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Monitoring and assessment of mercury pollution in the vicinity of a chloralkali plant. II Plant-availability, tissue-concentration and genotoxicity of mercury from agricultural soil contaminated with solid waste assessed in barley (*Hordeum vulgare* L.)

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Barley (*Hordeum vulgare* L.) was used to assess plant-availability, tissue-concentration and genotoxicity of mercury from the solid waste deposits of a chloralkali plant. Seeds of *H. vulgare*, presoaked in distilled water, were allowed to germinate and grow on agricultural soil mixed with solid waste containing 2550 ± 339 mg Hg kg^{-1} at different proportions (0·75, 1·5, 2·5 and 5%). Plants raised from germinating seeds on uncontaminated agricultural soil served as controls. On day 7, germination counts and seedling heights were recorded. The concentration of mercury in soil, and plant tissue (dry weight) were determined at different stages of plant growth from day 7 till maturity and harvest. The availability of mercury from the soil was assessed by extracting mercury at a range of pH values (2–6) and by chemical methods. The embryonic shoots excised at 36 h of germination were subjected to cytological analysis to determine mitotic index and frequency of mitoses with chromosomal aberrations. The pollen mother cells from anthers of young M_1-spikes were analysed to score meiotic aberrations. Subsequently, the pollen fertility and seed set were determined. Furthermore, M_2-seedlings were analysed for chlorophyll-deficient mutations. The cytogenetic analysis revealed the effects of mercury on the mitotic and meiotic chromosomes which were significantly correlated with soil-mercury. The bioconcentration of mercury in aerial tissues decreased with the age of the plant. Roots, in particular, were found to concentrate most of the mercury taken up by the plant. At the time of harvest, the bioconcentration of mercury in straw was at a minimum. The accumulation of mercury in grain, which was significant, did not increase with the increase in concentration of mercury in soil but maintained a plateau, indicating a restriction of transport of mercury through the phloem. The unique advantage with the use of *Hordeum* assay is that, besides assessing the germline toxicity, the assay takes into account the possible contamination of the agricultural food-chain.

INTRODUCTION

Solid waste disposed on surface land at the chloralkali site at Ganjam, India is the major source of terrestrial mercury in the region (Panda *et al.*, 1989, 1990). Mercury from solid waste deposits may contaminate the terrestrial ecosystem through many pathways (Van Hook, 1978). Irrespective of the ways, soil is the ultimate sink for all heavy metal emissions in the terrestrial environment. Terrestrial plants are important because they link soil with the terrestrial food chains (Martin & Coughtrey, 1981). The accumulation of heavy metals, particularly mercury, through the soil–plant–herbivore–carnivore food chain, allowing an

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intensification of their concentrations and effects, is well documented (Borg *et al.*, 1969; Haney & Lipsey, 1973; Bull *et al.*, 1977; Panel on Mercury, 1978). Chaney (1973) has reviewed the food chain effects of metals applied to land via sewage sludge and effluents and has suggested that cadmium, copper and zinc are a potential hazard to food chains via accumulation. Crop plants maize, sorghum and rape have been shown to be very useful in assessing plant-availability, uptake and phytotoxicity of several metals, such as cadmium, nickel, cobalt, lead and zinc (Singh & Norwal, 1984; Sikora & Wolt, 1986). In these studies considerable emphasis has been laid on crop plants because they represent a direct link for transportation of toxic metals from soil to man (Cataldo *et al.*, 1987).

Barley (*Hordeum vulgare*) is one of the well established genotoxicity assay systems available today (Constantin, 1978; Constantin & Nilan, 1982*a*,*b*). Following exposure of barley seeds to contamination levels, a number of biological endpoints at different stages of growth and development can be studied in this plant, viz: (a) germination, (b) seedling height, (c) mitotic index and mitotic chromosomal aberrations in embryonic shoot or root meristematic cells, (d) meiotic chromosomal aberrations in pollen mother cells (PMCs) of anthers from M_1-spikes and (e) M_2-seedling emergence and chlorophyll-deficient mutations in M_2-seedlings, that cover two generations. The unique advantages with *H. vulgare* as an assay system over other crop plants are that the former can assess genotoxicity based on chromosomal effects in mitoses and meiocytes, and chlorophyll mutations in M_2-seedlings. Furthermore, the genotoxicity can be correlated with plant-availability and tissue-concentration of the metal, having ecotoxicological implications.

In sequel to the short-term study based on *Allium* micronucleus (MNC) assay (Panda *et al.* 1989), an attempt has been made here to assess plant-availability, tissue-concentration and genotoxicity of mercury from agricultural soil contaminated with the solid waste of the chloralkali plant, using *H. vulgare* as the assay system.

