International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com

Research Article

Genotoxic Effects of Lead Acetate Employing *Allium sativum* Root Chromosomal Aberration Assay

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Abstract

The present study was planned to estimate the genotoxic potential of lead acetate using *Allium sativum* root chromosomal aberration assay. Root tips of *Allium sativum* were treated with different concentrations of lead acetate *viz.*, 0.2, 0.4, 0.6, 0.8 and 1 ppm. Various types of physiological (laggards, vagrants, c-mitosis, delayed anaphase/s and stickiness) and clastogenic (chromosomal breaks, chromatin bridges) aberrations were observed in *Allium sativum* root tip cells following treatment with lead acetate. The effect was found to be dose dependent and frequency of physiological aberrations ranged from 15.77 to 31.64% while clastogenic aberrations ranged from 1.07 to 2.85%. Total chromosomal aberrations frequency ranged from 17.27 to 34.50%.

Keywords: *Allium sativum;* lead acetate; genotoxicity; plant assay

Introduction

Recent advances in industrialization and technological revolutions have resulted in an ever growing negative impact on the environment in terms of its pollution and degradation. Industrialisation assisted and abetted both by needs and greed of man has resulted in an ever-increasing threats to environment and health. Various anthropogenic activities, such as manufacturing, processing, transportation and consumption besides depleting further stock of natural resources add stress to the environment by accumulating different hazardous substances (Oladele, et al 2013; Odjegba and Adeboye, 2013; Dubey *et al*., 2003). Environmental degradation which may become irreversible results in loss of labour productivity from ill- health and loss of crop output (Shukla and Dubey, 1997; De Luna and Hallam, 1987). In recent years, increasing use of pesticides, fertilizers, continuous air emission from industrial sources and vehicular traffic have contaminated environment with heavy metals. Lead has attained the attention of scientific fraternity of the world due to its toxic nature and widespread usage in different commercial products including batteries, paints, food containers and a wide range of products of every day use (Saini and Nagpal, 2011). Qian and Tiffany-Castiglioni, (2003) refers lead as nonophysiological trace element, one of the major elements of the antiquity and is a pervasive contaminant in the environment (Tchounwou *et al.*, 2004). Besides being toxic, subtle danger of presence of lead in the environment lies in its capacity to induce genetic damage which can lead to cancer and can also be passed on to future generations. In order to identify the genotoxic effects of toxic substances such as lead, other heavy metals and a wide range of organic and inorganic pollutants in the environment, a variety of test systems employing a wide range of organisms are commonly used (Matsumoto and Marin-Morales,

2004; Matsumoto *et al*., 2006). Among various plant bioassays, *Allium sativum* root chromosomal aberration assay is one of the most reliable bioassays which can be applied to detect wide range of genetic damages (Chauhan *et al.,* 2001; Saxena *et al.,* 2004; Saxena *et al.,* 2010).

Materials and Methods

The present investigation is aimed at estimating the genotoxic effects of lead acetate employing *Allium sativum* root chromosomal aberration assay. This test system was slected because of number of advantages it offers. These include easy availability and handling; availability of roots in a short duration (1-3 days) and large size and less number of chromosomes (2n = 16). The cloves of the *Allium sativum* were separated and the old roots and scales were removed. The denuded cloves were placed over test tubes containing distilled water and were incubated at 25 ± 1ºC for the root induction. The freshly emerged roots of about 0.5-1 cm length were treated by placing the rooted cloves on test tubes containing different concentrations of lead acetate corresponding to 0.2, 0.4, 0.6, 0.8 and 1 ppm of lead in solution for 3h. Distilled water was used as a negative control. Cytological investigations were carried out following the protocol of Saxena et al. (2004). 2 × 2 chi square contingency test was used to assess the significance of the effects of the treatment on the root tips of *Allium sativum.*

Results

Genotoxic potential of lead in *Allium sativum* root chromosomal aberration assay

