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Review Article

Studies on the Phyto-Toxicity of Heavy Metal Mercury in Wheat (*Triticum AestivumL*) I–Germination Percentage (GP) and Speed of Germination Index (SGI)

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Abstract

In the present investigation studies were made on the Phyto-toxicity of Heavy Metal Mercury (Hg) in four Wheat (*Triticum aestivumL*) varieties taking into consideration Germination Percentage (GP) and Speed of Germination Index (SGI) to bring out varietal tolerance behavior. It was observed that the germination decreased in the four wheat varieties viz., V1 Lok-1, V2 UP-2338, V3 PBW-154 and V4 PBW-502 with increasing concentration of Hg. A comparison with controls in the four varieties recorded in the controls: at 24 hours GP was lowest in the variety V1 (23.66%) and highest in V4 (26.66%); at 48 hours lowest in V1 (51.0%) and highest in V4 (54.66%); at 72 hours lowest in V1 (61.33%) and highest in V3 (66.66%); at 96 hours lowest in V2 (82.0%) and highest in V3 and V4 (84.33%) and at 120 hours lowest in V2 (86.0%) and highest in V3 (94.0%) respectively. On the other hand, in 100, 200 and 300ppm of mercury concentration treatments there was no germination found at all at 24 hours in all the four varieties while in 100ppm at 48 hours lowest GP was in V2 (16.0%) and highest in V3 and V4 (21.33%); at 72 hours lowest in V4 (31.33%) and highest in V2 (36.0%); at 96 hours lowest in V1 (44.0%) and highest in V2 (49.0%) and at 120 hours lowest in V4 (51.0%) and highest in V2 (53.66%) respectively. In 200ppm Hg concentration at 48 hours lowest GP was found in V4 (9.66%) and highest in V3 (13.66%) respectively; at 72 hours lowest in V4 (25.66%) and highest in V1 (37.0%); 96 hours lowest in V4 (37.33%) and highest in V1 (43.0%) and at 120 hours lowest in V3 (42.0%) and highest in V4 (48.33%) respectively. Lastly, in 300ppm at 48 hours lowest GP was in V4 (3.0%) and highest in V1 (5.0%); at 72 hours lowest in V2 (8.0%) and highest in V1 (12.0%); 96 hours lowest in V4 (17.0%) and highest in V1 (21.66%) and at 120 hours lowest in V2 (26.0%) and highest in V1 (32.33%) respectively. A comparison with controls in the four varieties recorded maximum average GP (65.13%) in control sets of var. V3 whereas minimum (11.2%) was recorded in the 300ppm Hg treatment sets in the var. V2. On the other hand, data on the basis of percent over control showed maximum GP (48.98%) in the var. V2 in 100ppm Hg treatment and the minimum (18.0%) was also recorded in the var. V2 in 300ppm Hg treatment. Showing a clear cut maximum decrease of (-81.99%) in the var. V2 in 300ppm Hg treatment and also minimum decrease of (-51.02%) in the var. V2 in 100ppm Hg treatment. With this SGI showed the trend as highest (811.66) in controls of V3 while lowest of (102.0) in V2 in 300ppm Hg treatment while data on percent over control basis showed highest (43.08) in 100ppm Hg treatment in the var. V1 while lowest (13.03) in 300ppm Hg treatment in the var. V2. Showing maximum decrease of (-86.96) in the var. V2 in 300ppm Hg treatment and minimum decrease of (-56.91) in the var. V1 in 100ppm Hg treatment.

Keywords: Wheat varieties; Germination percentage (GP); Speed of germination index (SGI); Mercury toleranc

Introduction

Unfortunately, land and water, the two precious natural resources on which relies the sustainability of agriculture and the civilization of mankind have been subjected to maximum exploitation and severely degraded/polluted due to anthropogenic activities. The pollution includes point sources such as emission [1-3], effluents and solid discharge from industries [4-7], vehicle exhaustion [1-2]. and metals from smelting and mining, and non-point sources such as soluble salts (natural and artificial) [8-14], use of insecticides/pesticides [15], disposal of industrial and municipal wastes in agriculture [7,16], and excessive use of fertilizers [17-19]. Contamination of agricultural land caused by heavy metal in and around industrial areas is a serious problem. According to several scientists such contamination is largely due to injudicious anthropogenic activities such as indiscriminate use of pesticides containing heavy metals in agriculture, discharge of untreated industrial wastes and effluents, faulty waste disposal, high rate of burning of fossil fuels, mining etc. [20-24].

Each source of contamination has its own damaging effects to plants, animals and ultimately to human health, but those that add heavy metals to soils and waters are of serious concern due to their persistence in the environment and carcinogenicity to human beings. They cannot be destroyed biologically but are only transformed from one oxidation state or organic complex to another [25,26]. Therefore, heavy metal pollution poses a great potential threat to the environment and human health [4-7,15,16,27-29].

Though, Lead is a ubiquitous poison known to man from biblical times Mercury became notorious in the recent past as a result of Minamata disease in Japan and has been found guilty as a harmful environmental pollutant. What worries us now and causes considerable concern is that this and other toxic metals are being found in an increasing amount in the human environment, in the air we breathe, in the water we drink and in the food we consume. Heavy metal toxicity, such as due to mercury in our environment, is seriously dangerous because these metals persist in the environment for years together [28]. The poor socio-economic conditions and lack of awareness in the agricultural community towards the hazards associated with waste water irrigation, it is practically impossible to put a stop to it. The magnitude of the danger of environmental pollution by heavy metal, especially mercury, was probably for the first time realized with the Minamata Disaster in Japan [6,28,30,31] when thousands of people suffered with mercury poisoning after consuming the fish caught in Minamata Bay contaminated by mercury released between 1953 and 1960 from the Chisso Corporation's Chemical Factory, Vinyl Chloride Plant [30]. Though, Minamata has made mercury famous but mercury has been around for a very long time. Metallic mercury in its liquid form is of little or no significance as a threat to health however, inhalation of metallic mercury vapours can cause acute or chronic health effects. Several of mercury salts are extremely toxic, the best known of which is mercuric chloride ($HgCl_2$). Organic compounds of mercury are the ones which are causing the greatest concern. Mercury is bio-transformed to Zinc mercuric compounds. This has been considered to be responsible for the Minamata episode [30].

The mercury cycle is a bio-geochemical cycle involving mercury. Mercury is notable for being the only heavy metal which is liquid at room temperature. It is volatile metal and evaporates, though it takes quite a while to do so. Most natural mercury occurs as cinnabar (HgS) where mercury (Hg^{2+}) is bound very tightly to sulphur but weathering slowly releases the mercury to the environment [32]. There are also trace amounts of mercury in coal. Mining mercury or burning coal results in releasing mercury. Volcanoes and forest fires are also sources of mercury. Chlorine factories, among other sources, release mercury into the atmosphere. This mercury is deposited back onto land and water. Inorganic mercury can be converted by bacteria into the organo-metallic cation known as methyl mercury (CH_3Hg^+) which bio-accumulates in fish. Over long periods of time, some mercury recombines with sulphur and is buried in sediments.

Then, the cycle repeats itself. Briefly, the bio-geochemical cycle of Hg starts with the evaporation of Hg from natural and anthropogenic sources, which is then oxidized to inorganic Hg. This element is spread by the rain. Once in the soil, Hg can be transformed into organic compounds by bacteria. Fish and shellfish have a natural tendency to concentrate mercury in their bodies, often in the form of methyl mercury. Species of fish that are high on the food chain, such as shark, swordfish, etc., contain higher concentrations of mercury than others. As mercury and methyl mercury are fat soluble, they primarily accumulate in the viscera, although they are also found throughout the muscle tissue.

When this fish is consumed by a predator, the mercury level is accumulated. Since fish are less efficient at depurating than accumulating methyl mercury, fish-tissue concentrations increase over time. Thus species that are high on the food chain amass body burdens of mercury that can be ten times higher than the species they consume called bio-magnification. Mercury poisoning happened this way in Minamata [33] therefore, called Minamata disease. Further, microorganisms in the water convert mercury to this highly toxic form methyl mercury and thus bacteria makes the mercury "bio-available" to be transported to fish and then to man.

Land and water pollution by heavy metals is a worldwide issue. All countries have been affected, though the area and severity of pollution vary enormously. In Western Europe, 1,400,000 sites were affected by heavy metals [34], of which, over 300,000 were contaminated, and the estimated total number in Europe could be much larger, as pollution problems increasingly occurred in Central and Eastern European countries [35]. In USA, there are 600,000 brown fields which are contaminated with heavy metals and need reclamation [36]. According to statistics, coal mine has contaminated more than 19,000 km of US streams and rivers from heavy metals, acid mine drainage and polluted sediments.

More than 100,000 ha of cropland, 55,000 ha of pasture and 50,000 ha of forest have been lost [37]. The problem of land pollution is also a great challenge in China, where one-sixth of total arable land has been polluted by heavy metals, and more than 40% has been degraded to varying degree due to erosion and desertification [38]. Soil and water pollution is also severe in India, Pakistan and Bangladesh, where small industrial units are pouring their untreated effluents in the surface drains, which spread over near agricultural fields. In these countries raw sewage is often used for producing vegetables near big cities [14-16].

