Informatics in Biology, Health, and Food

Interdisciplinary Explorations in Life Science

Volume 2. Number 1. Jun. 2025 DOI: 10.22034/ibhf.2025.519781.1023

Original Article

Cadmium and Copper Toxicity in *Matricaria chamomilla* L.: Dose-Dependent Mitodepression and Chromosomal Aberrations

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Received: 2025/04/28 **Accepted:** 2025/06/09 **Online Published:** 2025/06/12

Abstract

Environmental contamination by cadmium (Cd) and copper (Cu) threatens ecosystems and agricultural productivity due to their genotoxic impacts on plants. This study investigates the mitodepressive and clastogenic effects of Cd and Cu on *Matricaria chamomilla* L., a pharmacologically significant species with unexplored cytogenetic responses to heavy metals. Root meristematic cells were exposed to 50–200 ppm $CdCl_2$ and $CuCl_2$ to assess mitotic index (MI), chromosomal aberrations, and comparative metal toxicity. Mitotic abnormalities, including sticky chromosomes, laggards, bridges, micronuclei, and precocious movements, were analyzed alongside MI and Total Abnormality Percentage (TAP). Results revealed a dose-dependent decline in MI (Cd: 14.0 ± 0.36 to 7.15 ± 0.12; Cu: 15.13 ± 0.12 to 9.21 ± 0.18) and a rise in TAP (Cd: 3.37 ± 0.2 to 7.33 ± 0.14; Cu: 1.9 ± 0.23 to 5.51 ± 0.17). Cd exhibited greater toxicity, inducing higher abnormality frequencies than Cu. Chromosomal stickiness dominated anomalies, followed by laggards, bridges, and micronuclei, with Cd causing more severe disruptions to spindle dynamics and chromosome segregation. Control cells (MI: 16.51 ± 0.12) showed negligible aberrations. These findings underscore *M. chamomilla*'s sensitivity to metal-induced genotoxicity, validating its role as a bioindicator for pollution monitoring. The pronounced cytogenetic damage at elevated metal concentrations highlights ecological risks posed by Cd and Cu contamination. This study advances understanding of interspecific metal toxicity mechanisms and supports the integration of cytogenetic assays in environmental risk assessments, offering insights for phytoremediation strategies and heavy metal pollution management in agro-ecosystems.

Keywords: Chamomile, Genotoxic, Heavy metal, Mitotic Abnormalities.

Cite this article: Hajmoradi F. Cadmium and Copper Toxicity in Matricaria chamomilla L.: Dose-Dependent Mitodepression and Chromosomal Aberrations. *Informatics in Biology, Health, and Food*. 2025;2(1):100-106.
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Introduction

Environmental contamination by heavy metals has emerged as a major global concern due to their persistence, bioaccumulation, and detrimental effects on living organisms (1). Among these pollutants, cadmium (Cd) and copper (Cu) are particularly hazardous, with Cd being a non-essential, highly toxic element and Cu, though essential, becoming toxic at elevated concentrations (2). Both metals disrupt cellular homeostasis, interfere with enzymatic activities, and induce oxidative stress, leading to DNA damage and chromosomal instability (3). Their widespread presence in agricultural soils poses a significant threat to plant growth, crop productivity, and overall ecosystem health (4).

As primary producers, plants are highly sensitive to heavy metal toxicity, making them excellent bioindicators in environmental genotoxicity assessments (5). The root meristematic cells, due to their high mitotic activity, are particularly vulnerable to metal-induced cytogenetic damage (6). The mitotic index (MI) and chromosomal aberration assays are well-established biomarkers for evaluating the genotoxic potential of environmental pollutants (7). Heavy metals such as Cd and Cu interfere with spindle microtubule formation, DNA replication, and chromosome segregation, leading to abnormalities including sticky chromosomes, C-mitosis, micronuclei, laggard chromosomes, and chromosomal bridges (8). These aberrations not only impair plant growth but also serve as early warning signs of environmental toxicity (9).

Matricaria chamomilla L. (chamomile), a widely cultivated medicinal herb, is known for its pharmacological properties and sensitivity to environmental stressors (10). While previous studies have focused on its physiological and biochemical responses to heavy metals (11), limited research has explored its cytogenetic alterations under Cd and Cu stress (12). Given its economic importance and ecological relevance, understanding the mitotic disturbances in chamomile under metal toxicity is crucial for environmental risk assessment and development phytoremediation strategies (13).

