Effect of municipal wastewater treated by field-scale constructed wetlands on seed germination and their nutrient uptake

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Abstract

The present study aimed to evaluate the physiological effects of untreated and treated municipal wastewater from constructed wetlands on seeds of different edible crops. To find the construction wetlands can be the promising and alternative wastewater treatment technique, which usually require minimal inputs for installation, operation, and maintenance and also to mitigate the detrimental impacts of wastewater on the environment.

For this study, freshwater, untreated wastewater and Treated wastewater from constructed wetlands were utilized as water and wastewater resources. Further, the Seed germination bio-assay was conducted with selected edible seeds, namely-Zea mays (Maize), Cicer arietinum (Chickpea), Cajanuscajan (Pigeon pea), and Vignaunguiculata (Cowpea). Their vegetative growth parameters and the nutrient uptake by seedlings were analyzed in an optimum period of five days. The total experiment is conducted in three consecutive cycles and the multivariate statistical analysis is applied for the total obtained results.

The Treated wastewater has shown promoting results. Seedling growth parameters in Zea mays and Cajanuscajanwere observed to be highest with untreated wastewater than Treated wastewater. Conversely, the nutrient uptake (N, P, K, Mg, Ca, Na, and S) in both the crop species was found to be maximum with the treatment of Treated wastewater than with untreated wastewater. The present study reveals that, the bio-assay order of the crops tolerant to municipal wastewater can be *Zea mays>Cajanuscajan>Cicer arietinum>Vignaunguiculata*.

The study reveals that,thetreated wastewater can be used for irrigation to achieve more enriching benefits for agriculture. From wastewater analysis results, it can be suggested that constructed wetlands can treat wastewater effectively like other conventional treatment methods.

Keywords: Constructed wetlands, Edible crops, Germination, Municipal wastewater, Nutrient uptake, seed germination.

I. INTRODUCTION

Agriculture is the potential global consumer of freshwater, using about 70 % of available water resources. Due to the water scarcity problems, induced by population growth and increased demand for food and fiber, the share of freshwater for irrigation continues to compete with the domestic and industrial sectors [1]. The lack of freshwater is seeking an alternative source of irrigation for farmers. Hence, the reuse of wastewater for irrigation is an effective alternative method in arid and semi-arid regions of the world where the availability of freshwater is diminishing [2] and also provides a reliable water supply to the farmers. Higher crop productivity can be obtained from nutrient-rich municipal wastewater (organic, inorganic, suspended, and dissolved solids). All these are essential nutrients for plant growththereby, decreasing the usage of chemical fertilizers [3].

Due to the lack of a comprehensive sewage treatment and disposal infrastructure in developing countries like India, wastewater from urban settlements is disposed directly into the surface water bodies and/or to the agricultural fields without proper treatment [1]. Because of the presence of high concentrations of harmful microbes and heavy metals, the use of untreated wastewater for irrigation can have adverse effects on human health, crop, and soil fertility. Therefore, the use of municipal wastewater with proper treatment may increase the availability of water resources for irrigation to reduce health and environmental.

Conventional and advanced wastewater treatment techniques require expensive equipment, advanced technologies, and higher costs that have become severe constraints for developing countries. Hence, alternative eco-friendly, cost-effective, easily operational, and most effective treatment techniques like constructed wetlands (CWs) are suggested to treat municipal wastewater [4]. These resources mimic the natural processes and require less investment for treatment and maintenance but can remove pollutants from wastewater effectively like all other conventional treatment processes [5].

Cereals and legumes are the primary dietary sources of energy for the human population in many parts of the world. They provide significant amounts of energy, protein, amino acids, and macronutrients; hence they are included in the human diet to maintain good health [6]. Among different edible crops, cereals, and pulses (legume seeds), and fodder crops (grasses) are reported to be more tolerant towards wastewater irrigation [7].

As seed germination is considered as vital and a critical phase in the plant development; it can help to identify the right crop species that can be used and well adapted for wastewater irrigation. In this view, the experiment was conducted to assess the impact of untreated and treated municipal wastewater from constructed wetlands on seed germination, seedling performances, and nutrient uptake by Zea mays (Maize), Cicer arietinum (Chickpea), Cajanuscajan (Pigeonpea), and Vignaunguiculata (Cowpea).

II. MATERIALS AND METHODS

2.1 Sample collection and analysis

A field-scale constructed wetland (CWs) system was established at ICRISAT (The International Crops Research Institute for the Semi-Arid Tropics-Patancheru, Hyderabad, Telangana, India). Wastewater from the nearby urban residential colony was collected in the collection pond, which was later sent into the CW treatment facility for treatment.

The CWs system was planted with wetland plant species such as *Typhalati*z *folia* and *Ageratum conyzoides*(Fig 1). Untreated wastewater (UTWW) samples were collected from the inlet before treatment, and treated wastewater (TWW) samples were collected from the outlet of CWs after treatment.

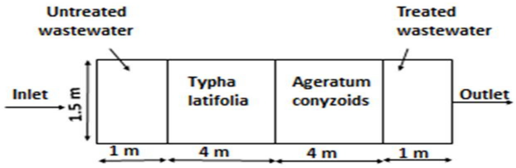

Figure 1. Top view of subsurface flow constructed wetland

Standard analytical procedures were followed for the analysis (Physical, chemical, and biological) of samples of water and wastewater [8].

2.2 Experimental procedure

The study was conducted with three types of water sources: Freshwater (CONTROL), untreated wastewater (UTWW), Treated wastewater (TWW) from CW. A completely randomized design (CRD) was framed as a template for the experiment with three replicates for each treatment. Seeds of *Zea mays* (Cultivar: HTMH 5404), *Cicer arietinum* (Cultivar: JG-11), *Cajanuscajan*(Cultivar: ICPL-88039), and *Vignaunguiculate*(Cultivar: Pusakomal)were treated with ethyl alcohol (0.1%) and washed with distilled water. All four types of certified seeds are provided by ICRISAT (The International Crops Research Institute for the Semi-Arid Tropics). Ten healthy seeds from each selected crop were placed in sterilized Petri dishes lined with whattman filter paper No.1 to maintain moisture throughout the experiment**.** Petri dishes were irrigated with 5mL of wastewater and incubated for five days at 25^{0}C. All vegetative growth parameters such as germination percentage, seedling length, seed vigor index, and seedling biomass were reported. After 5 days, seedlings were dried in an oven at 60^{0}C and then grounded into fine powder for nutrient up take (N, P, K, Mg, Ca, Na, and S) analysis. The total experiment is conducted in three consecutive cycles and the multivariate statistical analysis is applied for the total obtained results.

