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IMPACT OF INDUSTRIAL EFFLUENTS ON GERMINATION AND SEEDLING GROWTH OF *LENS ESCULENTUM* VARIETIES.

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Abstract

Effluent samples were collected from Koh-e-Noor textile mill, Marble Industry and Attock oil Refinery for present study. Different dilutions (0, 10, 20, 40, 60, 80 and 100%) were used to irrigate two varieties of lentil (M-93 & NARC-02-4). Koh-e-noor (textile) mill effluent was most injurious as its all concentrations caused a reduction in the length parameters of the two varieties. Roots were more affected than shoots. Whereas various concentrations of effluents from marble industry and refinery were stimulatory for the growth of both varieties. General growth of *Lens esculentum* var NARC-02-4 under normal conditions was better as compared to var M-93 but the percentage reduction in length parameters was less in case of M-93 as compared to NARC-02-4. Comparison of the two varieties showed that the overall fresh and dry weight of seedlings was more in case of M-93 as compared to NARC-02-4. The dry mater accumulation was comparable in the two varieties. Study concluded that effluents from Marble industry and Refinery could be used for irrigation purpose after dilutions as was evident from the length and weight parameter. The growth of both varieties of lentil was suppressed in textile effluent, but both could be grown in the other two effluents in variable concentrations for optimal growth.

Introduction

Pollution of the biosphere by heavy metals due to industrial, agricultural and domestic activities has created a serious problem for the safe and rational utilization of soils and water (Igwe *et al.,* 2005; Srivastava *et al.,* 2005). Heavy metal pollution is a serious threat to the environment due to the fact that they cannot be degraded, rather they persist and are accumulated, hence pose severe affects on all life forms. They can cause adverse toxic effects on the plants growing in the affected area leading to a decrease in agricultural productivity.

Other side of picture is that due to high cost and scarcity of chemical fertilizers, the land disposal of agricultural, municipal and industrial waste is widely practiced as a major and economic source of nutrients and organic matter for growing cereal crops by poor farmers in Pakistan (Younas & Shahzad, 1998; Jamal *et al.,* 2002). The use of such waste water in irrigation system definitely provide some nutrients to enhance the fertility of soil but also deposit toxicants that change soil properties in the long run. This necessitates a detailed scientific study before any specific waste can be used for irrigation for a particular crop and environmental conditions.

Since different crop species may have different tolerance to various pollutants. Seed germination and plant growth bioassays are the most common techniques used to evaluate phytotoxicity (Kapanen & Itavaara, 2001). Present study was designed for screening the suitability of various industrial effluents to use for irrigation purpose. Crop selected for this purpose was *Lens esculentum.*

Materials and Methods

Effluent samples were collected from Koh-e-Noor textile mill, Marble Industry and Attock oil Refinery (all are situated in Rawalpindi, Pakistan). They were subjected to various physico-chemical tests using the standard methods (Eaton *et al.,* 1998). For the germination experiments, certified seeds of *Lens esculentum* were obtained from NARC, Islamabad. Two varieties M-93 and NARC-02-4 were used for this study. Experimental setup was the same as described by Nawaz *et al.* (2006). Healthy and equally sized seeds of two varieties were sterilized with 0.1% $HgCl_2$. After repeated washings with sterilized distilled water, seeds were soaked in the same water for 4hrs. Then 20 sterilized seeds were arranged in sterilized Petri dishes, lined with double layer of Wattman filter paper No 1. Plates were labeled as per type, concentration of the effluent and variety of lentil. Different concentrations (0, 10, 20, 40, 60, 80,100%) of each effluent were made with distilled water. Respective effluent concentration were provided and incubated for three days at 26 $\pm 2^{o}$C for germination. Daily observations were made for the germinated seeds. On third day they were shifted to light (10kLux) for next seven days. Before shifting, 10 ml of nutrient solution (Hewitts, 1963) was provided with the same concentration of effluents. After seven days, seedlings were harvested; root, shoot & seedling lengths and fresh weights were recorded. For dry weights seeds were incubated at 60^{o}C for 24 hrs. Data was subjected to statistical analysis.

