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Germination and seedling growth of rice (*Oryza sativa* L.) as affected by varying concentrations of loom-dye effluent

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Abstract

Indiscriminate discharge of industrial effluent has become a serious problem for the agro-ecological environment in most of the areas of Bangladesh. The effects of loom-dye effluents on seed germination and early seedling growth of rice (*Oryza sativa* L.) were investigated by conducting an experiment in the laboratory of the Department of Agricultural Chemistry of Bangladesh Agricultural University, Mymensingh. Three types of loom-dye effluents were applied in sterilized petridishes at different loading ratios. Seven treatments (*i.e.*, T_0, T_1, T_2, T_3, T_4, T_5 and T_6 with 0, 5, 10, 25, 50, 75 and 100% effluent, respectively) of each effluent were used following completely randomized design (CRD) and replicated three times. Subsequently, Pb, Fe, Zn, Mn and Cr accumulation were also investigated in the harvested rice seedlings. Rice seed showed a significant difference in germination percentage with varying levels of effluent application at different days after setting of germination. The decreased seed germination rate and seedling growth of rice were observed with increased concentration of loom-dye effluents. The highest germination speed (97.8%) was obtained from control whereas the lowest germination speeds were obtained from T_5 of black, and T_6 of both black and violet effluents. Phytotoxic effects of loom-dye effluents on germination and radical length were extreme at 100% effluent concentration having the order of black > violet > pink. The maximum radical length (6.4 cm) and plumule length (7.5 cm) were observed with T_1 of pink dye effluent whereas the minimum length of radical and plumule were obtained from T_6 treatment of the effluents. The highest fresh weight (39.8 mg petridish^{-1}), dry weight (5.7 mg petridish^{-1}) and seedling vigor index (746.7%) were also observed from T_1 of pink dye effluent. The maximum uptake of Pb, Fe, Mn and Zn was 0.48, 3.81, 0.79 and 0.13 μg g^{-1}, respectively. The uptake of Cr was below the detectable limit. Total heavy metal accumulation in rice was in the following order: Fe>Mn>Pb>Zn>Cr. Results showed that the higher concentration of loom-dye effluent showed the higher toxic effects on different parameters of germination and early seedling growth compared with the lower effluent concentrations.

Introduction

Pollution of the natural environment by industrial effluent has become a matter of major concern because of containing a variety of metallic pollutants with harmful chemical constituents of undesirable concentration. Being an agricultural country, industrialization in Bangladesh is occurring in an expanding stage. Recently, the deterioration of water resources with the rapid growth of industries (sugar, paper, tannery, textile and dyeing industries) in the country has come into the discussion. It generally disrupts the habitats of the living organisms when discharged into the environment without proper treatment (David and Rajan, 2015). Due to poor enforcement of existing laws, the discharge of industrial effluent into water bodies consequently entering into agro-environment is going to become a major threat for agricultural crop cultivation. Industrial effluents are considered as one of the most important factors responsible for the low productivity of crops causing serious pollution to water and soil systems (Otokunefor and Obiukwu, 2005; Konwar and Jha, 2010). The effluents may affect photosynthetic organisms (Coulibaly *et al*., 2003; Stolt *et al*., 2006; Jamali *et al*., 2007) because of its composition of complex mixture of waste substances namely, organochlorine-based pesticides, heavy metals, pigments and dyes, harmful gases, and several organic and inorganic compounds (Balaji *et al*., 2012; Rohit and Ponmurugan, 2013).

