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Impact of treated wastewater on plant growth: leaf fluorescence, reflectance, and biomass-based assessment

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ABSTRACT

The study evaluated the impact of treated wastewater on plant growth through the use of hyperspectral and fluorescence-based techniques coupled with classical biomass analyses, and assessed the potential of reusing treated wastewater for irrigation without fertilizer application. Cherry tomato (*Solanum lycopersicum*) and cabbage (*Brassica oleracea* L.) were irrigated with tap water (Tap), secondary effluent (SE), and membrane effluent (ME). Maximum quantum yield of photosystem II (F_v/F_m) of tomato and cabbage was between 0.78 to 0.80 and 0.81 to 0.82, respectively, for all treatments. The performance index (PI) of Tap/SE/ME was 2.73, 2.85, and 2.48 for tomatoes and 4.25, 3.79, and 3.70 for cabbage, respectively. Both F_v/F_m and PI indicated that the treated wastewater did not have a significant adverse effect on the photosynthetic efficiency and plant vitality of the crops. Hyperspectral analysis showed higher chlorophyll and nitrogen content in leaves of recycled water-irrigated crops than tap water-irrigated crops. SE had 10.5% dry matter composition (tomato) and Tap had 10.7% (cabbage). Total leaf count of Tap/SE/ME was 86, 111, and 102 for tomato and 37, 40, and 42 for cabbage, respectively. In this study, the use of treated wastewater did not induce any photosynthetic-related or abiotic stress on the crops; instead, it promoted crop growth.

Key words: dry-weight matter, irrigation, performance index, physiological trait, spectral reflectance, treated wastewater

HIGHLIGHTS

- The treated wastewater used in this study did not induce photosynthetic-related stress on crops.
- Higher chlorophyll levels were observed in treated wastewater-irrigated crops.
- Without fertilizer application recycled water had a positive impact on crop growth.
- Treated wastewater could be a viable alternative water source for irrigation.

1. INTRODUCTION

Water is an essential resource vulnerable to qualitative deterioration and quantitative depletion. It is becoming a scarce resource globally (Kummu *et al.* 2016; Khalid *et al.* 2018). Climate variability, increased urbanization, and increased demand in freshwater for domestic, industrial, and agricultural purposes have been attributed as some of the causes for the scarcity (Ganjegunte *et al.* 2017; Liang *et al.* 2020). In the coming decades, several factors such as drought, population growth, pollution of freshwater sources, and climate change are expected to intensify the stress on water resources (Farhadkhani *et al.* 2018). The United Nations, through the Sustainable Development Goal 6, seeks to tackle water scarcity by increasing water-use efficiency, sustainable freshwater withdrawals, and increasing water recycling (United Nations 2015). Wastewater reuse or recycling is becoming an essential and reliable component of integrated and sustainable water resource management (Farhadkhani *et al.* 2018). It is regarded as a viable alternative with a wide range of applications, particularly in areas where natural water resources are limited (Malki *et al.* 2017). The utilization of treated wastewater for agricultural irrigation has already been suggested by some authors (Vergine *et al.* 2017; Ibekwe *et al.* 2018).

Many studies have been conducted on using treated wastewater for irrigation at laboratory and field scales (Bedbabis *et al.* 2014; Gatta *et al.* 2016; Ibekwe *et al.* 2018; Hussain *et al.* 2019; Ofori *et al.* 2021; Bakari *et al.* 2022; Singh *et al.* 2022). The reuse of treated wastewater for irrigation comes with pros and cons. It can contribute to the reduction of freshwater pollution and may make water available for irrigation (Becerra-Castro *et al.* 2015; Vergine *et al.* 2017). The practice allows for the recovery or utilization of nutrients in wastewater for plant growth (Tran *et al.* 2016; Vergine *et al.* 2017). Soil fertility can be significantly improved after irrigation with treated wastewater. Many researchers have reported significant increases in nitrogen, phosphorus, potassium, and micronutrient levels in soil after irrigation with treated wastewater (Galavi *et al.* 2010; Singh *et al.* 2012; Bedbabis *et al.* 2014). Organic matter/carbon that is essential for improving soil compactibility, soil buffering capacity, and nutrient availability could also be increased under-treated wastewater irrigation (Murphy 2015; Farhadkhani *et al.* 2018; Ofori *et al.* 2021). The use of treated wastewater for irrigation could also have serious adverse impacts on plants, soil, groundwater, and human health if not properly managed. A common problem associated with wastewater reuse is soil salinization, which is caused by the accumulation of salts. Various authors have reported an increase in soil salinity after treated wastewater irrigation (Kallel *et al.* 2012; Shakir *et al.* 2017; Farhadkhani *et al.* 2018). The presence of pathogens and other toxic substances could pose health risks to farmers, farmworkers, and consumers if not effectively managed (Khalid *et al.* 2018; Ofori *et al.* 2021). There is also the potential of increasing trace elements in soil and plants (Galavi *et al.* 2010; Kalavrouziotis *et al.* 2012). Supply of macronutrients and micronutrients in excess quantity by the water could induce toxicity to plants and inhibit their growth (Batarseh *et al.* 2011; Parveen *et al.* 2015; Ofori *et al.* 2021).

Generally, studies of the impact on plant growth are usually based on classical biomass assessment such as fresh mass, percentage dry matter composition, and yield (Bakari *et al.* 2022; Singh *et al.* 2022). These classical methods may not provide information on the health status and the photosynthetic activity of the plants during the irrigation period. However, the use of nondestructive reflectance and fluorescence-based approaches could provide useful information on plant growth and health status. Studies have shown that spectral reflectance has the benefits of easy data acquisition and provides in-depth information on crop's physiological traits (Zhu *et al.* 2020). Chlorophyll content, plant stress status, nitrogen deficiency, and photosynthetic activity could be assessed through these nondestructive measurement methods (Elvanidi *et al.* 2018; Zhu *et al.* 2020). Fluorescence-based approach has been actively used in plant studies for evaluating plant growth, health, and response to abiotic stress. The effect of abiotic stress on the photosynthetic activity of plants can be investigated effectively using fluorescence kinetics of chlorophyll *a* (Faseela *et al.* 2020). Changes in the photosynthetic apparatus of plants can be detected and quantified in a non-invasive manner (Faseela *et al.* 2020). It is a reliable tool to test plant's response to salinity stress, nutrient deficiency, and heavy metal-induced stress (Hniličková *et al.* 2017; Faseela *et al.* 2020). The aforementioned abiotic stresses have a direct relation with treated wastewater reuse for agricultural irrigation.

The present study sought to evaluate the impact of treated wastewater reuse on plant growth using hyperspectral and fluorescence-based techniques coupled with classical biomass analyses. In addition, the study also evaluates the potential of reusing treated wastewater for agricultural irrigation without the application of fertilizer and soil amendment. To the best of our knowledge, this is the first study of its kind where these techniques have been incorporated in a single study under wastewater irrigation. The study follows a multidisciplinary approach to wastewater reuse, remote sensing, and carpometry. It provides new insights into treated wastewater irrigation and contributes to narrowing the knowledge gap on water reuse. The study outcome is valuable for water experts and policymakers in assessing the suitability of treated wastewater for crop irrigation. The words 'crop' and 'plant', and 'treated wastewater' and 'recycled water'[1] are used interchangeably.

