International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:8, No:4, 2014

Use of Treated Municipal Wastewater on Artichoke Crop

Disciglio G., Gatta G., Libutti A., Tarantino A., Frabboni L., Tarantino E.

Abstract**—**Results of a field study carried out at Trinitapoli (Puglia region, southern Italy) on the irrigation of an artichoke crop with three types of water (secondary-treated wastewater, SW; tertiary-treated wastewater, TW; and freshwater, FW) are reported. Physical, chemical and microbiological analyses were performed on the irrigation water, and on soil and yield samples.

The levels of most of the chemical parameters, such as electrical conductivity, total suspended solids, Na^+, Ca^{2+}, Mg^{+2}, K^+, sodium adsorption ratio, chemical oxygen demand, biological oxygen demand over 5 days, NO_3 –N, total N, CO_3^{2}, HCO_3, phenols and chlorides of the applied irrigation water were significantly higher in SW compared to GW and TW. No differences were found for Mg^{2+}, PO_4-P, K^+ only between SW and TW. Although the chemical parameters of the three irrigation water sources were different, few effects on the soil were observed. Even though monitoring of *Escherichia coli* showed high SW levels, which were above the limits allowed under Italian law (DM 152/2006), contamination of the soil and the marketable yield were never observed. Moreover, no *Salmonella* spp. were detected in these irrigation waters; consequently, they were absent in the plants. Finally, the data on the quantitative-qualitative parameters of the artichoke yield with the various treatments show no significant differences between the three irrigation water sources. Therefore, if adequately treated, municipal wastewater can be used for irrigation and represents a sound alternative to conventional water resources.

Keywords**—**Artichoke, soil chemical characteristics, fecal indicators, treated municipal wastewater, water recycling.

I. Introduction

RE-USE of treated municipal wastewater in agriculture is widespread around the world. Indeed, unconventional water resources often represent an important contribution to the solving of the ever-increasing problems of water scarcity, particularly in the Mediterranean areas, and in the Puglia region (southern Italy). In Puglia, water re-use for agriculture needs to be a top priority, as more than 65% of the water resources are allocated to irrigation.

The use of unconventional water resources, such as wastewater, is essential in this sector, which suffers water shortages, and excessive and often uncontrolled groundwater exploitation, with the resulting sea water intrusion into the groundwater of the region. Municipal wastewater is potentially the most useable, because of its reliability as a supply (i.e., it is only slightly influenced by droughts), its allocation (in inland areas it is often available close to agricultural land), its composition (toxic compounds and salt concentrations are generally tolerable in various land and crop conditions), and the diffusion of treatment plants (imposed by regulations on effluent disposal) [1].

A large number of studies [2]-[8] have shown that microbiological contamination remains a crucial issue to ensure the safe use of municipal wastewater in agriculture. Many kinds of microorganisms, including viruses, bacteria and pathogenic protozoans/helminths, can pose significant risks; bacteria are the most common and numerous of the microbial pathogens found in recycled waters [9], [10]. To break down or otherwise reduce the contamination of municipal wastewater, and thus to minimize the risk of crop contamination, high-technology tertiary treatments and disinfection systems are essential, such as activated carbon, reverse osmosis, membrane filtration, chlorination, ozonation, and UV irradiation [11], to ensure that microbial populations remain below critical levels.

Several studies have been carried out on herbaceous crops in southern Italy environments, to evaluate the effects of the re-use of treated wastewater for irrigation on the physical, chemical and microbiological properties of the soil, and on the edible plant products. In a multi-year study carried out on a rotation of vegetable crops (processing tomato, fennel and lettuce) with two types of water (conventional and treated wastewater), no significant differences in crop production and accumulation of heavy metals in the soil and plants were detected [12].

Moreover, data have shown that the re-use of wastewater characterized by an *Escherichia coli* count that exceeds the threshold allowed by Italian law and applied through different irrigation methods has never resulted in fecal pollution of the soil and the marketable yield. Despite the bacterial contributions to treated wastewater, the contamination level of the soil is limited due to its natural and high capacity to reduce the bacterial load.

The probability of contracting an infection and/or an illness due to ingestion of vegetable products irrigated with urban treated wastewater, as calculated with the Beta-Poison model, was negligible and equal to 1 person every 100 million exposed people [13]. In another study on tomato and potato crops [14], the data showed that irrigation water quality had no impact on product quality. The two compared irrigation methods had no significant influences on marketable yield and microbiological pollution of edible parts of the vegetables and on the soil. Despite the increasing number of studies on wastewater re-use for herbaceous crops, no experimental data on artichoke are available.

Disciglio G., Gatta G., Libutti A., Tarantino A., Frabboni L., and Tarantino E. are with the Department of Science of Agriculture, Food and Environment, University of Foggia - 71122 Foggia, Italy (phone: +39 0881589127; e-mail of corresponding author: grazia.disciglio@unifg.it).

The main purpose of the present study was to monitor the physicochemical and microbial impact of drip irrigation with two different municipal wastewater qualities (secondary-treated wastewater, SW; and tertiary-treated wastewater, TW) compared to freshwater (FW) on both soil properties and artichoke yield in southern Italy.