A dyeing industry having 10 tons of production capacity generates nearly 100 to 150 m^3 of wastewater per hour (Haque, 2008). Effluent from loom-dye industries is considered as an important pollutant mixing with organic and inorganic compounds, acids, alkalis, suspended solids, heavy metals like lead, cadmium, chromium, iron, manganese, zinc and other materials. The heavy metal contamination by the effluents ends up

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infiltrating ecological compartments contaminating agriculture fields and rivers. The continuous irrigation of agricultural land with the effluent wastewater causes heavy metal accumulation in the grown crops (Sharma *et al*., 2007; Marshall *et al*., 2007). When this effluent discharged into the watercourses, they restrict the light penetration and inhibit the activities of aquatic lives (Parameswari and Udayasooriyan, 2013). On the other hand, effluent produced from the loom-dyeing industries is also a source of irrigation water since it contains nutrients having the potential use in agricultural crop cultivation, especially in the area of water scarcity. But, this practice of using the effluents for irrigation purpose has been unexpected because of the adverse effects of them on plants depending on the crop varieties, types and concentrations of toxic materials present (Hassan *et al*., 2013). Rice (*Oryza sativa* L.) is one of the most important cereal crops providing food for nearly a half of the world population (Panich-pat and Srinives, 2009) and contributing with one-fifth of the calories consumed by human's worldwide (Welch and Graham, 2005). About 90 percent of the total rice is cultivated in Asia (Salim *et al*., 2003). Recently, some studies on the effects of different industrial effluents on germination and seedling growth of rice had been reported in different parts of the world (Pandey *et al*., 2008; Medhi *et al*., 2008; Samuel and Muthukkaruppan, 2011; Mahesh *et al*., 2013; Raju *et al*., 2015). To the best of our knowledge, in Bangladesh, no published report is found on the effects of loom-dye effluent on germination and early growth of rice. Therefore, this research work was designed to study the effects of loom-dye effluent collected from various loom-dyeing industries of Sirajganj on germination and early seedling growth of rice as a preliminary step before the field trials.

Materials and Methods

Loom-dye effluents were collected from different dyeing industries of *Bhangabari*, *Dhukuriabera and Rajapur* of Belkuchi under Sirajganj district in 2 L plastic containers. The collected dye effluent samples were analyzed for their physico-chemical properties (Table 1) in the laboratory of Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh as per the standard methods (APHA, 2012). The detailed procedure was followed as described by Rahman *et al*. (2018). Germination experiment was performed in Postgraduate Research Laboratory of the Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh during the period of August to October 2017 and total sixty-three petridishes were lined with double layer of filter paper. Prior to the experimental setup, the petridishes were sterilized properly and incubated at 28±2°C for germination in dark (ISTA, 1985). Rice *var.* BRRI dhan28 collected from Bangladesh Agricultural Development Corporation (BADC), Mymensingh, was chosen as a test crop for this study. Fifteen healthy and uniform rice seeds were selected and thoroughly washed with distilled water to remove dust and fungal traces followed by sterilization with 0.1% $HgCl_2$.

Table 1. Physico-chemical properties of used loom-dye effluent

Parameters	Effluent-1 from *Bhangabari*	Effluent-2 from *Dhukuriabera*	Effluent-3 from *Rajapur*
Colour	Pink	Violet	Black
Odour	Slightly pungent	Pungent	Very pungent
Temperature (°C)	36.5	37.0	37.0
pH	12.86	12.74	12.89
Electrical conductivity (EC) (µS cm^{-1})	782	2947	4380
Total dissolved solids (TDS) (mg L^{-1})	1320	3584	5382
Calcium (mg L^{-1})	28.06	46.10	30.06
Magnesium (mg L^{-1})	6.42	5.50	2.48
Potassium (mg L^{-1})	9.52	9.58	19.39
Sodium (me L^{-1})	70.95	88.06	109.22
Sulphate (mg L^{-1})	69.67	69.16	68.25
Phosphate (mg L^{-1})	17.85	21.45	38.28
Iron (mg L^{-1})	3.78	13.37	8.18
Zinc (mg L^{-1})	0.15	0.41	0.38
Manganese (mg L^{-1})	0.43	1.72	0.18
Copper (mg L^{-1})	0.07	0.49	0.17
Lead (mg L^{-1})	0.63	1.14	1.12
Cadmium (mg L^{-1})	0.013	0.009	0.007

Seven treatment levels *viz*., T_0, T_1, T_2, T_3, T_4, T_5 and T_6 with 0, 5, 10, 25, 50, 75 and 100% loom-dye effluent were used following completely randomized design (CRD) with three replications. Seedlings were harvested at 7 days after sowing (DAS) of seed. The germination percentage was measured using the following formula (Raun *et al*., 2002).

