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EFFECTS OF INDUSTRIAL EFFLUENTS ON SEED GERMINATION AND GROWTH OF RADISH

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Abstract

The research work was conducted from October to December 2016 on the rooftop of Agriculture Faculty Building of Sylhet Agricultural University, Sylhet to assess the effects of industrial effluents on seed germination, seedling mortality and growth of radish (*Raphanus sativus* L.). Effluents were collected from common dumping-off areas of Narsingdi, Tongi, Hazaribagh, Alampur, Khadimnagar, Majortilla, Bhatiary, Bayazid bostami and Sagorika under Dhaka, Sylhet and Chittagong division of Bangladesh. Maximum concentration of Pb (2.038 mgl^{-1}), Cd (0.082 mgL^{-1}), Ni (0.237 mgl^{-1}) and Cr (0.172 mgl^{-1}) were obtained in Hazaribagh industrial effluents; Fe (7.171 mgl^{-1}) and Zn (1.938 mgl^{-1}) were highest in Khadimnagar. Both experiments were laid out in a CRD with three replications. Radish seedlings were significantly affected by the application of different industrial effluents. Maximum seedling mortality (22.78%) at 15 DAS was observed in Khadimnagar industrial effluents, whereas zero seedling mortality was observed in control. Shoot and root length of radish also affected by effluents. Due to toxicity of heavy metals shoot length (36.97%) and root length (61.38%) were reduced compared to control along with decrease in other growth parameters of radish. Maximum number of leaf $plant^{-1}$, leaf length, leaf width, fresh weight and dry weight were recorded in control whereas minimum values was recorded when Khadimnagar industrial effluents used as irrigation water followed by Hazaribagh. The overall results indicated that Khadimnagar and Hazaribagh industrial effluents were significantly contaminated with heavy metals namely Pb, Cd, Ni, Cr, Fe and Zn which have toxic effects on radish growth.

***Keywords*:** Industrial discharges, heavy metals, seedling mortality, development

Introduction

Bangladesh is a developing country, where massive urbanization, rapid rate of industrialization and intensive agriculture has accelerated the modernization of society. There is a progressive increase in industrial effluents due to the rapid industrialization; such effluents have been causing severe contamination to the nearby water and soils, and consequently polluting the environment. About 300 industries in Bangladesh including textile, garments, dandy-dyeing, plastics, metal fabrications, leather, tanning, BSCIC, steel mill discharge wastes and effluents, which containing heavy metals such as Pb, Cd, As, Zn, Cr, Sr, Ni, Li, Ag, Hg, Co and Se (Ahmed *et al.*, 2011). In most cases industries drained their effluents and wastewater without or any partial treatment into the nearby river or channel. As a result, the river water is badly polluted by these industrial discharges and thereby causes water pollution and led to gradual deterioration of its quality (Khaleel *et al.*, 2013). In Bangladesh river water largely used to irrigate agricultural crop field. Due to scarcity of freshwater, industrial effluents are used as an alternate water resource for irrigation of crops, mainly in peri-urban areas, due to its easy availability (Kumar *et al*., 2013; Vijayaragavan *et al*., 2011). Agricultural lands across the world are contaminated by heavy metals such as As, Cd, Cu, Cr, Ni, and Pb due to industrial effluents and faulty irrigation practices (Yadav, 2010). Increase in concentration of industrial effluents in irrigation water can cause a decrease in germination percentage of crops (Saravanamoorthy, 2007), inhibit seedling growth of crops and functions of essential enzymes (Nagada *et al*., 2006; Yousaf *et al*., 2010) also result in delayed fruiting and low yield (Uaboi-Egbenni *et al*., 2009). Excessive accumulation of heavy metals in agricultural soils through irrigation of untreated effluents, may not only cause soil contamination, but also lead to elevated heavy metal uptake by crops and thus affect food safety and quality (Muchuwetii *et al.*, 2006). The contamination of agricultural ecosystems by heavy metals has gained attention due to its effects on crop quality and human health (Kumar *et al*., 2013). Heavy metals accumulate in edible parts of leafy vegetables (Sinha *et al*., 2007). Vegetables are an important part of human diet as they are rich sources of vitamins, minerals, and fibers with beneficial antitoxidative effects. When vegetables are grown in contaminated soils, they can accumulate genotoxic compounds (Mathur *et al*., 2006) which constitutes a potential risk to animals and human health. There are few researches on effects of industrial effluents on vegetable crops in

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context of Bangladesh. Therefore, the study was undertaken to assess the effects of industrial effluents on seed germination, seedling mortality and vegetative growth of radish (*Raphanus sativus* L.) irrigated through industrial effluents or wastewater.

