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Effect of Reverse Osmosis Wastewater on Seed Germination and seedling performance of four Different Crops

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Abstract: Wastewater reclamation and its reuse for beneficial purposes is a common goal of many countries particularly in water stressed countries around the world. Cutting edge technologies such as reverse osmosis (RO), micro- and ultra-filtration are often used to purify underground water and wastewater generated from the industries. Wastewater is generated during the process of water purification with RO. However, the wastewater from these processes is usually laden with concentrated nutrients, salts, and other materials. Using RO rejection water as irrigation water for agriculture is a viable alternative. Hence, a study was conducted to assess the suitability of five different level of diluted RO wastewater along with control treatment (100% pure water) on seed germination and seedling performance of Amaranthus, cabbage, green gram and maize. This study was carried out in the laboratory with completely randomized design comprising three replicates. Purified water was used as control and used to prepare dilution levels of 20%, 40%, 60% and 80% along with RO wastewater. Seed germination percentage, germination time, shoot length, root length and fresh weight were recorded seven days after sowing and analyzed statistically. Significant differences ($p<0.05$) were found among treatments on germination percentage, germination time, root length, shoot length and fresh weight. Germination percentage decreased with the increasing percentage of RO rejected wastewater, while other parameters have shown slight fluctuations. Amaranthus and maize performed well with the combination of 40% wastewater and 60% purified water. Meanwhile, combination of 20% wastewater and 80% purified water showed positive effect on the germination of green gram (96.67%) and maize (100%). Furthermore, seven water quality parameters have shown a linear relationship with the fresh weight of sprouts. The results indicated that there is a possibility to use diluted reverse osmosis wastewater as source of irrigation water. Further, field studies needed to recommend the usage of RO rejected wastewater as part of irrigation water.

Keywords: Germination, Irrigation, Reverse osmosis, Wastewater

Introduction

A scarcity of fresh water limits sustainable agriculture development worldwide (Wang *et al.,* 2017). It is estimated that about 2/3 of world population will face a water deficiency at medium or high level by the year 2025.World total water resources is 1.4 billion m^3 and 1% of the water can only be used as drinking water (Bozdogan, 2015). Groundwater is the major source of water especially in rural areas, and it is estimated that about 72% of the rural

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population relies on groundwater for domestic use (Central bank report, 2010).

Eco-soft reverse osmosis system can be used to demineralize low to medium salinity water. Usually Eco-soft reverse osmosis plant is desalinated 10000 (± 10%) L/day. Reverse osmosis (RO) plants reject 15 - 20% of the feed water as waste of concentrated saline streams which considered as major environmental and economic drawback of the RO process (Ghamdi, 2016). Various alternative options used for the treatment and disposal of generated concentrate including deep well injection, evaporation ponds, disposal into surface water bodies, disposal through pipelines to municipal sewer systems, ion exchange procedures, shrimp breeding and hydroponic cultivation of salt tolerant plants (Qurie *et al.,* 2013).

Wastewater repossession and its reuse for beneficial purposes is a common aim of many countries particularly in water stressed countries around the globe. Cutting edge technologies such as RO, micro and ultra-filtration are often used to filter wastewater effluent generated in traditional systems. However, the rejected wastewater from these processes is usually laden with concentrated nutrients, salts, inorganic materials while it is rich in organic materials those are removed in the course of treatment (Shahalam *et al.,* 2010).

Using RO rejection water as irrigation water for agriculture is a viable alternative. In the present study RO rejected wastewater from an Eco-soft reverse osmosis plant was used as irrigation source with several dilutions with pure water to assess the suitability of Reverse Osmosis rejection water and its different dilution levels of water as source of irrigation for different crops such as Amaranthus, cabbage, green gram and maize.

Materials and Methods

Sample collection and preparation

The feed water was taken from the ground well, which is located in the Kilinochchi premises of the University of Jaffna. Reverse osmosis desalination (RO) plant is located in the Faculty of Agriculture, University of Jaffna, Kilinochchi premises. The RO plant consists of two membranes for desalination with a capacity of 6000 (±10 %) L/ day.Electric conductivity of the feed water is about 3.25 dS/m.

