliforms bacteria along the studied transect (150 cm) was recorded in the WWP and reaching a maximum value in the first (0-20 cm) soil layer (from 3.78 to 22.8 MPN/g dry weight (Figure 3). Taking into account the quality of the treated wastewater (fecal coliform bacteria concentration was equal to 1.1 10^3 MPN/100 ml) and that water was distributed to the orange grove daily during the irrigation period (an average of 2.46 mm/day), the measured contamination should be considered as very slight. As reported by Campos et al. (2000) 1 day after irrigation, fecal coliforms contamination in the soil will be considerably reduced (an abatement up to four logarithmic units) depending on the quality of the wastewater and the type of irrigation system. In addition, the soil seems to be able to reduce human bacteria contamination and their associated health risk following wastewater irrigation. From this point of view, land application could be considered as an efficient-means of wastewater disposal (Campos et al., 2000). On the other hand in GWP, soil fecal coliforms concentration was negligible (ranging from 1.3 to < 1 MPN/g dry weight). This low contamination could be ascribed to grazing, common in the experimental site, and considered as a non-point (diffuse) source of contamination together with roaming wild animals and birds and runoff from agriculture areas (Palese et al., 2009)

Fecal coliforms prevalence was detected especially in the upper soil layer (0-20 cm) particularly at the beginning point of the studied transect (0 cm/ dripper) (Figure 3a). Decreasing concentrations were observed according to soil depth and values measured at the deepest levels were negligible (ranging from 5 to < 1 MPN/g dry weight that is equivalent to no contamination). We infer this result in considering that soil matrix act as a filter, and so reducing the bacterial concentration in the deeper soil layers. These findings are in agreement with the results of Palese et al. (2009) and Oran et al. (2001). In particular, Oran et al. (2001) observed a gradual reduction of fecal coliform concentration through the soil profile, a silty clay type, and a complete disappearance of contamination beyond the limit of 25 cm, when the raw wastewater bearing 1000 CFU of coliforms per 100 ml was used for irrigation.

E. coli contamination followed the same trend in both wastewater and ground water used for irrigation soils even if in the former they showed higher contents (Figure 3b). Such enrichment was clearly due to the distribution of wastewater which, during the experimental period, equal to 9.1 10^2 MPN/100 ml. A very slight *E. coli* contamination (ranging from 1.3 to < 1 MPN/g dry weight soil) was recorded in soils sampled from the ground water plot (GWP).

As reported for fecal coliforms, *E. coli* was present particularly in the upper soil layers (0-20 cm) peaking at the beginning point of the studied transect (0 cm/dripper); in the other layers (20-40 and 40-60 cm) *E. coli* concentration tended to decrease with depth close to negligible values (always < 1 MPN/g dry weight in the deepest levels). Straining, depending on the soil pores and bacterial size, and adsorption onto soil particles are the most important factors influencing bacteria transport through the soil (Campos et al., 2000; Oran et al., 2001). Furthermore, the presence of channels due to plant root systems and earthworm burrows can strongly influence vertical migration of pathogens through the soil profile (Joergensen et al., 1998). On the other hand, the correct irrigation management (low water volumes distributed daily by a drip irrigation system according to soil hydrological and physical parameters and climatic pattern) and the intense water absorption by roots of both trees and cover crops, active in the wetted soil volume, excluded water logging by runoff and percolation to deeper soil layers avoiding aquifer pollution by fecal bacteria (Palese et al., 2009).

A significant increase ($p = 0.01$) in the concentration of fecal *Streptococci* was recorded in the WWP reaching a maximum at the beginning point of the studied transect (Table 4). This result probably reflects the high numbers of fecal bacteria present in the wastewater used for irrigation. It is believed that the use of less contaminant irrigation methods or better quality effluents might further reduce the risk of transmission of fecal bacteria pathogens (Al-Lahham et al., 2003).

