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Ecological response of Indian mustard and Boston fern to mercury contamination with special focus on soil nutrient availability

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Abstract
Heavy metals entry into the environment arises as a critical concern which posed major threat against human health and food safety. In a pot culture experiment, Indian mustard and Boston fern were grown in mercury spiked soil with different concentration variants of mercury with the objective of assessing the influence of different treatments on mercury tolerance of Indian mustard and Boston fern on soil available nitrogen, soil available phosphorous, soil available potassium and its correlation ship with soil mercury. Soil without mercury served as control for both tested plants. Our results indicated that nutrient content at different mercury concentration exhibited insignificant difference (p>0.05) compared to control. A significant difference (p< 0.05) was observed after 45 days of mercury treatment in soil available nitrogen. Maximum relative change of 6.06 and 5.85 per cent was observed in soil available nitrogen content of 20 mg kg^{-1} treated soil of Indian Mustard and 10 mg kg^{-1} treated soil of Boston fern, respectively. Parallel changes were recorded in phosphorous and potassium content. Plant response towards mercury contamination altered with growth period and the nutrient content tends to descend due to their mineralization and plant uptake.

Keywords: Mercury, contamination, soil nutrients, bioavailability

Introduction

Both Heavy metals and metalloids despite in trace quantities are responsible for significant toxic effects which resulted in an increased risk towards food safety, plant development and soil fertility. These heavy metals and metalloids are bio-accumulative and persistent in nature (Nagajyoti *et al.*, 2010) [10]. Soil nutrients plays a significant role in healthy plant growth and in maintaining cellular pH and osmotic potential. Requirement of macronutrients (nitrogen, phosphorous and potassium) were huge which is supplemented by the application of either bio or synthetic fertilizers. Phosphorous availability was very less or very slowly available. They occur in freshly precipitated forms or anions which can be easily exchanged from the adsorbed sites. The most important factor affecting the bioavailability of heavy metal to the plant is the soil pH. In order to ensure environmental protection, bioavailable pool of the trace elements should be studied to decide the soil quality criteria. Heavy metal enters the soil through various sources both natural and anthropogenic sources.

Mercury is considered as a potential neurotoxin and toxic in nature due to its persistence. Mercury occurs in various forms namely elemental, organic and inorganic mercury. Soil is reckoned to be a global sink for various contaminants which also ropes in mercury by means of bioaccumulation (Obrist *et al.*, 2018) [11]. Mercury accumulation in global soils was found to be 250-1000 Gg and they are of either natural (geological sources, forest fires and volcanic eruption) or anthropogenic (Chlor alkali plants, coal based thermal power plants, cement production, batteries, pesticides, mining, smelting ,etc.,) in origin (Obrist *et al.*, 2018) [11]. Both the soil physical (Soil texture, Particle size) and chemical properties (pH, CEC, organic matter type, clay type) influences the fate and transport of mercury. The dissolution of mercury in soil and its complexation with essential soil nutrients like nitrates and phosphates lowers the soil pH thereby rendering the nutrients inaccessible to microorganisms (Andrew and Jackson, 1996; Salam and Ishaq, 2019) [1 15]. Heavy metals such as zinc, copper, iron, and chromium are essential micronutrients that function as cofactors for many enzymes that play important roles in redox processes, molecule stabilisation through electrostatic interactions, and osmotic pressure regulation (Bruins *et al.*, 2000) [4].

On the contrary, non-essential heavy metals like lead, cadmium and mercury with no known biological functions become very toxic to soil microbes at higher concentrations. The presence of iron oxides in soil attributes towards Hg^{2+}sorption thereby reducing the concentration of inorganic mercury in soil (Selin, 2009) [16]. The presence of micronutrients like copper and zinc is directly proportional to Hg desorption in soil with Cu having a dominant role than Zn. This is because of the higher electronegativity of Cu than Hg and Zn in the competitive absorption at active sites. However, in soils with lower organic matter content, Zn was found to be readily absorbed than Hg. The soil Hg dynamics in relation to other nutrients depends on the relative orders of ionic properties like ionization potential, ionic radii, electronegativity, and reduction potential that serve as indicators for relative competition absorption capacities of Hg with other nutrients (Jing *et al.*, 2007; Qu *et al.*, 2019) [9, 13]. Similarly, the presence of Chloride ions also increased Hg desorption due to competitive absorption. Moreover, the presence of sulphate ions has also been found to reduce Hg absorption in soil (Zhang *et al.*, 2012) [19].

