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The nitrogen role in vegetables irrigated with treated municipal wastewater

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Abstract. The reuse of treated municipal wastewater for irrigation is an established alternative to conventional water, in many countries of the world, particularly where or when water resources are extremely limited. Wastewater reuse could represent a double benefit when used in agriculture, helping overcome any lack of water resources and additionally, enriching the soil with nutrients - especially nitrogen and phosphorus.

In the experimental site of Castellana Grotte (Apulia region, Southern Italy) during the 2012/13 and 2013/14 growing seasons, vegetable crops (fennel and lettuce) in succession were drip-irrigated with three different water sources. Two reclaimed water streams, obtained by applying different treatment schemes to the same municipal wastewater (an effluent from the full-scale treatment plant and an effluent from the Integrated Fixed-film Activated Sludge – Membrane BioReactor pilot plant) and a conventional source, to verify the crops response and nutrient contribution through wastewater supply.

Both lettuce and fennel yields were enhanced by the high content of nutrients in the effluent of one of the treatment plants, which had been operated for partial nitrogen removal. For Fennel 2013/14, wastewater-reuse led to a 54% reduction of nitrogen supply in relation to the other plots normally fertilized. In this way, an estimated saving of about 98.00 € ha^{-1} was achieved.

Crops irrigated with treated wastewater operated for partial nitrogen removal (IMBR) showed early ripening (8 days for lettuce and 35 days for fennel 2013/14) and better quality than others not similarly-treated. However, the wastewater presented a nitrate content in excess of legal limits (35 mg L^{-1}, D.M. 185/2003). Therefore, the contribution of nutrients increased production (47 vs 32 t ha^{-1} in IMBR and WELL 2012/13 fennel theses, 53 vs 31 t ha^{-1} in IMBR and WELL 2013 lettuce theses and 40 vs 31 t ha^{-1} in IMBR and WELL 2013/14 fennel theses respectively) and improved product quality, while simultaneously saving money for chemical fertilizers not supplied, producing less environmental impact.

Key words: irrigation reuse, nitrate, soil, nutrients, treated municipal wastewater, vegetables.

INTRODUCTION

Reclaimed wastewater and fertilizers

In recent years, the use of treated municipal wastewater for irrigation has become a very common practice in many countries of the world, especially in those with a dry climate where the water resource is extremely limited (Meli et al., 2002; FAO, 2010;

FAO, 2011). In many water-scarce countries such as Pakistan, Vietnam, Ghana and Mexico, wastewater is widely used for vegetable production (Pedrero et al., 2010).

The potential health risks and environmental impacts resulting from wastewater reuse in agriculture have already been studied (Angelakis et al., 2003). Sheikh et al. (1990) reported in Monterrey Wastewater Reclamation Study for Agriculture, food crops for raw consumption can be successfully irrigated with treated wastewater without adverse environmental or health effects. Furthermore, York et al. (2008), Lonigro et al. (2016) also demonstrated the safety and the suitability of reclaimed water use for agricultural irrigation.

Treated municipal wastewater reuse in agriculture may represent not only a resource to meet the growing water demand but also a cheap source of nitrogen and phosphorus nutrients (Chen et al., 2008; Disciglio et al., 2015).

Wastewater, containing macro and micro nutrients (e.g. nitrogen, phosphorus, potassium, calcium, and magnesium) that plants need to grow, can be considered a new and additional source of fertilizers leading to savings for an external supply. In addition, in some areas, it may be the only affordable source of fertilizers for poor farmers (Mateo–Sagasta & Burke, 2010).

Fertilizers are required for sustained food production, but their widespread and not rational use has aroused concern about resulting environmental pollution.

The nitrogen role

Nitrogen is essential to every living being. Nitrate (NO_3^-) is a naturally occurring form of nitrogen. Most crops require large nitrogen quantities to sustain high yields; it plays an essential role in plant biochemistry, participating in the formation of compounds essential to plant life such as amino acids, proteins, and nucleic acids. Nitrogen fertilization is an agronomic practice essential to meet the nutritional needs of crops. Nevertheless, the plant does not use all the nitrogen. In particular, nitrate is a soluble compound that, not being retained by the solid phase of the soil, can be easily leached from the soil by deep percolation to underground aquifers. Normally present in drinking water (World Health Organization (WHO) standards 50 mg L^{-1} and Italian Legislative Decree 31/2001), nitrate reaches high concentrations in plants (EC, 2011) and has always been considered potentially hazardous to human health.

