Assessment of phytotoxicity of treated water of Tabuk wastewater plant by different technologies on seed germination of chick pea (Cicer arietinum)

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ABSTRACT

The use of reclaimed water as an alternative source is a sustainable way forward for an arid country like The Kingdom of Saudi Arabia. The sewage contains organic and inorganic pollutants from households and industrial sources that may not be removed during treatment. In this study, seeds of Cicer arietinum were germinated using six different concentrations of treated water from the Tabuk wastewater treatment plant and tap water was used as control. The physicochemical properties such as total dissolved solids, electrical conductivity, total suspended solids, and turbidity values of treated water were higher which gradually decreased on dilution with tap water. The amount of ammonia, nitrite, nitrate, and phosphate was in higher concentration in treated water as compared to control. The use of 40% treated water (T3) improved the germination percentage, speed of germination and germination index of C. arietinum. The phytotoxicity test reveals that undiluted treated water (T6) is not fit for direct use on plants. All the investigated treatments confirmed that the use of more than 40% of treated water decreased the fresh weight and dry weight of the seedlings as compared to control. The results are encouraging and help in attaining water sustainability in the Tabuk region.

Key words: germination index, phytotoxicity, sustainable development, water pollution, water quality

HIGHLIGHTS

- In KSA agricultural practices is difficult to carry out due to water scarcity.
- To the best knowledge, it is the first report of the use of reclaimed water from Tabuk region.
- The use of more than 40% of treated water is not fit for direct use on plants.
- The results helps in attaining the water sustainability in the Tabuk region.
- It decreases the load of desalination of Red Sea and helps to achieve the VISION 2030 of KSA.

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INTRODUCTION

Water is indispensable to life, though 785 million people in the world have shortage to access it. As per the World Economic Forum, the water crisis is among the top five global risk in terms of impact to society (WHO & UNICEF 2017; World Economic Forum 2020). The availability of fresh water is becoming an increasingly scarce resource in many countries, due to population growth, enormous urbanization, poor agricultural and water management practices, water pollution and change in climatic pattern (Khaleel et al. 2013; Ganjegunte et al. 2017; Singh et al. 2019). This led to water scarcity even in those nations where water is present in abundance. The condition is critical in arid and semi-arid regions of the world. The Kingdom of Saudi Arabia (KSA) is the largest arid country on the Arabian Peninsula without permanent rivers or lakes. In KSA agricultural practices is very difficult to carry out, therefore domestic wheat production program was suspend in 2016. The key factor for the policy change was a deep concern over the exhaustion of the country’s scarce water reserves, as the wheat crop was 100% irrigated (World Grain 2016). The use of reclaimed water for irrigation and recharge of the aquifer is a sustainable way forward (Ibekwe et al. 2018; Abbas & Siddiqui 2020; Ofori et al. 2021).

The water recycling is a central aspect of water resource and environment management policies. Nowadays, partially treated wastewater is use as an artificial method for recharging groundwater as well as for irrigation (DHWA 2002; Raja et al. 2015; Shakir et al. 2017; Ofori et al. 2021). There are several advantages of the use of reclaimed water, it increases groundwater levels and protect underground freshwater in coastal aquifers against intrusion from the saline water. It also reduces pollution of rivers and canals and conserve micronutrients, organic matter, and vital NPK when used for irrigation (Hanjra et al. 2012; Ofori et al. 2021). Further, reclaimed water is highly attractive for poor farmers as it reduces the cost production of crops by acting as fertilizer (Otoo & Drechsel 2018; Ofori et al. 2021). Despite these advantages, there are several reasons that discourage its use as an alternative water resources. The treated wastewater may contains large variety of pollutants from different industries and household. Sewage contains composite concoctions of inorganic and organic complexes including pathogens and heavy metals that may not be degraded during treatment (European Commission Directive 2000, 2008; Sancey et al. 2011; Yi et al. 2011; Khaleel et al. 2013; Priac et al. 2017). The release of treated water contaminates groundwater, accumulates heavy metals and inorganic compounds in the soil. It also creates habitat
for harmful microorganism that could be a health hazard to farmers, farmworkers and users if not properly managed (Raja et al. 2015; Christou et al. 2017; Khalid et al. 2018; Diaz-Sosa et al. 2020; Ofori et al. 2021).