Comparative studies using model plants such as *Allium cepa* and *Vicia faba* have demonstrated that Cd and Cu induce dose-dependent reductions in MI and increase chromosomal aberrations (14,15). For instance, Arya and Mukherjee reported that cadmium toxicity in both *A. cepa* and *V. faba* and found that exposure to Cd caused a significant, dose-dependent increase in chromosomal aberrations, micronuclei frequency, and DNA fragmentation, accompanied by a reduction in mitotic index (MI) in root meristem cells (15).

This study aims to evaluate the mitodepressive and genotoxic effects of cadmium (Cd) and copper (Cu) on *M. chamomilla* by analyzing mitotic abnormalities and chromosomal aberrations at various concentrations (50–200 ppm). Additionally, it seeks to compare the relative toxicity of Cd and Cu and assess chamomile's suitability as a bioindicator for heavy metal pollution.

Material and Methods

Sample Preparation and Genotoxicity Assay

M. chamomilla seeds were used as the experimental material. For germination of root tip, sterilized and washed seeds were placed in a petridish lined with filter paper, then covered and incubated for 2-3 days at 22-25° C. Solutions of different concentrations (50 ppm, 100ppm, 150ppm, 200ppm) of cadmium and cooper were prepared using $CdCl_2$ and $CuCl_2$ by dilution method (16). After germination, the seedlings were treated with the respective metal solutions, while distilled water was used as the control for each treatment group. Then the root tips were washed thoroughly with distilled water and fixed in ethanol: acetic acid (1:3) for 24 hrs. The rootlets were removed from the fixing solution and transferred to 70% ethanol for further analysis. For cytogenetic examination, the root tips were hydrolysed in 1 N HCl for one minute, then stained with 4% acetocarmine under gentle heat, and the slides were prepared using the squash technique.

Cytogenetic Analysis

Mitotic index (MI) and total abnormality percentage (TAP) were assessed using a light microscope. MI was calculated by dividing the number of dividing cells by the total number of cells and multiplying by 100, as described by Love (1949). TAP was calculated by dividing

the number of abnormal cells by the total number of cells and multiplying by 100 (16).

Results

All the studied slides of *M. chamomilla* were diploid, with a chromosome number of 2n = 2x = 18. Mitosis in the control group appeared normal, and chromosomal anomalies were relatively negligible; however, the root meristematic cells of *M. chamomilla* at different concentration in all treated sets of $CdCl_2$ and $CuCl_2$, exhibited a concentration-dependent increase in mitotic abnormalities (Figure 1, Table 2).

The MI has been used as a parameter to assess the cyto/genotoxicity of metals. The MI for cadmium decreased from 14 ± 0.36 to 87.15 ± 0.12 and for cooper decreased from 15.13 ± 0.12 to 9.21 ± 0.18 with increase in heavy metals concentration. As it clear, with the increase in concentration factor, the mitotic index decrease (Figure 1, Table 2). The intensity of mitotic index was descending from copper to cadmium. Mitotic cells were analyzed during the study of Cd and Cu groups exhibited several types of chromosomal abnormality, such as stickiness, laggards, micronucleus, chromosome bridge and precocious movement of chromosome.