Materials and Methods

Collection and analyses of industrial effluents

Industrial effluents were collected from 9 (nine) common dumping-off areas of different industries of Dhaka, Sylhet and Chittagong division under the sub-locations viz. Narsingdi, Tongi, Hazaribagh, Alampur, Khadimnagar, Majortilla, Bhatiary, Bayazid bostami and Sagorika of Bangladesh. Effluents were collected in 30-L plastic containers at the points of discharge. The collection of samples was done following methods of APHA (2005). Subsamples were taken from all the samples for laboratory analyses. For the determination of Pb, Cd, Ni, Cr, Fe and Zn in effluents were read on atomic absorption spectrophotometer (AAS; iCE 3300, Thermo Fisher Scientific, USA). All analytical studies were done following the procedures as described by APHA (2005).

Experimental design and preparation of Petri dish

The experiment was designed in a Completely Randomized Design (CRD) with three replications from 20 October to 3 November 2016 at Sylhet Agricultural University, Sylhet, Bangladesh. Total number of treatments were 10 (ten); including 9 (nine) industrial effluents and control (Hoagland solution). The Hoagland solution is a hydroponic nutrient solution which provides all of the essential nutrients for plant growth and development (Hoagland and Arnon, 1950). The germination test of radish (BARI Mula-4) was conducted using the paper towel method in Petri dish as prescribed in ISTA rules (Arnon, 1999) by providing the optimum conditions. Total 30 sterilized glass petri dishes having the size of 125 mm × 15 mm were used and lined with filter papers moistened according to the treatments. Seeds of radish were sterilized by soaking in 0.1% $HgCl_2$ solution for 5 minutes and then washed with running tap water followed by deionized water. Then in each Petri dish, there were 40 (forty) seeds placed with maintaining minimum space. Petri dishes were irrigated with treatments at regular intervals after placing of seed.

Seedling test parameter

Seedling test parameters including seed germination (%), seedling mortality (%), shoot length (cm) and root length (cm) were recorded.

The equation used to calculate the germination percentage (%) of seed was;

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

The equation used to calculate the mortality percentage (%) of seedling was;

$$\text{Mortality percentage (\%)} = \frac{\text{Number of dead seedlings}}{\text{Total number of seedlings}} \times 100$$

Pot experiment

This experiment was also designed in a Completely Randomized Design (CRD) with three replications from 15 November to 31 December 2016 at Sylhet Agricultural University, Sylhet, Bangladesh. Same treatments were used as experiment no. 1. Total 30 plastic pots were collected having the size of 80 mm × 110 mm. Initial sand sample for radish cultivation was collected from nearby river. Then the sand was washed several times by distilled water to free of ionic constituents present in sand. After that, sand was air dried and sieved through a 2 mm mesh sieve and kept in a clean plastic bag. Plastic pots were filled with 0.5 kg of prepared sand and arranged maintaining sufficient spacing. Fifteen days old healthy seedlings of radish were transplanted in prepared pot. Before transplanting, radish seedlings were grown in a different seed bed where Hoagland solutions were applied as a nutrient solution for seedling growth. After that effluents were applied to each pot as per treatment. The irrigation was continued until the crops were matured for harvesting.

Growth parameter observed

Number of leaf, leaf length and width, fresh and dry weight of shoot and root of 40 day old Radish plant from each treatment was measured.

Chemical analyses of plant sample

The harvested plant samples were cleaned thoroughly with tap water and rinsed with 0.1 M HCL solution, followed by several rinses with distilled water for chemical analyses. Samples were dried in oven at 80ºC for about 72 hours. The dried plant samples were ground with a mechanical grinder and stored in polythene zipper bags separately. Then samples were digested by following procedure outlined by Tandon (1995) was used. Determination of different heavy metal content in aliquot were determined using atomic absorption spectrophotometer (iCE 3300, Thermo Fisher Scientific, USA) using the reagent blank.

Statistical analyses

The statistical analyses of the seedling test and growth data was done by using computer package program R software (Version 3.4.3). No statistical analysis was done for metals present in effluent samples and metals uptake by plant.

