The impact of treated and untreated municipal sewage water on growth and physiology of the desert plant *Calotropis procera*

Abdellah Akhkha, Ebtesam Salem Al-Radaddi and Abdul Khaliq Al-Shoaibi

Department of Biology, College of Science, Taibah University, Al-Madinah Al-Munawwarah, Kingdom of Saudi Arabia

**ABSTRACT**

The present study investigated the effect of irrigation with Al-Madinah Al-Munawwarah domestic sewage water on the desert shrub *Calotropis procera*. Five treatments including distilled, well water, untreated (T0), primary (T1), secondary (T2) and tertiary (T3) treated sewage waters were used to irrigate the plants. The chemical and physical properties of different sewage waters were determined. A number of growth parameters including % germination, plant height, leaf number, fresh and dry weights of leaves, stems, roots and whole plant. Root:Shoot ratio was also determined. Some physiological parameters such as photosynthetic rate, initial fluorescence $F_o$, maximum fluorescence $F_m$ and quantum yield $F_v/F_m$, and chlorophyll content index were measured. Most growth and physiological parameters were increased in response to irrigation with treated and untreated sewage waters. Carbohydrate content was increased under treatment with T0 but decreased under T2 and T3; while protein content was increased under most of sewage water treatments. In contrast, proline levels showed no change. The levels of heavy metals in sewage water were in trace amounts. The possibility of using sewage water as a potential and sustainable alternative water resource for irrigating non-crop plants for multiple uses is discussed.

**1. Introduction**

Water is considered the most important life support element for every organism, as it enters in the composition of the body, and plays a key role in its internal and external interactions. Water is a limiting factor in agriculture, especially in arid and semi-arid regions [1]. According to the FAO, water scarcity is one of the greatest challenges of our times and it is defined as an excess of water demand over available supply [2]. The United Nation defined water scarcity as “the capacity of a population to safeguard sustainable access to adequate quantities of acceptable quality water” [3].

Over the last four decades, Saudi Arabia has witnessed remarkable development in all areas of development. The increase in population, the expansion of urbanization, the improvement in the standard of living and the multiplicity of uses of water are among the most prominent outcomes of this development, which created a gap between supply and demand in terms of the provision of water for human use. The per capita water supply is expected to decrease from 277 m$^3$ in 2000 to 113 m$^3$ in 2025 [4].

Therefore, the Kingdom seeks to find new sources of renewable water for industrial and agricultural use in order to preserve the sources of potable water. Hence, the Kingdom’s interest in reuse of sewage water after treatment for non-drinking uses, including irrigation has increased with an ambition to produce 6.8 million m$^3$ per year, by 2035, of treated sewage water [5]. Sewage water or wastewater was defined by Dixit et al. [6] as: “A mixture of pure water with large number of chemicals (including organic and inorganic) and heavy metals which can be produced from domestic, industrial and commercial activities, in addition to storm water, surface water and ground water.” Sewage water can be treated in a series of operations summarized as follows:

1. Primary treatment (Physical): It involves removal of solid wastes of different sizes, and the deposition of suspended solid by the process of sedimentation.
2. Secondary treatment (Biological): Different types of bacteria are used to digest the organic material under both aerobic and anaerobic conditions.
3. Tertiary wastewater treatment (Chemical): This stage is based on the elimination of contaminated elements such as small granules and elements of phosphates and nitrite compounds. Chlorination or Ozone treatments are used to disinfect and purify water. Then treatment using chlorine to ensure the elimination of any microbes may remain. Carbon can also be used at this stage to adsorb harmful elements [7].
Wild plants are of great economic, social and ecological importance. They serve as a source of food, biofuel, medicine, and raw materials for many industries [8]. They also play a significant role in protecting the environment and preserving the biodiversity [9].

As a result of the rapid depletion of oil reserves, renewable energy sources, such as biomass, are of particular importance. Biomass is considered to be the fourth largest source of energy in the world, providing 14% of the world’s primary energy [10].

For example, the wild arid shrub *Calotropis procera*, is a potential source of hydrocarbons that can be used as biofuel after extraction by organic solvents such as petroleum ether and methanol [11]. The use of treated sewage water to irrigate plants to increase their biomass was increasingly considered. Song and Lee [12] reported that all types of trees studied showed an increase in height, chlorophyll content and photosynthesis when treated with 25% fertilizer and sewage water. In addition, Tabari et al. [13] observed that irrigation with sewage water increased the growth of *Pinus eldarica* trees, which can be used as stabilizers of soil and stands against dust storms especially in arid regions where there is a scarcity of vegetation. Such qualities that can be provided by sewage water of municipal origin to plant growth was reported to be due to high level of organic matter, micro and macro nutrients that can support plant growth [14]. Little work was carried out to investigate the effect of irrigation with sewage water on non-crop plants; most of the work was concentrated on the effect on crop plants such as the study carried out in our laboratory (Biology Department, College of Science, Taibah University) using Al-Madinah Al-Munawwarah treated and untreated domestic sewage water to investigate the impact on the growth of different tomato genotypes [15].