Fecal *Streptococci* prevalence was detected especially in the upper soil layer (0-20 cm). Decreasing concentrations were observed according to soil depth and fecal *Streptococci* values measured at the deepest levels which is negligible (Figure 3c). The observed stratification of microbial biomass according to soil depth corres-ponded and correlated generally to the decrease in C and N organic contents, as classically observed Lejon et al. (2007).

Conclusion

After more than 10 years of treated wastewater drip irrigation, and compared to soils irrigated with increase

Table 4. Fecal coliforms, *Escherichia coli* and fecal streptococci counts in soils from the studied sites.

Sample	Fecal coliforms (MPN .g $^{-1}$ dry weight soil)	*Escherichia coli* (MPN .g $^{-1}$ dry weight soil)	Fecal *Streptococci* (MPN .g $^{-1}$ dry weight soil)
WWH1P1	19±4.35^{a}	9.69±1.48^{a}	36.73±12.06^{a}
WWH1P2	15.52±4.45^{b}	8.73±2.52^{a}	30.39±12.32^{a}
WWH1P3	7.58±1.43^{cd}	6.63±0.62^{ab}	15.68±4.49^{bc}
WWH2P1	13.3±1.65^{b}	9.5±4.11^{a}	19±4.35^{b}
WWH2P2	9.89±1.51^{c}	7.50±1.42^{ab}	12.61±1.68^{bcd}
WWH2P3	3.00±1.10^{e}	4.9±1.19^{bc}	7.58±1.43^{cd}
WWH3P1	7.35±1.39^{cd}	5.42±1.15^{bc}	8.26±0.99^{cd}
WWH3P2	4.72±1.30^{de}	4.85±1.18^{bc}	9.31±2.20^{cd}
WWH3P3	4.48±2.61^{de}	2.61±1.05^{cd}	4.77±1.32^{d}
GWH1P1	2.26±0.34^{e}	0.88±0.38^{d}	1.54±0.30^{d}
GWH1P2	1.79±0.23^{e}	0.74±0.11^{d}	0.93±0.11^{d}
GWH1P3	0.95±0.23^{e}	0.49±0.17^{d}	0.75±0.11^{d}
GWH2P1	1.75±0.30^{e}	0.31±0.05^{d}	0.72±0.11^{d}
GWH2P2	1.89±0.40^{e}	0.13±0.22^{d}	0.48±0.17^{d}
GWH2P3	1.01±0.11^{e}	0.33±0.06^{d}	0.33±0.06^{d}
GWH3P1	1.19±0.30^{e}	0.22±0.19^{d}	0.47±0.16^{d}
GWH3P2	1.15±0.17^{e}	0.10±0.17^{d}	0.32±0.06^{d}
GWH3P3	0.69±0^{e}	0.23±0.20^{d}	0.29±0^{d}

(a, b, c, ...): For each count, means followed by the same letter are not significantly different according to the Student-Newman-Keuls test at P = 0.05. WW, wastewater; GW, groundwater; H1: 0 cm/drip, H2: 50 cm/drip and H3: 150 cm/drip, horizontal transect; D1: 0-20 cm, D2: 20-40 cm and D3: 40-60 cm; vertical transect. Each value is the mean of 3 replicates; ND = non-detectable.

groundwater, no negative changes have been observed in the evaluated soil properties with the exception of an in soil microbial biomass (heterotrophic bacteria and filamentous fungi) and in soil fecal indicator bacteria (coliforms, *Escherichia coli* and *Streptococci*) essentially around the emitter. This growth of microorganisms might be explained by the ready source of easily degradable compounds in the oligotrophic soil environment brought almost certainly by wastewater irrigation. Indeed, microorganisms are mainly heterotrophic and carbon-limited in soil and the observed differences could be due to a higher availability and quality of the carbon source supplied by wastewater irrigation. This would lead to a transitory increase in soil microorganisms with a significant resiliency due to the rapid decrease in the stock of fresh organic matter (Lejon et al., 2007).