The development of the intensive agriculture between 1960 and 1990 totally over passed the aspect connected with the negative impact of the toxic chemical compounds on the air, water and soil. As one of the consequences of heavy metal pollution in soil, water and air, plants are contaminated by heavy metals. Using chemical products as nutrients, fertilizers and pesticides, we believe that we attack our safety and we must know the effects of heavy metals from these compounds. Many researchers examined the inhibitory effect of heavy metal compounds on growth and the performance of photosynthetic apparatus of plants. There are two aspects on the interaction of plants with heavy metals:

(i) Heavy metals show negative effects on plants.

(ii) Plants have their own resistance mechanisms against toxic effects and for detoxifying heavy metal pollution.

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The effects of heavy metals on plants resulted in growth inhibition, structural damage, decline of physiological and biochemical activities, as well as of the function of plants. The effect of different metallic ions in the hydrolysis of ATP is studied in. Carotenes especially have attracted much attention in recent years for their biological function. Knowing the nutrients required to grow plants is only one aspect of successful crop production. Optimum yield also requires knowing the rate to apply, the method and time of application, the source of nutrients to use, and how the elements are influenced by soil and climatic conditions.

Mercury is an environmental pollutant which is mainly supplied via anthropogenic sources to the soil. It is harmful because of its toxicity, mobility, bio-accumulation, methylation process and transport in the atmosphere [39]. Plants living in Hg-enriched ecosystems can be adapted to field conditions by detoxification mechanisms, although Hg is a metal without known biological function in higher plants. Hg compounds are highly toxic to plants and concentrations in plant tissues increase with age. Soil Hg availability for plants is usually low because it is absorbed in the soil or precipitated in the soil solution and it is mainly accumulated in roots [40]. Nevertheless it reaches the shoots by translocation or foliar absorption. The most common chemical species in soils are Hg^0 and Hg^{++}. Long range atmospheric transport of mercury, in large part from coal combustion, can contribute up to 50% of total loading to humus rich soils. Humic matter forms strong complexes with Hg^{++}. In fact, transport of Hg in soil water is largely due to association with soluble humic matter. Chronic exposure due to consumption of methyl mercury in fish and other sea food with subsequent neurotoxicity is a human health concern [41].

Methyl mercury forms in anaerobic sediments of aquatic ecosystems and biomagnifies through tropic transfer to fish. The adverse effects of toxic chemicals on soil fauna and microbes are of the major foci in soil eco-toxicological assessments. There are several kinds of standardized plant toxicity tests, i.e., seed germination, root elongation and early seedling growth tests. Photosynthesis inhibition test and enzyme content fluctuation are also frequently used as endpoints for phyto-toxicity. The present study selected wheat for testing as it is the main staple cereal in the world.

Furthermore, germination (GP), speed of germination (SGI), root elongation is selected as a quantitative test endpoint in this study as the root accumulate more toxicants and is more sensitive than shoot. Recent reports on the toxic effects of heavy metals on wheat indicate that heavy metals inhibit GP and SGI with root and shoot growth [5,7,14,16,28,42,43] and also induce oxidative stress and lipid per-oxidation [5,14,28,44]. Various defense mechanisms adopted by wheat to avoid heavy metal toxicity have been reported by several researchers.

These include: alteration of antioxidant enzyme level [5,14,28,43-45] increase in the content of phyto-chelation [46,47] and increase generation of polyamine and ethylene [48]. High concentrations of heavy metals in soil can negatively affect crop growth, as these metals interfere with metabolic functions in plants, including physiological and biochemical processes [5,14,28,], inhibition of photosynthesis, and respiration and degeneration of main cell organelles, even leading to death of plants [25,49,50]. In order to cope up with heavy metal contaminated soils, various phyto-remediation approaches (phyto-stabilization, phyto-immobilization and phyto-extraction) can be applied. However, the choice will depend on many factors, such as plant tolerance to pollutants, soil physic-chemical properties, agronomic characteristics of the plant species, climatic conditions (rainfall, temperature), and additional technologies available for the recovery of metals from the harvested plant biomass. It appears that both chemical and biological approaches are passing through their infancy and need more efforts for their effective use in the future [5,7,14,16,27,28].

Significance of the Study

Environmental Pollution has emerged as a major epidemic endangering Life on earth. Due to unwise, unscientific and excessive use of natural resources eco-balance is disturbed. Industrialization and urbanization have also deteriorated the position. As such pollution can be considered the result of the growth of modern civilization. Hence effective Pollution control is the need of the hour. Episodes like the *Minamata* and *Itai-Itai* epidemics in Japan serve as a warning against the indiscriminate and careless use of toxic heavy metals. For effective control of heavy metal pollution, it is necessary to continuously monitor the environment for their presence, to initiate a system for biological monitoring for heavy metal exposure and to take appropriate steps to minimize and control heavy metal pollution.

Objectives

Thus, the objective of this work was to evaluate the toxicity of heavy metal mercury on wheat crop to provide information on the significance of seed GP and SGI, root/shoot ratio, dry and fresh weights of the seedlings, chlorophyll, carbohydrate and protein contents and enzymatic system in response to heavy metal stress and to determine the effects of heavy metal Hg on growth and metabolism of crop plants. The findings of present study would not only help in understanding the phyto-toxicity of heavy metal pollution but would also try to suggest the possibilities of selecting suitable varieties of crops for growing best under heavy metal polluted irrigation waters and soils and the control measures to overcome the heavy metal phyto-toxicity.

Material and Methods

a.Experimental Design

The four wheat varieties selected for the present investigation are most commonly grown in western U.P. particularly in Mathura and nearby areas. The seeds of the material involved in the present study were kindly provided by the Agriculture Research Centre Raya, Mathura. The following crop plants were chosen for experimentation: The following four wheat varieties (*Triticum aestivum* L) of the family Poaceae (Gramineae) were selected for experimentation:

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1) Lok1 (V1)

2) UP-2338 (V2)

3) PBW-154 (V3)

4) PBW-502 (V4)

The above crop varieties were screened for heavy metal Hg phyto-toxicity on the following parameters:

1. Germination Percentage (GP)

2. Seed Germination Index (SGI)

Certified seeds of the four local varieties of a cereal (Wheat) crop were screened for their relative tolerance to the Heavy metal mercury as $HgCl_2$ under varying concentration levels viz., 0, 100, 200 and 300ppm. Distilled water was used as control treatments. Observations on seedling growth were recorded as Germination Percentage (GP) Seed Germination Index (SGI) at 24 hours interval from 24 hours after sowing up to the end of 120 hours.

Preparation of Stock Solution:

Stock solutions for heavy metal mercury as $HgCl_2$ were prepared as follows:

1) 50ppm stock solution: dissolved 0.05g salt in 1000ml distilled water

2) 100ppm stock solution: dissolved 0.1g salt in 1000ml distilled water

3) 200ppm stock solution: dissolved 0.2g salt in 1000ml distilled water

4) 300ppm stock solution: dissolved 0.3g salt in 1000ml distilled water

Sterilization of seeds in HgCl2 salt treatment

Twenty seeds of each variety were surface sterilized by soaking in 0.1% (w/v) $HgCl_2$ for 2min and then rinsed twice with sterile distilled water. Sterilized seeds were subjected to heavy metal salt treatment in petri-dishes (for seed germination) containing autoclaved sterilized filter paper (Whatman No1) saturated with different concentrations of $HgCl_2$. Seed germination was calculated after 24, 48, 72, 96 and 120 hours. Three replications were taken for all the experimentation.

Screening of the crops for heavy metal Hg phyto-toxicity:

Screening of the crops for heavy metal phyto-toxicity was carried out after Garrads Technique [51] as modified by [52] and as per method of [53] and [9,10,54,55] Seed Germination analysis: Germination percentage (GP) at each interval at varying levels of heavy metal Hg concentration and control (distilled water) was observed along with the speed of germination index (SGI) was determined by following the formula of Carley & Watson [56]:

SGI = (5x1G + 4x2G +3x3G + 2x4G + 1x5G)

Where, 1G ———— 5G = Number of seeds germinated on the first (24 hours) to fifth (120 hours) day.

Statistical Analysis

All parameters with three replicates were analyzed by Analysis of Variance (ANOVA) by using window SPSS 2003. Data were expressed as the mean ± standard error of the mean. Critical differences at 0.01 and 0.05 per cent probability were calculated wherever the results were significant.

Results

Germination percentage (GP)

ANOVA analysis: As indicated in the Table 1 (ANOVA ANALYSIS-Germination Percentage) all the main effects viz., Variety, Treatment (Heavy metal), Duration and their interactions (A; B; C; A X B; A X C; B X C; A X B X C) were highly significant both at 0.01% and 0.05% level of probability as such significant differences were noticed in the germination percentage of the four crop varieties studied Tables 1-10 and Graphs 1-10. Results of all the main effects viz., Variety, Treatment (Heavy metal), Duration and their interactions (A; B; C; A X B; A X C; B X C; A X B X C) are described as follows:

Table 1: Anova table* germination percentage in the four wheat varieties.