The present work intends to make use of the local huge alternative water source treated municipal sewage water in Al-Khalil sewage water treatment station, Al-Madinah Al-Munawwarah, Kingdom of Saudi Arabia, in order to be used to irrigate wild plants that are candidates for biofuel production, medicinal products, pharmaceuticals and or stands against sandstorms that create a serious health and environmental hazard.

2. Materials and methods

2.1. Plant materials

The ripe Fruits of the *C. procera* plants were collected from Al-Madinah Al-Munawwarah Region, Kingdom of Saudi Arabia, during the summer. The ripe fruits were put in cardboard papers and kept under the room temperature; seeds were collected when the fruits were opened after two weeks of drying [16].

2.2. Well and sewage waters

The well water was collected from Chouran area in Al-Madinah Al-Munawwarah. Untreated T0 and treated sewage waters (primary T1, secondary T2 and tertiary T3) were obtained from Alkhalil sewage water treatment station in Al-Madinah Al-Munawwarah, Kingdom of Saudi Arabia. Physico-chemical analyses of sewage water followed the same procedures reported by Akkhia et al. [15].

2.3. Germination assay

To study the effect of different treatments of sewage water on germination, seeds were soaked after washing in sterilized distilled water for 24 h prior to germination. Seeds were then placed on Whatman No 2-filter paper saturated with sterilized distilled water in Petri dishes. Ten seeds were put in each dish, then a 5 ml of each sewage water treatment was added, then a 2 ml was added when needed to avoid drying. After incubation at 28 ± 2°C under darkness, germinated seeds were counted daily for 6 days.

2.4. Method of planting

The seeds were washed then soaked in water for 24 h to accelerate germination then planted in plastic pots containing each sandy soil: peat moss (3v:1v). After planting, pots were put in a controlled JSR Growth Chamber set at a temperature of 28 ± 2 °C, 40% ± 5% humidity and provided with fluorescent lighting lamps giving a light intensity of 200 µmol quanta m⁻² s⁻¹ at the plant level for 14 light hours. The pots were watered with tap water to the field capacity until the third leaves were fully expanded; after which three pots containing four plants each were designated for each sewage water treatment (a distilled water, well water, untreated sewage water, primary treated sewage water, secondary treated sewage water and tertiary treated sewage water). Plant were irrigated with one of the treatments by adding 200 ml daily [16].

2.5. Harvests

After two weeks of treatments with various well and sewage waters, the first harvest was carried out and the growth parameters, chlorophyll content index, chlorophyll fluorescence and photosynthesis rate were determined. A total of four harvests of each treatment were taken weekly.

2.6. Plant growth parameters

During plant growth, four harvests were obtained in order to determine the growth parameters including (plant height, number of leaves, leaf area, fresh and dry weights).
1. Plant Height: Plant height was measured in centimetres per each harvest by measuring the distance between the highest point of the plant (developing plant stem summit) and the soil level where stems meet roots.

2. Fresh Weight: After the end of each harvest, the root system was washed well and then dried with drying paper and then the root shoot systems were determined separately after which the total plant weight was calculated.

3. Dry Weight: The fresh shoots and roots were dried in an oven set at 80 °C for 48 h in order to determine dry weights.

4. Leaf Area: Leaf area was measured using a portable LI-COR leaf Area meter (LI-COR inc., NE, Lincoln, USA) by scanning each leaf separately after which the total leaf area was calculated.

2.7. Photosynthesis measurements

Gas exchange rates were measured in leaves using LI-COR 6400XT Infra-Red Gas Analyzer (IRGA) (LI-COR inc, NE, Lincoln, USA). Measurements followed that of Akhkha [16]. The photosynthetic rate measurements were conducted on fully expanded 5th leaves of six weeks old plants. Light intensities used in this experiment were 0, 50, 100, 500 750, and 1500 µmole quanta m\(^{-2}\) s\(^{-1}\).

2.8. Measuring chlorophyll content index

The chlorophyll content was measured as Chlorophyll Content Index in the fully expanded 5th leaves using a Chlorophyll Content Meter (Opti-Sciences, CCM-200, USA).

2.9. Measurements of chlorophyll fluorescence

To measure chlorophyll fluorescence in the fully expanded 5th leaves, Hansatech Chlorophyll Fluorometer (Hansatech Instruments, Narborough Road, Pentney, King’s Lynn, Norfolk, England PE32, 1JL, UK) was used as mentioned in Akkhha and Boutraa [17].