Nitrate is absorbed in the blood, and hemoglobin is converted into methemoglobin that does not carry oxygen efficiently to important vital tissues such as the brain. Severe methemoglobinemia can result in brain damage and death (Self & Waskom, 2013). This outcome is directly related to the intensive and improper use of mineral fertilizer and manure for agriculture, sometimes exceeding crop–nitrogen demand (Mateo-Sagasta & Burke, 2010). Most of the nitrate we consume is from our diets, particularly from raw or cooked vegetables. In fact, vegetables constitute the major dietary source of nitrate, generally providing from 30 to 94% of the dietary intake (Di Gioia et al., 2013). The leafy vegetables (especially lettuce and spinach), fennel, celery and rocket (Sagratella et al., 2011) are capable of holding the largest concentrations of nitrates (Gonnella et al., 2002).

Nitrate levels can also vary within species, cultivars, and even genotypes with different ploidy (Blom–Zandstra, 1989). An accumulation of nitrate in vegetables occurs when crops absorb more than they require for their sustainable growth (Anjana et al., 2007). The accumulation of nitrate in crops and their edible parts can depend on several factors such as species and cultivars, amounts, timing and source of fertilizers used, the weather conditions (temperature, intensity, and duration of exposure to light), the physical-chemical nature of the soil and the presence of water.

The recent revision of EU Regulation (EC, 2011) redefines and – compared to the previous EC Regulations (EC, 2006a; EC, 2006b) – raises the levels of nitrates in certain leafy vegetables (lettuce, fresh spinach) because of climatic differences found among the Member States. Some areas with low temperatures favor the presence and accumulation of nitrate in vegetables. The EC Regulation takes into consideration that nitrate accumulation in vegetables is higher when solar radiation is lower (Di Gioia et al., 2013).

In order to evaluate a strategy to reduce the excessive nitrogen fertilizer use and related negative environmental impact, the aim of this study was to compare the effects of two different types of treated municipal wastewater: 1) a traditional municipal treated effluent and 2) an effluent from a pilot treatment plant, respectively, with different nutrients content (NO_3^-) on fennel and lettuce crop-performance. In particular, the qualitative and quantitative aspects of fennel and lettuce crop productions and the level and accumulation of nitrate in vegetables were investigated. The results reported in this paper refer to a two years trial of irrigation on vegetable crops in succession.

MATERIAL AND METHODS

Treatments

The experimental trials were carried out in the countryside of Castellana Grotte (Bari, Southern Italy), near the municipal wastewater treatment plant. Three types of waters were compared in irrigation: the effluent from the full–scale treatment plant (EFF), the effluent from the IFAS-MBR pilot plant (IMBR) and conventional water drawn from a local well (WELL).

The full-scale municipal wastewater treatment plant 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. Subsequently, in the aerated reactor, oxidation of the organic fractions and nitrification occur. In the final settling tank, the produced sludge is separated from the liquid phase and partly recirculated to maintain the required biomass concentration. The secondary effluent is further treated through granular media sand filtration and chlorine disinfection, before being discharged on soil. During the experimental activities, a fraction of this effluent was split and used for irrigation at the test field located immediately outside the treatment plant.

The pilot-scale plant is based on the IFAS–MBR technology (Integrated Fixed–film Activated Sludge – Membrane BioReactor), it treats sewage after preliminary screening, where nitrates were intentionally not removed, to verify the effect on crops. The IFAS technology is based on the presence of suspended plastic carriers in the aerobic bioreactor (Fig. 1).

These carriers promote biomass accumulation in the form of a biofilm, and biological processes are carried out synergistically by the suspended biomass and the biofilm, resulting in limited biomass growth.

Figure 1. Schematic illustration of a plastic carrier (A) and particular of biofilm (B).

The combination between IFAS and 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. Furthermore, membrane separation results in high-quality effluent in terms of suspended solids, favoring the adoption of UV disinfection technologies 'on demand'. The end pipe of this plant is connected to a UV disinfection system that is activated when the irrigation line is switched on.

