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Cytological Abnormalities Induced by Mercury Water Pollutants on *Allium cepa* L.

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Mercury, a non-essential but highly toxic element for living organism, even at low concentration presents potential hazards due to enrichment in the food chain mainly through aquatic systems. Environmental concentration of mercury was reported to vary in different localities. Approximately 3800 water samples collected from more than 600 sites were analysed and found to be generally below the environmental quality standard of 0.5 ug mercury per litre (Forstner and Wittmann 1981). Mercury contained in the water bodies in and around Burdwan was between 6 ±0.8 ng ml^{-1} and 42 ±1.0 ng ml^{-1} (industrial effluents). Direct or food-mediated exposure to mercury compound was reported to be responsible for human chromosome breakage (Skerfving et el. 1970), chromosomal aberration (Popescu et al. 1979) and C-mitosis at higher concentrations (10 x 10^{-6} to 100 x 10^{-6}M) (Fiskesjo 1970) in blood cells. Such studies in plants were mainly confined to Allium root tip treated with 0.1% and 0.5% $HgCl_2$ (Nandi 1985) and Elodea densa with 2.5 x 10^{-7}M and 7.5 x 10^{-10}M, 7.5 x 10^{-9}M, 7.5 x 10^{-8}M methyl mercuric chloride (Czuba 1982). In Clarias batrachus, the effective concentration was 0.56 mg l^{-1} (Choudhuri and Choudhuri 1986).

In an earlier screening experiment different concentrations of $HgCl_2$, Hg_2Cl_2 and HgO have been used. The concentrations for genotoxicity seemed to lie in between 1 x 10^{-6}M and 1 x 10^{-2}M. Of these $HgCl_2$ and Hg_2Cl_2 which are used as fungicides (Veres and Hasty 1976) and HgO which comes from atmospheric fall out from power plants (Maxfield et al. 1974) and other industries, ultimately find their way as pollutant to the aquatic system.

Cytotoxic and genotoxic studies of these compounds on Allium root tip meristems have been worked out in detail to record genetic hazards (Ramel 1969).

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MATERIALS AND METHODS

A range of concentrations of $HgCl_2$, Hg_2Cl_2 and HgO were selected for studying cytological effects on the primary root meristem of Allium cepa L.(2n=16).

Aqueous solutions of 10^{-2}M, 10^{-3}M, 10^{-4}M, 10^{-5}M, 10^{-6}M of the compounds were prepared and pH of the solutions were adjusted to 5.4. The treatment was carried out for 30 minutes, 1,2,3,6 and 24 hours and subsequent recovery were done for 24, 48 and 72 hours in each case in darkness. Three parallel sets along with controls were maintained at 20 ±2°C. After treatment one set of roots were pretreated in bromonaphthalene for 2 hours at 25°C and fixed overnight to acetic ethanol(1:3). Another set was directly fixed overnight to acetic ethanol (1 : 3). Squash preparation were made following Feulgen schedule.

Approximately 1000 cells from at least 6-meristems from 3-replicates were screened for mitotic indices. Three hundred metaphases and 300 anaphases from 3-replicates were analysed for determining the frequency of aberrant cells. The mean percentage of aberrant cells ± S.D. values were represented in the bar graph (Fig. 1) and the aberrations have been documented with photomicrographs (Fig. 2-4). Scoring protocols of Hsu et al. (1981) and Brusick (1980) were followed. All types of chromosomal aberrations involving definite breaks were recorded and converted to a number of lesions per cells. Chromosomal breaks (breaks in the single chromatid and breaks in identical places in sister chromatids) and acentric fragments (chromosomes with no apparent centromere) were scored as single, unrepaired lesions. Chromosome rearrangements, such as exchanges and ring chromosomes were scored from at least two lesions. Since chromatid gap is the type of chromatid lesion which is very similar to a chromatid break but having the attenuated segment shorter than the breaks, it has been considered as single break. The 'baseline value' method (Au and Hsu 1979) was used as a mean of statistically evaluating the data. This value was established as two times the mean control value (i.e. any value more than two times of that observed as the mean of the control group was considered significantly different). Baseline values were used to compare mean number of lesions per cell, percentage of cells with aberrations and types of aberrant cells. Statistical analysis were made following LSD (least significant difference).