Results and Discussion

Three different industries i.e. Koh-e-Noor textile mill, Marble Industry and Attock oil Refinery were chosen for present study. Analysis of various physicochemical characters of these effluents is mentioned in the Table 1 (adopted from Nawaz *et al*., 2006). Color of the textile effluent was greenish, while other two were colorless. Seeds of the two lentil varieties were grown in different dilutions of the effluents for germination and growth. Rodosevich *et al.* (1997) considered seed germination a critical step as it ensures reproduction and controls the dynamics of plant population as well as probable crop productivity. In present study Lentil variety M-93 was germinating equally well (100%) from 0-40% effluent, whereas a little decrease (3-6%) was observed from 60-100% of all three effluents. Variety NARC-02-4 showed 100% germination in all concentrations of marble effluent and upto 60% concentration of ARL effluent, while showed a 3% decrease at 80 and 100% effluent. No particular pattern was observed in Kohinoor mill effluent as 10, 40 & 60% effluent caused a 3-6% decrease in germination as compared to control, but in rest of the concentrations, seeds were showing 100% germination.

Table. 1. Characterization of effluents used for germination and growth assay.

Parameters	Effluents from		
	ARL	KTM	MI
pH	7.06	6.80	7.96
Temperature ºC	26.4	26.1	25.5
DO (ppm)	1.5	5.23	6.46
Conductivity (µS/cm)	4.0	16	7
Turbidity (NTU)	5.86	61	59.6
TDS (ppm)	289	9.35	4.4
TSS (ppm)	0.09	11.76	56.27
Total hardness (ppm)	150	300	1666
Total alkalinity (ppm)	200	400	9000
Phosphates (ppm)	nil	nil	nil
Sulfates (ppm)	8	40	12
Nitrates (ppm)	45	42.8	3.76
Ammonia (ppm)	nil	nil	150.27

As sufficient water absorption is essential for proper seed germination without which seedling growth and development is severely affected (Kelly *et al.,* 1992; Debeaujan *et al.,* 2000). Under the environmental stress conditions, the energy forming molecules may be disturbed and subsequently carbohydrates and protein metabolites of the membrane are altered (Kannan & Upreti, 2008), which may lead to reduction in absorption of water by the seeds/seedlings. Some researcher related this delayed germination directly with the presence of higher salt/metal concentrations (Baruah & Das, 1997), while others with lower amounts of auxin production which is also a result of higher metal ion concentrations (Mukherji & Das, 1972). Inhibitory (Baruah & Das, 1997; Crowe *et al.,* 2002) as well stimulatory (Nawaz *et al.,* 2006; Yousaf *et al.*, 2010) effects of various effluents on the germination of a number of plant species were observed by many researchers.

Both varieties of *L.esculentum* were highly sensitive to the effluents of Kohi-e-noor mill. A regular decrease in the root, shoot and seedling lengths as observed with the increase in effluent concentrations (Table 2, 3 & 4). Variety M-93 showed 36.7-70.63% reduction in its seedling length as compared to variety NARC-02-4, where this reduction was 26.1-78% in 10-100% effluent concentration (Table 4). Same trend was observed in root and shoot lengths individually, though the pollution affected the roots more as compared to shoots. Wins & Murugan (2010) recommended the use of textile mill effluent at a concentration of 25% or lower after proper treatment as it could enhance the growth of Black gram. On the other hand Vijayakumari (2003) reported reduction in various growth parameters of soybean in various concentrations of textile dying effluents.

Marble industry effluent affected the seedlings of two varieties both in negative and positive way. Little reduction in seedling growth was observed in variety M-93 (3.4-5.1%) and NARC-02-4 (4.2-5.1%). Surprisingly the reductions were observed at 10% and then at 40-100% effluent concentration for variety M-93 and at 10 & 20% effluent for variety NARC-02-4. Variety NARC-02-4 showed improved growth at higher concentration of effluent. Maximum increase observed was 38% in 40% effluent. Comparison of root and shoot lengths showed a very negligible decrease in root lengths of M-93 up to 60% effluent and then an increase for 80-100% effluent, but lower concentrations of effluent enhanced the shoot growth (Table 2 & 3). In case of NARC-02-4, roots were promoted in 40-60% effluent concentration and there was a regular non-significant increase in all concentrations of Marble industry effluent. These effluents were rich in inorganic nutrients, which may have beneficial effects on the seedlings.

