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REUSE OF TREATED MUNICIPAL WASTEWATER FOR IRRIGATION IN APULIA REGION: THE "IN.TE.R.R.A." PROJECT

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Abstract

The use of non-conventional water resources including treated municipal wastewater has been increasing in the Mediterranean regions over the last decades to cope with water shortages and uneven rainfalls due to climate change.
The aim of this paper is to present the first results from two years of experimental field activities carried out in two different demo-places in Southern Italy: the municipal wastewater treatment plants of Noci and Castellana Grotte (Apulia region). In these sites different vegetable crops (cucumber, lettuce, melon, endive in Noci and fennel, lettuce, fennel in Castellana Grotte) were grown in succession and irrigated in parallel with treated wastewater and conventional water pumped from wells, for comparing the effects of the different water sources on soil and vegetables. Reclaimed water quality was monitored for chemical and microbial parameters and compared with conventional water. At harvesting time, microbial indicators were measured on edible part of crops and in soil.
Results show that the effluents produced by a full scale membrane bioreactor (MBR) treatment plant (Noci) comply with the stringent Italian standards for reuse in agriculture, and its microbiological quality is higher than the conventional well water. In Castellana Grotte the effluent quality of the two pilot plants was different according to the adopted technologies (MBR and tertiary cloth filtration), and sometimes depended on the quality of incoming wastewater.
As for the agronomic results, in both sites crop yields were higher in the plots irrigated with treated wastewater, and the microbial indicators *Escherichia coli* and *Salmonella* were never found, at harvesting time, on edible parts of crops and in the soil.

Key words: agricultural reuse, drip irrigation, treated wastewater, vegetable crops

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1. Introduction

Water stress in Mediterranean countries calls for solutions that should promptly provide responses to the effects of climate change and consequent decrease of available resources. Two approaches that should be combined to properly tackle the current water scarcity refer to (i) the improvement of resource management practices by balancing demand and supply, and (ii) the adoption of alternative water resources (e.g. desalination, wastewater reuse, rainwater harvesting, etc.)(GWP, 2012). Countries facing the Mediterranean have different priorities and constraints with respect to the improvement of freshwater availability, and strong differences exist among the regulations governing, for instance, treated wastewater reuse (Carp and Barbu, 2014; Kellis et al., 2013). In this context, several activities were carried out over the last decades to test and demonstrate the feasibility of wastewater recycling for producing a dedicated resource to activities requiring continuous water availability during the dry season (e.g. agriculture) (Bixio et al., 2006; Valipour et al., 2014). The concept of water production from wastewater treatment is implemented in Apulia (South East Italy), a region whose economy is based on agriculture despite its strong lack of water availability (Lopez et al., 2006). Here, the combination of dry weather conditions, absence of permanent rivers and natural lakes, and progressive

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groundwater salinization due to overexploitation requires the adoption of non-conventional resources. Previous experimental activities have shown the long term feasibility of adopting tertiary membrane filtration as a suitable tool for the upgrade of municipal effluents to comply with the stringent local standards for reuse (Lopez et al., 2010; Pollice et al., 2004). Different combinations of biological process and surface filtration were tested for the treatment of municipal sewage aimed at producing effluents suitable for agricultural reuse. The activity included the test of wastewater treatment process technologies, and the comparison of the produced effluents with conventionally treated tertiary effluents and with the well water normally used for irrigation by local farmers. This comparison was performed through agronomic experiments where test fields were cultivated and different types of vegetable crops were grown in succession.

In particular some relevant aspects were studied at the field scale, such as partial preservation of nutrients and "on-demand" disinfection. Moreover non-conventional water purification technologies were tested and compared to conventional treatments in terms of effluent quality and suitability for reuse practices. The innovative features of the proposed technologies will be briefly described here and evaluated with particular reference to effluent quality and suitability for reuse in irrigation of vegetable crops.

The results discussed in this paper refer to the activities performed at two demo sites: The municipal wastewater treatment plants of Noci and Castellana Grotte (Apulia region). At the first one, the effluent of a full scale membrane bioreactor (MBR) was monitored for chemical and microbiological quality and used for irrigation of a test field in comparison with local well water. At the second one, two demo-scale treatment plants were installed and operated for testing their potential. These were based on two non-conventional technologies: the IFAS-MBR, Integrated Fixed Film Activated Sludge - Membrane Bioreactor, and the GDF, Gravity Disk Filtration, respectively. Both plants were followed by UV disinfection systems operated "on demand".

The present paper reports some results of the experimental activities carried out within the projects In.Te.R.R.A. (funded by the Italian Ministry of Education and Research, PON 2007/2013). Goals of this project are to study, test and suggest innovative and sustainable technological and process strategies to promote reuse of treated municipal wastewater for irrigation.

2. Case study 1: The site of Noci

The full scale MBR wastewater treatment plant was monitored in terms of the chemical and microbiological suitability of its effluent for agricultural reuse and compliance with the local and national standards. The results reported in this paper refer the first two years of irrigation on four vegetable crops in succession.