2.10. Proline estimation

Proline was estimated through the addition of 5 ml of salisalicylic acid, 3% aqueous solution on a half gram of fresh weight of plant leaves from each treatment. Plant material was crushed using a pestle and a mortar, then filtrated using No 2 Whatman filter paper. 1 ml of the filtrate was then used and the method of Bates [18] was followed.

2.11. Total carbohydrates estimation

Total Carbohydrates were estimated using the method of Yemm and Willis [19] where 0.1 grams of fresh leaves of each treatment was used.

2.12. Total proteins estimation

The estimation of total proteins followed that of Bradford [20] where 0.5 grams of fresh leaves was used.

2.13. Statistical analyses

Four replicates were used per water treatment. The means, standard deviations and standard errors were calculated using Microsoft Excel 2016. Analysis of variance (ANOVA) was applied to data in order to determine the level of significance when comparing plants of different treatments. Minitab version 15 was used to handle statistical analyses.

3. Results

3.1. Sewage water characteristics

3.1.1. Physico-chemical analyses

Table 1 shows that all determined physical and chemical parameters were higher in untreated sewage water (T0) than all other treated sewage waters. The different parameters decreased as sewage water went through the primary treatment (T1) down to the tertiary treatment (T3). The levels of NH\(_3\)-N, NO\(_3\)-N, chloride and manganese also decreased with the sewage water treatments; however, T3 still showed slightly higher levels of NH\(_3\)-N and chloride compared to the acceptable standards. BOD and COD were higher in T0 (443 and 1066, respectively), and very low in T3 reaching 5 and 3.1 respectively. In the case of well water, most parameters were lower compared to that of sewage water, with the exception of TDS showing higher level than T1, T2 and T3. Heavy metals such as Cadmium, Lead and Arsenic were at sufficiently low concentrations as to be undetectable by the analytical method used in the present study; however, extensive use of sewage water for irrigation may lead to accumulation of the undetectable trace amounts.

3.2. Seeds germination

The response of seed germination of C. procera to treated and untreated sewage waters and the results are summarized in Table 2. The results showed that seed germination increased in all treatments from day 1 to day 6 when germination rate reached its maximum. Compared to distilled water, treatment with sewage water had a marked effect (\(p < .05\)) on germination rate reaching 20% increase in seeds treated with T0, T1
Inorganic chemical parameters

| Parameter | Distilled water | Well water | T0 | T1 | T2 | T3 | Water standards for irrigation |
|-----------|----------------|------------|----|----|----|----|-------------------------------|
| TDS       | 1              | 193        | 689| 160| 53 | 7  | 2000–2500                     |
| pH        | 7.0            | 7.55       | 7.47| 7.37| 7.60| 7.42| 6–8.4                        |

Organic chemical parameters

| Parameter | Distilled water | Well water | T0 | T1 | T2 | T3 | Water standards for irrigation |
|-----------|----------------|------------|----|----|----|----|-------------------------------|
| NH₃–N     | UD             | 1.76       | 39.0| 38.7| 25.0| 21.0| 5                             |
| NO₃–N     | UD             | 0.01       | 5.35| 4.72| 4.02| 2.30| 10                            |
| Chloride  | UD             | 75         | 209 | 201 | 176 | 180 | 100                           |

Table 1. Physico-chemical characteristics of well water, untreated and treated sewage water.

Table 2. Effects of sewage water treatments on % germination of *C. procera* (*n* = 4, Mean ± S.E.).

| Day | Distilled water | Well water | T0 | T1 | T2 | T3 | Water standards for irrigation |
|-----|----------------|------------|----|----|----|----|-------------------------------|
|     | 24± 2.8        | 45± 5.2    | 57± 5.0 | 61± 4.3 | 67± 4.5 | 76± 4.8 | 760–4.8                      |
|     | 26± 5.4        | 61± 5.7    | 70± 5.4 | 76± 4.5 | 86± 2.7 | 860± 2.7 | 760–4.8                      |
|     | 32± 8.4        | 78± 4.7    | 87± 3.7 | 91± 2.3 | 94± 1.6 | 95± 1.7 | 950± 1.7                     |
|     | 33± 6.5        | 76± 5.8    | 88± 3.6 | 91± 3.1 | 91± 3.1 | 91± 3.1 | 910± 3.1                     |
|     | 50± 3.9        | 86± 3.1    | 89± 2.8 | 92± 2.5 | 93± 2.1 | 95± 2.2 | 950± 2.2                     |
|     | 38± 6.6        | 66± 8.3    | 80± 3.7 | 84± 3.1 | 84± 3.1 | 85± 3.1 | 850± 3.1                     |

Note: Letters (a, b, c) indicate statistically significant differences between the means (*p* < .05).

and T2. However, T3 had no significant effect on seed germination (*p* > .05).

