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Effect of Hindon River Water on Seed Germination of *Mung Bean* (*Vigna radiata*), *Black Gram* (*Vigna mungo*) & *Wheat* (*Triticum aestivum*) *In vitro*

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ABSTRACT

Today quality of river water is an issue of serious concern, nowadays wastewaters from almost all the industries and domestic effluent are discharged untreated in to the rivers and ultimately agriculture fields are being generally irrigated from these polluted water resources loaded with harmful toxic substances which drastically affect the yield. Keeping this in mind, the aim of this study was to analyse Hindon River water for physico-chemical properties (pH, Electrical conductivity (EC), Total alkalinity, Total dissolved solids (TDS), Total suspended solids (TSS), Dissolved Oxygen(DO), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Chloride, Sulphate and Heavy Metals (Cr, As, Cu, Pb, Cd, Hg , Zn and Ni) to determine its water quality status in term of water quality index (WQI). In this study it is shown that water quality status of Hindon River deteriorated from very poor to unsuitable for drinking and agricultural practices. Additionally its effect on the germination of *Vigna radiata*, *Vigna mungo* & *Triticum aestivum* was investigated by treating with different concentrations 0% 25%, 50%, 75%, and 100%. It was found that concentration of 25% and 50% has stimulatory effects on germination rate, germination rate, seedling length, seedling vigour index and further increase in concentration beyond 50% showed inhibitory effects even on initial growth of these three plants. There was significant (at $p \leq 0.05$) differences in seed germination rate, root length, shoot length, fresh & dry weight and vigour index at different concentration of River water.

Keywords: Hindon River Water, Seed germination, Seedling length, Vigour Index, Heavy metal, WQI (Water quality Index), WQS (Water Quality Status).

INTRODUCTION

The Hindon River, is a potential source of water for different activities in the highly populated rural area of Western Uttar Pradesh region, is also utilized in different industrial activities taking place along its length. Indeed, the Hindon River and thereof two tributaries (Kali and Krishni River), together have different types of industrial manufacturing units and Municipal effluent drainages which discharge their waste water, often without treatment, directly into the Hindon River, and destroy the river's natural ability

(Bhardwaj, 2014). In suburban areas of many region of the world including India, it is common exercise to use the industrial or municipal waste water (Negi *et al.*, 2016; Rai *et al.,* 2011), Hindon river water is also being used for irrigation in the agriculture fields, as drinking water for livestock and for bathing. Chemical substance and heavy metals are particularly those which can enter in the food chain through infiltration in soil and then finally in plant (Oregani *et al.,* 2014; Soumare *et al.,* 2003 and Bernala *et al.,* 2006). As there is water shortage, farmers use these polluted waters for irrigation of their crops, but contained heavy metals which are detrimental could be transmitted to living beings through food chain (Pathrol *et al.,* 2015; Murtaza *et al.,* 2010). In contrast, Industrial effluent is also a potential source of irrigation water and nutrient sources to yield the crops, but the presence of the highly loaded nutrient and growth impeding substances may affect seed germination and productivity of a crop (Singh *et al.,* 2014), therefore, earlier to use in agriculture field, the industrial effluent should be tested and treated for its toxicity. Various researches have been carried out to study the effect of different industrial waste on different agricultural crop. The effluents from different industries utterly deter the seed germination and seedling growth, but this effect shows a discrepancy from one crop species to another (Kaushik *et al.,* 2005; Singh *et al.,* 2012, Mycin 2016).