2.1. The MBR treatment plant

The municipal MBR treatment plant of Noci (Apulia region, South Eastern Italy) is equipped with submerged hollow fiber membrane ultrafiltration modules (GE Water) providing solid/liquid separation and the desired biomass concentration in the final sections of the treatment train. Ultrafiltration is operated with the out-in mode and the modules are equipped with an air scouring cleaning system that prevents biomass accumulation on the membrane surface, limiting the effects of fouling and ensuring a membrane life of several years. The purifying performance is very high since it is possible to work with high concentration of biomass, and this promotes biological removal of COD and nutrients (the plant is operated for pre-denitrification). Moreover, according to the membrane pore size (0.04μm) the effluent quality is bacterial-free guaranteed.

2.2. The experimental field

The experimental field was located near the municipal wastewater treatment plant of Noci (40°47'56"N 17°04'63"E; altitude 360 m a.s.l.). The trial was carried out in a silt-loam soil (USDA classification) with a field capacity (-0.02MPa) of 25.2% dry weight (dw), a wilting point (-1.5 MPa) of 7.1% dw and a bulk density of 1.88 t m^{-3}. The main characteristics of the soil layer of the experimental site (0-0.4 m) characterized once before trial were the following: sand 35.7%; loam 60.0%; clay 4.3%; organic matter 1.67%; Olsen P_2O_5 15 mg kg^{-1}; Ac-extractable K_2O 80 mg kg^{-1}; total N 0.63‰ (Kjeldahl); pH 7.7; electrical conductivity 0.40 dS m^{-1}.

Four vegetable crops were grown in succession during two years of trials: cucumber, lettuce, melon and endive. The cucumber (*Cucumis sativus* L.), was transplanted on May 31st, 2012 in single rows, spaced at 1 m with plants 0.5 m apart from each other, realizing a plant density of 2 plants m^{-2}, and was harvested in 7 times, between July 10th and August 17th, 2012. Lettuce (*Lactuca sativa* L.) was transplanted on the same plots on September 26th 2012 in single rows, spaced 0.5 m with plants 0.3 apart from each other, realizing a plant density of 6.7 plants m^{-2}, and was harvested on January 22nd 2013. Melon (*Cucumis melo* L.) was transplanted on April 18th 2013 in single rows, spaced 2.0 m with plants 0.7 apart from each other, realizing a plant density of 0.7 plants m^{-2}, and was harvested in 7 times, between July 15th and August 28th 2013. Endive (*Cichorium endivia* L.) was transplanted on September 12th 2013 on the same plots, in single rows, spaced 0.5 m with plants 0.3 apart from each other, realizing a plant density of 6.7 plants m^{-2}, and was harvested on November 29th 2013.

Two types of water were compared for irrigation: MBR municipal effluent (MBReff) and conventional water pumped from phreatic well (Well1).

The experimental design used for all crops was a randomized block design with 4 replications, obtaining 8 plots of size 8 x 15 m (Fig. 1). For all crops, drip irrigation was used placing the dripping lines every other row.

The four crops were irrigated when the soil water deficit (SWD) in the root zone was 35% of the total available water (TAW). Irrigation was scheduled based on the evapotranspiration criterion, providing water to the crops when the following conditions were met (Eq. 1):

$$\sum_{1}^{n}\left(E_{tc}-R_{e}\right)=RAW \qquad (1)$$

where: *RAW* (Readily Available Water) = 30 mm for cucumber and melon; 25 mm for lettuce and endive, worked out for the soil in which researches were carried out ; *n* = number of days required to reach the SWDlim starting from the last watering; *Etc* = crop evapotranspiration (mm); *Re* = rainfall (mm). Evapotranspiration can be expressed by Eq. (2), where *E* is "class A" pan evaporation (mm); *Kc* is the crop coefficient; *Kp* is pan coefficient (0.8).

The experimental field was cultivated according to the agronomical practices (fertilization, pest and weed control) commonly adopted by the local farmers. The mean monthly main climate parameters recorded during the trial are reported in Table 1. These data were measured by a weather station located near the experimental area (http://www.agrometeopuglia.it).

$$E_{tc} = E*K_p*K_c \qquad (2)$$

2.3. Sampling and analyses

The irrigation water samples (treated effluent and conventional well water) were collected, under the dripper, on every watering and analyzed for chemical parameters according to standard methods (APHA et al., 2012). The measures parameters were: pH, electrical conductivity (EC), BOD_5, COD, N-NH_4, P-PO_4^-, Cl^-, SO_4^-, NO_3^-, F^-, Sodium Adsorption Ratio (SAR), Escherichia coli and Salmonella spp by the membrane filtration method. Soil samples were taken from each plot before and after every crop cycle at depths decreasing from 0 to 0.4 m, every 0.2 m.