RESULTS AND DISCUSSION

After 30 minutes treatment, $HgCl_2$, Hg_2Cl_2 and HgO showed

Table 1. Various aberrations following treatment with heavy metals in Allium cepa L.

Concentration	Single lesion									Double lesions					
	Acentric fragments			Chromatid breaks			Chromatid gaps			Ring Chromosomes			Exchanges		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Control	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
10^{-6}a	6	6	7	--	--	--	--	--	--	--	--	--	--	--	--
10^{-5}a	7	7	9	--	1	2	--	--	--	--	--	--	--	--	--
10^{-4}a	7	8	9	1	2	3	--	--	--	--	--	--	--	--	--
10^{-3}a	10	12	12	2	3	4	1	1	1	1	1	1	1	1	1
10^{-2}a	15	16	16	3	4	5	2	2	1	1	1	2	1	1	1
LSD at 5% level	0.25	0.96	0.92	0.75	0.98	0.68	0.36	0.28	0.62	0.01	0.03	0.54	0.03	0.07	0.42
10^{-6}b	11	11	11	--	1	2	--	--	--	--	--	--	--	--	--
10^{-5}b	11	12	12	1	2	3	1	1	1	1	1	1	1	1	1
10^{-4}b	17	19	23	2	3	5	2	2	1	1	1	2	1	1	1
10^{-3}b	25	25	26	4	5	6	2	3	2	1	2	3	2	3	2
10^{-2}b	26	26	27	6	7	8	2	3	3	2	3	3	3	4	4
LSD at 5% level	1.62	0.86	0.22	0.66	0.82	0.62	0.02	0.66	0.56	0.06	0.01	0.27	0.04	0.03	0.39

a=30 minutes treatment and 24 hours recovery ; b=6 hours treatment and 24 hours recovery. 1=$HgCl_2$; 2=Hg_2Cl_2 and 3=HgO