MATERIALS AND METHODS

Experimental soil

Soil obtained from local agricultural land near Berhampur University campus was used in the present experiments. The characteristics of the soil were: clay 17%, sand 71%, silt 12%, available organic carbon 0·35%, available P_2O_5 36 mg kg^{-1}, available K_2O 12 mg kg^{-1}, pH 7·4 and conductivity 0·12 mmho cm^{-1}. The natural level of mercury in the soil was 0·12 mg kg^{-1}. A solid waste sample from the chloralkali plant at Ganjam town, with a mercury concentration of 2550 ± 339 mg kg^{-1} at pH 8·6 ± 0·2, was used as the source of mercury for contamination of the experimental soil. Weighed soil (100 kg) samples were thoroughly hand-mixed with solid waste at 0·75, 1·5, 2·5 and 5·0% proportions (w/w) and placed in earthen pots (25 kg soil per pot), length 60 cm, breadth 30 cm and depth 26 cm; watered and left under natural field conditions for about two months for equilibration, prior to experimentation.

Hordeum assay

Samples (500 g) of contaminated and control agricultural soil from experimental pots described above were transferred to perforated polythene bags, and maintained at 90% water holding capacity in a culture room at temperature 24 ± 1°C. Seeds of a diploid ($2n$ = 14) hull-less, six-rowed barley, *Hordeum vulgare* L. cv. 292 were presoaked for 15 h and then sown for germination in the dark, on experimental soil samples taken from the polythene bags. The experiments were conducted in triplicate using 50 seeds per exposure. The percentage of germination was recorded after 36 h. At that time randomly selected 5–10 embryonic shoots per exposure were excised and fixed in 1 : 3 acetic acid : ethanol for mitotic chromosome analysis. The remainder of the germinating seeds were grown further under continuous illumination. On day 7, seedling height was recorded by measuring the length of the first leaf. The seedlings were then transplanted to the respective pots containing contaminated or control soil already kept ready in the field to raise M_1-plants. Transplanted seedlings were distributed in four pots per exposure with 30–35 seedlings per pot. Shoot samples were removed on day 7, 15, 30 and 50, and dried at 60°C to constant weight for analysis of the bioconcentration of mercury in shoots at different stages of plant development. Five to ten per exposure group of the first emerged M_1-spikes of suitable length were fixed in acetic acid : ethanol, 1 : 3 and preserved in 70% ethanol for meiotic chromosome analysis. The plants were thinned to 20–25 plants per pot. The plants took about 80 days to complete one life cycle, i.e. from the day of sowing of M_0-seeds to harvest of M_1-seeds. Following harvest, dry weighed samples of root, straw and M_1-seed (grain) were kept separately for analysis of tissue-concentrations of mercury. The remainder of the M_1-seeds were kept in a desiccator to raise the M_2-seedlings for the study of chlorophyll-deficient mutations.

Cytological analysis

For mitotic chromosome analysis, embryonic shoots were acid-hydrolysed in 1 N HCl at 60°C for 15 min, stained in Feulgen reagent (Darlington & La Cour, 1976) for 10–15 min, then treated with 4% pectinase solution for 5 min to ease maceration and were squashed under a coverslip in a drop of 0·5% acetocarmine on a glass slide. About 1000 cells and 100 anaphases from five slides representing five embryonic shoots were examined per exposure to determine mitotic index and fre-

quency of cells with chromosomal aberrations (Nicoloff & Gecheff, 1976; Panda, 1983). Mitotic index was calculated as percent of mitoses out of the total cells scored.

For meiotic analysis of pollen mother cells (PMCs), anthers from young M_1-spikes were stained and squashed in 1% acetocarmine. After mounting the coverslip, the excess of stain was blotted by gentle pressure under layers of filter paper. About 1000 PMCs at metaphase-I/anaphase-I stages, from anthers of at least five randomly chosen spikes from different M_1-plants, were examined in order to determine the frequency of PMCs with chromosomal aberrations (Panda, 1983).

Before cytological processing of embryonic shoots and anthers, the glass vials containing the fixed materials were coded. Temporary slides were read blind and representative observations were recorded photomicrographically using a Zeiss photomicroscope.

Pollen fertility and seed set

Pollen fertility was determined by squashing mature anthers from randomly chosen M_1-spikes in 2% acetocarmine. Fully stained pollen grains were regarded fertile, while empty, partially stained or shrivelled grains were considered sterile. A total of 1500–2000 pollen grains from ten anthers per spike, equivalent to a total of five spikes from each exposure group, were examined. Pollen fertility was calculated as the percentage fertile of the total pollen scored.

After harvest, about 25 M_1-spikes per exposure group were randomly selected, irrespective of replications, to determine the seed set. The total number of florets and seeds were counted in each of the spikes. Seed set was expressed as the percentage of seeds out of the total number of florets examined.