2. METHODS

2.1. Experimental design

The study involved irrigating potted cherry tomato (*Solanum lycopersicum* L.) and cabbage (*Brassica oleracea* L.) plants with three different irrigation water streams. The treatments consisted of SE (secondary effluent), ME (membrane effluent), and Tap (Tap water-control) irrigated crops. Tap water-irrigated plants (Tap) were the control group. Each treatment consisted of six pots, three for each plant. The pot has a height of 20 cm and a volume of 3.4 L. Cherry tomato and cabbage seeds

[1] *Recycled water in this case refers to treated municipal wastewater treatment plant effluent and the post-treated effluent/water, not treated greywater.*

were obtained from a commercial supermarket, and the seeding was done on filter paper placed in a Petri dish (Figure 1). The seeds were irrigated with the different irrigation water streams. After 3 days, the sprouted seeds were transferred to a commercial nursing substrate, ROOT!T (HydroGarden UK). The seedlings were later transplanted onto a soil media (loam soil) in a greenhouse. On average, the plants were irrigated once daily (≈50–100 ml depending on the crop's water requirement), and no fertilizer or soil amendment was applied. This was to enable the evaluation of plant growth based on the quality of the different irrigation water. The choice of tomato and cabbage for the study stems from their economic and nutritional importance in tropical and temperate regions.

2.2. Cultivation and growing conditions

Seeding and nursing were done in the laboratory at 23.5 °C and a relative humidity of 49.3%. Two Petri dishes each were used for the seeding of Tap, SE, and ME (Figure 1). Each lower-row Petri dish was divided into two sections, T and C. A paper media was placed in the lower-row dish and soaked with tap water, SE, and ME. Tomato seeds were placed in the section labelled T, while cabbage seeds were placed in the C section. The upper-row Petri dishes were used as lids to ensure a high-humidity environment for the germination of the seeds. The lids were labelled 1 (Tap), 2 (SE), and 3 (ME), representing the different treatments. Three days after seeding, the sprouted seeds were transferred to the ROOT!T nursing medium for an additional 10 days. The medium consisted of 24 rooting sponges. One sprouted seed was placed in each sponge according to the manufacturer's protocol.

The seedlings were then transferred to a greenhouse and cultivated under a tropically mimicked climate. Using two GIB Lighting Growth Spectrum Advanced 600 W lamps (Metal halide lamp) (GIB Lighting, Germany), a 12-hour daytime setting

Figure 1 | Seeding of tomato and cabbage seeds with tap water, secondary effluent, and membrane effluent. Lower-row Petri dishes contain the seeds, and the upper-row Petri dishes were used as lids to ensure a high humidity environment. Inside the lower-row Petri dishes are paper media on top of which lies the seeds. Each lower-row Petri dish is divided into two sections (T and C), C represents the section for the cabbage seeds and T is the section for the tomato seeds. The relatively bigger seeds are the cabbage seeds, and the relatively smaller seeds are the tomato seeds. All papers placed in the lower dishes were soaked with the respective irrigation water stream, tap water, secondary effluent, and membrane effluent before the seeds were placed on them. The labels 1 (Tap), 2 (SE), and 3 (ME) on the lids represent the Petri dish for Tap/SE/ME treatments, respectively.

of 7 a.m. to 7 p.m. was implemented from the first day of transplanting. The time setting was later reduced to a 9-h daytime setting of 7 a.m. to 4 p.m. after 66 days. The lamps heated the greenhouse to temperatures typical of tropical climates. Growing shades Adjust-A-Wings Defender reflectors (Adjust-A-Wings, Australia) were used to make efficient use of the light and heat generated by the lamps. The number of lamps was later reduced to one after it was observed that the temperature in the greenhouse was very high for the plants. At very high temperatures, sprinkling of water on the floor of the greenhouse was done to lower the temperature and increase the humidity. The cultivation bench is fitted with a flood bottom tray/top, which was regularly filled with water to increase humidity. Roof ventilation of the greenhouse also allowed air circulation and cooling down of extreme temperatures. The average temperature, relative humidity, and dew point in the greenhouse were 26 °C, 35%, and 10.4 °C, respectively. Aside from the lamps, the plants were also able to utilize sunlight for their growth due to the transparent nature of the greenhouse.

2.3. Application of irrigation water and quality

The SE was obtained from a municipal wastewater treatment plant (WWTP) with a population equivalent of about 1,000,000 PE. The inflow to the WWTP consists of wastewater from residential areas, business and office complexes, schools and commercial establishments, and stormwater. Wastewater to the WWTP is treated biologically using an activated sludge system, after physical and mechanical treatment. The treatment includes the dosing of ferric salt for the precipitation and removal of phosphorus and to help achieve lower chemical oxygen demand (COD). The influent to the WWTP is screened to remove large particles and grit. The partially treated influent is sent to the primary sedimentation tank for settling of suspended solids and then to the activated sludge tanks for the removal of nutrients and organics. After the biological treatment, the partially treated wastewater is sent for secondary sedimentation, after which the effluent (treated wastewater) is discharged through a canal. Sampling of the SE was done at the discharge canal, right after the secondary sedimentation. The effluent was collected in a 20-L reservoir, frozen to minimize changes, and applied to the crops in batches. Another 20-L portion of SE was collected, treated with a laboratory-scale ultrafiltration membrane, and applied to the crops in batches. The membrane module, ZeeWeed-1, is a submersible hollow fibre polyvinylidene difluoride membrane from GE Water (Ontario, Canada), with a pore size of 0.04 μm and a nominal membrane surface area of 0.093 m^2. It has a maximum transmembrane pressure of 62 kPa and a typical operating transmembrane pressure of 10–50 kPa (manufacturer's manual). Tap water was collected in a 10-L reservoir and applied in the same manner as the SE. In each irrigation cycle, the physicochemical characteristics of the different irrigation water were analysed twice, that is, before irrigation and then during the irrigation period. This was to consider any changes that might have occurred to the quality of the water during each irrigation cycle. The following parameters were measured in all the different irrigation water streams: alkalinity, pH, total suspended solids (TSS), conductivity, nitrate-nitrogen (NO_3-N), ammonium-nitrogen (NH_3-N), total nitrogen, phosphates (PO_4), sulphates (SO_4), calcium (Ca), magnesium (Mg), chlorides (Cl), potassium (K), boron (B), copper (Cu), zinc (Zn), arsenic (As), and lead (Pb) (Table 1). Potassium, boron, copper, zinc, arsenic, and lead were determined using atomic absorption spectroscopy (AAS). COD was determined by the colorimetry method using potassium dichromate and measured with a photoLab 7100 Vis series spectrophotometer (WTW GmbH, Germany) (APHA 2012). All other parameters were analysed using Thermo Fischer's Gallery Analyzer (Thermo Fischer Scientific Inc., Finland) except total nitrogen and TSS. Total nitrogen was determined photometrically using a spectroquant nitrogen cell test kit (Merck-Germany). The method is analogous to EN ISO 11905-1 and DIN 38405-9. TSS was determined by gravimetric method at 105 °C using a 0.45 μm filter paper (APHA 2012). The water was filtered to collect the solids, dried, and weighed.

