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Protecting groundwater in intensive agricultural areas through irrigation with treated wastewater: focus on nitrate, salt, and *Escherichia coli*

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ABSTRACT

A set of 4 soil column duplicates was irrigated with treated wastewater to study the possible leaching of nitrate, salt, and *Escherichia coli* to groundwater. The reclaimed water was a municipal secondary effluent, stored for 5 days to attenuate microbial contamination. It had nitrate concentration of 36.1±4.9 mgN/L, electrical conductivity of 1.6±0.1 mS/cm, and *E. coli* content between 36 and 918 MPN/100 mL (median value of 194 MPN/100 mL). Soil column tests were carried out over a period of 80 days, considering both the cultivation of a typical Mediterranean crop (pepper) and the edge case of non-cultivated soil. Nitrate and salt were up-taken by crops for around 90% and 50%, respectively, while they leached through non-cultivated soil according to linear relationships, with nitrate moving faster than salts. Due to its natural decay, *E. coli* never reached 66 cm depth. Crop irrigation with reclaimed water can be managed so as not to cause significant leaching of *E. coli* and nitrate, even though it may result in a small leaching of salt. Replacing groundwater with reclaimed water as an irrigation source should be considered as a possible action to protect aquifers, and especially those suffering from saline contamination, from the effects of overexploitation and overfertilization practices.

1. Introduction

In many areas worldwide, intensive farming is posing ever increasing risks on the sustainability of freshwater resources. On the one hand, if not correctly managed, the strong competition for the use of water often causes groundwater overexploitation, which may jeopardise groundwater quality and its usability in the long term, especially in coastal areas subject to salinity intrusion. In semi-arid regions the agricultural sector heavily contributes to this phenomenon, accounting for most groundwater withdrawals (www.worldbank.org). This scenario, aggravated by climate change, makes it urgent to find alternative irrigation water sources (WWAP, 2017; FAO, 2021). On the other hand, over-fertilization practices undermine the quality of underlying groundwater. Nitrate, if applied in excess with respect to plant requirement, accumulates into the root zone and it is easily leached by irrigation water and rainwater to the deeper soil layers (Libutti and Monteleone, 2017). Even though some measures were taken to deal with this problem (European Commission, 1991), overfertilization practices are still very common in intensive farming systems, so further mitigation strategies are required.

The use of treated wastewater for crop irrigation is widely recognized as an effective strategy to cope with water scarcity and is attracting global attention (Mishra et al., 2023; Tampo et al., 2022). In addition to saving freshwater resources, this practice has various advantages, including providing nutrients that may substitute chemical fertilisers (Vergine et al., 2017a), thus reducing the discharge of pollutants into sensitive water resources (European Commission, 2016). For this two-fold benefit, the use of treated wastewater in agriculture can be considered a good strategy to protect groundwater in intensive farming areas.

However, wastewater reuse has also potential risks associated with the residual presence of pollutants in the reclaimed water (Ofori et al., 2021), including the risk of groundwater contamination itself. Even if advanced wastewater treatments can produce effluents suitable for any type of use (Capodaglio, 2021), some pollutants, such as salts and microbial pathogens, can be removed only through relatively costly treatments that would reduce the competitiveness of reclaimed water with respect to freshwater. Desalination is a quite expensive process and, despite recent advances (Cohen, 2021), it is very rarely affordable within irrigational reuse schemes. Cost-effective disinfection technologies are available, but high dosages and duplication of the processes may be required to comply with some strict regulations and at the same time

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to cope with temporary decreases in the disinfection performance. Furthermore, nutrients, and in particular nitrate, are intentionally left in reclaimed water for their fertilising effect. Therefore, any realistic plan of irrigation with treated wastewater should evaluate the possible leaching of salts, nutrients, and pathogen indicators to groundwater. Other harmful substances can be contained in reclaimed water, including heavy metals and contaminants of emerging concern, but the scope of this study is limited to the most relevant and mobile pollutants.

To tackle nitrogen losses in agriculture, several actions were adopted to reduce the input of nutrients to the soil. Among these, acceptance limits for nutrient content in reclaimed water were established. However, their values are not supported by solid scientific evidence, since very few studies focused on the fate of nitrate in irrigation with treated wastewater (Lal et al., 2015). Due to the differences between this practice and those based on solid fertilisers, it is necessary to improve the knowledge on nitrate up-take by plants and on its transport through the soil when this is provided through reclaimed water.

Unlike nitrate, salinity in reclaimed water is mostly regarded, due to the risk of soil salinization. High levels of soil EC, exchangeable sodium and exchangeable sodium percentage can decrease soil productivity and crop yield, especially for vegetables that are particularly sensitive to soil salinity (Machado and Serralheiro, 2017; Mishra et al., 2023). When irrigating with saline water, farmers and agronomists usually focus on preventing the buildup of salinity in the soil (Minhas et al., 2020). The application of excess water beyond crop evapotranspiration is a management option to leach out salts accumulated in the root-zone, minimising yield reduction. On the other hand, this strategy results in drainage water enriched in salts that increases groundwater salinity. Possible solutions, such as tile drains (Singh, 2019), were proposed to deal with this problem, but they have still a limited application. The knowledge on the leaching of salts during irrigation with treated wastewater needs to be enhanced, as well as the risk of groundwater salinization should be assessed with respect to the environmental context.

