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TOXICOLOGICAL STUDY OF HEAVY METALS ON EARLY GROWTH RESPONSES OF SUNFLOWER (*Helianthus annuus* L.)

Muhammad Imran Atta[1], Allah Bakhsh Gulshan[2], Nazir Ahmad[2] and Sadia Saeed[3]
[1]Government Degree College for Boys, Block-17, Dera Ghazi Khan, Pakistan
[2]Government Post Graduate College, Dera Ghazi Khan, Pakistan
[3]Bahauddin Zakariya University, Multan, Pakistan
E-Mail: abgulshan12@gmail.com

ABSTRACT

Environmental contamination due to heavy metals is a major problem in recent time. We studied effects of Pb, Hg and Cr heavy metals on two sunflower varieties (Hysun-33 and SF-5009). Application of these heavy metals significantly ($p<0.05$) affected seed germination time and seedling growth. Plant tolerance index (T.I) also reflected negative impacts of heavy metals on plant vigor. In sunflower seedlings, Pb accumulation was less than Hg and Cr but its toxicity was more (Pb>Hg>Cr). Present study suggested heavy metals induced seed dormancy, phytotoxicity on meristemetic cell division and inhibited activity of growth hormones due to which seedlings attained stressed growth along metal concentration gradient.

Keywords: sunflower (*Helianthus annuus* L.), heavy metals, plant tolerance, phytotoxicity, dormancy, inhibited growth.

INTRODUCTION

Human activities are the major cause of environmental pollution due to significant release of heavy metals through mining, smelting, electroplating, agricultural practices, fuel production and untreated sewage drains (Nedel-koska and Doran, 2000). An increase in heavy metals in the soil and water results increased metal uptake by plants (Farooq *et al*., 2008). Lead (Pb) is adverse environmental pollutant generally used in gasoline to improve fuel efficiency. It pollutes urban environment after release from vehicle exhausts. For plants; Pb is a non-essential element, shows high tendency for its uptake and accumulation in different plant organs (Sharma and Dubey, 2005). Mercury (Hg) is considered as non-degradable pollutant and is being released in the atmosphere and water bodies' upto 7 x 104 tones/year. Chromium is another environmental pollutant due to its wide industrial use. It has two oxidation states i.e., Cr^{3+} and Cr^{6+}. Hexavalent Cr is suggested to be more toxic state than trivalent i.e., Cr^{3+} (Panda and Patra, 2000).

Industrial effluents enriched with such heavy metals are discharged in water bodies, used in crop irrigation and ultimately cause heavy metal pollution (Mussarat *et al*., 2007). In canola (*Brassica napus* L.), lead and Cr metals inhibited plant height, root and shoot length while an obvious loss in tomato growth and yield was noted due to Hg application (Ahmad *et al*., 2011; Shekar *et al*., 2011). In melon plants (*Cucumis melo* L.), decreased seed germination and seedling growth was attributable to Cr toxicity (Akinci and Akinci, 2010). In many plants, heavy metals produced nutritional abnormalities, chlorophyll and protein degradation and affected different physiological attributes (Trivedi and Erdei, 1992; Zengin, 2005; Ahmad and Wahid *et al*., 2011). To understand heavy metal pollution, present study was steered to assess toxicity of Pb, Hg and Cr metals in sunflower plants which are grown for its 40% seed oil in Pakistan (PARC, 2007).

MATERIALS AND METHODS

Petri plate experiment

Certified seeds of two commonly growing sunflower varieties (Hysun 33 = SF1 and SF-5009 = SF2) were purchased from agro-chemical market, Dera Ghazi Khan. To observe percent seed germination, germination time (days) and radicle length; healthy and uniform seeds of both test varieties were arranged on Petri plates furnished with two filter papers. For metal source, $Pb(NO_3)_2$, $HgCl_2$ and $K_2Cr_2O_7$ (Merck-Germany) were used. Varying nutrient free concentrations of Pb, Hg and Cr (50, 75, 100, 150, 200, 250 mg/L) were prepared with distilled water following two replicates treatment^{-1}. Seeds with one milimeter long radicle were implicit to be germinated. Germination time was counted for the day at which all seeds/treatment attained germination. Radicle length was measured at 10th day and data from all treatments was compared with control.