Seed germination parameters were calculated by using the following equations:

2.2.1 Germination percentage (G %) It is an estimation of viable seeds after 24hrs of incubation and was calculated by equation 1 [9].

$$G\% = \frac{SG}{ST} \times 100 \qquad (1)$$

Where,

SG is the number of seeds germinated; ST is the total number of seeds incubated.

2.2.2 Germination rate index (GRI) was calculated by using equation 2 [10].

$$GRI = \sum \frac{G_t}{T_t} \qquad (2)$$

Where,

G_t is the germination percentage at t^{th}day, T_t is the day of the germination test

2.2.3 The seedling length was calculated by equation 3.

Seedling length (cm) = Root length (cm) +Shoot length (cm) (3)

Plumule (shoot) length was measured from the base of the primary leaf to the base of the hypocotyl. Radicle (root) length was measured from the tip of the primary root to the base of the hypocotyl [10].

2.2.4 Seed vigor index (SVI) was calculated by using equation 4 and expressed in number (Abdul Baki and Anderson 1973)

SVI= Germination (%) X Seedling Length (4)

2.2.5 Biomass calculation (gm) was calculated by using following method.

Seedlings were dried in the oven at a constant temperature of 60 °C for 48 h to obtain their dry weights, and their dry weight was recorded by using an analytical balance [11].

2.3 Nutrient analysis procedures

The Dried seedling samples of four experimental crops ground into fine powder in a mortar and pestle to avoid chromium contamination from stainless steel. The analysis was conducted using the all the glassware was cleaned by soaking overnight in a 10% nitric acid solution and then rinsing three times with deionized water.Total N, P, K content in seedlings were determined by using the Sulfuric acid-Selenium Digestion method [12]. Digested seedling samples were analyzed for N and P using the auto-analyzer (Skalar SAN System, AA Breda, Netherlands). Potassium (K) was analyzed using atomic absorption spectrophotometer (AAS) (Savant AA, GBC Scientific Equipment, Braeside, VIC, Australia). Seedling samples were digested with nitric acid and were used for S and Na analysis by inductively coupled plasma optical emission spectrophotometer (ICP-OES) (Prodigy High Dispersion ICP, Teledyne Leeman Labs, Hudson, New Hampshire, USA; Wheal et al. 2011).

2.4 Statistical analysis

All statistical analyses were carried out using the Minitab 17 program, Version 6.1.7601. Data obtained from the seed germination bio-assay was statistically analyzed using ANOVA (Analysis of variance) as per Fisher's least significant difference test at $p < 0.05$ and found the F-ratio was significant.

III. RESULTS AND DISCUSSIONS

3.1 Municipal wastewater characterization

The Physico-chemical and biological characteristics of the water samples are represented in Table 1.

The pH of all water sources was observed to be in the neutral range. The EC of UTWW and TWW were recorded at 1.2 mS/cm and 1.1 mS/cm, respectively. Whereas, the same was obtained at 0.6 mS/cm for freshwater, as it contains fewer amounts of dissolved solids. The concentration of TDS and TSS in UTWW were obtained as1,113 mg/L and 38.6 mg/L, respectively. After treatment by constructed wetlands, the TDS and TSS of wastewater were found to be decreased to 1,029 mg/L and 28 mg/L, respectively. On the other hand, these parameters were observed in lower concentrations for freshwater (446 mg/L and 4.6 mg/L).

Table: 1. Physico-chemical and biological characteristics of water and municipal wastewater samples

Parameter	**Fresh water**	**Untreated wastewater**	**Treated wastewater**
pH	**7.44 ±0.07**	**7.46±0.17**	**7.02±0.26**
EC[a](ms/cm)	**0.63±0.06**	**1.22±0.01**	**1.10±0.01**
TDS[b](mg/L)	**445.6±3.52**	**1113±20.50**	**1029±7.33**
TSS[c] (mg/L)	**4.67±0.06**	**38.66±1.76**	**28±2.30**
COD[d](mg/L)	**29.3±2.6**	**99.22±9.69**	**54±1.15**
BOD[e] (mg/L)	**3.2±0.1**	**16.52±1.62**	**8.87±0.21**
Nitrate-N (mg/L)	**0.8±0.16**	**3.13±0.25**	**2.3±0.10**
Ammonium-N (mg/L)	**1.2±0.27**	**40.83±11.46**	**32.86±8.04**
Phosphates (mg/L)	**BDL**	**3.24±0.16**	**2.51±0.08**
Sulphates (mg/L)	**1.93±0.51**	**13.06±0.10**	**9.18±0.04**
Total Hardness(mg/L $CaCo_3$)	**134.67±17.37**	**333.66±20.16**	**291±19**
Total alkalinity(mg/L)	**94.7±4.05**	**256.66±12.77**	**252±27.49**
Chlorides(mg/L)	**47.02±5.9**	**57.33±2.66**	**51.66±6.22**
Calcium as Ca(mg/L)	**35.87±2.02**	**65.5±2.51**	**53.83±6.96**
Magnesium as Mg(mg/L)	**27.06±1.11**	**31.6±1.55**	**31.63±4.35**
Potassium as K(mg/L)	**8.39±0.63**	**14.33±1.45**	**13.69±2.78**
Sodium Na(mg/L)	**16.68±4.61**	**96.9±4.49**	**100.5±2.27**
Boron (mg/L)	**0.06±0.01**	**0.13±0.03**	**0.1±0.05**

[a] Electrical conductivity
[b] Total dissolved solids
[c] Total suspended solids
[d] Chemical oxygen demand
[e] Biochemical oxygen demand

Heavy metals were in below detectable range in all water samples.