Analysis for chlorophyll-deficient mutation in M_2-seedlings

M_1-seeds, totalling 3000 seeds per exposure group, were allowed to germinate on moist filter papers in petri dishes and M_2-seedlings were raised and grown under continuous illumination. The percentage of seedlings emerging was calculated (Konzak *et al.*, 1975). On day 10 the first leaves were examined for chlorophyll-deficient mutations.

pH-dependent leaching of solid waste mercury and chemical extraction of soil mercury

The leachability of mercury from solid waste was assessed by extraction of mercury in phthalate buffers (1 : 5 w/v) in the pH range 2–6 (Trefry & Metz, 1984). Plant-availability of mercury was assessed by chemical extraction methods using 10% HNO_3 (1 : 5 w/v), 0·025 M ammonium acetate/0·02 M ethylene diaminetetra acetic acid (NH_4Ac–EDTA) (1 : 5 w/v) and 0·05 M $CaCl_2$ (1 : 2·5 w/v), the details of which have been described earlier (Panda *et al.*, 1989).

Digestion and analysis for total mercury

The procedures for the digestion and analysis of samples of soil and filtered extracts have been described in detail earlier (Panda *et al.*, 1989). Weighed samples (dry wt) of root, shoot and grain (seed) were first wet-digested at room temperature (24 ± 2°C) in concentrated perchloric acid : HNO_3 (1 : 4 v/v) for 24 h for complete dissolution. This was followed by gentle heating in a Betheges apparatus until all the nitrogen oxide fumes were driven from the solution indicating completion of digestion. The above procedure avoids losses of mercury by volatilization (Bull *et al.*, 1977). After oxidation of the digests by potassium permanganate, total mercury was analysed by cold vapour atomic absorption spectrophotometry, following reduction with 20% $SnCl_2$ using a Mercury Analyser (MA 5800 D, ECIL, India) with a detection limit of 0·02 μg. Prior to analysis, the instrument was tested for non-specific absorption. $HgCl_2$ solutions in the range 0·01–0·1 mg litre^{-1} were regularly included as standards. The analyses gave a standard deviation of ±8% when subsamples from the same solution were analysed 12 times. The precision of the mercury analyses expressed as the coefficient of variation (CV) of replicate analyses was always < 5%.

Statistical analysis

The data on mitotic and meiotic cells with chromosomal aberrations were tested statistically to determine the levels of significance using the tables of Kastenbaum & Bowman (1970). For comparison the tissue-concentration of mercury in root, shoot, straw and grain was tested by the least significance difference (LSD) test. Furthermore, the biological endpoints, bioconcentrated as well as extractable mercury, were subjected to regression and correlation analysis against the soil-mercury levels (Gomez & Gomez, 1984).

RESULTS

Table 1 shows the regression and correlation analyses between soil-mercury and biological endpoints.

Table 1. Regression and correlation analyses between soil-Hg and biological endpoints in *Hordeum* assay

Biological endpoint (Y)	Soil-Hg (X)	
Germination	$Y = 97{\cdot}27 - 0{\cdot}18^{**}X$,	$r = -0{\cdot}96^{**}$
Seedling height	$Y = 15{\cdot}88 - 0{\cdot}07^{*}X$,	$r = -0{\cdot}88^{*}$
Mitotic index	$Y = 5{\cdot}11 - 0{\cdot}02^{*}X$,	$r = -0{\cdot}92^{*}$
Mitotic chromosome aberration	$Y = -3{\cdot}5 + 0{\cdot}36^{*}X$,	$r = 0{\cdot}96^{**}$
Meiotic chromosome aberration	$Y = 0{\cdot}6 + 0{\cdot}83^{*}X$,	$r = 0{\cdot}93^{*}$
Pollen fertility	$Y = 99{\cdot}41 - 0{\cdot}008X$,	$r = -0{\cdot}62$
Seed set	$Y = 87{\cdot}5 - 0{\cdot}075^{*}X$,	$r = -0{\cdot}94^{*}$
M_2-seedling emergence	$Y = 99{\cdot}34 - 0{\cdot}14X$,	$r = -0{\cdot}86$

Significant at 0·05 (*) or 0·01 (**) levels.

Table 2. Effect of soil contaminated with solid waste-Hg on germination, seedling height, mitotic index (MI) and mitotic chromosomes in embryonic shoot cells (ESCs) of *H. vulgare*

Conc. of Hg in soil (mg kg^{-1}) ± SD	Germination (%) ± SD	Seedling height (cm) ± SD	MI[a] ± SD	Total anaphases examined	Cells with aberrations	Aberrant cells (%)
0·12±0·04 (control)	95·62±3·4	14·43±3·15	5·2 ± 1·54	170	2	1·17
21·87±1·35	92·78±3·7	14·18±2·57	5·01±1·86	125	2	1·6
34·9±1·14	92·28±3·6	15·87±4·83	3·82±0·31*	116	10	8·62**
64·0±4·6	88·54±3·6	11·7±2·27**	3·85±0·69*	156	22	14·12**
103·3±10·4	76·87±2·4	8·05±1·32**	3·11±1·54*	87	33	37·93**

Significantly different from control at 0·05 (*), 0·01 (**) levels.