Thus, treated wastewater use in irrigation could have positive effects, not only in aspects of soil quality (organic content), but also in social terms, as it allows the maintenance of irrigated agriculture in areas where groundwater has been polluted by seawater intrusion. In these sites, treated municipal wastewater seems to be an alternative water resource for citrus tree irrigation with a correct salts management. However, studies of different types of wastewater and soils are needed before these results can be generalized, because changes in microbial community are also considerably influenced by soil type and certain agricultural practices.

ACKNOWLEDGEMENTS

The authors would like to thank Céline Faivre et Mélanie Lelièvre (UMR Microbiologie du Sol et de l'Environnement - INRA/Université de Bourgogne) for excellent technical assistance. The present study is a part of “EC2CO” program which is supported by the French Ministry of Research and INSU.

REFERENCES

Al-Lahham O, El Assi NM, Fayyad M (2003). Impact of treated wastewater irrigation on quality attributes and contamination of tomato fruit. Agric. Water Manage. 61(1): 51-62.

Al-Shammiri M, Al-Saffar A, Bohamad S, Ahmed M (2005). Wastewater quality and reuse in irrigation in Kuwait using microfiltration technology in treatment. Desalination 185(1-3): 213-225.

Angelakis AN, Marecos MHF, Bontoux L, Asano T (1999). The status of wastewater reuse practice in the Mediterranean Basin: need for guidelines. Water Res. 33(10): 2201-2217.

APHA (1998). Standards Methods for the Examination of Water and Wastewater. 20th ed. American Public Health Association, Washington DC.

Appelo CAJ, Postma D (1993). Groundwater and pollution, Geochem J. Rotterdam: Balkema. 536 p.

Campos C, Oron G, Salgot M, Gillerman L, Casals G (2000). Attenuation of microorganisms in the soil during drip irrigation with waste stabilization pond effluent. Water Sci. Technol. 11(42): 387-392.

Campos C (2008). New perspectives on microbiological water control for wastewater reuse. Desalination 218(1-3): 34-42.

Candela L, Fabregat S, Josa A, Suriol J, Vigués N, Mas J (2007). Assessment of soil and groundwater impacts by treated urban wastewater reuse. A case study: Application in a golf course (Girona,