Source of variation	DF	SS	MSS	F-value	Significance
Factor A (Variety)	3	63.35	21.117	5.18	*, **
Factor B (Treatment)	3	85064.58	28354.86	6965.37	*, **
Factor C (Duration)	4	72003.11	18000.78	4421.89	*, **
Factor A X B	9	250.58	27.84	6.84	), **
Factor A X C	12	224.85	18.73	4.6	*, **
Factor B X C	12	6592.79	549.39	134.96	*, **
Factor A X B X C	36	444.04	12.33	3.03	*, **
Error	160	651.33	4.07		
Total	239	165294.7			

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Table 2: Effect of heavy metal mercury ($Hgcl_2$) on germination percentage of four wheat varieties (variety).

Grand Mean = 32.925	SEM± 0.13	
Range of Germination	Lower Range = 32.66	Upper Range = 33.18

*Significant at 5% Level of Probability; ** Significant at 1% Level of Probability.

Variety	Germination Percentage	Range of Germination	
		Lower	Upper
V1 LOK1	33.68	33.16	34.19
V2 UP-2338	32.33	31.81	32.84
V3 PBW-154	33.08	32.56	33.59
V4 PBW-502	32	32.08	33.11
SEM±0.26			

V1 Lok1 (33.68%) > V3 PBW 154 (33.08%) > V2 UP 2338 (32.33%) > V4 PBW 502 (32.0%).

Table 3: Effect of heavy metals mercury [$Hgcl_2$] on germination percentage of four wheat varieties (treatment).

Heavy Metal Mercury ($HgCl_2$)	Germination Percentage	Range of Germination	
		Lower	Upper
Control DW 0ppm	63.48	62.96	63.99
100ppm	30.51	30	31.03
200ppm	25.25	24.73	25.76
300ppm	12.45	11.93	12.96
SEM±0.26			

Control (63.48%) > 100ppm (30.51%) > 200 (25.25%) > 300ppm (12.45%).

Table 4: Effect of heavy metal mercury ($Hgcl_2$) on germination percentage of four wheat varieties (duration).

Duration (hours)	Germination Percentage	Range of Germination	
		Lower	Upper
24hrs	6.41	5.84	6.99
48hrs	22.08	21.5	22.65
72hrs	34.27	33.69	34.84
96hrs	47.06	46.48	47.63
120hrs	54.79	54.21	55.36
SEM±0.291			

120hr (54.79%) > 96hr (47.06%) > 72hr (34.27%) > 48hr (22.08%) > 24hr (6.41%).

Table 5: Effect of heavy metal mercury ($Hgcl_2$) on germination percentage of four wheat varieties (Variety x treatment).

Variety	Heavy Metal Mercury ($HgCl_2$)	Germination Percentage	Range of Germination	
			Lower	Upper
V1 LOK1	Control DW 0ppm	62.33	61.3	63.36
	100ppm	30.46	29.43	31.49
	200ppm	27.73	26.7	28.76
	300ppm	14.2	13.17	15.22

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V2 UP-2338	Control DW 0ppm	62.2	61.17	63.22
	100ppm	30.93	29.9	31.96
	200ppm	25	23.97	26.02
	300ppm	11.2	10.17	12.22
V3 PBW-154	Control DW 0ppm	65.13	64.1	66.16
	100ppm	31	29.97	32.02
	200ppm	24.06	23.03	25.09
	300ppm	12.13	11.1	13.16
V4 PBW-502	Control DW 0ppm	64.26	63.23	65.29
	100ppm	29.66	28.63	30.69
	200ppm	24.2	23.17	25.22
	300ppm	12.26	11.23	13.29
SEM±0.521				

V1 LOK1-120hr (56.417%) > 96hr (47.750%) > 72hr (36.333%) > 48hr (22.000%) > 24hr (5.917%)

V2 UP-2338-20hr (53.167%) > 96hr (47.833%) > 72hr (33.417%) > 48hr (20.750%) > 24hr (6.500%)

V3 PBW-154-120hr (53.583%) > 96hr (46.833%) > 72hr (35.000%) > 48hr (23.417%) > 24hr (6.583%)

V4 PBW-502-120hr (56.000%) > 96hr (45.833%) > 72hr (32.333%) > 48hr (22.167%) > 24hr (6.667%).

Table 6: Effect of heavy metal mercury ($Hgcl_2$) on germination percentage of four wheat varieties (variety x duration).

Variety	Duration (hours)	Germination Percentage	Range of Germination	
			Lower	Upper
V1 LOK1	24hrs	5.91	4.76	7.06
	48hrs	22	20.85	23.15
	72hrs	36.33	35.18	37.48
	96hrs	47.75	46.6	48.9
	120hrs	56.41	55.26	57.56
V2 UP-2338	24hrs	6.5	5.35	7.65
	48hrs	20.7	19.6	21.9
	72hrs	33.41	32.26	34.56
	96hrs	47.83	46.68	48.98
	120hrs	53.16	52.01	54.31
V3 PBW-154	24hrs	6.58	5.43	7.73
	48hrs	23.41	22.26	24.56
	72hrs	35	33.85	36.15
	96hrs	46.83	45.68	47.98
	120hrs	53.58	52.43	54.73

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V4 PBW-502	24hrs	6.66	5.51	7.81
	48hrs	22.16	21.01	23.31
	72hrs	32.33	31.18	33.48
	96hrs	45.83	44.68	46.98
	120hrs	56	54.85	57.15
SEM±0.582				

Control-120hr Seedling (91.667) > 96hr Seedling (83.250) > 72hr Seedling (63.667) > 48hr Seedling (53.167) > 24hr Seedling (25.667)

100ppm-120hr Seedling (52.083) > 96hr Seedling (46.167) > 72hr Seedling (34.417) > 48hr Seedling (19.917) > 24hr Seedling (0.000)

200ppm-120hr Seedling (46.083) > 96hr Seedling (40.167) > 72hr Seedling (28.833) > 48hr Seedling (11.167) > 24hr Seedling (0.000)

300ppm-120hr Seedling (29.333) > 96hr Seedling (18.667) > 72hr Seedling (10.167) > 48hr Seedling (4.083) > 24hr Seedling (0.000).

Table 7: Effect of heavy metal mercury ($Hgcl_2$) on germination percentage of four wheat varieties (duration x treatment).

Duration (hours)	Heavy Metal Mercury ($HgCl_2$)	Germination Percentage	Range of Germination	
			Lower	Upper
24hrs	Control DW 0ppm	25.667	24.516	26.817
	100ppm	0	0	0
	200ppm	0	0	0
	300ppm	0	0	0
48hrs	Control DW 0ppm	53.167	52.016	54.317
	100ppm	19.917	18.766	21.067
	200ppm	11.167	10.016	12.317
	300ppm	4.083	2.933	5.234
72hrs	Control DW 0ppm	63.667	62.516	64.817
	100ppm	34.417	33.266	35.567
	200ppm	28.833	27.683	29.984
	300ppm	10.167	9.016	11.317
96hrs	Control DW 0ppm	83.25	82.1	84.4
	100ppm	46.167	45.016	47.317
	200ppm	40.167	39.016	41.317
	300ppm	18.667	17.516	19.817
120hrs	Control DW 0ppm	91.667	90.516	92.817
	100ppm	52.083	50.933	53.234
	200ppm	46.083	44.933	47.234
	300ppm	29.333	28.183	30.484
SEM±0.582				

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Table 8: Effect of different levels of heavy metal mercury (Hg) exposure on germination percentage in the four wheat varieties (variety x duration x treatment).

Variety	Duration (hours)	Treatment $HgCl_2$ Conc. (ppm)			
		Control DW 0ppm	100ppm	200ppm	300ppm
V1 LOK-1	24hrs	23.66	0	0	0
	48hrs	51	21	11	5
	72hrs	61.33	35	37	12
	96hrs	82.33	44	43	21.66
	120hrs	93.33	52.3	47.66	32.33
V2 UP-2338	24hrs	26	0	0	0
	48hrs	52.66	16	10.33	4
	72hrs	63.66	36	26	8
	96hrs	82	49	42.33	18
	120hrs	86.66	53.7	46.33	26
V3 PBW-154	24hrs	26.33	0	0	0
	48hrs	54.33	21.3	13.66	4.33
	72hrs	66.66	35.3	26.66	11.33
	96hrs	84.33	47	38	18
	120hrs	94	51.3	42	27
V4 PBW-502	24hrs	26.66	0	0	0
	48hrs	54.66	21.3	9.66	3
	72hrs	63	31.3	25.66	9.33
	96hrs	84.33	44.7	37.33	17
	120hrs	92.66	51	48.33	32
SEM±1.16					

Table 9: Effect of different levels of heavy metal mercury (hg) exposure on germination percentage in the four wheat varieties v x d x t (percent over control).