Treatment of *Allium sativum* roots with different concentrations of lead acetate revealed different types of chromosomal aberrations which were apportioned into physiological aberrations (c-mitosis, delayed anaphases, stickiness, laggards, vagrants, abnormal anaphases, abnormal metaphases) (Fig. 2) and clastogenic aberrations (chromatin bridges and chromosomal breaks) (Fig. 3). The physiological aberrations can be attributed to spindle inhibition, whereas clastogenic aberrations can be attributed to direct action on the chromosomes. The squash preparations of root tip cells of negative control cloves revealed a large number of normal dividing cells at different stages of mitosis. Whereas when root tips treated with different concentrations of lead acetate were squashed, a number of dividing cells with different kinds of aberrations such as c-mitosis, delayed anaphases, stickiness, laggards, vagrants, abnormal anaphases, abnormal metaphases, chromatin bridges and chromosomal breaks were observed. The frequency of physiological aberrations ranged from 15.77 % to 31.64 % while clastogenic aberrations ranged from 1.49 % to 2.85 % in root tip cells of *A. sativum* treated with different concentrations of lead acetate solution. Total percent aberrant cells were found to range from 17.27 % to 34.50 % (Table 1). Among all physiological aberrations observed, the frequency of cells with c-mitosis (9.89 %) was found to be maximum followed by delayed anaphases (9.15 %) and stickiness (5.49 %). While the frequency of other physiological aberrations like laggards and vagrants was quite low. Some of the aberrations which could not be accounted in any of the above mentioned physiological aberrations were considered under abnormal metaphases and abnormal anaphases. The spectrum of clastogenic aberrations included chromatin bridges and breaks. The frequency of cells with chromatin bridges was 2.19 % and that of chromosomal breaks was 0.65 %. The statistical analysis (2 X 2 chi square contingency test) revealed that the frequencies of chromosomal aberrations at all concentrations tested differed significantly from the control.

Discussion

The present study revealed a dose dependent increase in the total number of aberrations following treatment with different concentrations of lead acetate. The observed frequency of physiological aberrations was much more (31.64 % at highest dose) as compared to clastogenic aberrations (2.85 % at highest dose). Among different kinds of physiological aberrations, c-mitosis was found to be most common. C-mitosis is observed in root tip cells when any agent (physical/chemical) prevents the assembly of spindle microtubules by dissociating disulphide bonds leading to scattering of chromosomes in the cell. The term c-mitosis was coined by Levan (1938). It is probable that lead acetate used in the present study which induced an appreciable number of cells with c-mitosis may have acted by dissociating disulphide bonds of protein polymers and prevented the formation of any such new bond. Gul *et al.* (2006) found a similar trend of increase in the frequency of c-mitosis in root tip cells of *A. sativum* when treated with different

Table 1. Genotoxic potential of lead acetate employing *Allium sativum* root chromosomal aberration assay

Conc. (ppm)	**TDC**	**No. of Physiological aberrations (%)**								**No. of Clastogenic aberrations (%)**			**Total Aberrations PA + CA**	
		Cm	Da	St	Lg	Vg	Aa	Am	Total PA (%)	Bg	Bk	Total CA (%)	No.	%
NC	465	12 (2.58)	14 (3.01)	1 (0.21)	- (-)	- (-)	- (-)	3 (0.64)	30 (6.45)	- (-)	- (-)	- (-)	30	6.45
0.2	469	22 (4.69)	21 (4.47)	19 (4.05)	- (-)	1 (0.21)	2 (0.42)	9 (1.91)	74 (15.77)	7 (1.49)	- (-)	7 (1.49)	81	17.27*
0.4	463	27 (5.83)	31 (6.69)	17 (3.67)	- (-)	4 (0.86)	4 (0.86)	8 (1.72)	91 (19.65)	5 (1.07)	- (-)	5 (1.07)	96	20.73**
0.6	473	37 (7.82)	35 (7.39)	18 (3.80)	- (-)	4 (0.84)	6 (1.26)	7 (1.47)	107 (22.62)	7 (1.47)	3 (0.63)	10 (2.11)	117	24.73**
0.8	459	39 (8.49)	42 (9.15)	21 (4.57)	2 (0.43)	3 (0.65)	7 (1.52)	10 (2.17)	124 (27.01)	8 (1.74)	4 (0.87)	12 (2.61)	136	29.62***
1.0	455	45 (9.89)	41 (9.01)	25 (5.49)	2 (0.43)	5 (1.09)	12 (2.63)	14 (3.07)	144 (31.64)	10 (2.19)	3 (0.65)	13 (2.85)	157	34.50***

