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Indian Mustard and Boston Fern Exhibits Growth Tolerance to Increased Dose of Soil Spiked Mercury

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ABSTRACT

The current study sought to investigate the physiological and biochemical variations in response to various mercury concentration in Indian mustard (*Brassica juncea*) and Boston Fern (*Nephrolepis exaltata*). Results revealed a 17.3 and 10.4% reduction in chlorophyll content of *B. juncea* and *N. exaltata* between the 20 mg kg^{-1} Hg-treated plants and the control suggesting reduced photosynthetic rate. Albeit these parameters were affected, plants tolerated 20 mg kg^{-1} without any visual phytotoxicity symptoms. Gaseous parameters were inversely proportional to the mercury concentration whereas oxidative stress indicators and antioxidant enzymes exhibited a positive correlation. An average increase of 38% Proline was observed in both plants. In *B. juncea* and *N. exaltata*, average catalase activity and peroxidase activity ascended from 2.35 to 5.12 min^{-1} g^{-1} and 3.26 to 6.80 min^{-1} g^{-1} and 0.23 to 1.17 min^{-1} g^{-1} and 0.30 to 1.27 min^{-1} g^{-1} respectively. Thus, an effective metabolic defense and adaptation assures the phytoremediation potential of these plants in mercury contaminated soils.

Keywords Phytoremediation, Total chlorophyll, Gaseous exchange, Oxidative stress, Antioxidants.

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INTRODUCTION

Entry of heavy metals and metalloids into the environment and their escalating toxicity threatens the stability of the ecosystem. With the advancements in the field of science and technology, several physical and chemical technologies were employed in the contaminated site remediation. Unlike the physical and chemical approaches, phytoremediation is less expensive, less harmful and efficient in eliminating pollutants which switched the focus of scientific

community towards phytoremediation (Tangahu *et al.* 2011). Mercury is a ubiquitous environmental toxin whose high solubility in water and the versatility with which Hg shifts to the gaseous phase reflect the capacity and efficacy to travel in different environmental matrices and its persistence (Yang *et al.* 2008, Boening 2000, Clarkson and Magos 2006). Plants are capable of extracting a variety of metal ions from their growth substrates, including Hg. Exposure to mercury has led to substantial phytotoxicity followed by enzymatic and non-enzymatic defense activation (Su *et al.* 2009, Azevedo and Rodriguez 2012). The level of understanding about the mechanism and extent of Hg phytotoxicity is limited. It is essential to understand and define the magnitude of Hg-induced phytotoxicity because of the recurrence of Hg contamination and also the lack of expertise about the effects of this heavy metal in plants.

Indian mustard is an important oil seed crop in India belongs to the family Brassicaceae. Various literatures have reported Indian mustard as a potential candidate for mercury and other metal remediation because of its large biomass and high metal concentration in the above-ground tissues (Rathore *et al.* 2013, Rathore *et al.* 2019). Variations in the membrane lipid composition alongside with higher biomass makes it suitable for Hg phytoextraction and other heavy metals (Mahajan and Kaushal 2018, Tangahu *et al.* 2011, Hall 2002). Mustard has evolved different defense mechanism involving metal binding to cell wall, reducing cell membrane transport, Efflux (Hall 2002) apoplast storage (Boominathan and Doran 2003) chelation and vacuole compartmentalization, Volatilization and Intracellular Storage (Ma *et al.* 2005) Secretion of protective enzymes (Yadav 2010, Mani *et al.* 2013). Pteridophytes or Ferns are non-flowering vascular plants which have been speculated with high potential in remediating heavy metal polluted soils due to their inherent biological characteristics and also add aesthetic value to the site (Akomolafe *et al.* 2013). *Pteris vittata* with 2.8% of arsenic in its biomass has been identified as the first arsenic hyperaccumulator and other metal accumulators are *Nephrolepis cordefolia, Hypolepis muelleri, Pteris umbrosa, Pteris cretica* (Praveen and Pandey 2019). Ferns are efficient in adapting to metal stress conditions (Su *et al*. 2008). The present study was undertaken to study the effects of Mercury on physiological and biochemical response in Indian mustard and Boston Fern.