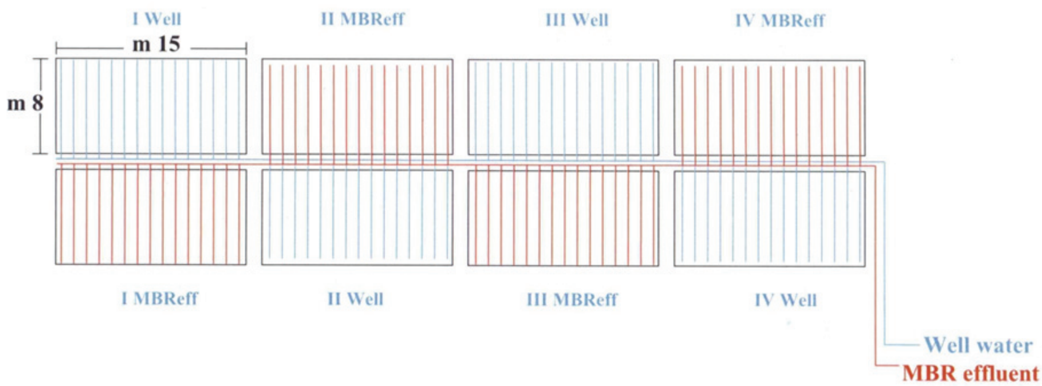

Fig. 1. The experimental randomized block design used for all crops with 4 replications

Table 1. Main climatic parameters recorded in Noci during the growing season of the four vegetable crops

Month	*[a]Climatic Parameters*							*T_{max} Long term average (°C)*	*T_{min} Long term average (°C)*	*P Long term average (mm)*
	T_{max} (°C)	*T_{min} (°C)*	*RH_{max} (%)*	*RH_{min} (%)*	*Ev (mm)*	*Ws ($m\ s^{-1}$)*	*P (mm)*			
Growing season Cucumber (april - august 2012)	28.07	12.93	93.28	35.53	5.44	1.99	129.20	23.55	14.91	180.30
Growing season Lettuce (september 2012 - january 2013)	17.76	7.56	99.38	63.40	1.89	2.11	436.80	15.70	9.51	362.10
Growing season Melon (april-august 2013)	24.42	10.59	89.49	38.81	4.72	2.11	162.40	23.55	14.91	180.30
Growing season Endive (september -december 2013	21.65	10.27	91.84	49.36	3.05	1.56	248.90	19.16	12.33	207.60

[a] T_{min}, T_{max}, monthly minimum, maximum air temperature; RH_{min}, RH_{max}, monthly minimum, maximum relative air umidity; P, total precipitation; W_s, monthly mean wind speed;E_v, total "class A"pan evaporation.

They were analyzed for Kjeldahl nitrogen (N), phosphorus (P_2O_5), organic matter (O.M.), pH and electrical conductivity according to standard procedures (Spark, 1996). Microbiological analyses (*E. coli* and *Salmonella spp*) were also carried out on soil samples at depth of 0-0.1 m according to standard methods (Woomer, 1994).

At harvesting time, the number of plants from each plot was counted and weighted to estimate total yield (TY, t ha^{-1}), marketable yield (MY, t ha^{-1}), and nonmarketable yield (NMY, t ha^{-1}). On 6 marketable samples from each plot, cucumber and melon fruits were also measured for: dry matter content (DM, % fresh matter) (AOAC 1990), average fruit weight, mean diameter (equatorial and longitudinal diameter) (D, cm), soluble solids content of the fresh (SSC; °Brix), titratable acidity (TA; g citric acid 100 mL^{-1} fresh juice) (AOAC, 1995) and texture. For lettuce and endive: number of plants and average clumps weight.

Microbiological analyses (*E. coli* and *Salmonella spp*) were also carried out on washing water of clumps and fruits according to standard methods (Sharf, 1966).

The measured data from each of the continuum variables relating to the qualitative/quantitative traits were processed statistically using analysis of variance (ANOVA). When significant effects were detected ($P \leq 0.05$), mean multiple comparisons were performed according to Tukey's tests. With reference to the analyzed qualitative parameters, the Bartlett test confirmed the homogeneity of the variance among the harvest data, so a combined statistical analysis was performed later. When the normality and the homogeneity of standard deviations about the distribution of the population from which the samples were drawn were not respected, Kruskall-Wallis non parametric test was performed.

2.4. Results

As reported in Table 1, during the growing season of four vegetable crops in succession the maximum temperature recorded, on average, was always higher respect to the long-term average and minimum temperature (always lower by about 2°C). Table 2 shows the average values of the main chemical and physical characteristics of the well water and the MBR effluent, as measured during the experimental trials. The overall quality of the two water sources was comparable, and complied with the Italian national standards (D.M. 185, 2003) and the Regional regulation for treated wastewater reuse (R.R. 8, 2012). MBReff was characterized by higher values of NH_4, PO_4^{3-}, SO_4^- and K^+ than Well1, which represent important nutrients to increase soil fertility, plant growth and crop yield.

As observed along the soil profile (0-0.40 m) over the research period, the use of MBReff for irrigation contributed to increase the values of O.M., N, and K_2O , if compared with the use of Well1 (Table 3).

The data about the influence of the type of water on the qualitative and quantitative crop parameters are shown in Table 4.

