PP-2

Modulation of cytogenetic effects of cadmium by humic acid; findings from a short-term plant-based bioassay

OThilomi Samarakoon[1,2], Takeshi Fujino[1]

[1]Graduate School of Science and Engineering, Saitama University, Japan,
[2]Department of Zoology and Environmental Management, University of Kelaniya, Sri Lanka

Saitama University 埼玉大学

Introduction

Cadmium (Cd) is a toxic heavy metal found as a contaminant in aquatic ecosystems as a result of both natural and mainly anthropogenic activities. In the environment, the toxicity of Cd can be altered by coexisting chemicals such as humic acid (HA): a naturally occurring organic compound ubiquitous in aquatic environments. Identification of such toxicity modifications is important for realistic ecological risk assessments to safeguard aquatic life.

Research purpose *Allium cepa* (common onion) bioassay is an effective test system for evaluating the cytotoxic and genotoxic potential of chemicals. Since it is well-known that Cd can affect living organisms at the cellular and genetic level, *A. cepa* bioassay can be effectively used to identify potential toxicity modulations by other chemicals in co-occurrence. Therefore, this study aimed to evaluate the influence of HA in modulating the toxicity of Cd using cytogenetic endpoints of *A. cepa* under a short-term water-borne exposure assessment to mixtures of Cd and HA.

Materials and Methods

Experimental design

Equal-sized healthy *A. cepa* bulbs were obtained from a local market. They washed properly with running tap water and placed over glass vials filled with aerated tap water for 24 h in the dark at 25 °C.

On the following day, bulbs with equal root lengths (~1 cm) were exposed to the following concentrations or concentration combinations of Cd and HA prepared using $CdCl_2$ (95% purity, first-grade) and HA (practical grade) obtained from FUJIFILM Wako Pure Corporation (Tokyo, Japan). Aerated tap water was used as the control and dilution water for treatments.

- **Control**
- **10 mg/L HA**
- **1 mg/L Cd**
- **1 mg/L Cd + 10 mg/L HA**
- **5 mg/L Cd**
- **5 mg/L Cd + 10 mg/L HA**

Fig. 1 Experimental setup of the *A. cepa* bulbs at the end of the exposure period of 48 h

Each treatment or control had 3 replicates (n=1 onion bulb per treatment). The exposure setup was placed at 25 °C in the dark for 48 h with a media renewal after 24 h with fresh ones.

Processing of roots

At the end of the exposure period, several root tips (1–2 mm) from each *A. cepa* bulb were excised and fixed in Carnoy's solution (1:3 v/v, glacial acetic acid: ethanol) overnight. After fixation, the root tips were transferred to 70% ethanol and stored at 4 °C until microscopic observations. At the time of microscopic observations, the root tips were hydrolyzed in 1 N HCl at 60 °C for 6 min, washed with ultra-pure water, and stained with acetocarmine for 15 min.

Microscopic observations and cytogenetic endpoints

The stained root tips were squashed and analyzed under a light microscope for cytogenetic endpoints to estimate the mitotic index and frequencies of nuclear abnormalities and chromosomal aberrations.

01. Mitotic Index (MI)	Calculated as the number of cells undergoing different stages of mitosis (dividing cells) (i.e., prophase, metaphase, anaphase, and telophase) per 1,000 cells in each *A. cepa* bulb.
02. Nuclear Abnormality (NA)	Estimated by scoring different nuclear abnormalities in 1,000 interphase cells (non-dividing cells) in each *A. cepa* bulb.
03. Chromosomal Abnormality (CA)	Estimated by scoring different chromosomal abnormalities in a total of at least 100 cells undergoing metaphase, anaphase, and telophase in each *A. cepa* bulb.

Statistical analysis

The modulation of Cd-induced cytogenetic effects by HA was statistically analyzed using IBM SPSS statistics 25.0 by the independent samples T-test between the Cd-treated A. cepa bulbs and corresponding combinations with HA.

Results and Discussion

Fig. 2 shows the normal appearance of *A. cepa* root meristematic cells in the interphase (non-dividing cells) and cells that are undergoing different phases of mitosis (i.e., prophase, metaphase, anaphase, and telophase) (dividing cells).

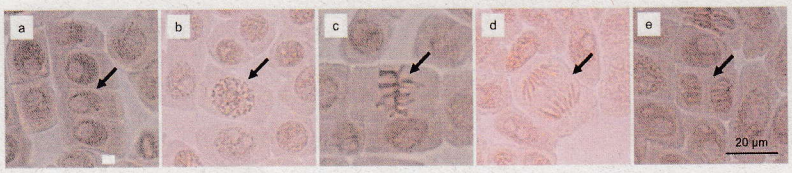
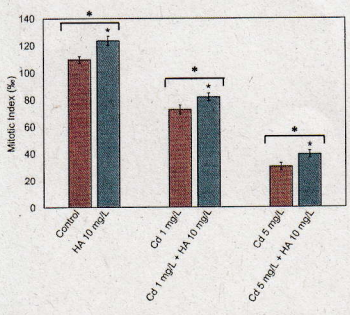


Fig. 2 (a) Normal interphase cells and (b–e) different stages of mitosis in *A. cepa* root tip cells (b) prophase (c) metaphase (d) anaphase, and (e) telophase



The ratio between the dividing and non-dividing cells (MI) is an important estimate of the capacity of cells to divide and the rate of cell division which indicates the cytotoxic potential of an agent.

Results indicated that HA can significantly increase the MI of the *A. cepa* root meristematic cells compared to the control bulbs (p<0.05) (Fig. 3). All Cd-treated *A. cepa* bulbs irrespective of the presence of HA showed decreased MIs. However, when *A. cepa* bulbs were exposed simultaneously to mixtures of Cd and HA, a significant increase in the MI was observed compared to their corresponding Cd-only treatments (p<0.05).

