Impact of Different Concentrations of Municipal Wastewater on Rice Seed Germination and Seedling Performance

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Abstract— *The major challenge faced by developing countries is to produce adequate food for their growing human population under the shortage of fresh water for agricultural activities. Laboratory experiment was conducted in a completely randomized design using sand medium with municipal wastewater and MR253 rice seed with the aim to evaluate effects of municipal wastewater (treated and untreated) at different concentrations (0, 2.5, 5, 10, 25, 50 and 100%) on seed germination and seedling performance. Significant (p<0.05) difference was observed between untreated and treated municipal wastewater for seedling length (SL), root volume (RV), seedling vigour index (SVI) and root: shoot ratio (R:S) while no difference were observed between untreated and treated municipal wastewater for germination percentage (GP) and the seedling phytotoxicity. The concentrations of N, P, K, Ca, Mg, Zn, Fe, Cu and Mn were higher in the untreated municipal wastewater compare to treated municipal wastewater. Seeds imbibed with untreated municipal wastewater have high seed germination and seedling performance compare to treated municipal wastewater. Inhibitory effect on chlorophyll content was observed at concentration >50% of both untreated and treated municipal wastewater while promoting effects were observed at lower (<25%) concentrations. However, all the nutrient elements were negatively and highly correlated with quantity of different municipal wastewater concentration. The study showed that seeds imbibed with untreated municipal wastewater have high seed germination and seedling performance compare to treated municipal wastewater. Municipal wastewater of <50% concentration could be recommended as a good source of water and nutrients for rice seed germination without affecting seedling performance.*

Keywords— Municipal wastewater, Seed germination, Seedling Performance, Oryza sativa, nutrient uptake, Chlorophyll content, Correlation.

I. INTRODUCTION

Potential means to secure rice productivity is to ensure that the quality of the seeds for sowing is good. A good and quality seeds are free of weed seeds, seed-borne diseases, pathogens, insects, or other matters and it possess high germination, vigor, viability and seedling performance (Chhetri, 2009). On the other hand, water is a major factor on earth and top priority for the existence of human life and crop production. The global demand for water in agriculture will have to increase with rising human population, escalating incomes, and deviations in nutritional favorites. Growing demands for water by industrial, urban users will deepen competition (de Fraiture and Wichelns, 2010). The use of domestic wastewater for agricultural production is increasing, especially as domestic wastewaters are rich in plant nutrients and organic matters which are essential for plant growth and development (Dash, 2012). The practice of using municipal wastewater will help reduce the pressure on fresh water for watering or irrigation of agricultural activities. Crops irrigated with wastewater have the potential to give a higher yield as the water helps to reduce the need for chemical fertilizers (Haussain et al., 2002). The macro and micro-nutrients in the wastewater assist as a good basis of plant nutrients and the organic constituents furnish helpful soil conditioning properties (Singh and Agrawal, 2008). Generally, wastewater (treated and untreated) is extensively utilized in farming because it is a rich basis of nutrients and provides all the moisture vital for crop growth. Water is a vital factor in agriculture and it plays an influential role in the growth and development of plants. Therefore, actions must be taken to reinforce irrigation farming, safeguard the rural environment and support water resource sustainability with good water management (de Juan *et al.*, 1999). Meeting the trials of feeding the ever-rising human populace, proficient uses of water and land resources is extremely vital in crop production. As the demand of wastewater is increasing, this study was conducted to evaluate the impact of untreated and treated municipal wastewater on rice seed germination and seedlings performance.

II. MATERIAL AND METHOD

2.1 Municipal Wastewater Source

Municipal wastewater used for this study was collected from Indah Water konsortium Berhad, wastewater treatment plant in Kuala Lumpur, Malaysia. Standard procedure (APHA, 1998) was followed during the collection and analysis of the wastewater samples. Some physico-chemical characteristics of the municipal wastewater were analyzed in the Department of Crop Science, Universiti Putra Malaysia by using Auto-Analyzer (Lachat 8000 Series) to analyzed nitrogen, phosphorous and potassium. Iron, zinc, calcium, magnesium, manganese, copper were analyzed by using atomic absorbtion spectrometer (Perkin Elmer AAnalyst 400) while cadmium and lead were analyzed by using inductively coupled plasma (Perkin Elmer, Optima 8300) at the Department of Land Management, Universiti Putra Malaysia. The analysis for biological oxygen demand (mg/l), chemical oxygen demand (mg/l), ammonium (ml/g), nitrate (ml/g), total suspended solid (mg/l), pH, oil and grease (ml/g) were done by Indah Water Konsortium Berhad, wastewater treatment plant Kuala Lumpur, Malaysia.

2.2 Experimental Procedures

A rice variety, MR253 was planted on sand medium in the Seed Science Laboratory, Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia. The rice seeds were watered with untreated (raw) and treated (processed) municipal wastewater samples.

2.3 Seed Preparation and Imbibition Treatment

Rice variety MR253 was sterilized with 70% of chlorox solution for fifteen minutes to remove microbes from the seeds. Double sterilized water was used to repeatedly wash the seeds. The seeds were then imbibed in different concentrations of untreated (raw) and treated (processed) municipal wastewaters and distilled water for six hours before planting. One Hundred healthy treated rice seeds were put in plastic germination boxes of 2.5kg of sterilized sands and the sands were moisturized with 500 mL of different concentration of wastewater before planting the seeds. Two hundred and fifty milli-liter (250 mL) of different concentrations of wastewater and distilled water were used for watering.

2.4 Experimental Design and Treatments

The experiments were conducted as factorial experiments using the completely randomized design with three replications with; untreated and treated municipal wastewater, seven concentrations of untreated and treated municipal wastewater diluted with distilled water (0, 2.5%, 5%, 10%, 25%, 50% and 100%) and one Malaysian Rice varieties (MR253). The boxes were placed in the laboratory at 25ºC under constant light. The germination test was conducted according to The International Seed Testing Association procedures (ISTA, 1999) and it was carried out for a period of two weeks.

