Environment & Ecology 27 (2A) : 927—930, 2009
 ISSN 0970-0420

Effect of Pig Iron Factory Effluent on Growth, Protein and Chromosome of *Lathyrus sativus* L.

ASIS KUMAR NANDI AND ANIRBAN MUKHERJEE

Department of Botany & Forestry, Vidyasagar University
Midnapore 721102, India
E-mail : asiskumarnandi@yahoo.com

Abstract

Effluent from the pig iron factory at Khargapore, West Bengal showed detrimental influence on seed germination, plant growth, cell division and chromosome morphology in the test plant *Lathyrus sativus* L. at different concentrations. Germination percentage exhibited gradual decrease with an increase in effluent concentration. Internode number and length were found to change not always in accordance with the increase of effluent concentration. Increase of effluent concentration caused a decrease in mitotic index, whereas an increase in chromosomal and cell divisional abnormality.
Key words : Chromosomal abnormalities, Effluent effect, *Lathyrus sativus*, Plant growth, Protein.

Industries dealing with the metal extraction purify the metals from ores and in the process various heavy metals pass to the nearby environment in the form of effluent, dump and dust. Effluents of metallic industries are good sources of heavy metals. Albeit the plants entailing some heavy metals in trace amount for their normal growth the inordinate presence of these elements in the surrounding environment impaired permeability of cell membrane, biochemical functions, division of cells and overall growth (1—3). Heavy metals adversely affect the electron transport during respiration and the growth by impairing the enzyme function partially or absolutely (4). Even these elements at a micro level leads to the anomalous mitotic division, chromosomal aberrations, intra-chromosomal mutation and micronucleus formation (5—7). Copper and mercury are known to prevent DNA synthesis and protein synthesis culminating to the mitotic and chromosomal anomalies (8). Effects of such heavy metal contaminants on live plants, seedling or adult plants (9—11) or seed (1, 12, 13) are quite abounding. The present study is aimed at revealing the impact of effluent of a pig iron factory on the growth of an agricultural plant species and some of the vital life processes of it namely, seed germination, seedling growth, cell division, chromosomal conformity and protein content.

Methods

The effluent was collected from the sewage outlet of the pig iron factory at Nimpura, near Khargapore of West Bengal. Collected effluent was considered as 100% concentration and other concentrations (6.25, 12.5, 25, 50% vol/vol) were made by dilution with double distilled water.

Lathyrus sativus L. was taken as test plant. Seeds of the species were collected from the Sate Seed Corporation of West Bengal. Fifty seeds of test species were soaked in 50 ml of each of the effluent concentrations and in the double distilled water, taken as control, for six hours. Seeds were then washed thoroughly with water and allowed to germinate and grow plants on sterilized soil-sand mixture. Germination percentage was studied till seventh day. Seedling growth was measured from 15-day old seedling growing in the pot. Growth was measured in terms of number of internodes per plant and average length of internode.

Cytological studies were carried out with the root tips of the treated plants fixed in 1 : 3 acetoethanol and stained with 2% aceto orcein following the squash technique. Quantitative estimation of buffer soluble protein was done by following Lowry method (14).

Table 1. Effect of effluent at different concentrations on the growth performance of *Lathyrus sativus* L.

	Control (0)	Concentrations (%) 6.25	12.5	25	50	100
Germination percentage	88.66 ±1.34	86.00 ±2.16	85.34 ±2.03	84.00 ±3.17	79.34 ±1.83	75.34 ±2.38
Internode Number	4.20 ± 0.83	3.46 ±1.02	3.65 ± 0.98	3.97 ±0.73	3.87 ±1.15	3.97 ±0.53
Internode Length (cm)	2.09 ±0.93	2.37 ±1.52	2.61 ±2.1	2.90 ± 0.85	2.38 ±1.31	2.28 ±1.62

Results and Discussion

Effluent of pig iron factory showed an inhibitory effect when present at a higher concentration, however 25% concentration of it showed better growth performance, in terms of internode length and number of internodes per plant (Table 1).

Seed germination percentage was found to decline in response of increasing concentrations of effluent (Table 1). Increased osmotic pressure due to high concentration of salt might be the possible cause of the inhibition of germination.