Field characteristics, agronomic conditions, and experimental design

The experimental field was located adjacent to the municipal wastewater treatment plant of Castellana Grotte (40°53'20"N 17°11'51"E; altitude 305 m a.s.l.) (Fig. 2). The trials were carried out in a loam soil, (USDA classification), with a field capacity (-0.02 MPa) 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) are as follow: sand 44.4%; silt 44.1%; clay 11.5%; organic matter 1.50%; P_2O_5 (Olsen) 19 mg kg^{-1}; extractable K_2O ($BaCl_2$) 70 mg kg^{-1}; total N 1.11 g kg^{-1} (Kjeldahl); pH 8.1; electrical conductivity (1:2.5 w/v) 0.22 dS m^{-1}.

Figure 2. Satellite view of the municipal wastewater treatment plant of Castellana Grotte (Bari, Italy) and experimental field (red circle) (https://earth.google.com.

Three types of water were compared to irrigation: the effluent from the full–scale treatment plant (EFF), the effluent from the pilot IFAS–MBR plant (IMBR) and conventional water drawn from a local well (WELL). A localized low–pressure drip irrigation system with flow of 4 L h^{-1} was used for the irrigation of vegetable crops (fennel and lettuce) grown in succession. Lettuce and fennel are two of the most important leafy vegetables regarding their cultivation area and consumption rate in the world, characterized by high tendency to accumulate nitrates. The soil was tilled to a depth of 0.40 m, and then its surface was grinded before transplanting. During the cropping season, nutrient intakes and other management practices were estimated from local farmers. Pest and weed control were performed according to common management practices.

The efficiency of the irrigation method adopted was 90%. Evapotranspiration can be expressed by Eq. (1), where E = 'class A' pan evaporation (mm); K_c = crop coefficient; K_p = pan coefficient (0.8).

$$E_{Tc} = E \cdot K_p \cdot K_c \tag{1}$$

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 evapotranspiration criterion providing water to the crops when the condition (2) for lettuce (a = 30 mm) and fennel (a = 25 mm) is met, where n = number of days required to reach the SWD_{lim} starting from the last watering; E_{tc} = crop evapotranspiration (mm); R_e = rainfall (mm); a = Readily Available Water

$$\sum_{1}^{n} (E_{Tc} - R_e) = a \tag{2}$$

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 (ASSOCODIPUGLIA, http://www.agrometeopuglia.it/opencms/opencms/Agrometeo/Meteo/Osservazioni/datiRilevati).

The experimental scheme adopted was the randomized block with four replicates realizing 12 large plot of the size of 20 x 20 m. During two years (2012/13 and 2013/14) of trials, three crops were grown in succession: fennel, lettuce, and fennel. Fennel 2012/13 (*Foeniculum vulgare* Mill) cv. Archimede 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 hand harvested on March 19th, 2013 in each plot. Pre-transplanting fertilization was applied to the soil by distributing 40 kg ha^{-1} N. Throughout the crop cycle, 110 kg ha^{-1} N were added through fertigation.

Lettuce (*Lactuca sativa* L.) cv. Iceberg, was transplanted in succession to fennel, 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 hand harvested on June 17th, 2013 in plots irrigated with IMBR water; after three days (June 20th) in plots irrigated with EFF and after eight days (June 25th) in plots irrigated with WELL water. Pre-transplanting fertilization was applied to the soil by distributing 40 kg ha^{-1}N. Throughout the crop cycle, 80 kg ha^{-1}N were added through fertigation. Fennel 2013/14 cv. Archimede, was transplanted, in succession to lettuce, on the same plots on August

30th, 2013 with the same modality of previous fennel and was hand harvested starting from December 10th 2013 (IMBR).

Table 1. Main climatic parameters recorded during the growing season of the three vegetable crops