2.4. Soil physical and chemical properties

Soil samples were air-dried, ground, and sifted through a 2 mm sieve. The basic chemical and physical properties (Table 2) were obtained using standard laboratory procedures under a constant laboratory temperature of 20 °C. Soil pH (pH_H_2O, pH_$CaCl_2$) was performed according to the ISO 10390 (2021) protocol. One portion of the soil sample was mixed with five portions of H_2O/$CaCl_2$ (1:5 w/v). The suspension was then shaken for about 1 h using a mechanical shaker. A calibrated pH meter was then used to measure the pH. Soil organic carbon content (Cox) was determined using the dichromate redox method (Skjemstad & Baldock 2008). The soil samples were wet oxidized by potassium dichromate. After the wet oxidation, ferrous ammonium sulphate was titrated against the oxidized samples. Exchangeable acidity (EA) was determined by titrating NaOH against soil extract in the presence of phenolphthalein. The endpoint volume of the NaOH was recorded and used for estimating the EA (Hendershot *et al.* 1993). Particle size distribution (fractions of clay, silt, and sand) was determined by the

Table 1 | Physicochemical water quality characteristics of the different irrigation water streams (tap water, secondary effluent, and membrane effluent) used in irrigating the tomato and cabbage plants

Water quality parameter	Tap water	Secondary effluent	Membrane effluent
pH	7.68 ± 0.69	7.90 ± 0.53	8.01 ± 0.43
Conductivity (mS/cm)	0.37 ± 0.14	0.74 ± 0.10	0.72 ± 0.13
Alkalinity (mg/L $CaCO_3$)	77.84 ± 8.62	120.76 ± 24.44	118.12 ± 20.20
TSS (mg/L)	0.14 ± 0.06	4.33 ± 0.67	0.29 ± 0.14
COD_{cr} (mg/L)	<20	<20	<20
NO_3-N (mg/L)	3.36 ± 0.57	9.00 ± 2.01	8.96 ± 2.33
NH_4-N (mg/L)	0.04 ± 0.02	1.44 ± 0.69	1.44 ± 0.65
Total nitrogen (mg/L)	4.12 ± 1.00	12.27 ± 0.50	12.57 ± 1.17
PO_4 (mg/L)	0.08 ± 0.02	0.72 ± 0.12	0.77 ± 0.19
SO_4 (mg/L)	81.16 ± 7.02	122.32 ± 43.69	129.73 ± 44.42
Ca (mg/L)	58.92 ± 4.64	79.89 ± 12.25	79.41 ± 10.37
Mg (mg/L)	7.56 ± 0.40	14.77 ± 2.85	14.99 ± 2.59
Cl (mg/L)	26.24 ± 2.00	114.46 ± 25.85	114.97 ± 26.50
K (mg/L)	4.97 ± 0.34	26.76 ± 2.72	27.86 ± 6.01
B (mg/L)	0.02 ± 0.003	0.07 ± 0.002	0.07 ± 0.001
Cu (mg/L)	<0.01	<0.01	<0.01
Zn (mg/L)	0.04 ± 0.01	0.13 ± 0.04	0.12 ± 0.05
As (mg/L)	<0.01	<0.01	<0.01
Pb (mg/L)	<0.05	<0.05	<0.05

Note: Concentrations are mean values expressed together with the standard deviation.

Table 2 | Soil characteristics (physicochemical and texture properties) of the soil used for the pot experiment

Soil parameter/soil property	Mean value ± standard deviation
pH_H_2O	6.74 ± 0.136
pH_$CaCl_2$	6.52 ± 0.077
ρ_s (g/cm^3)	2.550 ± 0.039
Salinity (μS/cm)	685 ± 0.5
HA (mmol$^+$/100 g)	0.66 ± 0.102
EA (mmol$^+$/100 g)	0.12 ± 0.086
CEC (mmol$^+$/100 g)	21.8 ± 0.520
Cox (%)	5.57 ± 0.078
NO_3-N (mg/kg)	32.22
PO_4 (mg/kg)	8.39
K (mg/kg)	1,350
Clay (%)	15.5
Silt (%)	38.6
Sand (%)	45.9

Note: Cox, organic carbon content; CEC, cation exchange capacity; HA, soil hydrolytic acidity; EA, exchangeable acidity; ρs, and particle density. The soil is classified as loam according to the United States Department of Agriculture (USDA) soil classification system. Nitrates, phosphates, and potassium were reported by Ofori et al. (2024).

hydrometer method (Gee & Or 2002) and particle density (ρs) by the pycnometer method (Flint & Flint 2002). The determination of cation exchange capacity (CEC) was based on the method of Bower & Hatcher (1966). Using ammonium acetate, the exchangeable sodium ions in the soil samples were removed into solution after the exchanged sites had been saturated with sodium. The saturation of the exchangeable sites was done using sodium acetate. The concentration of the exchangeable sodium ions in the solution was then measured using atomic absorption spectrophotometry (Bower & Hatcher 1966; Brodský *et al.* 2011). Soil hydrolytic acidity (HA) and salinity followed the procedures outlined by Klute (1996) and Rhoades (1996), respectively. Soil extract for nutrient analysis was obtained by using 0.01 M $CaCl_2$ extractant according to the procedure by Houba *et al.* (2000) and Motsara & Roy (2008). Nitrates and phosphates in the extract were determined by ThermoFischer's Gallery Analyzer and potassium by AAS.

2.5. Leaf fluorescence and reflectance measurement

Analyses of leaf reflectance and leaf fluorescence or plant photosynthetic activities were conducted on the matured tomato and cabbage plants. Five leaves were randomly selected from each plant in each pot and the spectral absorbance or reflectance was measured. Readings of 15 leaves of tomato and 15 leaves of cabbage were taken for each treatment (Tap/SE/ME). Leaf fluorescence and reflectance were measured using a portable fluorometer, FluorPen FP 110 (PSI spol s.r.o, Czech Republic), and ASD Fieldspec 4 high-resolution spectroradiometer (Malvern Panalytical-USA), respectively. The fluorescence measurements were performed on dark-adapted leaves by clipping the leaves with the dark-adapted clips for about 15–20 min before measuring the fluorescence with the FluorPen. Two indices were used for the evaluation, the maximum quantum yield of photosystem II (F_v/F_m) and performance index (PI). F_v/F_m is one of the most common parameters for identifying stress in plant leaves. It correlates with the maximum quantum yield of photosynthesis and reflects the high sensitivity nature of photosystem II to environmental stimuli (Murchie & Lawson 2013).

$$\frac{F_v}{F_m} = \frac{(F_m - F_o)}{F_m} \tag{1}$$

F_v is variable fluorescence, F_m is maximal possible value of fluorescence, and F_o is minimal level of fluorescence (Maxwell & Johnson 2000; Murchie & Lawson 2013; Guidi *et al.* 2019; Sánchez-Moreiras *et al.* 2020; Ruas *et al.* 2022). The PI is an indicator that could be used to evaluate a plant's vitality or homeostasis ability (Živčák *et al.* 2008; Ceusters *et al.* 2019). It is a very sensitive parameter, which provides quantitative information on the state of a plant/crop performance under stressful conditions (Ceusters *et al.* 2019; Faseela *et al.* 2020). It is the mathematical product of three parameters: the concentration of reaction centres per chlorophyll, primary photochemistry-related parameter, and a parameter related to electron transport (Strasser *et al.* 2004; Živčák *et al.* 2008; Kalaji *et al.* 2016; Kowalczyk *et al.* 2018; Ceusters *et al.* 2019).

Reflected spectra of the sampled leaves using the high-resolution spectroradiometer were measured within the range of 350–2,500 nm wavelength. It covered the full range of solar irradiance. A total of 2,151 readings per leaf sample were obtained, and the average of all 15 samples was used for computing the spectra diagram. In the case of tomato (Tap), 14 samples were used for computing the averages. The instrument has an 8 nm short-wave infrared spectral resolution at 1,400/2,100 nm (Malvern Panalytical 2022).