The different concentrations (20%, 40%, 60% and 80%) of wastewater (Table 1) were prepared by mixing reverse osmosis rejected water with pure water. Reverse osmosis wastewater (100%) and its dilutions (20%, 40%, 60% and 80%) were analyzed for electrical conductivity (EC), sodium (Na^+), potassium (K^+), hardness (calcium plus magnesium), alkalinity, phosphate and sulphate in triplicate.

Table 1: Treatments and different concentrations of RO wastewater

Treatment	Concentrations of RO wastewater
T_1 (control)	0%
T_2	20%
T_3	40%
T_4	60%
T_5	80%
T_6	100%

Germination tests

Germination test was carried out in laboratory conditions with three replicate and Completely Randomized Design. Seeds were surface-sterilized with 5% sodium hypochlorite ($NaOCl$) (Sauer and Burroughs, 1986) and washed thoroughly using distilled water. All petridishes were washed by tap water, followed by rinsing with distilled water. Hot air sterilizer was used for sterilization at 170^{o}C in 4 hours (Muhammad and Hussain, 2010).

Ten seeds were put in each petridish on one- layer filter paper. Initially, the seeds were immersed with 5 mL of appropriate solution in petri dish. Then, 1 mL of prepared solution was added to each Petri dish every day to provide moisture for the germination and was monitored daily.

Data collection

When the radical was two mm in length, it was considered as germinated seed (Panuccio *et al.,* 2014). Data were recorded at seven days after seed placement. The germination percentage, mean germination time, root length, shoot length and fresh weight of the germinated seeds were measured.

Statistical Analysis

The collected data were subjected to analysis of variance. Dunnett's test was used to compare mean at 5% probability level.

Moreover, correlation and regression analysis were performed to find the strength and relationship between the water quality parameters and seed germination and seedling performance.

Results and Discussions

Impact of different concentration of RO wastewater on seed germination and seedling performance

The highest germination percentage (80%), shoot length (2.16 cm), root length (1.25 cm) and fresh weight (0.06 g) were recorded in the (T_1) control treatment where the sole pure water was used as the irrigation source. Despite, the lowest mean germination time (2.33) was noted in the T_1. Amaranthus has shown significant difference ($p<0.05$) on germination percentage, shoot length, mean germination time and fresh weight.

However, there was no any significant ($p<0.05$) difference was recorded on the shoot length and fresh weight of sprout up to the combination of 40% wastewater and 60% pure water (T_3). These results indicated that 60% and 40% pure and RO wastewater combination were performed well for Amaranthus. This might be due to the undisturbed physiological activities of the seeds. Therefore that level could be used for irrigation without any impact on growth of germinated seed.

Salinity stress had a significant impact on the growth of different Amaranthus cultivars, where the yield was reduced from 17.8% to 25.2% for A0020 cultivar; from 6.9% to 28.3% for A0057 cultivar, from 3.6% to 8.6% for A211cultivar respectively for 3 dS/m and 6 dS/m compared to the control (0.92 dS/m). While, the same wastewater had been used since beginning of study, which ensures the suitability of diluted wastewater irrigation for the seed germination and seedling growth of Amaranthus (El Youssfi *et al.,* 2012)

Table 2: The effect of different dilution levels of RO wastewater on seed germination of Amaranthus.

