Original Article

INTERACTIVE EFFECTS OF SINGLE AND COMBINED HEAVY METALS ON GROWTH OF ISBGOL AND GARDEN CRESS GROWN ON HEAVY METAL CONTAMINATED SOIL

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Abstract

The toxicity of Cu, Cd, Hg and their interactions on Isabgol and Garden cress were determined. The effects were defined as antagonistic, additive, or synergistic, in accordance with the sign of difference between the tested hypothesis and the value of the observed toxicity at tested combinations. The type of toxic interaction at each tested metal combination was evaluated using independent t test and welch t test. The interactive effects of heavy metal on growth of Isabgol and Garden cress was evaluated by RRG values, % phytotoxicity and root-shoot ratio of young plants using descriptive statistics as well as inferential statistics.

Some of the findings are: (i) binary metal (Hg+Cd) combination has minimum Relative Root Growth in both younger and older Isabgol plant where as (cu+cd+Hg) has minimum Relative Root Growth in Garden Cress Plants. (ii) Single metal CuCl2 has minimum phytotoxicity in both younger and older Isabgol plant as well as in both younger and older Garden Cress Plants. From inferential statistics, it can be concluded that Garden Cress will have slow growth in pollutant soil in comparison of Isabgol. It can be also inferred that phytotoxicity has been highly increased in Garden Cress in comparison of Isabgol.

Keywords: heavy metals, toxicity, interactions, Isabgol, Garden cress, Kalmogorov – Smirnov test statistic, independent t test, welch t – test.

INTRODUCTION

In environment Many chemical mixtures, where concentrations of individual chemicals commonly exist at levels not considered toxic, are often present. More than on metals are present in environment where individual constituents are present at low, non-toxic concentrations may trigger toxicity due to additive or synergistic effects among the constituents (Rajapakse et al., 2002, Montvydienė D and Marčiulionienė D 2004). It is necessary to focus the rules of interactive toxic effects of metals (Olmstead and Leblanc 2005, Lin et al., 2005). The scope of this research included the evaluation of effects heavy metal interactions in Isabgol and Garden cress describing the toxic interactions of metals in the mixture through RRG values, % phytotoxicity and root-shoot ratio with their statistical analysis (The jamovi project 2021, R Core Team 2021).

MATERIALS AND METHODS

The earthen pots were filled with garden soil. The potted soil was contaminated with following heavy metal separately.200 ppm - 200 mg and 600 ppm - 600 mg $CuCl_2$/$CdCl_2$/$HgCl_2$ / kg soil
200 ppm (each) - 200 mg (each) and 600 ppm (each) - 600 mg (each) Cu+Cd/Cu+Hg/Hg+Cd /Cu+Cd+Hg/ kg soil

The pots without any addition of heavy metal were considered as control. 20 pots were kept for each treatment. Seeds of Isabgol(Plantago ovata Forsk var Guj 2) and Garden cress (Lepidium sativum L. var local) were sown in pots. Interactive effects of heavy metal on Isabgol and Garden Cress were studied regularly at the interval of 15 days, study period was 75 days. Methods of Gregory (1921, 1926) and Hunt (1978) were used to study the growth of the plants. The interactive effects of heavy metals were also evaluated with the help of following:

(a) Relative Root Growth (RRG)

(b) Percent Phytotoxicity

(c) Root Shoot (R/S) Ratio

(a) Relative Root Growth (RRG)

Relative Root Growth was calculated according to the formula given by Ouzounidou et. al., (1992). The formula is given below:

$$RRG = \frac{\text{Mean length of longest root in toxic solution}}{\text{Mean length of the longest root of control}} \times 100$$

(b) Percent Phytotoxicity

Percent Phytotoxicity was calculated following bioassay technique suggested by Chou and Muller (1972).

$$\text{Percent Phytotoxicity} = \frac{\text{Radical length of control} - \text{Radical length of test}}{\text{Radical length of control}} \text{X } 100$$

(c) Root Shoot (R/S) Ratio:

Root Shoot ratio was calculated using the following formula:

$$\text{Root-Shoot Ratio} = \frac{\text{Root length of particular treatment}}{\text{Shoot length of same treatment}}$$

DATA COLLECTION:

This research paper is based on experimental design. As per the above formula, % of RRG values, % phytotoxicity and root- shoot ratio of young plants for 15 days and 75 days of Isabgol and Garden Cress were observed and recorded as per the table1,table 2 and table 3.

DATA ANALYSIS AND RESULTS

Data was analysed using descriptive statistics and inferential statistics.