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds set for test}} \times 100$$

Germination energy and germination capacity were calculated in percentage of germinated seed at 3 and 7 DAS, respectively (Bam *et al*., 2006). Germination speed was measured by using the following formula (Krishnaswamy and Seshu, 1990).

Speed of germination (%)

$$= \frac{\text{Number of seed germinated at 72h}}{\text{Number of seed germinated at 168h}} \times 100$$

Relative toxicity of dye effluent on the germination over control was calculated by using the following formula (Chapagain, 1991).

$$\text{Relative Toxicity (\%)} = \frac{x - y}{x} \times 100$$

Where, x = Germination percentage in control at a particular hour of incubation; y = Germination percentage in the presence of effluent at the same hour of incubation.

Phytotoxicity of the dye effluent on seedling growth was calculated by using the following formula (Chou and Lin, 1976).

Phytotoxicity (%)

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

Seedling vigor index was calculated using the formula proposed by Abdul-Baki and Anderson (1973).

Vigor index = Germination percentage × Length of seedling

The seedlings were collected from each treatment and their fresh weights were measured. Then seedlings were dried in an oven at 60 ºC for 24 hours to measure dry weights. The results were presented as means ± SE (standard error of means) and these were analyzed using SPSS (20.0) and Microsoft Excel program.

Results and Discussion

Effects of loom-dye effluent on seed germination

Rice seed showed the significant difference of germination energy and capacity with varying level of effluent application at 3 and 7 DAS, respectively (Table 2). The maximum germination rate (100%) at 3 DAS was recorded from control treatment (T_0) followed by T_1 of pink and black dye effluent. The highest effluent concentration (T_6) resulted in the minimum germination percentage at 3 DAS. The increasing rate of effluent application significantly affected germination capacity of rice. The highest seed germination capacity (100%) was recorded at control and T_1 of the effluents whereas the lowest germination at 7 DAS was found from T_6 treatment of black dye effluent. Black dye effluent inhibited the germination capacity more than other effluents with the increased level of effluent application. The germination percentage of this study was supported by the findings of Mahesh *et al*. (2013) who found a decreasing rate of rice seed germination ranging between 100.00 – 41.50% among five cultivars. Previous researchers also found the same trend of germination percentage with the increasing levels of effluent (Singh *et al.*, 2007; Pandey *et al.*, 2008; Samuel and Muthukkaruppan, 2011; Rahman *et al.*, 2018).

Table 2. Effects of loom-dye effluent on seed germination parameters of rice (mean±SE)

Effluent	Germination energy (3 DAS)			Germination capacity (7 DAS)			Germination Speed		
Conc. (%)	Pink	Violet	Black	Pink	Violet	Black	Pink	Violet	Black
T_0	97.8±2.7	97.8±2.7	97.8±2.7	100±0.0	100±0.0	100±0.0	97.8±2.2	97.8±2.2	97.8±2.2
T_1	95.6±7.2	91.1±3.3	95.6±6.0	100±0.0	100±0.0	100±0.0	95.6±2.2	93.2±0.2	95.6±2.2
T_2	91.1±4.4	88.9±4.4	91.1±2.9	97.8±5.0	97.8±4.4	97.8±6.7	93.2±0.2	88.9±4.4	93.3±3.8
T_3	88.9±5.1	84.4±3.3	4.4±2.2	95.6±2.9	93.3±4.4	8.9±2.2	93.2±3.9	97.8±5.3	50.0±2.8
T_4	84.4±1.7	82.2±2.9	2.2±1.6	95.6±2.9	86.7±3.8	6.7±1.7	88.4±2.2	88.2±2.1	33.3±3.3
T_5	31.1±2.2	26.7±2.2	0±0.0	44.4±2.2	37.8±2.9	4.4±1.7	70.6±7.6	71.1±10.6	0.0±0.0
T_6	8.9±1.7	0±0.0	0±0.0	13.3±1.7	8.9±2.2	0±0.0	66.7±16.7	0.0±0.0	0.0±0.0

Legend: T_0= 0, T_1= 5, T_2= 10, T_3= 25, T_4= 50, T_5= 75 and T_6= 100 % loom-dye effluent

Suppression of germination might be caused due to the reduction of water absorption by seeds with high concentrations of effluent which ultimately affect the energy forming compounds (Kannan and Upreti, 2008) and the presence of a higher amount of total solids and heavy metals (Palanivel *et al*., 2004; Malla and Mohanty, 2005).