In Hindon River, dumping of waste water is one of the major issues. The disposal of effluent is mainly manage by different industries such as Sugar Mills, Paper Mills & Distilleries and Municipal waste carry domestic, hospital and small scale industrial wastes. There is a number of such small scale industries which are being operated illegally and do not follow waste disposal procedure. Singh & Yadav (2012) has studied that, sewage enriched Distillery Effluent has detrimental effect on growth of Wheat. Saini & Pant (2014) has investigated those inhibitory effects of Sugar mill waste on maize and wheat. Different workers had made efforts to conclude the impact of different industrial effluent on seed germination of a variety of crops such as Casuarinas (Kumar *et at.,* 2010); Cow pea (Pathrol & Bafna, 2015); Mung bean (Anbuselvam *et al.,* 2016; Vaithiyanathan *et al.,* 2016); Black gram (Vaithiyanathan *et al.,* 2014); Rice (Gassama *et al.,* 2015; Saini *et al.,* 2014; Sajani *et al.,* 2011; Dhanam, 2009); Wheat (Singh *et al.,* 2012; Bharti *et al.,* 2012; Pandey *et al.,* 2007).Thus the present study was done to appraise the effect of different concentrations of Hindon River water on biochemical & physical characteristics of Mung bean, Black gram and Wheat.

MATERIALS AND METHODS

Collection and Analysis of Hindon River water

For present study, the effluent samples were collected in a pre-cleaned glass bottles from Hindon River at Atali Village site, Muzaffarnagar district, (U.P) during June-Dec 2016. The collected water samples were stored at 4°C to maintain its original characteristics. These water samples were analyzed for Color, Odour, EC, and pH immediately at the sites of collection and TH, TA, DO, COD, BOD, TDS TSS, Chloride & Sulphate in the lab according to the method prescribed in APHA, 2012. These samples were also analyzed for concentration of Heavy Metals such as Cr, Pb, As, Ni, Cd, Cu, Hg & Zn by Inductively coupled plasma-mass spectrometer (ICP-MS).

Water Quality Index (WQI)

WQI provides single value that expresses the whole quality based on number of parameters. In this study, 8 parameters were selected for calculation of WQI on the basis of standard/specification recommended by WHO and BIS (Bureau of Indian Standard).

Calculation of WQI was done using the equation as stated below (Brown *et al.,* 1970):

$$WQI=\sum Q_n W_n / \sum W_n$$

$$Q_n=100[(V_n-V_i)/(V_s-V_i)],$$

Where,

W_n = Unit weight of nth parameter, Q_n = Quality rating of n^{th} parameter

V_n = Actual quantity of n^{th} parameter

V_i= Ideal value of the parameter, where $V_i = 0$, except for pH i.e. $V_i = 7$ and DO i.e. V_i = 14.6 mg/l

V_s = Standard permissible value for the nth parameter.

$$W_n=k/V_s,$$

$k=[1/\sum 1/V_{s=1,2,\ldots,n}]$= constant of proportionality

Table 1: The range of WQI, quality status and conceivable usage of water (Brown *et al.,* 1972)

WQI	Water quality status	Conceivable usage
0–25	Excellent	Irrigation, industrial and drinking
26–50	Good	Irrigation, industrial and drinking
51–75	Poor	Irrigation and industrial
76–100	Very poor	Irrigation
Above 100	Unsuitable for drinking, irrigation and aquaculture	Appropriate treatment required before taking in use

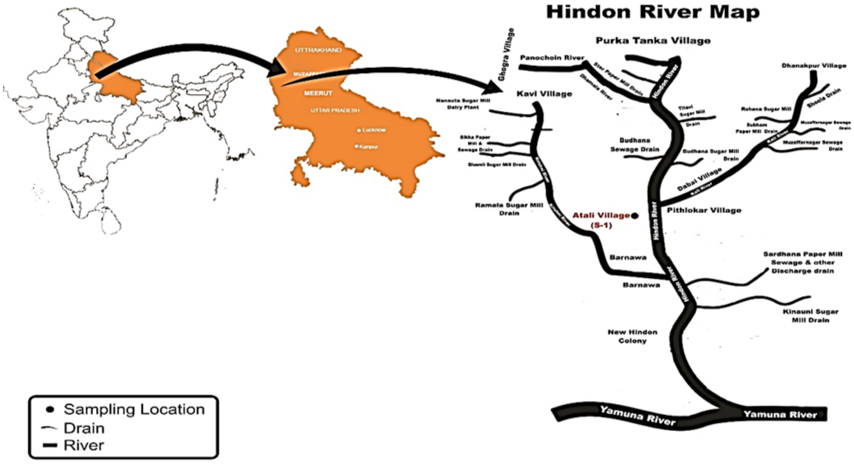


Fig. 1. Map of sampling location

Seed Material

Seeds of Vigna radiata, Triticum aestivum and *Vigna mungo* were purchased from market and homogeny was maintained on the subject of size, weight and color for better interpretation.