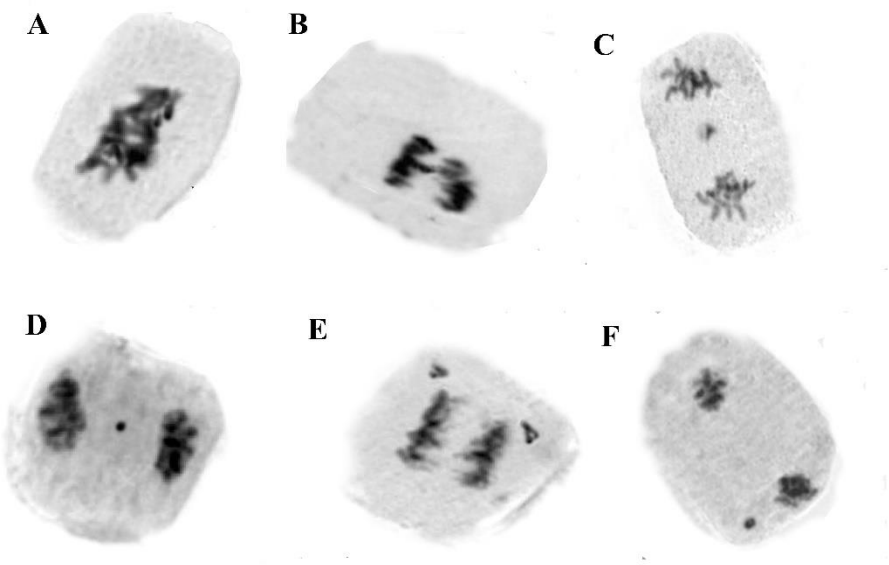

Figure 1:Different chromosomal abnormalities in root tip cells of heavy metal-induced ***M. chamomilla***; (A) sticky chromosome; (B) bridge; (C and D) laggard; (E) precious movement of chromosome; (F) micronucleus.

The total abnormality percentage (TAP) for cadmium increased from 3.37 ± 0.2 to 7.33 ± 0.14 (with increase of doses from 50 ppm to 200 ppm). For copper it increase from 1.9 ± 0.23 to 5.51 ± 0.17 over the same concentrations of cadmium treatment sets. Chromosome stickiness and C-mitosis were more frequently observed abnormalities across all treatment sets. The frequency of other abnormalities for cadmium and cooper treatment concentrations were as following order, alternatively:

For cadmium: laggards > bridge > micronucleous > precious movement.

For copper: bridge > precious movement > micronucleous > laggard.

The results of the present investigation indicated that both Cd and Cu can induce some abnormalities in the meristematic root cells of *M. chamomilla*, with notable effects on chromosomal morphology, including chromosome stickiness, laggard, anaphase bridge, micronucleus and precious movement. The effect of cadmium was found to be more toxic than copper.

Table 1. Frequency of chromosomal abnormalities at different mitotic stages in *M. chamomilla* root tip cells exposed to heavy metals.

Treatment			**Cd**		**control**
Concentration (ppm)	**50**	**100**	**150**	**200**	**-**
Sticky chromosome	2.18±0.15	2.42±0.20	2.81±0.23	3.39±0.08	-
Laggard	0.61±0.17	0.82±0.07	0.95±0.09	1.19±0.05	-
Micronucleus	-	-	0.67±0.25	0.85±0.09	-
Bridge	0.58±0.19	0.73±0.24	0.86±0.18	1.09±0.25	-
Precious movement	-	0.23±0.14	0.54±0.25	0.81±0.09	-
Treatment			**Cu**		**control**
Concentration (ppm)	**50**	**100**	**150**	**200**	**-**
Sticky chromosome	1.74±0.13	1.82±0.15	2.13±0.03	2.87±0.02	-
Laggard	-	-	0.35±0.09	0.52±0.19	-
Micronucleus	-	0.13±0.08	0.38±0.15	0.64±0.23	-
Bridge	0.16±0.11	0.38±0.18	0.58±0.17	0.81±0.02	-
Precious movement	-	0.18±0.23	0.35±0.19	0.67±0.18	-

Abbreviations: MI- Mitotic Index, TAB%- Total Abnormality Percentage.

Table 2. Effects of Cadmium and Copper on Mitotic Index (%) and Mitotic Abnormality (%) in Root Tips of *M. Chamomilla.*

Concentration (ppm)	**Mitotic abnormalities (Mean ± standard error**		**Mitotic Index (Mean ± standard error)**	
	cd	**cu**	**cd**	**cu**
control	-	-	16.51 ± 0.12	16.51 ± 0.12
50	3.37 ± 0.2	1.9 ± 0.23	14 ± 0.36	15.13 ± 0.12
100	4.2 ± 0.13	2.51 ± 0.4	11.23 ± 0.22	13 ± 0.2
150	5.83 ± 0.3	3.79 ± 0.1	9.11 ± 0.8	11 ± 0.16
200	7.33 ± 0.14	5.51 ± 0.17	7.15 ± 0.12	9.21 ± 0.18

Discussion

The present study elucidates the genotoxic effects of cadmium (Cd) and copper (Cu) on the mitotic cycle of *M. chamomilla*, a species widely recognized for its ecological sensitivity to environmental stressors. Our findings demonstrate that both metals disrupt mitosis in root meristematic cells in a concentration-dependent manner, with Cd exhibiting significantly higher genotoxicity than Cu. These results are consistent with increasing evidence that heavy metals induce chromosomal instability and mitotic depression in plants, posing risks to genomic integrity and ecosystem health (17, 18). Below, we contextualize these findings within existing literature, explore mechanisms underpinning metal-induced genotoxicity, and discuss implications for environmental monitoring and plant physiology.