In further analysis, the concentration of ammoniacal nitrogen and nitrate nitrogen in UTWW was recorded at 40.8 mg/L and 3.13 mg/L, but after treatment, they have decreased to 32.8 mg/L and 2.3 mg/L. In freshwater, the concentrations were found to be 1.2 mg/L and 0.8 mg/L respectively for the similar order of nitrogen forms. The phosphate concentration in UTWW and TWW was obtained at 3.24 mg/L and 2.51 mg/L, respectively, whereas it was below detection limit (BDL) in fresh water. In the studies of the organic matter content of water samples, the BOD of UTWW was obtained at 16.52 mg/L while it was determined as 8.87 mg/L and 3.2 mg/L in TWW and freshwater, respectively. The COD for UTWW was noticed as 99.22 mg/L, but after treatment, the COD value decreased to 54 mg/L. For freshwater, the COD level was analyzed as 29.3 mg/L.

In UTWW and TWW, the concentrations of chlorides were recorded as 57 mg/L and 51 mg/L, respectively. In freshwater, the chloride concentration was determined as 47.2 mg/L. The concentration of potassium in UTWW and TWW was recorded as 14.33 mg/L and 13.69 mg/L. The concentration of potassium detected in freshwater was 8.39 mg/L. In treated and untreated wastewater, boron and arsenic were found in trace amounts and heavy metals like Pb, Cr, Zn, and Fe were found BDL. In freshwater, all the micronutrients and heavy metals were also found BDL.

3.2 Seed germination percentage

Germination percentages of experimental crops observed during the experiment was presented in Fig. 2. UTWW and TWW recorded 100 % seed germination with *Zea mays* seeds, followed by 90% with Control. In *Cicer arietinum*, 100% germination was observed with TWW, 93% with Control and a minimum germination rate (83%) was obtained with UTWW. The *Cajanuscajan* seeds were germinated (100%) with TWW.

The lowest germination percentage of (86%) in *Cajanuscajan* was observed with UTWW, but it was statistically not different from Control (96%). In *Vignaunguiculata*, Control showed the highest germination percentage of 93%, followed by 86% with TWW, whereas the lowest germination percentage of 76% was recorded with UTWW.

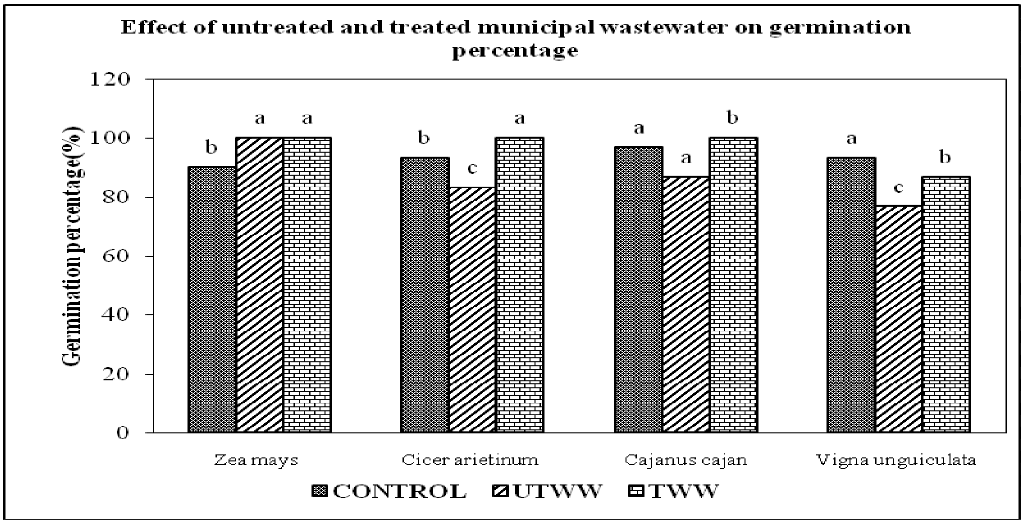

Figure 2: Effect of untreated and treated municipal wastewater on germination percentage of maize, chickpea, pigeon pea and cowpea. Means with the same letter are not significantly different at $P \leq 0.05$.

TWW showed a promoting effect on seed germination percentage in all crops. All required nutrients for plant growth may be present in it and available to plants in the form of dissolved salts and organic compounds in limited quantities. Hence, treated municipal wastewater might have enhanced seed germination and seedling growth, which is also following the results of several authors [1, 13, 14]. Lower concentrations of essential organic nutrients in untreated and treated wastewater can enhance prompting effects on seed germination by decreasing the negative impacts[1, 2, 10, 15].

Retardation in germination percentage was observed with UTWW in all crops (except in *Zea mays*). It might be due to the presence of high concentrations of solids as it can pose a toxic effect on plants [2, 10, 16]. High amounts of solids in water can change the osmotic pressure relationship between seed and water. This phenomenon may induce the seed to absorb less water, thereby the retarding germination rate [17, 18, 19].

Except for *Vignaunguiculata*, all the seeds have recorded higher germination percentage with TWW when compared to control. The minimum germination percentage in *Vignaunguiculata* seeds may be due to the saturation of seeds with water. These results show that *Vignaunguiculata* seeds were affected by both TWW and UTWW. *Zea mays*seeds were found to be more tolerant among all seeds under the present study. Huma et al. (2012) [19] inferred similar results, who found out that barley and fenugreek seeds were not affected by applying domestic wastewater, whereas fennel flower and coriander seeds were affected. They had stated that the selection of a suitable tolerant species played a vital role when wastewater was considered as the irrigation source.

Hence, in the present study, the tolerance levels of the crops to domestic wastewater can be arranged in the order as *Zea mays*>*Cicer arietinum*>*Cajanuscajan*>*Vignaunguiculata*. Similarly, Sankar et al. (2013) [13] has reported the tolerance levels of the chosen seeds with sewage water irrigation was Cicer arietinum>Pisumsativum. This pattern of analysis was also supported with the results of Mseddi et al. (2015) [20] pea> tomato with olive mill wastewater; Abu-Dieyeh et al. (2017) [21]tomato > barley > squash with distillery effluent; Salian et al. (2018) [10]Zea mays>Cajanuscajan>Cicer arietinum with brewery wastewater.

3.3 Germination rate index (GRI)

The results of GRI of all four crops were depicted in Fig.3.