Evidence of groundwater faecal pollution due to the irrigation with untreated wastewater or low-quality reclaimed water were widely reported (Vergine et al., 2015; Wu et al., 2020), as well as evidence of positive effects on soil microbiota (García-Orenes et al., 2015), but the knowledge about the effects of a residual but limited presence of faecal coliforms on the underlying subsurface water is still scarce.

Groundwater protection is particularly important in Apulia (Southeast of Italy) (Parisi et al., 2018). Like other coastal areas in the Mediterranean basin, Apulia is characterised by absence of relevant surface water bodies, due to its karstic nature (Polemio, 2018). Moreover, its economy is mostly based on irrigated agriculture. Intensive farming systems caused a serious groundwater degradation over the past decades: decrease of the piezometric level, salinization of coastal aquifers, and nitrate contamination were observed (Polemio, 2018; Serio et al., 2018). Irrigation with treated wastewater has a great potential to counteract this trend. Several studies showed the effectiveness of this practice to sustain the local agricultural sector, demonstrating also the suitability of nutrients recovery (Vergine et al., 2017a; Libutti et al., 2018; Vivaldi et al., 2022). Nevertheless, there is still a partial acceptance by farmers and other stakeholders, who ask for a higher involvement in the decision-making process and access to the results of demonstrative activities (Saliba et al., 2018). In some cases, water reuse strategies that can contrast saltwater intrusion are not implemented due to the overestimation of the possible negative effects. The knowledge about the risks of water reclamation and the definition of risk minimising strategies must be consolidated to allow for decisions that increase the overall sustainability of groundwater resources.

Within this framework, this study aims to evaluate the fate of nitrate, *E. coli* and salinity when a treated wastewater containing a residual content of these pollutants is used for irrigation. The leaching through the first metre of topsoil was studied under the edge case of non-cultivated soil. The up-take of salts and nitrate by a typical Mediterranean crop was also estimated over the entire cultivation period.

2. Materials and methods

2.1. Experimental set-up and conditions

Eight soil columns consisting of cylindrical containers with a diameter of 23 cm were filled with soil collected from a local vegetable farm and installed vertically on a steel structure, spaced about 50 cm from one another. The experimental set-up included 4 different treatments with two replicates each, as displayed in Fig. 1. Three treatments with the different heights of 33, 66, and 99 cm (we will refer to them as SC-33, SC-66, and SC-99, respectively) were chosen to study the distribution of pollutants along the first metre of soil. In another treatment 33 cm high (we will refer to as SC-33-P), pepper plants (*Capsicum annuum* L., cv Lamuyo) were grown to evaluate nitrate and salt up-take by plants.

Before the experiment started, a soil physical-chemical characterization was carried out. The particle size distribution was determined using the pipette-gravimetric method. According to the USDA classification (USDA, 1987), the soil had a sandy clay loam texture, with 49% sand, 26% silt and 25% clay. Water content at saturation, field capacity and permanent wilting point were determined using the Saxton pedotransfer function (Saxton et al., 1986) and their values were 43%, 28% and 16%, respectively. The containers had undulated walls (ring-like with amplitude of 5 mm and wavelength of 25 mm) to minimise preferential water flows along the column's edge. To reduce the lateral heating and possibly simulate an in-situ situation, each column was wrapped in aluminium foil and the whole set was protected with a lateral curtain for shielding from wind and direct sunlight.

The experiment was carried out in the open air, at the headquarters of the Italian Water Research Institute (IRSA CNR) in Bari (Italy). The meteorological data were acquired from the Regional Agrometeorological Network (Rete Agrometeorologico Regionale, ARIF - www.agrometeopuglia.it/), as measured by a weather station close to the experimental site over the trial period. The daily average values of meteorological data resulted as follows: air temperature between 16 and 33 °C; air humidity between 33% and 80%; wind speed between 8 and 24 km/h.

2.2. Irrigation test

The test started on May 20th, 2015, and ended on August 7th of the same year. Pepper seedlings were transplanted in SC-33-P duplicates (1 seedling per cylindrical container) at the beginning of week 2 (May 27th) and pepper fruit harvested, at full maturity stage, at the beginning of week 12 (August 5th). Soil columns were irrigated with tap water, reclaimed water, and deionized water (DIW) according to the following timeline:

- During the first 4 weeks, all the columns were irrigated with tap water to evaluate the background leaching of pollutants along the soil profile.
- From week 5–11, reclaimed water was used for irrigation. During this period, an additional amount of DIW was provided to SC-33-P to balance the loss of water due to plant transpiration. This ensured similar water content values in SC-33 and SC-33-P, which were checked weekly by weighing the columns. The additional DIW for SC-33-P did not increase the loads of salinity and nitrate.
- On week 12, just after pepper harvesting, two intense irrigations with DIW were applied in all columns to allow for the leaching of pollutants possibly accumulated into the soil.