Conc.- Concentration; NC- Negative control; TDC- Total Number of Dividing Cells; Cm-C-mitosis; Da- Delayed anaphase/s; St- Stickiness; Lg- Laggard/s; Vg- Vagrant/s; Aa- Abnormal anaphase/s; Am- Abnormal metaphase/s; PA-Physiological aberrations; Bg- Chromatin Bridge/s; Bk- Chromosomal Break/s; Ca- Clastogenic aberrations.
*significant at P<0.05, **significant at P<0.01, ***significant P<0.001 as compared to control

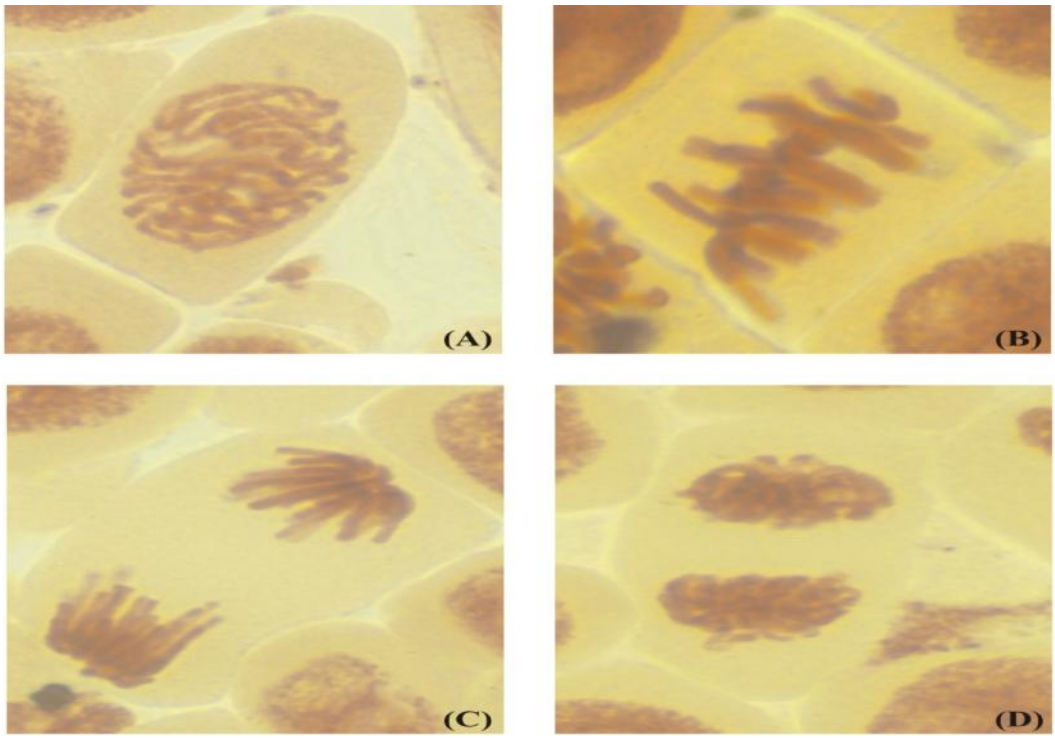

Fig. 1. Root tip cells of *Allium sativum* at Prophase (A); Metaphase (B); Anaphase (c); Telophase (D).