2.6. Determination of plant height, leaf count, and dry matter composition

Carpometry analyses were performed at different developmental stages of the plants, such as vegetative development and maturity stages. After 13 days of nursing, both the tomato and the cabbage seedlings were harvested and placed on a white paper. The height of the individual plants was measured with a measuring rule against the white background and recorded. The mean height was then computed for each treatment (Tap/SE/ME). Only the upper vegetative part of the plants was measured, excluding the root zone. Plant leaf count was manually performed by counting the leaves visually during the first 4 weeks after transplanting. It was done once a week, and only the well-developed leaves were included in the count.

Determination of the percentage dry matter composition of tomato and cabbage was based on the procedure outlined in the Organization for Economic Cooperation and Development's guidelines with slight modifications (OECD 2018). Only the edible parts of tomato (the fruits) and cabbage (the leaves) were used for analyses. The fresh edible parts were rinsed several times with distilled water and rapidly dried with tissue paper. The fresh samples were then placed on a clean dry Petri dish with a known mass and weighed on a scale to obtain the initial mass. After the initial mass was recorded, samples were oven dried for approximately 5 h at 70 °C to evaporate the moisture until a constant mass was obtained. Equation (2) is used to calculate the dry matter content based on the mass difference between the fresh and oven-dried samples.

$$\text{Percentage dry matter} = \frac{(C - A)}{(B - A)} \times 100\% \quad (2)$$

where A is the mass of the Petri dish without samples; B is the mass of the fresh samples plus the Petri dish; and C is the mass of the oven-dried samples plus the Petri dish (OECD 2018).

2.7. Data analyses

The data from the study were statistically analysed using Statistica (13.5.0.17) by TIBCO Software Inc. and Microsoft Excel 2019. Graphs were constructed using the mean and standard deviation. Analysis of variance (ANOVA) was used for parametric data and Kruskal–Wallis test was used to compare the means for non-parametric data. To establish the significance of the difference observed among the different treatments (Tap/SE/ME), a confidence level of 95% ($p < 0.05$) was adopted.

3. RESULTS AND DISCUSSION

3.1. The quality of the irrigation water

The results of the physicochemical characteristics of the different streams of irrigation water are presented in Table 1. The quality of the SE did not differ significantly from that of the effluent from the post-membrane treatment. Nitrate-N, ammonium-N, and total nitrogen were almost the same, indicating no significant impact of the post-treatment (ultrafiltration) process on the nutrient quality of the SE. No statistically significant difference ($p > 0.05$) was observed in all the analysed parameters between the two streams. This observation differed when SE and ME were compared to tap water. Except for pH, arsenic, and lead, all other quality parameters of tap water showed significant differences ($p < 0.05$) in relation to SE and/or ME. The percentage difference between Tap and SE is 66.7% for conductivity, 43.2% for alkalinity, 91.3% for nitrate-N, 30.2% for calcium, 64.6% for magnesium, and 40.5% for sulphates. Percentage differences between Tap and ME were 64.2, 41.1, 90.9, 29.6, 65.9, and 46.1%, respectively. Concentrations of nitrogen and phosphate in tap water were relatively lower, indicating lower nutrient potential. In this study, analysis of orthophosphate was used; however, in future research works, we suggest the inclusion of total phosphorus. This could help in providing a fair idea of the available phosphorus for the crops since the organic fraction of the phosphorus can be mineralized in the soil for plant uptake.

The pH of all three irrigation water streams was within the acceptable range of 6.6–8.4, making them suitable for crop irrigation. The salinity of tap water (0.37 ± 0.14 mS/cm) was more suitable for all types of crop irrigation, while that of SE and ME fell within slight to moderate restrictive usage for irrigation (FAO 2003). The test crop (tomato) has a salinity tolerance of 2–3 mS/cm; therefore, SE (0.74 ± 0.10 mS/cm) and ME (0.72 ± 0.13 mS/cm) were also suitable for irrigating tomato. Chloride content, which is an important indicator for evaluating the risk of crop ion toxicity, was below 4 meq/L (milliequivalent/litre) for all three irrigation streams (Tap = 0.74 meq/L; SE = 3.23 meq/L; ME = 3.24 meq/L) (FAO 2003). Also, the amount of organics in each stream was relatively low, less than 20 mg/L, with tap water having the lowest. The mean values of soluble cations and anions in the recycled wastewater streams were higher than tap water (Elliethy *et al.* 2022). The variability in the concentrations was significant ($p < 0.05$), which corresponded to the higher conductivity in both recycled water streams. The relatively high nutrient and salt content of the recycled water is characteristic of the source (influent). Wastewater is generally characterized by high nutrient load and salt content. However, the nutrient load of the recycled water in this study could be considered low compared to that of similar studies (Gatta *et al.* 2016; Heidari & Moradi 2019; Hussain *et al.* 2019). Consequently, this corroborates with the relatively small size and mass of the fruits produced.

3.2. Maximum quantum yield of photosystem II (F_v/F_m)

F_v/F_m is considered a strong indicator of the maximum yield of *PS II*. A F_v/F_m value of approximately 0.83 is highly consistent with unstressed leaves (Murchie & Lawson 2013). For this study, a range of ≥0.80 was considered for unstressed leaves based on the literature (Murchie & Lawson 2013). F_v/F_m values of tomato ranged between 0.78 and 0.80 (Figure 2(a)) for all the treatments. This suggests that the tomato plant leaves were slightly stressed. The stress cannot be attributed to the type of irrigation water used since Tap/SE/ME treatments all had values below 0.80. The stress might have been caused by environmental conditions such as temporal or intermittent drought. This assertion is supported by the view that the decline in F_v/F_m could be considered non-substantial. Baker & Rosenqvist (2004), and Murchie & Lawson (2013) noted that mild