Materials and Methods

The current study was carried out in Factorial Completely Randomized Design with two factors (Factor 1 – Plant and Factor 2 – Mercury dosage) which embraces total of 10 variants. Each treatment was provided in four replicates. Uncontaminated soil collected from Kodaikanal was used for the pot culture experiment and it 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}). 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. The experiment was carried out for 45 days. Initial soil was characterized for its physical, chemical and biological properties. The soil samples were collected at definite intervals *viz.*, 15^{th}, 30^{th} and 45^{th} days after mercury treatment and estimated for chemical parameters such as Available Nitrogen, Available Phosphorous and Available Potassium.

Water holding Capacity of the soil

Water Holding Capacity of the initial soil was measured based on the moisture content using pressure plate apparatus at Soil Physics Laboratory, Department of Soil Science and Agricultural Chemistry, TNAU, Coimbatore (Figure 1). The analysis is based on the principle that the probability of a pore to retain moisture under different pressure depends upon the size. When the saturated soil is subjected to a pressure greater than zero tension, some of the water will be drained away until the pores have a diameter corresponding to the applied pressure. Rubber rings were placed on the ceramic plate and it was filled with soil (sieved in 2mm sieve) kept in a tray containing water for overnight saturation. After saturation, excess water was drained and kept inside the extractor and it was closed immediately with lid. Then the pressure was set at 0.33 bar for Water holding capacity. Soil sample was allowed to attain equilibrium and release excess pressure. After the release of excess pressure, extractor was opened and the pressure plate was removed. Soil was transferred to pre-weighed aluminium containers and the moisture content was calculated using gravimetric method (Cresswel *et al.* 2008) [5].

Fig 1: Determination of water holding capacity of the initial pot soil using pressure plate apparatus

Soil Textural Composition

The textural composition of the soil was determined by using International Robinson Pipette Method (Robinson, 1922) [14] and is based on stokes law (Figure 2). According to this law, the rate of fall of a particle in the liquid is directly proportional to the square of its radius and is given as follows

$$V = \frac{2}{9} g r^2 \frac{(dp - d)}{\eta}$$

Where V=sedimentation velocity in cm/sec; g=acceleration due to gravity cm/sec^2; r=radius of the particles (cm); dp =density of the particle (g cc^{-1}); d=density of the liquid (g cc^{-1}) and η=viscosity of the liquid. In this method, the soil is first dispersed by destroying the binding agents with hydrogen peroxide and hydrochloric acid followed by treatment with dispersing agent. Clay and silt are separated by sedimentation and coarse and fine sand by sieving.

Fig 2: Determination of soil texture of the initial pot soil by International Robinson Pipette method

Major Nutrients

Available Nitrogen in the collected soil samples were analysed using alkaline permanganate method by Subbiah and Asija (1956) [17]. Available Phosphorous and Available Potassium were determined in the soil samples by Bray method (Bray and Kurtz, 1945) [3] and flame photometer method (Jackson, 1973) [8].

Statistical analysis

The data obtained from different experiments were analysed for the 'F' test of significance following the methods described by Panse and Sukhatme (1985) [12].

Results

Characteristics of initial soil

The pH and EC of the experimental soil was found to be 5.85

and 0.45 dSm^{-1}, respectively. Organic carbon content of the soil was 2.50 per cent. Available Nitrogen, Available Phosphorous, and Available Potassium recorded 225.50, 19.20, and 214 kg ha^{-1} respectively. Experimental soil recorded 22 per cent field capacity and the soil textural composition was found to be 25.4 per cent clay, 4.50 per cent silt and 67.4 per cent sand. The experimental soil did not contain any concentrations of mercury.