Pot experimental trial

During February-March, 2013; a pot experiment was designed in net house of Biology Department, Government Degree College for Boys, Dera Ghazi Khan. Garden clay mixed canal soil was used after careful sieving and mixing with humus by 3:1 ratio (soil and humus). The soil filled in empty, washed plastic pots of 12 inch diameter. Weighed doses for Pb, Hg and Cr (50, 75, 100, 150, 200, 250mg/kg of soil) were mixed with the pot soil. Four seeds from each variety-SF1 and SF2 were sown at equal distance and one inch deep in soil of corresponding pots. Pots were irrigated with tap water and data for seedling growth responses was taken at 25th day. Including control, each treatment was trailed by five replicates and a total of 70 pots were settled in a complete randomized block design (CRBD). Soil and seedlings were tested from soil and water laboratory, Dera Ghazi Khan. Soil characteristics were pH 7.66, EC 1.63 ds cm^{-1}, organic matter 7.0%, C 6.50%, N 1.29%, K 212ppm, P

16ppm. For heavy metal uptake study, ashed seedlings were subjected to digestion method using a dilute mixture of HNO_3 and HCl. Parkinson-Elmer spectrophotometer was then used for heavy metals uptake study in selected sunflower seedlings.

Plant tolerance index (T.I %)

Plant tolerance index (T.I %) was determined for total plant height using following formula.

$$T.I = \frac{G_t}{G_0} \square\ 100$$

G_t = Mean plant growth in metal enriched medium, *t* = metal treatment
G_0 = Mean plant growth in control treatment

Statistical analysis of data

The data was analyzed with one way ANOVA, standard deviation by SPSS-20 and MS-Excel 2003. Means among treatments were determined by DMRT at 0.05 alpha levels.

RESULTS

Effect on seed germination time and radicle length

Both test varieties were significantly influenced by Pb, Hg and Cr application. Percent germination was not affected but germination time period was increased than control. Due to Pb application, mean germination time was 6.17-6.68 days more than at Hg (5.17-5.5 days) and Cr (5.16-5.33 days). Pb and Cr mainly affected SF1 while Hg affected SF2 (Table-2). Radicle length imposed negative effect of applied heavy metals. The reduction pattern was less at lower metal doses (50-100mg/L) than at lower doses (150-250mg/L). In varieties SF1 and SF2, decline in radicle length due to Pb, Hg and Cr at 50-100mg/L was 5.8-15.89%, 3.7-14.9% and 1-13.16%, respectively. Rate of reduction at 150-250mg/L was 17.9-38.46%, 15.8-35.26% and 7.7-36.84%, respectively (Table-2). Figure-1 reflected Pb and Hg more toxic to variety SF2 and Cr for variety SF1.

Effect on seedling length and plant tolerance index

Although application of three metals affected seedling length, but Pb affected sunflower seedlings more than Hg and Cr. Decline in seedling length by Pb, Hg and Cr at 50-100mg/kg was 2.5-14%, 1.25-10.37% and 1.8-6.25%, respectively. At 150-250mg/kg reduction trend noted was 10.62-24.4%, 10-17.5% and 6.71-12.2%, respectively (Table-2). Figure-1 indicated Pb and Hg to be more toxic for variety SF2 and Cr for variety SF1. Plant tolerance index also varied along increasing metal concentration. Both varieties at 50-250mg/kg revealed reduced tolerance index by Pb, Hg and Cr up to 24%, 17.5% and 15%, respectively (Table-2).

DISCUSSIONS

Almost all heavy metals expose toxic effects when used in high concentration. Uptake of such noxious metals by forage plants caused a serious environmental and health risk problem through food chain (Nedel-koska and Doran, 2000; Kolke, 1980). Kabir *et al*., (2010) explored inhibitory effects of Pb on root, shoot and seedling length of *Thespesia populnea* L. various studies on Hg revealed it as toxic heavy metal. Hg developed adverse effects on seedling growth of rice and cucumber (Du *et al*., 2005). Mahmood *et al*., (2007) reported no significant effects of Hg on percent seed germination. Similarly, Atta *et al*., (2013b) also demonstrated no effect of nutrient free Cr solution on percent seed germination except that germination time was prolonged significantly. They also reported post flowering inhibition of sunflower seedling growth and tolerance due to Cr toxicity at 100-500mg/kg. Various growth parameters were improved at 50mg/kg Cr dose.