2.5 Procedure for nutrient analysis

Determination of nutrients was carried out using the modified method of Wolf (1982). The seedlings root and leaf samples were placed into envelop and then dried in the oven at 70ºC for 48 hours. The dried plant tissues were grinded and 0.25 g was used for the digestion. For the digestion process, the samples were transferred into clean digestion flasks and 5mL of concentrated H_2SO_4 was added to each flask for 2 hours. Thereafter, the flasks were heated for 45 minutes at 285ºC and 2ml of 50% (H_2O_2) was added to complete the process. The process was repeated several times until the samples became clear. The flasks were removed from digestion plate, cooled at room temperature and then diluted to make up to 100mL volume with distilled water. Then the macronutrients N, P and K were determined in the solution by using Auto-Analyzer (Lachat 8000 Series) while Ca, Mg and micronutrients Zn, Fe, Cu, Mn were determined in the solution by using Atomic Absorption Spectrometer (Perkin Elmer model 3110). Nutrients uptake by seedlings were determined as a function of the rice dry biomass production.

2.6 Chlorophyll Content

The chlorophyll content was measured according to the procedure described by Porra et al. (1989). The amount of 0.2 g of fresh leaf was homogenized in 80% acetone for 2 minutes and then was centrifuged at 2500 rpm for 20 minutes and supernatant was extracted. About 3.5 mL of samples were pipetted into microfuge and the chlorophyll content was measured by using scanning spectrophotometer (UV3101 PC). The samples were read at wavelength of 663 nm and 646 nm. The formulae of Lichtenthaler and Wellburn (1983) were used to calculate chlorophyll a and chlorophyll b contents.

2.7 Data Collection

Data was collected on the following parameters: germination percentage, seedling length, root volume, seedling vigour index, root:shoot ratio and seedling phytotoxicity.

According to the procedure of Abdul Baki and Anderson (1973).

Seedling Vigour Index (SVI) =Germination (%) x Seedling length (cm)

$$\text{Root: shoot ratio} = \frac{\text{Dry weight for root}}{\text{Dry weight for shoot}}$$

According to the procedure of Chou and Lin (1976).

$$\text{Seedling phytotoxicit} = \frac{\text{Radical length of control} - \text{Radical lenght of test}}{\text{Radical length of control}} \times 100$$

Root volume was measured by using the root scanner and analysis machine (Analyzer WinMagRhizo, Epson Expression 1680).

2.8 Statistical Analysis

The SAS statistical software (9.4 versions) was used to analyze the data including analysis of variance (ANOVA). Treatments means were compared using least significance difference (LSD) at $P<0.05$.

2.9 Toxicity Threshold in Crops

The municipal wastewater was checked for trace element, toxicity hazards, particularly when trace element contaminations were suspected. The maximum levels of trace element recommended for crop production is shown in Table 1 below.

TABLE 1
RECOMMENDED MAXIMUM LEVELS OF TRACE ELEMENTS FOR CROP PRODUCTION (FAO, 1985)

	Element	Recommended maximum concentration (mg/L)	Remarks
Cd	(cadmium)	0.01	Toxic to beans, beets and turnips at concentrations as low as 0.1 mg/l in nutrient solutions. Conservative limits recommended due to its potential for accumulation in plants and soils to concentrations that may be harmful to humans.
Cu	(copper)	0.20	Toxic to a number of plants at 0.1 to 1.0 mg/l in nutrient solutions.
Fe	(iron)	5.0	Not toxic to plants in aerated soils, but can contribute to soil acidification and loss of availability of essential phosphorus and molybdenum. Overhead sprinkling may result in unsightly deposits on plants, equipment and buildings.
Mn	(manganese)	0.20	Toxic to a number of crops at few-tenths to a few mg/l, but usually only in acid soils.
Pb	(lead)	5.0	Can inhibit plant cell growth at very high concentrations.
Zn	(zinc)	2.0	Toxic to many plants at widely varying concentrations; reduced toxicity at pH > 6.0 and in fine textured or organic soils.

III. RESULT AND DISCUSSION

3.1 Municipal wastewater characteristics

The analyses of both untreated and treated municipal wastewaters are shown in Table 2. The municipal wastewater had differences their chemical and physical characteristics. Municipal wastewater for both untreated and treated municipal wastewater was slightly acidic and alkaline in nature with pH range between 6.8 and 7.1. The characteristics of the wastewater used for watering showed that the presence of high amount of nutrients in untreated municipal wastewater than treated municipal wastewater.

TABLE 2
CHEMICAL AND PHYSICAL CHARACTERISTICS OF UNTREATED AND TREATED MUNICIPAL WASTEWATER

Parameters	Untreated wastewater	Treated wastewater
Color	Dark black	Clear
Nitrogen (mg/L)	24.2	14.5
Phosphorous (mg/L)	2.99	0.33
Potassium (mg/L)	5.45	0.35
✚ Iron (mg/L)	2.11	0.14
✚ Zinc (mg/L)	1.06	0.27
✚ Calcium (mg/L)	20.65	16.51
✚ Magnesium (mg/L)	3.86	1.59
✚ Manganese (mg/L)	3.02	1.87
✚ Copper (mg/L)	3.25	1.32
✚ Cadmium (mg/L)	0.02	0.01
✚ Lead (mg/L)	nil	nil
*Biological Oxygen Demand (mg/L)	247	6.9
*Chemical Oxygen Demand (mg/L)	436	32.2
*Ammonium (mg/L)	30	22
*Nitrate (mg/L)	3.2	1
*Total suspended solid (mg/L)	280	8.8
*Oil and grease (mg/L)	3.5	1.5
*pH	6.8	7.1

Sources: Analytical Lab, Department of Crop Science, Universiti Putra Malaysia
✚ *Analytical Lab, Land Management, Faculty of Agriculture, Universiti Putra Malaysia.*
**Indah Water Konsortium Berhad, wastewater treatment plant Kuala Lumpur, Malaysia.*