Seed Germination

Seeds of *Vigna radiata*, *Triticum aestivum* and *Vigna mungo* were washed with sodium hypochlorite (NaClO) solution having one percent active chlorine for 10 min. to sterilize from fungal contamination (Anbuselvam *et al.,* 2016) and then repeatedly washed with sterilized double distilled water. In this study, three replicates from each dilution were prepared by spreading 20 sterilized seeds of identical size on equal distance in each decontaminated petri dish coated with whatman paper. Then each petri dish were flooded with 5ml of different dilution of river water (0 %, 25%, 75 %, 50%, and 100%) and kept at 25±2^{0}C. Concentration of river Water were as follows: (a) 20 ml tap water i.e 0.00% concentration (Control) (b)15 ml tap water +5 ml river water i.e. 25% concentration (c) 10 ml tap water + 10 ml sewage water i.e. 50% concentration (d)5 ml tap water +15 ml sewage water i.e. 75% concentration (e) 0.00 ml tap water+20 ml sewage water i.e.100% concentration. Different growth parameters like germination %, seedling length & vigor index were observed after 12 h of cultivation and followed with subsequent observation after one day interval till 7th day of cultivation.

Germination (%)

Seed germination was calculated and expressed in percentage as given by ISTA rules (2016) and recorded every day for one week.

$$G\% = \frac{\text{Number of seed germinated}}{\text{Number of sampled}} \times 100$$

Seedling Length (cm)

Seedling lengths consist of the root and shoot length of the germinated seeds. The shoot length was measured as distance between the base of the primary leaf and the hypocotyl and the Root length was measured as distance between the tip of the primary root and hypocotyl.

Fresh weight and Dry weight (g)

Seedlings from each treatment were

collected and measured for their fresh weights with the help of an electrical balance. The same seedlings used for fresh weight were dried in hot air oven at 80˚C for 24 h and followed with keeping in desiccators for some time. Finally their dry weight is calculated with the help of an electrical balance (Kabir, 2008).

Seedling Vigour Index

Vigour index was computed by using formula i.e. Germination (%) X Seedling Length, given by Abul- Baki and Anderson (1973).

Statistical Analysis

The experimental data done in triplicate with 20 seeds per Petri plate was analyzed for each plant species by analysis of variance (ANOVA). Statistical significance between values was determined using Duncan's multiple comparison tests at different level (P≤0.05).

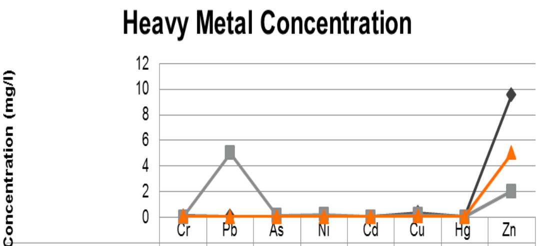
RESULTS AND DISCUSSION

Farming is the main occupation in Meerut & Muzaffarnagar (Uttar Pradesh) where about 75% of the populations are involved in agricultural activities for their income and cultivate two main crops such as Rabi crops i.e. Maize, rice, Sugarcane, jowar & bajra and Kharif crops i.e. wheat, pulses, mustard & rapeseed in this area. Wheat, Rice and Pulses are the major crops grown and eaten in this area.

In this study, water quality status of Hindon river water was analyzed (Table 2, 3 & 4) and effects of Hindon river water in different concentration (0%, 25%, 50%, 75%, 100%) on seed germination (%), seedling length, seedling weight and Vigor index of three different crops e.g. *Vigna radiata*, *Triticum aestivum* & *Vigna mungo* have been investigated as given in Table 5 & 6.