### 3.3. Plant growth parameters

A number of growth parameters were measured to find out how different treatments of sewage water affect plant growth; the results are summarized in Table 3.

The results showed that all growth parameters (plant height, leaf area and number, leaf fresh and dry weights, stem fresh and dry weights, root fresh and dry weights, total plant fresh and dry weights) of the *C. procera* plants were significantly increased (*p* < .05) in response to the treatments with untreated and primary treated wastewater with the latter being more effective in increasing plant growth compared to the control plants irrigated with distilled water or well water. In the contrary, secondary, tertiary treated sewage water and well water had no significant effect (*p* > .05) on any of the plant growth parameters measured except for leaf fresh weight that was significantly increased (*p* < .05) compared to plants irrigated with distilled or well water. Furthermore, the root:shoot ratio showed a significant decrease (*p* < .05) in plants irrigated with untreated sewage water, while no change was observed when plants were irrigated with T1.

### 3.4. Plant physiological parameters

A number of physiological parameters were measured to find out how different treatments of wastewater affect some plant physiological parameters; the results are summarized in Figure 1 and Table 4.

#### 3.4.1. The rate of photosynthesis

The rates of Photosynthesis in the leaves of *C. procera* irrigated with different treatments of well water, treated and untreated sewages, are plotted in Figure 1. There was a significant increase (*p* < .01) of photosynthesis in plants irrigated with well water. Treatment with T2 and T3 also increased significantly (*p* < .05) the rate of photosynthesis. In contrast, the irrigation with untreated or T1 did not lead to any significant change (*p* > .05).

#### 3.4.2. Chlorophyll content index

was measured as Chlorophyll Content Index using a portable Chlorophyll Meter (see Materials and Methods). The results in Table 4, showed that this parameter was increased significantly (*p* < .05) in all wastewater treatments and especially in the untreated (11.5 ± 1.0) and primary treated (13.2 ± 0.3) sewage water compared to that of the control plants (5.4 ± 0.5) irrigated with distilled water.

#### 3.4.3. Chlorophyll fluorescence parameters

Initial fluorescence *F₀*, maximum fluorescence *Fₓ*, variable fluorescence *Fᵥ*, and maximum quantum yield of photosystem II (*Fᵥ/Fₓ*), were measured in the fully expanded 5th leaf of plants treated with one of the sewage water treatments, distilled and well water.

The results in Table 4, showed that *F₀* was not affected by any of the sewage water treatments, which
Table 3. Effects of sewage water treatments on growth parameters of C. procera (n = 4, Mean ± S.E.).

| Parameter                      | Distilled water | Well water | T0          | T1          | T2          | T3          |
|--------------------------------|-----------------|------------|-------------|-------------|-------------|-------------|
| Plant height (cm)              | 19.5 ± 0.3      | 17.6 ± 0.6 | 21.3 ± 0.8  | 23.7 ± 0.6  | 19.4 ± 0.3  | 20.1 ± 0.1  |
| Leaf number                     | 11.3 ± 0.6      | 10.6 ± 0.6 | 12.6 ± 0.6  | 14.6 ± 0.6  | 12.0 ± 0.0  | 12.6 ± 0.6  |
| Leaf area (cm²)                 | 100.3 ± 18.3    | 87.2 ± 13.3| 177.3 ± 6.1 | 219.3 ± 7.8 | 116.5 ± 13.4| 105.2 ± 9.8 |
| Leaf fresh weight (g)           | 1.3 ± 0.3       | 1.3 ± 0.2  | 2.9 ± 0.1   | 4.2 ± 0.3   | 1.7 ± 0.2   | 1.4 ± 0.1   |
| Leaf dry weight (g)             | 0.1 ± 0.03      | 0.15 ± 0.01| 0.3 ± 0.02  | 0.4 ± 0.04  | 0.2 ± 0.03  | 0.2 ± 0.02  |
| Root fresh weight (g)           | 0.2 ± 0.02      | 0.1 ± 0.02 | 0.2 ± 0.01  | 0.3 ± 0.03  | 0.2 ± 0.06  | 0.2 ± 0.006 |
| Root dry weight (g)             | 0.05 ± 0.0001   | 0.03 ± 0.007| 0.04 ± 0.006| 0.07 ± 0.006| 0.04 ± 0.01 | 0.05 ± 0.002|
| Stem fresh weight (g)           | 0.9 ± 0.1       | 0.8 ± 0.09 | 1.6 ± 0.04  | 2.0 ± 0.1   | 1.1 ± 0.1   | 1.06 ± 0.05 |
| Stem dry weight (g)             | 0.1 ± 0.01      | 0.1 ± 0.01 | 0.2 ± 0.02  | 0.2 ± 0.03  | 0.1 ± 0.02  | 0.1 ± 0.01  |
| Whole plant fresh weight (g)    | 2.0 ± 0.6       | 2.4 ± 0.3  | 4.3 ± 0.2   | 7.0 ± 0.5   | 3.1 ± 0.4   | 2.9 ± 0.2   |
| Whole plant dry weight (g)      | 0.4 ± 0.05      | 0.3 ± 0.03 | 0.6 ± 0.04  | 0.8 ± 0.08  | 0.4 ± 0.07  | 0.4 ± 0.04  |
| Root:Shoot ratio                | 0.2 ± 0.02      | 0.2 ± 0.03 | 0.1 ± 0.008 | 0.2 ± 0.01  | 0.2 ± 0.05  | 0.2 ± 0.01  |