[a] $MI = \frac{\text{No. of mitoses}}{\text{Total cells}} \times 100.$

Germination, seedling height, mitotic index and chromosomal aberrations in embryonic shoot cells (ESCs)

The data on germination, seedling height, mitotic index and frequency of cells with chromosomal aberrations are presented in Table 2. Germination, seedling height and mitotic index all decreased with increasing concentrations of mercury in soil (Table 1). The frequency of chromosomal aberrations in ESCs induced by mercury-contaminated soil also followed a dose-response at concentrations ≥10 mg Hg kg^{-1} soil, indicated by the slope of the regression (plot not shown, Table 1). The chromosomal aberrations recorded were mostly anaphase with single, double or multiple bridge and anaphase with lagging or acentric chromosomes (Fig. 1(A),(B)).

Chromosomal aberrations in pollen mother cells (PMCs)

The type and frequency of PMCs with chromosomal aberrations following meiotic analysis are presented in Table 3. Regression analysis indicated a significant dose-dependent relationship between the frequencies of PMCs with chromosomal aberrations and mercury concentration of the soil (Table 1). The meiotic chromosomal aberrations recorded were chromosome bridge, fragments, lagging chromosome and non-congression of bivalents (Fig. 1 (C)–(F)). Of all the aberrations (Table 3), PMCs with non-congression of bivalents in metaphase I stage were observed most frequently.

Pollen fertility and seed set

The data on pollen fertility and seed set of M_1-plants grown on solid waste-contaminated soil are presented in Table 4. Pollen fertility was least affected by the contaminated soil at any of the mercury levels. On the other hand, seed set showed a negative correlation with the increase of mercury concentration of soil (Table 1).

M_2-seedling emergence and analysis of M_2-seedlings for chlorophyll-deficient mutations

The data on M_2-seedling emergence and analysis for chlorophyll-deficient mutations in M_2-seedlings of *H. vulgare* are presented in Table 5. Mercury-contaminated soil had no effect on M_2-seedling emergence nor did it induce any chlorophyll-deficient mutations in the M_2-seedlings.

Tissue-concentration of mercury

The tissue-concentrations of mercury in shoots of *H. vulgare* at different times, from day 7 to day 50, are presented in Fig. 2. The tissue-concentration of mer-

Table 3. Types and frequency of chromosomal aberrations in PMCs of *H. vulgare* grown on soil contaminated with solid waste-Hg from a chloralkali plant

Conc. of Hg in soil (mg kg^{-1})±SD	PMCs examined	Types of aberrations				Total aberrant PMCs	Aberrant PMCs (%)
		Br	Fr	NC	O		
0·12±0·04 (control)	1594	3	—	4	1	8	0·5
21·87±1·35	2490	5	2	9	6	21	0·84
34·9±1·14	1088	18	1	24	13	56	5·14**
64·0±4·6	1289	9	1	58	19	87	6·74**
103·3±10·4	1402	17	3	67	32	119	8·48**

Br, bridge; Fr, fragment; NC, non-congression; O, others include lagging chromosome(s).
Significantly different from control at 0·01 (**) level.

Table 4. Pollen fertility and seed set in *H. vulgare* grown on soil contaminated with solid waste-Hg from [illegible]oralkali plant at different levels

Conc. Hg in soil (mg kg^{-1}) ± SD	Pollen fertility[a] (%)	Seed set[b] (%)
0·12 ± 0·04 (control)	98·93±0·33	86·16±4·13
21·87±1·35	99·41±0·19	86·22±3·58
34·9±1·14	99·45±0·08	85·95±4·47
64·0±4·6	99·27±0·05	93·54±5·25
103·3±10·4	98·24±0·46	78·77±8·56

[a] Based on analysis of 1 500–2 000 pollen from ten anther/spike, five spikes.
[b] Based on analysis of florets from 25 spikes.

cury was found to be m[illegible]imal on day 7 followed by day 15, and gradually di[illegible]nished towards days 30 and 50, by which time it failed to show any correlation with soil-mercury. However, when compared individually with the control by the LSD test the concentration of mercury in tissues was still at statistically elevated levels on day 50 (Fig. 2).

The data on mercury concentrations in root, straw (dry shoot) and grain (M_1-seeds) are presented in Fig. 3. The levels of tissue-Hg in straw were low and failed to show any significant relationship with soil-Hg. The levels of tissue-Hg in roots, however, was found to exhibit a linear relationship with the levels of soil-Hg. The