3.2 Seed Germination

Different concentrations of untreated and treated municipal wastewater have significant difference ($p<0.05$) on rice seed germination. Germination percentage increased with increase in concentration up to 25% and thereafter decreased gradually for both untreated and treated municipal wastewater (Table 3). Maximum germination percentage was recorded at 25% concentration with 95.1% and 92.5% germination for untreated and treated wastewater, respectively. Lower wastewater concentration had promoting effect on seed germination while higher wastewater had reducing effect. Similar result on rice in previous studies was recorded when the seeds were imbibed in municipal wastewater (Gassama *et al*., 2015). Dash (2012) studied the impact of domestic wastewater on seed germination and physiological parameters of rice and wheat, and observed that at higher concentration, percentage germination of seeds were reduced. Similar result was also observed by Saravanamoorthy and Kumari (2007) in peanut. Furthermore, Singh et al., (2007) observed significant decrease in the percentage germination and seedling vigour of rice and wheat with an increase in spent wash concentration. The decrease may be due to the adverse effect of high toxicity of the wastewater at higher concentration (Ramana et al., 2002; Yousaf et al., 2010).

TABLE 3
MEAN COMPARISON OF DIFFERENT CONCENTRATIONS OF TREATED AND UNTREATED MUNICIPAL WASTEWATER ON PERCENTAGE GERMINATION FOR MR253 RICE SEEDLINGS

	CONC (%)	UTWW	TWW
MR253	0	84.0a	86.2b
	2.5	86.3b	85.0b
	5	87.6b	87.0b
	10	89.1b	85.3b
	25	95.1a	92.5a
	50	79.3c	82.3c
	100	76.6c	80.1c

Means within columns with common letters are not significantly (P>0.05) different
UTWW = Untreated wastewater
TWW = Treated wastewater
CONC = Concentrations

3.3 Seedling Length

Different concentrations of untreated and treated municipal wastewater on seedling length for variety MR253 was significantly different at p<0.05. Results showed that seedling length increased with increase in concentration of untreated wastewater up to 5% concentration followed by decline. On the other hand, seedling length increased with increase in concentration of treated wastewater up to 50% and further increase in the wastewater concentration, decrease in seedling length was observed (Table 4). Dash (2012) reported significant declined in seedling length when seeds were treated with sewage at higher than 75%. However, seed treated at 25%-50% wastewater concentration had increased seedling lengths in both rice and wheat. Nawaz *et al*. (2006) reported that seedling length of soybean in industry effluent decreased with increase in concentration of the effluent while in marble industry effluent, increase in seedling length was recorded. Dhanam (2009) observed that 100% concentration of dairy effluent inhibited rice seedling growth and suggested that this may be due to osmotic pressure caused by high effluent dose. The decrease in seedling length at higher concentration can be due to the presence of high amount of heavy metals at higher concentration in the industrial wastewater they were using. Increased in seedling length at lower concentrations of effluent might be due to the presence of nitrates and sulphates which stimulate the protein production and other organic molecules required for plant growth (Yousaf *et al.*, 2010).

TABLE 4
MEAN COMPARISON OF DIFFERENT CONCENTRATIONS OF TREATED AND UNTREATED MUNICIPAL WASTEWATER ON SEEDLING LENGTH FOR MR253 RICE SEEDLINGS

	CONC	UTWW	TWW
MR253	0	58.16c	47.88b
	2.5	74.78b	47.36b
	5	81.70a	49.79b
	10	73.29b	52.53a
	25	60.41c	53.23a
	50	56.60c	54.27a
	100	58.96c	46.95b

Means within columns with common letters are not significantly (P>0.05) different
UTWW = Untreated wastewater
TWW = Treated wastewater
CONC = Concentrations

3.4 Root Volume

Different levels of wastewater concentrations were significantly (p<0.05) different on root volume for MR253 rice seedlings. Results showed that root volume increased with increase in concentration of up to 25% followed by a decline in MR253 (Fig:

1). The result showed that lower concentration has stimulating effect on root volume while higher concentration has deleterious effect on root volume. Maximum root volume 0.25 cm^3 was recorded when watered with untreated municipal wastewater compared to treated municipal wastewater 0.19 cm^3 (Fig: 2). Similar result was also observed in previous study by Gassama *et al.*, (2015) in rice. Rehman *et al.* (2009) observed significant reduction in root volume of three vegetables crops when exposed to 100% concentration of effluent while increased was observed at lower effluent concentration. Dhanam (2009) observed that higher concentration of dairy effluent inhibited rice seedling growth and suggested that this may be due to the water toxicity caused by high effluent dose.

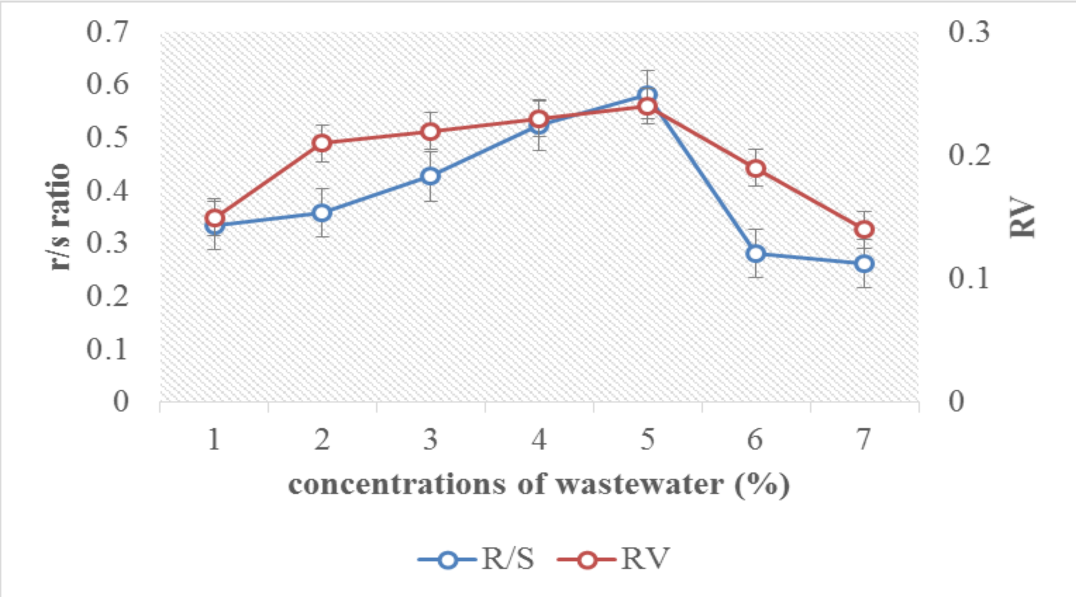

FIGURE 1: Effect of different concentrations of municipal wastewaters on root volume and root/shoot ratio for MR253 rice variety.