There is difference of opinions over the use of treated wastewater therefore, it is necessary to evaluate the toxicity of treated water before making any recommendations. Plant based bioassay using germination technologies are very popular due to its simplicity and easy application, it can be used \textit{in situ} and \textit{in vitro} condition. There is no constraint for major equipment and nominal maintenance costs is required. Seeds of higher plants can sustain without nutrients supply in the test water and are easily available. Moreover, small sample size is required that can be repeated multiple times in a short period (Mayer & Poljakoff-Mayber 1989; Wang & Freemark 1995; Salvatore et al. 2008; Rusan et al. 2015; Priac et al. 2017). Khaleel et al. (2013) evaluated the effect of wastewater treatments on seed germination and biochemical parameters of \textit{Abelmoschusesculentus} and recommended its use after proper dilutions. In case of assessment of olive mill wastewater (OMW) use on seed germination of barley, Rusan et al. (2015) propose 1:3 (OMW:Tap water) dilution of OMW. Authors further reported that microfiltration followed by reverse osmosis in addition to solar fenton oxidation and Jacto reactor is the most effective method in reducing the phytoxicity of OMW. Priac et al. (2017) applied germination and root elongation test parameters on \textit{Lactuca sativa} to assess the phytotoxicity of treated wastewater and reported differential sensitivity to treated wastewater among its cultivars. Shakir et al. (2017) highlighted the environmental and health hazards linked with the reuse of wastewater for irrigation. The poor socio-economic conditions of farmers compel them to use wastewater for irrigation and it leads to bioaccumulation of heavy metals in crops and vegetables (Raja et al. 2015). In view of the present scenario where opinions are divided about the use of treated wastewater, this study was carried out to screen the suitability of the use of treated water of Tabuk Waste Water Treatment Plant (TWTP). The identification of degree of dilution of treated wastewater of TWTP as a non-conventional water resource by using different technologies of seed germination.

**METHODS**

**Site of work**

Tabuk is among the top ten big cities of KSA in terms of population. In the last decade, it witnessed an increase of almost 150,000 people. The average daily wastewater generated in Tabuk city is around 186,000 m$^3$. This water is treated at three levels in a central plant located at the outskirt of the city (Figure 1) around 35 km away from University of Tabuk.

![Figure 1](http://iwaponline.com/wst/article-pdf/doi/10.2166/wst.2021.287/913575/wst2021287.pdf)
Assessment of water quality parameters

The treated water was collected as per American Public Health Association (APHA 2012) from the outlet point of WWTP. The treated water was diluted as per the Table 1 and tap water was used as control. The physicochemical parameters such as pH, turbidity, total suspended solids (TSS) total dissolved solid (TDS), alkalinity, nitrite, nitrate, ammonia, chloride, sulphate, sulfide, phosphate and iron of all the water samples were measured. The standard instruments of Hach such as DR900 colorimeter and DR3900 benchtop spectrophotometer along with titration accessories and standard guidelines were used to measure the physicochemical properties of treated and tap water and compared with the standard guidelines of water use of KSA (Table 2).

Model plant

The plant of our study was Cicer arietinum (Chick pea) of family Fabaceae. It is known for its high protein value and is one of the oldest cultivated legume of human civilization. It is very popular in Mediterranean, Middle East and Indian cuisine. Hummus, falafel, chana masala and chhole are the popular dishes enjoyed by the large population of these countries.

Experimental layout

The seed germination experiment was conducted at Department of Biology, University of Tabuk, Tabuk, Saudi Arabia. The C. arietinum seeds of same size and weight were surface-sterilized with 70% ethanol (2–3 min) followed by 50% bleach (5–15 min), rinsed for 1 min for four-seven times with distilled water. Thereafter, seeds were soaked overnight in different concentration of treated wastewater i.e. 10% (T1), 20% (T2), 40% (T3), 60% (T4), 80% (T5), 100% (T6) and in tap water (C). The germination test was conducted using 9-cm Petridishes containing one layer each of cotton and filter paper (Whatman No.1) moistened with 10 ml of respective concentration of treated water (Figure 2).

In each petri dishes fifteen seeds of C. arietinum were placed, and three petridishes were used for each water sample. It results in 45 seeds for each treatment distributed into three dishes. The whole set-up was irrigated with respective concentration of water at regular interval throughout the course of the experiment. The seeds were germinated under control conditions of incubator and following parameters of seed germination were recorded to prepare the results.