Effects of loom-dye effluent on germination speed

Types of effluents and their concentrations affected the germination speed of rice seed significantly (Table 2). Seed germination speed was inhibited by the increasing concentration of loom-dye effluent in comparison with control. The highest germination speed (97.8%) was obtained from control whereas the lowest germination speeds were found from T_5 and T_6 of black and T_6 of violet effluent. The speed of germination increased up to T_3 of pink dye effluent and was decreased with the increasing level of concentration of other effluents. A similar result was found by Rahman *et al*. (2018) in case of seed germination speed of red amaranth.

Relative toxicity of loom-dye effluent on seed germination

Relative toxicity of loom-dye effluents on seed germination of rice at 3 and 7 DAS have been presented in Fig. 1. Control treatment showed no toxicity. Among the treatment, the minimum relative toxicity was found with T_1 and the maximum was from 100% concentration (T_6) of the effluent. Above T_2, the relative toxicity was significantly increased and maximum relative toxicity was obtained from black dye effluent. Similar results of toxicity of different effluents were also found previously (Rani and Alikhan, 2007; David and Rajan, 2015; Rahman *et al*., 2018).

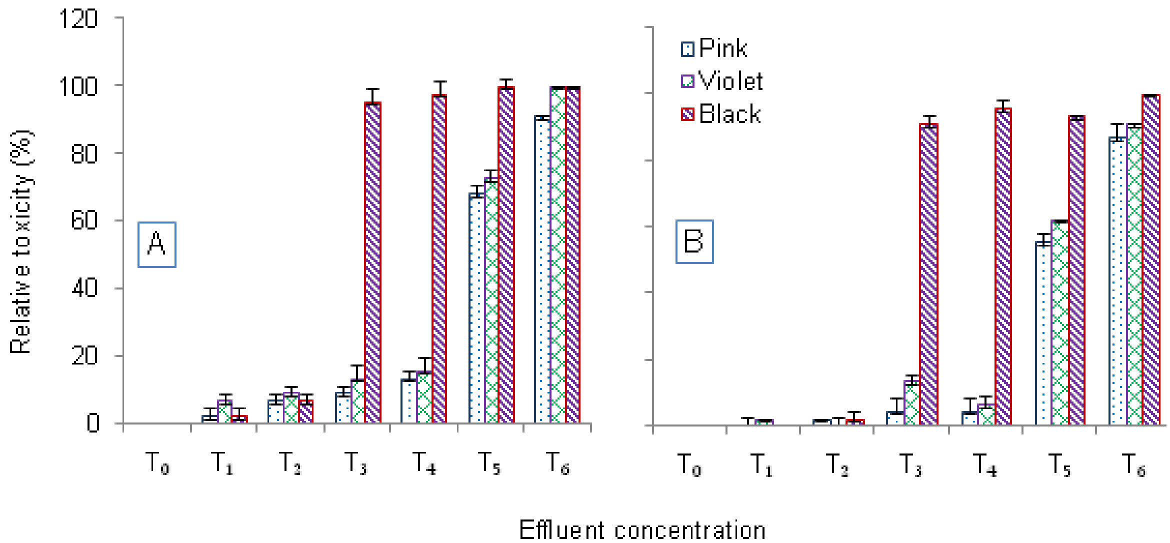

Fig. 1. Relative toxicity of loom-dye effluent on rice seed germination at 3 DAS (A) and 7 DAS (B). The error bars indicate the standard error of means