Analysis of Physico-chemical, Heavy metal and determination of WQI

Hindon River is one of the most polluted rivers in India due to the massive industrial and municipal discharge. Additionally it very much polluted with heavy metals and other harmful organic compounds. The values of different pollution parameters analyzed in collected water sample from Hindon River, along with prescribed tolerance limit as per BIS, CPCB & FAO are given in Table 2 and Figure 2.



	Cr	Pb	As	Ni	Cd	Cu	Hg	Zn
Hindon River Water	0.07	0.03	0.02	0.05	0.003	0.34	0.002	9.58
FAO Specification for Irrigation Water	0.01	5	0.1	0.2	0.01	0.2	0	2
BIS Specification for Drinking Water	0.05	0.01	0.01	0.02	0.003	0.05	0.001	5

Fig. 2. Heavy Metal concentration in Hindon River Water used for cultivation

Hindon River water used for irrigation was analysed and found light blackish in colour and had an unpleasant odour. A review of data presented in Table 2 showed that river water has very low level of DO (0.5) and high level of BOD (154) & COD (486). Such levels of BOD & COD indicate the high level of organic concentration and cause DO depletion which threat to aquatic life. Moreover TDS results showed high attribution to high concentration of Ca and Mg.

The concentration of TSS, TH, TA, Chloride and Sulphate was found beyond the permissible limit as per BIS and it also contained heavy metals above the permissible limit which caused toxic effect on plant growth. This study is also supported by the earlier finding of other researchers (Malik *et al.,* 2013; Sharma *et al.,* 2005; Jain *et al.,* 2003). Apart from this, Hindon river water was analysed in both seasons (pre-monsoon & post-monsoon) for 10 parameters and calculated WQI.

The high WQI scores for both season as shown in Table 3 & Table 4 are contributed mostly by different anthropogenic exercises like the direct disposal of sewerage from private and business foundations, absence of appropriate cleanness framework, horticultural run-off, transfer of untreated effluents from little scale enterprises and industrial facilities and persistent dumping of strong wastes by the groups dwelling close by the waterway and so on.

Effect of Hindon River Water on Seed germination of different Plants

Variation in seed germination, seedling length (shoot & root), fresh weight, dry weight and vigour index in different concentration of Hindon river water exhibited effects on plant growth. When river water treated and used for irrigation, maximum germination % was observed in 25% concentration and decreases steadily as the concentration increases. The high level of total dissolved solids absorbed by the seeds upsurge the salinity and conductivity which may cause the hindrance in the germination of seed after irrigation with high concentrated river water (Malaviya *et al.,* 2011).

Table 2: Concentration of Physico-chemical parameters of Hindon River Water and Tap Water used for Irrigation

Sl. No.	Parameters	Unit	Hindon River Water	Tap Water	BIS & CPCB* Specification
General Parameters					
1.	Colour	-	Light Black	Colorless	-
2.	Odour	-	Unpleasant	No odour	-
3.	pH	-	7.5	6.8	6.5-8.5
4.	EC	μs/cm	1560	81	300
5.	TA	mgL^{-1}	485	95	120
6.	TDS	mgL^{-1}	1235	0.0014	500
7.	TSS	mgL^{-1}	126	ND	500*
8.	TH	mgL^{-1}	355	5	300
9.	DO	mgL^{-1}	0.5	6.9	5*
10.	BOD	mgL^{-1}	154	3.1	5*
11.	COD)	mgL^{-1}	486	2.8	-
12.	Chloride	mgL^{-1}	118	14.52	250
13.	Sulphate	mgL^{-1}	59	8	150

Table 3: Calculation of WQI of Hindon River (Pre-Monsoon)