Note: Letters (a, b, c) indicate statistically significant differences between the means (p < .05).

The effect of sewage water on photosynthesis rate

Figure 1. Effect of sewage water treatments on photosynthetic rate in fully expanded 5th leaf of C. procera (n = 4, Mean ± S.E.).

suggests no effect on the integrity of the light harvesting complex of the PS II. However, well water treatment caused a slight but significant increase (p < .05) of \( F_0 \). Results showed that \( F_m \) was significantly increased (p < .05) as a response to all sewage water treatments. In addition, the results also showed that \( F_v/F_m \) which characterize the functional state of the photosystem II in dark-adapted leaves [21] were also increased in response to all sewage water treatments and well water.

3.4.4. Biochemical traits

The effects of wastewater treatments on some biochemical constituents were investigated and the results are summarized in Figures 2, 3 and 4.

Table 4. Effects of sewage water treatments on chlorophyll content index and chlorophyll fluorescence parameters: \( F_0 \), \( F_m \) and \( F_v/F_m \), of dark-adapted C. procera leaves (n = 4, Mean ± S.E.).

| Parameter                      | Distilled water | Distilled water | T0          | T1          | T2          | T3          |
|--------------------------------|-----------------|-----------------|-------------|-------------|-------------|-------------|
| Chlorophyll content index      | 5.4 ± 0.5       | 10.6 ± 1.6      | 11.5 ± 1.0  | 13.2 ± 0.3  | 10.3 ± 0.4  | 7.6 ± 0.7   |
| \( F_0 \)                       | 311 ± 5.5       | 343 ± 21.5      | 310.3 ± 5.8 | 330.6 ± 5.2 | 310.6 ± 6.6 |             |
| \( F_m \)                       | 1514 ± 5.0      | 1765 ± 40.3     | 1762 ± 41.6 | 1782 ± 29.1 | 1854 ± 14.3 | 1760 ± 91.6 |
| \( F_v/F_m \)                   | 0.795 ± 0.003   | 0.806 ± 0.008   | 0.824 ± 0.0005 | 0.826 ± 0.002 | 0.833 ± 0.001 | 0.823 ± 0.005 |

Note: Letters (a, b, c) indicate statistically significant differences between the means (p < .05).

3.4.5. Proteins content

Figure 2 shows that leaves of C. procera plants had significantly higher proteins content (p < .05) reaching 63%, 108% and 35% in leaves from plants irrigated with untreated, primary and secondary treated wastewaters respectively compared to that of plants irrigated with distilled water. In contrast, well water had no significant effect (p > .05) on protein content in leaves.

3.4.6. Carbohydrates content

The results presented in Figure 3, shows that carbohydrates content in leaves from C. procera plants was significantly higher (p < .01) reaching 87% when plants were irrigated with untreated wastewater compared to

Effects of sewage water treatments on chlorophyll content index and chlorophyll fluorescence parameters: \( F_0 \), \( F_m \) and \( F_v/F_m \), of dark-adapted C. procera leaves (n = 4, Mean ± S.E.).

...
Figure 2. Effect of sewage water treatments on protein content of *C. procera* (*n* = 4, Mean ± S.E.).

Figure 3. Effect of sewage water treatments on carbohydrate content of *C. procera* (*n* = 4, Mean ± S.E.).

that of distilled water irrigated plants. In contrast, secondary and tertiary treated wastewater caused a significant decrease (*p* < .01) in carbohydrates content of leaves reaching 30% and 52% respectively; with no significant effect (*p* > .05) of the primary treated wastewater or well water on carbohydrates content.