Soil Available Nitrogen

Soil available nitrogen level in the soil in Figure 3a. was found to be ranging from 208 (P_1T_4) to 218 (P_1T_1) kg ha^{-1} and 210 (P_2T_4) to 220 (P_2T_1) kg ha^{-1} after 15 days of mercury treatment. Soil nitrogen content tends to decline from 210 (T_1) to 195 (T_4) kg ha^{-1} till T_4 and increases in T_5 to 201 kg ha^{-1} in P_1 after 30 days of mercury treatment whereas in P_2, nitrogen content tends to reduce from T_1 (control soil) till T_3 and begins to ascend incase of T_4 and T_5. Meanwhile in 45 days after the mercury treatment, Soil available nitrogen content ranged from 186 (T_5) to 198 (T_1) kg ha^{-1} in P_1 and 192 (T_4) to 205 (T_1) kg ha^{-1} in P_2. Maximum mean nitrogen content was recorded in 209 T_1 and minimum in T_4 mean value in P_1 and P_2 reported with mean maximum of 213 kg ha^{-1} in T_1 and minimum in 202 kg ha^{-1} of T_4 soil. While weighing against P_1 and P_2, Mean Soil Available Nitrogen Content was recorded with 197 kg ha^{-1} as its minimum in P_1T_4 and 213 kg ha^{-1} as its maximum in P_2T_1. The interaction effect was not significant in 15 and 30 days whereas it was significant in 45 days after mercury treatment.

Soil Available Phosphorous

Soil available phosphorous indifferently varied emphasizing no significant difference with mercury concentration in Figure 3b. which reported from 17.3 (T_5) to 18.2 (T_4) kg ha^{-1} in P_1 and 17.2 (T_4) to 18 (T_3) kg ha^{-1} in P_2 after 15 days of mercury treatment. Highest soil available phosphorous of 16.8 and 16.7 kg ha^{-1} was witnessed in P_1T_4 and P_2T_3 and the lowest in P_1T_2 with 15.2 kg ha^{-1} and 15.6 kg ha^{-1} in P_2T_5 after 30 days of mercury treatment. Regarding 45 days after mercury treatment, Soil available phosphorous ranged from 13.9 (T_5) to 15.5 (T_3) kg ha^{-1} in P_1 and 14.2 (T_4) to 15.8 (T_1) kg ha^{-1} in P_2. P_1 reported maximum and minimum mean available phosphorous as 16.7 kg ha^{-1} in P_1T_3 and 15.5 kg ha^{-1} in P_1T_5 and P_2 as 16.8 kg ha^{-1} in P_2T_1 & P_2T_3 and 15.7 kg ha^{-1} in P_2T_4, respectively. Overall mean available phosphorous was highest at 16.8 kg ha^{-1} in P_2T_1 and P_2T_3 and the lowest in 15.5 kg ha^{-1} in P_1T_5.

Soil Available Potassium

In the present experiment, Soil available potassium content did not significantly differed with varying concentration of mercury in the soil with respect to different days after mercury treatment (Figure 3c.). Highest potassium content of 189, 185 and 173 kg ha^{-1} was registered in P_1T_1 and the lowest content of 181, 175 and 162 kg ha^{-1} was turned up in T_5, T_2 and T_2 after 15, 30 ad 45 days of mercury treatment in P_1 whereas in P_2, P_2T_1 recorded highest values (209, 206 and 197 kg ha^{-1}) and P_2T_4 (198, 195 and 192 kg ha^{-1}) with lowest along all the days after treatment. P_1 was documented with highest average potassium in T_1 with 182 kg ha^{-1} and the least with 172 kg ha^{-1} in T_2 whilst 204 kg ha^{-1} of P_2T_1 stands as the highest average potassium and the least average was witnessed in T_4 with 194 kg ha^{-1}. While comparing P_1 and P_2, P_2T_1 leads with 204 kg ha^{-1} mean available potassium content and the least was found in P_1T_2 with 172 kg ha^{-1}.