TABLE I
INITIAL PHYSICOCHEMICAL CHARACTERISTICS OF THE SOIL (0-30 CM DEPTH) AT THE EXPERIMENTAL FIELD

Sand (2.0> Ø <0.02 mm)	(%)	46.1
Loam (0.02> Ø <0.002 mm)	(%)	37.5
Clay (Ø < 0.002 mm)	(%)	16.4
pH (in H_2O)		8.1
EC_e	($dS\ m^{-1}$)	0.90
Ca^{2+}	($mg\ kg^{-1}$)	3510
Mg^{2+}	($mg\ kg^{-1}$)	380
Na^{+}	($mg\ kg^{-1}$)	1120
Sodium adsorption rate (SAR)		4.6
K^{+}	($mg\ kg^{-1}$)	820
NO_3-N^-	($mg\ kg^{-1}$)	7.93
NH_4-N	($mg\ kg^{-1}$)	3.81
P_2O_5 (Olsen)	($mg\ kg^{-1}$)	202
Total nitrogen (Kjeldhal)	(‰)	1,0
Organic matter (Walkley-Black)	(%)	1.9
Moisture at field capacity (- 0.03 MPa)	(%)	24.0
Moisture at wilting point (-1.5 MPa)	(%)	9.8
Bulk density	($t\ m^{-3}$)	1.25
Bulk density	($t\ m^{-3}$)	

II. MATERIAL AND METHODS

A. Site Description and Climate Parameters

The trial was carried out at Trinitapoli (Puglia region, southern Italy), with the aim to compare the effects of the three types of irrigation water sources (FW, SW and TW) on the soil during a cropping cycle (from May 2012 to May 2013) of globe artichoke (*Cynara cardunculus* L., subsp. *scolymus*), cv. Violetto of the Provenza type. The trial was carried out on a sandy clay loam soil (U.S.D.A. classification), and the main physical and chemical characteristics of the soil are reported in Table I.

The site is characterized by the typical Mediterranean climate, with a long-term average annual rainfall of 560 mm, with two thirds concentrated from fall to winter. The daily rainfall, minimum and maximum temperatures and Class "A" pan evaporation were monitored during the experimental period. The agro-climate data were supplied by *Consorzio di Bonifica della Capitanata* [15] as recorded at the nearest station, a few kilometers from the experimental site.

The monthly means of the minimum and maximum temperatures, total rainfall, and Class "A" pan evaporation monitored during the growing cycle of the artichoke are reported in Table II.

TABLE II
MONTHLY MAXIMUM AND MINIMUM TEMPERATURES, TOTAL RAINFALL AND TOTAL CLASS "A" PAN EVAPORATION DURING THE ARTICHOKE CROP CYCLE (MAY 2012 -MAY 2013)

	May 2012	June	July	Aug	Sept	Oct	Nov	Dec	Jan 2013	Feb	March	April	May
T_{max} (°C)	30.1	35.7	40.1	38.8	34.6	28.1	24.1	18.2	16.5	20.1	23.4	29.4	28.9
T_{min} (°C)	5.9	12.4	16.3	14.5	7.7	4.6	3.8	2.5	-0.8	-3.3	-2.18	3.2	6.2
Rainfall (mm)	9.2	0	10.2	0.5	145.7	31.0	134.2	80.7	59.2	43.7	54.8	32.2	66.7
E (mm)	118.0	151.9	156.8	142.4	75.4	43.2	18.3	13.5	14.89	23.0	47.7	81.4	108.9

During the trial, the monthly values of the mean of the maximum temperature varied between 16.5°C, in January 2013, and 40.1°C, in July 2012, whereas the means of the minimum temperatures varied from -3.3°C, in February 2013, to 16.3°C, in July 2012. As expected, the rainfall mainly occurred from September 2012 to May 2013. Total Class "A" pan evaporation was 995.3mm.

B. Water Sources, Irrigation Treatments and Other Cropping Practices

Three water sources were compared in the experiment: SW, TW that originated from a public plant [16], and FW. The SW was generated after screening and grit removal of influent wastewater. It was subsequently sent to primary clarifiers, and subjected to an activated sludge process and partial aerobic stabilization of the sludge, and finally for the chemical precipitation of phosphorus, denitrification and chlorination treatments. The TW was obtained from a membrane filtration public facility located near the experimental site, where the water was primarily collected in a 180m^3 tank and pumped to the sand filter section, including five tanks with the following filling materials: 1,150kg anthracite; 45,000kg quartz sand; and 2,040kg gravel support of different diameters. The second phase of the treatment included an ultra-filtration module equipped with hollow fiber membranes (nominal porosity, 0.2µm) with a cellulose triacetate double wall (diameter, 0.8mm) at an internal pressure of 0.8-1.0 bar. Periodically, all of the lines were automatically cleaned by back flushing. The FW was generated from the Marana Capacciotti dam, as usually used by farmers for irrigation.

Reclaimed wastewater was re-used under a controlled flow rate and distribution conditions specifically aimed to avoid contamination of bordering fields and the underlying groundwater.

The experimental design was a randomized block with the three irrigation treatments (SW, TW and FW), each one with three replications. Each plot was 77.5m^2 in area, and the sampling area was 12.5m^2. The artichoke was transplanted by offshoots on May 12, 2012, in rows 1.25m apart and with

1.25m spacing in the rows, resulting in a density of 6,410 plants ha^{-1}.

