Biochemical Responses of *Pisum Sativum* L. under Cadmium and Mercury Regimes

[1]S. MAHMOOD, [2]K. FARZANA, [3]M. ZIA-UL-HAQ*, [4]S. AHMAD, [1]F. RAIZ, [1]ABAIDULLAH
[1]*Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan.*
[2]*Department of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan.*
*[3]*Intellectual Property Organization, The Patent Office, Second Floor Kandawala building, M. A. Jinnah Road, Karachi, Pakistan.*
[4]*Department of Agronomy, Sargodha University, Sargodha, Pakistan.*

(Received 10th April 2007, revised 5th May 2007)

Summary: The effects of cadmium and mercury toxicity (30, 60, 90 and 120 μM) on seed germination, growth, pigments and total proteins of *Pisum sativum* (cv. Master Lee) were assessed. Seed germination was not affected by any of the metal tested, whereas initial growth was strongly inhibited by increasing concentrations of cadmium. Seedling biomass declined considerably in the presence of both metals. However, seedling growth was more influenced by varying levels of cadmium than mercury. Chlorophyll content showed a progressive decrease with increasing concentrations of cadmium and mercury. Protein content increased at 30 μM of mercury and at 60 and 90 μM of cadmium but decreased at the maximum level of both metals. Toxicity of both metals was expressed in terms of both morphological and biochemical variables. The accumulation of metals in root and shoot was concentration dependent. However, more cadmium deposited in shoots than in roots while the reverse was true for mercury. From these results, it was concluded that *P. sativum* had shown some tolerance towards various cadmium and mercury levels. It is likely that certain repair mechanisms can be attributed to metal tolerance in this species. Thus the species could be a useful phytoremediator for cadmium and mercury contamination.

Introduction

The existing industrial and agricultural activities have introduced a large amount of heavy metals into the environment. The toxicity and bioaccumulation of heavy metals are a potential threat to agriculture and environment [1]. Cadmium and mercury are the examples of heavy metals that have been classified as priority pollutants by the US Environmental Protection Agency (US EPA). These contaminants are among the conservative pollutants that are not biodegraded thus can persist indefinitely in soil and water. Moreover, their concentrations often exceed the permissible level. Cadmium pollution results from many industrial processes including electroplating leather tanning and pigment manufacturing [2]. While, mercury is released from metallurgical industries, metal finishing, and agrochemical industries [3].

The effluents from many industries are either transported to canals/ rivers through surface drains or spread near agricultural land. According to an estimate, 9000 million gallons of wastewater are daily discharged into water bodies from industrial sectors [4]. Long-term application of municipal wastewater also results in the accumulation of toxic levels of these metals in the soil.

Excessive concentrations of heavy metals have been reported to cause metabolic disorders and growth inhibition in a number of plant species [5-6]. However, the abilities of plant species to accumulate and tolerate toxic concentrations of specific metal vary widely [7]. Some leguminous species have been reported to possess excellent ability to acclimatize heavy metals [8-9]. *Pisum sativum* L. is an annual herb and is widely grown on arable irrigated land. The present study was aimed at evaluating noxious effects of these toxic metals on *P. sativum* (cv. Master Lee). The species was investigated for seed germination, seedling growth and for some biochemical parameters.

Results and Discussion

Seed germination of *P. sativum* was not statistically affected by any of the metals tested. Germination started on the fourth day and the maximum germination was observed after 7 days in

*To whom all correspondence should be addressed.

Table 1: Mean values (± S.E) for various attributes of *Pisum sativum* 21 days after growth under varying levels of Cadmium.