Growth of the species as portrayed in terms of internode number and internode length showed variable impact of effluent at different concentrations (Table 1). While the number of internodes per plant was greater in control plants than the plant under the influence of effluent, the length of internode was least in control plants. The number and length of internode uniformity increased up to the effluent concen tration of 25% but showed no regularity in response with further increase of effluent concentration. Better performance of plants regarding internode length with the increase of effluent concentration up to

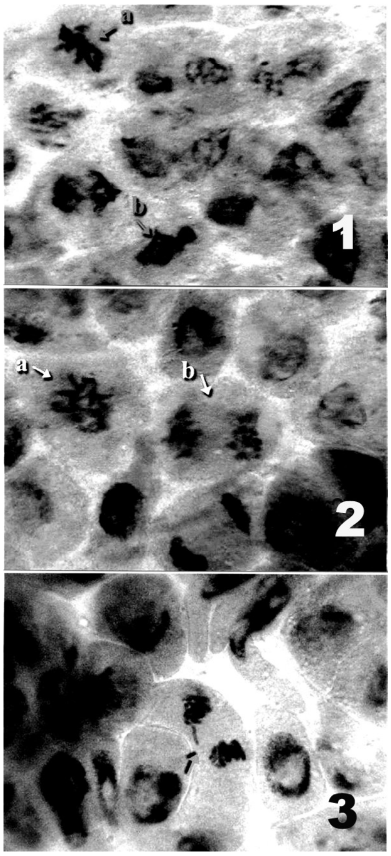

Figure 1. Chromosomal abnormalities at metaphase of *Lathyrus sativus* L. (a) Spindle disturbance and stickiness of chromosomes (b) Erosion and clumping of chromosome. **Figure 2.** Erosion of chromatin of *Lathyrus sativus* L. (a) Prophase (b) Ana-telophase. **Figure 3.** Laggard chromosome of *Lathyrus sativus* L. at anaphase.

Table 2. Effect of different concentrations of the effluent on cell division and chromosome in *Lathyrus sativus* L. P = Prophase, M=Metaphase, A = Anaphase, T = Telophase.

Effluent concentrations (%)	Div. cell (%)	Abnormal cell (%)	Abnormality percentages at different stages of cell division P	M	A	T
Control (0)	31.10 ± 2.12	0.52 ± 0.93	0.00 ± 0.00	15.58 ± 30.33	7.88 ± 19.55	4.04 ± 13.78
6.25	10.59 ± 4.35	0.58 ± 0.52	10.00 ± 29.49	21.25 ± 38.95	20.75± 39.36	17.00 ± 34.28
12.5	8.99 ± 3.89	0.62 ± 0.64	15.00 ± 35.90	16.25 ± 36.94	20.00± 38.99	13.75 ± 34.28
25	9.03 ± 3.14	0.79 ± 0.50	21.25 ± 39.48	26.25 ± 42.70	20.00± 35.74	17.50 ± 35.27
50	7.74 ± 2.81	0.90 ± 0.73	19.75 ± 35.66	22.34 ± 38.00	22.25± 35.96	16.25 ± 33.19
100	5.12 ± 2.51	1.68 ± 1.01	3.50 ± 12.10	33.87 ± 38.03	20.12± 27.94	27.70 ± 32.96

Table 3. Effect of different concentrations of the effluent on protein content in *Lathyrus sativus* L.

	Control (0)	Concentrations (%) 6.25	12.5	25	50	100
Protein content (ppm)	140.00 ±1.73	134.00 ±1.73	125.67 ±0.57	98.00 ± 4.58	89.33 ±8.08	73.67 ±5.13

a certain level might be due to the nutritive effects of heavy metals at a lower concentration as supported by Fernandes and Henriquies (3).

Different concentrations of effluent produced different degrees of impact on cell division and chromosomal morphology. With the increase in concentration of effluent the percentage of chromosomal abnormality also increased and the mitotic index decreased in the test plant (Table 2). Maximum chromosomal abnormality was noticed at 100% of concentration and the maximum mitotic index at control set. The kinds of anomalies met were mostly spindle disturbance, lagged chromosome, stickiness and clumping of chromosomes and erosion of chromatin contents (Figs. 1—3). Percentage of cytological abnormality was found to be proportional to the concentration of the effluent used. Reduction in mitotic index by heavy metals was witnessed in earlier works also (15). The formation of chromosomal aberration is assumed to be due to complex formation between the active agents and heavy metals presumably iron, within the chromosomes (16). A simultaneous negative influence on the division of cell and chromosome morphology extends support to the gross detrimental effect of the effluent.

A gradual decline in seedling protein content with the increase of the effluent (Table 3) concentration signifies impairment at the molecular level and the aberration in chromosomal complement is also expected to have enough reason for such implication.

Pearson correlation showed significantly positive relationship between effluent concentration and cytological abnormality and also between germination percentage and protein content (Table 4), but negative relationship between effluent concentration and germination percentage, effluent concentration and protein content, germination percentage and abnormality percentage and between abnormality percentage and protein content.