Potential out comes of present study strongly agreed these findings. Affected seed germination time and radicle length due to use of Pb, Hg and Cr predicted seed dormancy associated with inefficient membrane permeability, abnormal imbibition and embryonic growth process. Affected pre-mature sunflower seedling growth and metal tolerance indices suggested restricted meristemetic cell division and induction of growth inhibiting hormones. At 50mg/kg Cr metal application, early growth responses were not in agreement with Atta *et al*., 2013b suggested difference in growth stages and establishment of plants in metal enriched medium till maturity with passage of time. Metal accumulation (ppm) in seedlings increased with metal application (mg/kg) predicts development of more osmotic pressure and less nutrient uptake to retard seedling growth (Table-2). In affected seedlings, level of metal toxicity was Pb>Hg>Cr and order of metal accumulation was Cr>Hg>Pb. These findings indicate less metal accumulation/ mobility from soil to plant due to higher densities of Pb and Hg than Cr.

CONCLUSIONS

Present study exposes Pb, Hg and Cr heavy metals toxic to early plant growth. Plant tolerance data reveals these metals responsible to affect plant vigor. Due to greater density, Pb uptake in plants was less than Hg and Cr; but its (Pb) phytotoxicity found to be more than Hg and Cr. Lead and Hg mainly affected sunflower variety SF2 while Cr affcted SF1.

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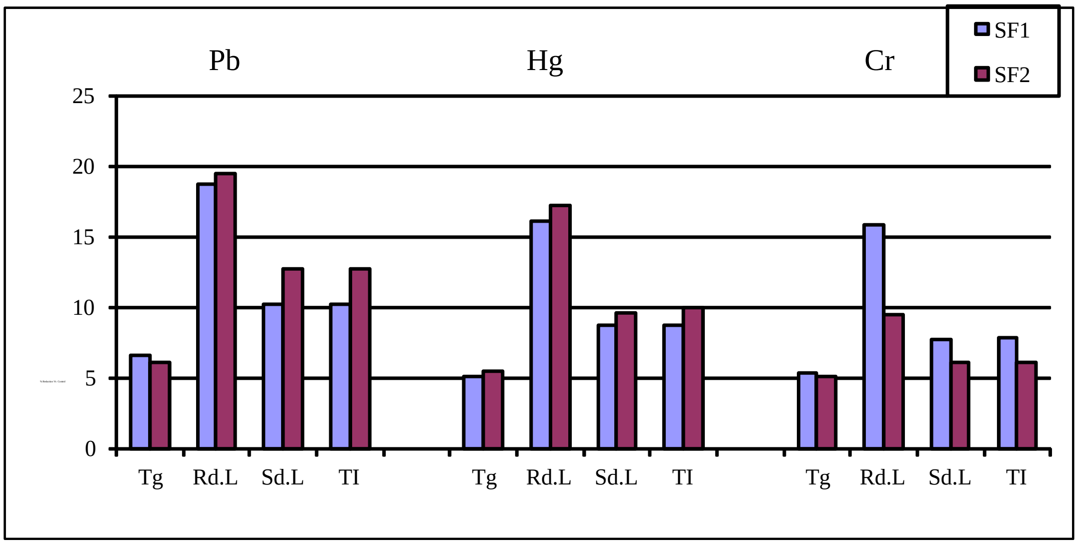

Figure-1. Mean reduction in various plant responses against heavy metals.

Table-1. One way ANOVA for plants various responses against heavy metals.

SOV		**DF**	**Pb**		**Hg**		**Cr**	
			MS	**F-value**	**MS**	**F-value**	**MS**	**F-value**
T.gr	SF1	20	12.877	18.41***	10.00	6.714**	9.510	10.03***
	SF2	20	6.001	4.001*	10.9	5.5**	5.851	15.11**
Rd. L	SF1	20	17.860	29.17***	15.91	21.3***	20.11	15.2**
	SF2	20	18.59	24.42***	17.01	20.5***	10.01	6.104**
Sd. L	SF1	34	6.185	25.13***	5.034	6.82***	4.812	7.315***
	SF2	34	8.65	35.16***	5.009	7.50***	4.785	7.652***
T.I	SF1	34	235.8	63.34***	227.16	70.92***	191.355	66.41***
	SF2	34	327.44	44.16***	191.54	81.61***	112.16	48.21***

Table-2. Analysis of plant responses against heavy metals.