- **Descriptive Analysis**

Table 1: Effect of single heavy metal and mixture of heavy metals as a soil pollutant on Relative Root Growth (RRG-%) of Isabgol and Garden Cress

HEAVY METAL TREATMENT	ISABGOL RRG(%)		GARDEN CRESS RRG(%)	
	GROWTH PERIOD-in days		GROWTH PERIOD-in days	
	15	75	15	75
Control	-	-	-	-
200 ppm				
$CuCl_2$	56	78	55	66
$CdCl_2$	50	68	45	57
$HgCl_2$	33	57	40	50
Cu+Cd	43	62	50	51
Cu+Hg	33	49	40	43
Hg+Cd	26	38	35	40
Cu+Cd+Hg	33	42	20	40
600 ppm				
$CuCl_2$	43	72	45	60
$CdCl_2$	36	57	25	36
$HgCl_2$	26	49	25	32
Cu+Cd	33	49	30	32
Cu+Hg	26	43	25	36
Hg+Cd	16	31	25	32
Cu+Cd+Hg	26	33	10	24

Table 1 shows the values of Relative Root Growth (RRG %) of Isabgol and Garden cress grown on heavy metal contaminated soil. The RRG values were minimum in 15days old as well as 75days old Isabgol grown with Hg+Cd. When RRG values in Hg+Cd, Cu+Cd+Hg treated plants were compared, values were higher in Cu+Cd+Hg treated plants than that in Hg+Cd treated plants, which indicates that ternary mixture slightly repaired injury. When single metal was used the lowering in RRG value was in the order of Hg>Cd>Cu. The interaction of Cu with Cd and Hg gave additive effects. Similarly, interaction of Hg with Cu and with Cd also gave additive effects. Thus, mixtures of metals were more adverse than single metal. The lowering of RRG values in Garden cress was in the order of Cu+Cd+Hg>Hg+Cd>Cu+Hg>Hg>Cu+Cd>Cd>Cu. In younger plants RRG values were higher in Cu+Cd than with Cd only. Thus, Cu acted as antagonistic element upto certain extent. When RRG values in young plants (15days) and old plants (75days) were compared it was found that values were higher in 75days old plants than that in 15days old plants suggesting physiological adaptation of root against heavy metals, with increasing age heavy metal may be translocated to the shoot and thus effect on root of older plants was less. RRG was used as a physiological parameter to evaluate the Cu, Cd and Hg toxicity in various crop plants (Ouzoundio *et. al.*, 1992, Vyas and Vedia 1999, Vyas *et. al.*, 2000, Vedia and Vyas 2002a, 2002b).

Table 2: Effect of single heavy metal and mixture of heavy metals as a soil pollutant on % Phytotoxicity of Isabgol and Garden Cress

HEAVY METAL TREATMENT	ISABGOL (% PHYTOTOXICITY) GROWTH PERIOD-in days		GARDEN CRESS % PHYTOTOXICITY GROWTH PERIOD-in days	
	15	75	15	75
Control	-	-	-	-
200 ppm				
$CuCl_2$	43	22	45	33
$CdCl_2$	50	31	55	41
$HgCl_2$	66	43	60	49
Cu+Cd	56	38	50	48
Cu+Hg	66	50	60	56
Hg+Cd	73	62	65	57
Cu+Cd+Hg	66	58	80	57
600 ppm				
$CuCl_2$	56	27	55	39
$CdCl_2$	63	38	75	63
$HgCl_2$	73	50	75	67
Cu+Cd	33	50	70	67
Cu+Hg	73	57	75	67
Hg+Cd	83	68	75	70
Cu+Cd+Hg	73	67	90	73

It can be seen from **Table 2** % phytotoxicity was higher in 600ppm treated plants than that in 200ppm treated plants, % phytotoxicity was also higher in younger plants than that in older plants which suggests acclimation under heavy metal stress. The acclimation was more significant with single metal than with mixture of metals. The maximum values were found in Hg+Cd treated plants of Isabgol and minimum values were found with Cu treated plants, % phytotoxicity values were higher in plants treated with binary mixture of Hg+Cd than that in plants grown with ternary mixture of Cu+Cd+Hg. In case of Garden cress maximum values were found with Cu+Cd+Hg followed by Hg+Cd. The values were minimum with Cu. The data indicates that interaction may be considered as additive interaction rather than antagonistic interaction of heavy metals.