Variety	Duration (hours)	Treatment $HgCl_2$ Conc. (ppm)			
		Control DW 0ppm	100ppm	200ppm	300ppm
V1 LOK-1	24hrs	100	0	0	0
	48hrs	100	41.2	21.56	9.8
	72hrs	100	57.1	60.32	19.56
	96hrs	100	53.4	52.22	26.31
	120hrs	100	56.1	51.07	34.64
V2 UP-2338	24hrs	100	0	0	0
	48hrs	100	30.4	19.61	7.59
	72hrs	100	56.5	40.83	12.56
	96hrs	100	59.8	51.62	21.95
	120hrs	100	61.9	53.46	29.99

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V3 PBW-154	24hrs	100	0	0	0
	48hrs	100	39.3	25.15	7.97
	72hrs	100	53	40	16.99
	96hrs	100	55.7	45.05	21.34
	120hrs	100	54.6	44.68	28.72
V4 PBW-502	24hrs	100	0	0	0
	48hrs	100	39	17.68	5.48
	72hrs	100	49.7	40.74	14.81
	96hrs	100	53	44.26	20.15
	120hrs	100	55	52.15	34.53
SEM±16					

Table 10: Effect of different levels of heavy metal mercury (hg) exposure on germination percentage in the four wheat varieties (percent over control) (data recorded after 72 hours of presoaking the seeds in test solutions).

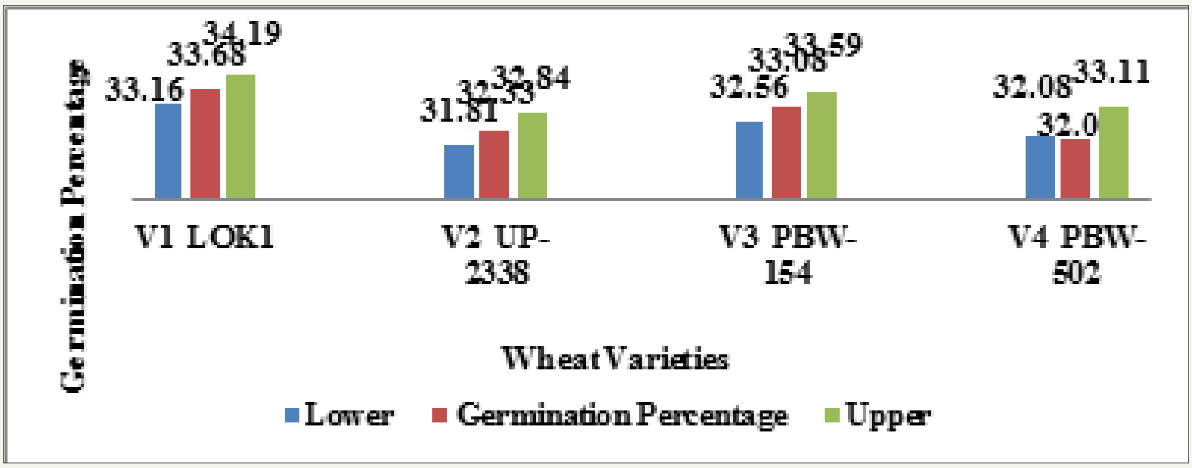
Variety	Treatment $HgCl_2$ Conc. (ppm)			
	Control DW ppm	100ppm	200ppm	300ppm
V1 LOK-1	100	57.06	60.3	19.56
V2 UP-2338	100	56.54	40.8	12.56
V3 PBW-154	100	52.99	40	16.99
V4 PBW-502	100	49.73	40.7	14.81
SEM±1.16				

V1 LOK1-Control (100%) > 200 (60.326%) > 100ppm (57.065%) > 300ppm (19.565%)

V2 UP-2338-Control (100%) > 100ppm (56.544%) > 200 (40.837%) > 300ppm (12.565%)

V3 PBW-154-Control (100%) > 100ppm (52.999%) > 200 (40.0007%) > 300ppm (16.999%)

V4 PBW-502-Control (100%) > 100ppm (49.734%) > 200 (40.741%) > 300ppm (14.814%)



Graph 1: Effect of heavy metal mercury [Hgcl2] on germination percentage of four wheat varieties (variety).

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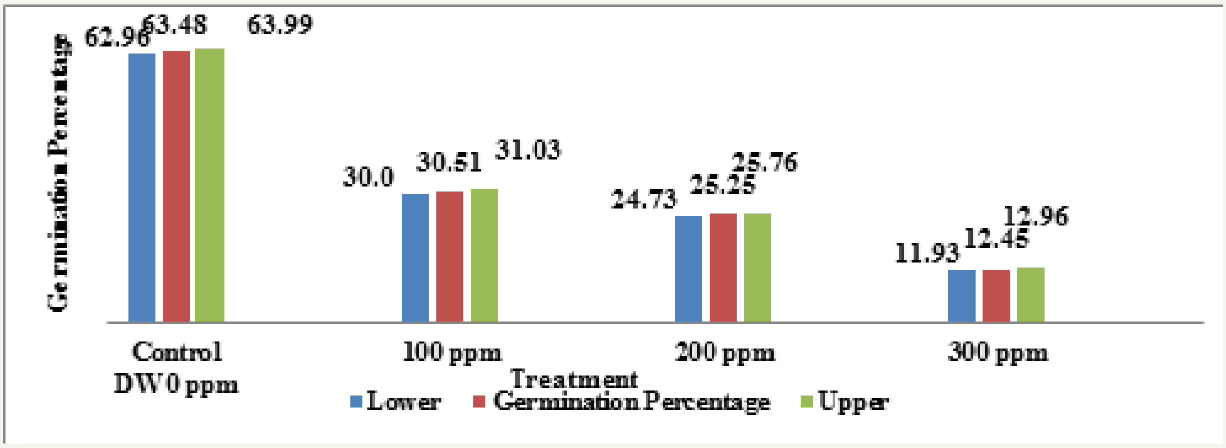
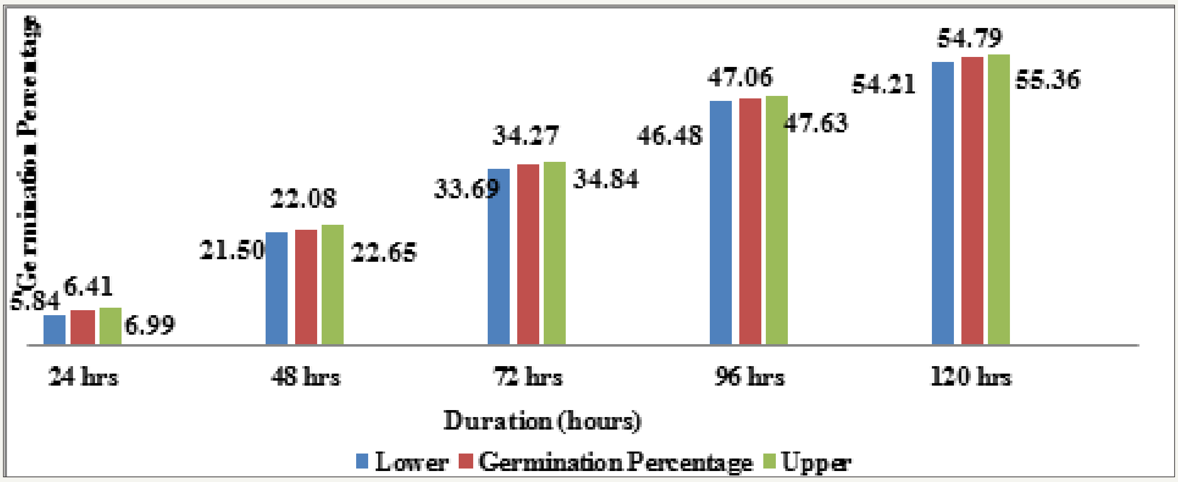
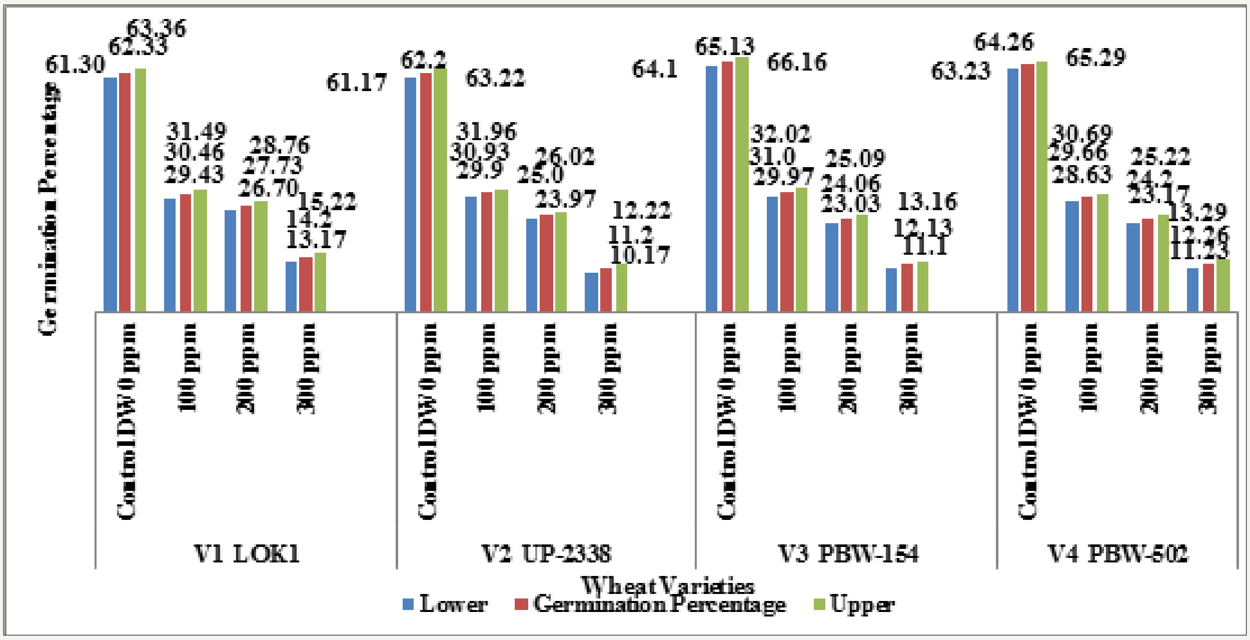


Graph 2: Effect of heavy metals mercury [$Hgcl_2$] on germination percentage of four wheat varieties (Treatment).