Parameter	Var	0	50	75	100	150	200	250
Pb								
T.gr (days)	SF1	3±0.0	4±0.8	6±0.8	6±1.0	7±1.0	8±0.7	9±0.85
	SF2	3±0.0	4±0.77	5±1.0	6±1.7	7±1.0	7±2.0	8±2.64
Rd. L (mm)	SF1	19±0.5	18±0.6	17.1±1	16.3±0.7	15.6±0.6	13.9±1.1	11.8±0.91
	SF2	19.5±2	18.1±0.61	17.4±0.6	16.4±0.53	15.8±0.4	14.3±0.3	12±0.88
Sd. L (cm)	SF1	16±0.2	15.6±0.61	15.2±0.5	14.4±0.43	14.3±0.3	13.8±0.6	12.8±0.5
	SF2	16.4±0.5	15.9±0.42	15.3±1	14.1±0.38	14.1±0.2	14±0.14	12.4±0.46
T.I (%)	SF1	100±0	97.5±1.5	95±2.1	90±1.87	89.9±1.21	86.2±1.3	80±3.53
	SF2	100±0	97±3.74	93.3±1.7	86±3.6	86±3.16	85.3±1.4	76±3.08
Pb uptake	SF1	-	11.7	13.9	19	29.4	38.2	47.8
(ppm)	SF2	-	12.4	14.1	19.9	30.2	40.1	49.3
Hg								
T.gr (days)	SF1	3±0.0	3±1	4±1	5±1	5±1.73	6±0.93	8±1.62
	SF2	3±0.0	4±0.85	4±2	5±0.91	5±1.18	7±0.79	8±0.96
Rd. L (mm)	SF1	19±0.5	18.3±0.3	18±0.3	16.4±0.4	16±0.6	14.6±0.53	12.3±0.49
	SF2	19.5±2	18.6±1.4	18.1±0.2	16.6±0.4	16±0.22	14.9±0.17	12.7±0.3
Sd. L (cm)	SF1	16±0.2	15.8±0.8	15.5±1.1	14.9±0.47	14.4±0.9	13.8±0.58	13.2±1.6
	SF2	16.4±0.2	16±0.33	15.5±1.6	14.7±0.67	14.6±0.7	14.1±0.41	13.6±0.56
T.I (%)	SF1	100±0	98.7±1.6	96.7±0.9	93±2.54	90±1.38	86.2±2.2	82.5±2.38
	F2	100±0	97.5±0.4	94.5±1.6	90±2.25	89±1.27	86±2.37	83±1.06
Hg uptake	SF1	-	11.2	12.7	19.2	30	35.6	45.2
(ppm)	SF2	-	13	13.2	18.4	29.5	39.3	48.1
Cr^{6+}								
T.gr (days)	SF1	3±0.0	4±1	4±1	5±0.14	6±1.73	6±1	7±0.48
	SF2	3±0.0	3±0.6	5±1	5±1	5±1	6±1	7±0.5
Rd. L (mm)	SF1	19±0.5	19±1	18±1	16.5±0.9	16±0.6	14.4±0.54	12±1.32
	SF2	19.5±2	19.3±2	18.7±0.7	18±1.6	18±0.43	16.2±0.62	15.4±0.8
Sd. L (cm)	SF1	16±0.2	15.7±0.6	15.5±0.61	15±0.75	14.7±0.9	14±0.21	13.6±0.9
	SF2	16.4±0.5	16.1±0.7	16.1±1	15.7±0.8	15.3±0.6	14.8±0.62	14.4±1.1
T.I (%)	SF1	100±0	98.1±1.2	96.8±0.7	93.7±2.5	92±1.38	87.5±2.6	85±1.82
	SF2	100±0	98.2±2.5	98.2±1.7	95.7±1.5	93.3±1.2	90.2±1.23	88±1.15
Cr uptake	SF1	-	14.5	18	24	33.8	40.2	46.7
(ppm)	SF2	-	13.7	18	23.5	33	40.4	46.1

Level of metal toxicity: Pb>Hg>Cr

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