Table 2. Average values of the main chemical, physical and microbiological parameters measured during the experimental period of trials in Noci, for the well water (Well1) and the effluent of the full scale municipal MBR (MBReff), used for vegetable crops irrigation

Water parameters	***Irrigation treatment***		***Significance***	***Italian/Regional limit values (R.R. 8/2012)***
	Well 1	***MBR_{eff}***		
EC (dS m^{-1})	0.77 ± 0.05	0.98 ± 0.02	**	3.00
pH	7.75 ± 0.07	7.48 ± 0.06	**	6.0 – 9.5
BOD_5 (mgO_2 L^{-1})	4.10 ± 1.2	9.69 ± 2.4	n.s.	20
COD (mgO_2 L^{-1})	17.8 ± 8.4	28.4 ± 6.3	n.s.	100
$NH_4^+ - N$ (mg L^{-1})	0 ± 0	5.1 ± 2.0	*	2 (15)[a]
$NO_3^- - N$ (mg L^{-1})	7.2 ± 3.0	8.6 ± 3.4	n.s.	35 (15)[b]
$PO_4^{3-} - P$ (mg L^{-1})	2.0 ± 4.2	4.6 ± 6.2	n.s.	10 (2)[b]
SO_4^- (mg L^{-1})	12.2 ± 3.3	38.9 ± 7.2	**	500
F^- (mg L^{-1})	0.28 ± 0.09	1.18 ± 0.37	*	1.5
Cl^- (mg L^{-1})	62.6 ± 12.5	129.3 ± 10.6	**	250
Na^+ (mg L^{-1})	36 ± 7	81 ± 6	**	
K^+ (mg L^{-1})	2 ± 0	12 ± 2	**	
Ca^{2+} (mg L^{-1})	71 ± 10	65 ± 7	n.s.	
Mg^{2+} (mg L^{-1})	19 ± 4	16 ± 3	n.s.	
SAR	1 ± 0	2 ± 0	n.s.	10
E. coli (CFU 100 mL^{-1})	0 ± 0	6 ± 4	n.s.	10
Salmonella (CFU 100 mL^{-1})	Absent	Absent		Absent

*Data are average ± standard error for each water sample analyzed between April 2012 and November 2013. **: Statistically significant at $P \leq 0.01$;*: Statistically significant at $P \leq 0.05$; n.s.: not significant. [a] Limit concentration for ammonium can be raised to the value in brackets upon special permission (R.R. 8/2012). [b] Limit concentrations for total nitrogen and total phosphorus (in brackets the limit concentrations for areas declared vulnerable to nitrate and phosphate pollution).*

Table 3. Average values of the main soil chemical parameters measured over the research period along the soil profile in Noci. EC and pH were measured on 1:2 (w/v) and 1:2.5 (w/v) aqueous soil extract, respectively

Soil parameters	Cucumber		Lettuce		Melon		Endive	
	Well1	MBR_{eff}	Well1	MBR_{eff}	Well1	MBR_{eff}	Well1	MBR_{eff}
EC	0.40	0.28	0.21	0.21	0.33	0.56	0.30	0.34
pH	7.68	7.68	8.02	8.04	8.13	8.15	8.28	8.21
OM (%)	1.7	1.8	1.5	1.6	1.9	2.2	1.3	1.7
N (g kg^{-1})	0.6	0.7	1.1	1.2	1.1	1.2	0.9	1.3
P_2O_5 (mg kg^{-1})	15	21	43	37	44	30	11	13
K_2O (mg kg^{-1})	77	89	84	91	82	88	83	92

Table 4. Average values of main quantity and quality crop parameters measured over the research period, at harvesting time in Noci

Crop parameters	Cucumber		Lettuce		Melon		Endive	
	Well1	MBR_{eff}	Well1	MBR_{eff}	Well1	MBR_{eff}	Well1	MBR_{eff}
Total Yield (t ha^{-1})	7.6 b	23.9 a	16.8 a	18.9 a	25.6b	35.2a	29.8a	35.2 a
Marketable Yield (t ha^{-1})	7.6 b	23.9 a	12.2a	13.7 a	25.48b	35.12a	29.8a	35.2a
Non Marketable Yield (t ha-1)			4.6a	5.2a	0.12a	0.08a		
Dry Matter (%FM)	3.4b	6.2a	4.9a	4.3a	11.2a	12.9a	5.9a	6.2a
SSC (°Brix)	3.2b	5.1a			9.9b	12.7a		
pH					6.36a	6.37a		
TA (g 100 mL^{-1})	0.1b	0.3a			1.10b	1.33a		
N of plant (m^{-2})	1	1	5.5	5.3	1.48	1.50	6.3	6.2
N. of fruits per plant	80b	229a			10.0a	12.4 a		
Average clumps weight (g)	294a	326a	221a	258a	411b	556a	477aa	556a

Letters represent significant differences between treatments ($p < 0.05$)

They show that the yield of different crops were significantly higher in plots irrigated with MBReff than in plots irrigated with Well1. Also, some quality crop parameters showed significant differences between MBReff and Well1 (D.M., SSC, pH and n. of fruits for cucumber crop and SSC for melon crops).

The microbiological analyses carried out on water show that faecal pollution indicator (*E. coli*) was never present in well water, while in MBR effluent, if present, was in very low concentration.

In soil and on crop samples *Escherichia coli* and *Salmonella* were never found.

3. Case Study 2: The site of Castellana Grotte

Another experimental investigation was carried out at Castellana Grotte (Apulia region, South Eastern Italy) to evaluate the feasibility of adopting the effluent of the local municipal wastewater treatment plant for agricultural reuse (irrigation of vegetable crops). Two non-conventional pilot-scale treatment plants were also installed and operated at the same site to test alternative treatment technologies based on surface filtration (membrane and cloth filtration, respectively). The demo-site includes a test field located immediately outside the treatment plant where vegetables can be grown for testing irrigation with different water sources. The results reported in this paper refer the first two years of irrigation on three vegetable crops in succession.