Effects of loom-dye effluent on radical and plumule lengths

The lengths of radical and plumule of rice varied with effluent types and their different concentrations (Fig. 2). The lengths of radical and plumule showed a significant reduction at 7 DAS with the increasing levels of the effluent. The maximum radical length (6.4 cm) and plumule length (7.5 cm) were observed with T_1 of pink dye effluent and the minimum lengths of radical and plumule were obtained from T_6 of the effluents and no radical was produced from T_6 of black dye effluent. The study showed clear inhibition of plumule and radical lengths, however, the radical length was more affected than the plumule by the application of comparatively higher concentrations of effluents (Oncel *et al*., 2000). The germinated seeds did not get enough oxygen for the toxicity of effluent solution and the radical continuously remained in direct contact with the effluent might be responsible for affecting cell multiplication or the growth (Kannan and Upreti, 2008). The lower effluent concentration might promote the growth because of containing plant nutrients (Augusthy and Annsherin, 2001). This result was in line with the findings of Mahesh *et al*. (2013) who obtained a decreasing length of radical (23.43–0.90 cm) and plumule (16.40–2.20 cm) of five paddy cultivars. Pandey *et al*. (2008) studied the toxicity level of distillery effluent on the early growth of rice and maize, and found a decreasing rate of root and shoot lengths with increasing effluent concentration. Many researchers observed inhibitory effects of various effluents on radical and plumule length of different plant species (Singh *et al*., 2006; Nawaz *et al*., 2006; Vijayaragavan *et al*., 2006; Yousaf *et al*., 2010; Vaithiyanathan and Sundaramoorthy, 2017; Rahman *et al*., 2018).

Effects of loom-dye effluent on seedling fresh and dry weights

The fresh and dry weights of rice seedling increased with lowering of the effluent concentration up to T_1 and decreased with higher concentration (Table 3). The highest fresh weight (39.8 mg petridish^{-1}) and dry weight (5.7 mg petridish^{-1}) were observed from T_1 of pink dye effluent. Black dye effluents lowered the fresh and dry weight of the seedlings significantly than others. The minimum fresh weight and dry weights were recorded from T_6 of the effluent. This result was in conformity with previous reports (Sarathchandra *et al*., 2006; Rani and Alikhan, 2007; Vaithiyanathan and Sundaramoorthy, 2017; Rahman *et al*., 2018). The promotion of seedling growth by lower concentration of effluents might be due to creating a favorable environmental condition for the germination utilizing the nutrients present in the effluent (Augusthy and Annsherin, 2001; Suresh *et al*., 2014; Vinod, 2014).

Phytotoxicity of loom-dye effluent on seedling growth

The increased levels of effluent concentrations inhibited the seedling growth of rice significantly (Table 3). The effluent application at the concentrations of $\geq T_2$ inhibited the total dry matter production, plumule and radical lengths of rice seedlings. The maximum phytotoxicity was observed with the treatment T_6 of black and the minimum was recorded from control. Except control, minimum phytotoxicity was obtained from T_1 followed by T_2 irrespective of the effluents. The highest phytotoxicity was observed from black dye effluent whereas, pink dye effluent showed less phytotoxicity. Due to the high pH, EC, TDS and metallic contents of loom-dye effluent, high level of phytotoxicity might be occurred. Similar toxic effects of industrial effluent were observed by David and Rajan (2015) and Rahman *et al*. (2018).