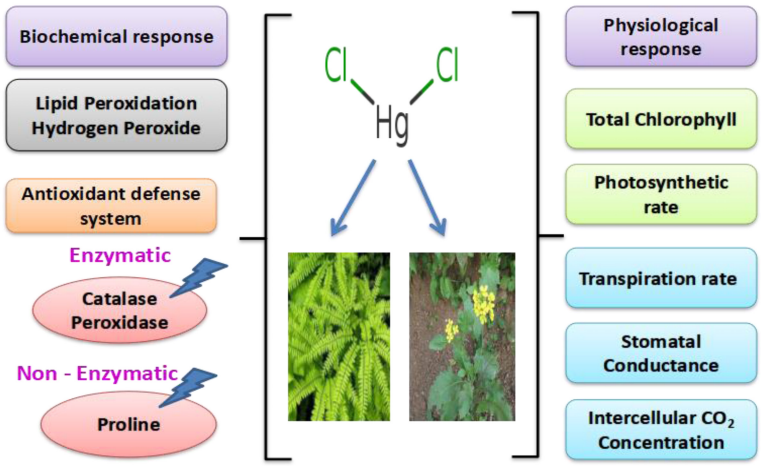
MATERIALS AND METHODS

The current study was carried out in Factorial Completely Randomized Design with two factors (Factor 1 – Plant (P_1,P_2) and Factor 2 – Mercury dosage (T_1, T_2, T_3,T_4,T_5)) which embraces a total of 10 variants as illustrated in Fig. 1 with four replicates each for 45 days. Uncontaminated soil collected from Kodaikanal is spiked with different known concentration of mercury viz., T_1 (0 mg kg^{-1}), T_2 (2.5 mg kg^{-1}), T_3 (5 mg kg^{-1}), T_4 (10 mg kg^{-1}) and T_5 (20 mg kg^{-1}) in the form of mercuric chloride salt on weight basis. The disease-free seeds of *Brassica juncea* var pusa tarak and 3 months old Boston Fern (*Nephrolepis exaltata*) were procured from Indian Agricultural Research Institute, New Delhi, India and Grass rootz Nursery, Coimbatore, India, respectively. Plant samples were collected at definite intervals such as 15th day, 30th day and 45th day after mercury treatment and were analyzed for physiological and biochemical parameters.

Total chlorophyll in *B. juncea* and *N. exaltata* was measured using chlorophyll content meter or SPAD meter. Gaseous exchange parameters of plants

Fig. 1. Overview of pot culture experiment and photosynthetic attributes measurement.

Graphical representation



like photosynthetic rate, vapour pressure deficit, intercellular CO_2 concentration were measured with the help of portable photosynthetic system, LC pro-SD. The measurement was performed within the time period 10.00-12.00 h maintaining the air temperature and air relative humidity at 25°C and 80-90% respectively. The content of proline was estimated in the sample as 520 nm. Lipid peroxidation and Hydrogen peroxide was quantified 532 nm and 390 nm as per the procedure alluded by Velikova *et al*. (2000). Catalase and peroxidase activity was determined at 240 and 420 nm according to the method given by earliar authors.

RESULTS AND DISCUSSION

Plants have divergent mechanisms to adapt to metal polluted environment which entails plant growth regulators, osmoprotectants and antioxidants (Emamverdian *et al.* 2015, Raj *et al.* 2020, Chen and Yang 2012, Zhang *et al.*2007, Isah 2019, Malar *et al*. 2016). Chlorophyll is an important indicator of photosynthetic potential and oxidative stress. Photosynthetic rate

Table 1. Effect of increasing mercury concentration on Total Chlorophyll and Photosynthetic rate of *B. juncea* and *N. exaltata.*

Plant species	Treatments	Total Chlorophyll 15 DAT	30 DAT	45 DAT	Mean	Photosynthetic rate (μ mol CO_2 m^{-2} s^{-1}) 15 DAT	30 DAT	45 DAT	Mean
P_1	T_1	10.6	16.0	26.5	17.70	3.76	9.53	15.60	9.63
	T_2	10.4	15.9	25.4	17.23	3.70	8.63	14.80	9.04
	T_3	10.2	16.1	24.8	17.03	3.06	8.73	15.20	9.00
	T_4	9.90	15.2	23.4	16.17	3.45	8.43	14.40	8.76
	T_5	9.90	14.8	23.8	16.17	2.90	8.63	13.60	8.38
	Mean	10.2	15.6	24.8		3.37	8.79	14.72	
P_2	T_1	4.70	5.20	6.50	5.47	1.24	3.52	4.61	3.12
	T_2	4.40	4.60	5.80	4.93	1.16	3.38	4.42	2.99
	T_3	4.50	4.70	6.10	5.10	1.21	3.28	4.31	2.93
	T_4	3.80	4.30	5.50	4.53	1.22	3.21	4.26	2.90
	T_5	3.70	4.40	5.20	4.43	1.18	3.13	4.22	2.84
	Mean	4.22	4.64	5.82		1.20	3.30	4.36	
P	SE(d)	0.039	0.078	0.084		0.016	0.046	0.040	
	CD	0.080	0.161	0.172		0.033	0.094	0.082	
T	SE(d)	0.062	0.124	0.133		0.025	0.072	0.063	
	CD	0.126	0.255	0.273		0.052	0.149	0.130	
PXT	SE(d)	0.087	0.175	0.189		0.036	0.102	0.089	
	CD	NS	0.358	0.386		0.073	0.21	0.183	