Results and Discussion

Concentration of heavy metals in industrial effluents

The industrial effluents used for irrigation were analyzed for its various heavy metal concentration and the results are shown in Table 1 as the average values of nine samples. The maximum concentration of Pb (2.038 mgl^{-1}) was observed in the effluents of Hazaribagh and the minimum amount of Pb (0.001 mgl^{-1}) was found in Major tilla industrial effluents. It was found that Pb in the sampling area was below the permissible limits (<5.0 mgl^{-1}) in effluents used for irrigation recommended by Ayers and Westcot (1985). According to Ayers and Westcot (1985), maximum recommended concentration of Cd in water used for irrigation was 0.01 mgl^{-1}. Considering this value as standard, among the samples, Hazaribagh industrial effluents (0.082 mgl^{-1}) exceeded the limit which might be not safe for long-term irrigation. The highest concentration of Ni (0.237 mgl^{-1}) and (Cr 0.172 mgl^{-1}) were observed in Hazaribagh industrial effluents which is exceeded the permissible limit among all samples and the lowest concentration was observed in Majortilla (0.035 mgl^{-1}) and Narsingdi (0.01 mgl^{-1}). The permissible limits of Ni and Cr in water used for irrigation are 0.20 and 0.10 mgl^{-1} (Ayers and Westcot, 1985). Maximum Fe (7.171 mgl^{-1}) and Zn (1.938 mgl^{-1}) concentration was found in Khadimnagar industrial effluents and minimum concentration of Fe (0.249 mgl^{-1}) and Zn (0.052 mgl^{-1}) was found in Hazaribagh and Bhatiary industrial effluents. Among the nine locations it was found that Fe concentration in Khadimnagar industrial effluents exceed the maximum recommendation limit (5.0 mgl^{-1}) and the Zn concentration in all sampling area was below the permissible limits (2.0 mgl^{-1}) recommended by Ayers and Westcot (1985).

Table 1. Heavy metal concentration in different Industrial effluents (mgl^{-1})

Location	Pb	Cd	Ni	Cr	Fe	Zn
Narsingdi	0.907±0.01	0.005±0.0	0.112±0.02	0.010±0.06	0.773±0.05	0.704±0.05
Tongi	0.041±0.01	0.009±0.0	0.042±0.01	0.011±0.04	0.282±0.04	0.801±0.02
Hazaribagh	2.038±0.03	0.082±0.001	0.237±0.03	0.172±0.06	0.249±0.04	0.863±0.04
Alampur	0.031±0.02	0.004±0.0	0.043±0.0	0.011±0.02	3.517±0.09	0.599±0.02
Khadimnagar	0.244±0.02	0.002±0.0	0.141±0.001	0.011±0.02	7.171±0.21	1.938±0.05
Majortilla	0.001±0.0	0.003±0.0	0.035±0.05	0.013±0.02	2.905±0.08	0.202±0.01
Bhatiary	0.010±0.0	0.003±0.0	0.100±0.04	0.012±0.01	1.515±0.03	0.052±0.01
Bayazid bostami	0.535±0.01	0.001±0.0	0.045±0.06	0.014±0.01	0.986±0.02	1.005±0.05
Sagorika	0.042±0.001	0.016±0.001	0.205±0.05	0.012±0.03	0.986±0.03	0.122±0.06

Seed germination

Seed germination of radish at different DAS was affected by industrial effluents is presented in Table 2. In case of all observations 1, 2, 3, 4 and 5 DAS maximum seed germination (88.67, 89.85, 91.67,92.22 and 92.72%, respectively) were observed in Control and minimum seed germination (0.00, 6.11, 52.56, 71.89 and 71.89%, respectively) was found when Khadimnagar industrial effluents were used as irrigation water followed by Hazaribagh industrial effluents. All the treatments, seeds were exhibited a successful germination. But the rate of germination was found different with various industrial effluents. At high concentration of numerous heavy metal constituents in effluents, there might be decrease of seed germination.

Heavy metals such as Pb, Ni, Cr, Fe, Cd, Cu and Zn in high concentration results in reduced seed germination of vegetable crops (Jadoon *et al.*, 2013). Ejraei (2013) and Shafiq *et al.* (2008) stated that Pb inhibits germination of seeds and decreases germination percentage of crops.