Drip irrigation was adopted. The crop was irrigated when the soil water deficit (SWD) in the root zone (~0.4m) was 30% of the total available water, which resulted in soil water deficit limits for irrigation (SWD_{lim}) of 30 mm. The irrigation schedule was based on the evapotranspiration criterion [17]. Water was provided to the crops whenever the condition was met, as in (1):

$$\sum_{1}^{n}(Etc - Re) = 30 \text{ mm} \quad (1),$$

where *n* is the number of days required to reach SWD_{lim} starting from the last watering, *Etc* is the evapotranspiration (mm), and *Re* is the rainfall (mm).

Crop evapotranspiration can be expressed as in (2):

$$Etc = kp \cdot kc \quad (2),$$

where *Etc* is the Class "A" pan evaporation (mm) provided by *Consorzio di Bonifica della Capitanata*, *kc* is the crop coefficient, as reported by Tarantino and Caliandro [18], and *kp* is the pan coefficient (0.8).

Fifteen watering events with a seasonal water volume of 3,000 m^3 ha^{-1} took place. All of the other agricultural practices (e.g., fertilization, weed and pest control) were those usually applied by local farmers.

As well as adopting drip irrigation, the measures taken to minimize possible risks to farm workers included general protective clothing, such as disposable gloves and boots. Any potential risk to the public was avoided by destroying the yield after sampling.

C. Water, Soil and Plant Sampling

Irrigation water samples of SW, TW and FW were taken and analyzed on six dates during the experimental period (May 23, September 28, October 16 and November 19, 2012; January 21, and May 13, 2013) to characterize their physicochemical and microbiological parameters.

The samples were collected randomly in three replications using 1000-ml sterile glass bottles, and they were transported in refrigerated bags until arrival at the laboratory for the analytical determinations. They were stored in a refrigerator at +4°C and examined within 24h of collection.

Soil samples were collected from each plot, using a soil auger, before transplanting time (on March 3, 2012) and during the artichoke crop cycle, on four dates (October 16, and November 19, 2012, January 21, and May 13, 2013), at 0 cm to 30 cm in depth (root density zone) in the areas wetted by the drippers.

Harvesting of the artichoke buds was scalar, at varied dates, and on a 15-day-interval basis, starting from November 11, 2012, until May 20, 2013. At each sampling in each plot, three basal leaves were also collected for microbiological testing.

D. Water and Soil Chemical Analyses

The irrigation water samples were analyzed in triplicate, according to the Italian standard methods [19] that refer to the common international methods [20], for the following physicochemical parameters: pH, electrical conductivity (EC_w; dSm^{-1}), total suspended solids (TSS; mg l^{-1}), biological oxygen demand over 5 days (BOD_5; mg l^{-1}), chemical oxygen demand (COD; mg l^{-1}), ammonium-nitrogen (NH_4-N; mg l^{-1}), nitrate-nitrogen (NO_3-N; mg l^{-1}), nitrite-nitrogen (NO_2-N; mg l^{-1}), phosphorus (PO_4-P; mg l^{-1}), sodium (Na^+; mg l^{-1}), calcium (Ca^{2+}; mg l^{-1}), magnesium (Mg^{2+}; mg l^{-1}), potassium (K^+; mg l^{-1}), carbonates (CO_3^{2-}; mg l^{-1}), bicarbonates (HCO_3^-; mg l^{-1}), sulfate (SO_4^-; mg l^{-1}), sodium adsorption ratio (SAR), phenols (mg l^{-1}), sulfates (mg l^{-1}), chlorides (mg l^{-1}) and fluorides (mg l^{-1}). The pH was measured with a GLP 22+ pH and Ion-Meter, CRISON, and the electrical conductivity with a GLP 31+ EC-Meter, CRISON. The sodium, calcium, magnesium and potassium levels were determined using ion-exchange chromatography (Dionex ICS-1100; Dionex Corporation, Sunnyvale, CA, USA). The TSS were determined after filtration of the water samples with a vacuum system through 0.45-µm pore size (47-mm-diameter) nitrocellulose membranes (Whatman, Maidstone, UK). The sodium adsorption ratio (SAR) was calculated using (3) [21]:

$$SAR = (Na^+)/[(Ca^{2+}+Mg^{2+})/2]^{1/2} \quad (3),$$

where Na^+, Ca^{2+} and Mg^{2+} are the sodium, calcium and magnesium concentrations, respectively, of the water samples, in meq l^{-1}.

The soil samples were air-dried and passed through a 2-mm sieve, and the main chemical parameters of agronomic interest were analyzed. Soil sub-samples from each depth were analyzed for pH, Ca^{2+}, Mg^{2+}, SAR, EC, nitrate-nitrogen (NO_3-N), ammonium-nitrogen (NH_4-N), available phosphorus (P_2O_5) potassium (K_2O) and organic matter (OM). The EC and pH were measured in 1:2 (w/v) and 1:2.5 (w/v) aqueous soil extracts, respectively. P_2O_5 was determined using the sodium bicarbonate method [22]. The concentrations of soluble Na^+, Ca^{2+}, Mg^{2+} and K^+ were analyzed using atomic absorption spectrometry (Perkin-Elmer Atomic Absorption Spectrophotometer – model 2380). Total organic carbon (TOC) was detected by oxidation with a potassium dichromate-titration of $FeSO_4$, according to the Walkley-Black method [23]. The OM was determined by multiplying the percentage of the organic carbon by the factor 1.724. Soluble NO_3-N and NH_4-N were determined according to Keeney and Nelson [24].