Table 2. Effect of increasing mercury concentration on Transpiration rate and Stomatal conductance of *B. juncea* and *N. exaltata.* Plants : P_1 - Indian Mustard , P_2 – Boston Fern.Treatments: T_1 - 0 mg kg^{-1}Hg, T_2 - 2.5 mg kg^{-1} Hg,T_3 - 5 mg kg^{-1} Hg,T_4 - 10 mg kg^{-1} Hg ,T_5 - 20 mg kg^{-1} Hg.

Plant species	Treatments	Transpiration rate (m mol m^{-2} s^{-1}) 15 DAT	30 DAT	45 DAT	Mean	Stomatal conductance (mol m^{-2} s^{-1}) 15 DAT	30 DAT	45 DAT	Mean
P_1									
	T_1	3.34	3.2	3.81	3.45	0.09	0.47	0.67	0.41
	T_2	3.2	3.15	3.25	3.20	0.08	0.45	0.65	0.39
	T_3	3.12	2.74	2.97	2.94	0.08	0.46	0.63	0.39
	T_4	2.84	2.16	2.38	2.46	0.07	0.44	0.58	0.36
	T_5	2.98	2.36	2.6	2.65	0.07	0.41	0.54	0.34
	Mean	3.10	2.72	3.00		0.08	0.45	0.61	
P_2	T_1	0.98	1.2	1.24	1.14	0.09	0.45	0.67	0.40
	T_2	0.95	1.18	1.21	1.11	0.08	0.45	0.65	0.39
	T_3	0.97	1.24	1.18	1.13	0.07	0.43	0.62	0.37
	T_4	0.94	1.16	1.1	1.07	0.08	0.41	0.53	0.34
	T_5	0.92	1.12	1.04	1.03	0.07	0.39	0.50	0.32
	Mean	0.95	1.18	1.15		0.08	0.43	0.59	
P	SE(d)	0.020	0.015	0.018		0.001	0.003	0.003	
	CD	0.042	0.039	0.036		NS	0.007	0.006	
T	SE(d)	0.032	0.023	0.028		0.001	0.005	0.006	
	CD	0.066	0.048	0.058		0.001	0.010	0.012	
PXT	SE(d)	0.046	0.033	0.04		0.001	0.007	0.007	
	CD	0.094	0.068	0.082		0.002	NS	0.013	