Seed germination

The seed germination is defined as the first emergence of the radicle (Redondo et al. 2004) and newly-germinated seeds were recorded after two, three, four and, five days and percentage seed germination was calculated according to Ruan et al. (2002).

\[
\text{% Seed Germination} = \frac{N_T \times 100}{N}
\]

where \( N_T \) = Number of seedlings emerging on day; \( N \) = Total Number of seeds.

Speed of germination

It was calculated as per the formula given by Krishnaswamy & Seshu (1990).

\[
\text{Speed of Germination} = \left( \frac{\text{Number of seed germinated at 72 hrs}}{\text{Number of seed germinated at 72 hrs}} \right) \times 100
\]

Table 1 | Different categories of diluted water samples of Tabuk Wastewater Treatment Plant

| Treatment                          | Code |
|-----------------------------------|------|
| Tap Water                         | C    |
| 10% Treated water + 90% Tap water | T1   |
| 20% Treated water + 80% Tap water | T2   |
| 40% Treated water + 60% Tap water | T3   |
| 60% Treated water + 40% Tap water | T4   |
| 80% Treated water + 80% Tap water | T5   |
| 100% Treated water                | T6   |
Table 2 | Water quality parameters of diluted water samples of Tabuk Wastewater Treatment Plant

| Parameters   | Unit | Control T1 | T2 | T3 | T4 | T5 | T6 | 2003-MMRA | 2006 MWE | 2003-MMRA | 2006 MWE |
|--------------|------|------------|----|----|----|----|----|-----------|----------|-----------|----------|
| TDS          | mg/L | 451.00     | 595.00 | 773.00 | 1,123.00 | 1,451.00 | 1,708.00 | 2,110.00 | 2,000.00 | 2,500.00 | 2,000.00 | 2,500.00 |
| Conductivity | μS/cm| 705.00     | 930.00 | 1,208.00 | 1,754.00 | 2,270.00 | 2,670.00 | 3,290.00 | –        | –        | –        | –        |
| pH           |      | 7.53       | 7.54 | 7.56 | 7.64 | 7.68 | 7.9 | 7.97 | 6.00–8.40 | 6.00–8.40 | 6.4–8.4 |
| TSS          | mg/L | 3.75       | 3.75 | 4.56 | 4.66 | 5.20 | 7.00 | 7.00 | 10.00     | 10.00    | 40.00    | 40.00    |
| Ammonia      | mg/L | 1.50       | 2.25 | 3.00 | 3.75 | 5.25 | 7.00 | 12.00 | 5.00     | 5.00     | 5.00     |
| Turbidity    | FAU  | 4.00       | 6.00 | 6.00 | 8.00 | 8.00 | 8.33 | 11.00 | –        | 5.00     | –        | 5.00     |
| Alkalinity   | mg/L | 182.00     | 184.00 | 212.00 | 229.00 | 246.00 | 268.00 | 307.00 | –        | –        | –        | –        |
| Nitrate      | mg/L | 0.40       | 0.80 | 0.90 | 1.60 | 2.10 | 2.75 | 3.30 | 10.00    | 10.00    | –        | 10.00    |
| Chloride     | mg/L | 700.00     | 387.00 | 561.00 | 589.30 | 617.70 | 642.55 | 670.95 | 100.00   | –        | –        | –        |
| Nitrite      | mg/L | 0.16       | 0.62 | 0.75 | 0.78 | 2.10 | 2.47 | 3.13 | –        | –        | –        | –        |
| Sulphate     | mg/L | 40.00      | 40.00 | 90.00 | 190.00 | 280.00 | 450.00 | 490.00 | 600.00   | –        | –        | –        |
| Phosphate    | mg/L | 2.40       | 3.10 | 3.10 | 5.20 | 5.80 | 7.80 | 9.10 | –        | –        | –        | –        |
| Sulfide      | mg/L | 0.001      | 0.004 | 0.005 | 0.006 | 0.006 | 0.01 | 0.018 | –        | –        | –        | –        |
| Iron         | mg/L | 0.06       | 0.04 | 0.05 | 0.07 | 0.11 | 0.17 | 0.30 | –        | 5.00     | –        | 5.00     |