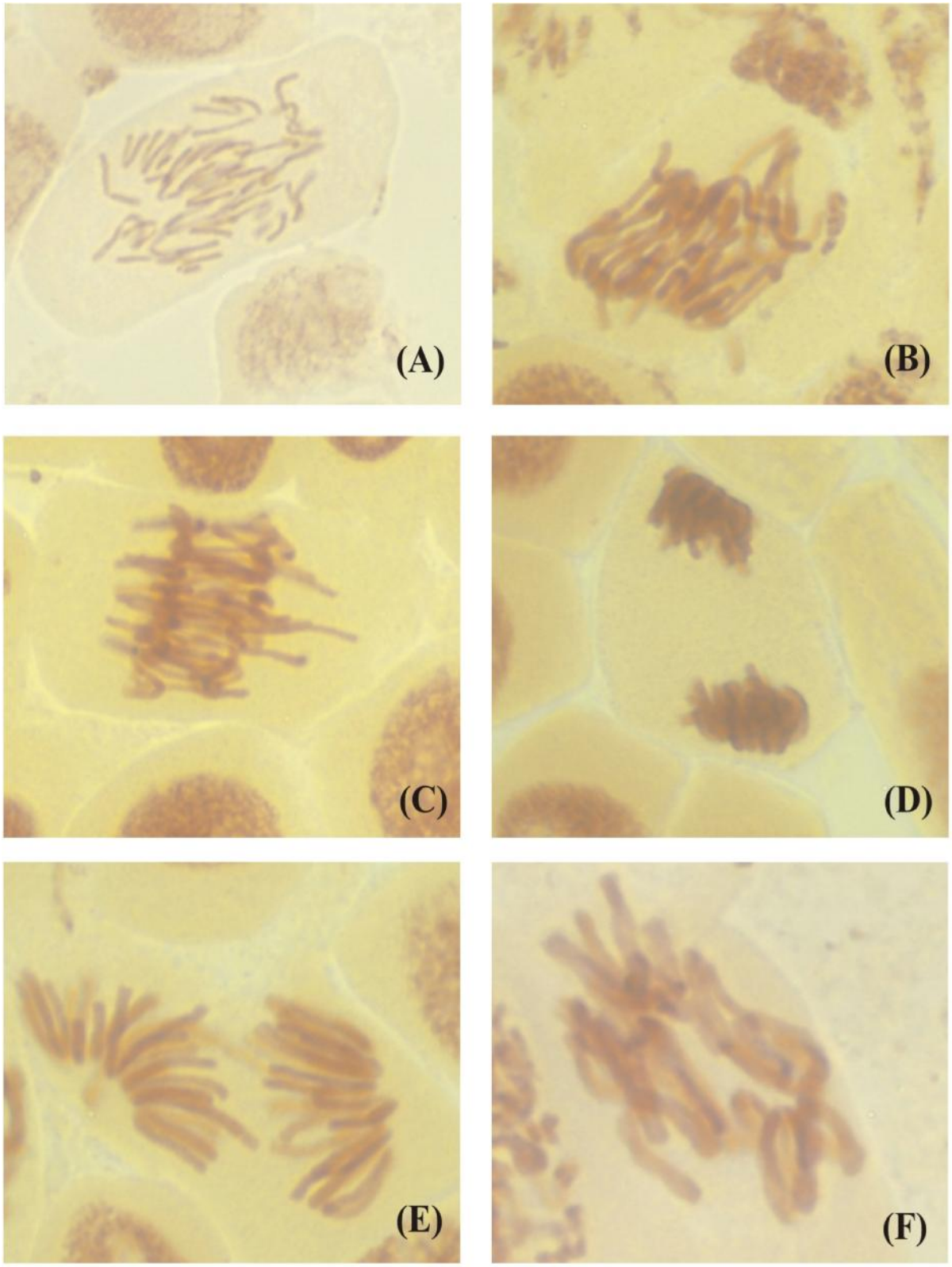

Fig. 2: Root tip cells of *Allium sativum* showing physiological aberrations: C-mitosis (A); Delayed anaphase (B and C); Stickiness at anaphase (D); Abnormal anaphase (E); Abnormal metaphase (F).