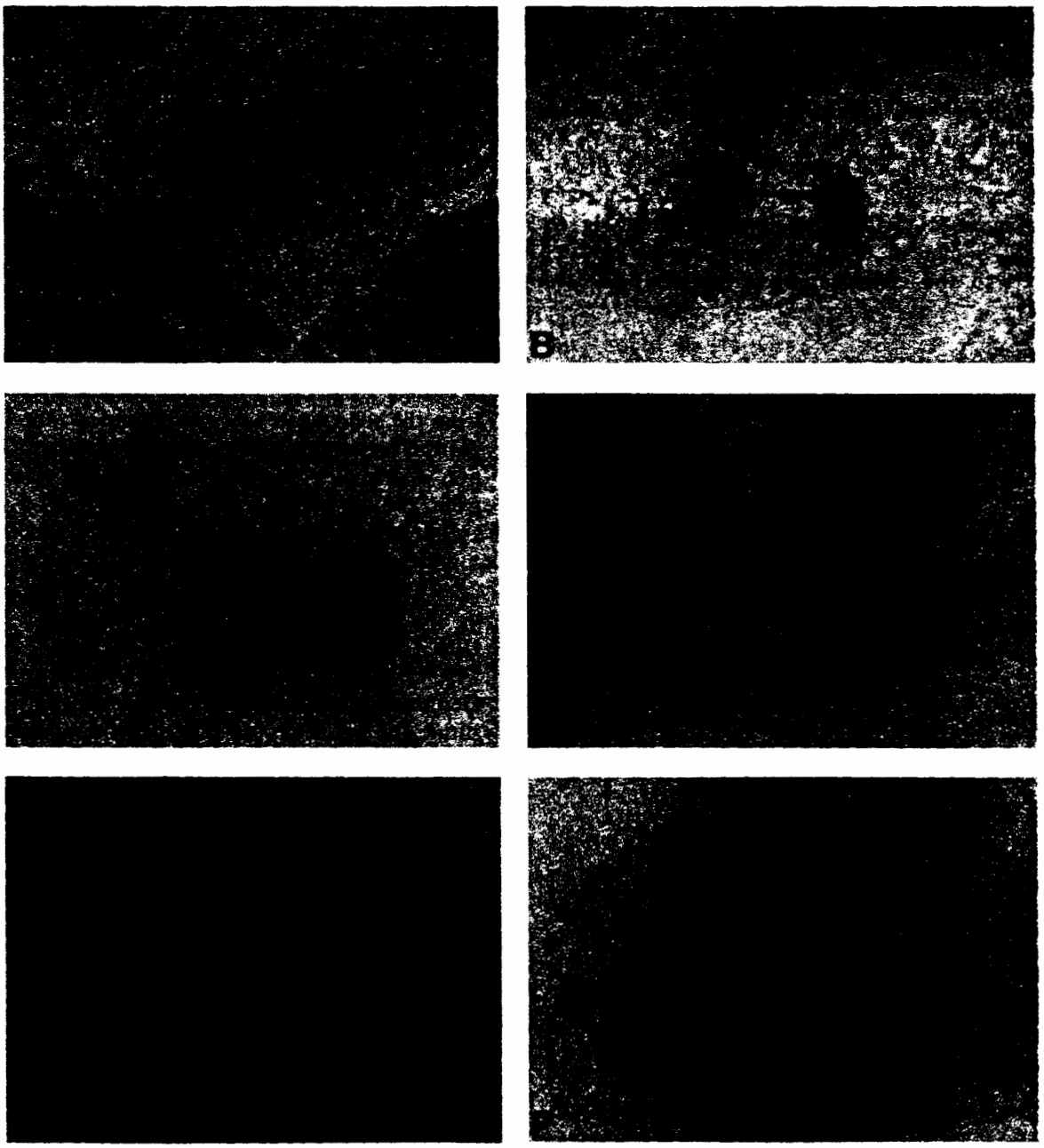

Fig. 1. Chromosome aberrations in ESC; (A) an acentric chromosome fragment in anaphase, (B) single bridge in anaphase. Chromosomal aberrations in PMCs; (C), (D) non-congression of bivalent in metaphase I, (E) acentric fragment in anaphase I, and (F) bridges in anaphase I.

Table 5. M_2-seedling emergence and analysis for chlorophyll-deficient mutations in M_2-seedling of *H. vulgare* following growth of M_1-Plants on soil contaminated with solid waste-Hg from a chloralkali plant

Conc. of Hg in soil (mg kg^{-1}) ± SD	No. of M_1-seeds sown	No. of M_2-seedlings emerged	M_2-seedling emergence (%)	Mutant(s)		
				Lethal[a]	Chlorophyll deficient	Chlorophyll deficient mutant/1000 M_2-seedlings
0·12±0·04 (control)	3000	2987	99·57	3	0	0
21·87±1·35	3000	2989	99·63	2	0	0
34·9±1·14	3000	2785	92·83	1	0	0
64·0±4·6	3000	2572	85·73	2	0	0
103·3±10·4	3000	2631	87·70	3	0	0

[a] Indicated by emergence of coleoptile but no leaf emergence following germination.

relationship between grain-Hg and soil-Hg was found to be non-linear but statistically significant (Fig. 3). An increase of grain-Hg was apparent with increase of soil-Hg at 5 mg kg^{-1}, but as the concentration of mercury in soil increased further, the levels of grain-Hg remained at a plateau, which was statistically elevated compared to the control, as analysed by LSD test ($p \leq 0{\cdot}01$).