The irrigation schedule included three events per week (Monday, Wednesday, and Friday), except for week 12, when two irrigations occurred over two consecutive days. With the aim of considering a

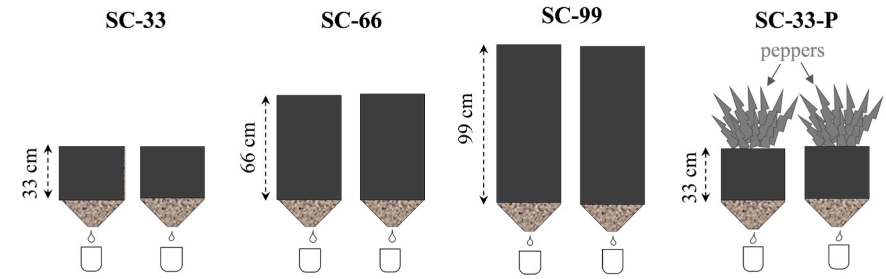

Fig. 1. Experimental set-up consisting of four treatments, each replicated twice: SC-33, soil column 33 cm high; SC-66, soil column 66 cm high; SC-99, soil column 99 cm high; SC-33-P, soil column 33 cm high planted with pepper.

worst-case scenario that emphasises the leaching of pollutants to groundwater, the amount of water supplied during the test was much higher than the crop water requirement (about four times as much). The volumes of rainfall that occurred during the experiment were measured on site. The outflow water from each column was collected and measured after each irrigation event.

Tap water was withdrawn from the drinking water distribution network. The reclaimed water was produced through a lab scale innovative secondary biological process treating real municipal wastewater and having filtration performance comparable to a full-scale treatment train that includes a tertiary filtration process (Salerno et al., 2017). The lab scale bioreactor was operated without denitrification. Before being used for irrigation, the bioreactor effluent (having *E. coli* content between $3.1 \cdot 10^3$ and $3.0 \cdot 10^5$ MPN/100 mL) was stored in a tank at room temperature for 5 days, with the aim of obtaining an *E. coli* concentration in the irrigation water in the order of 10^2 MPN/100 mL. The storage duration was set according to the results of preliminary *E. coli* decay tests carried out on treated wastewater samples.

2.3. Sampling and analyses

The irrigation water was analysed once per week for pH, electrical conductivity (EC), chemical oxygen demand, total suspended solids, nitrate, phosphate, and *E. coli*. After each irrigation event, the outflows of the soil columns were analysed for *E. coli*, nitrate, and EC. All the physical and chemical analyses were performed according to Standard Methods (APHA et al., 2005). Potassium chloride and sodium nitrate were used to verify the accuracy of EC and nitrate determinations, respectively. For the enumeration of *E. coli*, the Colilert®−18 (IDEXX Laboratories Inc.) was used. Its accuracy for wastewater samples was verified by three confirmation tests (Vergine et al., 2017b). The content of salts in the outflow of the soil columns was estimated by measuring the EC, according to the following relationship: salinity (mg/L) = EC (mS/cm) · 0.64 (Rhoades et al., 1992).

3. Results and discussion

3.1. Water quality and quantity

Irrigation water and rainwater characteristics are reported in Table 1. Tap water and rainwater had nitrate content close to zero, low EC and no *E. coli*. The nitrate content in the reclaimed water was much higher than tap water; the EC was also higher, but to a smaller extent (Table 1).

Table 1
Irrigation water and rainwater characteristics.

Parameter	Tap water	Rainwater	Reclaimed water
Total suspended solids (mg/L)	< 2	< 2	3.4±1.6
Chemical oxygen demand (mgO_2/L)	< 15	< 15	26.3±3.8
Nitrate (mgN/L)	0.2±0.0	0.5±0.3	36.1±4.9
Phosphate (mgP/L)	< 0.5	< 0.5	7. 3±0.8
pH (-)	7. 9±0.1	7. 6±0.2	7. 8±0.2
Electrical conductivity (mS/cm)	0.5±0.1	0.5±0.1	1.6±0.1
Escherichia coli (MPN/100 mL)	0	0	248±246

The reclaimed water was not disinfected, but it was stored for 5 days, simulating to some extent a lagoon system. This allowed for the decay of *E. coli*, whose concentrations after storage were between 36 and 918 MPN/100 mL, with a median value of 194 MPN/100 mL. With respect to *E. coli*, this reclaimed water would fit within the class C of the European regulation on minimum requirements for water reuse, which allows for cultivation of raw food crops using drip irrigation, excluding root crops and other crops where the edible part is in direct contact with reclaimed water (European Union, 2020).

As regards the salinity, the reclaimed water can be considered as “slightly saline”, according to FAO irrigation and drainage paper 48 (Rhoades et al., 1992), with limitations for crops that are sensitive or moderately sensitive to salinity (Grieve et al., 2012). Most of the cultivated vegetables species are characterised by low tolerance to the irrigation use of saline water for long term. To this regard, pepper is classified as “moderately sensitive” to salinity due to its ability to tolerate EC values in irrigation water up to 1.0 mS/cm without any significant yield reduction (Grieve et al., 2012). However, crop salinity tolerance depends also on plant growth stage and cultural practices. Generally, at earlier growth stages (seedling, establishment) plants are more sensitive to salinity. Moreover, appropriate irrigation methods and scheduling and the application of a leaching fraction can mitigate the effects of saline water by influencing water-use efficiency, salt accumulation and distribution in the soil. Considering the salinity of the reclaimed water used in this study, full yield potential is still possible by taking actions to maintain soil salinity within the tolerance of pepper crop along the different growth stages.