r/s= root/shoot ratio; RV= root volume;

Vertical bars on line represent error bars with percentage (±)

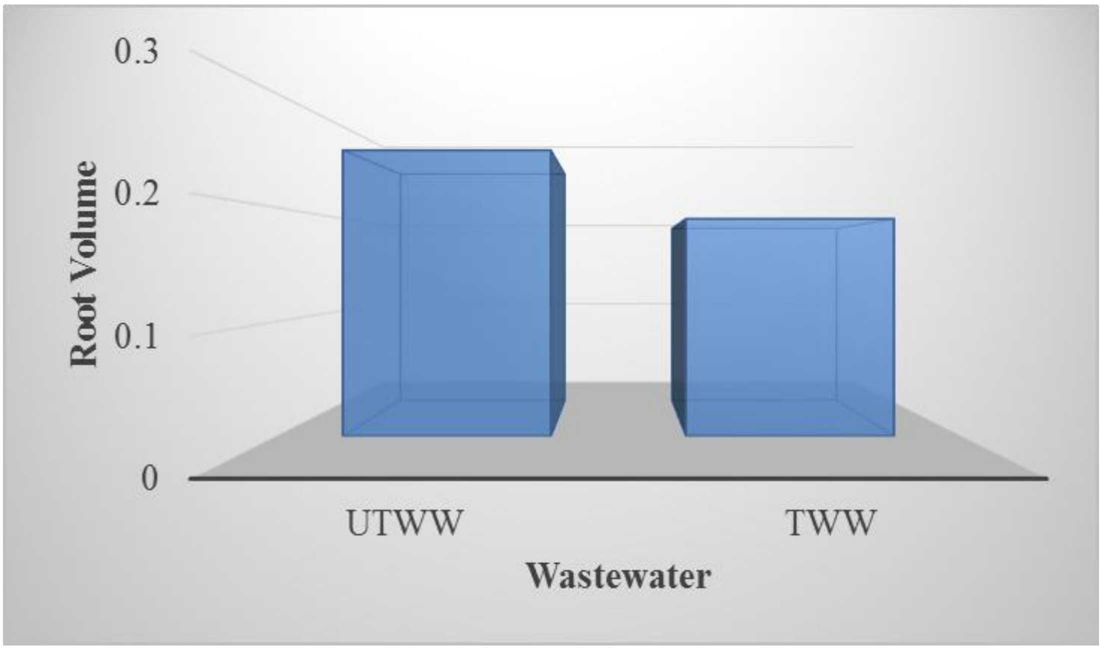

FIGURE 2: Effect of treated and untreated municipal wastewater on root volume (RV) for MR253 rice seeds.

3.5 Root/Shot Ratio

Result showed that MR253 rice seedlings watered with different concentration of municipal wastewater showed significant ($p<0.05$) difference for root:shoot ratio. Rice seedlings irrigated with distilled water (control) gave normal root/shoot ratio and any changes from this normal level either up or down would be an indication of a change in the overall health of the crop. Maximum root/shoot ratio was recorded at 25% concentration for MR253 rice seedlings. Furthermore, increasing the concentrations to 50% and 100% of the wastewaters saw reduction in the root:shoot ratio MR 253 rice seedlings (Fig: 1). Increase in root/shoot ratio was observed at lower concentration of municipal wastewater for MR253 rice seeds while decreased ratio was observed at higher concentrations of the wastewaters. An increase in root:shoot ratio is an indication of a healthier plant while decrease in root:shoot ratio is an indication of deterioration in the health of the crops. The direct use of the raw wastewater resulted in decreased benefits whereas diluted concentrations noticed higher seed quality parameters because of lesser toxicity and better utilization of plant nutrients by the seedlings (Manunatha, 2008).

3.6 Seedling Vigour Index

The data pertaining to the seedling vigor index for MR253 rice seedlings as influenced by different concentrations of municipal wastewater was significantly ($p<0.05$) (Fig. 3). It was observed that the gradual but steady increase in various concentration levels of municipal wastewaters from 0% to 25% increased the seedling vigor index. Further increase in the municipal wastewater concentration from 50% to 100% saw significant decline in seedling vigor index for seeds. However, seeds watered with 25% concentration of municipal wastewater were the most vigorous seedlings with SVI of 6850 and seeds watered with 100% concentration of wastewater had the lowest seedling vigor index of 4533. Similar result has been recorded in previous studies when rice seeds were imbibed in municipal wastewater (Gassama *et al*., 2015). The seeds that gave the highest seedling vigor index for MR253 rice seeds are considered to be most vigorous (Abdul-Baki and Anderson, 1973). When the wastewaters are diluted in disparate concentrations, the toxicity of the wastewater's constitution goes on weakening and at an optimum concentrations larger utilization of nutrients takes place (Manunatha, 2008).