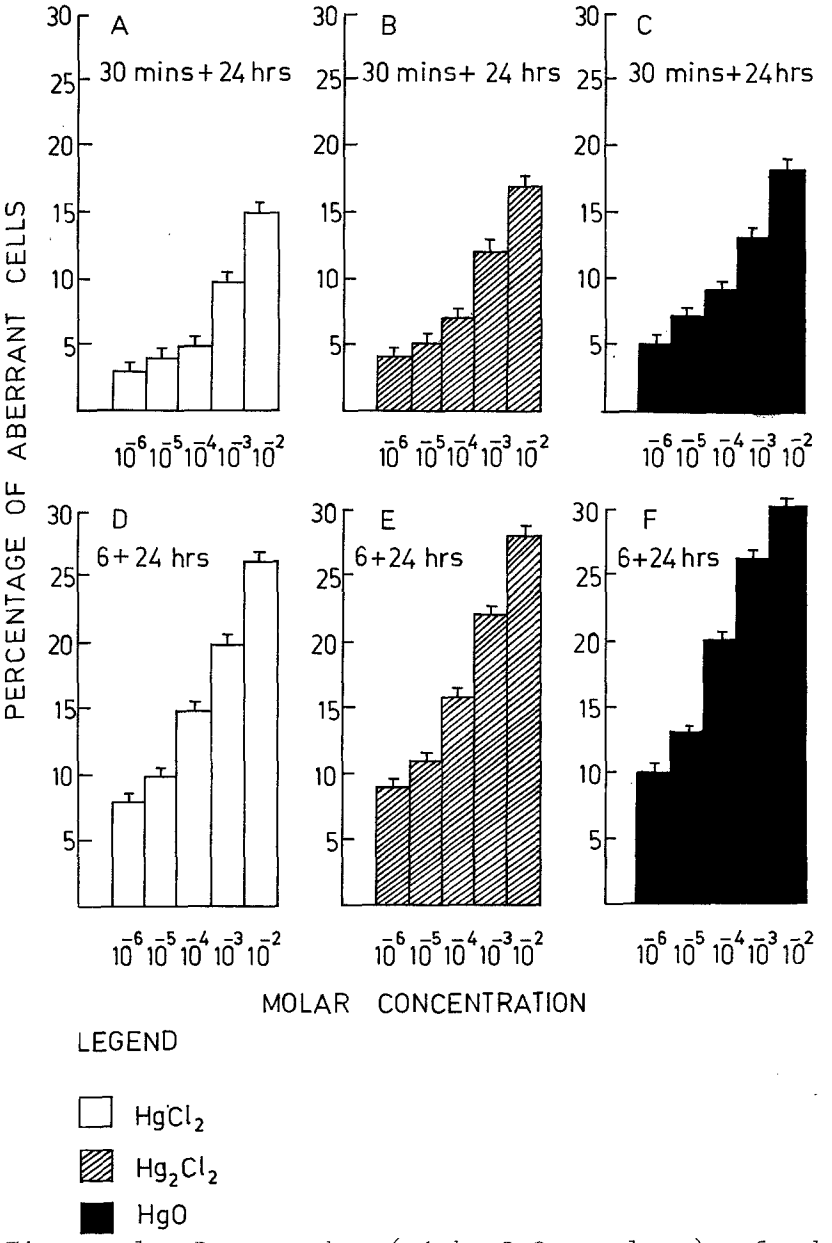

Figure 1. Bar-graphs (with S.D. values) of aberrant cells in different concentrations and in different time-periods of $HgCl_2$ (A & D), Hg_2Cl_2 (B & E) and HgO (C & F).

much lower mitotic index than control which indicated that the chemicals were mitodepressant. When the treatment time was increased together with the decrease of concentration, the mitotic index increased inversely. Among the different treatment times, the toxicity ranged, as indicated by aberrant cells, between 30

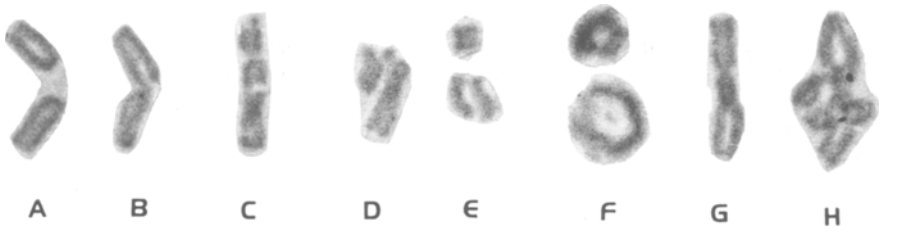

Figure 2

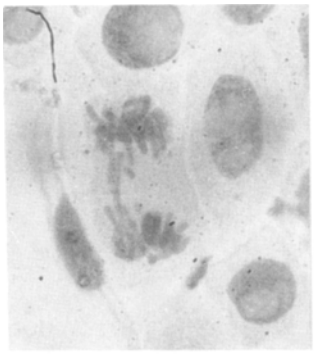

Figure 3

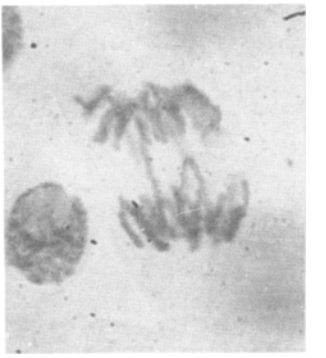

Figure 4

Figure 2. A-H Different types of chromosomal abnormalities observed in metaphase-spread.
A-isochromatid gap, B-chromatid gap, C-chromatid break, D-chromosome break, E-acentric fragments, F-ring-chromosome, G-dicentric chromosome, H-exchanges
Figure 3. Anaphase bridge with accentric fragment.
Figure 4. Anaphase bridge with criss-cross arrangement.

minutes and 6 hours. So the data of the lowest (30 minutes) and the highest (6 hours) level of toxicity were only considered and shown in Table 1.