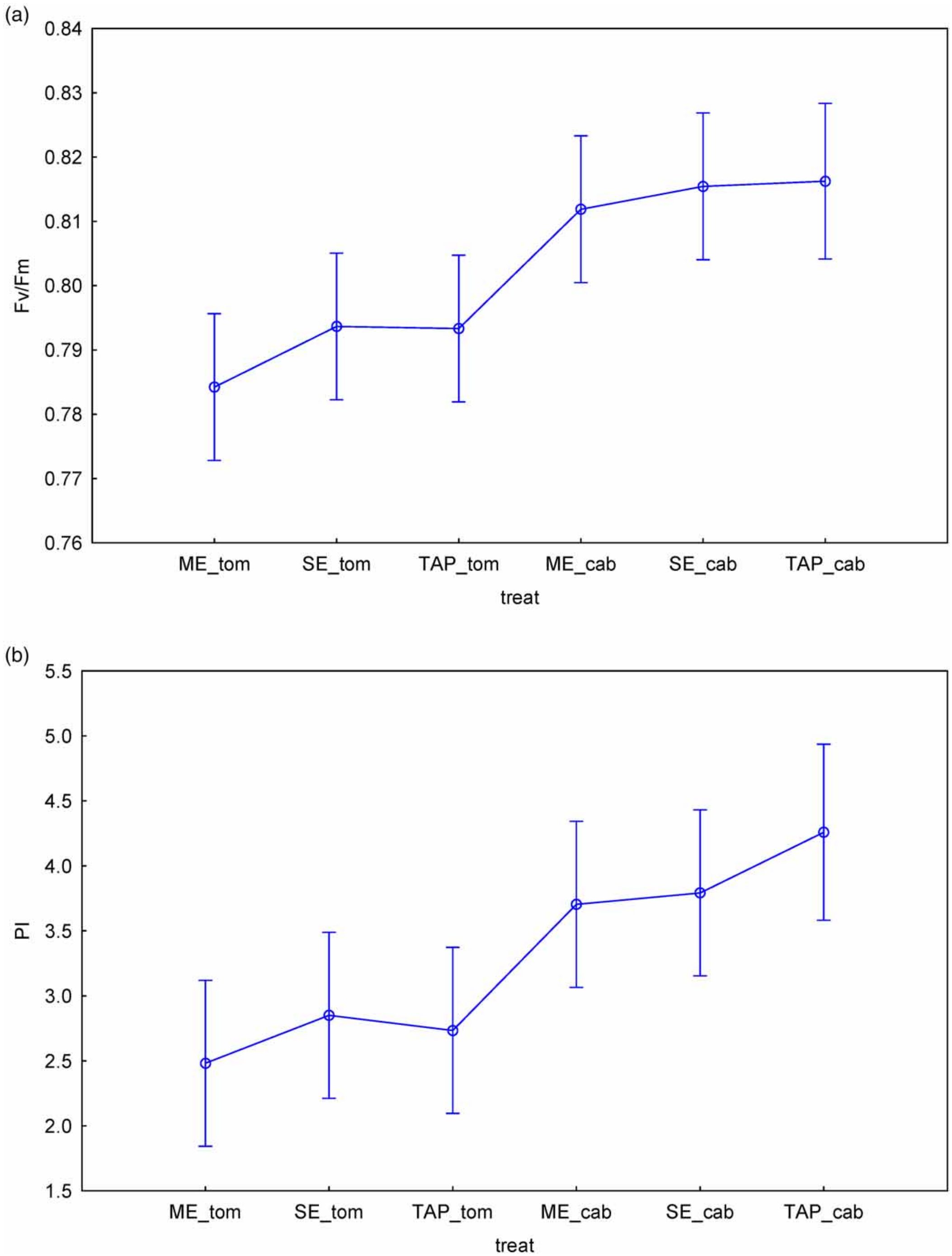

Figure 2 | Effect of tap water, secondary effluent, and membrane effluent on (a) maximum quantum yield of PSII and (b) performance index of tomato (tom) and cabbage (cab) plants, respectively. Vertical bars denote 95% confidence intervals. No significant difference was observed within the individual treatments of tomato and cabbage plants. A cross-comparison between tomato and cabbage treatments showed a significant difference ($p < 0.05$).

drought accompanied by the closure of leave stomata would not necessarily lead to a substantial decline in F_v/F_m value. During the experiment, the tomato plants experienced occasional drought due to a high evapotranspiration rate, leading to temporal curling of leaves. The heat from the lamps in the greenhouse increased the rate of water loss from the soil leading to the temporal drought. However, the plants quickly recovered after irrigation.

On the other hand, the cabbage did not experience any stress in the leaves. F_v/F_m values of Tap/SE/ME treatments were between 0.81 and 0.82, well within the range of unstressed leaves or plants. The results indicate that the irrigation water did not induce any leaf-related stress on the cabbage plant. It must be stated that since F_v/F_m evaluates only the maximum quantum yield of *PS II*, any other stress that might have been experienced by the plants other than the leaves may not have been detected (Murchie & Lawson 2013).

3.3. Performance index

The plant vitality indicator showed no significant difference among all three treatments (Tap/SE/ME) for both tomato and cabbage (Figure 2(b)). ME had the lowest PI in both crops, with SE and Tap having the highest for tomato and cabbage, respectively. Our results show that the type of irrigation water did not impactfully influence the crop's ability to undergo homeostasis in the event of stress or environmental stimuli.

The PI of the cabbage plants was significantly higher than the tomato plants (except SE_{Tom} and ME_{Cab}). This observation is attributed to the occasional mild drought, which the tomato plants experienced, and not the type of water used for the irrigation. This is because PI is very sensitive to water deficit and can express the effect of such stress on plants' vitality (Živčák *et al.* 2008). The expression of the effect of water stress or drought is usually characterized by a reduction in the index value. In the work of Ceusters *et al.* (2019), a decrease in the PI was observed when the test crop was subjected to drought.

Generally, the PI of the tap water-irrigated plants was relatively high compared to the treated wastewater-irrigated plants. The high quality of the tap water (Table 1) such as the low salinity and low heavy metal content might have contributed to this observation. PI is known to be very sensitive to abiotic stress such as salinity, osmotic stress, and heavy metal-induced stress (Faseela *et al.* 2020). A slight induction of such stress could result in a reduction of the index due to its sensitivity. This might be the reason for the relatively low index value of the treated wastewater-irrigated plants when compared to the tap water-irrigated plants. However, the difference is not statistically significant ($p > 0.05$) as shown in Figure 2(b). Therefore, it is concluded that the use of the treated wastewater did not significantly have an adverse effect on the photosynthetic efficiency and plant vitality of the tomato and cabbage plants.

3.4. Spectral reflectance characteristics of Tap, SE, and ME

The results of the hyperspectral reflectance of Tap/SE/ME treatments are presented in Figure 3. The spectral trends of the reflection peaks and absorption valleys for the different treatments were the same for tomato and cabbage. The order of reflectance within the visible light spectrum was SE > Tap > ME and Tap > SE > ME for tomato and cabbage, respectively. This observed order remained unchanged throughout the entire spectrum in the case of cabbage. On the other hand, tomato had a variation in the reflectance order. It changed from SE > Tap > ME to Tap > SE > ME in the near-infrared band (≈812 to ≈1,375 nm) and later changed to Tap > ME > SE in the ≈1,954 to 2,500 nm band (Figure 3(a)). Differences in chlorophyll and moisture content of the leaves are attributed as the possible cause for the changes. The variation occurred within the chlorophyll absorption band, main absorption band, and water absorption bands (Zhu *et al.* 2020). Generally, the difference in the reflectivity was not wide except within the main absorption band. The opposite was observed for cabbage, where Tap/SE/ME treatments had a relatively lower reflectance difference within the main absorption band (Figure 3(b)). In all the treatments, high light absorption occurred within the blue and green zones (visible region) of the spectrum leading to lower reflectance. This was partly due to chlorophyll, which absorbed a significant portion of the light energy.

Chlorophyll is a plant pigment that plays an essential role in light energy absorption, transformation, and transmission. It is an important pigment for photosynthesis, accumulating energy, and storing substances needed for plant growth (Zhu *et al.* 2020). From the results, the type of irrigation water did not adversely affect the tomato crops' light absorption capacity to undergo photosynthesis. ME had high light absorption capacity within the visible range of the spectrum, with Tap and SE having almost the same capacity. The reflectance was between 10 and 20%.

In the case of cabbage, a wide difference was not seen between SE and ME within the green zone band. The difference in the light absorption capacity was not wide since the reflectance was 19 and 17%, respectively. Tap had a relatively high reflectance of 23%, suggesting that SE and ME could absorb more light energy for photosynthesis than Tap. It was evident that the recycled water did not induce any photosynthetic-related stress that affected the crops' ability to absorb light for photosynthesis.