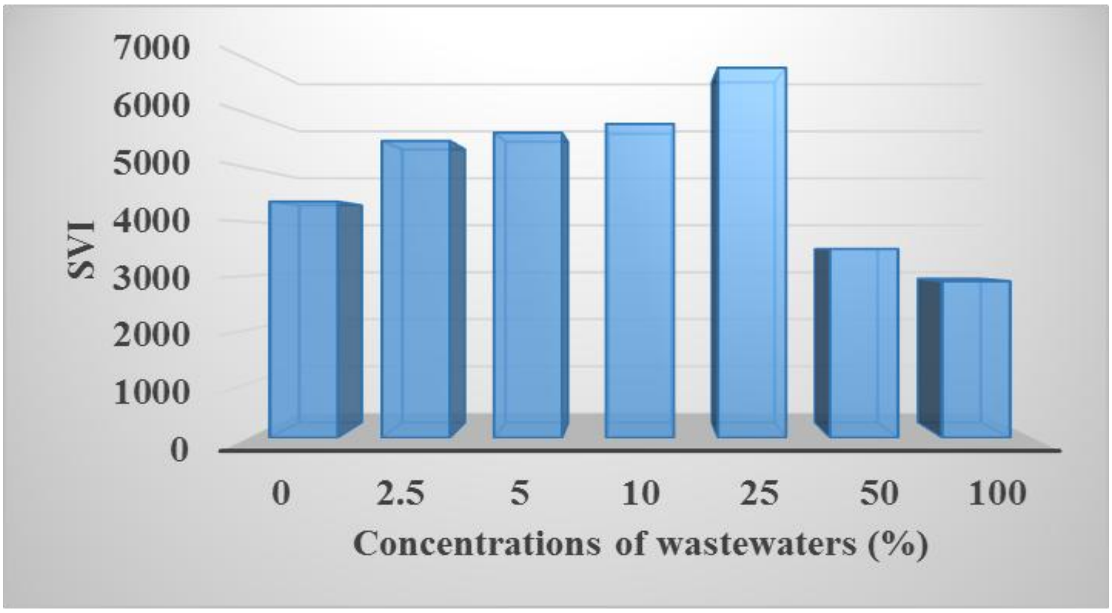

FIGURE 3: Effect of different concentrations of municipal wastewaters on seedling vigour index for MR253 rice variety.

3.7 Seedling Phytotoxicity Index

Data showed significant ($p<0.05$) difference on phytotoxicity for MR253 rice seedlings when imbibed in different concentrations of treated and untreated municipal wastewater. Lower wastewater concentrations (<25%) had lower phytotoxicity on the rice germination process that then gave better response of the MR253 rice seeds to the wastewater. However, further increase in the concentrations of the wastewater from 50%-100% resulted in higher phytotoxicity on the rice germination process that led to retarded growth for MR253 rice seeds (Fig: 4). The wastewater at lower concentrations had less toxicity that was unable to hinder the growth and development of the crops while at higher concentrations its toxicity was high enough to reduce growth and development of the crops. Higher wastewater concentrations have high phytotoxicity. Higher concentration of wastewater effluent decreases activities of dehydrogenase (Murkumar and Chavan, 1987) and acid phosphatase (De Leo and Sacher, 1970) which are important enzymes during early germination process and also involved in mobilization of nutrient reserves (Flinn and Smith, 1967). The low amount of oxygen in dissolved form due to high concentration of dissolved solids in the effluent reduces the energy supply through anaerobic respiration causing retardation of growth and development of seedling (Saxena *et al.*, 1986). The enhancement of seed quality by the lower concentrations of the wastewater was due to the presence of low toxic activities in the wastewater.

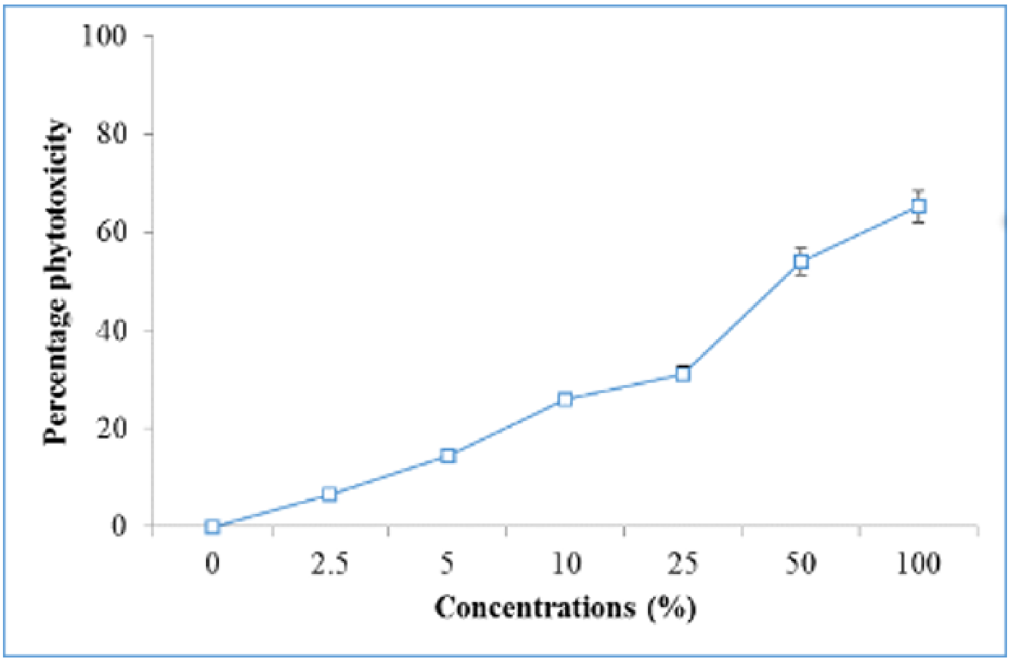

FIGURE 4: Effect of different concentrations of municipal wastewaters on seedling phytotoxicity for MR253 rice variety.