In *Zea mays* seeds, the highest GRI (0.71) was observed with TWW, and it was not statistically different with UTWW (0.7), whereas the lowest GRI (0.61) was noticed with Control. In *Cicer arietinum*, the highest GRI (0.73) was noted with TWW followed by 0.68 with Control, and the lowest GRI of 0.64 was observed with UTWW. In *Cajanuscajan*, the highest value of GRI was noticed in TWW (0.78), whereas with UTWW treatment has shown the lowest value (0.71), which was statistically not significant from Control (0.76). *Vignaunguiculata* seed recorded less germination percentage compared to other type if seeds in the experiment hence less GRI values are recorded among all the other types of seeds. The highest value of GRI was observed with Control (0.71), with TWW 0.66 and the lowest value was with UTWW (0.58) respectively. The higher salt concentrations in wastewater might have contributed in the delay of seed germination and thus resulted in lesser GRI rates [2, 22, 23].

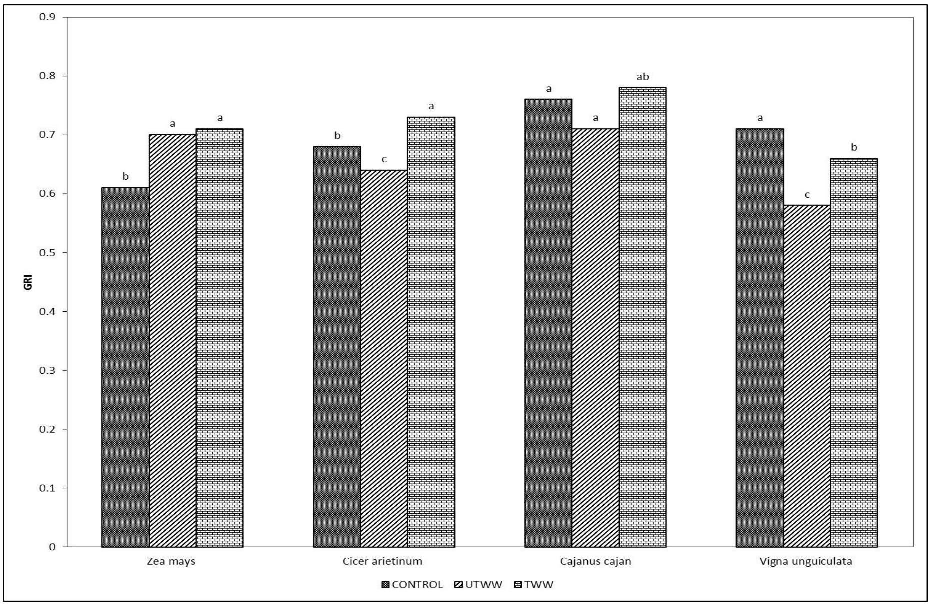

Figure 3: Effect of untreated and treated municipal wastewater on germination rate index (GRI) of maize, chickpea, pigeon pea and cowpea. Means with the same letter are not significantly different at $P \leq 0.05$.

3.4 Seedling length (cm)

Zea mays seeds measured a maximum seedling length of 16.9 cm with UTWW followed by 15.27 cm with TWW, and a minimum length of 12.13 cm was recorded with Control. In *Cicer arietinum*, the seedling length was maximum with TWW (15.9cm), which was higher than Control (6.97 cm), whereas the lowest value of 4.8 cm was recorded with UTWW. The highest value for seedling length in *Cajanuscajan* was recorded with UTWW (7.4cm) followed by TWW (6.10 cm), the least value of seedling length was recorded with Control (5.22cm). In *Vignaunguiculata*, maximum seedling lengths were recorded with TWW (8.4 cm) followed by Control (7.1 cm) while the lowest value of 3.39 cm was observed with UTWW. The results of seedling lengths of all four crops were depicted in Fig.4

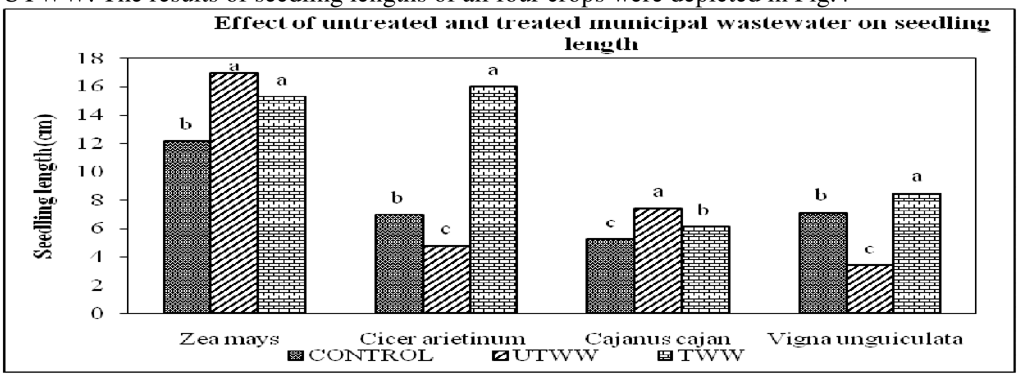

Figure 4: Effect of untreated and treated municipal wastewater on seedling length of maize, chickpea, pigeon pea and cowpea. Means with the same letter are not significantly different at $P \leq 0.05$.

TWW comprised of higher amounts of nutrients than freshwater, which was substantial for seedling growth and development [1, 14]. Hence, all crops showed significant seedling lengths with TWW when compared to Control. *Zea mays* and *Cajanuscajan*, seedling lengths were highest with UTWW than TWW. The promoting effect on seedling lengths might be due to a decrease in nutrient load and organic compounds in TWW after treatment by constructed wetlands. A similar result was encountered by Huy and Iwai (2018) [24], they concluded that municipal wastewater without dilution showed a positive effect on seed growth parameters than diluted wastewater. They found that dilution of wastewater might reduce the nutrients required for plants and hence will be less effective on seedling growth.