Graph 3: Effect of heavy metals mercury [$Hgcl_2$] on germination percentage of four wheat varieties (Duration).



Graph 4: Effect of heavy metal mercury (Hg) on germination percentage of four wheat varieties (Variety X treatment).

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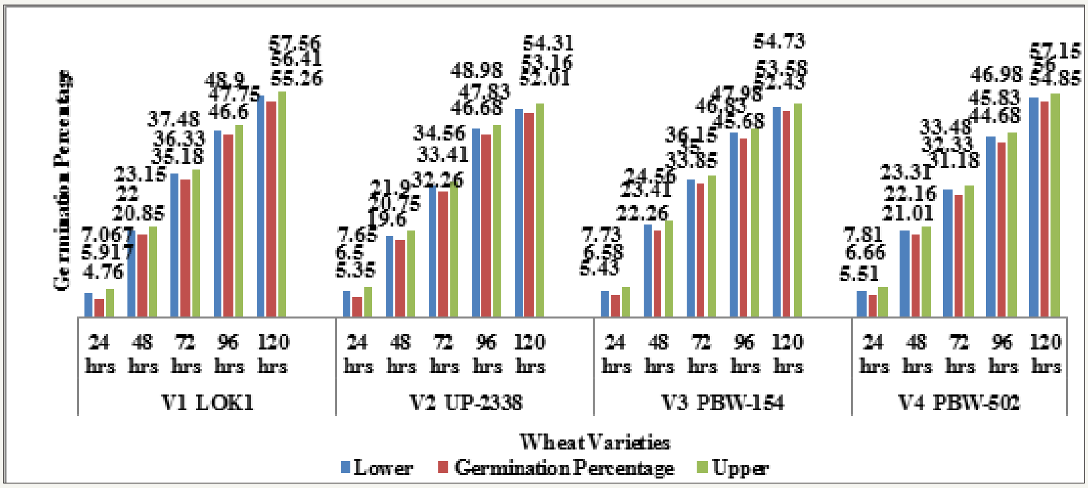
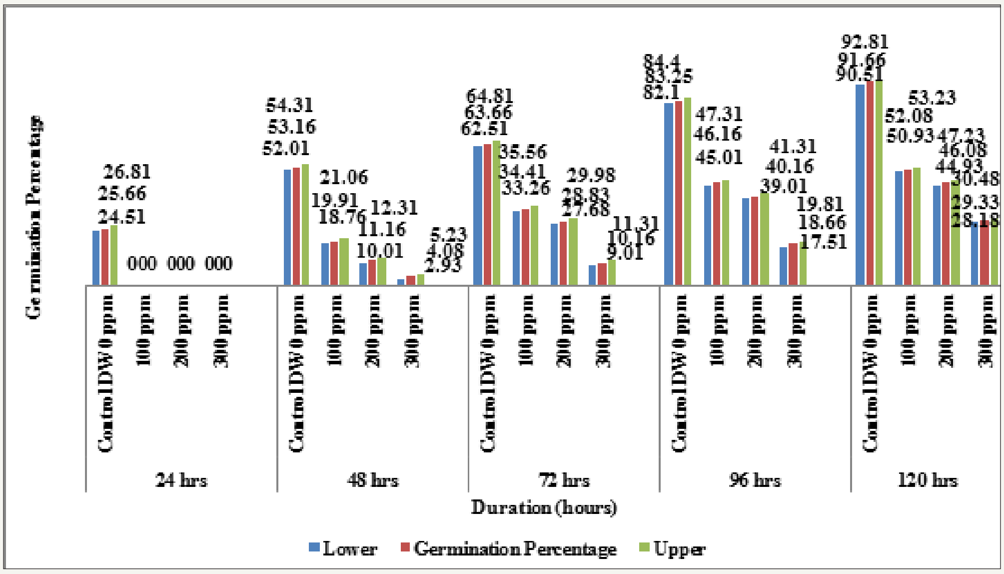
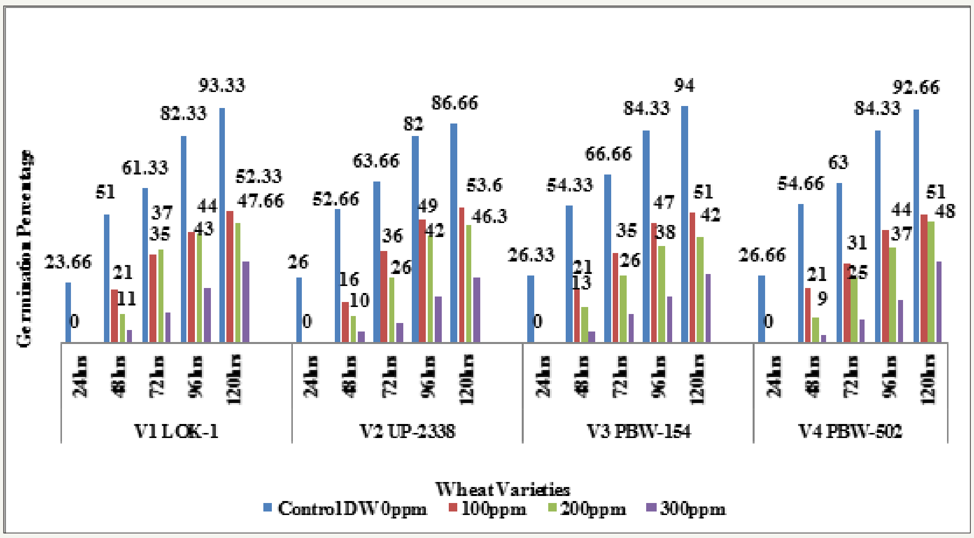


Graph 5: Effect of heavy metal mercury [hgcl$_2$] on germination percentage of four wheat varieties (variety x duration).



Graph 6: Effect of heavy metal mercury [hgcl$_2$] on germination percentage of four wheat varieties (duration x treatment).



Graph 7: Effect of different levels of heavy metal mercury (hg) exposure on germination percentage in the four wheat varieties (variety x duration x treatment).