Water Quality Index (WQI)						
Parameters	*K* $k=[1/\sum 1/V_{s=1,2,\ldots,n}]$	*Vs*	W_n Wn=k/Vs	V_n	Q_n $Q_n=100[(Vn-Vi)/(Vs-Vi)]$	Q_nW_n
pH		8.5	0.214	7.5	33.33	7.15
EC		300	0.006	1364	454.67	2.76
TDS		500	0.004	745	149	0.54
TSS		500	0.004	123	24.6	0.09
TA	1.822	120	0.015	443	369.17	5.61
TH		300	0.006	374	124.67	0.76
DO		5	0.364	1.1	140.63	51.24
BOD		5	0.364	154	3080	1122.35
Chloride		250	0.007	110	44	0.32
Sulphate		120	0.015	54	45	0.68
$WQI=\sum Q_nW_n/\sum W_n$						1191.5
WSQ WQI value >100, it comes under "Unsuitable " category						

Table 4: Calculation of WQI of Hindon River (Post-Monsoon)

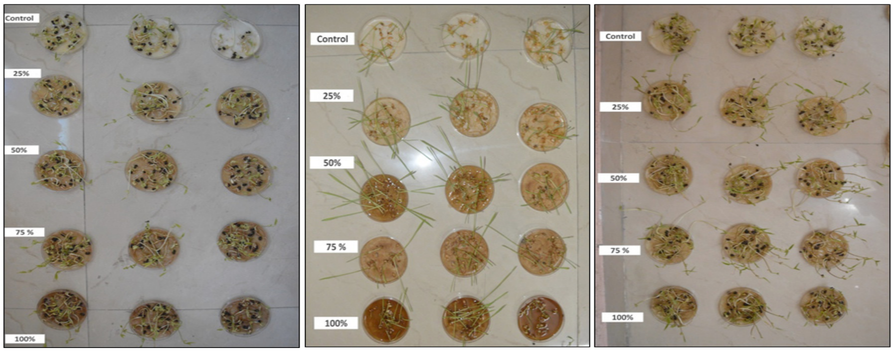
Water Quality Index (WQI)						
Parameters	*K* $k=[1/\sum 1/V_{s=1,2,\ldots,n}]$	*Vs*	W_n Wn=k/Vs	V_n	Q_n $Q_n=100[(Vn-Vi)/(Vs-Vi)]$	Q_nW_n
pH		8.5	0.214	7.1	6.67	1.43
EC		300	0.006	1154	384.67	2.34
TDS		500	0.004	821	164.2	0.6
TSS		500	0.004	110	22	0.08
Total Alkalinity)	1.822	120	0.015	138	115	1.75
Total Hardness		300	0.006	423	141	0.86
DO		5	0.364	1.2	139.58	50.86
BOD		5	0.364	173	3460	1260.82
Chloride		250	0.007	124	49.6	0.36
Sulphate		120	0.015	62	51.67	0.78
$WQI=\sum Q_nW_n/\sum W_n$						1319.88
WSQ WQI value >100, it comes under "Unsuitable " category						

High concentration of heavy metal in Hindon river water also caused retard of seed germination and growth pattern of plant. (Solanki, 2011) investigated the effect of Zinc and copper on *Vigna mungi* (L) and (Abraham, 2013) discussed the effect of Cd, Pd and Cu on seed germination of *Arachis hypogeae*. L.