In reflectance spectral studies, the reflectance within the green zone of the visible region of the spectral band could be used as a proxy in the estimation of chlorophyll content. The higher the reflectance, the more likely the chlorophyll content would

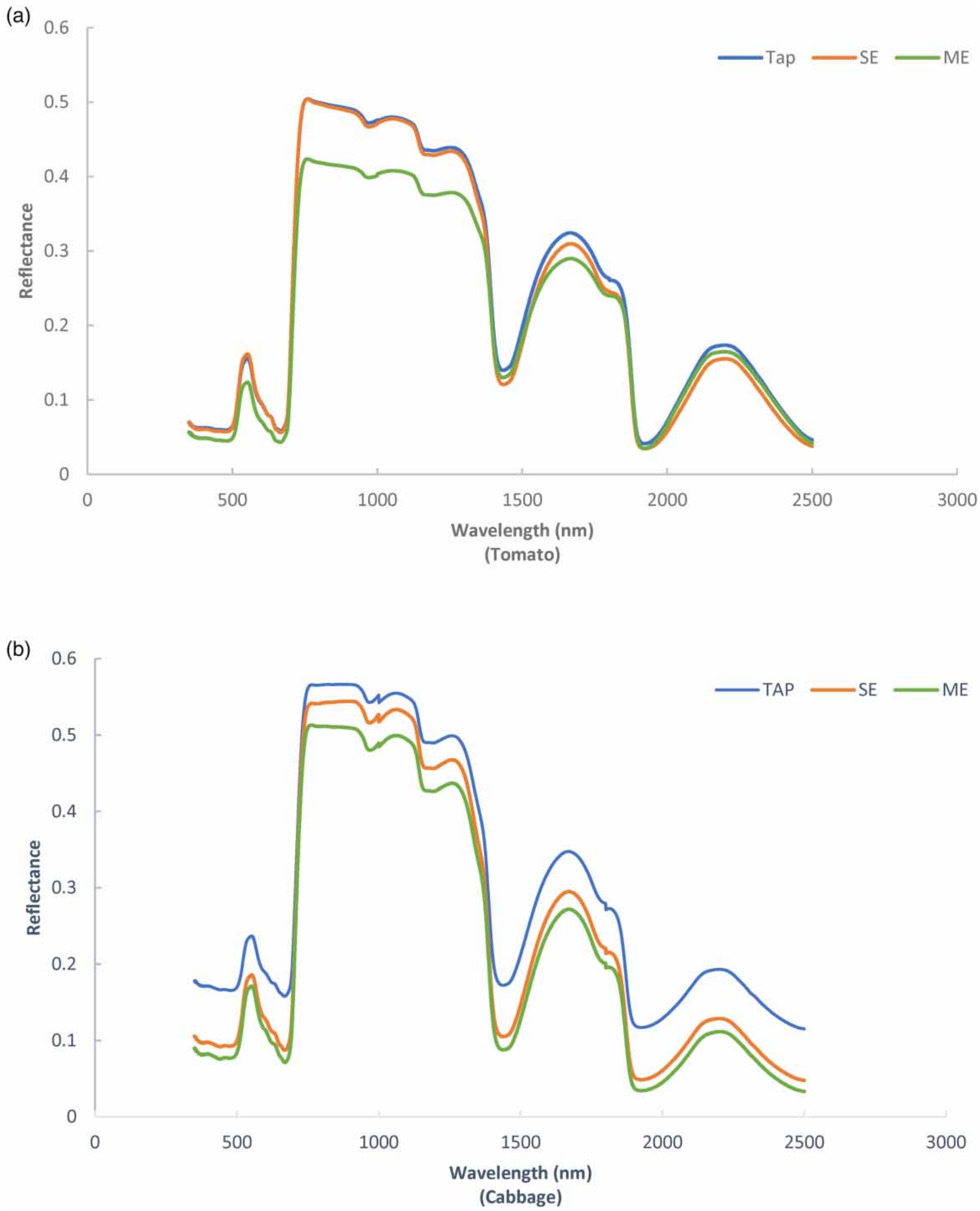

Figure 3 | Hyperspectral reflectance characteristics of (a) tomato and (b) cabbage leaves showing the impact of tap water, secondary effluent, and membrane effluent irrigation on physiological and morphological growth traits of plants. The measurement occurred within the range of 350–2,500 nm wavelength, covering the full range of solar irradiance. Blue, orange, and green spectral lines represent the spectral reflectance of tap water, secondary effluent, and membrane effluent irrigated plants, respectively. Spectral trends of the reflection peaks and absorption valleys are similar for both tomato and cabbage plants.

be lower. Lin *et al.* (2015) found that leaves with smaller chlorophyll content showed higher reflectance in the visible band of the spectrum. They concluded that a stronger negative relationship exists between chlorophyll concentration and reflectance in the visible light region. In another study, the authors previously confirmed this inverse relationship and stated that an increase in reflectivity within the visible range indicates a decrease in chlorophyll content (Gitelson *et al.* 2003). In the present study, the spectral reflectance indicates that SE and ME treatments had higher chlorophyll content than Tap. The reflectance within the 500–700 nm band was generally lower for SE and ME treatments. Therefore, the recycled water may have enhanced the synthesis of chlorophyll within the test crops due to the high magnesium content. Since magnesium is an essential element for chlorophyll synthesis (Coleby-Williams 2014), the crops might have benefited from the high magnesium content of the recycled water. The synthesized chlorophyll, in turn, might have boosted the photosynthetic potential of SE and ME treatments, thereby promoting their growth.

The reflectance data within the near-infrared region suggest that SE and ME treatments were richer in nitrogen than Tap. Research has shown that reflectance in this region of the spectral band could provide information on the nitrogen content of plants. In a study involving tomato plants, Elvanidi *et al.* (2018) found a direct link between nitrogen content and reflectance in the 750–1,000 nm band. They observed high reflectance for nitrogen-deficient tomato crops within the above spectral region. The reflectance of Tap (for tomato and cabbage) was higher than SE and ME for the above-stated band, as shown in Figure 3. Therefore, it suggests that the crops under recycled water irrigation had higher nitrogen content than those irrigated with tap water. This is attributed to the relatively high nitrogen content of the recycled water. In Table 1, the nitrogen content of the latter was thrice that of tap water. Considering the mobile nature of nitrates, SE and ME might have had access to a high amount of nitrogen to boost their growth. In conclusion, the reflectance spectra showed that SE and ME exhibited better physiological and morphological growth traits than Tap.

3.5. Impact on plant height and leaf development

The height of the test plants at the time of transplanting is shown in Figure 4. Tap had the highest mean height of 3.5 ± 0.3 and 5.6 ± 0.6 cm for both tomato and cabbage, respectively. SE had the same height as Tap for tomato but recorded the lowest height (5.0 ± 0.3 cm) for cabbage. The highest individual plant height was recorded by SE for tomato and Tap for cabbage at 4.2 and 7.7 cm, respectively. At the early stages of growth (germination and nursery), SE and ME treatments of cabbage showed relatively lower height than Tap. The percentage variation (decrease) in height relative to Tap was 11.6 and 8.0%, respectively. This might have been due to growth stress caused by the salinity of the respective irrigation water, which is consistent with the literature (El-Shaieny 2015). In a study involving cowpea, the shoot of the seedlings decreased in height due to the salinity of the irrigation water (El-Shaieny 2015). However, no statistically significant difference was observed in plant height. This suggests that the impact of the different irrigation water on plant height was similar. The growth pattern changed after transplanting. SE and ME treatments began to show higher growth rates than Tap, which continued in most cases until the end of the experiment. This growth improvement could be attributed to the fertilizing effect of the treated wastewater and the plant's adaptation to the quality characteristics of the water. The recycled water might have