Table 3 represents the Root Shoot Ratio values were also affected by single metal and mixture of metals. In 15days old plants of Isabgol 200ppm of Cd, Cu+Hg, Hg+Cd, Cu+Cd+Hg increased root shoot ratio values. When concentration was severe i.e. 600ppm, Cu+Cd, Cu+Hg, Cu+Cd+Hg increased the values. Higher root shoot values indicated that heavy metal may disturb stem elongation also. Thus root shoot ratio was higher than control. In 75days old plants Cu+Hg, Hg+Cd, Cu+Cd+Hg remarkably increased the value. Higher values indicate that either root elongation was not much affected or stem elongation was highly reduced. When concentration was 600ppm Cd, Cu+Hg, Hg+Cd, Cu+Cd+Hg increased the values in 15days old plants of Isabgol but in 75day old plants Hg, Cu+Hg, Hg+Cd and Cu+Cd+Hg gave very high values. The data indicate that not only root but stem elongation was also stunted. Root shoot values were lower in heavy metal treated Garden cress plants than that in control plants. The root shoot ratio values were almost similar in 15days and 75days old plants.

Table 3: Effect of single heavy metal and mixture of heavy metals as a soil pollutant on Root Shoot Ratio of Isabgol andGarden Cress

HEAVY METAL TREATMENT	ISABGOL R/S RATIO		GARDEN CRESS R/S RATIO	
	GROWTH PERIOD-in days		GROWTH PERIOD-in days	
	15	75	15	75
Control	0.23	0.37	0.28	0.22
200 ppm				
$CuCl_2$	0.21	0.34	0.15	0.16
$CdCl_2$	0.25	0.36	0.16	0.17
$HgCl_2$	0.2	0.4	0.16	0.19
Cu+Cd	0.21	0.38	0.23	0.17
Cu+Hg	0.25	0.47	0.19	0.16
Hg+Cd	0.26	0.5	0.21	0.17
Cu+Cd+Hg	0.33	0.73	0.095	0.19
600 ppm				
$CuCl_2$	0.18	0.44	0.2	0.24
$CdCl_2$	0.22	0.4	0.12	0.15
$HgCl_2$	0.2	0.86	0.16	0.15
Cu+Cd	0.2	0.43	0.17	0.13
Cu+Hg	0.26	0.72	0.16	0.14
Hg+Cd	0.25	0.82	0.16	0.14
Cu+Cd+Hg	0.38	1	0.062	0.15

- **Inferential Analysis**

Statistical Inferential analysis of data is done using software JAMOVI version 2.2. As the data follows assumption of parametric test, to compare statistically significant difference between two concentrations for RRG, phytotoxicity and root shoot ratio, independent t test is used. If assumption of homogeneity is violated then welch's t test is used instead of independent student's t test. In this paper we have tried to measure effect of 200ppm and 600ppm heavy metal contaminated soil on Isabgol and Garden Cress for 15 days as well as 75 days.

- **Statistical Analysis For RRG**

Table 4: Difference of heavy metal treatment on Relative Root Growth of Isabgol and Garden Cress

		Mean		Test of Normality		Test of Homogeneity		Mean difference	Student's t statistic	p -value
Plant	N	200ppm	600ppm	Kolmogorov-Smirnov statistic	p -val ue	Leven's statistic	p-value			
ISABGOL 15days	7	39.1	29.4	0.94	0.41	0.79	0.39	9.71	1.85	0.089
ISABGOL 75days	7	56.3	47.7	0.94	0.48	0.06	0.81	8.57	1.13	0.282
GARDEN CRESS 15days	7	40.7	26.4	0.93	0.33	0.16	0.69	14.29	2.47	0.03
GARDEN CRESS 75days	7	49.6	36	0.88	0.06	0.02	0.90	13.57	2.42	0.032

It can be observed from table 4 that p value of Kolmogorov-Smirnov test statistic is greater than 0.05. So assumption of normality is satisfied. Even p value of Leven's statistic is also greater than 0.05 i.e. test of homogeneity is also satisfied. To teststatistically significant difference of treatment 200ppm and 600ppm

metals on soil for Relative Root Growth of Isabgol and Garden Cress, independent student's t test is applied and the results for plants of Isabgol indicate that p values for Isabgol 15 days as well as Isabgol 75 days are greater than 0.05. So it can be concluded that relative root growth for plant of Isabgol is not affected due to heavy metal concentration. Isabgol can grow with pollutant soil. But the results for plant of Garden Cress indicate that p values for Garden Cress 15 days as well as Garden Cress 75 days are less than 0.05. So it can be interpreted that relative root growth for plant of Garden Cress is affected due to heavy metal concentration. In other word, Garden Cress willhave slow growth in pollutant soil.