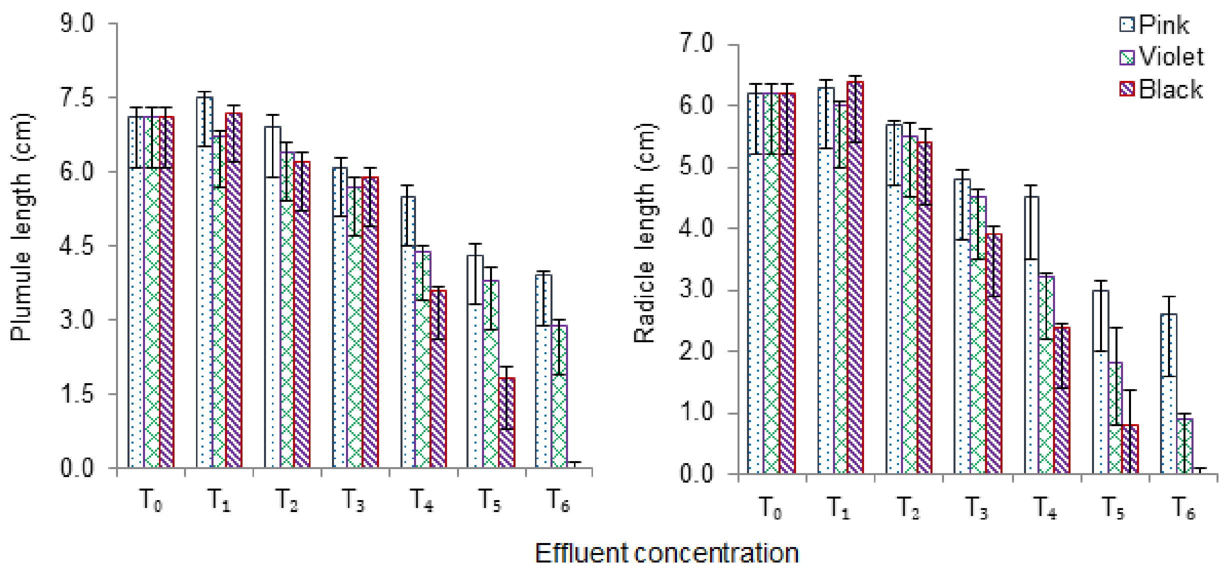

Fig. 2. Effects of loom-dye effluent on plumule and radical lengths of rice. The error bars indicate the standard error of means

Table 3. Effects of loom-dye effluent on seedling growth parameters of rice and phytotoxicity of the effluents (mean±SE)

Effluent	Fresh weight (mg petridish^{-1})			Dry weight (mg petridish^{-1})			Phytotoxicity (%)		
Conc. (%)	Pink	Violet	Black	Pink	Violet	Black	Pink	Violet	Black
T_0	39.3±1.13	39.3±1.13	39.3±1.13	5.5±0.31	5.5±0.31	5.5±0.31	0.0±0.00	0.0±0.00	0.0±0.00
T_1	39.8±0.94	35.5±1.34	34.6±1.30	5.7±0.29	5.1±0.13	5.2±0.21	3.8±1.97	2.2±1.45	6.6±4.13
T_2	37.9±0.88	32.8±1.75	33.2±1.99	5.3±0.47	4.7±0.52	4.8±0.58	7.1±1.09	9.8±3.79	11.5±0.95
T_3	32.4±2.44	28.7±1.10	28.5±1.23	4.7±0.59	4.1±0.38	4.1±0.42	20.8±2.38	26.8±2.38	36.1±2.50
T_4	28.4±2.38	25.1±0.72	25.7±2.26	4.1±0.54	3.6±0.32	3.8±0.55	26.2±3.41	47.5±0.95	60.1±2.38
T_5	22.2±1.44	17.6±1.12	14.3±0.67	3.2±0.34	2.5±0.24	2.06±0.21	50.3±2.38	71.0±9.29	86.9±3.28
T_6	17.1±0.82	9.2±1.97	0.0±0.00	2.5±0.19	1.35±0.21	0.0±0.00	57.9±4.76	84.2±5.21	100.0±0.00

Legend: T_0= 0, T_1= 5, T_2= 10, T_3= 25, T_4= 50, T_5= 75, and T_6= 100 % loom-dye effluent

Effects of loom-dye effluent on seedling vigor index

The decreased vigor index (SVI) of rice seedling observed with the increasing level of effluent concentrations has been presented in Fig. 3. The highest seedling vigor index (746.7%) was observed with T_1 of pink dye effluent whereas, the lowest was recorded from T_6 of the effluents at 7 DAS. The variation of SVI might be due to the stress levels of different effluent concentrations. Black and violet effluents imposed more inhibition on SVI than the pink dye effluent. Previous reports also showed higher SVI with a lower effluent concentration (Rani and Alikhan, 2007; Singh *et al.*, 2007; Rahman *et al*., 2018).