Table 5: Effect of Hindon River water on three different plant species

Attribute	Treatment	Mung Bean (Vigna radiata)	Black Gram (Vigna mungo)	Wheat (Triticum aestivum)
Germination (%)	Control (0%)	86.67 ±2.36a	85.00±4.08a	76.67± 2.36ab
	25%	76.67±2.36b	86.67±2.36a	68.33± 2.36bc
	50%	86.67±2.36a	88.33±2.36a	80.00± 4.08a
	75%	68.33±2.36c	73.33±2.36b	78.33± 2.36a
	100%	63.33±2.36c	61.67±2.36c	66.67± 4.24c
	F-Value	22.013	44.898	4.727
Shoot Length (cm)	Control (0%)	8.76±0.08 ab	7.95±0.2ab	6.38±0.21ab
	25%	9.67±0.79ab	9.21±0.44ab	8.60±0.72ab
	50%	11.35±0.90a	9.87±0.52a	9.76 ±0.51a
	75%	9.72±0.60ab	8.49±0.44ab	7.01±0.70ab
	100%	7.96±0.36b	6.83±0.65ab	5.61±0.40ab
	F-Value	2.361	1.067	1.511
Root Length (cm)	Control (0%)	3.91 ±0.21b	3.15 ±0.15c	2.83 ±0.27d
	25%	5.84±0.04a	3.62±0.17b	6.48 ±0.29a
	50%	6.83±0.62a	4.66±0.16a	5.43 ±0.29b
	75%	6.07±0.98a	4.10±0.14b	4.22±0.26c
	100%	3.28±0.12b	2.98±0.36c	2.65 ±0.13d
	F-Value	13.551	20.681	83.841
Fresh Weight (gm)	Control (0%)	2.40±0.14b	2.61±0.11b	1.80±0.04a
	25%	3.12±0.11a	3.29±0.15a	2.06±0.06ab
	50%	3.45±0.14a	3.57±0.09a	2.25±0.37ab
	75%	3.18±0.18a	3.46±0.17a	1.93±0.05a
	100%	2.44±0.18b	2.63±0.23b	1.59±0.13b
	F-Value	16.531	13.464	3.587
Dry Weight (gm)	Control (0%)	0.26±0.03b	0.29±0.03c	0.32±0.01c
	25%	0.35±0.02a	0.39±0.02ab	0.45±0.03b
	50%	0.36±0.01a	0.43±0.03a	0.63±0.04a
	75%	0.32±0.01a	0.41±0.02a	0.37±0.01bc
	100%	0.21±0.01b	0.35±0.02bc	0.39±0.05bc
	F-Value	19.932	7.555	24.63

For each attribute the mean values with the same superscript letters in same column are non-significant at 5% level of probability ($P<0.05$) (Duncan's multiple comparisons test).



(a) Vigna Mungo (b) Triticum aestivum (c) Vigna Radiata

Fig 3: Effect of Hindon River Water on seed germination of three crops

The seedling (root and shoot) length and fresh & dry weight were parameters for growth characteristic of Plants. In this study, plant showed irregular growth characteristic on treatment with Hindon river water and studied growth parameters analysed statistically using ANOVA showed significant difference, where in case of root length, it was as: *Vigna radiata* (F=13.551, p<0.05), *Vigna mungo* (F = 20.681, p<0.05) and *Triticum aestivum* (F = 83.841, p<0.05). For shoot length, *Vigna radiata* (F=2.361, p<0.05), *Vigna mungo* (F = 1.067, p<0.05) and *Triticum aestivum* (F =1.511, p<0.05). For fresh weight, it was as: *Vigna radiata* (F=16.531, p<0.05), *Vigna mungo* (F = 13.464, p<0.05) and *Triticum aestivum* (F = 3.587, p<0.05) and for dry weight, it was as: *Vigna radiata* (F=19.932, p<0.05), *Vigna mungo* (F = 7.555, p<0.05), and *Triticum aestivum* (F = 24.630, p<0.05).

Table 6: Vigour Index (VI) for three different plant species

Treatment	Vigna radiata)	Vigna mungo	Triticum aestivum
Control (0%)	1097.80±50.95	942.93±53.55	705.28±38.33
25%	1187.67±59.69	1111.52±42.21	1029.03±27.31
50%	1575.43±139.97	1282.85±46.96	1212.42±55.39
75%	1083.53±188.39	921.98±11.74	877.90±69.68
100%	711.03±26.63	604.17±47.17	549.85±56.42

In case of *Vigna radiata*, root and shoot length were found in range of 3.28-6.83 cm and 7.96-11.35 cm respectively (Table 5). Maximum root length (6.83 cm) was investigated at 50% concentration followed by 75% (6.07 cm) > 25% (5.84 cm) >0% (3.91cm)> 100% (3.28 cm). The growth of shoot length at different concentration was as: 50% (11.35 cm) > 75% (9.72 cm) > 25% (9.67 cm) > 0% (8.76 cm) > 100% (7.96 cm). The fresh weight (g) and dry weight (g) lied in the ranges of 2.40-3.45 and 0.21-0.36, respectively. The highest fresh weight was observed at concentration of 50% (3.45 g) followed by 75% (3.18 g) > 25% (3.12 g) > 100% (2.44 g)> 0% (2.40 g). Likewise, Dry weight (g) increases from 50% (0.36 g) > 25% (0.35 g) > 75% (0.32 g)>0% (0.26 g)> 100% (0.21 g). It has been observed that the concentration at 100% significantly reduced the growth of plant.