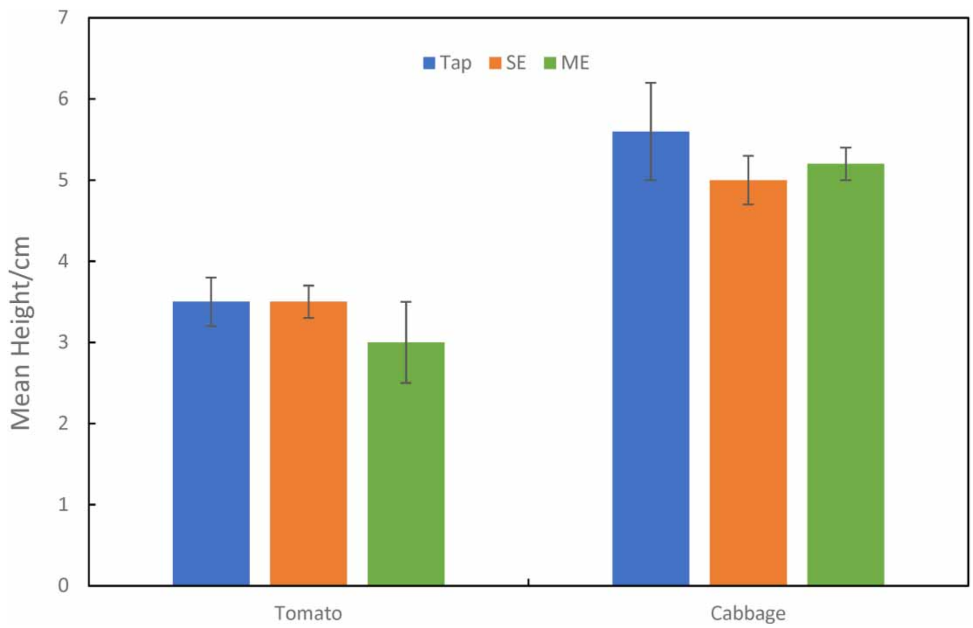

Figure 4 | Impact of tap water, secondary effluent, and membrane effluent on plant height (mean) of tomato and cabbage seedlings. Error bars represent the standard deviation, and no statistical difference was observed among individual treatments. Test of significant difference (ANOVA) was at a 95% confidence interval ($p < 0.05$).

supplied more nutrients to boost vegetative development and biomass production than the tap water. This observation from the study is in line with findings from similar studies (Gatta *et al.* 2016; Heidari & Moradi 2019).

The order of growth after 3 weeks of transplanting using leaf count was SE > ME > Tap and ME > SE > Tap for tomato and cabbage, respectively (Figure 5). The total leaf count of tomato at the time of transplanting was almost the same for all treatments. After the first week, the variation in the number of leaves began to widen, with treated wastewater-irrigated

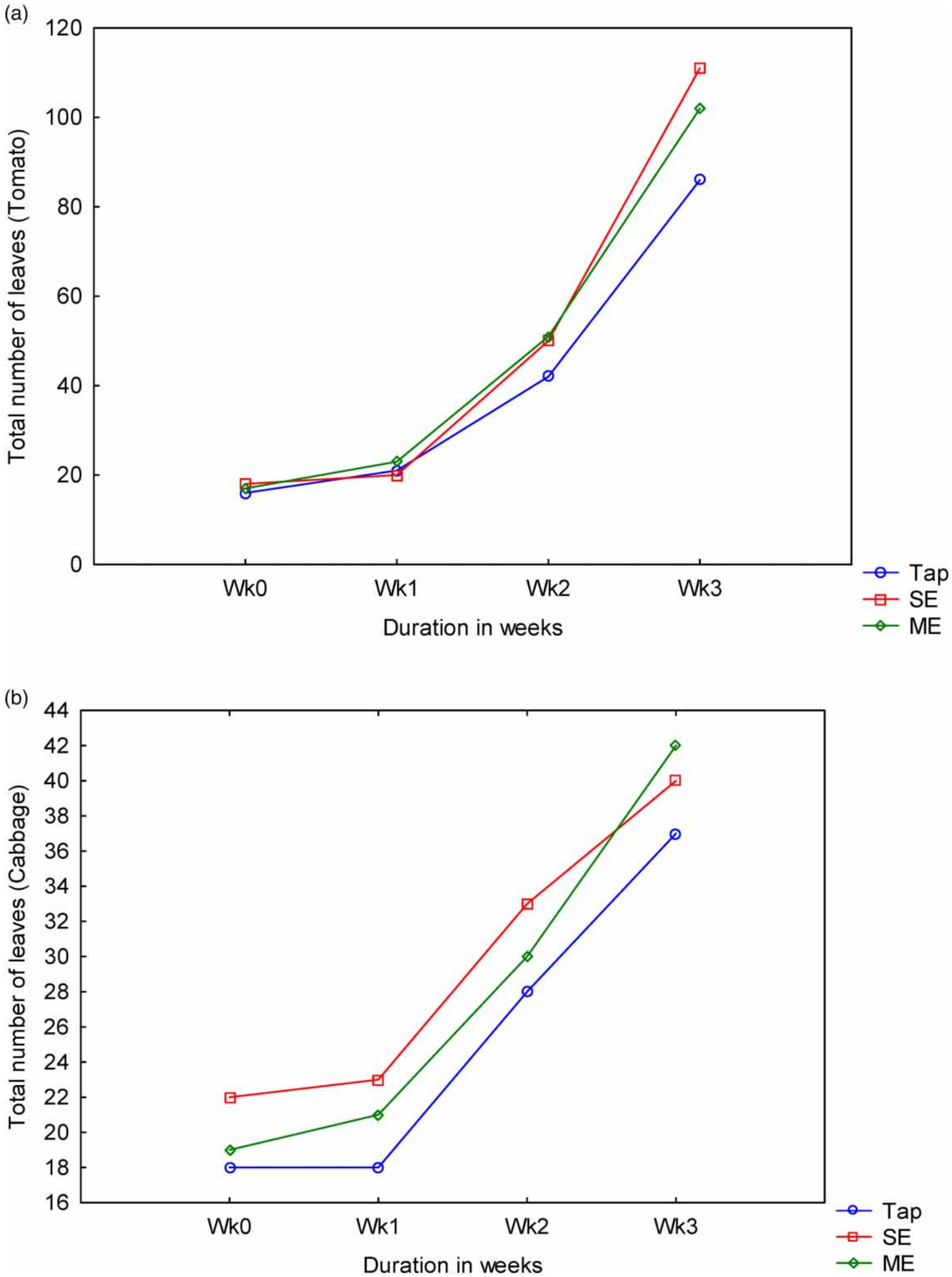

Figure 5 | Impact of tap water and treated wastewater on leaf development of (a) tomato and (b) cabbage plants. Leaf count was done 3 weeks after transplanting and once a week. Only well-developed leaves were counted. The leaf count at week zero is the total number of leaves at the time of transplanting. Blue line represents leaf counts of tap water-irrigated crops, the green line represents leaf counts of membrane effluent irrigated crops, and the red line represents leaf counts of secondary effluent irrigated crops.