The present study also revealed that UTWW had a negative influence on *Cicer arietinum* and *Vignaunguiculata* seedling growth. Similar results were also stated by several authors in their studies on germination bio-assays with different types of wastewaters [3,18]. Egbuikwem.et al (2020) [25] observed that treated municipal wastewater has a promoting effect on edible crops. Still, when they were irrigated with untreated wastewater, adverse effects on seed germination and root growth and elongation were observed. Essential nutrients present in wastewater can show beneficial or adverse effects on plants that might depend on the total nutrient composition of sewage and also on the ability of plant species to tolerate those nutrient levels. Thus, *Cicer arietinum* and *Vignaunguiculata* were less tolerant to UTWW than *Zea mays* and *Cajanuscajan*; and hence lesser seedling lengths were achieved in former than latter respectively.

3.5 Seedling vigor index (SVI)

Results of the seedling vigor index calculated during the germination experiment for all four types of crops were depicted in Fig.5.The Highest SVI of 1699 was calculated in *Zea mays* seeds with UTWW followed by 1394 with TWW and 1102 with Control respectively. *Cicer arietinum* seeds imbibed with TWW showed the highest SVI (1598), followed by Control 651 and UTWW recorded lowest SVI of 398.Among *Cajanuscajan* seedlings, the highest SVI of 742 was with UTWW, 526 with TWW and the lowest SVI (503) was recorded with control seedlings. In *Vignaunguiculata*, the highest SVI of 731 was obtained with TWW followed by 664 for Control, whereas the lowest SVI of 260 was recorded with UTWW. In line with seedling lengths, SVI values were also impacted by UTWW in *Cicer arietinum* and *Vignaunguiculata*. In contrast, SVI values in *Zea mays* and *Cajanuscajan* were not affected by wastewater treatment

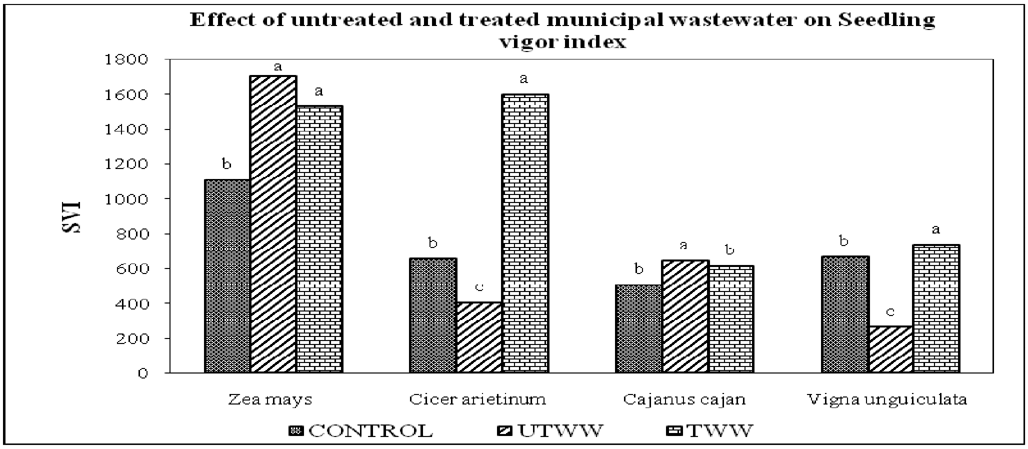

Figure 5: Effect of untreated and treated municipal wastewater on Seedling vigor index (SVI) of maize, chickpea, pigeon pea and cowpea. Means with the same letter are not significantly different at $P \leq 0.05$.

Macronutrients present in the wastewater can enhance the growth of seedlings. In addition to the macronutrients, wastewater also contains various micronutrients such as Fe, B, Mn, and Zn, which can improve crop growth and yield. Similar comparable results, with the proven positive effect of wastewater on seedling growth parameters and SVI values, were also documented by Essa et al. (2019) [26]. Untreated and treated wastewater with low levels of heavy metals doesn't show any ill effect on the plant and physiological parameters; instead, it promotes the growth [13, 15, 24].

3.6 Biomass of seedlings

Dry weights of seedlings recorded during the germination experiment are presented in Fig. 6a & 6b. TWW has contributed to gain in biomass as the highest dry weight values in *Zea mays* (3.65 gm), *Cajanuscajan*(0.87 gm), and *Vignaunguiculata* (1.51) gm. But in *Cicer arietinum*, the highest biomass was recorded with Control (2.31gm) followed by TWW (2.31 g). UTWW contributed to lower dry weights in *Zea mays* (2.58 g), and *Cicer arietinum* (1.93 g) might be due to chlorides and sodium of UTWW, which led to a decrease in their dry weights. Lafragueta et al. (2014) [22] stated that in some plant species, the embryo of the seeds is protected to external factors like salinity by seed coat. But, after seed germination and it might be stimulated by the concentration of salts present in wastewater, thereby reducing seedling growth. But in *Cajanuscajan* and *Vignaunguiculata*, these values were either higher than or equal to control; thus, showing their ability to resist untreated wastewater. Similar results of positive dry weights in earlier studies with both untreated and treated wastewater application were reported in okra plants, sunflower, mustard, and in three different varieties of tomato like Toufan, Heinz and Bouzina [14]. A positive effect on the dry weight of crops with sewage sludge application was also indicating that sewage effluent could be considered as one of the potential and alternate sources for freshwater irrigation [1,11,17].