In the effluent taken from Attock oil refinery, seedling of M-93 remained unaffected at 10% concentration and then an increase of 6.3-11.64% was observed for 20-60% effluent concentration. Maximum growth was observed at 40% effluent concentration. Whereas variety NARC-02-4 showed 0.5-12.31% promotion in seedling length under all stress conditions as compared to control. In this variety root growth was actually enhanced under all concentrations of effluent and was more pronounced in 60-100% effluent, whereas shoot showed a non-significant increase at lower concentration and then a decrease at higher concentrations of effluents (Table 3). Augusthy and Sherin (2001) indicated that length of root system and number of lateral roots of *Vigna radiata* increased by low concentrations of effluent. Similar results had been reported by Bera & Kanta, (1999) and Sundaramoorthy and Lakshami, 2000.

We may relate the reduction in seedling (root & shoot) lengths with the elevated amounts of total dissolved solids at higher concentrations. This could also be related to the fact that some of the nutrients present in the effluents are essentials but at above a particular concentration, they become hazardous. Study of Panaskar & Pawar (2011a & b) also showed that textile effluents were not inhibitory at low concentrations but with the increase in concentration growth of seedlings was affected. Hussain *et al.* (2010) found that tannery effluents caused a reduction in germination, growth of sunflower parameters along with other parameters like chlorophyll content, protein and carbohydrate content etc.

No particular relationship could be developed with the increase in the effluent concentration and the weight parameters. Seedling of both varieties showed a reduction in fresh weight with the increase in textile mill effluent, while they showed a mixed behavior in marble industry and Refinery effluents (Table 5). Like fresh weight, no particular trend was seen for the dry weight parameter. Both increases and decreases were observed at certain treatments (Table 6). In almost all cases (few exceptions for variety NARC-02-4) dry weight was enhanced as compared to the normal condition (Table 6) and the increase was quite significant. Powel *et al.,* (1996) reported reduction in fresh weights of seedlings, while dry weights remained unaffected under the pollution stress. Both increases and decreases in the weight parameters were also reported by Nawaz *et al.* (2006) and Yousaf *et al.* (2010).

Dry matter accumulation is an important parameter which tells about the accumulation/deposition of heavy metal and other ions inside the body, which may help in combating the stress. Variety NARC-02-4 showed an increase in dry matter upto 40% (textile effluent), 20% (marble effluent) and 80% (ARL) effluent concentration after that decreases were observed in this parameter, whereas variety M-93 accumulated more upto (KNM, MI) and 10% (ARL) effluent concentrations. In refinery effluent accumulation was almost constant from 20-80% concentration (Table 7).

Table 2. Effect of three industrial effluents on the root length (cm) of Lentil varieties.

Sites	*Lens esculentum* var M-93							*Lens esculentum* var NARC-02-4						
	0%	10%	20%	40%	60%	80%	100%	0%	10%	20%	40%	60%	80%	100%
Koh-e-Noor Mill	8.92 ± 0.40	4.49 ± 0.30	3.78 ± 0.30	1.63 ± 0.14	1.24 ± 0.08	0.89 ± 0.05	0.63 ± 0.04	10.96 ± 0.87	6.98 ± 0.66	2.67 ± 0.01	0.94 ± 0.03	0.92 ± 0.03	0.61 ± 0.08	0.19 ± 0.08
Marble Industry	8.92 ± 0.40	8.78 ± 0.25	8.7 ± 0.49	8.49 ± 0.66	8.58 ± 0.60	9.18 ± 0.41	9.82 ± 0.79	10.96 ± 0.87	10.07 ± 0.78	9.71 ± 0.42	17.29 ± 0.55	11.43 ± 0.17	10.65 ± 0.19	10.57 ± 1.01
Attock Refinery	8.92 ± 0.40	8.92 ± 0.40	9.95 ± 0.51	11.06 ± 1.1	10.48 ± 0.75	9.77 ± 0.16	9.14 ± 0.51	10.96 ± 0.87	12.35 ± 0.47	11.83 ± 0.43	11.32 ± 0.65	13.69 ± 0.99	12.63 ± 0.74	12.92 ± 0.75

Table 3. Effect of three industrial effluents on the shoot length (cm) of Lentil varieties.