E. Yield and Buds Qualitative Analysis

At harvest, the buds of the first and second order were analyzed. At each harvest date, the marketable yield, as the total number of buds (No.) and the number of buds per plant (No. $plant^{-1}$), were counted. Also, on a sample of ten buds from each plot, the dry matter (%), mean weight (g), equatorial and longitudinal diameters (cm), and nitrate content were measured.

F. Microbiological Analysis

Microbiological analysis of the irrigation water sources (FW, SW and TW) was performed for *E. coli*, fecal coliforms, and *Salmonella*, as useful indicators for contamination, because of their resistance to disinfection and environmental factors and their ability to survive for long periods in the environment [25]-[27].

Water samples were analyzed by the membrane filtration method. Triplicate aliquots of 100, 10, 1 and 0.1ml of each water sample were filtered through 0.45-µm pore size (47mm diameter) nitrocellulose membranes (Whatman, Maidston UK). For *E. coli* enumeration, the membranes were placed on TBX agar (Oxoid, London, UK) and incubated at 37°C for 24h. For fecal coliforms, the membranes were placed onto Slanetz and Bartley agar (Oxoid, UK) and incubated at 37°C for 48 h.

Bacteriological indicators of the irrigation water, soil leaves and bud samples, included *E. coli*, fecal coliforms, and *Salmonella* spp. were determined. The analyses were conducted using the spread plate method, as follows: 25.0 g of each sample was weighted and diluted in 225.0 ml buffered peptone water (BPW), placed in a stomacher, homogenized for 180 s, and stored at room temperature for 30 min, to allow bacterial cell recovery. Then, serial ten-fold dilution in BPW were spread onto agar plates, containing C-EC agar (Biolife) for fecal coliforms, and TBX for *E. coli*. The plates were incubated with different incubation temperatures, as follows: 37°C for *E. coli*, and 44°C for fecal coliforms. Incubations were for 24h for *E. coli* and 48 h for fecal coliforms.

The same water samples were also analyzed for *Salmonella* spp. Detection was performed following the UNI EN ISO 19250:2013 procedure.

The densities of colonies were expressed as CFU 100 ml^{-1} for water, CFU g^{-1} for soil, and CFU g^{-1} for buds.

G. Statistical Analysis

All of the data were analyzed by analysis of variance (ANOVA) followed by Tukey's *post-hoc* tests for significant values. Standard errors (SEs) were also calculated. Values of $p<0.05$ were considered as statistically significant. All of the analyses were performed using the JMP software [28].

III. Results And Discussion

A. Irrigation Water Characteristics

Table III shows the mean values, standard errors and significances of the different irrigation water characteristics (FW, SW and TW) and the parameter limits allowed under Italian law [29] for wastewater re-use in agriculture.

The physical and chemical characteristics varied considerably among the three sources of irrigation water used. As expected, the levels of most of the chemical parameters, such as EC, TSS, Na^+, Ca^{2+}, SAR, COD, BOD_5, total N, CO_3^{2-}, HCO_3^-, phenols, and chlorides were significantly higher in SW compared to GW and to TW. No differences were found for Mg^{2+}, PO_4-P and K^+ between SW and TW. The other analyzed parameters showed similar concentrations in all of the three kinds of irrigation water.

Moreover, the values for the main physicochemical characteristics of SW and TW meet the Italian standard for wastewater re-use, except for NO_3-N (3.8 and 2.5mg l^{-1}, respectively), phenols (0.7 and 0.3mg l^{-1}, respectively) and BOD_5 (only for SW, as 30.7mg l^{-1}).

The presence of phenols in SW and TW does not seem to be a limiting factor for the wastewater use, since they are quickly degraded by soil microorganisms. With regard to the constant presence of fertilizing elements, such as nitrogen, phosphorus and potassium in SW and TW, their supply is beneficial as plant nutrients and thus they deserve to be taken into account in the fertilization practice.

TABLE III
MEAN VALUES AND STANDARD ERRORS OF PHYSICAL AND CHEMICAL PROPERTIES OF IRRIGATION SECONDARY-TREATED WASTEWATER (SW), TERTIARY-TREATED WASTEWATER (TW), FRESHWATER (FW) AND THE ITALIAN THRESHOLD VALUES FOR WASTEWATER IRRIGATION REUSE (MD 152/06)