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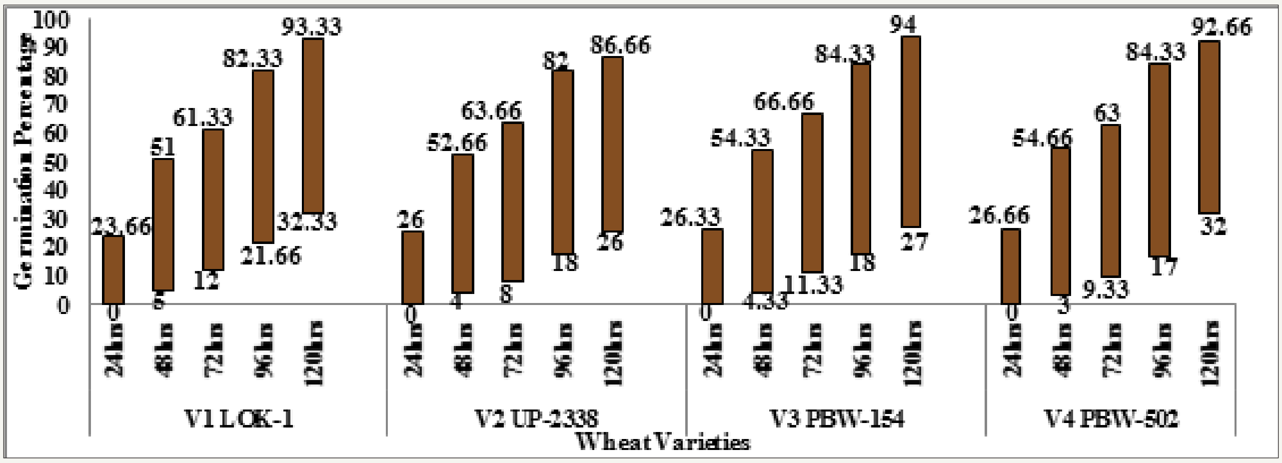
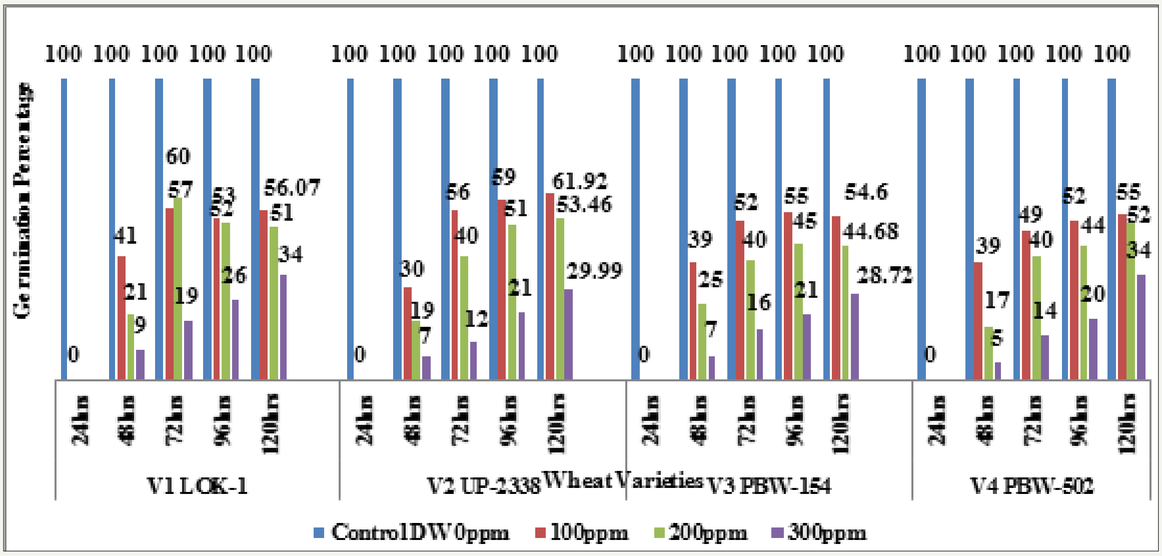
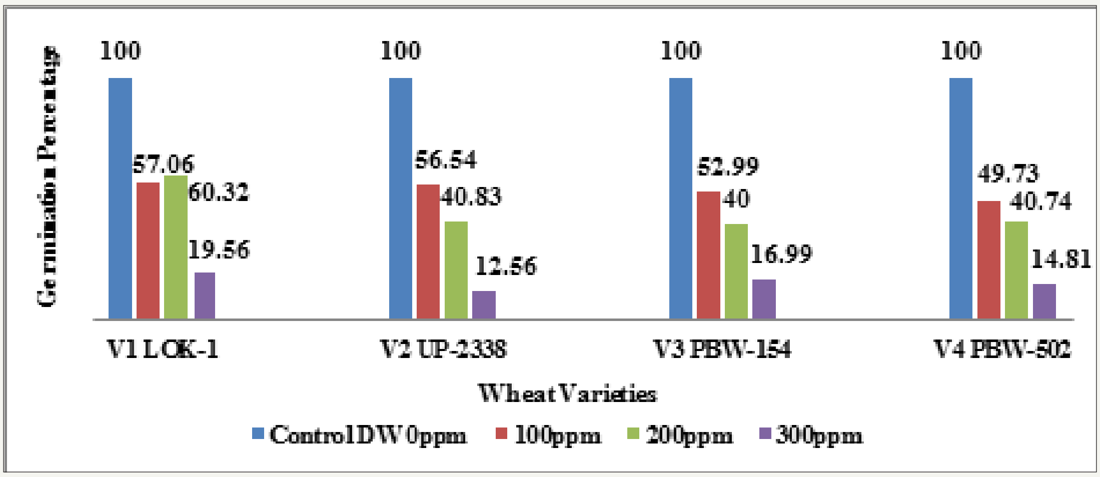


Graph 8: Effect of different levels of heavy metal mercury (hg) exposure on germination percentage in the four wheat varieties (variety x duration x treatment).



Graph 9: Effect of different levels of heavy metal mercury (hg) exposure on germination percentage in the four wheat varieties (percent over control).



Graph 10: Effect of different levels of heavy metal mercury (hg) exposure on germination percentage in the four wheat varieties (percent over control)(data recorded after 72 hours of presoaking the seeds in test solutions).

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Main effect variety: The highest mean germination percentage (33.68%) was recorded in the variety V1 followed by V3, V2 and lastly V4 with the lowest germination percentage (32.0%). The arieties were arranged in the following descending order on the basis of their respective germination percentage irrespective of the treatment and seedling age (Variety) (Table 2 & Graph 1). A significant reduction in germination percentage with increasing heavy metal levels was observed irrespective of variety, salt and seedling age (Treatment). The reduction in germination percentage was more pronounced after 200ppm of Hg treatment. Further, with Hg treatment germination percentage was highest in controls (63.48%) while lowest (12.45%) with 300ppm (Table 3 & Graph 2) and the treatments were arranged in the following descending order. The significant interaction of varieties with treatment (Variety X Treatment) is depicted in Tables 4 & 5 and Graph 4**.** All the four varieties showed a decrease in germination percentage with increasing salt treatment however, the genotypic variations were quite evident and were arranged in the following descending order on the basis of their respective germination percentage irrespective of the treatment.

All the crop varieties showed an increase in germination percentage (interaction Variety X Duration) exhibiting marked differences in their early seedling growth with advancement in seedling age and the effect of salt declined, i.e., in general, tolerance to heavy metal increased (Table 6 & Graph 5) and were arranged in the following descending order on the basis of their respective germination percentage irrespective of the treatment

Interaction duration X treatment: The interaction of Duration X Treatment (Table 7 & Graph 6) shows that with increasing salt concentration level the deleterious salt effect was clearly observable which, however, declined with seedling age. Initially at 24 hours seedling age control sets showed 25.667% germination but no germination was observed in the Hg treated sets of 100, 200 and 300ppm. The treatments were arranged in the following descending order

Interaction Variety X Duration X Treatment: The results in relation to the effect of different concentrations of $HgCl_2$ on germination performance measured in terms of percent germination after 24, 48, 72, 96 and 120 hours of sowing have been shown in Tables 1-10 and Graphs 1-10. The germination percentage was significantly inhibited by Hg in all the four wheat varieties. The degree of inhibition varied depending on the concentration of heavy metal Hg. A review of the final interaction (Variety X Duration X Treatment) reveals that irrespective of salt concentration levels the germination percentage had increased with seedling age and that the salt treatments had their individual effect depending upon the varying treatment levels (Table 8 and Graph 7 & 8).

The germination decreased with increasing concentration of mercury in the four wheat varieties studied viz., V1 Lok1, V2 UP-2338, V3 PBW-154 and V4 PBW-502 (Tables 1-10 & Graphs 1-10). A comparison with controls in the four varieties studied showed in the controls: at 24 hours lowest in the variety V1 (23.66%) and highest in V4 (26.66%); at 48 hours lowest in V1 (51.0%) and highest in V4 (54.66%); at 72 hours lowest in V1 (61.33%) and highest in V3 (66.66%); at 96 hours lowest in V2 (82.0%) and highest in V3 and V4 (84.33%) and at 120 hours lowest in V2 (86.0%) and highest in V3 (94.0%) respectively, whereas in 100, 200 and 300ppm of mercury there was no germination found at all at 24 hours in all the four varieties studied; in 100ppm at 48 hours lowest in V2 (16.0%) and highest in V3 and V4 (21.33%); at 72 hours lowest in V4 (31.33%) and highest in V2 (36.0%); at 96 hours lowest in V1 (44.0%) and highest in V2 (49.0%) and at 120 hours lowest in V4 (51.0%) and highest in V2 (53.66%) respectively; at 200ppm at 48 hours lowest in V4 (09.66%) and highest in V3 (13.66%) respectively; at 72 hours lowest in V4 (25.66%) and highest in V1 (37.0%); 96 hours lowest in V4 (37.33%) and highest in V1 (43.0%) and at 120 hours lowest in V3 (42.0%) and highest in V4 (48.33%) respectively and at 300ppm at 48 hours lowest in V4 (03.0%) and highest in V1 (5.0%); at 72 hours lowest in V2 (08.0%) and highest in V1 (12.0%); 96 hours lowest in V4 (17.0%) and highest in V1 (21.66%) and at 120 hours lowest in V2 (26.0%) and highest in V1 (32.33%) respectively (Table 8 and Graph 7 & 8).