	^{a} Climatic Parameters							T_{max}	T_{min}	P
Month	T_{max} (°C)	T_{min} (°C)	RH_{max} (%)	RH_{min} (%)	Ev (mm)	Ws (m s^{-1})	P (mm)	Long term average (°C)	Long term average (°C)	Long term average (mm)
September 2012	27.80	16.10	95.30	34.80	3.73	2.51	8.60	24.13	15.07	59.60
October 2012	22.48	12.11	99.37	48.40	2.25	2.35	55.60	18.87	10.87	66.90
November 2012	16.77	9.83	98.56	67.70	1.17	2.92	151.60	15.00	7.87	80.10
December 2012	10.50	3.87	99.19	60.61	0.78	3.69	64.30	10.83	4.90	77.10
January 2013	10.49	3.28	99.45	62.84	0.91	3.62	96.80	9.43	3.47	72.80
February 2013	10.33	1.79	99.78	61.00	1.28	3.03	89.30	10.57	3.60	63.00
March 2013	13.44	5.68	99.23	57.87	1.93	4.42	62.20	12.57	5.03	63.00
Growing season Fennel	**15.97**	**7.52**	**98.70**	**56.17**	**1.72**	**3.22**	**528.40**	**14.49**	**7.26**	**68.93**
April 2013	19.31	8.48	89.13	37.23	3.29	3.33	11.00	16.53	7.63	44.70
May 2013	23.56	11.35	92.30	28.44	4.51	2.91	17.00	21.57	11.63	37.50
June 2013	26.20	14.50	82.50	31.46	5.07	2.44	11.20	25.57	15.23	31.00
Growing season Lettuce	**23.02**	**11.44**	**87.98**	**32.38**	**4.29**	**2.89**	**39.20**	**21.22**	**11.50**	**37.73**
August 2013	30.60	18.19	83.00	32.42	5.00	2.18	47.20	28.53	17.80	24.70
September 2013	26.20	14.45	92.54	35.07	3.64	2.01	9.60	24.13	15.07	59.60
October 2013	21.64	12.40	97.35	55.45	2.13	2.34	112.90	18.68	10.87	66.90
November 2013	15.13	7.99	98.17	67.20	1.19	2.55	145.60	15.00	7.87	80.10
December 2013	11.56	2.71	98.36	63.42	0.90	2.20	114.00	10.83	4.90	77.10
January 2014	11.36	4.96	98.37	70.29	0.87	3.38	58.60	9.43	3.47	72.80
Growing season Fennel	**19.42**	**10.12**	**94.63**	**53.98**	**2.29**	**2.44**	**487.90**	**17.77**	**10.00**	**381.2**

$^{a}T_{min}$, 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; Ev, total ‘class A’ pan evaporation.

Differently from the first fennel crop, the IMBR plots did not receive any dose of fertilizer in fertigation to evaluate the efficacy of the contribution of nutrient uptakes and fertilizer practices in more detail. Three harvestings were performed from December 2013 to January 2014, on the days after transplanting of 102, 107, 137 for IMBR, EFF and WELL plots, respectively. Marketable yield (t ha^{-1}), average weight (g) and clumps dry matter (%) were measured at harvesting time.

Water, soil, vegetable sampling and analysis

WELL, IMBR and EFF water samples were collected under the dripper at every watering throughout the crop irrigation period to quantify the main physicochemical parameters according to standard methods (APHA, 2012). The water samples were collected in triplicate in 1,000 mL PE bottles and transported to the laboratory in a refrigerated box. The samples were then kept in a refrigerator at +4 °C and examined within 24 h of their collection. The measured parameters were: pH, electrical conductivity (ECw, dS m^{-1}), BOD_5 (mg L^{-1}), COD (mg L^{-1}), ammonium-nitrogen (NH_4–N, mg L^{-1}), nitrate-nitrogen (NO_3–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}), sulphate (SO_4^-, mg L^{-1}), Sodium Adsorption Ratio (SAR). The anions and cations content were determined by ion-exchange chromatography (Metrohm mod. 883 Basic IC plus, Switzerland).

Soil samples were collected under the dripper in triplicate from each plot before and after every crop cycle (harvesting time) at depths decreasing from 0 to 0.4 m, every 0.2 m and they were air-dried, crushed, and passed through a 2 mm sieve before the chemical analysis. Nitrogen Kjeldahl (N), phosphorus available (P_2O_5), potassium exchangeable (K_2O), organic matter (O.M.), pH and electrical conductivity were routinely analyzed according to standard procedures (Spark, 1996).

Lettuce and fennel samples were collected at harvesting time, in triplicate from each treatment plot by picking all of the marketable size plants. The freshly collected plant samples were introduced in PE bags and immediately chilled to +4 °C and kept as such during transport to the laboratory for chemical analyses. On the marketable edible parts of vegetable crops were counted the number of plants and weighted to estimate total yield (TY, t ha^{-1}). On marketable samples from each plot, dry matter content (DM, % fresh matter) (AOAC, 1995) and an average weight of plants were also measured.