In *Vigna mungo*, root and shoot length fall under the ranges of 2.98-4.66 and 6.83-9.87cm respectively (Table 5). The root length observed as follow: 50% (4.66 cm) > 75% (4.10 cm) > 25% (3.29 cm)>0% (3.15 cm)> 100% (2.98 cm). Likewise, shoot length showed trend as 50% (9.87 cm) > 25% (9.21cm) > 75% (8.49 cm)>0% (7.95 cm)> 100% (6.83 cm). The fresh and dry weight (g) falls between 2.61-3.57 and 0.29-0.43 respectively. The highest fresh weight (3.57 g) was found at 50% concentration, followed by 3.46 g (at 75% concentration) and 3.29 g (at 25% concentration). The minimum was at 100 % & 0% concentration. Similarly, the maximum dry weight (0.43g) was at 50% concentration, followed by 0.41g (at 75% concentration) and 0.39 g (at 25% concentration). The fresh and dry weight continue rise with increasing concentration, but the concentration of 100% revealed abrupt reduction in length and weight of plants.

Triticum aestivum plants showed impact of different concentration of river water on growth and development of plants (Table 5). However, better growth was noted at low concentration. Fresh and dry weight ranged 1.59-2.25 and 0.32-0.63 respectively. The highest root length (6.48 cm) was witnessed at 25% concentration, followed in the order 5.43 (at 50%) > 4.22 (at 75%) > 2.83 (at 0%) and 2.65 (at 100%). The shoot length was found in the range of 5.61-9.76 cm. The highest shoot length of 9.76 cm was witnessed at 50% concentration, followed by 8.60 (at 25%), 7.01 (at 75%), 6.38 (at 0%) and 5.61 (at 100%). The fresh weight showed as follow 2.25 (at 50%) > 2.06 (at 25%) > 1.93 (at 75%) > 1.80 (at 0%) > 1.59 (at 100%). Likewise, dry weight follows a trend of 0.63 (at 50%) > 0.45 (at 50%) > 0.39 (at 100%) > 0.37 (at 75%) > 0.32 (at 0%). As Hindon water strength get increases, the growth of plant keeps on decreasing.

CONCLUSION

In this study, The WQI values of water samples collected and analyzed in pre-monsoon and post-monsoon were 1191.50 & 1319.88 respectively, indicated that the Hindon river water quality highly worsened and extended just because of continuous discharge of contaminants mainly heavy metals and nutrient which affected the river fitness and moreover, endangered the fitness of Hindon water for many purposes. Hindon River water used in irrigation of plant would be useful alternate resources to fresh water after proper treatment. As revealed by three crops i.e. *Vigna radiata*, *Triticum aestivum* & *Vigna mungo* when was irrigated with Hindon River water Fig. 3, it can be recommended that Hindon river water is a potential source of different nutrients. Therefore, Hindon water can be used for the purpose to irrigate agricultural field after proper dilutions. This study showed that seed germination of the entire test species were not so sensitive like shoot and root elongation and different species show different levels of tolerance to Hindon river water. The deterioration of water quality due to waste produced by growing population and industrial evolution became a concern and imperilled public health (Anonymous, 1971). The collected water sample holds different ions that can be useful for development & growth of plant but its extreme concentration can cause adverse effects and can hinder the plant growth. Industrial wastes from different manufacturing units contribute to water pollution, which considerably polluted whole food chain. It is also recommended that treatment of effluent is indispensable to minimize the pollution loads which either directly or indirectly enter food chain.

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