Parameters	Metal concentration (μM)						
	Control	30	60	90	120	F value	Significance
Germination Percentage	99.5 ± 0	98.3 ± 0.08	98.7 ± 0.018	98.1± 0.085	87.5 ± 0.026	0.79	ns
Fresh biomass FW g	6.28 ± 0.01a	4.26 ± 0.11b	3.34 ± 0.08c	3.02 ± 0.02c	2.19 ± 0.06d	2215.69	**
Dry biomass DW g	3.28 ± 0.10a	2.28 ± 0.02b	2.03 ± 0.12b	1.37 ± 0.05c	1.31 ± 0.07c	322.48	**
Photosynthetic area (cm^2)	5.40 ± 1.68a	3.78 ± 0.27b	3.38 ± 0.10b	2.92 ± 0.02c	1.99 ± 0.41d	9.74	**
Chlorophyll a (mg g^{-1} F W)	1.60 ± 0.05a	1.36 ± 0.05b	1.41± 0.04b	1.22 ± 0.06c	1.10 ± 0.02d	798.62	**
Chlorophyll b (mg g^{-1} F W)	0.72 ± 0.05a	0.45 ± 0.09b	0.42 ± 0.03b	0.34 ± 0.05c	0.33 ± 0.03c	514.16	**
Total chlorophyll (mg g^{-1} F W)	2.43 ± 0.07a	1.87 ± 0.17b	1.89 ± 0.24b	1.40 ± 0.11c	1.29 ± 0.19d	439.02	**
Protein content mg g-1 FW	22.97 ± 1.97a	18.21± 0.22b	23.15 ± 0.15a	24.24 ± 0.23a	16.32 ± 0.32c	487.91	**
Metal accumulation in root (μg g-1 DW)	0a	188 ± 6b	226 ± 11c	319 ± 21d	440 ± 27e	594.54	**
Metal accumulation in shoots (μg g-1 DW)	0a	110±4b	529±17c	628±11d	635±21e	600.07	**

Statistical analysis determined by ANOVA. **=Significant at 0.01 % of probability.
Mean sharing same letters are not significantly different by DMRT ($P < 0.05$).

the control solution. The maximum inhibition (12.06 %) was observed at 120 μM of both the metals (Table 1).

Both the metals had an inhibitory effect on fresh biomass of seedling of *P. sativum*. The biomass showed the maximum decline (65.12 %) at 120 μM of cadmium. Elevated levels of mercury had also caused an inhibition of fresh biomass but the extent of decline was lower (32.32 %) at 120 µM mercury as compared to cadmium. The dry biomass also exhibited a similar trend as shown by fresh biomass. The maximum decrease was observed at the highest level of cadmium than mercury (Table- 1 and 2).

Various concentrations of cadmium and mercury influenced the photosynthetic area and caused chlorosis of leaves, which led to the reduction in photosynthetic capability thereby causing retarded growth of the seedlings. These findings are in accordance with Chatterjee and Chatterjee [10] who reported chlorotic effects of some heavy metals in plants.

The observed changes in the pigment content of the plants suggested significant effect of increasing concentrations of the tested metals on green pigments. Chlorophyll content showed a progressive decrease with increasing concentrations of both the metals. The maximum inhibition of chlorophyll a, b and total chlorophyll was observed at 120 μM of cadmium (Table- 1). Decline in photosynthetic pigment could be attributed to the possible degradation of chloroplast. Chlorophyll degradation has been reported earlier in Cu treated plants [12]. Reduction may also be caused by inhibition of chlorophyll biosynthesis. Such inhibition has also been reported for lead, mercury, chromium and cadmium due to their effect on enzymes that are responsible for biosynthesis of chlorophyll [13].

The results of the study showed stimulatory effect on protein content up to 30 μM of mercury and 60 and 90 μM of cadmium but the maximum inhibition was observed at 120 μM of mercury (Table- 2). The protein content showed an increase initially under varying levels of the metals

Table- 2: Mean values (± S.E) for various attributes of *Pisum sativum* 21 days after growth under varying levels of Mercury.