Treatment	Germination %	Mean germination time	Root length (cm)	Shoot length (cm)	Fresh weight(g)
T_1 (100% control)	80	2.33	1.25	2.16	0.06
T_2 (80% pure:20% RO)	70	3.45	0.72	1.61	0.06
T_3 (60% pure:40% RO)	70	3.41	0.79	0.96	0.05
T_4 (40% pure:60% RO)	66.67	3.45	0.81	0.81*	0.04*
T_5(20% pure:80% RO)	53.33	3.85*	0.93	0.73*	0.04*
T_6 (100% RO)	40*	5.36*	1.18	0.39*	0.03*
F test	**	**	NS	**	**

*Dunnett's test significant at α = 0.05 level is indicated by *; F test significant at α = 0.05 level is indicated by **; NS- Not significant*

Table 3: The effect of different dilution levels of RO wastewater on seed germination of Cabbage

Treatment	Germination %	Mean germination time	Root length (cm)	Shoot length (cm)	Fresh weight(g)
T_1 (100% control)	83.33	2.68	10.35	3.45	1.77
T_2 (80% pure:20% RO)	73.33	3.05	9.19	2.83	1.06
T_3 (60% pure:40% RO)	73.33	3.53*	8.32*	2.53	1.08
T_4 (40% pure:60% RO)	66.67	3.79*	8.58*	2.85	0.94*
T_5 (20% pure:80% RO)	66.67	4.30*	7.52*	2.05	0.97*
T_6 (100% RO)	66.67	4.13*	7.33*	1.47*	0.78*
F test	NS	**	**	**	**

*Dunnett's test significant at α = 0.05 level is indicated by *; F test significant at α = 0.05 level is indicated by **; NS- Not significant*

Table 3 shows the germination parameters of cabbage. The significant difference ($p<0.05$) was observed for all the parameter except germination percentage. The highest germination percentage (83.33%), shoot length (10.35 cm), root length (3.45 cm) and fresh weight (1.77 g) were recorded in the T_1. However, there was no any significant difference ($p<0.05$) observed up to combination of 80% and 20% of pure water (T_2) and RO wastewater respectively for all the parameters. Kiziloglu1 *et al.* (2007) stated that cabbage could perform well under wastewater irrigated field (3510 ± 54.2 kg ha^{-1}) than non-wastewater irrigated land (2780 ± 42.1 kg ha^{-1}). If cabbage can survive with the wastewater application as irrigation source at the germination and seedling stage, it would provide beneficial agronomic and yield

Table 4: The effect of different dilution levels of RO wastewater on seed germination of green gram.

Treatment	Germination %	Mean germination time	Root length (cm)	Shoot length (cm)	Fresh weight(g)
T_1 (100% control)	100	1.03	6.78	16.48	3.97
T_2 (80% pure:20% RO)	96.67	1.07	5.7	15.16	3.87
T_3 (60% pure:40% RO)	83.33	1.2	4.52	9.66*	3.73*
T_4 (40% pure:60% RO)	80	1.62*	5.28	8.33*	3.23*
T_5 (20% pure:80% RO)	80	1.62*	5.62	7.57*	3.23*
T_6 (100% RO)	80	3.14*	3.37*	5.81*	3.43*
F test	NS	**	**	**	**

*Dunnett's test significant at α = 0.05 level is indicated by *; F test significant at α = 0.05 level is indicated by **; NS- Not significant*

attributes at the reproductive and harvesting stage. Hence, application of diluted wastewater could enhance the plant growth, reduce the requirement of fertilizer application and enhance the productivity of marginal soil.

The significant difference ($p<0.05$) was observed in germination parameter of green gram (Table 4). The lowest performance was recorded in the T_6, where the 100% of wastewater was used as the treatment that inhibits the physiological process of the green gram. Moreover, there was no any significant difference ($p<0.05$) observed up to combination of 80% pure water and 20% RO wastewater (T_2) for all the parameters. In this treatment, the germination percentage of maize seeds and shoot length of sprouts have recorded as 96.67% and 15.16 cm, respectively.

Similar results have reported from the previous studies, where the germination percentage was dropped due to the raising concentration of sugar mill effluent. Higher amount of solids found in the effluent leads to manipulations in osmotic relationship of seed and water, which leads to reduction in absorption of water by the seed. Furthermore, this can be altered by the effluent salinity as well (Baskaran *et al.,* 2009). Moreover, Divya *et al.* (2015) reported that the maximum germination percentage and seedling performance could be achieved at lesser dilutions of 10–30% for the wastewater.

