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ORIGINAL ARTICLE

BIO-ACCUMULATION AND RELEASE OF MERCURY IN *VIGNA MUNGO* (L.) HEPPER SEEDLINGS

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Effect of mercury on the seedling of *Vigna mungo* seedlings was studied by culturing the seedlings in Hoagland medium artificially contaminated with 5 and 10μM Mercuric Chloride. Histochemical localization of the mercury in shoot and root tissues was done by staining with dithizone and quantitative analyses of mercury content accumulated in root, stem and leaf tissues were done using mercury analyser. Localization of mercury was observed as coloured masses in the cells of root and stem. Stem tissues of seedlings showed anatomical modification in the epidermal cells as trichomes. Patterns of bioaccumulation of mercury was root> stem> leaves revealing feeble translocation to the shoot system. A comparison of residual mercury content retained in the growth medium after sample harvesting and quantity accumulated in the plant body reveals that some quantity of mercury is lost presumably through the trichomes developed on the stem and/ or through stomata of the leaves.

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Plants live in diverse environment that provide essential nutrients as well as non nutrient metals inclusive of mercury and the level of mercury in the soil ranges from low to high depending on the nature of environmental conditions. Mercury can persist indefinitely in the soil posing an ever increasing threat to natural plant growth. Toxicity of mercury pertaining to plants is complex and depends on plant species, concentration and chemical form of the metal and composition of the soil. Even trace quantity of mercury can have detrimental effect on plant growth and development (Woolhouse, 1983; Lindenberg, 1987; Kabata-Pendias and Pendias, 1992; Lenka *et al.,* 1993; Syamala and Rao, 1999; Fodor, 2002; Pilon-Smits, 2005).

Compared to the extended and systematic studies on uptake and accumulation of many heavy metals (Hendry *et al.,* 1992; Boumik and Sharma, 1999) much fewer data are available on mercury. Although some studies have been conducted on

bioaccumulation of mercury (Woolhouse, 1983; Baker and Walker, 1990; Velasco-Alinsug et al., 2005) this area of research is fraught with confusion and controversy. So the present investigation is proposed to study the anatomical/histochemical aspects and to estimate the quantity of mercury absorbed/ translocated/ accumulated in the root, stem and leaves of *Vigna mungo* seedlings grown in Hoagland medium artificially contaminated in the mercuric chloride ($HgCl_2$).

MATERIALS AND METHODS

Seeds of Black gram, *Vigna mungo* (L.) Hepper were obtained from Tamil Nadu Agricultural University, Coimbatore. Seeds were surface sterilized with 0.1% mercuric chloride and after thorough washing in distilled water seeds were germinated in sterilized Petri dishes lined with filter paper wetted with distilled water. Healthy seedlings of 48 hrs old were transplanted to nutrient solution prepared according Epstein (1972) as described by Taiz Zeiger (1991) containing two concentrations 5µM and 10µM of mercuric chloride. Nutrient solution without mercuric chloride served as control. All experimental set up was maintained at room temperature (29±3ºc). Nutrient solution was replaced with fresh solution at every 24 hrs interval. Anatomical/histochemical studies and estimation of mercury were conducted at various intervals i.e., 12, 24, 48, 72 and 168 hrs after transplantation to the culture medium.

Histochemical study

Both root and stem cuttings were collected and fixed in Formalin Acetic acid-Alcohol mixture (FAA), dehydrated through TBA series and paraffin infiltrated tissues were cut at 10µ. Deparaffinised sections were stained with dithizone to localize mercury according to Pears (1972). Photomicrographs were taken using Nikon microscope (Model ECLIPSE 400) fitted with Nikon camera (Model DXM).

Estimation of Mercury

Mercury content of root, stem, cotyledons and leaves were analyzed using air dried materials. Samples were wet digested (Allan, 1969) and mercury was analyzed using Mercury Analyzer (Model MA 5800 E of Electronic Corporation of India Ltd.). The reaction mixture contained 8ml of 10% (v/v) HNO_3, 2ml of 20% (w/v) $SnCl_2$ in concentrated HCl and 2ml of aliquote. Aqueous solution of $HgNO_3$ was used as a standard.

Mercury content of the nutrient solution (residual) after sample collection also was analyzed using the procedure as described above. All experiments were repeated a minimum of three times each keeping duplicate sets of culture. Data was analyzed statistically and tests of significance were also done.