Figure 4. Effect of sewage water treatments on proline content of *C. procera* (*n* = 4, Mean ± S.E.).
3.4.7. Proline content
In the case of proline content (Figure 4) that was determined in order to investigate whether C. procera plants irrigated with different sewage water treatments were under any kind of stress. The results showed that none of the treatments caused any significant changes in the proline content of leaves.

4. Discussion
The present study investigated the impact of different sewage water treatments, T0, T1, T2 and T3 in comparison with distilled and well water, on growth and physiology of the wild desert plant C. procera as a potential sustainable water resource to irrigate wild plants for the purpose of increasing vegetation in the desert, combating desertification, decreasing sand movement, and producing biofuels and pharmaceutical compounds.

In the present study, treatment with different sewage waters increased markedly germination of C. procera seeds. Such increase was observed in many crop plants including, tomato, radish, carrot and onion [22]. The stimulatory effect of sewage water was due to an increase of the activity of certain enzymes responsible for germination such as amylase, invertase and protease [23]. In contrast, high sewage water concentrations were found to inhibit germination in a number of crop plants such as tomato [15], mustard, rapeseed, coriander and barley [24].

When irrigated with untreated and primary treated sewage waters, C. procera showed an increase of almost all plant growth parameters. However, T2 and T3 had no effect on plant growth. Furthermore, the root:shoot ratio decreased under T1 treatment, suggesting that there was an increase in the growth of shoot system over the root system. However, T1 treated plants showed similar root:shoot ratio to that of the control plants irrigated with distilled or well water, indicating proportional changes in shoot and root systems under such treatment. In the case of landscape plants such as Euca- lyptus sp., Forsythia sp., Medicago arborea, Buddleia variabilis and Nerium oleander, Pedrero et al. [25] reported that the growth of plants irrigated with treated sewage water was significantly increased, owing possibly to the beneficial effect of the nutrients present in the treated sewage water. The positive effect of untreated and T1 wastewaters was possibly due to high levels of nutrients recorded by wastewater analysis, especially ammonia (NH₃–N), which was higher than the recommended limit.

Irrigation with treated or untreated wastewater was also found to impair the physiology of plants irrigated with wastewater. While untreated and T1 had caused no change in the rate of photosynthesis, T2 and T3 lead to an increase. This finding did not go inline with the changes in plant growth parameters. Such increase was also observed in our laboratory using the same sewage water treatments from the same source and the same butch but with a crop plant (Tomato, A1 genotype). In contrast, the same study revealed a decrease in the rate of photosynthesis in A1 genotype; however, changes in the rate of photosynthesis in P genotype depended on the sewage water treatment [15].

The present study showed that untreated and treated sewage waters were able to increase chlorophyll content index in C. procera leaves. Zeid and Abou El Ghate [23] reported similar results when Phaseolus vulgaris plants were treated with sewage waters, owing such increase to Mg²⁺ and other nutrients present in sewage water. Similarly, Thaplyal et al. [26] observed the enhancing effect of untreated and treated sewage water on the content of chlorophyll a and b. This was in contrast with the findings by Akhkha et al. [15] that sewage water treatments inhibited chlorophyll content in tomato leaves, which suggests the ability of C. procera to respond well to irrigation with sewage waters compared to some crop plants. Similarly, in a study carried out by Manisha and Angloorbala [27], they observed a maximum decrease in chlorophyll a, chlorophyll b and total chlorophyll contents when plants were treated with undiluted and 50% diluted sewage water.

In the attempt to investigate the impact of sewage water on the integrity of photosystem II, chlorophyll fluorescence parameters were determined. Untreated and treated sewage waters were found to alter most chloro- phyll fluorescence parameters by increasing Fm, Fv/Fm and Fv/Fo. Such increase was only reflected in the photosynthesis when plants were irrigated with T2 and T3. The increase in Fv/Fm was interpreted as an adaptation capability of C. procera to sewage water treatments as suggested by Jägerbrand and Kudo [28]. In contrast, similar sewage water treatments had no effect on Fm but caused a decrease of Fv/Fm in dark adapted tomato leaves [15], suggesting the ability of the C. procera as a wild plant to withstand any stress may be caused by sewage water treatments.

The present study, investigated also the effect of sewage waters on proteins and carbohydrates content. It was concluded that sewage waters increased Carbohydrates content in the leaves of C. procera when plants were irrigated with T0. In contrast, the other sewage treatments (T1, T2, T3) caused a decrease. In the case of proteins content, T0, T1 and T2 caused an increase. Such increase was reported also by Akhtar et al. [29] when wheat plants were irrigated with sewage water. Similarly, proteins and carbohydrates contents were increased in shoots of maize [30] and two Mulberry varieties [31] irrigated with sewage water. Proline content showed no change under any of the treatments, indicating that plants irrigated with different sewage water treatments were under no stress. Proline content accumulation was reported by many authors when plants suffer from abiotic stress in order to limit damage caused by such stress [32,33]. In cases where
sewage water increased proline content in plants, the main cause was due to high levels of heavy metals that sewage water may contain [33].