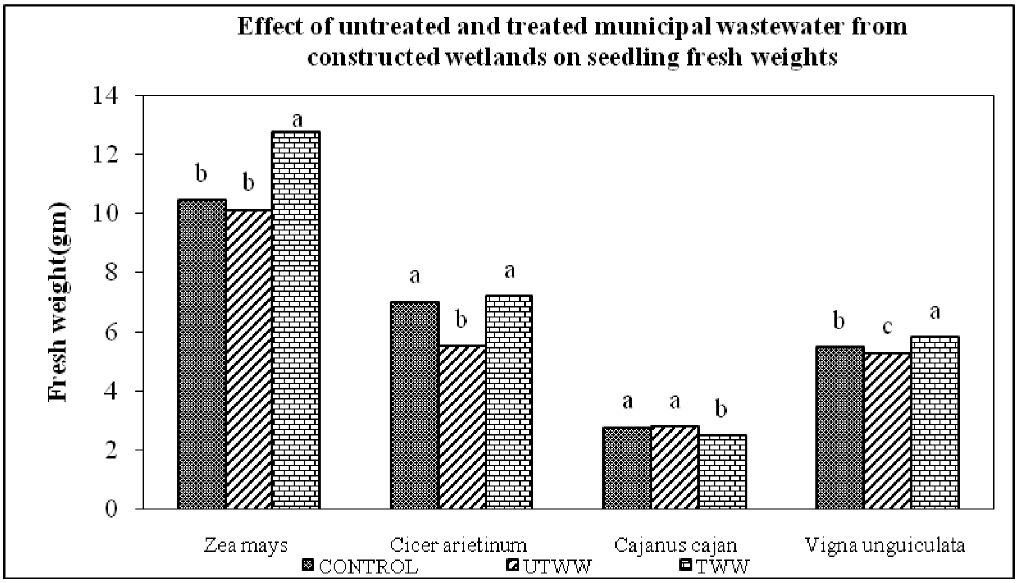

Figure 6a: Effect of untreated and treated municipal wastewater from constructed wetlands on fresh weights of maize, chickpea, pigeon and cowpea. Means with the same letter are not significantly differentat $P \leq 0.05$.

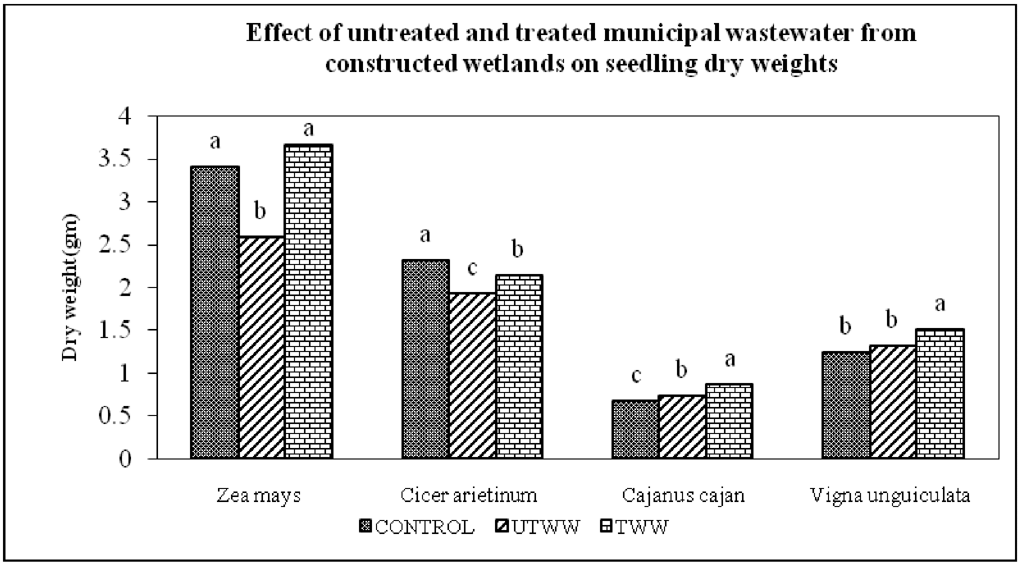

Figure 6b: Effect of untreated and treated municipal wastewater from constructed wetlands on dry weights of maize, chickpea, pigeon and cowpea. Means with the same letter are not significantly different at $P \leq 0.05$.

3.7 Nutrient uptake by seedlings

Municipal wastewater contains high levels of nutrients which can contribute to plant growth but the nutrients present in the wastewater are essential for plant growth only up to a certain threshold, beyond which they become hazardous to the crops [26].

The statistical analysis was performed to the results obtained during the study, the nutrient uptake concentrations of the four seedlings with the control, untreated wastewater (UTWW) and Treated wastewater (TWW) sources were furnished in the Tables 2, 3 and 4 respectively.

The data was presented as, the range of the nutrient uptake (minimum and maximum), the mean with standard deviation, Skewness and kurtosis. Skewness is a measure of the asymmetry and kurtosis is a measure of 'peakedness' of a distribution. In the present study both skewness and kurtosis found statistically significant.

The macro nutrientslike N, P, K, Ca and Mg uptake in seedlings observedis the Nitrogenuptake found higher in *Cicer arietinum* using TWW and lower levels in *Cajanuscajan* using control water. Phosphorus uptake was found higher in *Zea mays* using TWW and the lower levels in *Cajanuscajan* using control water. Potassium uptake was found higher in *Vignaunguiculata* using TWW and lower levels in *Cajanuscajan* using control water. Calcium uptake was found higher in *Cicer arietinum* using UTWW and the lower levels in *Zea mays* using control water. Magnesium uptake was found higher in *Zea mays* using TWW and lower levels in *Cajanuscajan* using control water. In the above all the four seedlings, the higher nutrient uptake was observed in TWW compared to other two waters. In the other hand the nutrients like Na, S and Boron were followed the same trend.In this present study the nutrient uptake using the seedlings with TWW was showing significant results comparing with other waters.

Table: 2: Statistical analysis for Nutrient uptake of four seedlings in Control water