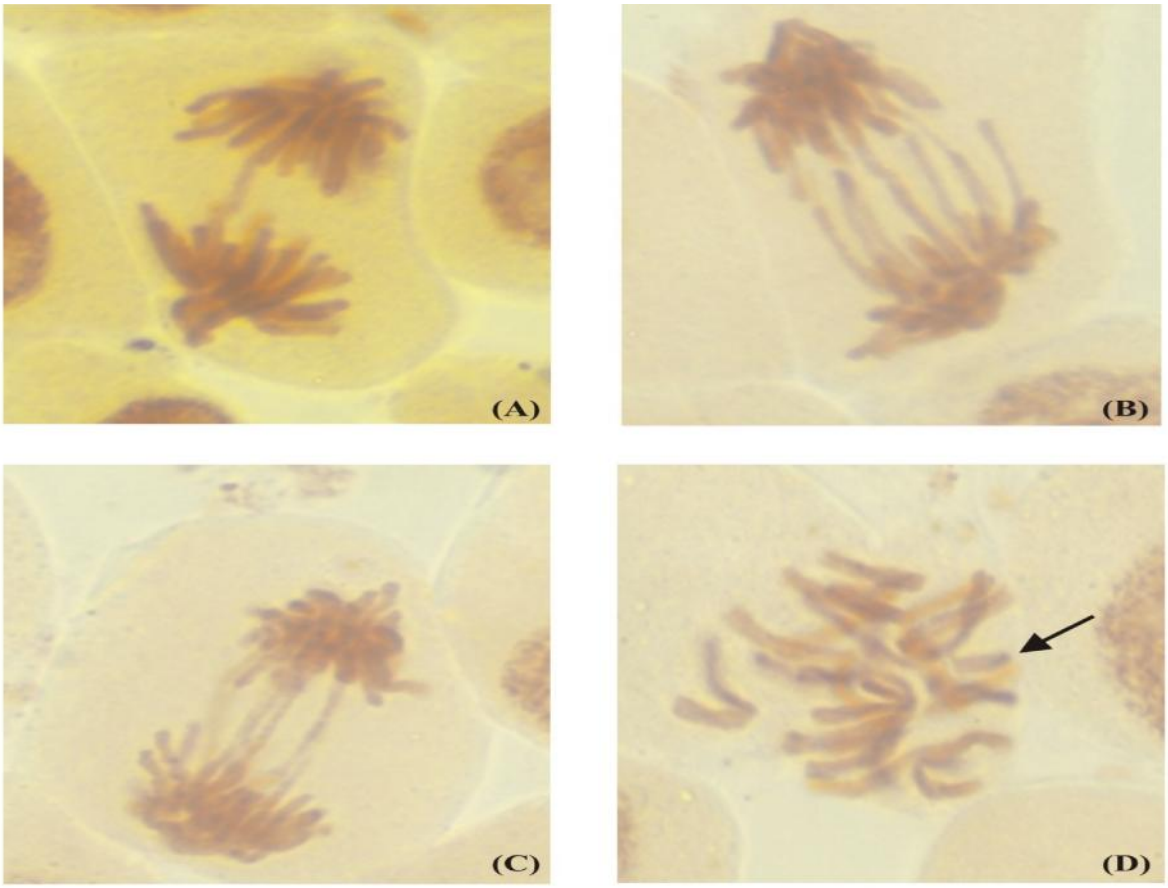

Fig. 3. Root tip cells of *Allium sativum* showing clastogenic chromosomal aberrations: Single chromatin bridge (A), Double chromatin bridges (B), Multiple chromatin bridges (C) and Chromatin fragment (D).

Fig. 3. Root tip cells of *Allium sativum* showing clastogenic aberrations: Single chromatin bridge (A), Double chromatin bridges (B), Multiple chromatin bridges (C) and Chromatin fragment (D).

concentrations of Avenoxan (herbicide) for different time intervals. Liu *et al.* (2009) reported the occurrence of c-mitosis when root tip cells were treated with copper sulphate. A high frequency of delayed anaphase configurations in which the two anaphasic groups of chromosomes lie close to each other near equatorial plate was observed in all the concentrations of lead used in the present study. The frequency of delayed anaphases was observed to be more than that of c-mitosis at 0.4 ppm of lead and was highest (9.15 %) at 0.8 ppm of lead. Such delayed anaphase like configurations have also been reported in *Allium cepa* by Fiskesjo (1979, 1981) following treatment with selenium and MNNG respectively and by Nagpal and Grover (1994) following treatment with systemic pesticides, carbaryl and thimet. Stickiness is one of the physiological aberrations which may be the result of agglutination and association of chromosomes caused by heterochromatin (Reiger and Michaelis, 1972). This may also arise from improper folding of the chromosomal fibers which have resulted in intermingling of fibers and chromosomes become attach to each other by means of sub-chromatid bridges (McGill *et al.*, 1974; Klasterska *et al.*, 1976). In the present study, stickiness has been found in all the concentrations of lead. The highest frequency of stickiness (5.49) was observed at the highest concentration of lead. In consistent with present report, stickiness has also been observed in *Allium sativum* following treatment with herbicide avenoxan