A key observation in this study is the progressive decline in MI with increasing concentrations of Cd and Cu. The MI decrease from 14.0% to 8.15% under Cd treatment and 15.13% to 9.21% under Cu treatment, emphasizes the mitodepressive nature of these metals, likely due to their interference with cell cycle checkpoints and DNA replication machinery. Such mitotic inhibition has been documented in other plant models exposed to heavy metals, including *Allium cepa* and *Vicia faba* (15, 19). The suppression of MI at higher concentrations may stem from metal-induced oxidative stress, which disrupts tubulin polymerization and spindle formation, thereby arresting cells in interphase or early mitotic stages (20). Notably, Cd's stronger mitodepressive effect compared to Cu aligns with its higher redox activity and affinity for sulfhydryl (-SH) groups in structural proteins, exacerbating cellular damage (21).

The spectrum of chromosomal aberrations observed—stickiness, laggards, bridges, micronuclei, and precocious movement—reflects distinct mechanistic disruptions. Chromosome stickiness, the most frequent abnormality in Cd-treated cells, is often linked to DNA-protein crosslinking or partial chromatin decondensation caused by metal interference with topoisomerases (22).

Anaphase bridges and lagging chromosomes indicate unresolved DNA strand breaks or faulty sister chromatid separation, likely due to oxidative DNA lesions from reactive oxygen species (ROS) overproduction (7). Cd, a non-redox metal, indirectly induces ROS via glutathione depletion, while Cu, a redox-active metal, directly participates in Fenton reactions, generating hydroxyl radicals (23). Paradoxically, Cu-treated cells exhibited fewer aberrations than Cd-treated ones, possibly due to Cu's essential role in cellular enzymes (e.g., cytochrome oxidase), which may activate detoxification pathways at sublethal doses (24).

Micronuclei formation, a hallmark of clastogenic damage, arises from acentric chromosome fragments or whole chromosomes failing to incorporate into daughter nuclei. The higher micronucleus frequency in Cd-treated cells reinforces its clastogenic potency, consistent with studies on *Triticum aestivum* (25).

The heightened genotoxicity of Cd over Cu observed here corroborates prior comparative studies (17, 19). This divergence may arise from multiple factors:

1. Cellular uptake and binding affinity: Cd's ionic mimicry of Ca^{2+} and Zn^{2+} allows it to hijack membrane metal transporters, leading to rapid accumulation (26). Its strong binding to nucleic acids and histones disrupts chromatin structure, whereas Cu primarily targets mitochondria and chloroplasts (27).
2. Antioxidant system overload: Cd depletes glutathione (GSH) and inhibits antioxidant enzymes like superoxide dismutase (SOD), exacerbating oxidative damage. Cu, though pro-oxidant, is regulated by metallothioneins and phytochelatins, mitigating its toxicity (23).

The sensitivity of *M. chamomilla* to metal-induced chromosomal damage positions it as a potential bioindicator for soil pollution. Its rapid root growth and clear cytogenetic endpoints make it a practical tool for ecotoxicological assessments, complementing traditional models like *A. cepa* (22). Furthermore, the dose-dependent response underscores the risk of agricultural productivity loss in metal-contaminated soils, where even sublethal metal concentrations may reduce crop viability through genotoxic stress (24).

While this study provides robust evidence of Cd/Cu genotoxicity, further molecular analyses are needed to unravel specific pathways. For instance, transcriptomic profiling could identify metal-responsive genes (e.g., ATM, ATR) regulating cell cycle arrest. Additionally, field studies assessing *M. chamomilla*'s bioindicator efficacy in real-world contaminated sites would strengthen its applicability.

In summary, Cd and Cu induce significant mitotic aberrations in *M. chamomilla*, with Cd exhibiting greater genotoxicity due to its disruptive effects on spindle formation, redox balance, and DNA repair. These findings highlight the urgent need for monitoring heavy metal pollution and developing phytoremediation strategies using metal-sensitive species. Future research integrating cytogenetic, molecular, and ecological

approaches will deepen our understanding of plant-metal interactions.

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