PARAMETERS §	MD 152/06	SW	TW	FW	Significance
pH	6-9.5	7.8 ± 0.1	8.0 ± 0.03	8.0 ± 0.1	ns
ECw (dS m^{-1})	3	13.4 ± 0.8	1.3 ±9.5	0.62 ± 19.6	*
TSS (mg l^{-1})	10	34.6 ± 11.1	5.8 ± 1.2	4.6 ± 0.9	*
Na^+ (mg l^{-1})		126.9 ± 11.2	119.6 ±10.9	48.2 ± 6.9	*
Ca^{2+} (mg l^{-1})		68.6 ± 2.5	67.7 ± 2.3	58.6 ± 4.4	*
Mg^{2+} (mg l^{-1})		19.6 ± 1.4	21.0 ± 1.5	13.6 ± 1.0	*
SAR	10	3.2 ± 0.4	3.0 ± 0.2	1.3 ± 0.4	*
COD (mg l-^{1})	100	59.1 ± 8.8	35.6 ± 3.57	11.5 ± 3.3	*
BOD_5 (mg l^{-1})	20	30.7 ± 7.3	17.4 ± 2.6	8.0 ± 2.3	*
NO_3-N (mg l^{-1})	2	3.8 ± 1.2	2.5 ± 0.46	0.1 ± 0.03	*
NH_4-H (mg l^{-1})		0.2 ± 0.03	0.3 ± 0.1	1.1 ± 0.2	ns
Total N (mg l^{-1})	35	23.5 ± 4.6	19.2 ± 4.4	1.9 ± 0.2	*
Phenols (mg l^{-1})	0.1	0.7 ± 0.1	0.3 ± 0.02	0 ± 0.0	*
CO_3^- (mg l^{-1})		340.4 ± 19.4	311.6 ± 9.9	175.7 ± 7.8	*
HCO_3^- (mg l^{-1})		528.2 ± 33.8	459.0 ± 15.5	238.7 ± 7.2	*
PO_4-P (mg l^{-1})	10	6.2 ± 0.4	7.3 ± 0.75	0.2 ± 0.0	*
K^+ (mg l^{-1})		22.6 ± 4.7	22.8 ± 4.8	0.3 ± 0.6	*
Sulphates (mg l^{-1})	500	86.3 ± 8.7	85.0 ± 8.9	77.3 ± 9.1	ns
Chlorides (mg l^{-1})	1200	438.7 ± 137.1	375.1 ± 110.38	73.8 ± 11.8	*
Fluorides (mg l^{-1})	1.5	0.4 ± 0.05	0.4 ± 0.03	0.6 ± 0.0	ns

§ The mean values (and standard errors) for each trait were determined on 18 samples for each type of irrigation water
* Statistically significant at p 0<0.05 level of significance

TABLE IV
MEAN VALUES OF MAIN CHEMICAL PARAMETERS MEASURED ON SOIL SAMPLES COLLECTED AT A 30-CM DEPTH IN THE TEST FIELD IRRIGATED WITH SECONDARY-TREATED WASTEWATER (SW), TERTIARY-TREATED WASTEWATER (TW) AND FRESHWATER (FW)

Treatments	pH (in H_2O)	EC (dS m^{-1})	NO_3-N (mg/kg)	NH_4-H (mg/kg)	P_2O_5 (mg/kg)	OM (%)
SW	8.2	1.1	13.1	10.8	284.0	2.1
TW	8.2	0.9	11.9	10.2	280.8	2.1
FW	8.3	0.8	12.8	10.0	236.2	1.9
Significance	ns	ns	ns	ns	ns	ns

ns, not significant difference, according to Tukey's test (ANOVA)

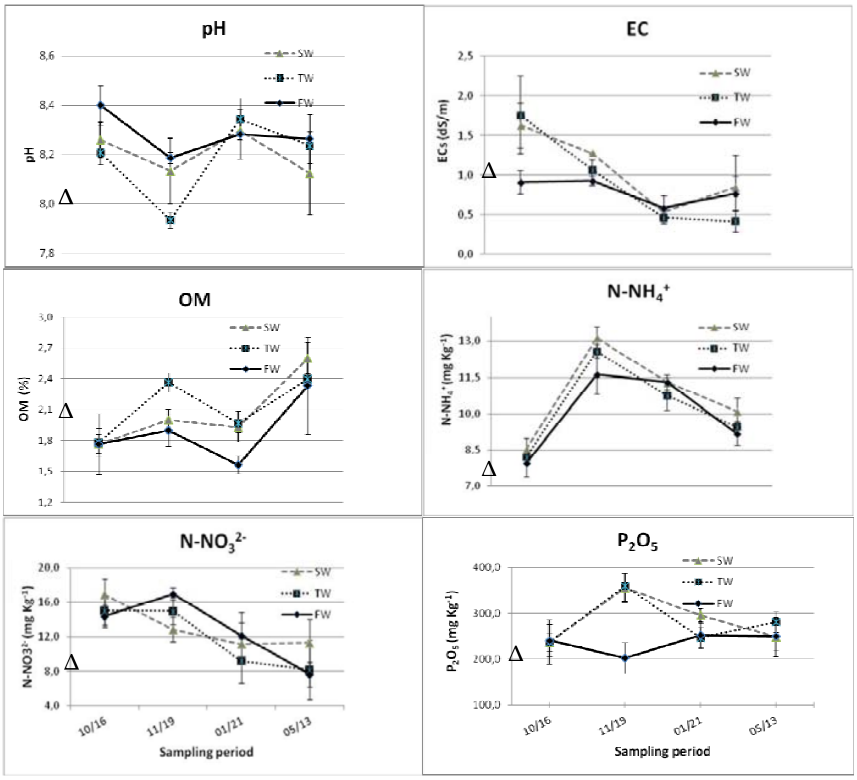

Fig. 1 Variations of the physicochemical parameters in soil before the crop cycle and irrigation season on March 16, 2012 (Δ values reported on the ordinate axis) and during the growing cycle of the artichoke for four dates (October 16, November 11, 2012; January 21, May 13, 2013) irrigated with the three types of water (FW, SW and TW)

B. Effect of Irrigation Water Type on Chemical Characteristics of Soil

As previously reported, the three types of water (FW, GW and TW) were provided to the test field by drip irrigation.