In terms of nitrogen, according to the Integrated Production Disciplinary of the Apulia Region (BURP n° 22/2020, https://burp.regione.puglia.it/), the requirement for pepper crop can be defined within the optimal range of 160–200 kgN/ha, which may be totally fulfilled by the irrigation with the reclaimed water used in the study. Indeed, during weeks 5–11, the supply of about 5000 m^3/ha of reclaimed water having an average nitrate content of 36.1±4.9 mgN/L resulted in an overall nitrate load of 170 kgN/ha.

Fig. 2 shows the amounts of water supplied to each soil column duplicate and the corresponding volumes in the outflow. Non-cultivated soil columns reached a relatively constant outflow starting from week 5. Between weeks 5 and 11, the flux throughout these columns was quite stable, despite a continuous but very small decrease of the outflows due to the seasonal rise in air temperature between June and July (data not shown). Unlike non-cultivated soil columns, SC-33-P had quite variable outflow along the test period, because of the variability in the plant transpiration and in the water volumes supplied accordingly. During weeks 6 and 7, the outflow from SC-33-P was almost absent (Fig. 2). This may have generated an accumulation of pollutants in the soil. With the supply of the additional DIW, from the 8th week onwards there was a significant outflow from SC-33-P, favouring the release of the pollutants that may have accumulated in the soil.

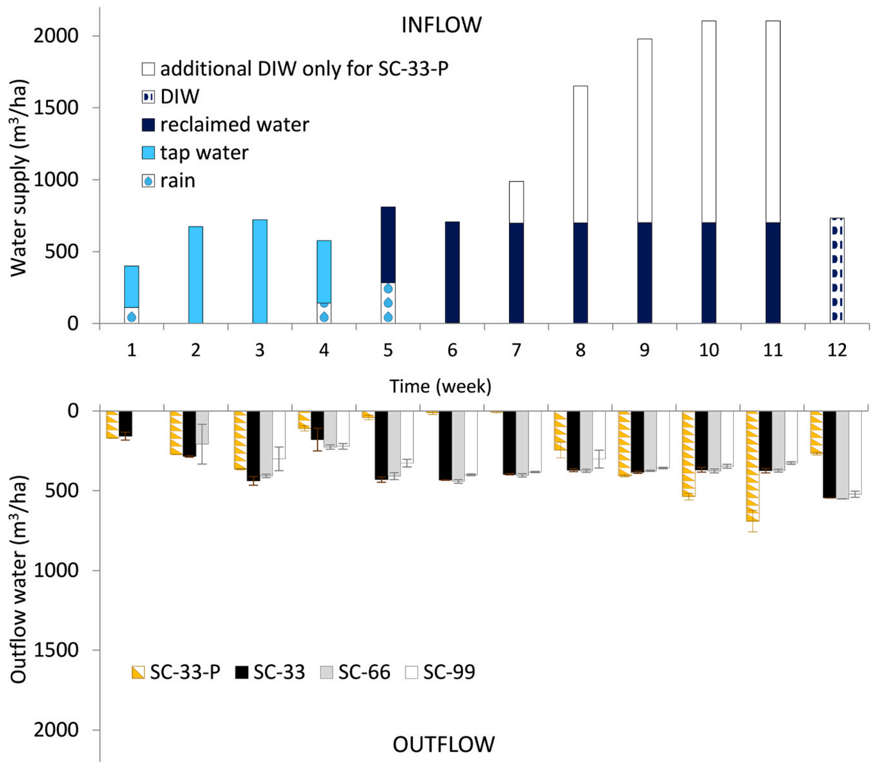

Fig. 2. Water balance. Water amount applied weekly to all columns (top) and corresponding outflow from each soil column duplicate (bottom).

Table 2 reports the water balance, divided into three periods characterised by different water sources. During weeks 5–11, the evaporation was around 50% in non-cultivated soil columns, with small differences at the different heights: 46%, 46%, and 52% for SC-33, SC-66, and SC-99, respectively. In SC-33-P plant transpiration had a relevant influence on the overall water balance. Indeed, during weeks 5–11 the overall evapotranspiration in SC-33-P was 90% and reached a maximum of 99% during week 7.

Table 2
Mass balance of water, nitrate, and salt during the irrigation with tap water (weeks 1–4), reclaimed water (weeks 5–11), and DIW (week 12). Loads of nitrate and salt are the product between concentrations in water and water volumes sampled. Average values of duplicates.