Nitrogen content of vegetables

Therefore, each sample consisted of a pool of 10 plants of a commercial size. In order not to affect the analytical determination, from each plant non-edible and damaged outer leaves were removed. The samples were not subjected to washing as this might result in the reduction of the levels of nitrates. Fresh weight was detected on the edible portion of the samples and then, after drying in a thermo-ventilated stove at 65 °C until the constant weight (dry weight) was reached. The dry substance thus obtained was finely ground with the micrometric mill and then subjected to quantitative analysis of nitrate using the method reported by Parente et al. (2002). For the determination of nitrate an ionic chromatograph Metrohm (Switzerland) mod. 883 Basic IC plus was used.

The determination of nitrate was carried out on the dry matter, while the analytical data was expressed in fresh weight.

Statistical analysis

Before to processing data with the analysis of variance, normal distribution was verified on all the experimental data, the Bartlett test was applied to verify the homogeneity of the error variance. When the data were normal and the Bartlett test was significant, the analysis of variance was performed with a nonparametric test to one classification criterion (The Kruskal-Wallis test). For multiple comparisons, it was applied the Nemenyi-Damico-Wolfe-Dunn test. In other cases, the F-test was performed for the analysis of variance (ANOVA) and the SNK test to compare the means.

RESULTS AND DISCUSSION

Irrigation water quality

In Table 2 the chemical–physical characteristics of the water used during the experimental irrigation period are reported.

Table 2. Means of the main chemical-physical parameters measured during the experimental period of trials, for the well water (WELL), full scale municipal wastewater treatment plant (EFF) and MBR pilot plant (IMBR) used for vegetable crops irrigation

Water parameters	U.M.	FENNEL 2012/13				LETTUCE 2013				FENNEL 2013/14				Italia Law Limit Values (D.M. 185/2003) (R.R. 8/2012)
		WELL	EFF	IMBR	p-value	WELL	EFF	IMBR	p-value	WELL	EFF	IMBR	p-value	
EC	(dS m^{-1})	0.94 ± 0.006	1.60 ± 0.37	0.99 ± 0.11	0.12	1.17 ± 0.14	1.13 ± 0.14	0.99 ± 0.02	0.27	0.66 ± 0.14	0.88 ± 0.03	0.94 ± 0.03	0.09	3.00
pH		7.71 ± 0.054	7.85 ± 0.03	7.79 ± 0.16	0.60	7.48 ± 0.07	7.67 ± 0.02	7.51 ± 0.05	0.09	7.30 ± 0.14	7.62 ± 0.05	7.36 ± 0.09	0.26	6–9.5
BOD$_5$	(mgO$_2$ L^{-1})	0 ± 0 **C**	10.3 ± 0.33 **B**	19.3 ± 2.4 **A**	0.001	1.35 ± 0.77 **B**	4.35 ± 0.32 **A**	4.30 ± 0 **A**	0.009	5.25 ± 0.72	7.27 ± 1.82	8.17 ± 2.51	0.51	20
COD	(mgO$_2$ L^{-1})	0 ± 0 **C**	13.5 ± 3.17 **B**	37.3 ± 4.3 **A**	< 0.001	2 ± 1.15 **B**	18 ± 0.58 **A**	16.5 ± 0.29 **A**	<0.001	8.5 ± 0.29	21.7 ± 2.70	22 ± 5.57	0.05	100
Na$^+$	(mg L^{-1})	55 ± 21.4	175 ± 61.2	66.3 ± 4.4	0.09	42 ± 13.8	74.5 ± 2.02	189.5 ± 68.4	0.16	12.5 ± 0.29 **B**	74.7 ± 3.33 **A**	62.7 ± 6.67 **A**	0.0007	
K$^+$	(mg L^{-1})	0 ± 0 **b**	3.5 ± 2.02 **b**	13.6 ± 3.2 **a**	0.03	1.5 ± 0.86 **b**	17.5 ± 0.29 **ab**	38 ± 11.5 **a**	0.05	0.5 ± 0.29 **B**	12 ± 1.52 **A**	13.3 ± 1.76 **A**	0.0047	
Ca^{2+}	(mg L^{-1})	45.8± 20.3	85.5 ± 3.17	78.3 ± 26.9	0.35	83.5 ± 5.48	48 ± 1.15	348 ± 169.1	0.18	60.5 ± 19.3	69.3 ± 13.2	95.7 ± 50.7	0.62	
Mg^{2+}	(mg L^{-1})	25 ± 5.2	27 ± 6.35	10.3 ± 3.75	0.23	39.5 ± 3.17**AB**	5.5 ± 1.44 **B**	59.5 ± 13.5 **A**	0.034	20 ± 11.6	8 ± 4.62	23.3 ± 19.1	0.47	
NH$_4^+$	(mg L^{-1})	3 1 ± 1.73 **B**	5 ± 2.89 **B**	19 ± 1.15 **A**	0.01	0 ± 0	0 ± 0	0 ± 0		0 ± 0	0 ± 0	11.3 ± 6.64	0.16	2 (15)^a
Cl$^-$	(mg L^{-1})	79 ± 25.4	357 ± 144.9	92.6 ± 10.3	0.09	106.5 ± 41.3	122.5 ± 7.21	120.5 ± 14.7	0.91	16.9 ± 2.39 **B**	91.3 ± 12.3 **A**	91.3 ± 5.36 **A**	0.0017	250
NO$_3^-$	(mg L^{-1})	6.7 ± 3.75 **c**	33 ± 4.04 **b**	85 ± 9.45 **a**	0.02	13 ± 0 **B**	33.5 ± 3.17 **B**	73 ± 19.1 **A**	0.001	7.85 ± 0.09	34.7 ± 18.6	99.3 ± 36.2	0.06	35*(15)^a
P$_2$O$_4^{3-}$	(mg L^{-1})	1.3 ± 1.3	0.66 ± 0.67	8.7 ± 4.7	0.23	0.01 ± 0.01 **b**	21 ± 6.24 **a**	15.3 ± 2.9 **a**	0.02	0 ± 0	15 ± 13	12.7 ± 7.22	0.34	10 (2)^b
SO$_4^{2-}$	(mg L^{-1})	50 ± 24.2 **B**	129 ± 5.19 **A**	3.3 ± 3.3 **B**	0.008	6 ± 1.73 **B**	61 ± 11.5 **A**	31.5 ± 7.8 **B**	0.01	4.5 ± 2.59 **b**	61 ± 11.1 **a**	19.3 ± 13.4 **b**	0.034	500
SAR		1.79 ± 0.7	4.1 ± 1.27	1.93 ± 0.2	0.16	0.93 ± 0.28 **b**	2.71 ± 0.04 **a**	2.45 ± 0.35 **a**	0.03	0.44 ± 0.12 **b**	2.41 ± 0.43 **a**	1.96 ± 0.55 **a**	0.03	10