Despite the differences in the chemical parameters of the three types of irrigation water sources, few effects on the soil were observed. On average, the main chemical values of the soil were not significantly different among the three irrigation treatments (Table IV); conversely, very small differences were noted among the treatments at each sampling date (Fig. 1). This could be due to the influence of climate and agronomic practices, including fertilization and irrigation during the crop cycle, which led to temporary differences in the values of the chemical parameters in the soil.

Moreover, EC and NO_3-N generally tended to decrease slightly during the growing cycle of the artichoke, due to leaching of salts into the soil caused by the winter rainfall, and also to the absorption of NO_3-N by plants. Variable trends in the other parameters during the crop cycle were observed.

C. Effect of Irrigation Water Type on Quantitative-Qualitative Traits of Artichoke Yield

Table V gives the results of the effects of the irrigation waters on the productive traits of the artichoke crop. Such traits are related to all of the harvest dates (cumulative yield).

As it is clearly revealed, the three water irrigation sources did not significantly affect the quantitative-qualitative traits. The marketable yield was on average 95,420 buds per hectare and 15.4 buds per plant.

TABLE V
EFFECTS OF IRRIGATION WATER TYPE WITH SECONDARY-TREATED WASTEWATER (SW), TERTIARY-TREATED WASTEWATER (TW) AND FRESHWATER (FW) ON SOME PRODUCTIVE TRAITS OF ARTICHOKE

Treatments	MY (No. buds ha^{-1})	MYP (No. $plant^{-1}$)	DM (%)	MW (g)	ED (cm)	LD (cm)
SW	95470	15.7	11.7	102.3	5.6	8.9
TW	95390	15.0	12.1	93.6	5.2	87
FW	95400	15.4	12.2	101.5	5.1	9.0
Significance	ns	ns	ns	ns	ns	ns

MY, marketable yield; MYP, marketable yield per plant; DM, dry matter content; MW, mean weight; ED, equatorial diameter; LD, longitudinal diameter of buds. Ns, not significantly different for $p<0.05$ (Tukey's test)

D. Effect of Microbiological Indicators on Soil, Leaves and Buds

Regarding the microbial characteristics of the used irrigation water sources (SW, TW and FW), considerable differences among them was observed.

In Italy, the guidelines for crop irrigation allow the use of municipal wastewater with a contamination of *E. coli* of less than 100 colony forming units (CFU) $100ml^{-1}$ in 80% of samples, and/or 1000 CFU $100ml^{-1}$ as the maximum value of samples, whereas the presence of *Salmonella* spp. is not permitted (Decree of the Ministry for the Environment, No. 152/2006). Under the aforesaid Italian legislation, no indication is given about the maximum allowable concentration of fecal coliforms.

As expected and reported in Table VI, the mean *E. coli* number in SW was very high (1.3×10^6 $100ml^{-1}$), and above the Italian standard for wastewater re-use. Contamination of fecal coliforms in this wastewater was equally very high (1.4×10^6 CFU $100ml^{-1}$). Both *E. coli* and fecal coliform contamination of SW varied considerably during the trial period (as shown by the standard error reported in Table VI) with much higher values during the spring-summer period, with respect to the fall-winter one.

In TW, very low *E. coli* and fecal coliforms were detected (0.7×10 and 1.2×10^2, respectively), which were always below the Italian threshold values for wastewater re-use in irrigation, thereby confirming that to avoid risks to human health, for municipal wastewater reuse the tertiary treatment is recommended.

Finally, *Salmonella* spp. was always absent for all of the three irrigation water sources. Regarding the microbiological parameters of the soil, both *E. coli* and *Salmonella* spp. were never detected in all of the plots irrigated with the three water sources, whereas the plots irrigated with SW were heavily contaminated by fecal coliforms, with an mean of 6.6×10^4 CFU $100g^{-1}$. Moreover, comparing the contamination levels in water and soil, a notable reduction was observed in the soil due to the breakdown by soil. Similar data have been reported in other studies [30], [31].

On the contrary, the plots irrigated with TW and FW showed higher fecal coliforms contamination than the respective water sources, with mean values of 8.6×10^2 and 1.5×10^3 CFU $100g^{-1}$ of soil, respectively.

These data suggest that fecal coliform contamination of soil is occasional and might be attributable not only to irrigation water contamination, but also to other factors, such as roaming animals, birds and run-off [32]. Some studies [33] have verified that the weather influences the transport and dissemination of microbial agents via the rainfall and run-off, and the survival and/or growth through such factors as temperature. During the fall-winter period, which corresponds to harvesting time for artichoke, the rainfall and the low air temperature might reduce the level of contamination of the buds.

Regarding the microbiological analysis of leaves and the yield, no *E. coli* was isolated on any of the harvest dates for all of the treatments, and the level of fecal coliforms was found to be increasingly low passing from leaves to buds. In particular, these values ranged, on average, from 1.3×10^3 in FW to 1.5×10^5 in SW treatment for leaves, and from 2.6×10^2 in SW treatment and 9.8×10^2 CFU $100g^{-1}$ in TW for buds, which was not directly influenced by the water used for irrigation. Those data might be due to the lack of contact between the irrigation water and the plants. The presence of fecal coliforms on leaves and buds is likely to be due to environmental pollution, a secondary source of contamination, and to accidental contamination occurring during the sampling.