RESULTS

Mercury was localized as brown coloured masses in stem and root cells. The staining intensity was very feeble at low concentrations and the localization was clearly seen in the seedlings treated with 5µM and 10µM $HgCl_2$ sampled after 168hrs (Fig 1). In roots mercury content was observed as coloured masses filled in the vessels of vascular tissue (Fig. 1-B1, B2). Sections of stem samples treated with 10µM mercury showed some modifications in the epidermal cells as some cells are enlarged and projected outwards which appeared as trichomes and their shape was different from that of other epidermal cells (Figure 1-D1, D2) whereas the epidermal cells of control stem did not show any trichome cells in the epidermis (Fig. 1 C1,C2) This type of trichome cells were observed in 5 and 10µM treatments but the distribution of trichomes was abundant in the stem treated with 10µM mercury. Similarly more dithizone sensitive

brown coloured masses were observed in the trichomes. Feebly stained patches were observed near the trichomes which appeared as stained mercury complex escaping from the stem through these specialized trichome structures.

Mercury was found to translocate to the stem and leaves of seedlings but maximum content was present in the roots; the quantity of which was increased significantly with the enhancement of mercury in the nutrient solution (Table 1, 3). Hence the stem tissue contained only low mercury content compared to the root. However, increase in the Hg accumulation was increased proportional to amount of $HgCl_2$ in the growth medium. The leaf tissue contained only very low quantity of mercury compared to the stem and root. However there was a gradual increase as the concentration increased in the medium.

Residual mercury content present in the nutrient media at each interval showed an increase or remained unchanged since the nutrient solution containing $HgCl_2$ was replaced at every 24 hrs intervals (Table 2). There was a significant difference in the mercury content when a comparison in made between the amounts of $HgCl_2$ added to the medium and the sum of the residual Hg present in the medium plus Hg accumulated in the seedling (Table 2).

Table 1. Bioaccumulation of mercury in different tissues of *V.mungo* seedlings ($\mu g\ g^{-1}$ dry tissue) during growth

Tissue	Treatment	Interval (hour)					
		0	**12**	**24**	**48**	**72**	**168**
Root	Control	ND	ND	ND	ND	ND	ND
	5µM		2.53 ± 0.031	5.35 ± 0.0032	11.13 ± 0.044	17.36 ± 0.019	35.41 ± 0.47
	10µM		11.15 ± 0.046	15.94 ± 0.058	21.27 ± 0.118	30.96 ± 0.017	78.65 ± 0.063
Stem	Control	ND	ND	ND	ND	ND	ND
	5µM		0.36 ± 0.015	0.45 ± 0.003	1.26 ± 0.0053	2.84 ± 0.073	3.02 ± 0.0074
	10µM		0.65 ± 0.019	0.67 ± 0.0085	2.19 ± 0.0062	3.02 ± 0.0047	21.30 ± 0.074
Leaf	Control	ND	ND	ND	ND	ND	ND
	5µM		0.059 ± 0.002	0.146 ± 0.00096	0.40 ± 0.0042	0.776 ± 0.0014	1.02 ± 0.0081
	10µM		0.25 ± 0.019	0.516 ± 0.0028	1.06 ± 0.028	1.87 ± 0.039	3.20 ± 0.032
Cotyledon	Control	ND	ND	ND	ND	ND	ND
	5µM		0.094 ± 0.004	0.37 ± 0.0017	0.506 ± 0.011	0.916 ± 0.024	1.12 ± 0.0088
	10µM		0.12 ± 0.0014	0.50 ± 0.0008	0.95 ± 0.0115	1.52 ± 0.012	1.78 ± 0.039

Values are mean of six replicates ± SE ND - not detected

Table 2. Mercury content in nutrient solution (residual) after sample collection at each interval ($\mu g\ L^{-1}$)

Treatment	Interval (hour)					
	0	**12**	**24**	**48**	**72**	**168**
Control	0	0	0	0	0	0
5µM	0	935.94 ± 5.52 (416.97)	874.62 ± 3.11 (474.05)	901.33 ± 3.46	912.09 ± 2.43	876.74 ± 7.35
10µM	0	1932.64 ± 5.59 (765.19)	1906.23 ± 6.81 (786.14)	1898.27 ± 4.66	1839.61 ± 8.20	1881.42 ± 6.84

Values are mean of six replicates ± SE (values in parenthesis shows the difference between added mercury content in the nutrient solution and sum of the mercury absorbed by the plant and that retained in the nutrient solution.

Table 3. Bioaccumulation percentage of mercury in different tissues of *V.mungo* seedlings during growth

Tissue	Treatment	Interval (hour)					
		0	**12**	**24**	**48**	**72**	**168**
Root	Control	0	0	0	0	0	0
	5µM		83.14	84.71	83.71	79.30	87.27
	10µM		91.62	90.43	83.51	82.85	74.89
Stem	Control	0	0	0	0	0	0
	5µM		11.83	7.12	9.48	12.97	7.44
	10µM		5.34	3.80	8.60	5.26	20.37
Leaf	Control	0	0	0	0	0	0
	5µM		1.94	2.31	3.01	3.54	2.51
	10µM		2.05	2.93	4.16	3.26	3.05
Cotyledon	Control	0	0	0	0	0	0
	5µM		3.09	5.86	3.81	4.18	2.76
	10µM		0.99	2.84	3.73	2.65	1.69