and chlorophyll levels in the leaves of *B. juncea* and *N. exaltata* significantly decreased with increasing Hg concentration compared to control ($F=52.71$, $p<0.05$ and $F=19.41$, $p<0.05$). However, it does not show any visual toxicity symptoms. Average Total Chlorophyll content significantly reduced from 17.70 (T_1) to 16.17 (T_5) and 4.43 (T_5) to 5.47 (T_1) and average photosynthetic rate declined from 9.63 (T_1) to 8.38 (T_5) and 3.12 (T_1) to 2.84 (T_5) in *B. juncea* and *N. exaltata,* respectively (Table 1). With increasing Hg doses, photosynthesis impairment and fall in gaseous exchange measurements were observed (Gill *et al.* 2012). It could be because mercury inhibits Fe and induces chlorosis in leaves, which has a deleterious effect on chlorophyll metabolism and diminishes micronutrients. In certain cases, parts of chlorophyll can be transformed to pheophytin. Sanmartin *et al.* (2011) described pheophytins as compounds produced during chlorophyll degradation due to the loss of magnesium ions from chlorophyll (Gomes *et al.* 2016, Mobin and Khan 2007). As far as gaseous exchange parameters are concerned, they are inversely proportional to the increasing mercury concentration except for intercellular CO_2 concentration. Mean Transpiration rate decreased from 3.45 (T_1) to 2.65 (T_5) and 1.14 (T_1) to 1.03 (T_5) and Mean Stomatal conductance reduced from 0.41 (T_1) to 0.34 (T_5) and 0.40 (T_1) to 0.32 (T_5) in *B. juncea* and *N. exaltata,* respectively (Table 2). Average intercellular CO_2 concentration varied from 578 (T_1) to 472 (T_5) ppm and 484 (T_1) to 473 (T_5) was recorded in *B. juncea* and *N. exaltata*, respectively (Fig. 2). These findings are consistent with those of Januskaitiene (2010), who found that with heavy metal stress in pea plants, gaseous exchange parameters such as photosynthetic rate, intercellular CO_2 concentration and so on decreased (Boening 2000). Carboxylation efficiency is derived as the ratio of photosynthetic rate to the intercellular CO_2 concentration. Carboxylation efficiency exhibited a gradual decline in Fig. 3 ranging from 2.97 (T_1) to 2.76 (T_5) and 0.96 (T_1) to 0.88 (T_5) in *B. juncea* and *N. exaltata* after 45 days of mercury treatment, respectively. Heavy metal toxicity resulted in a decreased carbon assimilation due to disruption of chloroplast structure and reduced Photosytem II photochemical efficiency, which affects plant development (Parmar *et al.* 2013, Asgher *et al.* 2015). However an increasing trend was observed in the analyzed parameters with respect to days after mercury treatment.

The primary response of plants exposed to stress

lead to the formation of free radicals. ROS intermediates are reduced forms of atmospheric oxygen (O_2) (Sharma *et al*. 2012, Gimenez *et al.* 2018 , Hasanuzzaman *et al.* 2020). Present study also showed the increased level of H_2O_2 with increasing Hg doses. This might be largely attributable to the destabilization of membrane in plants with accelerating metal stress. Since this plants were spotted to accrue more metal with increasing its doses. In plant cells, ROS formed as a result of oxidative stress induces a range of negative effects, including photosynthetic inhibition, ATP inhibition, lipid peroxidation and DNA damage (Zhang *et al.* 2007b, Jiang *et al.* 2010, Niu *et al.* 2014). Inordinate accretion of reactive oxygen species (ROS), such as free radicals and H_2O_2, has been linked to mercury-induced cellular oxidative damage in plants. H_2O_2 is vital in terms of plant growth and resistance as a signal molecule, but exceedingly high levels of H_2O_2 combined with ROS can elicit lipid peroxidation by attacking membrane lipids. TBARS, which are formed when certain primary and secondary lipid peroxidation products decompose, can be used as a marker of lipid peroxidation in tissues. Mercury exposure resulted in a substantial accumulation of H_2O_2 (Chen *et al*. 2009, Shiyab *et al.* 2009, Kapoor *et al.* 2014). Mean Proline content, Mean Lipid peroxidation and Mean Hydrogen peroxide content of 0.19 (T_1) to 0.32 (T_5) µ mol proline g^{-1} tissue, 0.39 (T_1) to 0.62 (T_5) and 0.13 (T_1) to 0.22 (T_5) µmol g^{-1} fresh weight, 4.31 to 5.79 and 0.44 to 0.54

