EFFECTS OF CADMIUM AND ZINC ON CELL DIVISION AND CHROMOSOMAL ABERRATIONS IN *ALLIUM SATIVUM*

ANITA MUKHERJEE and ARCHANA SHARMA
Department of Botany, University of Calcutta, 35, Ballygunge Circular Road, Calcutta 700 019, India.

ABSTRACT

The cytotoxic effect of cadmium and a combination of cadmium with the essential element zinc in *Allium sativum* was studied on cell division and chromosomal aberrations. The percentage of chromosomal aberrations was significantly higher in combined cadmium chloride and zinc chloride treatment than in the individual metals. The combined treatments showed an additive effect with respect to the parameters studied.

INTRODUCTION

One of the common sources of heavy metal pollution is the discharge of industrial effluents which are continuously released into the environment[1]. The biological effect of pollutants may be greatly influenced by their antagonistic or synergistic interactions[2,3]. Cadmium ranks close to lead and mercury in toxicologic importance[4] and can induce a wide range of toxicity[2,5]. Although industrial operations are major sources of cadmium, disposal of metal-rich sewage sludge on land renders plants a health hazard if consumed by man and animals[6]. Cadmium is unique since it is always found in association with zinc in ore and soil[7]. However, zinc is an essential trace element in living cells. Cadmium, though found in tissues and biological fluids, has no known useful biological function[8].

Interaction between cadmium and zinc on metabolic processes has been widely studied in different organisms. The different aspects reviewed include carcinogenesis, cytotoxicity, teratogenesis and other physiological aspects[9]. Many of the manifestations of chronic exposure to cadmium resemble those of zinc and copper deficiencies[2]. Pretreatment with or simultaneous administration of excess zinc also protects against cadmium damage[10]. These observations are known mainly with regard to physiological changes in animals. Data on the effect of interaction between the metals on their clastogenic and mitostatic effects are relatively rare in animals and more so in higher plants. A majority of the studies on the action of metallic salts in combination on plants, principally deal with germination and growth parameters[11,12]. Cadmium and zinc are also reported to interact in uptake by plants. High zinc levels enhance the uptake of cadmium in radish leaves[13-16] while a short-term uptake of cadmium by roots of rye grass was suppressed considerably by calcium, manganese and zinc[17].

The present investigation was undertaken to study the cytotoxic effects of cadmium and zinc metals individually and in combination on cell division and chromosomal aberrations in *Allium sativum* roots.

MATERIALS AND METHODS

$CdCl_2$ (Loba Chemical Co., India) and $ZnCl_2$ (British Drug House, India), analar grade, were dissolved in Knop's nutrient solution and stored at 7°C.

A. sativum bulbs were chosen as the test system due to their sensitivity to changes in environmental conditions[18]. Seven experimental sets, each with 10 bulbs were kept with roots immersed in solutions containing (i) control—only Knop's solution; (ii) 1 ppm of $CdCl_2$; (iii) 1 ppm of $ZnCl_2$; (iv) 100 ppm of $CdCl_2$; (v) 10 ppm of $ZnCl_2$; (vi) combination of (ii) and (iii), and (vii) combination of (iv) and (v). Uniform pH (neutral) and temperature (30 ± 2°C) were maintained.

Root tips were excised at intervals of 1, 3, 6 and 24 hr and fixed in acetic ethanol (1 : 3). Slides were prepared following the acetic orcein squash schedule[19]. At each interval approximately 1000 cells from each set were scanned for the dividing and aberrant cells. The mean values with standard deviation are given in tables 1 and 2. Duncan's multiple range test was used to determine the significance at 95% confidence limits[13].

RESULTS

1. The frequency of dividing cells at the lower concentration (1 ppm) of $CdCl_2$ and $ZnCl_2$ was not significantly different from that of control over

24 hr of treatment. Following treatment with the higher concentration of $ZnCl_2$ (10 ppm) or $CdCl_2$ (100 ppm), the frequency of dividing cells was not significantly different from the control till 6 hr of treatment. At 24 hr, the frequency of dividing cells was significantly ($P < 0.05$) less than the control.

