Meiotic Abnormalities and Tetraploidy (by Lead Nitrate) Induced by Heavy Metals (Pb, Cu, Hg, Zn) on *Helianthus annuus* L.

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Summary Cytological effects of some (Pb, Cu, Zn, Hg) heavy metals were tested on meiotic studies of *Helianthus annuus*. The used heavy metals did not show much variations in the type of induced abnormal PMCs. The abundant meiotic irregularities stickiness, lagging, bridges, unequal separation, fragmentation *etc.* were observed. Mercury was the most toxic of all used heavy metals since it produced highest percentage of abnormal PMCs. Lead nitrate is a mutagen which induce tetraploidy.

Key words Heavy metals, Meiotic abnormalities, *Helianthus annuus*, Tetraploidy.

Heavy metals Pb, Cu, Zn, Hg and their salts may exert toxic effects on living tissues at different levels-cellular, sub-cellular and/or molecular. The impact of toxic agents on plants and animals is currently an area of intense interest due to ubiquity of exposure. Most of them involve some form of genetic toxicity. Chromosomal alterations caused different degree of damages. Change in mitotic index or chromosomes or spindle behavior indicates, most metals when administered to higher organism are clastogenic in nature at certain doses and duration of treatment. Heavy metals in higher amount even nutritionally essential metals are toxic to plants, disturb biological equilibrium in the soil which in turn unfavourably influences soil fertility, plant development and yield (Vallee and Ulmer 1972). Mercury, a non-degradable toxic heavy metal, is most harmful both in organic and inorganic compounds. Lead is a non-essential toxic element.

The effects of heavy metals on plant cells have been worked out in *Vicia faba* Amoore (1961), *Pisum sativum* Khilman (1975), *Oryza sativa* Mukerjee and Moitre (1976), Singh and Sharma (1980) have also shown the effects of certain metals on cell division and chromosome structure of plants and animals.

Among all these heavy metals, lead nitrate was found to be a mutagenic agent and it induced tetraploidy in sunflower. Lead is a pollutant and suspect carcinogen. Various organic and inorganic salts of this metal have been subjected to a variety of mutagenicity tests by O'Riordan and Evans (1974), Nishioka (1975), Singh and Sharma (1980, 1982) *etc.* Lead has been found to inhibit spindle formation and showed c-mitotic effects. Some have reported the clastogenicity in lead treated plants but results were controversial. Clastogenic, mitotoxic and cytotoxic effects of metallic salts Hg^{2+} , Pb^{2+} and Cd^{2+} were studied on root meristem of *Allium cepa* (Sathaiah and Reddy 1984). They have reported that the maximum degree of mutagenicity is caused by Lead.

The effect of heavy metals on different plants have been experimented by different workers but to the best of my knowledge, these effects have so far not been studied on *H. annuus*.

Materials and methods

Fresh and healthy seeds of *H. annuus* cv. Morden were procured from Directorate of Oil Seed

Research, Rajendra Nagar, Hyderabad. Seeds were irrigated with water containing different heavy metals Zinc chloride, Mercury chloride, Copper chloride, Lead nitrate. The impact of these chemicals on the meiotic cell division of plants were studied.

For cytological investigations, young flower buds were fixed in freshly prepared Carnoy's fixative. Squash preparations of anthers were made in 2% aceto-carmin. Best stages of division were photographed.

Results

The cytological effects of pollutants on somatic cells have been estimated on the basis of induced abnormalities. The pollutants inhibited cell division as shown in tables. Various types of abnormalities have been observed at metaphase, anaphase and telophase stages. These abnormalities were due to the effects of pollutants in spindle apparatus. All studied heavy metals induced same types of meiotic irregularities.

The examination of 430, 519, 317 and 413 pollen mother cells (PMCs) showed that majority of PMCs were normal but 20.9%, 19.5%, 20.5% and 22.5% of them were abnormal (Table 1). Studies of different stages of meiotic division show that almost all the stages of division were affected to different degrees. In diakinesis (Fig. 1), different configurations of bivalents were observed, maximum in copper chloride (26%) and minimum in lead nitrate (10%) (Table 3). Stickiness was a common and temporary effect since the percentage of stickiness decreased gradually from the early stages of the 1st division to the late stages of 2nd division except in the case of zinc chloride. Stickiness was found to increase up-to 80.6% in anaphase II in zinc chloride (Table 2). In metaphase I (Fig. 2) stickiness varied from multivalent formation to clotting of all the bivalents in one mass. In diakinesis chromosomes were arranged in one mass. Quadrivalents and multivalents were also observed. Quadrivalents were commonly observed in lead nitrate treated plants. In telophase I stickiness was observed in the form of tripolar condition (Fig. 7).