Table 2. Effects of different industrial effluents on seed germination of radish

Treatment	Seed Germination (%)				
	1DAS	2DAS	3DAS	4DAS	5DAS
Narsingdi	79.44 c	80.00 c	82.78 b	83.67 c	83.67 c
Tongi	86.44 ab	87.78 ab	90.56 a	90.56 b	90.56 b
Hazaribagh	76.11 c	77.22 c	78.89 c	78.89 d	78.89 d
Alampur	85.55 ab	85.55 b	88.33 a	88.33 b	88.33 b
Khadimnagar	0.00d	6.11 d	52.56 d	71.89 e	71.89 e
Majortilla	86.11 ab	86.67 b	88.89 a	88.89 b	88.89 b
Bhatiary	85.56 ab	86.67 b	88.89 a	88.89 b	88.89 b
Bayazid bostami	86.89ab	88.33 ab	90.55 a	90.55 b	90.55 b
Sagorika	85.00 b	86.11 b	88.89 a	88.89 b	88.89 b
Control	88.67 a	89.85 a	91.67 a	92.22 a	92.22 a
CV%	2.60	2.14	2.58	1.68	1.68
LS	**	**	**	**	**

In a column, figure(s) followed by the same letter(s) do not differ significantly at 1% level of significance. CV = Coefficient of variation, LS = Level of Significance, ** = Significant at 1% level of probability.

Seedling mortality and industrial effluents

The effects of industrial effluents on seedling mortality of radish at 9, 12 and 15 DAS was significantly different from one treatment to another treatment showed in Table 3. There were no effects of industrial effluents on seedling mortality of Radish at 3 and 5 DAS. At 9, 12 and 15 DAS, maximum seedling mortality (11.11, 15.56 and 22.78%) was observed in Khadimnagar industrial effluents, which is followed to Hazaribagh industrial effluents at 12 and 15 DAS (7.78 and 12.78%). On the other hand, lowest seedling mortality (0.0%) was found in Control for all observation. Seedling mortality was increased in Khadimnagar industrial effluents followed by Hazaribagh with day by day which might be toxicity of heavy metals like Pb, Ni, Fe etc. Under Fe stress, the inhibition of germination was observed (Rasafi *et al.*, 2016) and accumulation of toxic heavy metals in plant living cells results in various deficiencies, reduction of cell activities, stunted of plant growth (Shafiq *et al.*, 2005).

Shoot and Root length

The shoot and root length of radish seedlings at 15 DAS under different industrial effluents are represented in Fig. 1. Shoot and Root length were higher in Control compared to other treatment. Shoot length (12.12 cm) of Radish observed maximum in Control and minimum (7.64 cm) in Khadimnagar industrial effluents, decreased with 36.97%. On the other hand, highest root length (7.64 cm) was found in Control and lowest (2.95 cm) was observed due to use of Khadimnagar Industrial effluents as irrigation water in Radish, which is decreased with 61.38%, followed by Hazaribagh industrial effluents 39.52% compared to Control. Generally, the reduction in shoot and root length could be due to the adverse effects of heavy metal toxicity of industrial effluents. Mishra and Choudhari (1998) observed that Pb inhibits the growth of seedlings; decreased root and shoot length. This result is in agreement with the findings of Juwarkar and Shende (1986) that Pb also inhibits root and stem elongation *Raphanus sativa*. Under Fe stress condition plants suffer increased oxidative damage, shoot and root length was decreased as the concentration of Fe increased (Verma and Pandey, 2015).

Table 3. Effects of different industrial effluents on seedling mortality in radish

Treatment	Seedling Mortality (%)				
	3DAS	6DAS	9DAS	12DAS	15DAS
Narsingdi	0.00	0.00	2.78 cd	3.89 cd	7.22 c
Tongi	0.00	0.00	2.78 cd	5.00 c	7.78 c
Hazaribagh	0.00	0.00	3.89 c	7.78 b	12.78 b
Alampur	0.00	0.00	3.33 cd	3.33 cd	6.67 c
Khadimnagar	0.00	0.00	11.11 a	15.56 a	22.78 a
Majortilla	0.00	0.00	2.22 cd	3.89 cd	5.00 d
Bhatiary	0.00	0.00	1.67 de	2.22 d	4.44 d
Bayazid bostami	0.00	0.00	3.89 c	4.44 c	7.78 c
Sagorika	0.00	0.00	6.11 b	7.22 b	7.33 c
Control	0.00	0.00	0.00 e	0.00 e	0.00 e
CV%	0.00	0.00	30.49	18.44	11.26
LS	-	-	**	**	**

In a column, figure(s) followed by the same letter(s) do not differ significantly at 1% level of significance.
CV = Coefficient of variation, LS = Level of Significance, ** = Significant at 1% level of probability.