Fig. 3 Mitotic indices (‰) in *A. cepa* root meristematic cells after 48 h exposure to Cd and HA alone and in mixtures. * indicates a statistically significant difference between the two pairs (independent samples T-test, p<0.05)

NAs defined as morphological alternations in the nuclei in the interphase cells are also crucial endpoints in determining the cytotoxic and genotoxic potential of chemical substances. In this study, five prominent types of NAs (i.e., condensed nuclei, nuclear buds, binuclei, micronuclei, and blebbed nuclei) were recognized (Fig. 4).

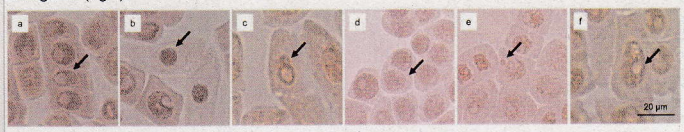


Fig. 4 (a) Normal interphase cells and (b–f) nuclear abnormalities observed in interphase cells in root tips of *A. cepa* bulbs exposed to Cd (b) condensed nucleus (c) nuclear bud (d) bi nucleus, and (e) micronucleus and (f) blebbed nucleus

CAs are characterized by changes in either chromosomal structure or in the total number of chromosomes. The most common CAs scored in this study are shown in **Fig. 5**.

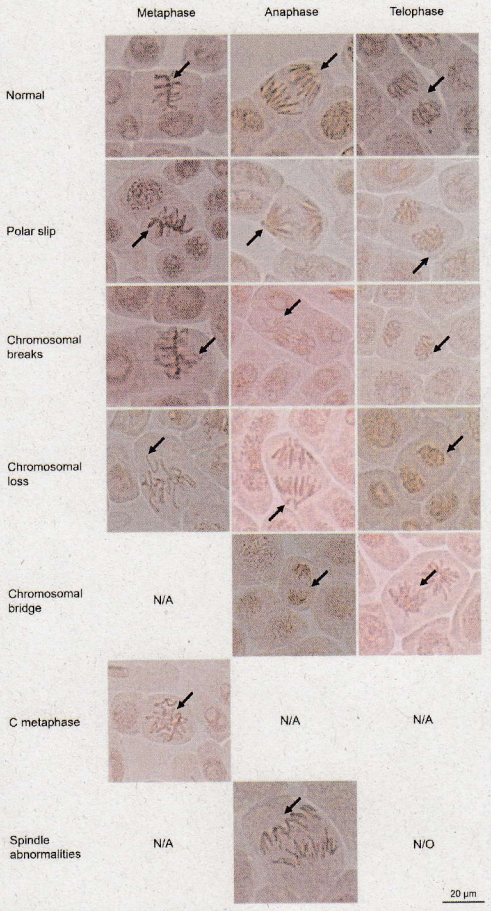


Fig. 5 Normal metaphase, anaphase, and telophase and different types of chromosomal aberrations observed in each phase in root tip cells of *A. cepa* bulbs exposed to Cd. NA: not applicable, NO: not observed

According to the results, the NA and CA observed in the HA-treated *A. cepa* bulbs were insignificant compared to the control bulbs (Fig. 6 (a) and (b)). Increased MI and negligible occurrence of NA and CA in the HA-treated *A. cepa* bulbs portray the nutrient quality of the HA as it can be a source of nutrients such as carbon, nitrogen, etc. All Cd-treated *A. cepa* bulbs irrespective of the presence of HA showed increased frequencies of NAs and Cas. However, when *A. cepa* bulbs were exposed simultaneously to mixtures of Cd and HA, significant decreases in NAs and CAs were observed compared to their corresponding Cd-only treatments.

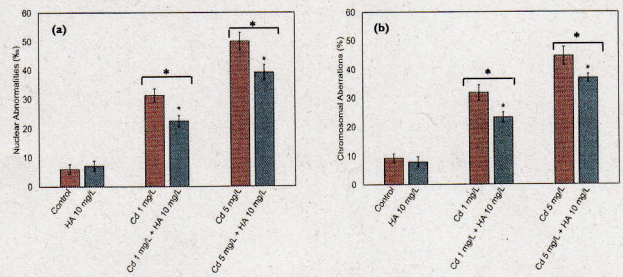


Fig. 6 Frequencies of **(a)** nuclear abnormalities (‰) and **(b)** chromosomal aberrations (%) in *A. cepa* root meristematic cells after 48 h exposure to Cd and HA alone and in mixtures.
* indicates a statistically significant difference between the two pairs (independent samples T-test, p<0.05)

Conclusion and Recommendations

The findings of this study demonstrate the protective role of HA against the toxicity of Cd in *A. cepa* root meristematic cells. This could be due to a possible complexation reaction between Cd and HA to reduce the bioavailability of Cd. Further studies are recommended with natural HAs isolated from the environment as well as with other types of humic substances such as fulvic acid because they possess a complex chemistry that depends upon their type and origin.

References

Leme DM, Marin-Morales MA. (2009). *Allium cepa* test in environmental monitoring: a review on its application. Mutation Research 682(1):71-81.

Seth CS, Misra V, Chauhan LK, Singh RR. (2008). Genotoxicity of cadmium on root meristem cells of *Allium cepa*: cytogenetic and Comet assay approach. Ecotoxicology and Environmental Safety 71(3):711-6.

Unyayar S, Celik A, Cekiç FO, Gözel A. (2006). Cadmium-induced genotoxicity, cytotoxicity, and lipid peroxidation in *Allium sativum* and *Vicia faba*. Mutagenesis 21(1):77-81.