The negative effects of sewage water on plant growth reported in some studies were owed to the high levels of heavy metals present in sewage waters [34,35]. In the present investigation, the levels of heavy metals in different treated and untreated wastewaters were lower than the limits set by the Saudi Ministry of Water and Electricity [36] and the Ministry of the Saudi Municipal and Rural Affairs [37] for irrigation water. Such low or below the norm levels of heavy metals in sewage water used in the present study, explains why plant growth and physiological parameters were not affected negatively; instead there was an increase in most plant growth parameters under untreated and primary treated sewage water and physiological parameters under all sewage water treatments. Although, the level of heavy metals was not determined in soils after irrigation and with the possibility of accumulation, there was no sign of any negative impairment of any parameters in C. procera; this was not the case in some tomato genotypes as reported by Akhkha et al. [15]. Similarly, TDS which was under the permissible limit for irrigation water, did not affect negatively the growth or physiology of C. procera even though plants were extensively irrigated with such waters with the possibility of increasing the level of salt in the soil. However, C. procera is a well-known plant species that withstand high levels of salinity [17].

5. Conclusion

Irrigation with Al-Madinah Al-Munawwarah domestic treated and untreated sewage water led to an increase in most growth parameters of C. procera. Physiological parameters such as chlorophyll content, \( F_{m} \) and \( F_{v}/F_{m} \) were also increased in all sewage water treatments, except photosynthetic rate, which showed an increase only under T2 and T3 treatments. Biochemical parameters such as carbohydrate and protein contents were increased under some of the sewage water treatments with the exception of proline that showed no change. We can conclude from the present results that Madinah domestic sewage water showed below the norm levels of heavy metals but increased levels of nutrients which makes it suitable for irrigation of wild plants that can have an environmental value and can play a role in the protection of the environment by using such plants as barriers against sand storms, source of medicinal compounds or biofuels or simply to increase the vegetation cover.

Acknowledgements

This research was supported by the Department of Biology, College of Science, Taibah University, Al-Madinah Al-Munawwarah, Kingdom of Saudi Arabia. Special thanks to the General Directorate of Water in Al-Madinah Al-Munawwarah, Ministry of Environment, Water and Agriculture for providing the permission to obtain the treated and untreated wastewater samples from Al-Khaleel Wastewater Treatment Plant.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Abdellah Akhkha http://orcid.org/0000-0001-9498-7181
Ebtesam Salem Al-Radaddi http://orcid.org/0000-0002-9623-1270
Abdul Khaliq Al-Shoaibi http://orcid.org/0000-0002-4327-5616

References

[1] Tilman D. Global environmental impacts of agricultural expansion: The need for sustainable and efficient practices. Proc Natl Acad Sci. 1999;96:5995–6000.
[2] FAO. Coping with water scarcity: an action framework for agriculture and food security. FOA Water Reports. 2012:38.
[3] UN-Waters. Water Security and the global water agenda. policy and analytical briefs. Hamilton, Ont., United Nations University (UNU); 2013.
[4] Khouri J. Sustainable development and management of water resources in the Arab region. Dev Water Sci. 2003;50:199–220.
[5] KAUST (King Abdullah University of Science & Technology). KAUST industry and collaboration program (KICP), the KICP annual strategy study: Promoting wastewater reclamation and reuse in the Kingdom of Saudi Arabia: Technology trend, innovation needs, and business opportunities. Jeddah: KAUST; 2011.
[6] Dixit A, Dixit S, Goswami CS. Process and plants for wastewater remediation: a review. Sci Rev Chem. Commun. 2011;11:71–77.
[7] Adnan A. Methods of wastewater treatments. Biotech Articles. 2010;20(18):10, views 4123. Date: 2010-08-20.
[8] Baydoun SA, Kanj D, Raafat K, et al. Ethnobotanical and economic importance of wild plant species of Jabal Moussa Bioserve, Lebanon. J Ecosyst Ecogr. 2017;7(3):245. DOI: 10.4172/2157-7625.1000245.
[9] Bidaka LM, Kamal SA, Halmy MWA, et al. Goods and services provided by native plants in desert ecosystems: examples from the northwestern coastal desert of Egypt. Glob Ecol Conserv. 2015;3:433–447.
[10] Balat M, Ayar G. Biomass energy in the world, use of biomass and potential trends. J Energy Sources. 2005;27(10):931–940.
[11] Rathore M, Meena RK. Potential of utilizing Calotropis procera flower biomass as a renewable source of energy. J Phytof. 2010;2(1):78–83.
[12] Song U, Lee E. Ecophysiological responses of plants after sewage sludge compost applications. J Plant Biol. 2010;53:259–267.
[13] Tabari M, Salehi A, Mohammadir J. Impact of municipal waste water on growth and nutrition of afforested Pinus eldarica Stands. In: Sebastin F., Einschlag G. (eds) Waste Water - Evaluation and Management. pp. 303–312; 2011.
[14] Gupta AP, Narwal RP, Amtal RS. Sewer water composition and its effect on soil properties. Bioresource Technol. 1998;65:171–173.
The Influence Of Al-Madinah Al-Munawwara treated And untreated domestic wastewater On growth And physiology Of three tomato (Lycopersicon Esculentum Mill.) genotypes. Pak. J. Bot. 2017;49(3):879–890.