Sites	*Lens esculentum* var M-93							*Lens esculentum* var NARC-02-4						
	0%	10%	20%	40%	60%	80%	100%	0%	10%	20%	40%	60%	80%	100%
Koh-e-Noor Mill	5.93 ± 0.45	4.91 ± 0.42	4.36 ± 0.39	4.25 ± 0.40	4.10 ± 0.30	4.11 ± 0.18	3.73 ± 0.31	6.42 ± 0.37	5.85 ± 0.39	5.29 ± 0.52	4.43 ± 0.23	4.05 ± 0.29	3.97 ± 0.14	3.68 ± 0.31
Marble Industry	5.93 ± 0.45	6.16 ± 0.53	7.4 ± 0.57	5.85 ± 0.29	5.41 ± 0.44	5.48 ± 0.37	5.17 ± 0.20	6.42 ± 0.37	6.57 ± 0.47	6.78 ± 0.44	6.76 ± 0.52	6.61 ± 0.42	6.77 ± 0.51	6.77 ± 0.49
Attock Refinery	5.93 ± 0.45	5.93 ± 0.34	5.84 ± 0.34	5.52 ± 0.41	5.77 ± 0.44	5.10 ± 0.48	5.07 ± 0.38	6.42 ± 0.37	6.86 ± 0.56	6.53 ± 0.38	6.74 ± 0.53	5.81 ± 0.14	4.96 ± 0.41	4.57 ± 0.48

Table 4. Effect of three industrial effluents on the seedling length (cm) of Lentil varieties.

Sites	*Lens esculentum* var M-93							*Lens esculentum* var NARC-02-4						
	0%	10%	20%	40%	60%	80%	100%	0%	10%	20%	40%	60%	80%	100%
Koh-e-Noor Mill	14.85 ± 0.85	9.40 ± 0.72	8.14 ± 0.69	5.88 ± 0.54	5.34 ± 0.38	5.0 ± 0.23	4.36 ± 0.35	17.38 ± 1.24	12.83 ± 1.05	7.96 ± 053	5.37 ± 026	4.97 ± 0.32	4.58 ± 0.22	3.82 ± 0.39
Marble Industry	14.85 ± 0.85	14.94 ± 0.78	16.10 ± 1.06	14.34 ± 0.95	13.99 ± 1.04	14.66 ± 0.78	14.69 ± 0.99	17.38 ± 1.24	16.64 ± 1.25	16.41 ± 0.86	24.05 ± 1.07	18.04 ± 059	17.42 ± 0.70	17.34 ± 1.50
Attock Refinery	14.85 ± 0.85	14.85 ± 0.74	15.79 ± 0.85	16.58 ± 1.51	16.25 ± 1.19	14.87 ± 0.64	14.21 ± 0.89	17.38 ± 1.24	19.21 ± 1.03	19.36 ± 0.81	19.06 ± 1.18	19.50 ± 1.13	17.59 ± 1.15	17.49 ± 1.23

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Table 5. Effect of three industrial effluents on the fresh weight per seedling (mg) of Lentil varieties.

Sites	*Lens esculentum* var M-93							*Lens esculentum* var NARC-02-4						
	0%	10%	20%	40%	60%	80%	100%	0%	10%	20%	40%	60%	80%	100%
Koh-e-Noor Mill	148.40 ± 7.73	102.16 ± 5.18	99.35 ± 7.96	94.51 ± 5.25	84.60 ± 7.93	83.28 ± 7.9	76.91 ± 4.56	108.08 ± 3.42	85.32 ± 4.67	82.25 ± 6.98	66.53 ± 5.23	70.47 ± 6.07	75.31 ± 5.24	76.07 ± 4.15
Marble Industry	148.40 ± 7.73	144.83 ± 12.24	116.61 ± 4.89	111.88 ± 8.37	113.45 ± 5.86	123.65 ± 6.42	125.12 ± 8.44	108.08 ± 3.42	102.74 ± 6.95	100.25 ± 8.85	111.01 ± 4.67	118.34 ± 9.09	129.06 ± 12.38	115.64 ± 9.35
Attock Refinery	148.40 ± 7.73	121.54 ± 3.22	111.77 ± 9.23	91.82 ± 6.62	112.74 ± 5.2	114.89 ± 8.44	118.34 ± 10.07	108.08 ± 3.42	113.43 ± 6.66	117.88 ± 9.22	101.62 ± 7.13	98.43 ± 5.52	94.50 ± 7.72	95.33 ± 4.69