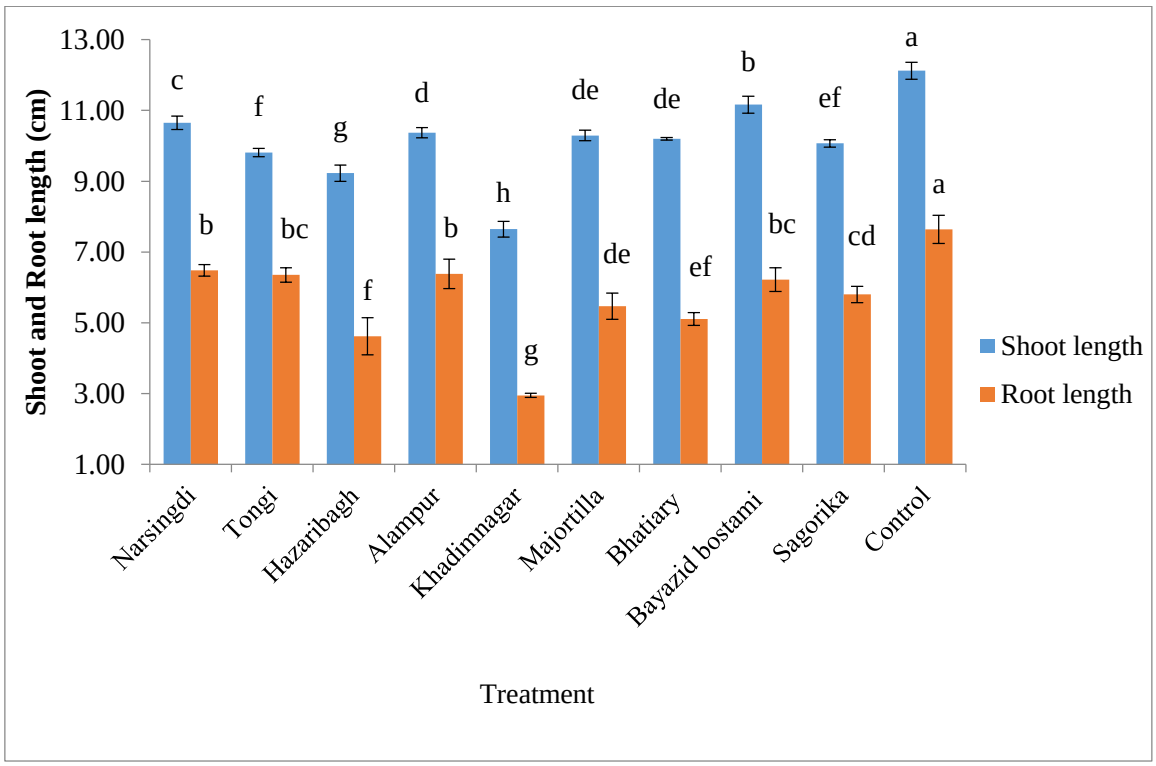

Fig. 1. Effects of industrial effluents on shoot and root growth in radish.

Table 4. Effects of different industrial effluents on number of leaves, leaf length and leaf width in radish

Treatment	Number of leaf plant^{-1}		Leaf length (cm)		Leaf width (cm)	
Narsingdi	10.89	bc	14.2	bc	3.84	b
Tongi	9.44	cd	14.07	bc	3.39	cd
Hazaribagh	8.89	d	12.93	d	3.04	de
Alampur	9.84	b-d	13.68	b-d	4.01	ab
Khadimnagar	6.22	e	9.61	e	2.95	e
Majortilla	10.22	b-d	14.57	ab	4.01	ab
Bhatiary	10.44	b-d	14.23	bc	3.93	ab
Bayazid bostami	11.22	ab	13.96	bc	3.65	bc
Sagorika	10.56	b-d	13.35	cd	3.73	bc
Control	12.67	a	15.46	a	4.26	a
CV%	9.96		3.85		6.14	
LS	**		**		**	

In a column, figure(s) followed by the same letter(s) do not differ significantly at 1% level of significance. CV = Coefficient of variation, LS = Level of Significance, ** = Significant at 1% level of probability.