Effects of loom-dye effluent on heavy metal uptake by seedlings

Heavy metal uptake by rice seedlings has been presented in Fig. 4. The loom-dye effluent collected from different industries showed increased heavy metal accumulation in seedlings compared with fresh water treatment. Increased uptake of heavy metals was found with increasing effluent concentrations up to ≤ T_5 of dye effluents. It could be due to the adverse effect of the higher concentration of effluents on plant cells which impaired the tissues leading to lower uptake of metals (Balakrishnan *et al*., 2008; Farook *et al*. 2008; Yousuf *et al*. 2010; Oguntade *et al*., 2014).

Lead (Pb) contents in rice seedlings varied significantly with different doses of effluent application. The highest Pb content (0.48 µg g^{-1}) in rice seedling was obtained from T_5 of the black dye effluent and the lowest from the control. This was comparable to average Pb concentrations of 0.25 µg g^{-1} in field rice sites in China (Williams *et al*. 2012). The highest Fe uptake was 3.81 µg g^{-1}, which was obtained from seedlings grown with T_4 of violet dye effluent. The maximum Mn and Zn uptakes were 0.79 and 0.13 µg g^{-1}, respectively obtained from T_3 of violet dye effluent. The uptake of Cr was completely absent in all the treatments. The order of total heavy metal accumulation in the crop was in the following order: Fe>Mn>Pb>Zn>Cr.

This finding was in line with the previous reports (Kisku *et al*., 2000; Echem, 2014; Farooq *et al*., 2008; Singh *et al*., 2010; Oguntade *et al*., 2014). The concentrations Cr, Mn, Pb and Zn in typical agricultural crops were from 0.2–1.0, 15.0–100.0, 0.10–10.0, 15.0–200.0 mg kg^{-1} dry weight, respectively proposed by Allaway (1968).

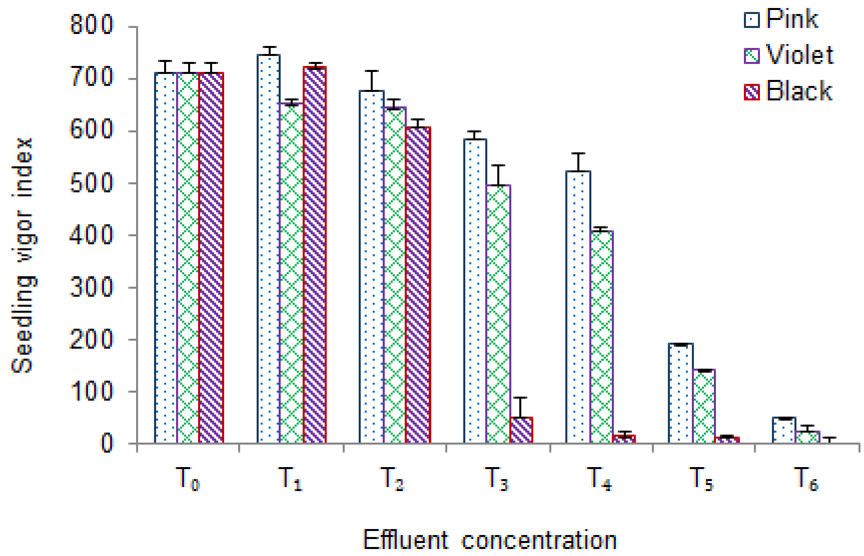

Fig. 3. Effects of loom-dye effluent on vigor index of rice seedling. The error bars indicate the standard error of means