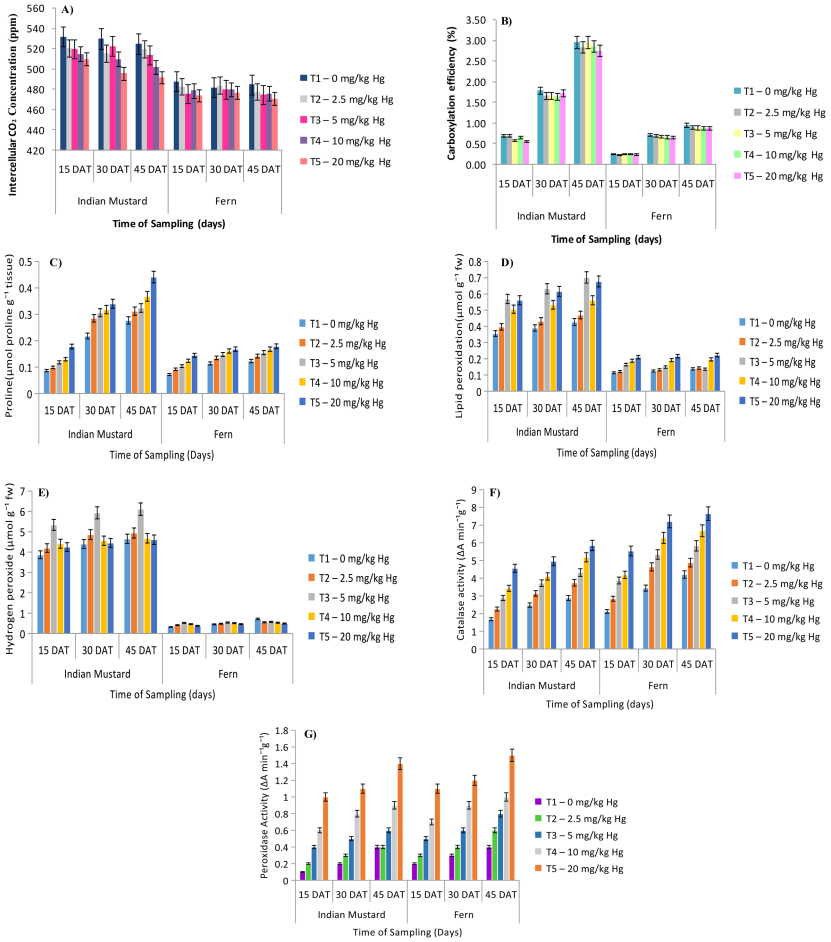


Fig.2. Effect of increasing mercury concentration on A) Intercellular CO_2 Concentration, B) Carboxylation efficiency, C) Proline, D) Lipid peroxidation, E) Hydrogen peroxide, F) Catalase and G) Peroxidase activity of *B. juncea* and *N. exaltata*.

Table 3. Linear regression model to assess the influence of Hg on Physiological and Biochemical parameters of *B. juncea* and *N. exaltata.* *NS – Not significant.

Parameter	Regression equation		Standard error		Coefficient R^2	
	Indian Mustard	Boston Fern	Indian Mustard	Boston Fern	Indian Mustard	Boston Fern
Total Chlorophyll	17.46 - 0.086 Hg	5.244 - 0.047 Hg	0.314	0.240	0.86	0.76
Photosynthetic rate	9.360 - 0.053 Hg	3.044 - 0.012 Hg	0.207	0.061	0.84	0.75
Transpiration rate	3.239 - 0.040 Hg	1.137 - 0.006 Hg	0.286	0.016	0.62	0.90
Stomatal conductance	0.382 + 0.001 Hg	0.366 + 0.002 Hg	0.004	0.006	0.88	0.90
Intercellular CO_2 concentration	570.70-5.664 Hg	485.023 – 0.57 Hg	21.95	1.268	0.84	0.94
Proline	0.208 + 0.006 Hg	0.114 + 0.003 Hg	0.014	0.013	0.93	0.78
Lipid peroxidation	0.447+ 0.010 Hg	0.127 + 0.005 Hg	0.089	0.010	0.49	0.95
Hydrogen peroxide	4.243 + 0.068 Hg	0.470 + 0.003 Hg	0.289	0.032	0.71	0.62
Catalase	2.701 + 0.131 Hg	3.730 + 0.167 Hg	0.315	0.445	0.93	0.92
Peroxidase	0.249 + 0.043 Hg	0.338 + 0.048 Hg	0.070	0.053	0.98	0.98

µmol g^{-1} fresh weight was recorded in *B. juncea* and *N. exaltata*, respectively (Fig. 2). Proline is generally referred as stress enzyme and a sensitive plant marker of oxidative stress caused by biotic or abiotic factors. Significant difference was observed in the production of proline after 15 days ($F=61.13$, $p<0.05$), 30 days ($F=82.76$, $p<0.05$) and 45 days ($F=86.83$, $p<0.05$) in response to mercury treatment with highest content of 0.441 µ mol proline g^{-1} tissue in T_5 and the least in T_1 with 0.277 µ mol proline g^{-1} tissue (Fig. 2). From the simple linear regression represented in Table 3, it could be witnessed that proline, lipid peroxidation and hydrogen peroxide are positively correlated with accelerating mercury dosage whereas Table 4 depicts the correlationship among all the variables and reveals the inter relationship among the variables. Plant's ability to mitigate heavy metal toxicity or to endure stress helps them to thrive under such environments. Similarly, metal treatment induced increased activities of catalase and peroxidase enzymes, which aided in the scavenging of free radicals. Significant parallel changes were observed in antioxidant enzymatic activity between mercury treated *B. juncea* and *N. exaltata* and control (Catalase: After 15 day $F=20.61$, $p<0.05$, 30 day $F=86.60$, $p<0.05$, 45 day $F= 10.70$, $p<0.05$ and Peroxidase after 45 days $F=119.96$, $p<0.05$). In *B. juncea* and *N. exaltata,* Mean catalase activity accelerated from 2.35 (T_1) to 5.12 (T_5) min^{-1} g^{-1} and 3.26 (T_1) to 6.80 (T_5) min^{-1} g^{-1}, respectively while mean peroxidase activity increased 0.23 (T_1) to 1.17 (T_5) min^{-1} g^{-1} and 0.30 (T_1) to 1.27 (T_5) min^{-1} g^{-1}, respectively (Fig. 2). There was no significant difference in peroxidase generation was observed up to 30 days but 45 days after mercury treatment marked