Treatment	Pb	Cd	Ni	Cr	Fe	Zn
Narsingdi	0.016	0.002	0.034	0.001	145.32	33.34
Tongi	0.003	0.002	0.006	0.001	105.41	38.21
Hazaribagh	0.720	0.013	0.069	0.075	102.5	41.02
Alampur	0.012	ND	0.006	0.001	181.11	24.04
Khadimnagar	0.021	0.003	0.051	0.001	351.72	68.48
Majortilla	ND	ND	0.007	0.003	175.01	17.88
Bhatiary	ND	0.001	0.045	0.001	142.35	9.26
Bayazid bostami	0.012	ND	0.001	0.002	147.22	39.74
Sagorika	0.010	ND	0.024	0.002	137.22	16.86
Control	ND	ND	ND	ND	129.44	18.72

Table 5. Mean concentrations (mg/kg) of heavy metals in radish shoot irrigated with industrial effluents
ND=Not Detected

Number of leaf, leaf length and leaf width

Number of leaf, leaf length and leaf width of radish at 40 DAS under different industrial effluents are represented in Fig. 4. All observation was higher in Control compared to other treatment. Number of leaf plant^{-1} (12.67), leaf length (15.46 cm) and leaf width (4.26 cm) of radish were highest in Control and lowest in Khadimnagar industrial effluents (Number of leaf plant^{-1} 6.22, leaf length 9.61 cm and leaf width 2.95 cm) decreased with 50.91, 37.84 and 30.75%, respectively followed by Hazaribagh industrial effluents 29.83, 16.36 and 28.63% compared to Control. This indicated that increased effluent concentrations in irrigation water reduced growth in radish. Niroula (2003) has reported, reduced crop performance in response to high concentrations of industrial effluents of different sources and types. Increasing levels of Pb concentration affect leaf growth (Kevresann *et al*., 2001) and inhibit leaf expansion of *Raphanus sativus* (Juwarkar and Shende, 1986). Higher level of Zn showed a significant decrease in leaf area (Chaves *et al*., 2011).