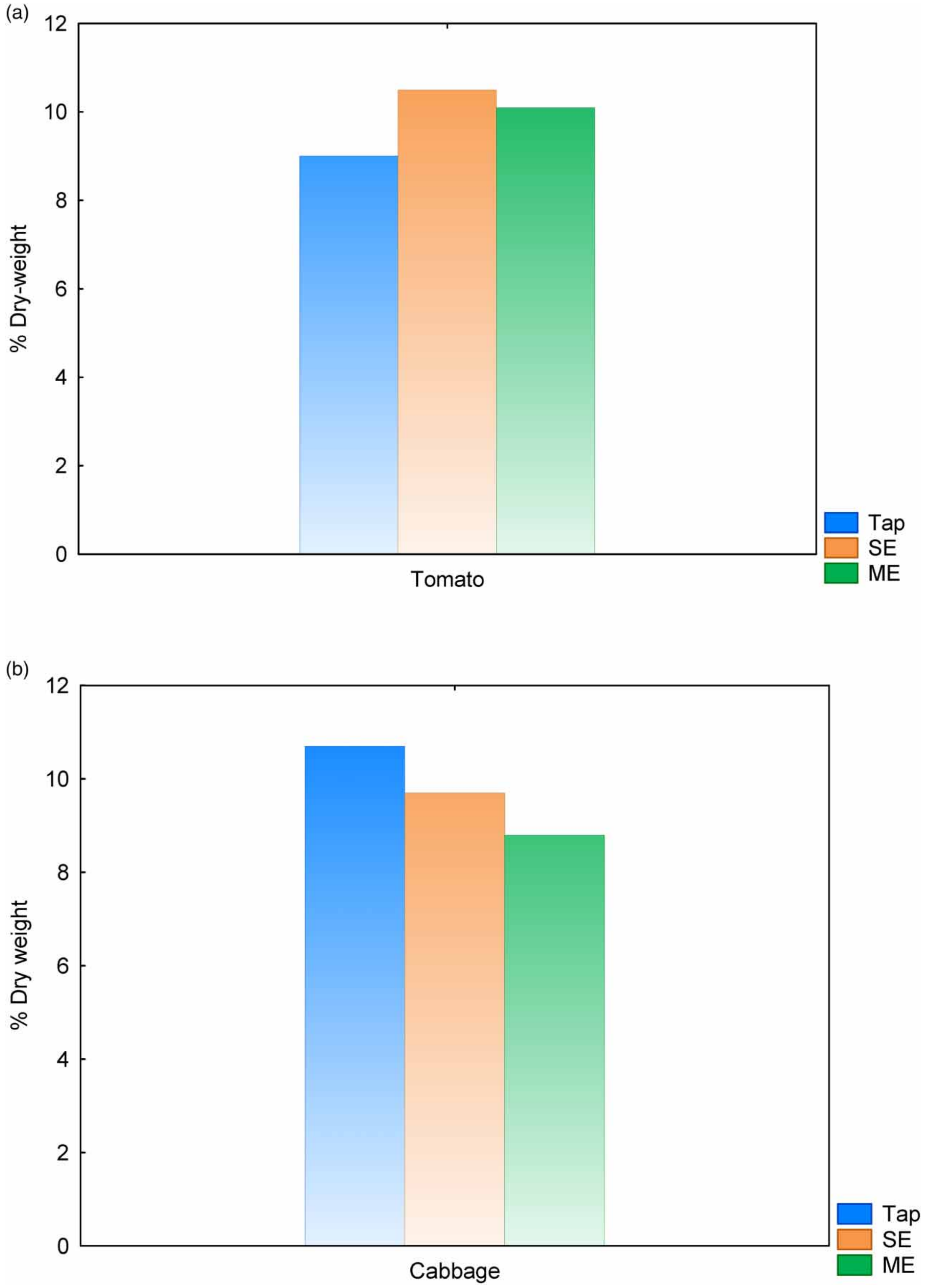

Figure 6 | Effect of tap water, secondary effluent, and membrane effluent irrigation on percentage dry matter composition of (a) tomato and (b) cabbage plants. The blue, orange, and green bar graphs represent tap water, secondary effluent, and membrane effluent irrigated plants, respectively. Recycled water-irrigated plants had higher dry matter content for tomato, while tap water-irrigated plants had the highest dry matter content in the case of cabbage.

plants recording higher counts than tap water-irrigated plants. Tap/SE/ME treatments had 86, 111, and 102 well-developed leaves at the end of the third week, respectively. This represents an increase of 29.1 and 18.6% for SE and ME treatments relative to Tap, respectively. The leaf count of cabbage was significantly lower compared to tomato. The leaf count of Tap/SE/ME treatments for cabbage was 37, 40, and 42 leaves, respectively. SE and ME treatments had 8.1 and 13.5% increase in total leaf count relative to Tap, respectively. Wastewater-irrigated cabbage plants showed better leaf development than tap water-irrigated cabbage plants. The authors of a similar study found that recycled water-irrigated maize plants had better leaf development than the control (Cakmakci & Sahin 2021). Their findings align with the findings of the current study. However, in another study, the findings on leaf development were contrary to the present study (Jagathjothi & Mohamed Amanullah 2018).

Nutrient supply is essential for the development or formation of leaves, an important part of photosynthesis (Yang & Kim 2019). The relatively high leaf count coupled with well-developed leaves of SE and ME could be attributed to the high nitrogen content of the recycled water. Nitrogen is known to promote the growth of strong and healthy leaves (Coleby-Williams 2009). More nitrogen might have been supplied by the treated wastewater to boost the growth and development of the leaves and other vegetative parts of the crops.

3.6. Age dry mass of biomass

Tap had the highest proportion of dry matter composition (10.7%) for cabbage (Figure 6). This unexpected result could be ascribed to the salinity effect of the recycled water. Cabbage seems susceptible to the salt content of the water, as was evident in the plant height at the initial growth stage (Maggio *et al.* 2005; Sardar *et al.* 2023). As expected, the dry matter composition of SE and ME was higher than Tap for tomato fruits. SE had the highest percentage dry matter of 10.5% of the total mass of fruit. This implies that tomato fruits produced under recycled water irrigation had more biomass than tap water due to the high availability of nutrients. The works of Zema *et al.* (2012) and Gatta *et al.* (2016) reported higher biomass production under wastewater irrigation compared to their respective controls. Zema *et al.* (2012) reported a mean dry biomass yield of 63% higher than the control in other plant species, such as the broadleaf cattail (*T. latifolia*). The authors attributed the reason for such a high yield to the fertilizing effect of the wastewater. The study results suggest that the tomato fruits produced from recycled water might produce a better paste and be a good nutrient source because of the high biomass production.

4. CONCLUSION

This study evaluated the impact of treated wastewater reuse on plant growth using reflectance and fluorescence-based techniques coupled with biomass production assessment. The evaluation was based on the morphological and physiological traits of the test crops, that is, tomato and cabbage. F_v/F_m assessment indicated mild stress in both recycled and tap water-irrigated tomato plants. This was caused by mild drought and not the type of irrigation water used. Cabbage had F_v/F_m of more than 0.80 in all treatments and did not experience any photosynthetic-related stress. PI indicated that treated wastewater did not significantly have an adverse effect on the photosynthetic efficiency and plant vitality of the tomato and cabbage plants. Hyperspectral data revealed higher chlorophyll and nitrogen content in leaves of recycled water-irrigated crops than in tap water-irrigated crops. Biomass production was relatively high for crops irrigated with treated wastewater, especially for tomatoes. The results of the study imply that treated wastewater may not induce photosynthetic-related stress to crops nor adversely affect the crop's ability to undergo homeostasis.

The study outcome is consistent with the existing literature on recycled water reuse. It supports the assertion that recycled water could be a potential water source for agricultural irrigation. The results highlight the nutritional benefits that can be harnessed from recycled water and the insignificant adverse effect on plant growth and photosynthetic activities. Considering these outcomes, recycled water or treated wastewater with similar physicochemical characteristics to that of the present study could be used for crop irrigation after disinfection. Studies on evaluating the health risks such as antibiotic resistance genes and pathogens dissemination (which is outside the scope of the current study) associated with recycled water use are encouraged. Such studies could contribute to providing a comprehensive view of the reuse of treated wastewater for crop irrigation.

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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 STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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