Another characteristic phenomenon was the occurrence of lagging chromosomes (Fig. 5) in various percentage during anaphase I in all used chemicals. The presence of single lagging bivalent in metaphase I was common occurrence. Lagging of single univalent was a common phenomenon in anaphase I. In some cells more than one lagging chromosomes (Fig. 4) were observed. The number of lagging chromosomes was more in metaphase II. Lagging chromosomes might be responsible for the formation of micronuclei.

Chromatin bridges were also observed in some cells (Fig. 8). The percentage of formation of bridges were maximum in lead nitrate 19.3% and minimum in copper chloride 9.2%. In case of copper and zinc chloride the percentage of bridges in anaphase II did not exceed than those of anaphase I but in the case of lead nitrate and mercury chloride it was just reverse. Sometimes, 1 or even 3 bridges and bridges along with lagging chromosomes were formed.

Unequal separation of chromosomes was observed at anaphase (Fig. 6). Sometimes unequal

Table 1. Number and percentage of the abnormalities after treatment of 4 heavy metals

Heavy metals	No. of observed PMCs	No. of abnormal PMCs	%	Percentage of different types of abnormalities				
				Stickiness	Laggards	Bridges	Fragmentations	Unequal separation
Control	250	—	—	—	—	—	—	—
Lead nitrate	430	90	20.9	20.5	32.9	19.3	12.1	15.2
Copper chloride	519	101	19.5	25.6	18.3	9.2	30.6	16.3
Zinc chloride	317	65	20.5	13.6	16.6	15.9	26.3	27.6
Mercury (II) chloride	413	93	22.5	31.5	30.6	16.2	17.1	4.6

Figs. 1–10. 1) Disturbed polarity at diakinesis $\times 940$. 2) Stickiness of chromosomes in 2 groups at metaphase I $\times 840$. 3) Stickiness of chromosomes at anaphase I $\times 1300$. 4) Lagging of chromosomes at anaphase I $\times 1120$. 5) Lagging of chromosomes and bridge at anaphase I $\times 850$. 6) Unequal separation of chromosomes at anaphase I $\times 1060$. 7) Tripolar condition at telophase I $\times 840$. 8) Bridge at telophase II $\times 900$. 9) Control (diploid) showing $2n=34$ chromosomes $\times 1200$. 10) Tetraploid of lead nitrate treated plants showing $2n=68$ chromosomes $\times 860$.

number of chromosomes moved to more than 2 poles leading to multipolar condition.

Chromosome fragments were also observed. Fragments were maximum in copper chloride and minimum in lead nitrate (Table 2). 1 or 2 fragments were observed per cell. Heavy fragmentation was observed in lead nitrate treated plants.

Another phenomenon which was rarely observed, the appearance of univalents in diakinesis in

Table 2. Percentage of different types of abnormalities caused by heavy metals

Heavy metals	Metaphase I			Anaphase I			Telophase I			Metaphase II			Anaphase II			Telophase II								
	Stick.	Lag.	Frag.	Stick.	Lag.	Bridge Frag.	Uneq. sep.	Stick.	Lag.	Frag.	Uneq. sep.	Stick.	Lag.	Bridge Frag.	Stick.	Lag.	Bridge Frag.	Stick.	Lag.	Bridge Frag.				
Control	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—				
Lead nitrate	36.4	53.3	10.3	21.4	47.0	20.4	1.1	10.1	56.6	1.9	39.4	2.1	22.2	71.2	6.6	—	6.7	20.2	71.9	1.2	7.5	—	1.5	1.0
Copper chloride	59.3	30.7	10.0	10.0	19.0	60.0	5.0	6.0	5.6	—	4.4	—	18.0	60.6	13.8	7.6	6.6	64.0	26.6	2.8	8.0	—	1.0	5.5
Zinc chloride	62.4	37.6	—	6.6	20.3	55.5	—	17.6	—	—	—	—	34.2	65.8	—	—	80.6	11.1	8.3	—	8.2	—	1.8	—
Mercury (II) chloride	26.8	73.2	—	20.0	55.0	20.0	—	5.0	71.8	—	28.2	—	26.8	65.8	7.4	—	—	28.5	57.1	14.4	5.0	—	1.0	1.3

Stick. =Stickiness, Lag. =Laggards, Frag. =Fragmentation, Uneq. sep. =Unequal separation.