TABLE VI
AVERAGE ENUMERATION OF BACTERIAL INDICATORS AND STANDARD ERRORS OF WATER USED, SOIL, LEAVES AND BUDS OF THE THREE IRRIGATION TREATMENTS. THE MEAN VALUES (AND STANDARD ERRORS) FOR EACH TREATMENT WERE DETERMINED ON 18 SAMPLES FOR WATER AND ON 12 SAMPLES FOR SOIL, LEAVES AND BUDS

Bacterial indicators	SW		TW		FW	
Water source (CFU 100 ml^{-1})						
E. coli	1.3×10^6	$\pm5.8\times10^5$	0.7×10	$\pm0.4\times10$	0	
Fecal coliforms	1.4×10^6	$\pm5.1\times10^5$	1.2×10^2	$\pm8.3\times10$	0.2×10^2	$\pm0.5\times10$
Soil (CFU 100 g^{-1})						
E. coli	0		0		0	
Fecal coliforms	6.6×10^4	$\pm6.2\times10^4$	8.6×10^2	$\pm4.3\times10^2$	1.5×10^3	$\pm4.8\times10^2$
Artichoke leaves (CFU 100 g^{-1})						
E. coli	0		0		0	
Fecal coliforms	1.5×10^5	$\pm1.0\times10^4$	1.6×10^4	$\pm1.4\times10^4$	1.3×10^3	$\pm9.1\times10^2$
Artichoke buds (CFU 100 g^{-1})						
E. coli	0		0		0	
Fecal coliforms	2.6×10^2	$\pm 2.3\times10$	9.8×10^2	$\pm7.7\times10^2$	6.8×10^2	$\pm 2.2\times10^2$

IV. Conclusion

Although the three types of irrigation water (FW, SW and TW) applied to the artichoke crop have different physicochemical and microbiological characteristics, they did not show significant differences among the irrigated plots for most of the soil and yield characteristics.

The quantitative-qualitative yield of the artichoke crop irrigated with SW and TW was not significantly different from that irrigated with FW. Regarding the microbiological parameters, although the effluent showed high contamination, as in the case of SW, there was no soil contamination, due to the high soil capacity to break down the wastewater bacterial load. As for the yield contamination, the drip irrigation method was effective because it reduced the direct contact of the water with the plants; also, its high efficiency makes it possible to use small amounts of water, so as to avoid any pollution of deep percolation and surface water run-off.

Consequently, drip irrigation combined with wastewater re-use offers the most effective and efficient way to cope with water shortage in agriculture.

Acknowledgments

The authors wish to thank the Italian Ministry of University and Research (MIUR) for its financial support under the Project In.Te.R.R.A. (contract No. 01_01480) co-funded within the Italian Programme "PON/*Ricerca e Competitività* 2007-2013".

References

[1] S. Barbagallo, G.L. Cirelli, S. Indelicato, "Wastewater reuse in Italy", *Water Science and Technology,* n$_o$ 43, pp.45-50, 2001.

[2] R.K.X. Bastos, D.D. Mara, "The bacterial quality of salad crops drip and furrow irrigated with waste stabilization pond effluent: an evaluation of the WHO guidelines", *Water Sci. Technol.,* n$_o$ 12, pp. 425-430, 1995.

[3] B. El Hamouri, A Handouf, M. Mekrane, M. Touzani, A. Khana, K. Khallayoune, T, Benchokroun, "Use of wastewater for crop production under arid and saline conditions: yield and hygienic quality of the crop and soil contaminations", *Water Sci. Technol*., vol. 33, n$_o$ 10-11, pp. 335-344, 1994.

[4] A. Lopez, A. Pollice, A. Lonigro, S. Masi, A.M. Palese, G.L. Cirelli, A. Toscano, R. Pasino, "Agricultural wastewater reuse in southern Italy", *Desalination,* vol. 187, pp. 323-334, 2006.

[5] A. Lopez, A. Pollice, G. Laera, A. Lonigro, P. Rubino, "Membrane filtration of municipal wastewater effluents for implementing agricultural reuse in southern Italy", *Water Science &Technology,* n$_o$ 625, pp. 1121-1128, 2010.

[6] M.L. Ndiaye, S. Niang, H.R. Pfeifer, R. Peduzzi, M. Tonolia, Y Dieng, "Effect of irrigation water and processing on the microbial quality of lettuces produced and sold on markets in Dakar (Senegal)". *Irrigation Drainage,* n$_o$ 60, pp. 509-517, 2011.

[7] S.R. Petterson, N.J Ashbolt, A. Snarma, "Microbial risks from wastewater irrigation of salad crops: a screening-level risk assessment", *Water Environment Research,* n$_o$ 73, pp. 667-672, 2011.

[8] A. Pollice, A. Lopez, G. Laera, P. Rubino, A. Lonigro, "Tertiary filtered municipal wastewater as alternative water source in agriculture: a field investigation in Southern Italy", *Science of the Total Environmental* , n$_o$ 324, pp. 201-210, 2004.

[9] S. Toze, "Microbial pathogens in wastewater". *Literature review for urban water system multi-divisional research program CSIRO LAND and WATER Technical Report,* n$_o$ 1/97, 1977

[10] S. Toze, "PCR and the detection of microbial pathogens in water and wastewater", *Water Research,* vol. 33, n$_o$ 17, pp. 3545-3556, 1999.