Vertical bars on line represent error bars with percentage (±)

3.8 Plant Nutrient Concentration

The results showed that Cu and Fe concentration were toxic to the rice seedlings while the concentration of Mn and Zn were within limit for the rice seedlings. Furthermore, the result revealed that N concentration was deficient while P, K and Mg were sufficient for the rice seedlings but Ca was found to be at a critical level in the rice seedlings (Table 5). Nutrients contents in the rice seedling samples analyzed were compared with the critical nutrients range at tillering required by rice as reported by Dobermann and Fairhurst (2000). The analysis shows that P, K, Mg, Mn and Zn were sufficient for the rice seedling at 14 days of planting while N and Ca were far from optimum level for rice but Cu and Fe were excess and toxic in the rice seedlings (Table 6).

TABLE 5
NUTRIENT CONCENTRATIONS IN RICE SEEDLINGS AFTER 14 DAYS OF PLANTING WITH DIFFERENT CONCENTRATIONS OF MUNICIPAL WASTEWATER

Concentration of wastewater	%					mg kg^{-1}			
	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
0	1.39	0.21	1.02	0.11	0.17	32.88	468	218.44	193.33
2.5	1.41	0.24	2.41	0.15	0.17	60.22	1096	695.78	317.33
5	1.42	0.23	2.12	0.17	0.17	52.88	987.33	662.44	291.33
10	1.48	0.25	2.59	0.17	0.18	56.44	952	643.78	289.33
25	1.55	0.24	2.39	0.17	0.17	68.44	1507.33	622.44	290
50	1.66	0.24	2.49	0.18	0.17	45.55	997.33	591.78	288
100	1.49	0.25	2.08	0.19	0.18	52.66	1026.67	498.44	256.67

TABLE 6
OPTIMUM RANGES AND CRITICAL LEVELS FOR OCCURRENCE OF MINERAL DEFICIENCIES OR TOXICITY IN RICE TISSUE

Elements	Growth stage	Plant part	Optimum range	Critical level for deficiency	Critical level for excess or toxicity
N	Tillering -PI	Y Leaf	2.9-4.2%	<2.5%	>4.5%
	Flowering	Flag Leaf	2.2-2.5%	<2.0%	
	Maturity	Straw	0.6-0.8%		
P	Tillering -PI	Y Leaf	0.20-0.40%	<0.10%	>0.50%
	Flowering	Flag Leaf	0.20-0.30%	<0.18%	
	Maturity	Straw	0.10-0.15%	<0.06%	
K	Tillering -PI	Y Leaf	1.8-2.6%	<1.5%	>3.0%
	Flowering	Flag Leaf	1.4-2.0%	<1.2%	
	Maturity	Straw	1.5-2.0%	<1.2%	
Ca	Tillering	Y Leaf	0.2-0.6%	<0.15%	>0.7%
	Tillering-PI	Shoot	0.3-0.6%	<0.15%	
	Maturity	Straw	0.3-0.5%	<0.15%	
Mg	Tillering -PI	Y Leaf	0.15-0.30%	<0.12%	>0.50%
	Tillering-PI	Shoot	0.15-0.30%	<0.13%	
	Maturity	Straw	0.20-0.30%	<0.10%	
Cu	Tillering -PI	Y Leaf	7-15 mg kg^{-1}	<5 mg kg^{-1}	>25 mg kg^{-1}
	Maturity	Straw		<6 mg kg^{-1}	>30 mg kg^{-1}
Fe	Tillering	Y Leaf	75-150 mg kg^{-1}	<70 mg kg^{-1}	>300 mg kg^{-1}
	Tillering	Shoot	60-100 mg kg^{-1}	<50 mg kg^{-1}	
Mn	Tillering	Y Leaf	40-700 mg kg^{-1}	<40 mg kg^{-1}	>800 mg kg^{-1}
	Tillering	Shoot	50-150 mg kg^{-1}	<20 mg kg^{-1}	
Zn	Tillering -PI	Y Leaf	25-50 mg kg^{-1}	<20 mg kg^{-1}	>500 mg kg^{-1}
	Tillering -P	Shoot	25-50 mg kg^{-1}	<10 mg kg^{-1}	>500 mg kg^{-1}

Source: Dobermann and Fairhurst (2000)
**** mg kg^{-1} = ppm***

3.9 Plant Nutrient uptake in Seedlings

The interaction among variety, different levels of concentrations and wastewaters was significantly ($p<0.05$) different for N, P, K, Ca and Mg nutrients for MR253 rice seedlings. The concentration of N, P and K nutrients in seedlings increased when a certain concentration of treated wastewater <25% was used for imbibition. As the concentration of treated wastewater increased beyond a critical point >50%, the concentration uptakes of N, P and K decreased. The concentration of N, P and K nutrients in seedlings increased when <25% concentration of untreated wastewater was used for imbibition for MR253, thereafter, as the concentration of the untreated wastewater increased beyond a critical point >50%, the concentration of N, P and K decreased for MR253 (Table 7). The concentration of Ca and Mg nutrients in seedlings increased when a certain concentration of treated wastewater <10% and <25% respectively for MR253 was used for imbibition. As the concentration of treated wastewater increased beyond a critical point >25% concentration for Ca and >50% concentration for Mg, decreased was observed. The concentration of Ca and Mg nutrients in seedlings increased when 10% and 25% concentration of untreated wastewater was used for imbibition respectively and thereafter, as the concentration of the untreated wastewater increased beyond a critical point >25% and >50%, the concentration of Ca and Mg decreased respectively (Table 7).