Table 3. Number and percentage of the abnormalities after treatment

Heavy metals	Diakinesis			Metaphase I			Anaphase I			Telophase I			Metaphase II			Anaphase II			Telophase II			
	No. of counted PMCs	No. of PMCs	ab. %	No. of PMCs	ab. %	No. of PMCs	No. of PMCs	ab. %	No. of PMCs	No. of PMCs	ab. %	No. of PMCs	No. of PMCs	ab. %	No. of PMCs	No. of PMCs	ab. %	No. of PMCs	No. of ab. %	No. of PMCs		
Control	250	30	—	—	—	—	40	—	—	25	—	—	55	—	—	20	—	—	10	—	—	
Lead nitrate	430	40	4	10	100	25	25	80	20	25	50	6	12	80	20	25	60	13	22	20	2	10
Copper chloride	519	19	5	26	101	19	19	140	38	27	55	5	9	90	20	22	59	8	14	55	6	11
Zinc chloride	317	20	3	15	50	11	22	60	16	27	20	3	15	75	18	24	72	12	17	20	2	10
Mercury(II) chloride	413	22	5	23	59	17	29	62	21	34	29	4	14	110	23	21	90	20	22	41	3	7

ab. = Abnormal, PMCs = Pollen Mother Cells.

Table 4. Different chromosomal associations in diploid and tetraploid plants

	Chromosomal status	No. of cells analysed	Chromosomal associations			
			I	II	III	IV
Control ($2n=34$)	2x	100	—	17.0	—	—
Tetraploid ($2n=68$)	4x	100	3.2	29.4	—	1.4

the plants treated with copper chloride.

The percentage of abnormalities in metaphase I, II and anaphase I, II were higher than telophase I and II (Table 3).

The effect of different chemicals was almost the same but there was considerable variations in the percentage of the induced abnormal PMCs. The presented results showed that mercury chloride was the most toxic to the plants. Lead nitrate stood next to mercury chloride in toxicity followed by copper chloride and zinc chloride which were least toxic. Apart from these abnormalities studies of cell division show that some of lead nitrate treated plants were tetraploid.

Tetraploidy induced by Lead nitrate. A number of diploid and treated plants were analysed. The diploids revealed 17 bivalents and normal tetrads at diakinesis and metaphase I (Fig. 9). Cytological studies of treated plants revealed that some plants were tetraploids. This was confirmed by the number of bivalents and chromosome number $2x=34$ (Fig. 10). In the diploids there were normal separation of 17 chromosomes to each pole at anaphase I but in tetraploids meiotic division was disturbed. Table 4 depicts different chromosomal configurations per cell seen at diakinesis and metaphase I. The variable number of univalents, bivalents and multivalents were formed.

Discussion

From these results, it has become clear that the metallic salts used in the present investigation are potentially capable of inducing various types of cell and chromosomal abnormalities, spindle disturbances and chromosomal irregularities. The frequency of cell division was also effected. Various types of meiotic abnormalities might have arisen by the genetic and physiological disturbances.

Stickiness might be due to disturbances in cytochemical balance reaction (Rao and Lakshmi 1980). Multivalent associations have been attributed to pairing due to translocations and inversions (Shaikh and Godward 1972). Lagging of chromosomes may be due to the hindrance of movement of the bivalents to the equatorial plate at metaphase I. Lagging of single univalent was the most common phenomenon. More than 1 lagging chromosomes in meiosis might be due to hindrance of the prometaphase movement of chromosomes accompanied by adhesion of the centromeric to the adjacent inner surface of plasma (Barthelmess 1957). Univalents might have originated due to early disjunction in some of the partially homologous chromosomes, as a consequence of which they were unable to form normal bivalents.

Occurrence of multivalent association is a common feature in treated plants arising mainly due to the presence of more than 2 homologous chromosomes. Number of bridges was formed. The origin of bridges was primarily due to fusion between broken chromosomes (Coldecott and Smith 1952). Fragments were a common phenomenon. It was inferred that many of the breaks were in the distal region of the chromosomes and others extremely close to the ends of the arms (Walters 1950).