Period	Parameter	Inflow	Outflow SC-33-P	Outflow SC-33	Outflow SC-66	Outflow SC-99
Weeks 1–4	Water (m^3/ha)	1831	732.4 ±65.5	959.5 ±7.0	841.2 ±3.6	522.2 ±65.5
	Nitrate (kg/ha)	0.3	9.0±2.3	9.3±2.0	8.0±5.4	2.2±0.2
	Salt (kg/ha)	558.6	277.9 ±32.0	606.3 ±114.6	440.4 ±0.2	216.4 ±86.7
Weeks 5–11	Water (m^3/ha)	5163.6*	1919.0 ±132.4	2780.4 ±35.3	2831.7 ±52.7	2496.0 ±132.4
	Nitrate (kg/ha)	170.1	19.3 ±0.4	170.8 ±4.3	158.2 ±14.7	99.2 ±4.8
	Salt (kg/ha)	4769.4	1611.5 ±119.6	3385.1 ±211.3	2632.6 ±33.4	1769.1 ±289.3
Week 12	Water (m^3/ha)	732.5	266.9 ±8.7	544.8 ±0.7	551.6 ±1.4	522.0 ±18.9
	Nitrate (kg/ha)	0.0	0.0±0.0	63.5 ±0.5	59.5 ±2.6	40.3 ±2.4
	Salt (kg/ha)	0.0	226.3 ±9.5	959.4 ±67.4	804.4 ±18.1	601.0 ±35.2

*19730 m^3/ha for SC-33-P, due to additional DIW.

3.2. Nitrate

Fig. 3 shows the nitrate content in the outflow from each soil column duplicate. During the first 4 weeks, a significant presence of nitrate was observed in the outflow from all soil columns, despite during the same period the nitrate content in the irrigation water was close to zero (Table 1). Subsequently, when the reclaimed water was used for irrigation, the nitrate content in the outflow from each non-cultivated soil column steadily increased day by day. On the contrary, no relevant changes in the nitrate content were observed in the outflow from SC-33-P.

The increases in SC-33, SC-66 and SC-99 were similar, irrespective of the soil column height, as shown by the linear relationships parallel to

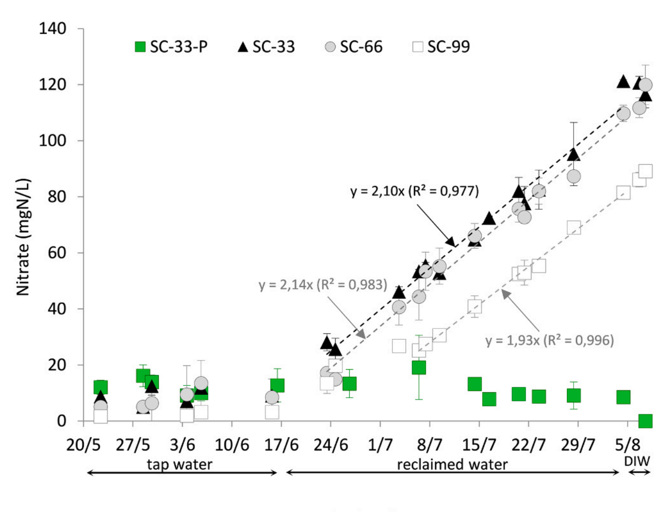

Fig. 3. Concentrations of nitrate in the outflow from each soil column duplicate.

each other, with a slope of around 2 mgN/L (Fig. 3). It can be assumed that, if we continued irrigating with reclaimed water under a constant application rate, the nitrate content in each outflow would achieve a stationary value, as indicated by previous studies that modelled nitrogen dynamics in soil columns irrigated under unsaturated conditions (Dayanthi et al., 2008; Jing and Zang, 2021). The nitrate brought to non-cultivated soil by irrigation was easily leached along the soil profile due to the poor adsorption by soil particles and the consequent highly solubility and mobility within the soil water solution.

Fig. 4 shows the weekly loads of nitrate in and out from all soil columns, calculated multiplying the weekly volumes of water by the average concentrations of nitrate. The sharp discontinuity in the inflow nitrate load due to the change of irrigation water source (from week 5) resulted in a progressive increase in the outflow load only for non-cultivated soil columns (Fig. 4). The nitrate balance reported in Table 2 shows that the whole nitrate load supplied with the reclaimed water leached out from the SC-33 during weeks 5–11, whereas, during the same period, only 11% of it came out from SC-33-P. With respect to previous studies investigating nitrate leaching to groundwater in pepper cultivation, this finding is consistent with the results of other fertigation practices (Romic et al., 2003), whereas agronomic practices based on solid fertilisation cause much higher nitrate contamination (Flores et al., 2005; Dahan et al., 2014).

Moreover, the two intense irrigations with DIW performed during week 12 caused a relevant leaching of nitrate in non-cultivated soil columns, whereas the same events did not cause any leaching in soil columns where peppers were grown (Table 2). This allows to exclude the accumulation of nitrate in cultivated soil. Therefore, the nitrate supplied with the reclaimed water that has not leached out from SC-33-P during weeks 5–11 had been up-taken by crops or possibly removed through denitrification promoted by crops in the root zone (Rummel et al., 2021). Temporary accumulation of nitrate in SC-33-P did not even occur in correspondence of the period with scarce outflow (weeks 6 and 7), as shown by absence of peaks of nitrate leaching during the subsequent weeks (Fig. 4).