(Gul *et al.,* 2006); copper sulfate (Liu *et al.,* 2009); insecticide carbofuran (Saxena, 2010). Lagging chromosomes/chromosome fragments (also known as laggards) are a type of physiological aberration which arises because of failure of whole chromosome or acentric fragment of a chromosome to get attached to the spindle fiber. In the present study, few cells with lagging chromosomes were observed with lead acetate treatment corresponding to 0.8 and 1 ppm of lead. Lagging chromosomes have been observed in root tips cells of different plant species following treatment with several chemicals including pesticides like rogor in *Vicia faba* (Amer and Farah, 1974); thimet in *H. vulgare* (Singh *et al.,* 1977). Lagging chromosomes in root tip cells of *Allium cepa* were reported by (Gul *et al.,* 2006; Saxena, 2010). Cells having vagrant chromosomes were also observed in root tip cells when treated with all the concentrations of lead acetate used in the present study. The frequency of vagrant chromosomes was minimum (0.21) at 0.2 ppm while it was maximum (1.09) at 1 ppm. Fiskesjo (1979) attributed the occurrence of vagrant chromosomes to week c-mitotic effect of a chemical. Chromosomal breaks and chromatin bridges observed in the present study constituted spectrum of clastogenic aberrations. Chromosomal breaks were observed in low frequency as compared to chromatin bridges and their frequency is maximum (0.87) at 0.8 ppm. A chromosomal break is considered to involve DNA molecule responsible for the linear continuity of the chromosome and may be due to unfinished or misrepair of DNA (Evans, 1977). The formation of chromatin bridges may be the result of unequal exchanges resulting in the formation of dicentric chromosomes which are pulled equally to both poles at anaphase. Chromatin bridges were observed following treatment with all the concentrations of lead acetate. The maximum frequency of chromatin bridges was 2.19 % at 1 ppm concentration. A number of workers have reported the induction of chromatin bridges in different plant species following treatment with different pesticides (Mohandas and Grant, 1972; Mishra and Sinha, 1979). The presence of chromatin bridges in *Allium sativum* root tip cells were also reported (Saxena *et al.,* 2010; Liu *et al.,* 2009).

References

Amer SM, Farah OR (1974). Cytological effects of pesticides. VI. Effect of insecticide rogor on the mitosis of *Vicia faba* and *Gossypium barbadense*. *Cytologia*. 39: 507-514.

Chauhan LKS, Saxena PN, Gupta SK (2001). Evaluation of cytogenetic effects of isoproturon on the root meristem cells of *Allium sativum. Biomedical and Environmental Sciences.* 14: 214–219.

De Luna JT, Hallam TG (1987). Effects of toxicants on populations: a qualitative approach IV. Resource - Consumer - Toxicant models. *Ecological Modelling.* 35: 249-273.

Dubey B, Upadhyay RK, Hussain J (2003). Effects of Industrialization and Pollution on Resource Biomass: A Mathematical Model. *Ecological Modelling.* 167: 83–95.

Evans HJ (1977). Molecular mechanism in the induction of chromosome aberrations. In: Scott, D., Bridges, B. A. and Sobels, F. H. (eds.). *Progress in Genetic Toxicology*. Elsevier/North- Holland and Biomedical Press. Amsterdam. pp. 57.

Fiskesjo G (1979). Mercury and selenium in a modified *Allium* test. *Hereditas*. 91: 169-178.

Fiskesjo G (1981). Benzo (a) pyrene and N-methyl-N'-nitro-N-nitrosoguanidine in the *Allium cepa* test. *Hereditas*. 95: 155-162

Gul T, Kaymak F, Fulya D, Muranli G (2006). Genotoxic Effects of Avenoxan on *Allium cepa* L. and *Allium sativum* L. *Caryologia.* 59: 241-247.