* Limit related to total nitrogen; [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); data are means ± standard error for each water analysed between September 2012 and January 2014; capital letters (A, B and C) represent significant differences at *$P < 0.01$;* lower case letters differences at *$P < 0.05$.*

In fennel 2013, the organic matter content expressed as BOD and COD, and the nitrate content present in IMBR water were doubled compared to EFF. For potassium, the value was just higher than EFF. Whereas lettuce 2013 showed in IMBR a content of nitrate and potassium twice compared with EFF. Regarding fennel 2013/14, the content of nitrate of IMBR was three times the EFF.

The higher NO_3^- levels in IMBR respect to EFF and WELL indicate that IMBR represents the major source of nutrient for the plants and the soil, and can contribute to crop growth (Gatta et al., 2014) (Generally it is not taken into account by farmers when applying fertilizer).

As reported in Jensen et al. (2006), from an agronomic perspective wastewater irrigation represents an opportunity for accessing 'free' nutrients which if realized contribute towards the inter–related objectives of productivity maximization, nutrient capture and wastewater reclamation and reuse. The resulting nitrogen excess in the soil is then particularly vulnerable to the risk of leaching, thus increasing the environmental problem of nitrate pollution (Gatta et al., 2014). Consequently, the use of wastewater for irrigation helps to reduce downstream health and environmental impacts that would otherwise result if wastewater was discharged directly into surface bodies (Mateo–Sagasta & Burke, 2010).

Table 3 shows main parameters of soil irrigated during the trial. No significant differences were found.