TABLE 7
MEAN COMPARISON OF NUTRIENTS UPTAKE AS AFFECTED BY DIFFERENT CONCENTRATIONS, MUNICIPAL WASTEWATERS AND VARIETIES FOR N, P, K, CA AND MG FOR MR253 RICE SEEDLINGS (mg/g)

		N		P		K	
Variety	**Concentrations of wastewater**	**TWW**	**UTWW**	**TWW**	**UTWW**	**TWW**	**UTWW**
MR253	0	0.28d	0.32d	0.3b	0.34b	0.35d	0.35d
	2.5	3.90b	4.60b	0.5a	0.5a	2.90b	3.90b
	5	4.20b	5.70b	0.5a	0.6a	3.60a	4.60a
	10	5.20b	6.10a	0.5a	0.7a	4.70a	5.20a
	25	6.80a	6.50a	0.9a	0.8a	5.80a	5.80a
	50	3.70c	2.90c	0.2b	0.3b	2.01c	2.30c
	100	2.70c	2.50c	0.2b	0.2b	2.50c	2.01c
		Ca		**Mg**			
Variety	**Concentrations of wastewater**	**TWW**	**UTWW**	**TWW**	**UTWW**		
MR253	0	0.02d	0.02d	0.03d	0.02d		
	2.5	0.20b	0.50a	0.30b	0.40a		
	5	0.20b	0.60a	0.30b	0.40a		
	10	0.40a	0.60a	0.50a	0.60a		
	25	0.10c	0.50a	0.70a	0.70a		
	50	0.10c	0.20b	0.10c	0.20b		
	100	0.10c	0.10c	0.10c	0.10c		

**** Means within columns with common letters are not significantly (P>0.05) different***
****TWW= Treated wastewater; UTWW= Untreated wastewater.***

The interaction among variety, different levels of concentrations and wastewaters was significantly ($p<0.05$) different for Mn and Zn for MR253 rice seed. The concentration of Mn and Zn nutrients in seedlings increased when the seeds were imbibed in a certain concentration of treated wastewater (<25%). As the concentration of treated wastewater increased beyond a critical point (>50%), the concentration of Mn and Zn decreased. The concentration of Mn and Zn nutrients in seedlings increased when (<25%) concentration of untreated wastewater was used for irrigation and thereafter, as the concentration of the untreated wastewater increased beyond a critical point (>50%), the concentration of Mn and Zn decreased (Table 8).

The interaction among variety, different levels of concentrations and wastewaters was significantly ($p<0.05$) different for Cu and Fe for MR253 rice seed. The concentration of Cu and Fe nutrients in seedlings increased when the seeds were imbibed in a certain concentration of treated wastewater (<25%). As the concentration of treated wastewater increased beyond a critical point (>50%), the concentration of Mn and Zn decreased. The concentration of Cu and Fe nutrients in seedlings increased when (<25%) concentration of untreated wastewater was used for irrigation and thereafter, as the concentration of the untreated wastewater increased beyond a critical point (>50%), the concentration of Cu and Fe decreased (Table 8).

TABLE 8
MEAN COMPARISON OF NUTRIENTS UPTAKE AS AFFECTED BY DIFFERENT CONCENTRATIONS, WASTEWATERS AND VARIETIES FOR Mn, Zn, Cu AND Fe FOR MR253 RICE SEEDLINGS (mg/g)

		Mn		Zn	
Variety	**Concentrations of wastewater**	**TWW**	**UTWW**	**TWW**	**UTWW**
MR253	0	0.013c	0.040b	0.021b	0.023b
	2.5	0.120b	0.140a	0.063a	0.074a
	5	0.130b	0.140a	0.066a	0.075a
	10	0.220a	0.160a	0.069a	0.084a
	25	0.091c	0.180a	0.091a	0.086a
	50	0.046c	0.084b	0.037b	0.032b
	100	0.041c	0.053b	0.028b	0.028b
		Cu		**Fe**	
	Concentrations of wastewater	**TWW**	**UTWW**	**TWW**	**UTWW**
Variety	0	0.018c	0.010c	0.020c	0.050b
	2.5	0.066a	0.140a	0.310a	0.200a
	5	0.076a	0.120a	0.320a	0.220a
	10	0.077a	0.130a	0.370a	0.350a
	25	0.096a	0.170a	0.390a	0.450a
	50	0.051b	0.075b	0.120b	0.150a
	100	0.032c	0.037b	0.049c	0.064b

**** Means within columns with common letters are not significantly (P>0.05) different***
****TWW= Treated wastewater; UTWW= Untreated wastewater.***

These results above are consistent with the findings of Mojiri and Aziz (2011) who reported that irrigation with wastewater increased Fe, Mn and Zn in roots and leaves of *Lepidium sativum.* Arora *et al.* (2008) also observed similar results that concentrations of macro and micronutrients were higher in wastewater irrigated vegetables than in fresh water irrigated plants. The nutrients in the municipal wastewaters at the lower concentration were within the permissible limits for plant development and below those that are toxic to plant growth (Varadarajan, 1992). Increase in the concentration of different minerals in the rice seedlings subjected to different municipal wastewater irrigation was different between untreated and treated wastewater. Untreated municipal wastewater was found to have more mineral nutrients content than treated wastewater. The rice variety had more nutrient uptake when imbibed with untreated municipal wastewater compared to when imbibed with treated municipal wastewater. This might be due to the more nutrient in the untreated municipal wastewater application which might be influencing the physiological process that leads to increase in growth as compared to treated wastewater (Singh and Bhati, 2003). Furthermore, higher concentration of untreated wastewater which inhibit nutrients uptake might be due to cells that have contact with concentrated wastewater which may have high level of cell abnormalities thereby lower nutrient uptake under concentrated wastewater (Abu and Ezeugwu, 2008). Khan and Sheikh (1976) clarified in a way that significant reduction of nutrients uptake under high concentrated effluents might be due to decrease in water uptake at higher level of salinity in view of toxicity of high osmotic pressure due to high soluble salts. Similar phenomenon may have happened in this study. Crop scientists have reported that use of treated and untreated wastewater increased yield parameters of field crops to a certain concentration of wastewater and suggested that treated wastewater can be used for producing better quality crops with higher yields (El-Nahhal et al., 2013). In the present study, irrigation with both treated and untreated wastewater at lower concentration (<25%) increased nutrients uptake which helped in rice seedling growth and development.

3.10 Chlorophyll Content in Seedlings

The interaction among variety, different levels of concentrations and wastewaters was significantly (p<0.05) different for chlorophyll a and b content in the leaves of the seedlings derived from MR253 rice seed. Chlorophyll a and b contents increased at lower wastewater concentrations in leaves for MR253 rice seeds, while decrease in chlorophyll a and b contents was observed at higher wastewater for both treated and untreated municipal wastewater. Lower wastewater concentration (<25%) had promoting effect on chlorophyll a and b contents while higher wastewater concentration (50%-100%) had deleterious effect on chlorophyll a and b content for MR253 rice seedlings (Table 9).