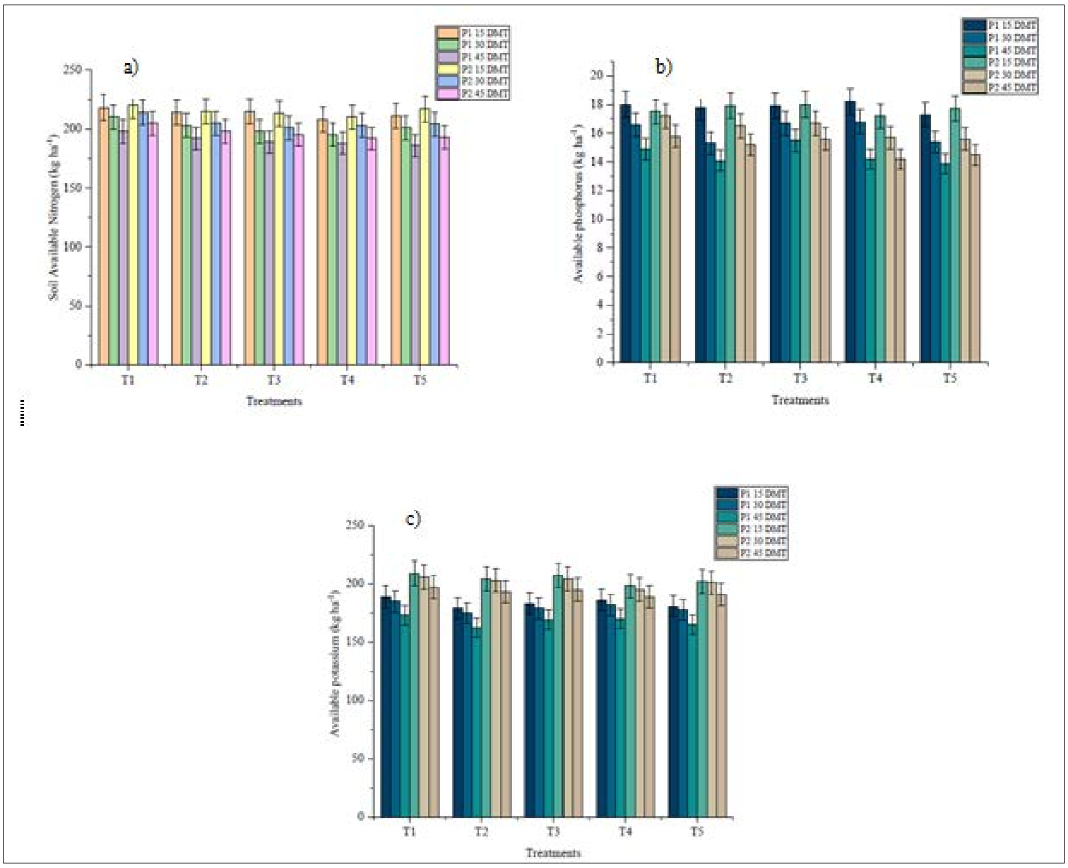

Fig 3: Effect of Soil Hg on Macronutrient availability (a) Soil Available Nitrogen b) Soil Available Phosphorous and c) Soil Available potassium) in Indian Mustard and Boston Fern

Discussion

From Table 1., results of relative changes in the soil available nitrogen, soil available phosphorous and soil available potassium content. Relative change express the per cent variation of the treated plant from control. Maximum relative change of 6.06 and 5.85 per cent was observed in soil available nitrogen content of 20 mg kg^{-1} treated soil of Indian Mustard and 10 mg kg^{-1} treated soil of Boston fern, respectively. Parallel changes were recorded in phosphorous and potassium content. While comparing three major nutrients, relative change was higher in phosphorous content in treated soil than control soil. The soil available nitrogen is found to be decreased with increase in time and mercury application to soil. The report of Dai *et al.* (2004) [6] could be correlated that the N mineralization are negatively associated with the increase in heavy metal concentration. Similarly, Zhou *et al.* (2016) [20] examined the changes in the soil nitrogen and organic carbon (OC) contaminated with the metals of Cd, Pb, Cu, Zn where the effect of heavy metals on the soil OC (-4.95%) and Total N (-17.9%) concentrations were observed in highly polluted by metals due to mineralization and the decrease in the nutrients ultimately reduced the vegetation. The Phosphate anion ($H_2PO_4^-$) strongly compete with heavy metal anions, such as arsenate and selenate, which results in the desorption of the metals (Bolan *et al.*, 2003) [2]. Even though the interaction effect is not significant, there is a decline in phosphorous content. Minimal decrease in potassium concentration over the mercury spiked soils were noticed in our study. Similarly, the level of potassium was at lower level along the dumping site of heavy metals Pb, Zn, Ni, Cu, Cd, Mn, Cr, Fe and As and vegetation area nearby compared with the adjacent areas (Tahar and Keltoum, 2011) [18]. Pearson correlation matrix table illustrates the relationship among different variable subjected to the experiment. Thus soil mercury and macro nutrients exhibit inverse relationship which can be witnessed in Table 2.