- **Statistical Analysis for Phytotoxicity**

Table 5: Difference of heavy metal treatment on Phytotoxicity of Isabgol and garden Cress

		Mean		Test of Normality		Test of Homogeneity		Mean difference	Student's t statistic	p -value
Plant	N	200ppm	600ppm	Kolmogorov-Smirnov statistic	p -value	Leven's statistic	p-value			
ISABGOL 15days	7	60	64.9	0.91	0.16	0.64	0.44	-4.86	-0.66	0.53
ISABGOL 75days	7	43.4	51	0.94	0.39	0.00	0.96	-7.57	-0.97	0.35
GARDEN CRESS 15days	7	59.3	73.6	0.93	0.33	0.16	0.69	-14.29	-2.47	0.03
GARDEN CRESS 75days	7	48.7	63.7	0.87	0.06	0.01	0.92	-15	-2.73	0.02

It can be observed from table 5 that p value of Kolmogorov-Smirnov test statistic is greater than 0.05. So, assumption of normality is satisfied. Even p value of Leven's statistic is also greater than 0.05 i.e. test of homogeneity is also satisfied. To test statistically significant difference of treatment 200ppm and 600ppm metals on soil for Phytotoxicity of Isabgol and Garden Cress, independent student's t test is applied and the results for plants of Isabgol indicate that p values for Isabgol 15 days as well as Isabgol 75 days are greater than 0.05. So it can be depicted that Phytotoxicity for plant of Isabgol is not affected due to heavy metal concentration. But the results for plant of Garden Cress indicate that p values for Garden Cress 15 days as well as Garden Cress 75 days are less than 0.05. So it can be portrayed that Phytotoxicity for plant of Garden Cress is affected due to heavy metal concentration. Phytotoxicity has been highly increased in Garden Cress.

- **Statistical Analysis for Root Shoot Ratio**

It can be observed from table 6 that p value of Kolmogorov-Smirnov test statistic is greater than 0.05. So assumption of normality is satisfied and p value of Leven's statistic is greater than 0.05 for plants considered except Isabgol 75 days. i.e. test of homogeneity is not satisfied for Isabgol 75 days. So to test statistically significant difference of treatment 200ppm and 600ppm metals on soil for Root Shoot Ratio of Isabgol and Garden Cress, independent student's t test (welch's t test for Isabgol75 days) is applied and the results for plants of Isabgol as well as Garden Cress indicate that p values are greater than 0.05. So it can be concluded that there is no significant effect of metals as a pollutant on root shoot ration of both Isabgol and Garden Cress.

Table 6: Difference of heavy metal treatment on Root Shoot Ratio of Isabgol and Garden Cress

		Mean		Test of Normality		Test of Homogeneity		Mean difference	Student's t/ welch's t statistic	p -value
Plant	N	200ppm	600ppm	Kolmogorov - Smirnov statistic	p - value	Leven's statistic	p-value			
ISABGOL 15days	7	0.24	0.24	0.23	0.48	0.59	0.46	0.00	0.09	0.93
ISABGOL 75days	7	0.45	0.67	0.11	0.99	5.83	**0.03**	-0.21	(welch) - 2.0293	0.10
GARDEN CRESS 15days	7	0.17	0.15	0.19	0.71	0.01	0.92	0.02	0.98	0.35
GARDEN CRESS 75days	7	0.17	0.16	0.33	0.10	1.72	0.21	0.02	1.06	0.31

CONCLUSION:

Finally it is concluded that Isabgol and Garden cress were sensitive to Cu, Cd, Hg and their binary and ternary interactions. Binary interactions of each metal gave additive effects on growth and metabolism of both the crops, the intensity of the effects depend upon nature of the metal, as Hg and Cd were more phytotoxic than Cu. As a cost-effective and eco-friendly approach Phytoremediation could be a successful mitigation measure to revegetate heavy metal-polluted soil.

On the basis of concentration 200 ppm as well as 600 ppm, it can be concluded that relative root growth for plant of Isabgol is not much affected due to heavy metal concentration. But the relative root growth for plant of Garden Cress is more affected due to heavy metal concentration. In other words, Garden Cress will have slow growth in pollutant soil in comparison of Isabgol. It can be also inferred that phytotoxicity has been highly increased in Garden Cress in comparison of Isabgol. Concentration of 200 ppm or 600 ppm of the metals in soil has no any significant effect on root shoot ratio of Isabgol as well as Garden Cress. In other words, lower concentration of 200ppm also shows high adverse effect on root shoot ration in both younger and older plants of Isabgul as well as Garden Cress.

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