3.1. The municipal wastewater treatment plant

The full scale municipal wastewater treatment plant of Castellana Grotte is based on a pre-denitrification process scheme. The sewage, after pre-screening and primary settling, is sent to the first anoxic reactor where the nitrate recirculated from the following aerobic tank is removed from the liquid phase through biological denitrification. In the subsequent aerated reactor, oxidation of the organic fractions and nitrification occur. The produced sludge is separated from the liquid phase in the final settling tank and partly recirculated to maintain the required biomass concentration. The secondary effluent is further treated through granular media filtration and disinfection (chlorination), before being discharged on soil. During the experimental activities described here, a fraction of this effluent was diverted and used for irrigation at the test field located immediately out of the treatment plant.

3.2. The IFAS-MBR pilot plant

The first pilot plant was based on the IFAS-MBR technology (Integrated Fixed film Activated Sludge – Membrane BioReactor), and treated sewage after preliminary screening. The IFAS technology is based on the presence of plastic carriers in the aerobic bioreactor. These carriers promote biomass accumulation in the form of biofilm, and biological processes are carried out synergistically by the suspended biomass and the biofilm, resulting in limited biomass growth. Coupling the IFAS with an MBR has further potential benefits, since the membrane bioreactor allows optimal control of suspended biomass in terms of sludge retention time, possibly resulting in reduced production of partially stabilized sludge. This plant was operated to promote the nitrification of ammonia, in order maintain the nitrogen concentration of treated wastewater and evaluate the nutrient contribution to the growth of vegetables. Moreover membrane separation results in high quality effluent in terms of suspended solids, favoring the adoption of UV disinfection technologies. In fact the outlet of this plant was connected to a UV disinfection system that was activated when the irrigation line was switched on ("on demand" disinfection).

3.3. The GDF pilot plant

The second pilot plant was based on the GDF technology (Gravity Disk Filter). It treated a fraction of the secondary effluent taken downstream to the secondary settling tank of the main wastewater treatment plant. Therefore, the GDF provided a tertiary physical treatment allowing for improved removal of residual suspended solids. The system was based on a cloth filtration process that was operated through a set of disks submerged into the tank. Also in this case a UV system was placed downstream and treated the effluent "on demand", i.e. when irrigation was performed.

3.4. Experimental trials

The experimental field was located near the wastewater treatment plant of Castellana Grotte (40°53'20"N 17°11'51"E; altitude 305 m a.s.l.). The trials were carried out in a loam loose soil (USDA classification) with a field capacity (- 0.02MPa) of 24.4% dry weight (dw), a wilting point (-1.5 MPa) of 6.7% dw and a bulk density of 1.7 t m^{-3}. The main characteristics of the soil layer of the experimental site (0-0.4 m) characterized once before trial are as follow: sand 44.4%; loam 54.1%; clay 1.6%; organic matter 1.50%; Olsen P_2O_5 19 mg kg^{-1}; $BaCl_2$ extractable K_2O 70 mg kg^{-1}; total N 1.11‰ (Kjeldahl); pH 8.1; electrical conductivity 0.22 dS m^{-1}.

Four types of irrigation water were compared: the effluent from the full scale plant (EFF), the effluent from the pilot IFAS-MBR plant (IMBR), the effluent from the GDF pilot plant (GDF), and the water pumped from a local phreatic well (Well2). For all crops, drip irrigation was used placing the dripping lines every other row. During two years of trials three vegetable crops were alternated: fennel, lettuce and fennel.

Fennel (*Foeniculum vulgare* Mill) was transplanted on September 29th, 2012 in single rows, spaced 0.2 m with plants 0.5 apart from each other, realizing a plant density of 10 plants m^{-2}, and was harvested on March 19th, 2013.

Lettuce (*Lactuca sativa* L.) was transplanted on the same plots on April 18th 2013 in single rows, spaced 0.5 m with plants 0.3 apart from each other, realizing a plant density of 6.7 plants m^{-2}, and was harvested on June 17th 2013 in plots irrigated with IMBR water; after three days (June 20th) in plots irrigated with EFF and GDF, and after eight days in plots irrigated with Well2.

Fennel (*Foeniculum vulgare* Mill) was transplanted on the same plots on August 30th 2013 in single rows, spaced 0.2 m with plants 0.5 apart from each other, realizing a plant density of 10 plants m^{-2} and was harvested on .

Marketable yields data (t ha^{-1}), average weight (g), and clumps dry matter (%) were measured at harvesting time, i.e. after 102 (December 10th 2013) , 109 (December 17th 2013), 127 and 137 days (January 14th 2014) from transplanting in plots irrigated with IMBR water, EFF water, GDF and Well2, respectively. The experimental design used for all crops was a latin square design obtaining 16 plot of size 20 x 20 m (Fig. 2).