Statistical analysis for Nutrient uptake of four seedlings in Control water									
		ca	mg	Na	P	N	K	S	B
maize (*Zea mays*)	**Mean**	1.19	4.86	1.97	1.14	56.48	19.43	4.59	0.03
	SD	0.05	0.02	0.02	0.02	0.40	0.15	0.25	0.01
	Min	1.14	4.84	1.94	1.12	55.98	19.20	4.32	0.02
	Max	1.24	4.89	1.99	1.16	56.82	19.58	4.85	0.05
	Skewness	0.16	1.29	-0.96	-0.60	-0.61	-1.03	0.10	0.53
	Kurtosis	-2.56	2.35	-0.58	0.37	-2.98	1.05	-2.86	-1.49
Chickpea (*Cicer arietinum*)		ca	mg	Na	P	N	K	S	B
	Mean	6.57	4.50	2.05	0.80	75.56	24.40	5.48	0.03
	SD	0.18	0.23	0.02	0.04	0.85	0.39	0.17	0.01
	Min	6.42	4.28	2.02	0.76	74.52	23.98	5.02	0.02
	Max	6.85	4.86	2.08	0.85	76.58	24.88	5.42	0.04
	Skewness	1.13	1.10	0.18	0.73	-0.20	-0.08	-0.52	0.60
	Kurtosis	-0.14	0.85	-0.53	-0.93	-1.93	-2.17	-1.90	-3.32
Pigeonpea (*Cajanuscajan*)		ca	mg	Na	P	N	K	S	B
	Mean	1.59	1.26	0.52	0.21	22.38	10.87	1.16	0.07
	SD	0.01	0.01	0.02	0.01	0.08	0.25	0.03	0.01
	Min	1.58	1.24	0.50	0.20	22.28	10.64	1.12	0.06
	Max	1.59	1.28	0.54	0.22	22.47	11.24	1.20	0.07
	Skewness	-0.48	-0.32	0.00	0.59	-0.14	0.66	0.17	-0.61
	Kurtosis	-3.11	1.70	-3.00	-3.31	-2.11	-0.40	-0.38	-3.33
Cowpea (*Vignaunguiculata*)		ca	mg	Na	P	N	K	S	B
	Mean	1.96	3.45	1.11	0.63	55.52	19.45	3.16	0.02
	SD	0.02	0.02	0.01	0.02	0.04	0.02	0.01	0.01
	Min	1.92	3.43	1.10	0.62	55.48	19.43	3.14	0.02
	Max	1.98	3.48	1.12	0.66	55.58	19.48	3.18	0.04
	Skewness	-1.24	0.45	-0.59	1.26	1.01	0.07	0.12	2.24
	Kurtosis	1.60	0.67	-0.39	0.33	1.61	-1.82	1.96	5.00

Cicer arietinum, *Cajanuscajan*, and *Vignaunguiculata* seedlings have absorbed maximum amounts of N & K compared to Zea *mays*. In *Cicer arietinum* seedlings, the highest amount of N (79.98 mg), K (25.12 mg) and in *Zea mays* (1.29 mg) uptake was identified using TWW. Zea mays seedlings irrigated with TWW were accumulated with more significant amount of P than other three types of seedlings.

Table: 3: Statistical analysis for Nutrient uptake of four seedlings in Untreated Wastewater

Statistical analysis for Nutrient uptake of four seedlings in Untreated Wastewater									
		ca	mg	Na	P	N	K	S	B
maize (*Zea mays*)	**Mean**	1.68	4.02	2.31	0.95	45.47	15.45	3.43	0.05
	SD	0.01	0.01	0.06	0.02	0.15	0.10	0.03	0.02
	Min	1.76	4.02	2.22	0.92	45.28	15.35	3.40	0.03
	Max	1.79	4.04	2.38	0.98	45.68	15.58	3.48	0.08
	Skewness	-1.43	2.21	-0.84	0.62	0.36	0.26	0.91	0.59
	Kurtosis	2.21	4.91	2.29	1.71	0.16	-2.61	-0.20	-0.02
Chickpea (*Cicer arietinum*)		ca	mg	Na	P	N	K	S	B
	Mean	6.86	4.26	2.32	0.67	68.37	21.28	4.50	0.04
	SD	0.02	0.04	0.04	0.02	0.42	0.30	0.17	0.01
	Min	6.84	4.22	2.26	0.64	67.89	20.98	4.23	0.02
	Max	6.89	4.32	2.36	0.69	68.98	21.69	4.69	0.06
	Skewness	0.61	0.07	-0.82	-0.03	0.45	0.43	-1.06	1.25
	Kurtosis	-2.58	-1.83	2.37	-0.42	-0.23	-1.74	1.70	1.62
Pigeon pea		ca	mg	Na	P	N	K	S	B

(*Cajanuscajan*)	Mean	2.16	1.51	0.61	0.23	25.25	12.30	1.40	0.07
	SD	0.04	0.04	0.04	0.04	0.30	0.17	0.17	0.02
	Min	2.12	1.46	0.56	0.18	24.92	12.14	1.28	0.04
	Max	2.22	1.56	0.66	0.28	25.64	12.56	1.64	0.09
	Skewness	0.94	0.23	-0.02	-0.01	0.12	0.97	0.88	-0.11
	Kurtosis	-0.67	-0.49	-1.89	1.26	-1.62	0.50	-1.69	-1.21
Cowpea (*Vignaunguiculata*)		ca	mg	Na	P	N	K	S	B
	Mean	2.67	3.89	1.78	0.72	58.96	22.81	3.53	0.02
	SD	0.02	0.13	0.08	0.07	0.81	0.52	0.08	0.01
	Min	2.64	3.68	1.68	0.63	57.58	22.33	3.44	0.02
	Max	2.69	3.99	1.88	0.82	59.63	23.62	3.64	0.04
	Skewness	-0.83	-1.27	0.02	0.42	-1.74	1.10	0.64	2.24
	Kurtosis	-0.87	1.22	-2.18	0.46	3.20	0.47	-1.30	5.00

Traces of heavy metals were not detected in seedlings due to BDL levels in the water sources used for the study.A related study on *sorghum* and *sunflower* seeds carried out by Ahmed (2009) [27] showed that *sorghum* seeds were able to accumulate higher concentrations of Mn and Zn than sunflower seeds. On the other hand, Sunflower seeds absorbed a significantly higher concentration of Cr when compared to the s*orghum* crop.

The uptake of Ca, Mg, and Na in the seedlings has increased when they were irrigated with TWW. Seed germination was delayed due to high SAR values for both UTWW and TWW, but after germination, seedlings showed good progress in seedling growth than Control. Similar studies showing that the micronutrients such as Fe, Zn, B, & S had improved crop yield when *canola* seedlings were incubated with treated municipal wastewater. Irrigation of Maize crop with UASB treated textile water shown positive results on the growth and yield of the crop [28].Elevated levels of Fe, Mn, and Zn were detected in vegetables [29] when they are irrigated with treated wastewater and also their positive impact on seedling development.