DISCUSSION

Bioaccumulation of mercury is maximum in the roots and the quantity of which is increased with the enhanced concentration of $HgCl_2$ in the nutrient medium and longer period of exposure. Since the roots are directly in contact with mercury present in the medium, maximum content is absorbed and very feeble translocation of mercury results in more accumulation. According to Woolhouse (1983), Lenka et al., (1993) Khanna and Rai (1995) and Velasco-Alinsug et al., (2005) mercury translocation from root to stem is very slow and most of the mercury gets deposited in the root tissue. Stunted growth of root system due to the abundant accumulation is the most visible symptom of mercury toxicity in plants (Morishita and Boratynski, 1992). Based on these views mercury adsorbed in the root causes maximum toxicity to the root system because the absorbed/ absorbed Hg^{2+} ions are not distributed or translocated evenly to all parts of the plant.

Owing to the interference of heavy metals like mercury with cell division and cell elongation (Davies, 1991) differentiation is inhibited in plants. In the case of *Vigna mungo* Hg^{2+} ions inhibit cell division and differentiation resulting in stunted growth and partial disfunctioning of root system. Nevertheless, limited translocation of Hg^{2+} to the tissue can not be ruled out since stem and leaf showed the presence of mercury. An important histochemical observation is the deposit of dithizone stained masses in the roots (Fig. 1 B1, B2) and the absence in control (Fig. 1 A1, A2). Since most of the conducting vessels in the root system is occluded with mercury it is apparent that the transport of mercury if at all occurring is through apoplast which is an open lattice of polysaccharide, through which mineral ions diffuse readily and since all cells are inter connected by cell walls, ions can diffuse across a tissue entirely through the cell wall space (apparent free space) without entering the cytoplasm (Taiz and Zeiger, 2002).

Structural modifications are observed in the epidermal cells of the stem (Figure 1-D1, D2). Some epidermal cells are modified into gland like trichomes which are absent in the control plants (Fig. 1 C1, C2).

The glandular trichomes are secretary in function (Fahn, 1982) and these modified cells release aqueous solution, gums and lipophilic secondary products including essential oils, volatile compounds and quinines. The distribution of these epidermal trichomes on the stem is varied among the treatments. Maximum number of trichomes is present in plants treated with 10μM mercury compared to low concentrations. Occurrences of dithizone positive stained patches inside the trichomes indicate the escape of mercury through these trichomes (Fig. `1 D1, D2).

Data on the residual mercury analysis in the nutrient solution after sample collection at 12 and 24 hrs and comparison against the total uptake by seedlings showed that more than 1/3 part of the total mercury applied in each treatment is lost during growth (Table 2). An important source of the mercury loss is presumed to be the escape through the glandular trichomes on the stem and this observation and concept are in conformity with the suggestion of Orcutt and Nilson (2000) who suggested that plants may form volatile organic derivatives of mercury to remove it from the tissue. The brown coloured patches inside the trichomes confirm the association of mercury with trichomes. A single transverse section of the stem showed the presence of many trichomes, and hence innumerable number of trichomes may be present in the entire stem and the loss of about 1/3 amount of mercury supplied to the nutrient medium can be accounted towards the escape through the trichomes.

When a comparison is made between the mercury content added to the medium on one hand and the sum of the balance amount in the residual solution and accumulated content on the other, it is obvious that significant amount of mercury is lost during growth of seedling and the disparity can be correlated with or adjusted against the apparent loss of mercury due to the escape through the trichomes of the stem and / or throught the stomata (Sahadevan, 2002). Hence some sort of 'cycling' of mercury occurs from the nutrient medium to the plant and from the plant to the atmosphere – a phenomenon comparable to soil plant atmosphere continuum (SPAC) concept of water relations in plants.

The present investigation reveals that *Vigna mungo* plant is not tolerant to mercury because at highest concentration applied (10μM) the survival of the plant was only for limited period. Not withstanding, the plant is neither an 'excluder' (Fitter and Hay, 1983; Turner, 1994; Orcutt and Nilson, 2000) nor an accumulator of mercury (Baker and Walker, 1990; Turner, 1994). Similarly this plant never exhibit strategy like avoidance and biochemical tolerance (Berry, 1986) because mercury enter the plant to some extent and internal detoxification or biochemical tolerance are not apparent. However, the strategy of response shown by *Vigna mungo* towards mercury toxicity may be considered as amelioration. According to Fitter and Hay (1983), Turner (1994), Taiz and Zeiger (2003) amelioration means plant absorb the toxic ions and act up on it in such a way as to minimize that effect variously and this may involve chelation, dilution, localization or excretion. In *Vigna mungo* the 'cycling' of mercury between growths media, plant and atmosphere involves absorption, chelation and localization in the roots and/ or excretion through trichomes.

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