Table 1: Relative change in soil nutrients in response to increasing mercury concentration

Plant	Spiked mercury concentration	Relative change in soil available nitrogen (%)				Relative change in soil available phosphorous (%)				Relative change in soil available potassium (%)			
		15 DMT	30 DMT	45 DMT	Mean	15 DMT	30 DMT	45 DMT	Mean	15 DMT	30 DMT	45 DMT	Mean
P1	T_2	1.83	3.33	3.03	2.87	1.11	7.83	5.37	4.85	5.29	5.41	6.36	5.49
	T_3	1.38	5.71	4.55	3.83	0.56	0.60	4.03	1.21	3.17	3.24	2.31	2.75
	T_4	4.59	7.14	5.05	5.74	1.11	1.20	4.70	0.61	1.59	1.62	1.73	1.65
	T_5	3.21	4.29	6.06	4.78	3.89	7.23	6.71	6.06	4.23	3.78	4.62	3.85
	Mean	2.75	5.12	4.67	4.31	1.67	4.22	5.20	3.18	3.57	3.51	3.76	3.43
P2	T_2	2.27	4.21	3.41	3.29	2.29	4.07	3.80	1.79	2.39	1.46	2.03	1.96
	T_3	3.18	6.07	4.88	4.69	2.86	2.91	1.27	0.00	0.96	0.97	1.02	0.98
	T_4	4.55	5.14	6.34	5.16	1.71	8.72	10.13	6.55	5.26	5.34	4.06	4.90
	T_5	1.36	4.67	5.85	3.76	1.14	9.30	8.23	5.36	3.35	2.43	3.05	2.94
	Mean	2.84	5.02	5.12	4.23	2.00	6.25	5.85	3.42	2.99	2.55	2.54	2.70

Plants: P_1 - Indian Mustard, P_2 – Boston Fern; DMT – days after mercury treatment
Treatments: T_1 - 0 mg/kg Hg, T_2 - 2.5 mg/kg Hg, T_3 - 5 mg/kg Hg, T_4 - 10 mg/kg Hg, T_5 - 20 mg/kg Hg

Table 2: Pearson Correlation matrix illustrating the relationship between soil mercury and nutrient content in a) Indian mustard and b) Boston fern

a)	Mercury	AN	AP	AK
Mercury	1			
AN	-0.72	1		
AP	-0.55	0.22	1	
AK	-0.27	0.37	0.72	1
b)	*Mercury*	*AN*	*AP*	*AK*
Mercury	1			
AN	-0.49	1		
AP	-0.77	0.55	1	
AK	-0.62	0.72	0.94	1

Conclusion

Phytoremediation of mercury contaminated ecosystem has gained attention and considered as a promising technique for its remediation and resilience of the ecosystem. Various macro and micro nutrient availability gets influenced by the interference of heavy metals. In some cases, e presence of certain micronutrients enhances the uptake of contaminant into the plant system. From the present study results, the extent of influence on soil macro nutrient availability was well discussed highlighting the maximum relative change in available phosphorous content rather than available nitrogen and available potassium in the soil. Results from Pearson correlation matrix indicates that the soil mercury negatively influence the macro nutrients present in the soil.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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