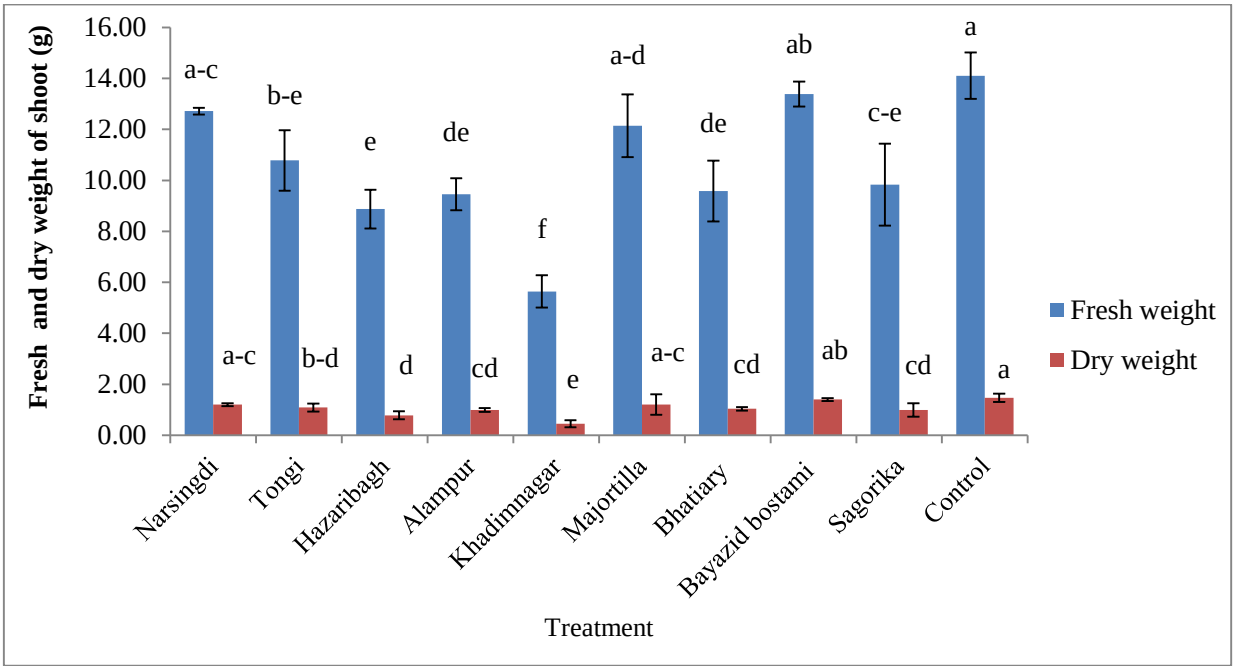

Fig. 2. Effects of industrial effluents on shoot fresh and dry weight in radish

Shoot fresh and dry weight

The effects of industrial effluents on fresh and dry weight of shoot of Radish at 40 days found significant difference from one treatment to another treatment showed in Fig. 2. Maximum fresh weight (14.11 g) and dry weight (1.46 g) was obtained in Control. Whereas, minimum fresh weight (5.64 g) and dry weight (0.45 g) was found in plants irrigated with Khadimnagar industrial effluents decreased with 60.03 and 69.18% fresh and dry weight respectively followed by Hazaribagh industrial effluents 37.13 and 46.58% compared to Control. Effluents from textile, garments, dandy-dyeing, leather, tanning and various industries contain heavy metals that have negative impacts on vegetative growth. Jadoon *et al.* (2013) reported that untreated textile effluents decrease biomass of root and shoot. The seedling dry weight also significantly reduced at high concentration of Pb and Cd (Shafiq *et al.*, 2008). Similar results were found in *Pisum sativum* (Kevresan *et al.*, 2001) and increasing Fe concentration reduced dry matter yields (Olaleye *et al.*, 2001).

Heavy metals content in radish

The effects of different industrial effluents on heavy metal uptake in radish showed in Table 5. Contaminations of heavy metals in radish indicated that the concentration of Pb, Cd, Ni and Cr were obtained maximum (0.720, 0.013, 0.069 and 0.075 mg kg^{-1}, respectively) due to use of Hazaribagh industrial effluents as irrigation water followed by Khadimnagar industrial effluents (Pb 0.021, Cd 0.003 and Ni 0.051 mg kg^{-1}, respectively). In case of Fe (351.72 mg kg^{-1}) and Zn (68.48 mg kg^{-1}), highest uptake was detected in Khadimnagar industrial effluents followed by Alampur (Fe 181.11 mg kg^{-1}) and Hazaribagh (41.02 mg kg^{-1}). Generally, heavy metal concentrations in shoots of radish potted irrigated with effluents were higher than those of control water grown radish. The heavy metal content in radish with respect to concentrations of effluents was in the order of Hazaribagh > Khadimnagar > control (no effluent). The results clearly indicated higher concentration of metals present in industrial effluents which led to high accumulation in radish due to irrigation with effluents. The prolonged irrigation by industrial effluents increased heavy metal accumulation in the vegetables which leads to contamination of food crops. Arora *et al.* (2008) found higher concentrations of heavy metals in radish, spinach, turnip, brinjal, cauliflower and carrot grown under waste water irrigation as compared to those at clean water irrigated site. Yousaf *et al.* (2010) who reported that in higher plants roots are the first organs that come in contact with toxic metal concentrations and root tips are the key site of injury, leading to inhibited root growth, reduced uptake of water, stunting and then reduction in yield.

Conclusion

Heavy metal toxicity is a serious threat to the global agricultural systems, which is augmented by industrial discharge. The use of untreated industrial effluents for irrigation of radish caused heavy metal toxicity and affects the vegetable especially when applied of Khadimnagar and Hazaribagh industrial effluents. It is concluded from the results of the study that high Concentration of heavy metals, like Pb, Cd, Ni and Fe presence in Khadimnagar and Hazaribagh industrial effluents had adverse effects on seed germination, seedling mortality, shoot and root length, also growth characters of radish. Due to these effluents fresh and dry biomass was also greatly reduced 60.03% and 69.18% in radish. The long-term effluents irrigation led to the accumulation of heavy metals in soil and consequently into the radish, which may cause of economic loss of radish production and also human health risk due to consumption of Radish grown in the area having long term uses of untreated industrial effluents for irrigation.

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