Klasterska I, Natrajan AT, Ramel C (1976). An interception of the origin of sub-chromatid aberrations and chromosome stickiness as acategory of chromatid aberrations. *Hereditas*. 83:153-162.

Levan A (1938). The effect of colchicne on root mitosis in *Allium. Hereditas*. 24: 471-486.

Liu D, Jiang W, Meng Q, Zou J, Gu J, Zeng M (2009). Cytogenetical and ultrastructural effects of copper on root meristem cells of *Allium sativum* L. *Biocell.* 33: 25-32.

Matsumoto ST, Mantovani MS, Malaguttii MIA, Dias AL, Fonseca IC, Marin-Morales MA (2006). Genotoxicity and mutagenicity of water contaminated with tannery effluents, as evaluated by the micronucleus test and comet assay using the fish *Oreochromis niloticus* and chromosome aberrations in onion root-tips. *Genetics and Molecular Biology*. 29: 148-158.

Matsumoto ST, Marin-Morales MA (2004). Mutagenic potencial of the water of a river that receives tannery effluent using the *Allium cepa* test system. *Cytologia.* 69: 399-408.

McGill M, Pathak, S, Hsu TC (1974). Effects of ethidium bromide on mitosis and chromosomes: A possible material basis for chromosome stickiness. *Chromosoma*. 47: 157-167.

Mishra GM, Sinha SP (1979). Effect of malathion on mitotically dividing onion (*Allium cepa*) root tip cells. *Indian Journal of Experimental Biology*. 17: 716-717.

Mohandas T, Grant WF (1972). Cytogenic effects of 2,4-D and aminotriazole in relation to nuclear volume and DNA content in some higher plants. *Canadian Journal of Genetics and Cytology*. 14: 773-783.

Nagpal AK, Grover IS (1994). Genotoxic evaluation of systemic pesticides in *Allium cepa*. L. Mitotic effects. *The Nucleus*. 37: 99-105.

Odjegba VJ, Adeboye RO (2013). Oxidative stress and DNA damage to root cells of *Allium cepa* L. by Copper and lead induction. *Indian Journal of Innovations and Developments.* 2: 828-834.

Oladele EO, Odeigah PGC, Taiwo, IA (2013). The genotoxic effect of lead and zinc on bambara groundnut (*Vigna subterranean*). *African Journal of Environmental Science and Technology*. 7: 9-13.

Qian Y, Tiffany-Castiglioni E (2003). Lead-induced endoplasmic reticulum (ER) stress responses in the nervous system. *Neurochemical Research*. 28: 153–162.

Reiger R, Michaelis A, (1972). Radiomimetic effects of herbicides on the progress of mitosis in pea (*Pisum sativum*) in comparison with those of chemo-mutagens and gamma rays. *Genetic Polanica*. 60: 301-311.

Saini S and Nagpal A (2011). Lead toxicity and genotoxicity- An overview. *Bioscience Guardian.* 1: 561-570.

Saxena PN, Chauhan LKS, Chandra S, Gupta SK (2004). Genotoxic effects of diuron contaminated soil on the root meristem cells of *Allium sativum*: a possible mechanism of chromosome damage. *Toxicology Mechanisms and Methods.* 14: 281–286.

Saxena PN, Gupta SK, Murthy RC (2010). Carbofuran induced cytogenetic effects in root meristem cells of *Allium cepa* and *Allium sativum*: A spectroscopic approach for chromosome damage. *Pesticide Biochemistry and Physiology.* 96: 93–100.

Shukla JB, Dubey B (1997). Modelling the Depletion and Conservation of Forestry Resources: Effects of Population and Pollution. *Journal* of *Mathematical Biology*. 36: 71–94.

Singh BD, Singh Y, Singh RB, Singh RM, Sarma NDRK, Singh J (1977). Cytogenetic aberrations and morphological changes induced by insecticide treatments of barley seeds. *Indian Journal of Experimental Biology*. 15: 688-691.

Tchounwou PB, Centeno JA, Patlolla AK (2004). Arsenic toxicity, mutagenesis and carcinogenesis. A health risk assessment and management approach. *Molecular and Cellular Biochemistry*. 255: 47–55.