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

Soil parameters	Fennel 2012/13			Lettuce 2013			Fennel 2013/14		
	WELL	EFF	IMBR	WELL	EFF	IMBR	WELL	EFF	IMBR
EC	0.22	0.20	0.21	0.38	0.35	0.43	0.16	0.25	0.26
pH	8.10	8.07	8.04	8.03	8.15	8.05	7.88	8.09	8.02
O.M. (%)	1.61	1.77	2.02	1.72	1.84	2.03	1.69	1.76	1.91
N (g kg^{-1})	1.11	1.11	1.14	1.08	1.21	1.39	1.11	1.25	1.40
P_2O_5 (mg kg^{-1})	82	87	133	43	47	77	36	38	43
K_2O (mg kg^{-1})	132	134	148	111	116	120	73	82	84

Harvest and yields

The harvest of fennel 2012/13 was performed after 171 days at once for all treatments (IMBR, EFF, and WELL). Data collected were a marketable yield (t ha^{-1}), average weight (g), dry matter of clumps (%) and nitrate concentration (mg kg^{-1}fw). The results obtained for the nitrates are respectively 169 mg kg^{-1} for WELL, 754 mg kg^{-1} for EFF and 1,040 mg kg^{-1} for IMBR.

The harvest of lettuce took place after 60, 63 and 68 days respectively for the theses IMBR, EFF and WELL. Data collected were a marketable yield (t ha^{-1}), average weight (g), dry matter of heads (%) and nitrate concentration (mg kg^{-1} fw). The data obtained show an average concentration of nitrate content of 244 mg kg^{-1} in the thesis irrigated with WELL, of 477 mg kg^{-1} in the thesis EFF and 804 mg kg^{-1} for the IMBR. The harvest of fennel 2013/14 was performed after 102, 109 and 137 days respectively for the thesis IMBR, EFF and WELL. Data collected were the same as the previous year trial. In this case, the results obtained for the nitrates are respectively 140 mg kg^{-1} for WELL, of 743 mg kg^{-1} for EFF and 245 mg kg^{-1} for IMBR. The levels of nitrate (mg kg^{-1} fresh weight) in the considered plants, the yield data (t ha^{-1}), the average weight (g), dry matter (%), the number of days from transplanting to harvesting and inputs of nitrogen (mg ha^{-1}) are reported in Table 4. The result is given as the average of 4 replicates.

The results show that, even for breeding crops particularly prone to a high accumulation of nitrates, the values obtained are well below the limits permitted by law. In fact, in none of the samples analyzed the nitrate concentration found exceeded the limits set by EU Regulation No. 1258/2011 (EC, 2011) (Fig. 3).

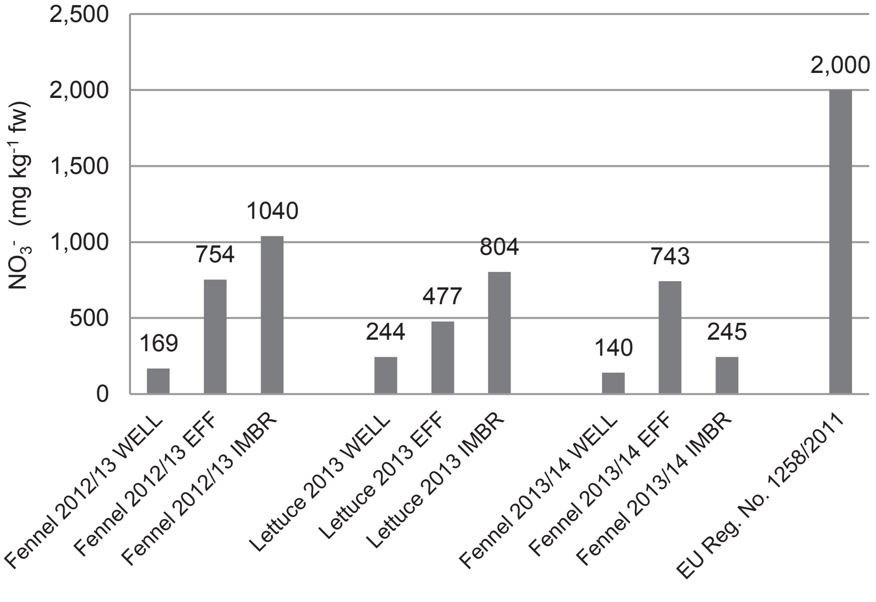

Figure 3. Comparison of average values of nitrates found in all the theses and limit defined by EU Regulation No. 1258/2011.

The use of wastewater to fertigate fennel and lettuce had positive effects on fertilizer management. Although excessive doses of nitrogen (mineral fertilization plus IMBR nitrate intake) were tested, the nitrate content of lettuce was found to be well below the limits allowed by law (2,000 mg kg^{-1}). Perhaps this is due to the effects of the climatic conditions of the experimental site located in Southern Italy, which may contribute to a content of nitrates lower compared to northern regions. Fennel 2013/14, grown in the same IMBR plots of lettuce and fennel 2012/13, did not get any doses of fertilizer. The only input of nitrates was from wastewater (74.8 kg ha^{-1}), and N supplied at transplanting.