Parameters	Metal concentration (μM)						
	Control	30	60	90	120	F value	Significance
Germination percentage	99.5 ± 0	97.9 ± 0.056	98.2 ± 0.052	97.5 ± 0.067	87.6 ± 0.093	2.91	ns
Fresh biomass FW g	6.28 ± 0.01a	6.07 ± 0.24a	5.35 ± 0.15b	5.23 ± 0.17b	4.25 ± 0.16c	2417.9	**
Dry biomass DW g	3.28 ± 0.10a	3.02 ± 0.11a	2.59 ± 0.31b	2.56 ± 0.43b	1.73 ± 0.21c	498.82	**
Photosynthetic area (cm^2)	5.40 ± 1.68a	7.06 ± 0.21b	6.00 ± 0.4c	5.20 ± 0.21ac	4.11 ± 0.14d	114.17	**
Chlorophyll a	1.60 ± 0.05a	1.41 ± 0.04b	1.43 ± 0.04b	1.41 ± 0.05b	1.26 ± 0.07c	240.54	**
Chlorophyll b	0.72 ± 0.05a	0.59 ± 0.05b	0.54 ± 0.08b	0.45 ± 0.02c	0.36 ± 0.08d	4461.01	**
Total chlorophyll	2.43 ± 0.07a	2.14 ± 0.09a	1.97 ± 0.22a	1.62 ± 0.31b	1.53 ± 0.21b	439.02	**
Protein content mg g-1 FW	22.97 ± 1.97a	24.12 ± 2.65a	18.18 ± 0.32b	17.19 ± 0.12b	15.31 ± 0.24c	392.5	**
Metal accumulation (μg g-1 DW) in root	0a	128 ± 7b	328 ± 16c	478 ± 24d	9395 ± 47e	1188.54	**
Metal accumulation (μg g-1 DW) in shoots	0a	94 ± 2b	113 ± 13b	159 ± 16b	220 ± 31c	3515.51	**

Statistical analysis determined by ANOVA. **= Significant at 0.01 % of probability.
Mean sharing same letters are not significantly different by DMRT (P <0.05).

tested. This may be an indicative of initiation of stress proteins synthesis [14]. However, the reduction in protein content at higher concentrations of these metals might be due to lipid peroxidation leading damage to membrane thus reducing membrane proteins. Further oxidative stress in the presence of heavy metals can also denature or damage proteins including some enzymatic proteins [15].

Plant treated with cadmium and mercury accumulated appreciable amount of them. The accumulation of metals was concentration dependent. The results presented clearly indicated that more mercury was deposited in roots while significantly higher content of cadmium were found in shoots. Earlier reports indicated that cadmium and mercury content varied widely in different plant parts owing to differential transport mechanism [7]. These results also signify different transport mechanism of these metals to different plant parts thus are in agreement to the above workers.

Experimental

Methodology

Preparation of Sample

The seeds of *P. sativum* L. (cv. Master Lee) were surface sterilized with dilute solution of sodium hypochlorite (2 %). The stock solution (1000 μM) for cadmium and mercury was prepared in Hoagland nutrient solution [10]. The cadmium was applied as cadmium carbonate $CdCO_3$ (Merck), while mercury was used as mercury sulphate Hg_2SO_4 (Merck). The concentrations 30, 60, 90, 120 μM were prepared by diluting 1000 μM stock. The Hoagland solution was used as control and was without cadmium and mercury.

Germination and Seedling Experiment

Thirty appropriately labeled Petri plates (8 cm internal diameter) lined with filter paper (Whatman No. 1) then 20 seeds were placed into

each Petri dish. Each treatment was replicated three times and 20 ml of treatment solutions were applied appropriately. The experiment was conducted in a growth chamber at 25 °C, 12 hours light and dark period, (illumination of 2500 lux, Philips T2 40W/ 33 lamp). The seeds were observed each day and germination was recorded. A seed was considered to be germinated when both radicle and plumule had emerged (0.2 cm).