The germination parameters of maize is given in table 5. The significant difference ($p<0.05$ was observed for all the parameter except germination percentage in the germination study of maize. The highest germination percentage 100%), shoot length (16.7 cm), root length (9.94 cm) and fresh weight (11.31 g) were recorded in the treatment 1.

However, there was no any significant difference ($p<0.05$) observed up to

Table 5: The effect of different dilution levels of RO wastewater on seed germination of maize.

Treatment	Germination %	Mean germination time	Root length (cm)	Shoot length (cm)	Fresh weight(g)
T_1 (100% control)	100	1.77	16.7	9.94	11.31
T_2 (80% pure:20% RO)	100	2.07	15.32	9	10.54
T_3 (60% pure:40% RO)	100	2.87	14.21	8.24	9.51
T_4 (40% pure:60% RO)	100	3.17*	10.77*	6.02*	10.5
T_5 (20% pure:80% RO)	100	3.27*	7.84*	4.23*	9.5
T_6 (100% RO)	96.67	3.37*	11.80*	3.97*	8.64*
F Test	NS	**	**	**	**

combination of 60% and 40% of pure water and RO wastewater respectively for all the parameters. Comparison of corn yields obtained from plots irrigated with effluent (1.46 dS/m) and fresh water (0.51 dS/m) demonstrated that the high quantity of corn gained from the plot which was irrigated by the effluent. This could be due to the availability plant nutrient and dormancy breaking substances ability in the source of irrigation. (Hassanli *et al*., 2009)

Measures of relationships between water quality parameters and seed germination and seeding performance

Table 6 shows the chemical quality of the different combination of the RO wastewater and the pure water. Electric conductivity was increased with increasing percentage of reverse osmosis rejected water. Electric conductivity of control treatment and 100 % of RO rejected water were recorded as 0.38 dS/m and 11.34 dS/m, respectively. Total sodium, potassium, phosphate and sulphate were increased with increasing concentration of RO wastewater which may be due to presence of salt ions in the water. Water hardness and alkalinity were increased with increasing RO wastewater amount due to presence of calcium, magnesium and carbonates in the water.

To study the patterns of the relationships between the water quality variables and germination and seedling performance of Amaranthus, cabbage, maize, and green gram, the Pearson's correlation

Table 6: Water quality parameters of different treatments

Treatment	EC (dS/m)	Na^+ (ppm)	K^+ (ppm)	Hardness (ppm)	Alkalinity (ppm)	PO_4^{3-} (ppm)	SO_4^{2-} (ppm)
T_1	0.38	51.24	22.52	55	0	3	1
T_2	2.91	153.73	23.51	850	84	9.57	38
T_3	5.13	237.31	24.01	1500	132	11.57	59
T_4	7.37	316.92	24.5	1950	184	12.71	90
T_5	9.34	355.72	24.75	2075	246	13.86	127
T_6	11.34	419.4	25.25	2550	384	14.43	161

coefficient (r) was used. The correlation coefficient (Table 7) shows the linear relationship between initial growth of germinated seeds and seven water quality parameters. No significant relationships ($p<0.05$) were seen between root length of Amaranthus and water quality parameter except concentration of PO_4^{3-}, while this was shown weak negative correlations, respectively. Root length of cabbage has shown strong but negative correlation with EC, hardness, alkalinity and concentration of cation and anion of RO wastewater.

Furthermore, EC and concentration of anions and cations of wastewater have moderately and negatively correlated with root length of green gram, while strong negative relationships were recorded in between the hardness and alkalinity of the water with root length of green gram. There were strong negative relationships were found in between the root length of maize and EC, Na^+ and K^+ concentration, hardness and concentration of SO_4^{2-} of the irrigated wastewater. Whereas PO_4^{3-} concentration and alkalinity moderately but negatively related with root length of maize. Strong positive relationships were observed in between the quality of the irrigation water and time taken for the seed germination of four different crops.