Table 6. Effect of three industrial effluents on the dry weight per seedling (mg) of Lentil varieties.

Sites	*Lens esculentum* var M-93							*Lens esculentum* var NARC-02-4						
	0%	10%	20%	40%	60%	80%	100%	0%	10%	20%	40%	60%	80%	100%
Koh-e-Noor Mill	16.55 ± 1.38	18.08 ± 0.62	17.95 ± 0.44	17.60 ± 0.85	18.97 ± 0.55	17.73 ± 0.38	16.42 ± 0.79	13.59 ± 0.30	13.64 ± 0.54	14.45 ± 0.59	14.86 ± 0.46	14.42 ± 0.32	14.66 ± 0.78	12.75 ± 1.12
Marble Industry	16.55 ± 1.38	17.75 ± 0.79	17.55 ± 0.62	17.29 ± 0.55	18.05 ± 0.61	17.15 ± 0.61	18.75 ± 1.26	13.59 ± 0.30	12.9 ± 0.43	14.77 ± 1.33	13.72 ± 0.27	13.22 ± 0.19	13.85 ± 0.64	15.28 ± 1.14
Attock Refinery	16.55 ± 1.38	17.53 ± 0.44	17.10 ± 0.79	18.01 ± 0.67	17.09 ±0.60	18.01 ± 0.60	17.22 ± 0.21	13.59 ± 0.30	14.73 ± 1.03	13.62 ± 0.86	13.25 ± 0.62	13.29 ± 0.71	12.78 ± 0.71	13.25 ± 0.49

Table 7. Effect of three industrial effluents on the dry matter accumulation (mg/gm) of Lentil varieties.

Sites	*Lens esculentum* var M-93							*Lens esculentum* var NARC-02-4						
	0%	10%	20%	40%	60%	80%	100%	0%	10%	20%	40%	60%	80%	100%
Koh-e-Noor Mill	120.2 ± 11.7	178.09 ± 8.91	183.96 ± 3.93	189.09 ± 8.39	228.95 ± 16.4	218.29 ± 19.3	215.13 ± 12.6	129.74 ± 1.57	168.86 ± 8.41	178.58 ± 2.31	226.17 ± 11.4	208.86 ± 16.7	195.99 ± 9.96	148.55 ± 12.4
Marble Industry	120.2 ± 11.7	124.78 ± 10.1	149.72 ± 1.92	156.312 ± 7.20	159.69 ± 4.64	159.40 ± 4.32	150.16 ± 4.67	129.74 ± 1.57	137.53 ± 10.2	147.51 ± 5.61	121.96 ± 4.95	113.27 ± 6.7	110.20 ± 11.2	133.68 ± 10.7
Attock Refinery	120.2 ± 11.7	193.83 ± 14.5	153.67 ± 8.82	153.07 ± 3.27	152.21 ± 5.78	156.54 ± 10.2	148.46 ± 11.5	129.74 ± 1.57	130.46 ± 8.73	131.73 ± 7.79	131.25 ± 5.41	136.65 ± 11.5	141.72 ± 10.4	138.44 ± 9.29

From the present study it could be concluded that the textile effluent was more toxic as compared to the other two effluents. Effluents from Marble industry and Refinery could be used for irrigation purpose after dilutions as was evident from the length and weight parameter. Both varieties of lentil almost behaved similarly in textile effluent, but both could be grown in the other two effluents in variable concentrations for optimal growth.

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