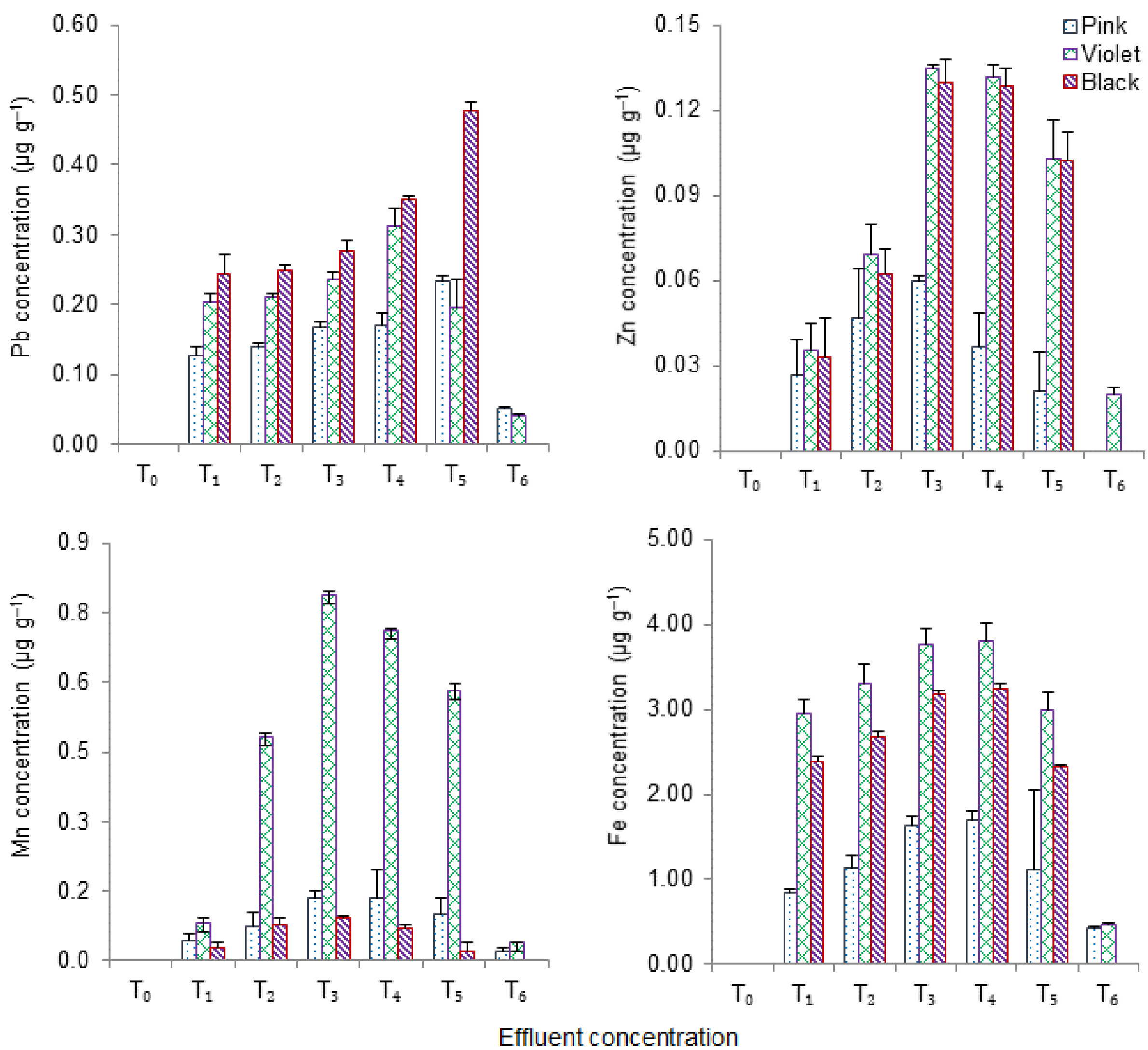

Fig. 4. Heavy metal accumulations of rice seedlings harvested at 7 DAS. The error bars indicate the standard error of means

Conclusion

Based on the experimental observations, it was found that the declined rate of germination percentage, germination speed, radical length, plumule length and seedling vigor index were found with increasing effluent concentrations. The pink dye effluent having lower EC and TDS comparatively imposed a less adverse effect on different parameters. The overall results suggested that the loom-dye effluent should not be used for seed germination and early seedling growth of rice without proper treatment.

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