Table 4. Pearson correlation matrix illustrating the relationship among the variables. *Hg – Mercury, TC – Total Chlorophyll, PR – Photosynthetic rate, TR – Transpiration Rate, SC – Stomatal conductance, ICC - Intercellular CO_2 Concentration, CE - Carboxylation Efficiency, CAT – Catalase, POX – Peroxidase, PROLINE – Proline, LP - Lipid Peroxidation, HP - H_2O_2.

Hg	TC	PR	TR	SC	ICC	CE	CAT	POX	PRO	LP	HP
Hg	1.00										
TC	-0.08	1.00									
PR	-0.08	1.00	1.00								
TR	-0.16	0.98	0.98	1.00							
SC	-0.44	0.27	0.24	0.29	1.00						
ICC	0.28	0.74	0.72	0.70	0.55	1.00					
CE	-0.13	0.99	0.99	0.97	0.19	0.65	1.00				
CAT	0.82	-0.57	-0.57	-0.62	-0.63	-0.25	-0.59	1.00			
POX	0.98	-0.23	-0.23	-0.31	-0.52	0.13	-0.27	0.91	1.00		
PRO	0.45	0.83	0.82	0.74	0.05	0.82	0.79	-0.06	0.31	1.00	
LP	0.26	0.90	0.89	0.85	0.08	0.77	0.88	-0.24	0.12	0.96	1.00
HP	0.30	0.98	0.98	0.96	0.19	0.71	0.98	-0.50	-0.17	0.86	0.95	1.00

a significant difference. The results of the simple linear regression analysis are listed in Table 3 which reveals the relationship between Hg and the attributes and the per cent variation. These findings align with those of Doganlar *et al.* (2012).The plant's antioxidant capacity was increased in a dose-dependent manner. Catalase scavenges H_2O_2 directly, switching it to H_2O and O_2. Peroxidases like ascorbate peroxidase and peroxidase indirectly scavenge H_2O_2 by pairing it with antioxidants like ascorbate (Sofo *et al.* 2015, Sytar *et al.* 2013) or transfer of electrons to various donor molecules such as phenolic compounds, lignin precursors, or secondary metabolites (Kim *et al*. 2010). As the concentration of Hg in the plant tend to increases, plant cells generate greater amounts of those enzymes (Sahu *et al.* 2012, Kapoor *et al*. 2014).

CONCLUSION

Since mercury is a critical pollutant, several studies has been carried out to get insights into the ecotoxicity of mercury. This study documents a reduction in the physiological functions (Photosynthetic and Gaseous exchange parameters) in *B. juncea* and *N. exaltata* with increasing Hg concentration leading to slower metabolism in association with various factors and development of antioxidant defense system against ROS generation. Even though ROS has an indispensable role in plant system (For instance, as signal molecules for stomatal closure), generation of larger quantity would result in phytotoxicity. However *B. juncea* and *N. exaltata* exhibited tolerance up to 20 mg kg^{-1} without any toxic symptoms which might be due to the antioxidant defense system. In addition, Proline significantly increased from 0.27 (control) to 0.44 (20 mg kg^{-1}) Sand 0.12 (control) to 0.18 (20 mg kg^{-1}) µ mol proline g^{-1} tissue in *B. juncea* and *N. exaltata* which acts as an osmoprotectants. While comparing, Proline, catalase and peroxidase was higher in *B. juncea* than *N. exaltata* which highlight the ability of *B. juncea* to tolerate the Hg contaminated soil.

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