Twenty pre germinated seeds were transferred to thirty labeled plastic beakers filled with 100 ml of different concentration of cadmium and mercury. While, nutrient solution (Hoagland) served as control. An equal quantity of polystyrene beads was added to each beaker and during the course of seedling growth beakers were aerated. Seedlings were allowed to grow for 21 days then they were taken out from the solutions and washed carefully. Fresh biomass was determined for seedling then plants were oven dried at 80 ° C for 48 hours and dry weight measurements were taken. Metal content were determined using oven dried plant material digested with concentrated nitric acid. Hg levels were measured mercury analyzer (SP3D, Nippon Instrument Co., Japan) following Park method [16]. Cadmium was analyzed by atomic absorption spectroscopy (Varian AAS, 1475). The chlorophyll and carotenoids content were determined by following Arnon method [17] while total protein estimation was carried out by the method of Lowry [18]. The data were analyzed statistically by one-way analysis of variance (ANOVA) to confirm its validity.

Conclusion

Although the toxicity was expressed in terms of some morphological and physiological variables and plants accumulated appreciable amount of metals. Nevertheless, *P. sativum* plants sustained growth under metal contamination even though biomass, pigment and protein content showed a slight decrease or increase at varying levels of these metals. This may be an indicative of certain repair mechanism that induced metal tolerance particularly initiation of stress protein synthesis at low concentrations of these metals. Higher concentrations, however, had caused more drastic effect on plant growth and caused toxicity symptoms. It may thus be concluded that *P. sativum* has a threshold for cadmium and mercury and could be a potential phytoremediator like other leguminous species.

References

1. J. M. Horsfall and A. I. Spiff, *Electronic J. Biotechnol.*, **8**, 3417 (2005).
2. M. Faisal and S. Hasnain, *African J. Biotechnol.* **3**, 610 (2004).
3. J. C. Igwe, C. U. Iroh and A. A. Abia, *J. Environ. Managt.* **4**, 1109 (2005).
4. M. A. Saleemi, In *Proceedings of the CEWRE international symposium*,, EPA Bulletin, Lahore, p. 63 (1993).
5. L. C. Claire, D. C. Adriano, K. S. Sajwan, S. L. Abel, D. P. Thoma and J. T. Driver, *Water, Air and Soil Poll.*, **59**, 231 (1991).
6. H. Dahmani-Mullar, F. Van Oort and M. Balbane, *Environ. Poll.*, **109**, 231 (2000).
7. M. Vojtechova and S. Leblova, *Biol. Plant.*, **33**, 386 (1991).
8. S. N. Jamal, M. Z. Iqbal and M. Athar, *Int. J. Environ. Sci. Tech.*, **3**, 53 (2006).
9. U. N. Joshi, S. S. Rathore and S. K. Arora, *Indian. J. Environ. Protect.*, **19**, 745 (1999).
10. J. Chatterjee and C. Chatterjee, *Environ. Poll.*, **109**, 69 (2000).
11. C. M. Luna, C. A. Gonzalez and V. S. Trippi, *Plant Cell Physiol.* **35**, 11 (1994).
12. P. Vajpayee, R. D. Tripathi, U. N. Rai, M. B. Ali and S. N. Singh, *Chemosphere.* **41**, 1075 (2000).
13. R. D. Tripathi,P. Vajpayee, N. Singh, U. N. Rai, A. Kumar, M. B. Ali, B. Kumar and M Yunus, *Chemosphere.* **54**, 1581 (2004).
14. J. L. Hall, *J. Exp. Bot.*, **53**, 1 (2002).
15. D. R. Hoagland and D. I. Arnon, California Agricultural Experiment Station Circular, University of California, Berkley. **347**, 1 (1950).
16. H. J. Park, M. Kim; S. M. Shim and G. H. Kim, *Bull. Environ. Contam. Toxicol.*, **74**, 470 (2005).
17. D. I. Arnon, *Plant Physiol.*, **24**, 1 (1949).
18. O. H Lowry, N. J. Rosebrough, A.L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).