Akhkha A, Boutraa T. Effect of salinity on chlorophyll fluorescence and chlorophyll content of the desert shrub Calotropis procera. J Int Environ Appl Sci. 2010;5(4):556–565.

Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water-stress studies. Plant Soil. 2005;39(1): 205–207.

Yemm EW, Willis AJ. The estimation of carbohydrates in plant extracts by anthrone. Biochem J. 1954;57(3):508–514.

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochem. 1976;72: 248–254.

Vassilev A, Manolov P. Chlorophyll fluorescence of barley (H. vulgare L.) seedlings grown in excess of Cd. Bulg J Plant Physiol 1999;25(3–4):67–76.

Ravindran B, Kumari SKS, Stenstrom TA, et al. Evaluation of phytotoxicity effect on selected crops using treated and untreated wastewater from different configurative domestic wastewater plants. Env Techn. 2016;37(14):1782–1789.

Zeid IM, Abou El Ghate, HM. Effect of sewage water on growth, metabolism and yield of bean. J Biol Sci. 2007;7:34–40.

Humaz Z, Naveed S, Rashid, A, et al. Effects of domestic and industrial waste water on germination and seedling growth of some plants. Curr Opin Agric. 2012;1(1):27–30.

Pedreroa GF, Kalavrouziotisb I, Alarcóna JJ, et al. Use of treated municipal wastewater in irrigated agriculture – review of some practices in Spain and Greece. Agric Water Manag. 2010;97:1233–1241.

Thaplyal A, Vasudevan P, Dastidar MG, et al. Irrigation with domestic wastewater: Responses on growth and yield of ladyfinger Abelmoschus esculentus and on soil nutrients. J Environ Biol. 2011;32:645–651.

Manisha P, Angoorbala B. Effect of sewage on growth parameters and chlorophyll content of Trigonella foenum-graecum (Methi). Int Res J Env Sci. 2013;2(9):5–9.

Jägerbrand AK, Kudo G. Short-term responses in maximum quantum yield of PSII (Fv/Fm) to ex situ temperature treatment of populations of bryophytes originating from different Sites in Hokkaido, Northern Japan. Plants. 2016;5(2):1–7.

Akhtar N, Inam A, Inam A, et al. Effects of city wastewater on the characteristics of wheat with varying doses of nitrogen, phosphorus, and potassium. Recent Res Sci Technol. 2012;4(5):18–29.

Abdel Latef AA, Sallam MM. Changes in growth and some biochemical parameters of maize plants irrigated with sewage water. Austin J Plant Biol. 2015;1(1):1004.

Chikkaswamy BK, Prasad MP, Paramanik RC. Effect of sewage irrigation on physio-biochemical characterization of two mulberry varieties. J Chem Pharm Sci. 2014;4:30–32.

Ashraf M, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot. 2007;59:206–216.

Kausar S, Faizan S, Haneef I. Nitrogen level affects growth and reactive oxygen scavenging of fenugreek irrigated with wastewater. Trop Plant Res. 2017;4(2):210–224.

Mangabeira P, Almeida AA, Mielke M, et al. Ultrastructural investigations and electron probe X-ray microanalysis of chromium treated plants. Proc. VI COBTE, Guelph, p. 555; 2001.

Jomova K, Morovic M. Effect of heavy metal treatment on molecular changes in root tips of Lupinus luteus L. Czech J Food Sci. 2009;27:S386–S389.

MWE. Technical guidelines for the use of treated sanitary wastewater in irrigation for landscaping and agricultural irrigation. Ministry of Water and Electricity, Kingdom of Saudi Arabia; 2006.

MMRA. Technical guidelines for the use of treated sanitary wastewater in irrigation for landscaping and agricultural irrigation, first ed. Ministry of Municipal and Rural Affairs – Deputy Ministry for Technical Affairs – General Department for Infrastructure, Kingdom of Saudi Arabia; 2003.