Disturbances in metaphase II and anaphase II might be due to the disturbance of the spindle apparatus so the chromosome spread irregularly in the cell. Unequal separation of chromosomes at anaphase I might be responsible for the presence of unequal chromosome number in the second equatorial plates of metaphase II.

Mercury chloride caused significant increase in frequency of aberrations. The clastogenicity of mercury compounds has been ascribed to the formation of chelate complex of mercury with DNA (Poojary and Manohar 1984). Lead nitrate inhibited the spindle formation and c-mitotic effects resulting in arrested metaphase with scattered and clarified chromosomes. Conceivably lead might act on spindle fibres and bring about meiotic disturbances culminating in c-mitotic effects. Further it is interesting that lead caused mitodepression despite metaphase accumulation. Lead nitrate seemed to be most potent in inducing waxy locus mutants. The mutation rates produced by the metal depicted an appropriate linear dose-response relationship.

The results of the present study clearly indicate the ability of the chemicals to induce cytological abnormalities in cell division.

During the present investigation both chromosomal aberrations and spindle abnormalities were observed. In the present case dissolved chemicals in water might be responsible for producing abnormalities in cell division.

It was apparent from the present observations that both lead nitrate and mercury chloride were toxic for the chromosomes of *Helianthus annuus*. Between the two mercury chloride was more toxic than lead nitrate. Among the four lead nitrate was found to be mutagenic agent and it induced tetraploidy in sunflower.

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References

- Amoore, J. E. 1961a. Arrest of mitosis in root. *Proc. Roy. Soc. (London)* **154**: 95–108.
- Barthelmess, A. 1957. Chemisch induzierte multipolare Mitosen. *Protoplasma* **48**: 546–561.
- Coldecott, R. S. and Smith, L. 1952. A study of X ray induced chromosomal aberrations in barley. *Cytologia* **17**: 224–242.
- Khilman, B. A. 1975. Root tips of *Vicia faba* for the study of the induction of chromosomal aberration. *Mutat. Res.* **31**: 401–412.
- Mukerjee, S. and Moitra, P. 1976. Toxic effects of lead on growth and metabolism of germinating rice (*Oryza sativa* L.) seeds and on mitosis of onion (*A. cepa* L.) root tip cells. *Ind. J. Exp. Biol.* **14**: 519–521.
- Nishioka, H. 1975. Mutagenic activities of metal compounds in bacteria. *Mutat. Res.* **31**: 185–189.
- O'Riordan, M. L. and Evans, H. J. 1974. Absence of significant chromosome damage in persons occupationally exposed to lead. *Nature* **247**: 50–53.
- Poojari, M. D. and Manohar, H. 1984. Synthesis and X-ray structure of a Hg(II) complex with adenine N(1)-oxide. A model for mercury binding to DNA. *Inorg. Chim. Acta.* **93**: 153–160.
- Rao, N. B. and Lakshmi, N. 1980. Gamma ray induced meiotic abnormalities in *Capsicum annum* L. *Caryologia* **33**: 509–518.
- Sathaiah, V., Prasanna, A. and Tummala, R. P. 1984. Clastogenic and Mutagenic Activity of Certain Heavy Metals in *Allium cepa* and *Hordium vulgare*. In: Manna, G. K. and Sinha, U. (eds.). *Perspective in Cytology and Genetics* **4**: 265–272.
- Shaikh, M. A. Q. and Godward, M. B. E. 1972. The meiotic consequences of radiation induced chromosome breaks in *Lathyrus sativus* and *Vicia ervilia*. *Cytologia* **37**: 497–505.
- Singh, O. P. and Sharma, A. 1980. Effects of certain metallic pollutants on plant genetic system. *The Nucleus* **23**: 15–29.
- and — 1982. Effects of Mercury, Cadmium and Lead on Plant Chromosomes. *Today and Tomorrow Printers, Prof. S. M. Sircar Memorial Volume*. New Delhi. pp. 305–312.
- Vallee, B. L. and Ulmer, D. D. 1972. Biochemical effect of Hg, Cd, Pb. *Ann. Rev. Biochem.* **41**: 91–128.
- Walters, M. S. 1950. Spontaneous breakage and reunion of meiotic chromosome pairs in the hybrid *Bromus tritici* × *B. maritimus*. *Genetics* **35**: 11–37.