Chemical extractability of mercury from contaminated soil

Mercury from contaminated soil was extracted by 0·05 M $CaCl_2$, NH_4Ac–EDTA and 10% HNO_3, the extractability of which were in the order 10% HNO_3 > NH_4Ac–EDTA > 0·05 M $CaCl_2$. The CV of the extractions were 2·64% (0·05 M $CaCl_2$), 3·02% (NH_4Ac–EDTA) and 4·52% (10% HNO_3) indicating the precision of mercury extraction by the respective extractants. The extraction of mercury by all the extractants showed a linear relationship with soil-Hg which were statistically significant (Fig. 4).

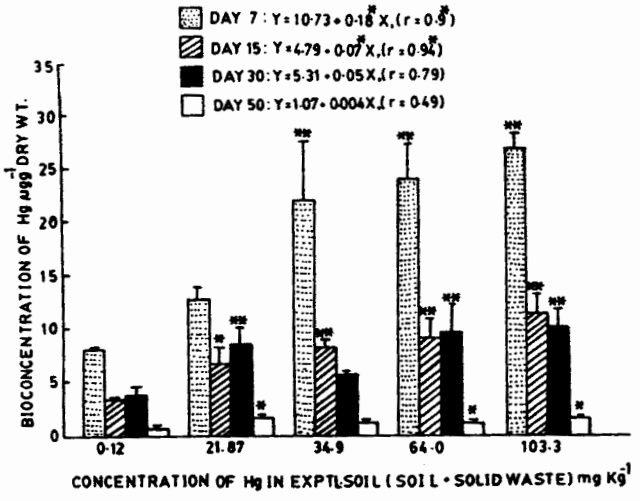

Fig. 2. Concentration of mercury in shoot (dry wt) at different days of plant growth in relation to the levels of total mercury in soil contaminated with solid waste. Significant at 0·05 (*) and 0·01 (**) levels.

pH-dependent leachability of mercury from solid waste

The solid waste, containing 2550 ± 339 mg Hg kg^{-1}, and used in all these experiments for contamination of experimental soil, was subjected to leaching of mercury at a range of pH values (2–6), using phthalate buffer. It is evident from Fig. 5 that the leachability of mercury from the solid waste was pH-dependent, the lower the pH the higher was the leaching of mercury. The fraction of mercury leached from the solid waste ranged from 71·8 ± 3·49 mg kg^{-1} at pH 2·3 to 13·6 ± 1·3 mg kg^{-1} at pH 6·3, representing 2·8% and 0·5% of the total solid waste mercury, respectively.

DISCUSSION

Germination, cell division and seedling growth constitute the initial processes of plant development. The present results indicate that the three processes were affected by the mercury-contaminated soil to different degrees. The effects were found to be dose-dependent and showed a negative relationship with soil-Hg. Such initial responses to metal stress have been well documented in plants (Lepp, 1981).

The mitotic or ESC analysis further indicated the induction of anaphases with single or multiple chromosomal bridges that followed a dose–response relationship. The present results indicate the possible value of ESC analysis of *H. vulgare* as a short-term screening assay to test genotoxicity of complex environmental mixtures, such as solid wastes, and is therefore in agreement with an earlier report (Constantin *et al.*, 1982).

The meiotic or PMC analysis indicated a germline effect of mercury-contaminated soil. Of the types of aberrations recorded, PMCs with the non-congression