Spain). Sci. Total Environ. 374(1): 26-35.
Cohen S, Svrjcek A, Durborow T, Barnes L (1999). Water quality impacts of golf courses. J. Environ. Qual. 28: 798-809.
Dère C, Lamy I, Jaulin A, Cornnu S (2006). Long term fate of exogenous metals in a sandy Luvisol subjected to intensive irrigation with raw wastewater. Environ. Pollut. 15: 1-10.
Gloaguen TV, Forti MC, Lucas Y, Montes CR, Gonçalves RA, Herpin UW, Melfi AJ (2007). Soil solution chemistry of a Brazilian Oxisol irrigated with treated sewage effluent. Agric. Water Manage. 88(1-3): 119-131.
Haas C (1996). How to average microbial densities to characterize risk, Water Res. 30(4): 1036-1038.
Haruvy N (2006). Reuse of wastewater in agriculture-economic assessment of treatment and supply alternatives as affecting aquifer pollution. NATO Security though Sci. Series. 5: 257-262.
Hidri Y, Bouziri L, Maron P, Anane M, Jedidi N, Hassen, Ranjard L (2010). Soil DNA evidence for altered microbial diversity after long-term application of municipal wastewater. Agron. Sustain. Dev. 30 (2): 423-431.
Huertas E, Salgot M, Hollender J, Weber S, Dott W, Khan S, Schafer A, Messalem R, Bis B, Aharoni A, Chikurel H (2008). Key objectives for water reuse concepts. Desalination. 218(1-3): 120-131.
Joergensen RG, Kuntzel H, Scheu S, Seitz D (1998). Movement of fecal indicator organisms in earthworm channels under a loamy arable and grassland soil. Appl. Soil Ecol. 8(10): 1–10.
Leckie SE, Prescott CE, Grayston SJ, Neufeld JD, Mohn WW (2004). Comparison of chloroform fumigation-extraction, phospholipid fatty acid, and DNAmethods to determine microbial biomass in forest humus. Soil Biol. Biochem. 36(3): 529-532.
Lejon DPH, Chaussod R, Ranger J, Ranjard L (2005). Microbial community structure and density under different tree species in an acid forest soil (Morvan, France). Microb. Ecol. 50: 614-625.
Lejon DPH, Sebastia J, Lam I, Nowak V, Chaussod R, Ranjard L (2007). Relationship between soil organic status and microbial density and genetic structure in two agricultural soils submitted to various organic managements. Microb. Ecol. 53: 650-653.
Martinez-Beltran (1999). Irrigation with saline water: benefits and environmental impact. J. Agric. Water Manag. 40(2-3): 183-194.
Mohamed MJ, Mazahreh N (2003). Changes in soil fertility parameters in response to irrigation of forage crops with secondary treated wastewater. Comm. Soil Sci. Plant Anal. 34(9-10): 1281-1294.
NAWM (2001). The national agency for wastewater management. Yearly report of the exploitation of purification stations. p. 52.
NAWM (2002). The national agency for wastewater management. Yearly report of the exploitation of purification stations. p. 44.
Oron G, Armon R, Mandelbaum R, Manor Y, Campos C, Gillerman L, Salgot M, Gerba C, Klein I, Enriquez C (2001). Secondary wastewater disposal for crop irrigation with minimal risks. Water Sci. Technol. 43(10): 139-146.
Palese AM, Pasquale V, Celano G, Figliuolo G, Masi S, Xiloyannis (2009). Irrigation of olive groves in Southern Italia treated municipal wastewater: Effects on the microbiological quality of soil and fruits. Agriculture Ecosys. and Environ. 129(1): 43-51.
Ramirez-Fuentes E, Lucho-Constantino C, Escamilla-Silva E, Dendooven L (2002). Characteristics, carbon and nitrogen dynamics in soil irrigated with wastewater for different lengths of time. Bioresource Technol. 85(2): 179-187.
Ranjard L, Lejon DPH, Mougel C, Schehrer L, Merdinoglu D, Chaussod R (2003). Sampling strategy in molecular microbial ecology : influence of soil sample size on DNA fingerprinting analysis of fungal and bacterial communities. Environ. Microb. 5: 1111-1120.
Rusan MJM, Hinnawi S, Roussan L (2007). Long term effect of wastewater irrigation of forage crops on soil and plant quality parameters. Desalination. 215(1-3): 143-152.
Sheng Z (2005). An aquifer storage and recovery system with reclaimed wastewater to preserve native groundwater resources in El Paso, Texas. J. Environ. Manag. 75(4): 367-377.
Shetty S (2004). Treated wastewater use in Tunisia: Lessons Learned and the Road Ahead. The World Bank. Jakarta. Indonesia. 10: 163-172.
Takashi A (1994). Irrigation with treated sewage effluents. Adv. Series in Agricultural Science. 22: 199-228.
Tarchouna LG, Merdy P, Raynaud M, Pfeifer H, Lucas Y (2010). Effects of long-term irrigation with treated wastewater. Part 1: Evolution of soil physico-chemical properties. Appl. Geochem. 25: 1703-1710.
Weber S, Khan S, Hollander J (2006). Human risk assessment of organic contaminants in reclaimed wastewater used for irrigation. Desalination 187: 53-64.
WHO (1998). Health guidelines for the use of wastewater in agriculture and aquaculture: Report of a WHO Scientific Group. WHO Technical Report Series 778. World Health Organization, Geneva, Switzerland. p. 74.