Table: 4. Statistical analysis for Nutrient uptake of four seedlings in Treated Wastewater

Statistical analysis for Nutrient uptake of four seedlings in Treated Wastewater									
maize (*Zea mays*)		ca	mg	Na	P	N	K	S	B
	Mean	1.78	5.16	2.18	1.29	56.22	19.43	4.29	0.04
	SD	0.11	0.28	0.05	0.12	0.57	0.41	0.26	0.02
	Min	1.56	4.84	2.12	1.14	55.42	18.96	3.98	0.02
	Max	1.82	5.48	2.24	1.48	57.02	19.84	4.58	0.06
	Skewness	0.20	0.19	-0.21	0.57	0.01	-0.45	-0.16	-0.65
	Kurtosis	-2.54	-2.47	-1.24	1.22	1.97	-2.96	-2.27	-0.21
Chickpea (*Cicer arietinum*)		ca	mg	Na	P	N	K	S	B
	Mean	5.53	4.15	2.47	0.79	79.35	24.62	6.57	0.02
	SD	0.28	0.02	0.18	0.12	0.61	0.37	0.19	0.01
	Min	5.24	4.12	2.21	0.68	78.51	24.16	5.20	0.02
	Max	5.98	4.18	2.68	0.98	79.98	25.12	5.68	0.04
	Skewness	1.17	0.59	-0.57	1.53	-0.47	0.21	-0.85	2.24
	Kurtosis	1.71	-0.83	-0.17	2.68	-1.40	-0.40	0.61	5.00

		ca	mg	Na	P	N	K	S	B
Pigeon pea (*Cajanuscajan*)	**Mean**	**2.61**	**1.79**	**0.74**	**0.25**	**30.05**	**14.72**	**1.52**	**0.10**
	SD	**0.24**	**0.18**	**0.06**	**0.02**	**0.15**	**0.13**	**0.10**	**0.02**
	Min	**2.38**	**1.58**	**0.68**	**0.22**	**29.84**	**14.58**	**1.38**	**0.08**
	Max	**2.98**	**1.98**	**0.82**	**0.28**	**30.22**	**14.89**	**1.66**	**0.12**
	Skewness	**1.22**	**-0.13**	**0.18**	**-0.52**	**-0.56**	**0.52**	**0.16**	**-0.05**
	Kurtosis	**1.19**	**-2.59**	**-1.54**	**-0.50**	**-0.04**	**-1.84**	**0.91**	**-2.33**
Cowpea (*Vignaunguiculata*)		**ca**	**mg**	**Na**	**P**	**N**	**K**	**S**	**B**
	Mean	**2.98**	**4.26**	**1.77**	**0.63**	**66.04**	**25.15**	**3.60**	**0.03**
	SD	**0.12**	**0.25**	**0.13**	**0.10**	**0.12**	**0.19**	**0.36**	**0.02**
	Min	**2.86**	**4.02**	**1.56**	**0.52**	**65.84**	**24.98**	**3.12**	**0.02**
	Max	**3.16**	**4.68**	**1.88**	**0.78**	**66.14**	**25.46**	**3.98**	**0.06**
	Skewness	**0.64**	**1.52**	**-1.16**	**0.73**	**-1.53**	**1.43**	**-0.50**	**1.91**
	Kurtosis	**-0.24**	**2.60**	**0.58**	**0.74**	**2.26**	**2.22**	**-1.63**	**3.75**

Long term irrigation of wastewater can increase soil nitrogen levels because domestic wastewater is rich source of urea and nitrogen.Urea is also an alternate source of N which other hand decreases the use of chemical fertilizers, thereby abating the ecological and economic concerns. The uptake of all types of nutrients was higher in all fourexperimental crops treated with UTWW than control and TWW,which might be due to the presence of higher concentrations of nutrients in UTWW. Application of such nutrient rich UTWW might have influenced the physiological process during germination and growth of seedlings, thus leading to an upsurge in the seedling growth than TWW [30]. Contrary, the excess nutrient content in wastewater can be toxic to seed germination and seedling growth, resulting in adverse effects on plant growth and health [17].

UTWW might be beneficial in the initial stages of plant growth, but in later stages, it may cause toxicity leading to lesser growth and finally to lower possible yields. Hence it was recommended to treat wastewater before its use for irrigation [31]. Detrimental impact on seed germination of *Triticumaestivum* seeds due to accumulation of Cu, Zn, and in *tomato* by higher levels of Cr with the domestic effluent has been reported [10].

Dilution of wastewater must be considered even after treatment, to avert adverse effects on soil properties and plant growth [10]. Thenutrients in wastewater act as stimulants in lower concentrations and might be toxic at higher levels. Various other authors also suggested for the dilution of wastewater for irrigational purposes [7, 26].

Seed germination plays a crucial role in determining the later stages of seed development, such as the emergence of seedlings, overall growth, and survival of crops. It thus helps to control the dynamics of the plant population [14]. Seed germination experiments provide fast, cost-effective, and appropriate acute phytotoxicity bio-assay, offering a broad range of benefits such as sensitivity, suitability tests at the initial stages of crop growth using unstable chemicals, water, or wastewater samples [7]. Hence, the selection of suitable crop species for wastewater applications can be selected with this simple germination test. Based on the bio-assay results of the present study, crops tolerant to municipal wastewater can be arranged as *Zea mays>Cajanus cajan>Cicer arietinum>Vigna unguiculata*.

IV. CONCLUSIONS AND RECOMMENDATIONS:

At the outset, TWW from constructed wetlands had a promoting impact on seed germination rate, seedling parameters, and nutrient absorption. *Zea mays*, *Cicer arietinum*, and *Cajanus cajan*seeds exhibited good results when they were imbibed with both UTWW and TWW. Whereas in *Vigna unguiculata*, untreated and treated municipal wastewater inhibited germination percentage, and it might be due to salt stress (accumulation of nutrients). However, after germination, they recorded maximum seedling lengths by absorbing nutrients from TWW. While UTWW has also shown promoting effects on seedling growth of all crops, it cannot be recommended for long-term use, as it could have negative impacts on the environment. Thus, TWW can be used for irrigation to achieve more enriching benefits for agriculture. From wastewater analysis results, it can be suggested that constructed wetlands can treat wastewater effectively like other conventional treatment methods. However, promising wastewater treatment techniques like constructed wetlands are highly recommended, which usually requires minimal inputs for installation, operation, and maintenance. This could also mitigate the detrimental impacts of wastewater on the environment. Hence, the present study highlights the reuse of treated wastewater for irrigation.

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