[11] T. Asano, A.D. Levine, "Wastewater reclamation, recycling and reuse: an introduction", in: *Wastewater Reclamation and Reuse,* vol. 10, T. Asano Ed., CRC Press. Boca Raton, Fla, pp. 1-56, 1998.

[12] A. Lonigro, M. Catalano, P. Rubino, "Impiego in agricoltura di acque reflue urbane depurate nel rispetto della sostenibilità ambientale", *Ital. J. Agron/Riv. Agr*., n$_o$ 2, pp. 217-259, 2007.

[13] P. Rubino, A. Lonigro, "Municipal treated wastewater irrigation: Microbiological risk evaluation", *Italian J. Agron.,* n$_o$ 2, pp. 119-124, 2008.

[14] M. Solimando, "Acque reflue per i campi, una pratica da incrementare". *Agricoltura*, pp. 87-89, 2008.

[15] *www.consorzio.fg.it.* Agroclimatic data: 2012-2013.

[16] P. Soldo, G. Tucci, "Riutilizzo in agricoltura delle acque reflue urbane depurate del Comune di Trinitapoli", in *Acque reflue della Capitanata. La sperimentazione di Trinitapoli* (a cura di G. Arcangelo), pp. 67-95, 2005.

[17] R.G Allen, L.S., Pereira, D. Raes, M. Smith, "Crop Evapotranspiration Guidelines for Computing Crop Water Requirements". In *Irrigation and Drainage Paper*, n$_o$ 56, Food and Agriculture Organization of the United Nations (FAO), Rome, 1998.

[18] E. Tarantino, A. Caliandro, "Studio sulla distribuzione dei consumi idrici durante il ciclo colturale del carciofo nel Metapontino", *Atti del 3° Congresso Internazionale di Studi sul Carciofo*, pp. 505-523, Bari, 1981.

[19] APAT, IRSA-CNR, "Analytical methods for water", *and guidelines*, 2003. Available from: http://www.irsa.cnr.it.

[20] APHA, AWWA, WEF, "Standard Methods for the Examination of Water and Wastewater", *XXI ed. American Public Health Association*, Washington, D.C., 2005.

[21] L.A. Richards, "Diagnosis and Improvement of Saline and Alkali Soils". *USDA Agriculture Handbook,* n$_o$ 60, Washington D.C., 1954.

[22] S.R. Olsen, C.V. Cole., F.S. Watanabe, L.A Dean, "Estimation of available phosphorus in soil by extraction with sodium bicarbonate". *USDA Circular* 939, Washington, D.C., pp. 1-19, 1954.

[23] A. Walkley, I.A. Black, "An examination of the Degtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents", *Soil Sci.,* n$_o$ 63, pp. 251-263, 1934.

[24] D.R Keeney, D.W. Nelson, "Nitrogen inorganic forms". *Methods of soil analysis.* Part. 2 2nd ed. Agr. Monogr. 9 ASA and SSA. Madison, USA, pp. 643-698, 1982.

[25] P. Tallon, B. Magagna, C. Lofranco, K.T. Leung, "Microbial indicators of faecal contamination in water: a current perspective", *Water Air Soil Pollut.,* n$_o$ 166, pp. 139-166, 2005.

[26] M. Salgot, E. Huertas, S. Weber, W Dott, J. Hollender, "Wastewater reuse and risk. Definition of key objectives", *Desalinitation*, n$_o$ 187, pp. 29-40, 2006.

[27] A. Pourcher, F. Picard-Bonnaud, V. Ferré, A. Gosinska, V. Stan, G. Moguedet,. "Survival of faecal indicators and enteroviruses in soil land-spreading of municipal sewage sludge", *Appl. Soil Ecol.,* n$_o$ 35, pp. 473-479. 2007.

[28] JMP, Statistical software (Version 8.1). SAS Institute. Inc, Cary, North Caroline 27513, 2008.

[29] Decree of Ministry of the Environmental, N$_o$ 152, April 3, 2006. Gazzetta Ufficiale n. 88, Italian Guidelines for water reuse, Rome, April 14, 2006.

[30] A.M Palese, V. Pasquale, G. Celano, G. Figliolo, S. Masi, C. Xiloyannis, "Irrigation of olive groves in Southern Italy treated municipal wastewater: effects on microbiological quality of soil and fruits. Agriculture", *Ecosystems and Environment,* n$_o$ 129, pp. 43-51, 2009.

[31] G.A. Vivaldi, S. Camposeo, P. Rubino, A. Lonigro,. "Microbial impact of different types of municipal wastewaters used to irrigate nectarines in Southern Italy", *Agriculture, Ecosystems and Environment,* n$_o$ 181, pp. 50-57, 2013.

[32] J.L Mawdsley, R.D. Bardgett, R.J Merry, B.F. Pan, M.K. Theodorou, "Pathogens in livestock waste, their potential for movement through soil and environmental pollution". *Applied Soil and Ecology,* n$_o$ 2, pp. 1-5, 1995.

[33] J.B. Rose, P.R. Epstein, EK Lipp, B.H. Sherman, S.M. Bernard, J.A. Pat, "Climate variability and change in the United States: potential impacts on water and foodborne diseases caused by microbiological agents"; *Environmental Health Perspectives*, vol. 2, n$_o$ 109, pp. 211-221, 2001.