However, average germination time of green gram seeds has given moderately positive relationship with PO_4^{3-} concentration of water. There was significant relationship observed at 0.05 levels of significance of water quality variables and shoot length of seeds of cabbage, maize, Amaranthus and green gram, which could be recorded with strong negative relationship. The fresh weight of the germinated seeds of four crops were strongly but negatively correlated with water quality parameters. Meanwhile, Zhao *et al.* (2014) stated that the concentration of Na^+ had significantly impact on the germination of *Sorghum bicolor* (L.).

Influence of the water quality on fresh weight of sprout

Electrical Conductivity (EC):

Fresh weight of sprouts of Amaranthus, cabbage, maize and green gram have slightly decreased with increasing EC. The significance ($p<0.05$) of EC on fresh weight of sprouts of all the crops have illustrated in figure 1, while the maize and cabbage have exhibited the highest and the lowest sensitivity to the increasing EC of irrigation water, respectively. The principal effect of increasing EC of irrigation water on crop productivity is the incapability of the plant to compete with ions in the soil solution for water. The negative effect of EC on germination was reported by many authors from several plant species (Cavalcante *et al.,* 2005; Mostafa *et al.,* 2012).

Salt stress:

The negative effect of Na^+ and K^+ are given in figure 2 and figure 3, respectively. Salinity can enhance the osmotic pressure, which disrupts the absorption of water at the completion of cell division and differentiation, and length of plumule and radicle could be decreased due to the rising Na^+ and K^+ concentration of irrigation water (Eskandari

Table 7: Correlation coefficient between water quality parameters and seed germination and seedling parameters

Parameter	*Amaranthus*				Cabbage				Green gram				Maize			
	Mean germination time	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Mean germination time	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Mean germination time	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Mean germination time	Root length (cm)	Shoot length (cm)	Fresh weight (g)
EC (dS/m)	0.8844	0.0301	-0.9639	-0.9684	0.9678	-0.9549	-0.9148	-0.8493	0.8346	-0.7634	-0.9636	-0.8554	0.9543	-0.8343	-0.9787	-0.8606
Na^+ (ppm)	0.8673	-0.0525	-0.9822	-0.9506	0.9693	-0.9568	-0.8913	-0.8820	0.8017	-0.7754	-0.9750	-0.8723	0.9721	-0.8372	-0.9633	-0.8498
K^+ (ppm)	0.8836	-0.1200	-0.9855	-0.9384	0.9579	-0.9647	-0.8992	-0.9205	0.7875	-0.7999	-0.9621	-0.8476	0.9614	-0.8144	-0.9427	-0.8625
Hardness (ppm)	0.8608	-0.1170	-0.9938	-0.9277	0.9584	-0.9578	-0.8793	-0.9066	0.7780	-0.8043	-0.9781	-0.8588	0.9773	-0.8135	-0.9356	-0.8554
Alkalinity (ppm)	0.9556	0.1404	-0.9234	-0.9848	0.8978	-0.9240	-0.9531	-0.8241	0.9227	-0.8298	-0.9135	-0.7397	0.8846	-0.7110	-0.9409	-0.8926
PO_4^{3-} (ppm)	0.8085	-0.3607	-0.9637	-0.8270	0.9261	-0.9543	-0.8389	-0.9685	0.6265	-0.7619	-0.9112	-0.8129	0.9295	-0.7999	-0.8576	-0.8286
SO_4^{2-} (ppm)	0.9103	0.0866	-0.9377	-0.9806	0.9498	-0.9458	-0.9359	-0.8316	0.8651	-0.7587	-0.9353	-0.8199	0.9201	-0.8102	-0.9792	-0.8668