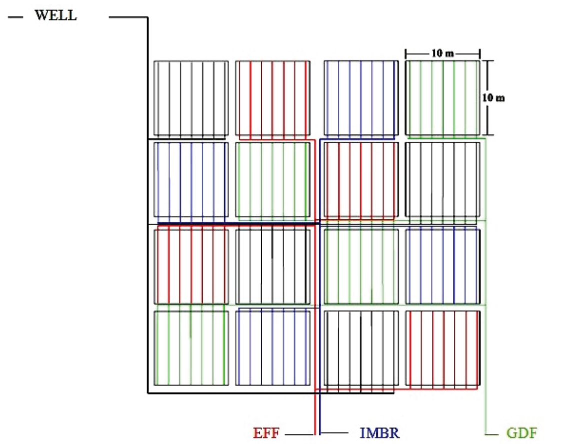

Fig. 2. Latin square experimental design used for all crops

The three crops were irrigated when the soil water deficit (SWD) in the root zone was 35% of the total available water (TAW). Irrigation was scheduled based on evapotraspiration criterion providing water to the crops when the following conditions were met (Eq. 3):

$$\sum_{1}^{n}\left(E_{tc} - R_e\right) = 30 \text{ mm for lettuce; 25 mm for fennel} \quad (3)$$

The experimental field was cultivated according to the agronomical practices (fertilization, pest and weed control) commonly adopted by the local farmers.

The mean monthly main climate parameters recorded during the trial are reported in Table 6. These data were measured by a weather station located near the experimental area (ASSOCODIPUGLIA).

3.5. Sampling and analyses

Irrigation water samples of the different types (EFF, IMBR, GDF and Well2) were collected under the dripper on every watering and analyzed for chemical parameters according to standard methods (APHA et al., 2012). The measured parameters were: pH, electrical conductivity (EC), BOD_5, COD, N-

NH_4, P-PO_4^-, Cl^-, SO_4^-, NO_3^-, F^-, Sodium Adsorption Ratio (SAR), *Escherichia coli* and *Salmonella spp* by the membrane filtration method (Table 5).

Soil samples were taken from each plot before and after every crop cycle at depths decreasing from 0 to 0.4 m, every 0.2 m. They were analyzed for nitrogen Kjeldahl (N), phosphorus (P_2O_5), organic matter (O.M.), pH and electrical conductivity according to standard procedures (Spark, 1996). Microbiological analyses (*E. coli* and *Salmonella spp*) were also carried out on soil samples at depth of 0-0.1 m according to standard methods (Woomer, 1994). At harvesting time, the marketable edible parts of vegetable crops were counted and weighted to estimate total yield (TY, t ha^{-1}), marketable yield (MY, t ha^{-1}) nonmarketable yield (NMY, t ha^{-1}). On 6 marketable samples from each plot, were also measured dry matter content (DM, % fresh matter) (AOAC, 1990), average fruit weight and number of plants. Microbiological analyses (*E. coli* and *Salmonella spp*) were also carried out on washing water of clumps and fruits according to standard methods (Sharf, 1966). Statistical analysis was performed just as for the site of Noci (see paragraph 2.3).

3.6. Results

Climatic conditions in the experimental period September 2012 - January 2014 (Table 5) show that the maximum temperatures were higher than the long-term average, while minimum temperatures have been in accordance with the long-term average. The average values of the main chemical and physical characteristics of the irrigation waters during the experimental trials are reported in Table 6. COD, NO_3, K^+, Na^+ and Cl^- are the only parameters significantly different between the various types of water. However, if compared with Well2, all treated wastewaters (IMBR, EFF and GDF) were also characterized by higher average values of NH_4, PO_4^{3-}, SO_4^- and Ca^{2+}, which represent important nutrients to increase soil fertility, plant growth and crop yield.

Data observed along the soil profile (0-0.40 m) over the research period, show no differences in terms of soil fertility (Table 7). About the influence of the different types of water on the qualitative and quantitative crop parameters, results are shown in Table 8. They show that the yield of different crops are significantly higher in plots irrigated with IMBR than Well2.

The microbiological analyses conducted on water, reported in Table 6, show that faecal pollution indicator (*E. coli*) was never present in well water; in IMBR, when present, was in very low concentration and not significantly different from the well water, while in EFF and GDF, *E. coli* values exceeded respect to the Italian law value and result significantly different respect to Well2. *Salmonella* was never found in waters. In soil and on crop samples *Escherichia coli* and *Salmonella* were never found.

4. Discussion

The MBR technology (full-scale MBR at Noci, pilot scale IFAS-MBR at Castellana Grotte) showed the potential to produce an effluent that complies with the local limits for reuse in agriculture. On the contrary GDF and EFF did not allow to respect the limit related to the faecal indicator *E. coli*. All types of treated wastewater had on average higher values of important nutrients for the crops, such as NO_3, NH_4, PO_4^{3-}, SO_4^-, K^+ and Ca^{2+}. This resulted, in most of the cases and for different type of crops, in a significant increase of crop yields.

The pilot scale IFAS-MBR was operated only for nitrification to supply nutrients to plants. The higher content of nitrates in IMBR, if compared with all the other sources of water used in this study, not only increased crop yields, but also resulted in a early ripeness. In fact for all crops grown there was a maturity advance variable from 8 to 30 days between IMBR and Well2 plots, respectively. The IMBR provided, constantly at each watering, small but still significant amounts of nutrients to a soil, not very fertile, as that of Castellana Grotte.

The soil fertility and the quality of the crops was not systematically improved by the use of treated wastewater for irrigation.