Control-Tap Water, T1 (10%), T2 (20%), T3 (40%), T4 (60%), T5 (80%), T6 (100%).
MMRA: Ministry of Rural Affairs and Housing; MWE: Ministry of Water and Electricity.
Germination index

It was calculated as per the formula given by Tao & Zheng (1990).

\[
\text{Germination Index} = \frac{\% \text{ Seed germination}}{\text{Germination days}}
\]

Phytotoxicity index (PI)

The phytotoxicity bioassay rests on calculating the root elongation in the germinating seeds as per the formula given by Mekki et al. (2007).

\[
PI = 1 - \frac{R_{LT}}{R_{LC}}
\]

Where \( R_{LT} \) = Root length I the treated seed; \( R_{LC} \) = Root length in the control seeds.

The values of the PI ranges between 0 and 1, a higher value means a toxic effects and the lower values signifies a stimulatory effects of the treated water.

Fresh weight and dry weight of seedlings

After 10 days of growth (Figure 3) fresh weight of each seedling was recorded. The samples were then placed in an oven maintained at 60 °C for 48 hrs and thereafter dry weight of seedlings were recorded.

Statistical analysis

In one treatment, a petri dish was treated as one replicate and all the treatments were repeated five times. The data were expressed as means ± standard error, and analysed statistically with IBM SPSS Statistics 20 software (SPSS Inc., Chicago, IL, USA). Means were statistically compared by Duncan’s multiple-range test (DMRT) at the \( p<0.05 \) level.

RESULTS AND DISCUSSION

The use of reclaimed water has several advantages and disadvantages at environmental level along with issues of public health. It depends upon the quality of the treated effluent and other external factors. The quality of treated wastewater is determined by various water quality parameters like pH, turbidity, TDS, conductivity etc. and other factors include the frequency of irrigation, climatic conditions and soil properties (Elgallal et al. 2016; Ofori et al. 2021). In our study water quality parameters of all the water samples were measured and compared with standard guidelines of KSA for reuse of water. It was found that the values of TDS, conductivity, turbidity and chloride were more than the permissible limits (Table 2). Due to
dilution, these values were decreased and found to be near optimum in T4 treatments. The amount of nitrate, iron, total suspended solids and the value of pH was recorded within the permissible limits. Our results showed due to dilution almost all studied parameters were in the permissible limits of the standard guidelines of KSA for reuse of water. In another study, Balkhair et al. (2013) conducted a field study in KSA and conserved 60% of ground water by using six different wastewater qualities of treated wastewater for irrigation.

The seed germination is a very complex process and begins with water uptake and transition of a quiescent dry seed to a metabolically active state. It is the basis that forecast plant growth, development and productivity. In the present work, different technologies of seed germinations were assessed to determine the efficiency of different concentration of treated wastewater as an alternative source for water management. The results shows that after five days of treatment maximum seed germination was recorded in T3 (96%) and minimum was in T6 (65%) (Figure 4). The negative effect of the T6 water on seed germination of Cicer may be attributed to the high TDS, conductivity and chloride content. There are reports that

**Figure 3** | Germinated seeds of *Cicer arietinum* in different concentrations of treated water, C (Tap Water), T1 (10%), T2 (20%), T3 (40%), T4 (60%), T5 (80%), T6 (100%).

**Figure 4** | Seed Germination of *C. arietinum* (%) in different concentrations of treated water Control-Tap Water, T1 (10%), T2 (20%), T3 (40%), T4 (60%), T5 (80%), T6 (100%). Values are means ± standard errors of 5 replicates; within each column, means followed by the same letter are not significantly different at p ≤ 0.05 according to DMRT.
suggests seed germination is negatively affected by salinity through osmotic effects (Zhang et al. 2010; Krmz & Bell 2012), by ion toxicity (Hampson & Simpson 1990) or by a combination of the two (Huang & Redmann 1995).