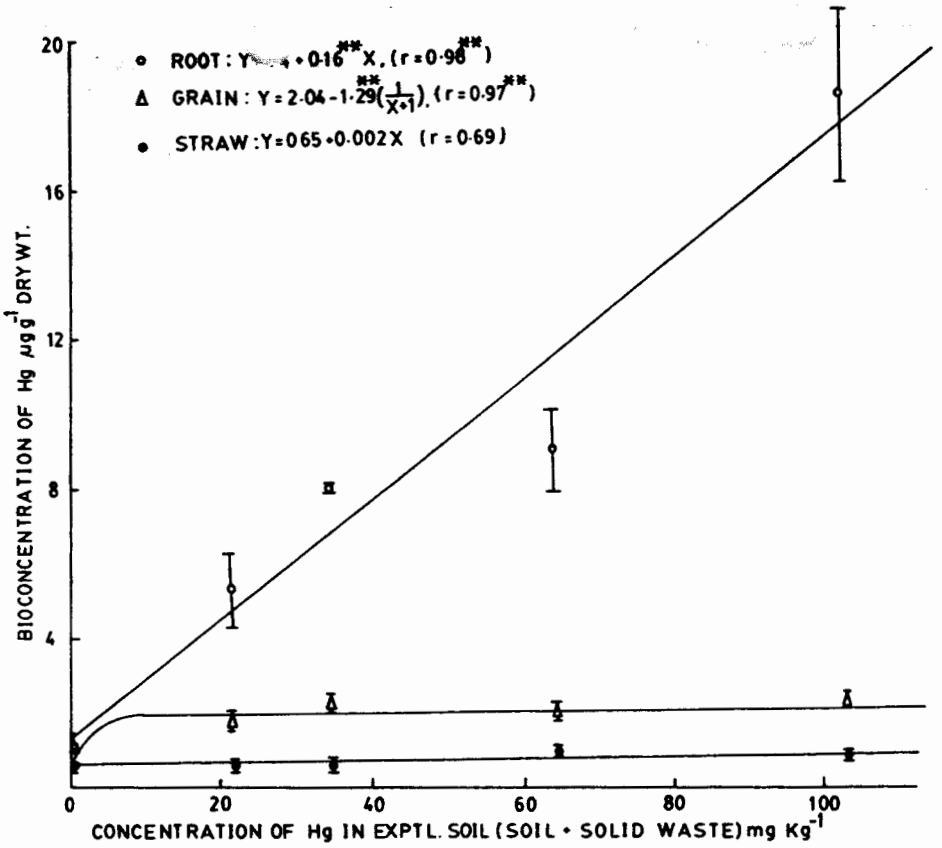

Fig. 3. Concentration of mercury in root, shoot and grain (dry wt) after harvest in relation to the levels of total mercury in soil contaminated with solid waste. Significant at 0·05 (*) and 0·01 (**) levels.

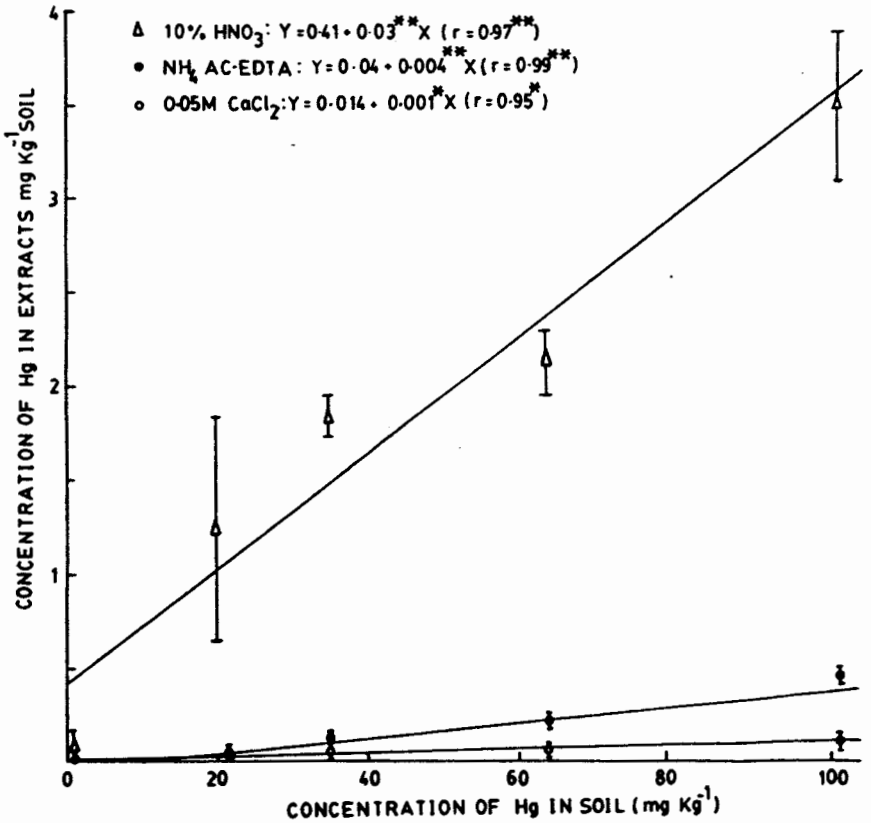

Fig. 4. Concentration of extractable-mercury from soil contaminated with solid waste. Significant at 0·05 (*) and 0·01 (**) levels.

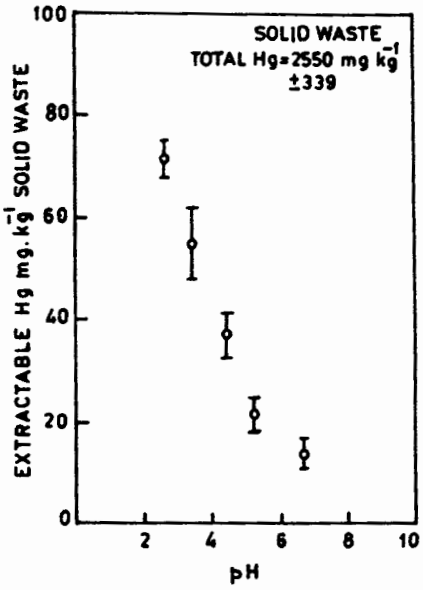

Fig. 5. Selective leaching of mercury from solid waste as a function of pH values (2–6).

of one or more bivalents in metaphase-I were very frequent, followed by the bridges in anaphase I (Table 3). Chromosomal bridges recorded in the ESC and PMC analyses were earlier thought to be the result of stickiness (Bale & Hart, 1973*a,b*), but later were interpreted as clastogenic manifestations (McGill *et al.*, 1974; Klasterska *et al.*, 1976). Induction of non-congression of bivalents in PMCs can be attributed to the impairment of spindle function. Mercury is well known to impair spindle function through interaction with tubulin-SH (Panda *et al.*, 1989). Non-congression of bivalents in PMCs may result in numerical chromosomal aberration or aneuploidy (Khush, 1973) and therefore has the potential for use as a system to detect environmental chemicals for induction of aneuploidy (Zimmermann *et al.*, 1979; Dellarco *et al.*, 1986). A survey of literature, however, indicated that the above system has not been utilized for this purpose (for review see Sandhu *et al.*, 1986).