Table 5. Main climatic parameters recorded in Castellana Grotte during the growing season of the three vegetable crops

Month	*[a] Climatic Parameters*							*T_{max} Long term average (°C)*	*T_{min} Long term average (°C)*	*P Long term average (mm)*
	T_{max} (°C)	*T_{min} (°C)*	*RH_{max} (%)*	*RH_{min} (%)*	*Ev (mm)*	*Ws (m s^{-1})*	*P (mm)*			
Growing season Fennel (september 2012- march 2013)	15.97	7.52	98.70	56.17	1.72	3.22	528.4	14.49	7.26	68.93
Growing season Lettuce (april – june 2013)	23.02	11.44	87.98	32.38	4.29	2.89	39.2	21.22	11.50	37.73
Growing season Fennel (august 2013 - march 2014)	17.88	8.71	95.49	55.19	2.16	2.67	559.1	16.22	8.58	63.40

[a]T_{min}, T_{max}, monthly minimum, maximum air temperature; RH_{min}, RH_{max}, monthly minimum, maximum relative air humidity; P, total precipitation; W_s, monthly mean wind speed; E_v, total "class A" pan evaporation

Table 6. Means of the main chemical-physical and microbiological parameters measured during the experimental period of trials in Castellana Grotte, for the well water (Well2), the full scale municipal wastewater treatment plant effluent (EFF), the IFAS-MBR pilot plant effluent (IMBR) and the GDF pilot plant effluent (GDF), used for vegetable crops irrigation

Water parameters	*Irrigation treatmen*					*Italian Law Limit value (DM 185/2003) (R.R. 8, 2012)*
	Well2	*EFF*	*IMBR*	*GDF*	*p-value*	
EC (dS m^{-1})	0.87 ± 0.01	1.02 ± 0.09	0.95 ± 0.03	0.88 ± 0.01	0.103	3.00
pH	7.53 ± 0.10	7.62 ± 0.06	7.59 ± 0.09	7.62 ± 0.08	0.949	6.0 – 9.5
BOD_5 (mgO_2 L^{-1})	2.64 ± 1.05	9.55 ± 1.73	9.60 ± 2.71	5.28 ± 0.71	0.075	20
COD (mgO_2 L^{-1})	4.40 ± 1.53 B	49.71 ± 12.45 A	24.20 ± 2.21 A	20.22 ± 2.43 A	0.004	100
NH_4^+-N (mg L^{-1})	0.78 ± 0.72	9.89 ± 4.01	10.11 ± 3.53	2.09 ± 2.09	0.057	2 (15)[a]
NO_3^--N (mg L^{-1})	1.50 ± 0.46 B	5.68 ± 1.14 B	22.17 ± 3.72 A	9.68 ± 3.06 B	0.0001	35 (15)[b]
PO_4^{3-}-P (mg L^{-1})	5.71 ± 5.23	8.76 ± 3.03	11.64 ± 4.17	9.13 ± 4.21	0.427	10 (2)[b]
SO_4^- (mg L^{-1})	18.19 ± 12.40	35.59 ± 9.71	45.75 ± 11.52	32.02 ± 8.08	0.407	500
F^- (mg L^{-1})	0.69 ± 0.36	0.59 ± 0.25	1.43 ± 0.52	0.71 ± 0.31	0.285	1.5
Cl^- (mg L^{-1})	56.34 ± 25.24 B	135.03 ± 31.72 A	102.41 ± 5.99 A	87.37 ± 5.32 AB	0.018	250
Na^+ (mg L^{-1})	31.52 ± 12.6 B	87.58 ± 13.26 A	94.13 ± 17.15 A	82.51 ± 6.48 A	0.015	
K^+ (mg L^{-1})	0.71 ± 0.42 B	20.69 ± 3.93 A	21.14 ± 3.26 A	17.74 ± 1.99 A	0.001	
Ca^{2+} (mg L^{-1})	63.66 ± 10.8	59.56 ± 5.58	112.96 ± 42.14	70.78 ± 9.73	0.932	
Mg^{2+} (mg L^{-1})	23.86 ± 6.73	9.41 ± 2.32	18.07 ± 6.71	6.92 ± 1.88	0.208	
SAR	0.71 ± 0.42 B	2.94 ± 0.32 A	2.57 ± 0.20 AB	2.77 ± 0.23 A	0.0023	10
E. coli (CFU 100 mL^{-1})	2 ± 2 B	1389 ± 477A	9 ± 4 B	431 ± 189 A	3.183 E^{-6}	10
Salmonella (CFU 100 mL^{-1})	Absent	Absent	Absent	Absent		Absent

Data are means ± standard error for each water analyzed between September 2012 and January 2014. Capital letters represent significant differences between treatments ($p < 0.01$); [a] Limit concentration for ammonium can be raised to the value in brackets upon special permission (R.R. 8, 2012); [b] Limit concentrations for total nitrogen and total phosphorus (in brackets the limit concentrations for areas declared vulnerable to nitrate and phosphate pollution)

Table 7. Average values of main soil chemical parameters measured over the research period along the soil profile in Castellana Grotte. EC and pH were measured on 1:2 (w/v) and 1:2.5 (w/v) aqueous soil extract, respectively