The effluent from metal factory proves to have the potential to encumber the normal development of plants by affecting the efficiency of cell division, chromosomal conformity, protein contents, ability of germination and also the growth in general. Since growth of plant is rather a complex attribute, affected by various factors, the expression at the level of internode number and internode length becomes confounding with respect to the effects revealed at the level of protein content or chromosome. The plants seemingly enjoy a better nutritional effect of effluent contents at certain concentrations of it, even surpassing the detrimental effect to cell division and chromosomal entity and thus do not corroborate with the cytological and biochemical results. The impaired cell division efficiency and chromosomal structure due to the action of effluent contents seem to provide enough reason for explaining the decline in protein content, seed germination and also to some extent the plant growth, in general ; still then some other finer causes at the molecular level, not revealed in the present study, might also be playing vital role in contributing the unusual expressions of the plants under the influence of effluent.

Table 4. Correlation between different parameters of *Lathyrus sativus* L. and effluent concentrations. * Significant at the 0.05 level, **Significant at the 0.01 level.

	Internode No.	Internode length	Mitotic index	Abnormality (%)	Protein	Treatment
Germination	– 0.083	0.016	0.699	– 0.931**	0.944**	– 0.977**
Internode no.		– 0.255	0.528	0.229	– 0.233	0.211
Internode length			– 0.532	– 0.112	– 0.211	– 0.104
Mitotic index				– 0.536	0.679	– 0.583
Abnormality (%)					– 0.871*	0.984**
Protein						– 0.919**

References

1. Claire L. C., D. C. Adriano, K. S. Sajwan, S. L. Abel, D. P. Thoma and J. T. Driver. 1991. Effect of selected trace metals on germinating seeds of six plant species. Water, Air and Soil Poll. 59 : 231—240.
2. Dimitrova I. and E. Ivaova. 2003. Effect of heavy metal soil pollution on some morphological and cytogenetical characteristics of flax (*Linium usitatissum* L.). J. Balkan Ecol. 4 : 212—218.
3. Fernandes J. C. and F. S. Henriques. 1991. Biochemical, physiological, and structural effects of excess copper in plants. Bot. Rev. 57 : 246—273.
4. Larcher W. 1995. Physiological plant ecology. Springer Verlag. 3 : 424—426.
5. Fiskesjo G. 1997. Allium test for screening chemicals ; evaluation of cytological parameter. Pages 308—333 *in* Plants for environmental studies. CRC Press, LLC, New York, USA.
6. Kovalhuk O., V. Titov, B. Hohn and I. Kovalchuk. 2001. A sensitive transgenic plant system to detect toxic inorganic compounds in the environment. Nat. Biotechnol. 6 : 568—572.
7. Njagi C. and H. Gopalan.1981. Mutagenicity testing of herbicides, fungicides and insecticides 1. Chromosomal aberration in *Vicia faba.* Cytologya 46 : 169—172.
8. De Flora S., C. Bennicelli and M. Bagnasco. 1994. Genotoxicity of mercury compounds : A review. Mutant Res. 317 : 57—79.
9. Chatterjee J. and C. Chatterjee. 2000. Phytotoxicity of cobalt, chromium, and copper in cauliflower. Environ. Pollut. 109 : 69—74.
10. Oncel I., Y. Kele and A. S. Ustun. 2000. Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. Environ. Pollut. 107 : 315—320.
11. Pichtel J., K. Kuroiwa and H. T. Sawyer. 2000. Distribution of Pb, Cd, and Ba in soils and plants of two contaminated sites. Environ. Pollut. 110 : 171—178.
12. Vojtechova M. and S. Leblova. 1991. Uptake of lead and cadmium by maize seedlings and the effects of heavy metals on the activity phosphoenolpyruvate carboxilase isolated from maize. Biologia Plantaram 33 : 386—394.
13. Xiong Z. T. 1998. Lead uptake and effects on seed germination and plant growth in a Pb hyperaccumulator *Brassica pekinensis* I. Rupr. Bull. Environ. Contam. Toxicol. 60 : 285—291.
14. Lowry O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with folin-phenol reagent. J. Biol. Chem. 193 : 265—275.
15. Staykoval T. A., E. N. Ivanoval and I. G. Velcheva. 2005. Cytogenetic effect of Heavy metal and cyanide in contaminated waters from the region of southwest. Bulgaria. J. Cell and Mol. Biol. 4 : 41—46.
16. Kihlman B. A., T. Merz and C. P. Swanson. 1957. Experimentally induced chromosome aberrations in plants J. Biophys. and Biochem. Cytol. 3 : 381—390.