Similar results have been reported by Garg and Kaushik (2008) in sorghum cultivars treated with textile mill wastewater. The present study was also in conformity with Pathrol and Bafna (2013) who reported decreased in chlorophyll content in *Trigonella foenumgraecum* with decrease in the dilutions of the sewage water. These findings were also similar to the findings of Khan *et al*. (2011) who suggested that higher concentrations of wastewater are inhibitory to the synthesis of chlorophyll molecules particularly chlorophyll *a*. In wheat, Liu et *al.* (2002) observed a decline in chlorophyll level when seedlings were irrigated with sewage water.

Chlorophyll a and b contents in the leaves of the seedlings decreased in response to high concentration of wastewater. Reduction in chlorophyll content induced by wastewaters could be associated with higher concentration of heavy metals (Gadallah, 1995). Some of the possible reasons for any decreases may be due to: (a) Formation of enzymes such as chlorophyllase which is responsible for chlorophyll degradation (Majumder et al., 1991: Rodriquez et al., 1987: Sabater and Rodriquez 1978). (b) Retardation of chlorophyll synthesis under the effect of heavy metals present in wastewaters or due to changes in the endogenous cytokinins in leaves (Cizkova, 1990) which were reported to be responsible for stimulation of chlorophyll synthesis (Banerji and Laloraya, 1967). (c) Enhancement of chlorophyll loss due to increases in the endogenous ABA in the leaves (Cizkova, 1990) which were reported to accelerate the chlorophyll destruction (Mittelheuser and Van Steveninck, 1971) and inhibit plastid differentiation and chlorophyll synthesis (Le Page-Degivery et al., 1987). Similar phenomenon may have happened in this study. Rice seedlings exposed to higher concentrations of wastewater (>50%) had inhibitory effect on the chlorophyll content in the leaves of the rice seedlings which can be as a result of the heavy metals in the wastewater at higher concentration which are toxic to the leaves of the seedlings. However, seedlings imbibed in lower concentration of wastewater (<25%) had stimulated effect on the chlorophyll content in the leaves of the rice seedlings. In the lower concentrations of wastewater there might be no toxic effect in the leaves of the rice seedlings rather it has some beneficial effect.

TABLE 9
MEAN COMPARISON OF CHLOROPHYLL A AND CHLOROPHYLL b (µg/ml) CONTENT AS AFFECTED BY DIFFERENT CONCENTRATIONS, MUNICIPAL WASTEWATERS AND VARIETY FOR MR253 RICE SEEDLINGS

		Chl a		Chl b	
Variety	**Concentrations of wastewater**	**TWW**	**UTWW**	**TWW**	**UTWW**
MR253	0	20.34b	20.06b	12.08b	20.40b
	2.5	26.01a	27.97a	11.83b	21.43b
	5	26.06a	29.29a	13.46b	23.33a
	10	27.62a	29.40a	15.46b	23.59a
	25	29.01a	30.75a	23.26a	32.15a
	50	21.28b	20.46b	12.57b	17.13c
	100	14.54c	12.37c	7.738c	17.87c

**** Means within columns with common letters are not significantly (P>0.05) different***
****TWW= Treated wastewater***
****UTWW= Untreated wastewater***
****Chl a = Chlorophyll a***
****Chl b = Chlorophyll b***

3.11 Correlation Analysis

A negative and highly significant correlation was indicated for all the nutrients elements when correlated with quantity of municipal wastewater used for rice seeds imbibition (Table 10). Negative correlation indicated that when one variable is increasing the other variable has tendency to decrease. Therefore, the increase in the concentration of the municipal wastewater (>50%) saw a decrease in the nutrients elements in the rice seedlings while decrease in the concentration of the municipal wastewater (<25%) saw increase in the nutrients elements in the rice seedlings. Similar finding was also observed for rice at maximum tillering stage which showed negative correlation between P and N (Islam et al., 2008).

TABLE 10
THE VALUES OF CORRELATION BETWEEN NUTRIENTS OF RICE SEEDLINGS AS AFFECTED BY MUNICIPAL WASTEWATER

	Con	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
Con	1									
N	-0.709**	1								
P	-0.737**	-0.878**	1							
K	-0.544**	-0.717**	-0.685**	1						
Ca	-0.562**	-0.736**	-0.850**	-0.596**	1					
Mg	-0.740**	-0.883**	-0.944**	-0.693**	-0.861**	1				
Cu	-0.723**	-0.848**	-0.864**	-0.614**	-0.747**	-0.846**	1			
Fe	-0.411**	-0.343*	-0.441**	-0.183**	-0.341*	-0.454**	-0.418**	1		
Mn	-0.716**	-0.845**	-0.955**	-0.665**	-0.900**	-0.950**	-0.834**	-0.470**	1	
Zn	-0.743**	-0.857**	-0.885**	-0.692**	-0.777**	-0.953**	-0.829**	-0.412**	-0.884**	1

Level of significance ** = p < 0.001, * = p < 0.01, NS = Not significant
**** Con=concentration of wastewater***

IV. CONCLUSION

The use of wastewater in plant nourishment would be beneficial water resources for irrigation due to its nutrient contents. Municipal wastewater contains essential nutrients for plant growth and development. The promotion of seeds and seedlings quality parameters at lower concentrations of the wastewater is due to the presence of optimum levels of plant nutrients in the wastewater. Seedlings imbibed with lower concentration of untreated municipal wastewater showed better seedling performance compare to treated municipal wastewater although untreated wastewater contains some hazardous toxic elements. Thus, municipal wastewaters can be used for irrigation purposes in agricultural practices after proper dilutions. It is also suggested that, treatment of municipal wastewaters is necessary to minimize the pollution effects before irrigating the crops.

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