Table 2 also shows that, at the end of the experiment, the nitrate leached out from SC-33 (9.3 + 160.8 + 63.5 = 243.6 kgN/ha) was much higher than overall load supplied (0.3 + 170.1 + 0.0 = 170.4 kgN/ha). Therefore, a significant amount of nitrate contained in the soil before the test leached from non-cultivated soil columns, whereas this did not occur in cultivated soil. Most of the release of nitrate from non-cultivated soil columns was observed in correspondence of the heavy irrigations with DIW that simulated rainfall events. These findings suggest that using reclaimed water to simultaneously manage plant irrigation and fertilisation could mitigate nitrogen losses in agricultural fields that had been previously overfertilized.

3.3. Salinity

Fig. 5 shows the EC in the outflow from each soil column duplicate. Fig. 6 shows the weekly loads of salinity in and out, calculated by multiplying the weekly volumes of water by the average concentrations of salinity, with the latter estimated on the basis of the EC values (Rhoades et al., 1992). As with nitrate concentration, during the irrigation with reclaimed water, the EC increased in the outflow from non-cultivated soil columns according to linear relationships parallel to each other. However, temporal distances among EC trends related to SC-33, SC-66 and SC-99 in Fig. 5 were higher than those related to the corresponding nitrate trends in Fig. 3. This indicates a slower transport of salt through the soil compared to nitrate.

Unlike nitrate, relevant variations in the EC trend were observed also in the outflow from soil columns where pepper plants were grown. The

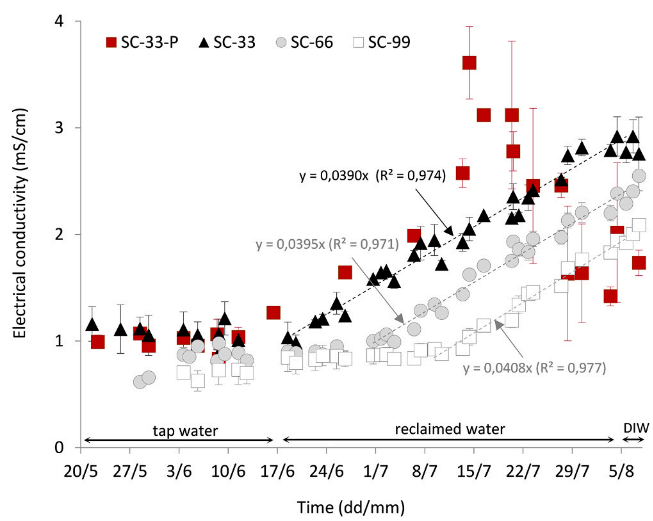

Fig. 5. Electrical conductivity in the outflow from each soil column duplicate.

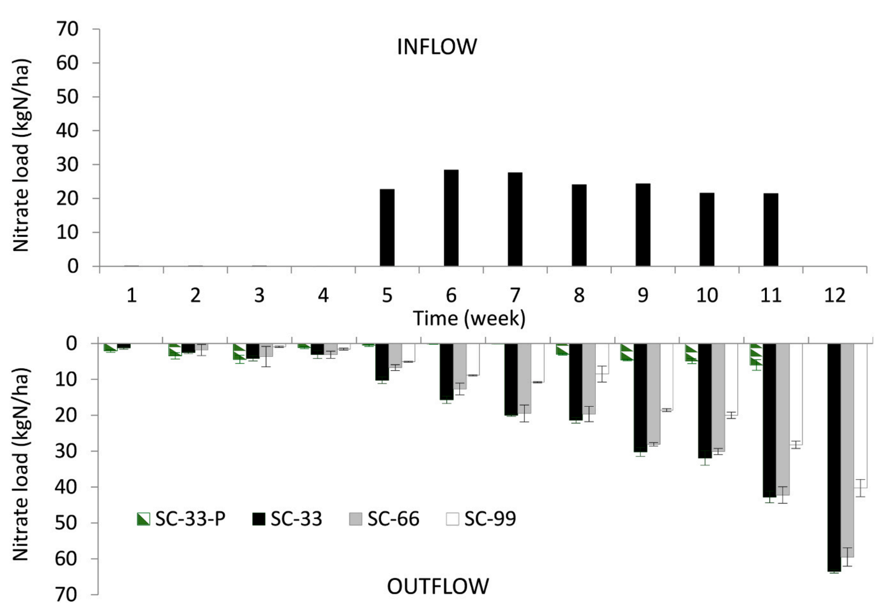

Fig. 4. Nitrate mass balance. Nitrate load applied weekly to all columns (top) and corresponding load in the outflow from each soil column duplicate (bottom).