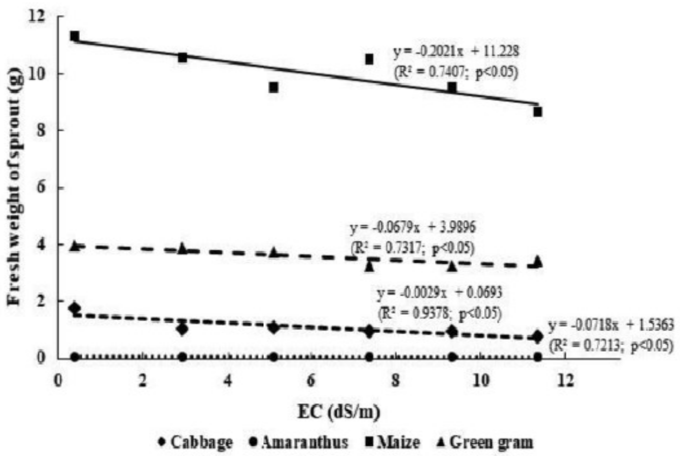

Figure 1. Fresh weight of sprout as affected by the EC of water

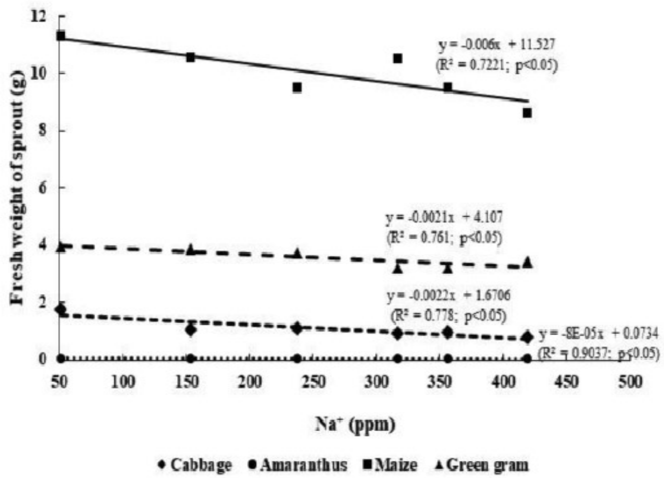

Figure 2. Relationship between fresh weight of sprout and Na^+

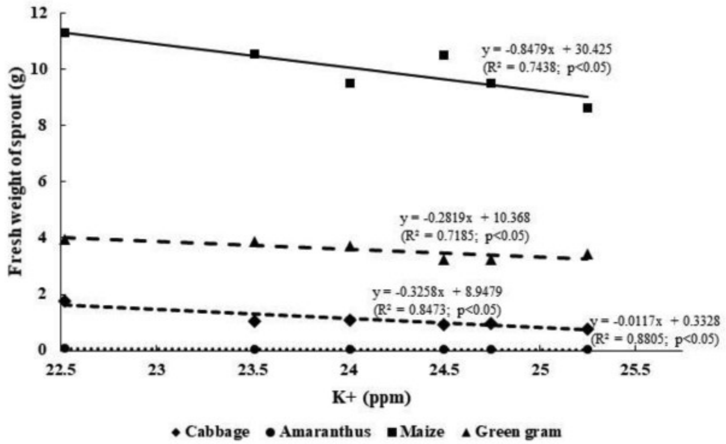

Figure 3. Relationship between fresh weight of sprout and K^+ concentration

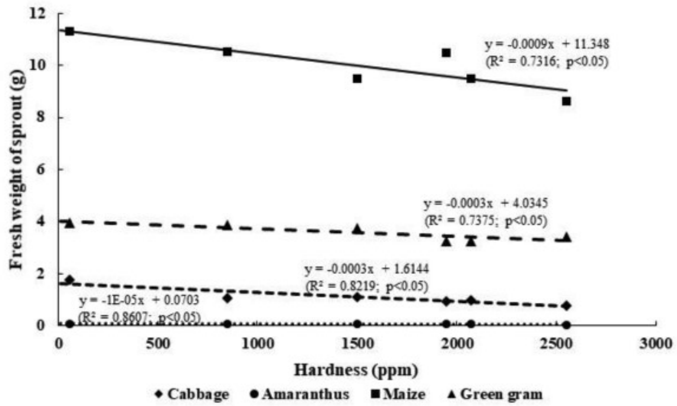

Figure 4. Relationship between fresh weight of sprouts and hardness of water

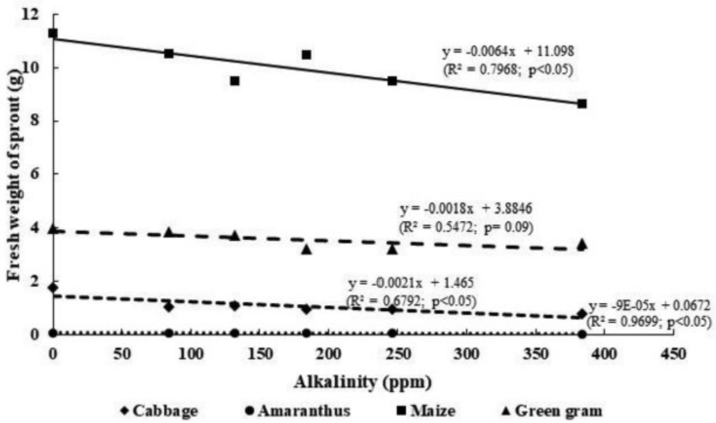

Figure 5. Relationship between fresh weight of sprouts and alkalinity of water

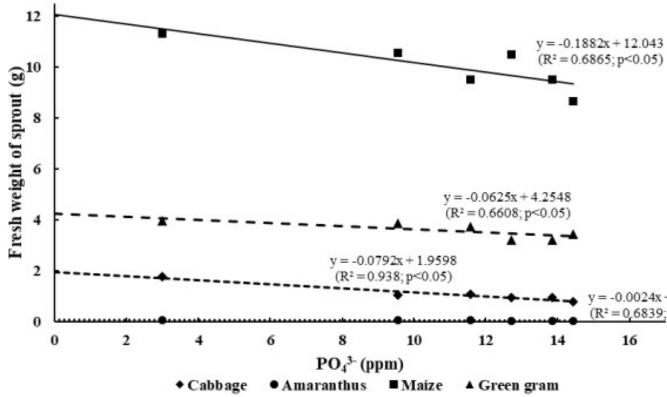

Figure 6. Relationship between fresh weight of sprouts and PO_4^{3-} concentration

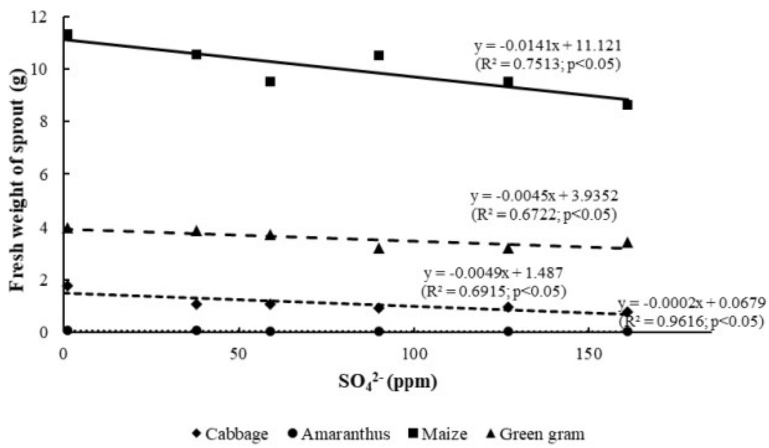

Figure 7. Relationship between fresh weight of sprouts and SO_4^{2-} concentration

and Kazemi, 2011). Among seed of four different plant species, fresh weight of germinated maize seeds have considerably decreased with increasing Na^+ (y = -0.006x + 11.527) and K^+ (y = -0.8479x + 30.425) concentrations. On the other hand, sprouted seeds of Amaranthus have shown slight decrease of fresh weight with increasing concentration of Na^+ and K^+, where the increase of one unit concentration of Na^+ and K^+ have reduced the fresh weight by 8.45×10^{-5} and 0.0117, respectively. From previous studies, similar relationship had been reported on the fresh weight of alfalfa sprouts (*Medicago sativa* L.) with salinity (Zhang *et al*., 2017).