The principal effects on the chromosomes were of two types: (i) on the spindle leading to non-disjunction, diplochromatid, metaphase arrest, stickiness, early and unequal separation, and (ii) on the chromosomes shown by breaks, lesions and exchanged portions. The percentage of aberrant cells scored was, however, significantly higher ($P \leq 0.05$) than that of control and was dose and duration-dependent.

2. *Combined treatment with $CdCl_2$ and $ZnCl_2$*

(i) *1 ppm of $CdCl_2$ and $ZnCl_2$*: The frequency of dividing cells decreased significantly at 24 hr of combined treatment and was less than that of control and only Cd or Zn treated groups. The aberrations induced were significantly higher in the combined treatment than that of control or individual salts. The increased frequency of the aberrant cells showed that the effect of combined treatment on plant by these metals is additive. The differences were statistically significant ($P < 0.05$) as calculated from Duncan's multiple range test (table 1).

(ii) *100 ppm of $CdCl_2$ and 10 ppm of $ZnCl_2$*: Following combined treatment, the percentage of

Table 1 *Effect of $CdCl_2$ (1 ppm) and $ZnCl_2$ (1 ppm) on root tip cells of Allium sativum*

	Mean of aberrant cells (%) ± S.D.			
	Period of treatment (hr)			
Mode of exposure	1	3	6	24
Control	$3.11^a \pm 1.34$	$3\ 17^a \pm 0.79$	$4.54^a \pm 0.87$	$4.83^a \pm 0.91$
Cd	$9\ 18^b \pm 1.51$	$10.72^b \pm 1.73$	$12.43^b \pm 1.20$	$13\ 37^b \pm 1\ 54$
Zn	$8.85^b \pm 1.68$	$10.51^b \pm 1\ 43$	$11.57^c \pm 2\ 60$	$12\ 51^b \pm 3.75$
Cd + Zn	$20.41^c \pm 4.31$	$22\ 86^c \pm 1.87$	$23.60^d \pm 2.32$	$29.03^c \pm 2.16$

Values in a vertical column followed by the same letter are not significantly different at 5% level as determined by Duncan's multiple range test. Each value is the mean of 10 samples ± S.D.

Table 2 *Effect of $CdCl_2$ (100 ppm) and $ZnCl_2$ (10 ppm) on root tip cells of Allium sativum*

	Mean of dividing cells (%) ± S.D.				Mean of aberrant cells (%) ± S.D.			
	Period of treatment (hr)				Period of treatment (hr)			
Mode of exposure	1	3	6	24	1	3	6	24
Control	$7.74^a \pm 1.41$	$6.96^a \pm 1.66$	$6.03^a \pm 1.68$	$7.20^a \pm 1.61$	$3.11^a \pm 0.92$	$4.83^a \pm 0.69$	$4.54^a \pm 1.40$	$3.11^a \pm 0.70$
Cd	$7.17^a \pm 1.04$	$6.53^a \pm 1.30$	$6.00^a \pm 0.72$	$5\ 46^b \pm 0.98$	$16.35^b \pm 1.42$	$17.32^b \pm 3.20$	$19.10^b \pm 3.21$	$26.28^b \pm 4.32$
Zn	$7.01^a \pm 1.20$	$7.13^a \pm 1\ 23$	$6.74^a \pm 2.13$	$5.54^c \pm 1.67$	$12.55^c \pm 1.98$	$14.35^b \pm 1.05$	$13.34^c \pm 2\ 91$	$18.82^c \pm 1.43$
Cd + Zn	$6.99^a \pm 1.46$	$6.17^a \pm 0.93$	$5.14^b \pm 1.40$	$3.66^d \pm 1.50$	$27.50^d + 4.39$	$29.37^d \pm 3.68$	$34.28^d \pm 3.45$	$39.71^d \pm 4.26$

Values in vertical column followed by the same letter are not significantly different at 5% level as determined by Duncan's multiple range test. Each value is the mean of 10 samples ± S.D.

dividing cells decreased gradually with the period of treatment. It was statistically significant when compared to control and individual Cd or Zn treated groups from 6 hr onwards.