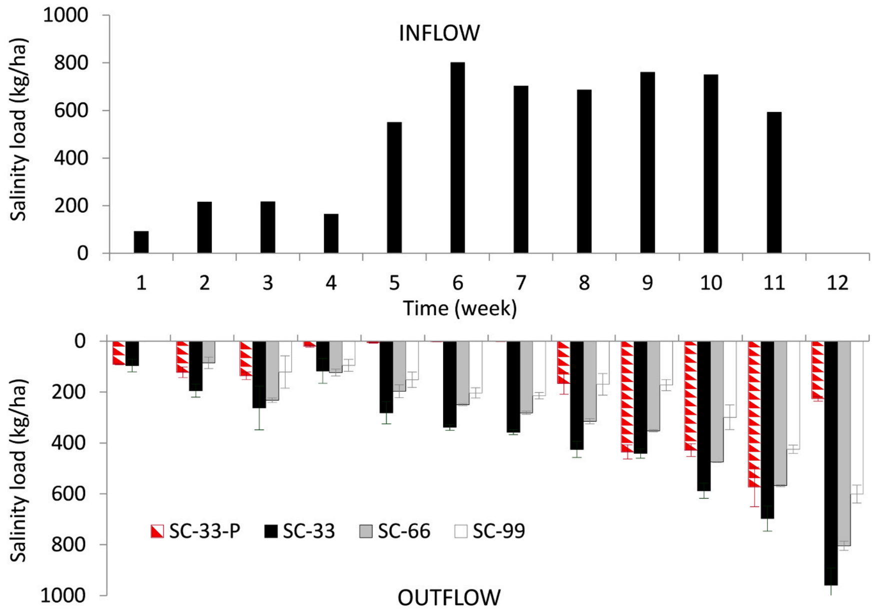

Fig. 6. Salt mass balance. Salinity load applied weekly to all columns (top) and corresponding load in the outflow from each soil column duplicate (bottom).

EC in SC-33-P outflow (Fig. 5) started to increase as soon as reclaimed water was used for irrigation and it had a peak just after the period characterised by scarce leaching (weeks 6 and 7), suggesting the release of salts previously accumulated into the soil. The salt balance reported in Table 2 shows that 71% of the salts supplied with the reclaimed water leached out from SC-33 during weeks 5–11 and a further 20% after the subsequent irrigations with DIW. During weeks 5–11, the loads of salt in the outflow of SC-33-P were about half of SC-33 (Table 2). Moreover, at the end of the test the outflow EC values (Fig. 5, from day 28/7) and the outflow salinity loads (Fig. 6, week 12) were much lower in SC-33-P than in SC-33, indicating a higher accumulation of salt in non-cultivated soil. These findings confirm that salt up-take is relevant in irrigation with reclaimed water (Zalacáin et al., 2019), but also that a significant leaching can occur. However, it's important to contextualise the phenomenon with respect to the underlying aquifer. In some cases, the salinity in groundwater is much higher than in reclaimed water. This is relatively common in coastal areas suffering from saline contamination. As an example, despite the high differences due to variability of the geomorphological characteristics, groundwater salinity in the Apulia region is on average quite high. A recent study, which covers 22 sampling campaigns over 24 years, reported an average EC value of 4.0 mS/cm on the entire regional monitoring network (341 wells and 20 springs) (Masciale et. al, 2021). This value is even higher than the local limit for water reuse in irrigation (3.0 mS/cm - Legislative Decree 152/2006, 2006). In contexts like this, characterised by severe aquifer salinization, replacing groundwater with reclaimed water as an irrigation source is highly recommended to contrast saline intrusion.

3.4. *E. coli*

The irrigation water and the outflows from non-cultivated soil columns were analysed for *E. coli* at every irrigation event. The results related to tap water and reclaimed water are displayed in Fig. 7 as boxplots. As for the irrigations with DIW performed on week 12, *E. coli* was not detected in any of the outflows. This makes it possible to exclude a significant accumulation in the soil during the irrigation with reclaimed water.

During the first 4 weeks, despite the *E. coli*-free inflow, *E. coli* was occasionally detected in the outflow from SC-33 (Fig. 7). As previously observed (Forslund et al., 2012), wild animals may have caused the faecal contamination of the soil before this was taken from the open field or while the test was running. This result highlights the need to properly consider the background faecal pollution, such as that originated from natural sources, when setting up regulatory limits for wastewater reuse

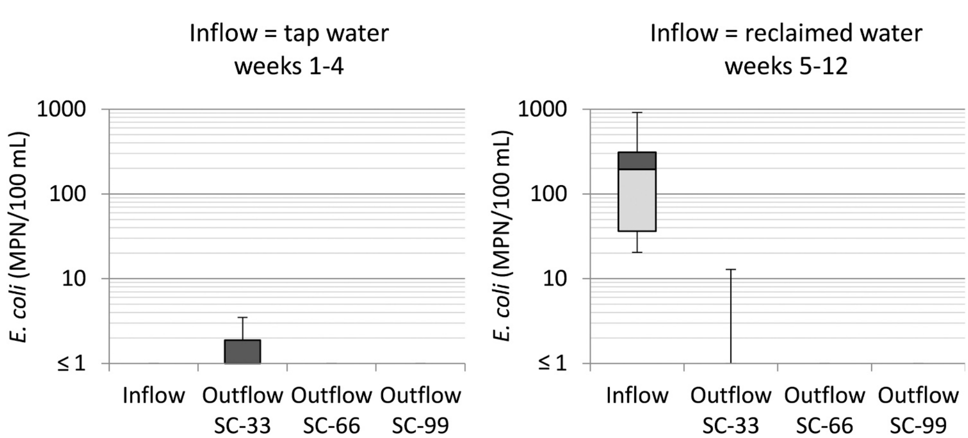

Fig. 7. Boxplots of *E. coli* concentrations in the inflow and outflow of non-cultivated soil columns during the irrigation with tap water (left) and reclaimed water (right).

in agriculture. In addition to that caused by wildlife, the pollution present in conventional irrigation water sources should be considered to establish the acceptable risk in the irrigation with reclaimed water (Allende and Monaghan, 2015).