Soil parameters	*Cucumber*				*Lettuce*				*Fennel*			
	Well2	*IMBR*	*GDF*	*EFF*	*Well2*	*IMBR*	*GDF*	*EFF*	*Well2*	*IMBR*	*GDF*	*EFF*
EC	022	0.21	0.20	0.20	0.38	0.43	0.40	0.35	0.16	0.26	0.23	0.25
pH	8.10	8.04	8.14	8.07	8.03	8.05	8.16	8.15	7.88	8.02	8.09	8.09
OM (%)	1.77	1.61	1.52	2.02	1.84	2.03	1.74	1.72	1.69	1.91	1.76	1.76
N (g kg^{-1})	1.11	1.14	1.04	1.11	1.21	1.08	1.12	1.39	1.40	1.25	1.08	1.11
P_2O_5 (mg kg^{-1})	87	133	19	32	43	77	48	47	14	13	12	8
K_2O (mg kg^{-1})	132	148	96	134	120	116	108	111	73	82	79	84

Table 8. Average values of main quantity and quality crop parameters measured over the research period, at harvesting time in Castellana Grotte

Crop parameters	*Cucumber*				*Lettuce*				*Fennel*			
	Well2	*IMBR*	*GDF*	*EFF*	*Well2*	*IMBR*	*GDF*	*EFF*	*Well2*	*IMBR*	*GDF*	*EFF*
Total Yield (t ha^{-1})	32.2b	47.8a	31.1b	46.1a	34.2b	53.6a	34.9b	38.5b	31.6a	38.7a	35.7a	29.8a
Marketable Yield (t ha^{-1})	32.2b	47.8a	31.1b	46.1a	34.2b	53.6a	34.9b	38.5b	31.6a	38.7a	35.7a	29.8a
Dry Matter (%FM)	7.85a	7.37a	7.87a	7.42a	4.93a	4.01a	4.66a	4.79a	8.98a	7.67a	8.18a	8.70a
N. of plant m^2	140.5	139	127	138	154	163	158	163	144	159.2	160.3	175
Average clumps weight (g)	441.2b	667.7a	477.6b	656.1a	556.4b	826.0a	553.3b	591.8b	373.8a	487.0a	442.6a	403.0a

Letters represent significant differences between treatments ($p < 0.05$)

This occurred in the experimental site of Noci, whereas not relevant differences in terms of soil fertility and quality crop parameters were observed in the site of Castellana Grotte.

In both the experimental sites, in soil and on crop samples *Escherichia coli* and *Salmonella* were never found. These results are in agreement with Lonigro et al. (2007), Benami et al. (2013), which assessed soil irrigated with treated wastewater and with fresh water. Other studies have reported that the level of other faecal indicators in soil irrigated with raw or treated wastewater can significantly differ from that with freshwater application (Malkawi and Mohamad, 2003; Travis et al., 2010). It also needs to be considered that in the study of Malkawi and Mohamad (2003) there were no fecal coliforms recorded in the soil before the irrigation with fresh water, and thus sources other than water can affect this indicator. With Travis et al. (2010), the levels of *Faecal Coliforms* in soil irrigated with untreated or treated gray water was always below 100 CFU g^{-1}.

Considering that *E. coli* was not isolated from any of the soil samples in the present study, it is possible that the die-off, or at least the loss of cultivability, of this important indicator in the present field study occurred faster than previously reported (Lang et al., 2007; Van Elsas et al., 2011). Analogous data were obtained for the edible portion of plants, in which no *E. coli* was isolated from every plots. These data are in agreement with other studies (Cirelli et al., 2012; Lonigro and Rubino, 2005), in which only fruit samples (tomato and eggplant) directly in contact with the soil where contaminated by faecal bacteria. Wood et al. (2010) showed that the decline in *E. coli* on the surface of spray-irrigated spinach was considerably rapid (3–5 log reduction in 72 h, and no isolation after 6 days). Another study reported that in the summer months, which are characterized by higher sunlight exposure of the crops, there was a more rapid decay of both the indicator and pathogenic microorganisms (Sidhu et al., 2008). In the present study, irrigating with treated wastewater (EFF) with an average value of 600 CFU 100 mL^{-1} of *Escherichia coli*, at harvesting time was not registered any presence of fecal contamination on lettuce crop.

We would thus argue that the good microbial quality of vegetable crops, (no *E. coli*, no *Salmonella spp*.), can be seen as the positive consequence of several factors: among the principal ones, drip irrigation method, that does not dirty the leaves, the summer period with increased UV radiation exposure of lettuce leaves surfaces. Also, the interval time between irrigation and harvesting may have contributed to reduce the effect of treated wastewater on the microbial load of vegetables.

5. Conclusions

Demo-scale research activities were conducted in Southern Italy for studying the effects of irrigation with treated municipal wastewater on vegetable crops, in terms of their quality and safety. The different wastewater treatment technologies tested produced effluent quality mostly within the stringent local standards and suitable for irrigation. Crop yields obtained with treated wastewater were higher than those of well water, due to the positive effect of higher water-borne nutrient concentrations.

Microbial indicators *E. coli* and *Salmonella* were never found in soil and on plants. Treated wastewater reuse in semiarid areas allows relevant fresh water savings and limits pollution due to fertilizers.

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