The number of aberrant cells increased with the duration of treatment and was statistically significant at this combination set, an addition of Zn caused an increase in the percentage of aberrant cells over the single Cd-treated set (table 2).

In both sets of combined treatment, $ZnCl_2$ enhanced the cytotoxic effect of Cd as measured by the decrease in mitotic index and increase in aberrant cells.

DISCUSSION

The combined effects of $ZnCl_2$ and $CdCl_2$ were more toxic than those of a single metal as shown by the frequencies of dividing and aberrant cells were additive. Within one cell cycle (22 hr) Cd and Zn when given singly at 1 ppm concentration did not affect cell division even at 24 hr of treatment, and at higher concentrations till 24 hr of treatment. A combination of the two significantly reduced the frequency of dividing cells, the effect being seen at 6 hr and 24 hr in the combined treatment sets (ii) and (i) respectively with the higher concentrations used. The number of aberrant cells increased both in single and combined treatments. Earlier studies have shown that the action of Cd is localized in the G_2 phase or later[22] which accounts for the increase in aberrations with no significant change in cell division[23]. Therefore, $CdCl_2$ and $ZnCl_2$ appear to act independently of one another in *A. sativum*. No antagonism was observed as was recorded for animal systems[23]. The toxic action of $CdCl_2$ could not be eliminated in plants through redistribution or by metallothionein-like proteins as was observed in animals where the mechanism of detoxification is well worked out[10,24].

Similar additive interactions have been observed in plants between Pb and Fe[25], Hg and Se[26], Cd and Se[23] and synergistic interaction between the metals Pb and Cd[27], or between the metals Pb, Cd, Hg, Cu in aquatic plants[28]. That the effects were more or less physical was confirmed when the addition of one metallic ion to another metal accentuated the toxicity further[28]. Supportive evidences can be drawn from the hydroponic experiments with corn, where the toxic effects of Cd were ameliorated by the addition of Zn to the nutrient solutions[28]. Despite low uptake of Cd in the combination treatment, cytotoxicity was further enhanced in the combination of even relatively lower (1 ppm) concentrations. Probably, metals when given in combination, disrupt cell division directly or indirectly through acting on other metabolites or by leading to ionic imbalance.

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ANNOUNCEMENTS

NATIONAL WORKSHOP ON SCREENING FOR ENVIRONMENTAL MUTAGENS AND CARCINOGENS IN MAMMALS AND MAN

The above Workshop will be held during February 10–25, 1988 at the Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Hyderabad. Scientists from various parts of India and abroad are expected to participate. The number of participants is fifteen and registration is free. The morning sessions include lectures, and practicals will be conducted in the afternoons on mutagenic and carcinogenic evaluation of chemicals. The candidates should posses M.Sc. degree and experience in the field, and a permanent post in the Institution. Those interested should send the application along with biodata to: Dr P. Rita, Organizing Secretary of the Workshop on or before 31 December 1987.

For details please contact Dr P. Rita, Secretary, Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Begumpet, Hyderabad 500 016.

THE SECOND WINTER SCHOOL ON MOLECULAR REACTION DYNAMICS

The Second Winter School on Molecular Reaction Dynamics to be held during December 27, 1987 to January 9, 1988, is sponsored by the Department of Science and Technology, New Delhi and organized by the Department of Chemistry, Indian Institute of Technology, Kanpur.

The aim is to introduce the modern area of molecular reaction dynamics to young researchers in the country with the hope of initiating extensive research in this area.

The applicants must be members of teaching or research staff or research fellows in a University/Institute and have Ph.D. or M.Sc., degree in Chemistry (or Physics). Preference will be given to applicants below the age of thirty five years. Total number of participants will be restricted to a maximum of twenty.

Further particulars may be had from: The Convenor; Prof. N. Sathyamurthy, Department of Chemistry, Indian Institute of Technology, Kanpur 208 016.