In this case, wastewater reuse led to a reduction of 54% of nitrogen fertilizer in relation to the other plots normally fertilized. All this resulted in an advance of maturity (harvest made 35 days before conventional), better quality (marked green color of the leaves and more resilient post-harvest), a lower nitrate content than the average reported in the literature and a significant savings of chemical nitrogen fertilizers.

Table 4. Effects of water irrigation treatments (WELL = conventional water; EFF = effluent from full scale treatment plant; IMBR = effluent from pilot treatment plant) on yield, average plant weight, dry matter, levels of nitrate (on fresh weight), number of days from transplanting to harvest and nitrogen inputs at harvesting time

							N supply (kg ha^{-1})				
Crop	Treatments	Yield (t ha^{-1})	Average weight (g)	Dry matter (%)	NO_3^- (mg kg^{-1} fw)	Harv. time	Seasonal Irrigation Volume (m^3 ha^{-1})	Pre-transplanting fertilization $HPO_4(NH_4)_2$	Fertirrig. NH_4NO_3	N in the water	N TOTAL
	WELL	32.16 B	441 B	7.95 a	169 B	171	800	40	110	0	150.0
	EFF	46.09 A	656 A	7.43 a	754 A	171	800	40	110	4.8	154.8
	IMBR	47.80 A	668 A	7.38 a	1040 A	171	800	40	110	6	156.0
	WELL	31.28 B	556.41 B	4.93 a	244 B	68	1,800	40	80	4.1	124.1
	EFF	38.49 B	591.84 B	4.80 a	477 AB	63	1,800	40	80	15.5	135.5
	IMBR	53.64 A	826.01 A	4.01 b	804 A	60	1,800	40	80	69.2	189.2
	WELL	31.63 B	373.80 b	8.98 a	140 b	137	1,100	40	110	1.7	151.7
	EFF	29.82 C	380.01 b	8.70 a	743 a	109	1,100	40	110	12.4	162.4
	IMBR	39.97 A	487.02 a	7.67 b	245 b	102	1,100	40	0	34.8	74.8

Capital letters represent significant differences at $P < 0.01$; lower case letters differences at $P < 0.05$.

Usually, wastewater–watering protracts the crop development pattern, prolonging ripening, delaying flowering and reducing the economic fraction (marketable yield) (Jensen et al., 2006). In the case of leafy vegetables, high N–availability in wastewater promoting vegetative growth (= increasing production), indeed represents an important benefit. Moreover, the continuous supply of nitrogen with irrigation is, without doubt, the most important factor – especially in autumn–winter seasons – limiting nitrogen losses from gasification and leaching thereby improving the efficiency of fertilization.

Wastewater contains nutrients in many forms (ammonia, phosphates, nitrate, etc.), with a daily and seasonal variation in concentrations. Therefore, wastewater irrigation could contribute to reduced nutrients from the environment being high enough to at least partly fulfill crop nutrient requirements. Nonetheless, a total application of organic and mineral fertilizers is excessive.

Farmers frequently oversupply nutrients (Evers et al., 2006). Wastewater nutrient content is sufficient to partly meet crop nutrient requirements per growing season, but farmers often use wastewater only as a source of water and do not consider it as a source of nutrients. This conduct is frequently due to a lack of information on nutrient management and wastewater-quality from institutional organizations. With careful planning and management, the use of wastewater for agriculture can be beneficial to farmers, cities and the environment (Mateo–Sagasta & Burke, 2010).

CONCLUSIONS

This study focuses on agronomic aspects of wastewater and its nutrient opportunity, a resource still very undervalued and unexploited in Italy. According to the data obtained, it is possible to conclude that treated municipal wastewater without nutrient-removal influences the crop cycle and represents an alternate and relevant source of nutrients intake. In particular, the high nitrogen content enhances vegetative growth, promotes crop development and sustains both economic and environmental benefits. By the current market price of nitrogen fertilizer, the estimated savings is about 98.00 € ha^{-1}.

These results should encourage achievement of a more-sustainable agriculture through the use of treated municipal wastewater not deprived of nutrients, thereby limiting the use of higher-quality water, thus saving fertilizers and money. All this can be accomplished while respecting and protecting the environment.

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