Seeing overall results, it was recorded that the lowest germination percent (3.0%) was found in 300ppm of Hg solution at 48 hours of germination in the variety V4 whereas highest (94.0%) was recorded in Controls at 120 hours in the variety V3. Thus, it has been observed that the varieties V1 and V4 behaved better, even in 300ppm of Hg concentration at 120 hours of seedling growth. Thus, varieties show overall germination % as - V1>V4>V3>V2. Germination percentage as percent over control showed lowest rate (5.48%) at 48 hours in 300ppm in the Variety V4 and the highest of (61.92%) at 120 hours in 100ppm Hg in the variety V2 (Table 9 & Graph 9). On the basis of percent over control varieties are placed as - V1> V4 >V2 > V3. Data recorded after 72 hours of presoaking the seeds in test solution have shown highest rate of germination (60.32%) in 200ppm in the variety V1 and lowest (12.56%) in 300ppm in the variety V2 and the overall better performance of the variety V1 in all the treatments (V1>V3>V4>V2) (Table 10 & Graph 10) and were arranged in the following descending order:

Speed of germination index (SGI)

The results for Speed of Germination Index (SGI) have been depicted in the Tables 11-13 & Graphs 11-13. The four wheat varieties at 100, 200 and 300ppm Hg treatment concentrations showed a gradual decrease in SGI from highest (811.66) in the Var. V3 PBW-154 in the Control Sets while lowest (102.0) was recorded in the Var. V2 UP-2338 in the treatment sets of 300ppm. When SGI was calculated under varying concentration levels of heavy metal Hg Treatment as percent over Control, the lowest SGI was recorded in the Var. V2 UP-2338 (13.03) at 300ppm whereas highest was in the Var. V1 LOK-1 (43.08). The decrease (-) in SGI of treated seeds over control minimum decrease (-56.91) was found in the Var. V1 LOK-1 at 100ppm and maximum decrease (-86.96) was in the Var. V2 UP-2338 at 300ppm heavy metal conc. followed by Var. V4 PBW-502 (-86.78), V3 PBW-154 (-85.91) and V1 LOK-1 (-82.77) also in 300ppm. Compared to SGI, GP shown as percent over Control had recorded lowest of 18.0% in the Var. V2 UP-2338 at 300ppm whereas the highest GP of 48.98% was also found in the same variety at 100ppm (Table 12 & Graph 11).

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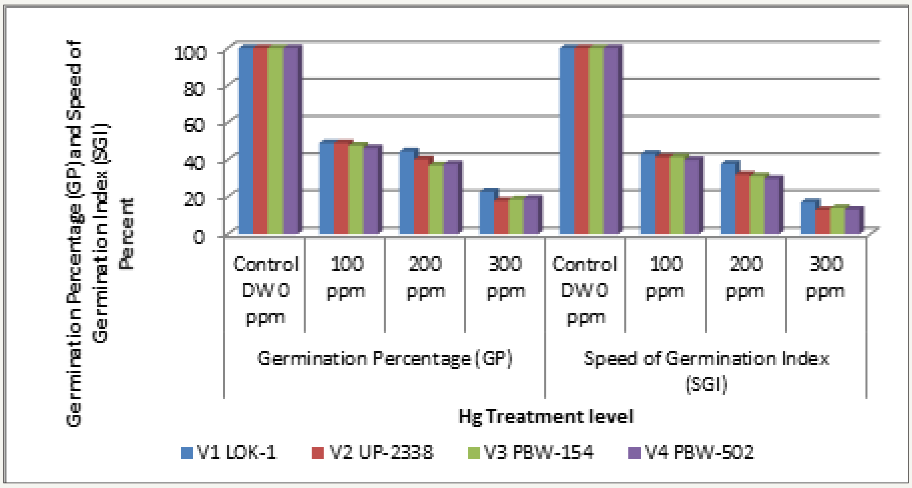
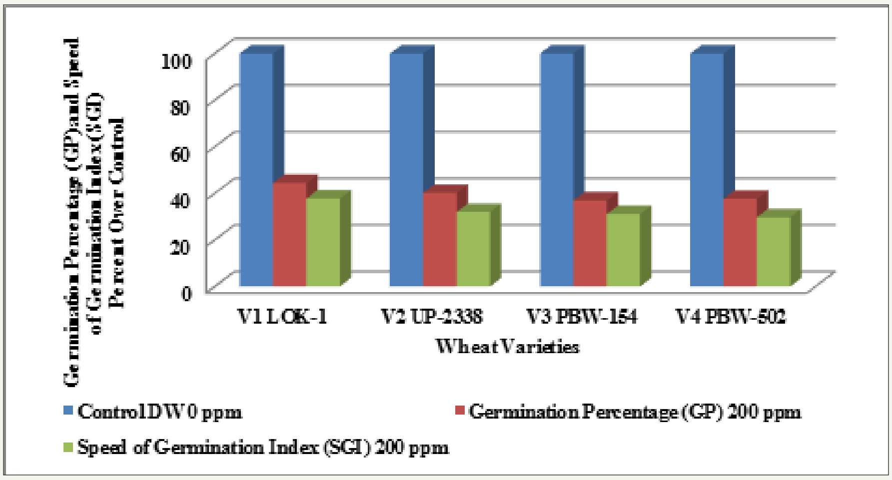
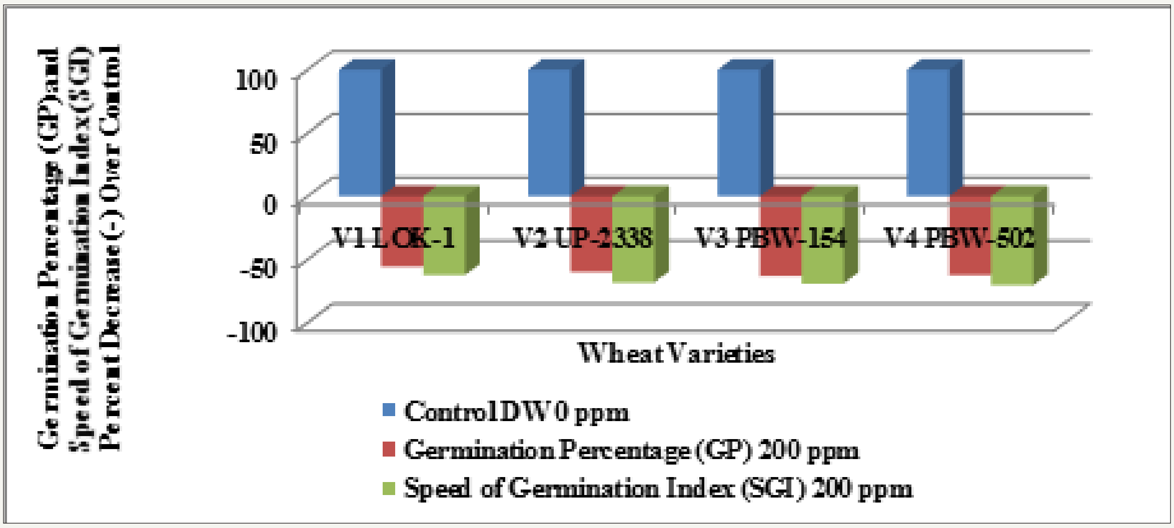


Graph 11: Effect of varying heavy metal mercury concentration on germination percentage (gp) and speed of germination index (sgi) in the four crops (percent over control).



Graph 12: Effect of varying heavy metal mercury concentration on germination percentage (gp) and speed of germination index (sgi) in the four crops [percentover control] at critical level of 200ppm.



Graph 13: Effect of varying heavy metal mercury concentration on germination percentage (gp) and speed of germination index (sgi) in the four crops [percent decrease (-) over control] at critical level of 200ppm.

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Table 11: Effect of varying heavy metal mercury concentration on speed of germination index (sgi) in the four crops.

Wheat Varieties	Speed of Germination Index (SGI)			
	Treatment $HgCl_2$ Conc. (ppm)			
	Control DW 0ppm	100ppm	200ppm	300ppm
V1 LOK-1	764.33	329.33	288.66	131.66
V2 UP-2338	782.33	323.66	250.33	102
V3 PBW-154	811.66	336.66	252.66	114.33
V4 PBW-502	802.33	319.66	238.66	105.99

Table 12: Effect of varying heavy metal mercury concentration on germination percentage (gp) and speed of germination index (sgi) in the four crops (percent over control).

Wheat Varieties	Germination Percentage (GP) Per cent Over Control				Speed of Germination Index (SGI) Per cent Over Control			
	Control DW 0ppm	100ppm	200ppm	300ppm	Control DW 0ppm	100ppm	200ppm	300ppm
V1 LOK-1	100	48.97	44.49	22.78	100	43.08	37.76	17.22
		*(-51.02)	*(-55.5)	*(-77.22)		*(-56.91)	*(-62.23)	*(-82.77)
V2 UP-2338	100	48.98	40.19	18	100	41.37	31.99	13.03
		*(-51.02)	*(-59.8)	*(-81.99)		*(-58.62)	*(-68.00)	*(-86.96)
V3 PBW-154	100	47.59	36.94	18.62	100	41.47	31.12	14.08
		*(-52.40)	*(-63.05)	*(-81.37)		*(-58.52)	*(-68.87)	*(-85.91)
V4 PBW-502	100	46.16	37.65	19.08	100	39.84	29.74	13.21
		*(-53.83)	*(-62.34)	*(-80.91)		*(-60.15)	*(-70.25)	*(-86.78)

*Values in Parenthesis show Percent Decrease (-) over Control in GP and SGI

Percent over control

GP: V1 LOK-1 (44.49%)> V2 UP-2338 (40.19%)> V4 PBW-502 (37.65%)> V3 PBW-154 (36.94%)

SGI: V1 LOK-1 (37.76%)> V2 UP-2338 (31.99%)> V3 PBW-154 (31.12%)> V4 PBW-502 (29.74%)

Percent decrease (-) over control

GP: V1 LOK-1 (-55.5%)< V2 UP-2338 (-59.8%)< V4 PBW-502 (-62.34%)< V3 PBW-154 (-63.05%)

SGI: V1 LOK-1 (-62.23%)< V2 UP-2338 (-68.0%)< V3 PBW-154 (-68.87%)< V4 PBW-502 (-70.25%).