Hardness:

Hard water contains high quantity of dissolved calcium and magnesium (Sengupta, 2013). Compare to other water quality parameters, hardness of irrigation water has less effect on the fresh weight of the sprouts, where the increment of one unit hardness drop the fresh weight by less than 0.001 g. Fresh weight of maize sprouts have shown a significant ($p<0.05$) linear relationship (y = -0.0009x + 11.348; R^2 = 0.73) with the water hardness (Figure 4). Meanwhile, similar pattern of relationship have observed on fresh weight of cabbage and green gram with water hardness as y = -0.0003x + 1.6144 and y = -0.0003x + 4.0345, respectively. Although, Salahshoor and Kazemi (2016) found that the impact of calcium on reducing the salt stress in seed germination and early growth stage of *Festuca ovina* L., the salinity can dominate than the concentration of calcium, where the seed germination and early growth could be altered even with increasing hardness of irrigation water (hardness 2). Furthermore, considerable abnormalities in seedlings of Haloxylonammodendron had recorded while treating with Mg^{2+} salts. This had happened due to the disruption of membrane permeability and functions of the plasma membrane and cell wall (Tobe *et al*., 2004).

Alkalinity:

The fresh weights of sprouts have significantly ($p<0.05$) decreased with alkalinity stress except fresh weight of green gram sprouts (Figure 5).The highest and the lowest sensitivity have noted on fresh weights of maize and Amaranthus, respectively with the alkalinity.

Furthermore, relationship of alkalinity with fresh weight of germinated seeds of maize and Amaranthus can be estimated by using linear equations $y = -0.0064x + 11.098$ and $y = -9 \times 10-5x + 0.0672$, respectively. Some sesame cultivars also have given the similar trend for the alkalinity stress, where the dry weight of sesame cultivars have declined with rising alkalinity stress (Mahdavi, 2016). The impact of alkaline stress generally added the impact on high pH, which can obstruct the ionic uptake and ionic balance of plant cells (Lin *et al.,* 2012).

Concentration of PO_4^{3-} and SO_4^{2-}:

The concentration of PO_4^{3-} and SO_4^{2-} of wastewater are given in figure 6 and figure 7, respectively. Sprout fresh weights of Amaranthus, cabbage, maizeand green gram have decreased with increasing concentration of PO_4^{3-} and SO_4^{2-}. Fresh weight of germinated seeds of Amaranthusis minimally threatened by both PO_4^{3-} ($y = -0.0024x + 0.0776$) and SO_4^{2-} ($y = -0.0002x + 0.0679$) concentration, while fresh weight of maize sprout has been adversely altered by the concentration of both anions (PO_4^{3-}; $y = -0.1882x + 12.04$, SO_4^{2-}; $y = -0.0024x + 0.0776$). This could be due to the occurrence of osmotic imbalance with high concentration of salts (PO_4^{3-} and SO_4^{2-}), which leads to poor germination and seedling performance.

Conclusions

The study showed that the combination of 40% of RO rejected wastewater and 60% pure water recorded good results on germination and seedling growth of Amaranthus and maize. At the same time green gram and cabbage showed good germination and seedling growth in the combination of 20% of RO rejected wastewater and 80% pure water.

Furthermore, the chemical analysis of the treatments indicated that the addition of pure water to the sole RO rejected wastewater helps to reduce the harmful effect of chemical parameters of the RO rejected wastewater. Meanwhile, the estimated relationships between water quality parameters and seed germination and seedling growth variables have given an idea to find the appropriate concentration of RO wastewater for the irrigation purposes.

The findings indicated that the possibility of using RO rejected water as a part of irrigation water source. The germination and seedling stage are the most critical period in the plant growth cycle. However, seeds and seedlings of four different plant species have showed good performance in germination and seedling growth. Further, field studies needed to commercialize the usage of RO rejected wastewater as irrigation water.

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