After 30 minutes treatment, 10^{-2}M of $HgCl_2$, Hg_2Cl_2 and HgO showed the mean mitotic index of 10.1, 9.7 and 9.3 respectively. Within similar time period 10^{-6}M of $HgCl_2$, Hg_2Cl_2 and HgO showed the mean mitotic index of 14.6, 13.9, 13.4 respectively whereas the control set showed 17.5. From the above observation it was clear that the mitotic index increased inversely with the increase in concentration. As all the divisional stages of mitosis were observed and cytokinesis was normal, it indicated that these concentrations showed inhibitory but non-lethal effects.

The mean mitotic index decreased after 6 hours treatment in 10^{-2}M $HgCl_2$ (5.2). From this observation it was clear that the increase in treatment time resulted in decrease in mitotic index. Similar observations were

revealed from Hg_2Cl_2 and HgO.

Maximum frequency of 30% aberrant cells was noted in 10^{-2}M HgO (Fig.1). Among the aberrant cells the acentric fragments of variable size and number (Table 1) were noted in all treatments. Occurrence of both single and double lesions (Table 1) in high frequency in presence of the tested chemicals indicated their clastogenicity. Types of aberrations (Fig. 2-4), percentage of aberration (Fig. 1) and lesions per cell were significantly increased according to the baseline rule. The range of mean lesion per cells were from 0.03-0.26 in $HgCl_2$ treated cells, 0.04-0.28 in Hg_2Cl_2 treated cells and 0.05-0.32 in HgO treated cells. Similar observations were made in small mammals, Peromyscus leucopus and Sigmodon hispidus which were used as indicators of environmental mutagenesis to study water polluted with a complex mixture of various agents (McBee et al. 1987).

Treatment with $HgCl_2$ was found to be less lethal than Hg_2Cl_2 and HgO probably because the former contained 0.74 gram of mercury while the later two, in sequence,had 0.85 gram and 0.93 gram per gram of compound. However, the lower concentration showed non-lethality though the level of mercury caused various cytological aberrations (Table 1). Mercuric chloride is known to be more stable and requires presence of sunlight and organic matter for decomposition into Hg_2Cl_2 and metallic mercury (dis-proportionation-reaction) in presence of water (Sharvelle 1961). Possibly the accumulation of Hg becomes higher in Hg_2Cl_2 treated cells. As a result of this Hg accumulation and the conversion of Hg_2Cl_2 to $HgCl_2$ (which is more poisonous (Dutt 1979) than Hg_2Cl_2) the Hg_2Cl_2 treated cells showed more toxic effects than $HgCl_2$ treated cells. All the three tested water-pollutants were clastogens and produced a high rate of chromosome breaks. In a previous study organic and inorganic mercuric fungicide produced fragments when treated for shorter period (Nandi 1985). As the fragments and anaphase bridge indicated dicentrics or exchanges of chromosome at metaphase, the condition might have resulted from unequal or from lateral fusion after breaks at the same locus. All such chromosomal exchange might lead to chromosomal mutations and in extreme cases, such mutations might cause death to the cells. Ring chromosome indicated deletion at G_1 phase similar to those induced by X-ray irradiation (Auerbach 1976). Hence these pollutants seemed to be radiomimetic in nature. Chromatid breaks are known to cause chromosomal mutation because of deletion and duplication. Thus, various cytological abnormalities from various water pollutants produce deleterious effects on living cells.

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