Table 13: Effect of varying heavy metal mercury concentration on germination percentage (gp) and speed of germination index (sgi) in the four crops [percent decrease (-) over control] at critical level of 200ppm.

Wheat Varieties	Control DW 0ppm	Germination Percentage (GP) 200ppm	Speed of Germination Index (SGI) 200ppm
V1 LOK-1	100	44.49	37.76
		*(-55.5)	*(-62.23)
V2 UP-2338	100	40.19	31.99
		*(-59.8)	*(-68.0)
V3 PBW-154	100	36.94	31.12
		*(-63.05)	*(-68.87)
V4 PBW-502	100	37.65	29.74
		*(-62.34)	*(-70.25)

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Finally, comparing the tolerance behavior of four varieties of wheat to heavy metal concentration at the Critical level of 200ppm Hg as percent over control with percent decrease (-) over control on the basis of GP and SGI the four varieties showed the following trend (Table 13 and Graphs 12 & 13).

Discussion

A variety of abiotic stresses including heavy metals causes molecular damage to plant cells either directly or indirectly through the formation of reactive oxygen species (ROS) [57,58]. Protonation of O_2^* can produce the hydroperoxyl radical (OH^*, H_2O_2), which can convert fatty acids to toxic lipid peroxides, destroying biological membranes. The measurement of antioxidative enzymes could be useful for the level of antioxidant. The effects of heavy metals on plants resulted in growth inhibition, structural damage, a decline of physiological and biochemical activities, as well as of the function of plants. Our measurements showed that both growth and photosynthetic pigments are affected by the presence of heavy metals.

Rodriguez et al. [39] stated that mercury, a widely distributed environmental pollutant mainly supplied *via* anthropogenic sources to the soil, can induce toxicity in living organisms, including higher plants. Its toxicity, mobility, bioaccumulation, methylation process and transport in the atmosphere make Hg harmful and one of the best known toxic metals discharged from human activities. In the past couple of years the environmental protection agencies have expressed increasing concern over the release of mercury to the environment. Mercuric chloride ($HgCl_2$) is dominant among all mercury forms. Shriapanahi & Anderson [59] found elevated levels of mercury in the upper layer of soil following long term application of municipal wastewater resulting in the accumulation of toxic levels of heavy metals in the vegetables grown in these areas.

The response of crop species to heavy metals reporting mechanisms responsible for their tolerance or sensitivity have been reported [59-64]. The toxic effect of mercury on germination, growth and yield has been studied on different plants [13,44,65-68]. Parameters such as percentage of germination [69] and shoot and root lengths have been used as an indicator of heavy metal toxicity in plants [70,71]. Seeds treated with Hg showed lower germination percentage and that inhibition in seed germination at higher concentration of heavy metals was mainly caused by ion toxicity which is associated to changes in cellular permeability, inhibition of protein activity and/or direct toxicity to the embryo and seedling [72,73].

Reduction in germination of seeds in presence of Hg has been reported in rice [74] and in other plants [75-77]. The percentage of germination and % DFC clearly indicate the inhibitory effects of Hg on germination. The results indicate that %DFC increase with increasing concentration of metal solutions and higher values of % DFC suggest the greater susceptibility to Hg at higher concentration. In low level Hg concentration treatments, percentages germination were not much inhibited, showing that they were well within the tolerable range of seedlings. However, in high level treatments, germination percentages were detrimentally affected, implying that higher concentration of Hg was not conducive to seed germination. This may be attributed to depression of oxygen uptake and physiological disturbance in mobilization of reserve seed food materials [78].

Our results showed that seed germination of all the four wheat varieties was inhibited at all the concentrations of mercury as compared to controls. The degree of inhibition varied depending on the concentration of heavy metal. Seeing overall results it was recorded that the lowest germination percent (3.0%) was found in 300ppm of Hg solution at 48 hours of germination in the variety V4 whereas highest (94.0%) was recorded in Controls at 120 hours in the variety V3. It has been observed that the varieties V1 and V4 behaved better, even in 300ppm of Hg concentration at 120 hours of seedling growth. Thus, varieties show overall germination % as - V1 > V4 >V3 > V2.

Seeds treated with Hg showed lower germination percentage. Several workers [7,27,44,73] reported that inhibition in seed germination at higher concentration of heavy metals was mainly caused by ion toxicity. Ion toxicity is associated to changes in cellular permeability, inhibition of protein activity and/or direct toxicity to the embryo and seedling [44,68]. It has also been observed in *Vigna ambacensis* by Mohammad Nasser Al-Yemeni (2001). Reduction in germination of seeds in presence of Hg has been reported in rice [74] and in other plants [68,75-77]. Results also showed that V1 was more tolerant for heavy metal Hg in comparison to other wheat varieties.

Conclusion

Environmental Pollution has emerged as a major epidemic endangering Life on earth. Due to unwise, unscientific and excessive use of natural resources eco-balance is disturbed. Industrialization and urbanization have also deteriorated the position. As such 'pollution' can be considered the result of the growth of modern civilization. Hence 'effective Pollution control' is the need of the hour [7]. Episodes like the *Minamata* and *Itai-Itai* epidemics in Japan serve as a warning against the indiscriminate and careless use of toxic heavy metals. The concept that chemicals in the environment are transported through a cyclic process is not always re-assuring when one considers the time necessary for operations of the cycles to take place. These time lags may permit build up of specific chemicals to concentrations never before experienced.

Organisms, both plant and animal, have been shown to be capable of bio-accumulating toxic metals. Some of these enter the food chain and pose direct threat to man. One would like to know the complete natural history of occurrence, transport, transformation, accumulation and degradation for all toxic metals. Can environmental toxicology, combined with ecological considerations, provide information to prevent or minimize the hazards due to such events? A genuine appreciation of the problem of toxic metals in the environment is yet to be generated. There is no reason why with adequate understanding of the problem, one should not be able to continue to use toxic metals safely. It may be

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necessary to prescribe safe limits of exposure for the toxic metals and ensure that no exposure beyond these levels occur either occupationally or in the community.

The Water Pollution Act does not permit the discharge of toxic metals into water ways above certain limits. We are to ensure that these standards are adequately implemented. Similarly the atmospheric emission of toxic metals has also to be controlled below permissible limits that are likely to be recommended in the near future. For effective control of heavy metal pollution, it is necessary to continuously monitor the environment for their presence, to initiate a system for biological monitoring for heavy metal exposure and to take appropriate steps to minimize and control heavy metal pollution [7,54].

Therefore, heavy metal pollutants are the main concern of new agricultural productions. Industrial products and using synthetic materials lead to drastically increase in concentration of different heavy metals in the environment. Heavy metals are largely used in electronic industries thus the wastewater of factories could pollute agricultural lands. Different heavy metal solutions were investigated for their effects on seed germination characteristics and phyto-remediation potential of a cereal crop (wheat). The wheat seeds germinated after treatments in solutions containing varying concentrations of heavy metal mercury showed that in all treatments the percentage of seed germination, root and shoot length decreased as concentrations of solution increased.

The present work compiling valuable material on Biological Control of Mercury Pollution supported by scientific experimental analysis of the issues and substantially based on the authoritative reference material derived from the writings of the eminent scientists in the field. The worker hopes that this work will prove an authoritative research work for students, scholars and academics in the field of heavy metal pollution besides policy planners, environmental scientists, laboratory technicians and environmental activists.

Thus, the objective of this work is to evaluate the toxicity of heavy metal mercury on wheat crop to provide information on the significance of seed germination rate, root/shoot ratio, dry and fresh weights of the seedlings, chlorophyll, carbohydrate contents, protein contents and oxidative enzymatic system of catalase, peroxidase and superoxide dismutase enzyme activities in response to heavy metal stress and to determine the effects of heavy metal Hg on growth and metabolism of crop plants [79-86].

Recommendations

In wheat (*Triticum aestivum*), Hg phytotoxicity contributed significantly towards reduction in percent seed germination. Planting crops in mercury-contaminated soil can produce significant health risks to consumers. Therefore, it is highly recommended that crops with short rooting systems should not be cultivated in mercury stress areas. Moreover a comprehensive public awareness through media and active participation of local youth is needed for avoiding such mercury induced toxicity problems in contaminated areas for growing agricultural crops.

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