During the irrigation with reclaimed water, a limited presence of *E. coli* was observed in the outflow from SC-33, with a maximum concentration of 13 MPN/100 mL (Fig. 7). Considering median values, the *E. coli* content decreased from 194 MPN/100 mL in the inflow to 0 MPN/100 mL in the outflow from SC-33, corresponding to a removal of 2 logs or higher. Previous studies indicate that the natural die-off is the main process influencing *E. coli* concentration during the percolation through the soil (Wilkinson et al., 2011; Vergine et al., 2015). In this study, the time *E. coli* needed to cross the first 33 of soil can be roughly estimated to be equal to 10 days or longer, considering that dissolved salts leached at a speed of about 33 cm every 10 days (see the time distances between the linear relationships in Fig. 5) and that in a similar experiment salt resulted to cross the soil a bit faster than *E. coli* (Vergine et al., 2015).

Finally, Fig. 7 shows that the considerably different *E. coli* contents in the two inflow water sources did not result in significant differences in the corresponding outflows. Both with tap water and reclaimed water, *E. coli* was never found in the outflows from SC-66 and SC-99. Therefore, under the conditions applied in the present study, the risk of groundwater pollution due to the residual presence of faecal bacteria in the reclaimed water can be considered not relevant. Further studies should be carried out to assess the influence of those parameters, such as crop type, *E. coli* content, and soil texture, that can play an important role in the migration of *E. coli* through unsaturated soils (Wen et al., 2017).

4. Conclusions

A treated municipal wastewater, characterised by a high content of nitrate (36±5 mgN-NO_3/L), a slight salinity (1.6±0.1 mS/cm), and a residual faecal contamination (*E. coli* median value of 194 MPN/100 mL) was used as irrigation water during soil column experiments. From 80 days of tests performed on 4 duplicate columns, the following conclusions can be drawn:

– Irrigation with reclaimed water having a limited faecal pollution (*E. coli* below 1000 MPN/100 mL) has no relevant risks to cause faecal pollution to groundwater. *E. coli*, due to their natural decay during the time needed to cross the soil, never reached 66 cm depth, even in non-cultivated soil.
– There is no risk of significant nitrate leaching to groundwater when crops, from the vegetative stage onward, are irrigated with reclaimed water. When the reclaimed water was used to irrigate soils where peppers had been grown for already 3 weeks, plant up-take was a crucial factor for both nitrate and salts. Nitrate was quickly and almost completely up-taken by pepper plants, avoiding both the accumulation in the soil and the leaching toward the underlying layers. Salts were also relevantly assimilated, but about half of the initial salinity load reached 33 cm depth and temporary accumulations in the soil were also observed.
– Irrigating non-cultivated soils with reclaimed water may cause the release of nitrate and salts to groundwater. Under the irrigation schedule applied in this study (700 m^3/ha per week), which was much higher than crop water needs to emphasise leaching, nitrate and salts moved fast through the topsoil. In about 8 weeks, the whole influent loads of nitrate and salts reached 33 cm depth and a relevant part of them arrived at 99 cm depth.

Focusing on the pollutants selected in this study, these findings indicate that crop irrigation with a treated municipal wastewater can be managed as not to cause significant risks of groundwater pollution, except for a limited release of salts. Therefore, replacing groundwater with reclaimed water as an irrigation source should be considered as a possible strategy to protect groundwater and soil in coastal areas characterised by intensive farming. While the possible leaching of pollutants from reclaimed water is minimal, this strategy can relevantly contrast the effects of overexploitation and overfertilization practices, so overall attenuating the concentration of salts and nitrates in groundwater and soil. First, it reduces groundwater extraction, and consequently saline intrusion. Secondly, it mitigates soil salinization, caused by the irrigation with groundwater interested by saline intrusion. Finally, if municipal wastewater treatment plants are operated without denitrification, nitrate can be fully recovered up to entirely fulfil nitrogen requirement for plant growth. This would lead to avoid the use of chemical fertilisers and the corresponding leaching to groundwater. However, attention must be paid to the use of reclaimed water, or to its nitrate content, when irrigating non-cultivated soils (e.g., pre-sowing irrigation) and, reasonably, also during the plant growth stages where nitrate up-take is low (e.g., seedling and ripening). Furthermore, any plan of irrigation with reclaimed water should carefully consider site-specific conditions and make an assessment, at least at catchment scale, on the possible environmental and socio-economic changes induced by its implementation.

CRediT authorship contribution statement

Vergine Pompilio: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Berardi Giovanni:** Formal analysis, Data curation, Conceptualization. **Libutti Angela:** Writing – review & editing, Writing – original draft. **Salerno Carlo:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis, Data curation. **Casale Barbara:** Writing – original draft, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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