The decrease in seed set may be an indication of germline effects that the pollen fertility analysis failed to detect. Because of the small sample size of M_1-seeds used, the M_2-seedling emergence and chlorophyll mutation analysis constituted only a preliminary study which failed to provide evidence that the mercury-contaminated soil could be genotoxic at specific gene-loci (Constantin, 1978; Constantin & Nilan, 1982*a*). Not even a single chlorophyll-deficient M_2-seedling mutant was recorded. Further investigation, however, is warranted to confirm the above observation.

The tissue-concentration of mercury in shoots of *H. vulgare* at different stages of development in the M_1-generation was found to be dependent on soil-concentration of mercury. It is of interest to note that the concentration of shoot-Hg decreased with increased age of the plants, which obviously could be attributed to the increase of relative biomass or to some mechanism involving an increased mobilization of mercury from soil during germination or during an early stage of plant development (Ferguson & Bollard, 1976). At the time of harvest it was noted that roots carried most of the mercury, depending on the concentration of soil-Hg (Fig. 3). This may indicate a limitation of translocation of mercury to the aerial plant parts, such as shoot and grain. Shoot-Hg showed a linear relationship with soil-Hg till day 15, but failed to show a correlation at later stages (Fig. 2). It was noteworthy that tissue-accumulation of mercury in straw was almost negligible and is therefore of little ecotoxicological consequence. The concentrations of grain-Hg, on the other hand, exhibited a non-linear but significant correlation with soil-Hg (Fig. 3) indicating that mercury transport into grain through the phloem may be limited. The accumulation of mercury in grain is of ecotoxicological concern because it may enter and biomagnify in food chains, an ecological phenomenon which is well known for a variety of metals and toxic chemicals (Panel on Mercury, 1978; Hughes, 1981; Martin & Coughtrey, 1981; Moriarty, 1983). It would be of interest to identify and quantify the species of Hg in the grain, because of its wider ecotoxicological implications.

Chemical extraction of mercury from the contaminated soil was carried out in an attempt to assess the plant-availability of the metal (Leschber *et al.*, 1985), the significance of which has been discussed earlier (Panda *et al.*, 1989). Correlation analysis between extractable-Hg and tissue-Hg indicated that, whereas $CaCl_2$-extractable-Hg was found to be best correlated with 7-day-old shoot-Hg (r^2 = 0·98), 10% HNO_3-extractable-Hg was best correlated with root-Hg (r^2 = 0·96).

The results on pH-dependent leaching (Fig. 5) demonstrated that the leachability of mercury from the solid waste decreased with increase of pH, which was in agreement with the observation made earlier with respect to sediment mercury (Panda *et al.*, 1990). The leaching of mercury from the solid waste at the dump sites of the chloralkali plant is therefore expected to be minimal at its present pH of 8·6 ± 0·2. The increase of pH at the abandoned dump sites by liming may further improve the situation in reducing the leachability of solid waste mercury.

Determination of a permissible limit for mercury in soil is a pre-requisite to developing effective environmental management of terrestrial mercury pollution. Attempts have been made to determine an acceptable limit for soil-Hg by different methods (Bashor & Turri, 1986; Revis *et al.*, 1990). From the present studies it was evident that mercury-contaminated soil exerts no significant genotoxicity at concentrations ≤22 mg Hg kg^{-1} soil, as indicated by the mitotic as well as meiotic chromosomal analyses, although slopes of regression

were apparent below this level (Tables 1, 2 and 3). The analysis of tissue-mercury further reveal that the levels of residual mercury in grain harvested from plants grown on soils contaminated at different mercury-levels, were significantly higher than the control, but did not increase with the increase of soil-Hg beyond a certain level, i.e. 5 mg Hg kg^{-1} soil, in the present study. Thus, present studies failed to pinpoint a permissible level for mercury in agricultural soil. The results, nevertheless, unequivocally demonstrated that the soil containing mercury of levels of 5 mg kg^{-1} or more may be considered as 'not safe' for agricultural use for a crop such as *Hordeum*. It must, however, be kept in mind that the above inference may not be extrapolated to other crops or other soil conditions, as metal-uptake, as well as metal-tolerance, is expected to vary from crop to crop and from soil to soil. The unique advantage of the use of the *Hordeum* assay is that, besides assessing the germline toxicity, it takes into account the possible contamination of the agricultural food-chain at source, that presently no other single assay can do.

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