The speed of germination and germination index can be used as an indicator of phytotoxicity. The speed of germination was fast in the initial 72 hrs as compared to 120 hrs in all the treatments except control. The speed of germination in T6 was lowest after 120 hrs (Figure 5). After 2 days the germination index was higher in T3 whereas in T6 it reached maximum only after three days (Figure 6). Naseri et al. (2012) reported that due to salinity germination rate index, root length and root weight reduce significantly. Osmotic pressure of the effluent increases at higher concentrations of total salts making imbibition more difficult and retard germination efficiencies (Nagda et al. 2006; Khan et al. 2011). In another report, Rahman et al. (2008) argues salinity disturb the

**Figure 5** | Speed of Germination of *C. arietinum* seeds in different concentrations of treated water Control-Tap Water, T1 (10%), T2 (20%), T3 (40%), T4 (60%), T5 (80%), T6 (100%). Values are means ± standard errors of 5 replicates; within each column, means followed by the same letter are not significantly different at $p \leq 0.05$ according to DMRT.

**Figure 6** | Germination index of *C. arietinum* seeds in different concentrations of treated water Control-Tap Water, T1 (10%), T2 (20%), T3 (40%), T4 (60%), T5 (80%), T6 (100%). Values are means ± standard errors of 5 replicates; within each column, means followed by the same letter are not significantly different at $p \leq 0.05$ according to DMRT.
imbibition process and water intake by seeds decreases it is followed by osmotic and ion toxic effects that prevent the seed germination and reduces germination rate. Further, salt concentration reduces the germination rate (Sabir & Ashraf 2005; Akbari et al. 2007) which confirms our results of % of seed germination, speed of germination and germination index.

The phytotoxicity index measures the difference of values in root elongation of treated and control water during seed germination. The PI values after 5 days were lower in all treatments thereafter it gradually increased in seedlings. It was lowest in T3 (0.133) and maximum in T6 (0.90) after 10 days of treatments (Figure 7). The nutrient uptake by plant is motivated by the root system and concentration of nutrients at the root surface. The nutrients are generally taken up in their ionic form (Jones & Olson-Rutz 2016; Ofori et al. 2021). Our results showed that in the root system, the harmonious concentration of nutrients were resulted in low PI value in T3 treatment as compared to T6. The fresh weight and dry weight of the seedlings were significantly different in the treatments (Figure 8). The maximum fresh weight was recorded in T3 (48.93 g/45 seedlings) and
minimum in T6 (34.712 g/45 seedlings). Similarly, the maximum dry weight was recorded in T3 (14.99 g/45 seedlings) and minimum in T6 (11.65 g/45 seedlings).

Germination is a very sensitive and vulnerable plant process. It is affected by water availability, salinity and to the toxic compounds of the growth medium (Khan et al. 2002). In T6 water sample, TDS, Conductivity, Ammonia, and chloride concentrations were higher than the permissible values. There are reports that suggest salt stress results in reduced cell turgor and depressed rate of root and leaf elongation (Fricke et al. 2006). It was visible in the fresh and dry weight of the Cicer seedlings.

As stated earlier, use of treated wastewater has both positive and negative implications on plant growth and development (Ofori et al. 2021). It was clearly visible in our study; in different concentrations of treated water, the nitrogen was available in the form of nitrates, nitrite and ammonia whereas, phosphate is the source of phosphorus in the treated water. This makes the accessibility of nitrogen and phosphorus uptake very easy for plant and it improved the growth and yield (Aziz & Farissi 2014; Vergine et al. 2017) by providing nutrients in easy accessible form upto T3 treatments. However, at higher concentrations of treated water (T4 onwards) a sharp decrease in the growth and yield was recorded. The possible reasons are the excess nutrients (Mccauley et al. 2011), heavy metals (Becerra-Castro et al. 2015), trace elements (Galavi et al. 2010), organic xenobiotic compounds like pharmaceuticals, personal care products, endocrine disrupting compounds (Fatta-Kassinos et al. 2011). The accumulation of these compounds affects biochemical processes of plants and inhibits growth and development and results in reduce yield.

CONCLUSION

In the present study, it was found that use of treated wastewater for irrigation purposes require caution and management. The phytotoxicity test reveals that undiluted treated water (T6) is not fit for direct use on plants. All the investigated treatments confirmed that the use of more than 40% of treated water decreased the fresh weight and dry weight of the seedlings as compared to control. To our best knowledge, this is the first report of the use of reclaimed water of Tabuk wastewater treatment plant on a seed germination experiment. The results are encouraging and help in attaining water sustainability in the Tabuk region. It also decreases the load of desalination of Red Sea and helps to fulfill the sustainable development goals of VISION 2030 of KSA.

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DATA AVAILABILITY STATEMENT

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

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