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Original Article

Accumulation of Heavy Metals and Their Genotoxic Potential in Medicinal Plant *Verbacsum speciosum* Schrad.

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Abstract

Background: Nowadays, the contamination of soil, water, and plants by heavy metals (HMs) presents a notable environmental problem.

Methods: Lead (Pb) and zinc (Zn) levels in soil and *Verbacsum speciosum* populations were measured using atomic absorption spectroscopy, and meiosis in pollen mother cells (PMCs) was examined through the squash technique to evaluate genotoxic effects.

Results: The results showed that Pb and Zn concentrations in the soil and, roots, and above-ground portions of all *Verbacsum speciosum* populations within the mining area exceeded permissible thresholds significantly. Particularly, the contaminated soil displayed average Pb and Zn concentrations of 3527.4 ± 0.7 and 1671.4 ± 0.5 mg/kg, respectively. The roots of *Verbacsum speciosum* plants closest to the mine exhibited the highest levels of Pb and Zn, measuring 204 ± 0.05 mg/kg and 570 ± 0.16 mg/kg, respectively. Additionally, the above-ground parts of the plants showed Pb and Zn concentrations of 312 ± 0.12 mg/kg and 519 ± 0.17 mg/kg, respectively. Moreover, the transfer factor (TF) for both Pb and Zn was found to be above one, indicating substantial accumulation of these metals in the above-ground portions. The presence of Pb and Zn also resulted in abnormalities during meiosis. As the distance from the mine increased, a decrease in the occurrence of meiosis abnormalities and an increase in the meiotic index (MI) were observed.

Conclusion: These results highlight the potential risks to human health posed by industrial wastewater and the natural presence of HMs in soil. The ability of these metals to accumulate in plants and interfere with their meiosis underscores the importance of addressing and mitigating these hazards.

Keywords: Heavy metals, Medical plant, Meiosis abnormality, Plant pollution, Transfer factor, *Verbascum speciosum*

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Introduction

One of the most significant environmental concerns today is soil pollution caused by heavy metals (HMs). The accumulation of HMs in plants, animals, and the environment directly and indirectly affects human health.[1] Mines release their tailings and effluents into nature, leading to soil pollution and the subsequent transfer of pollutants to plants, animals, and humans. Plants absorb these HMs from contaminated soil and environments and store them in their edible parts.[2-4] If medicinal plants and spices are collected from areas contaminated with HMs, they can become important sources of transmission for this type of pollution to humans. Lead (Pb) and zinc (Zn) are the most commonly found HMs. While plants require a certain amount of Zn for natural growth, higher concentrations can be extremely toxic. Pb, as a hazardous anthropogenic HM, is typically absorbed through the roots and transported to the aerial parts of the plant at a rate of several percent. The presence of large quantities of Pb and Zn compounds in liquid, solid, and gaseous waste can have significant negative impacts on biological and ecological systems.[5]

With the rise in mining activities in Iran, medicinal plants are increasingly exposed to the risk of HM accumulation. *Verbascum* L., belonging to the family Scrophulariaceae, is a medicinally valuable plant with a wide distribution in Iran, comprising 41 species. These species are significant medicinal plants used by the public for treating lung infections, bronchitis, whooping cough, pancreatitis, diarrhea, hemorrhoids, and urinary tract infections. Additionally, drugs derived from this plant are used as anti-cough and expectorant medications. *Verbacsum speciosum* Schrad., a well-known medicinal species for treating infectious diseases, is abundant in Iran's meadows. This plant is native to Eastern Europe and Western Asia and is known as Gole Mahur in Iran. It is a biennial plant characterized by large rosette leaves, an upright stem that can grow over 1 meter in height, and large clusters of flowers.[6] In a previous study, Güleryüz et al[7] investigated the levels of HMs and concluded that this plant species is suitable as a bio-indicator for monitoring specific HMs in the environment. Arslan et al[8] demonstrated that as the amount of HMs in the soil increases, their concentration also rises, suggesting that this plant can serve as a bio-indicator species. In another study, Shah et al[9] examined *V. thapsus* under HMs stress and found that plant-based biomaterials offer a promising alternative for the biosorption of HMs.

Meiosis is an intricate process that involves a specific set of genes at each stage. Alongside genetic factors, environmental factors also contribute to coordinating the various stages of meiosis.[10] Elevated levels of HMs have been found to have harmful effects on chromosomes, leading to mutations in a wide range of plant species.[11-13] In recent decades, studies have investigated the effects of Pb and other HMs on meiotic and mitotic disturbances and abnormalities in various plants.[5,14-16] This study represents the first report on the risk posed by the accumulation of Pb and Zn HMs in causing meiotic abnormalities in the medicinal plant *Verbacsum speciosum*. In the Zeh Abad Pb and Zn mine located in the Qazvin province, mineral tailing materials, along with the produced wastewater, are transported to a tailings dam site situated 1 km away from the mine. After deposition, a portion of the wastewater is reused in the processing. *Verbacsum speciosum* grows in this mining area, and despite its location, local people harvest it for its medicinal value, posing potential health risks. The current study aims to evaluate:

1- The concentrations of HMs (Pb and Zn) in five populations of *Verbacsum speciosum* and their respective soils in the polluted mine area using atomic absorption spectroscopy.
2- The comparison of HM concentrations in roots and stems to determine their distribution.
3- The cytological damage caused by Pb and Zn on pollen mother cells (PMCs) within the studied plant.

Material and Methods

Site Description

The Zehabad-e-Qazvin Mine is situated in a mountainous area on the southern side of the central Alborz mountain range, approximately 85 km from the Qazvin-Rasht road. It is located near the Zeh Abad village at Latitude = 36° 28' 13" and Longitude = 49° 25' 02". The mine is located 3 km away from Zeh Abad village and has an approximate operating area of 9.5 km^2.[17] Due to the presence of Pb and Zn deposits in the mining area and the extraction of these two minerals, Pb and Zn were chosen to investigate HM contamination. To determine the concentration of Pb and Zn in the soil and plants, samples were collected from five localities surrounding the mine (a to e localities, Figure 1). The control locality was located approximately 12 km

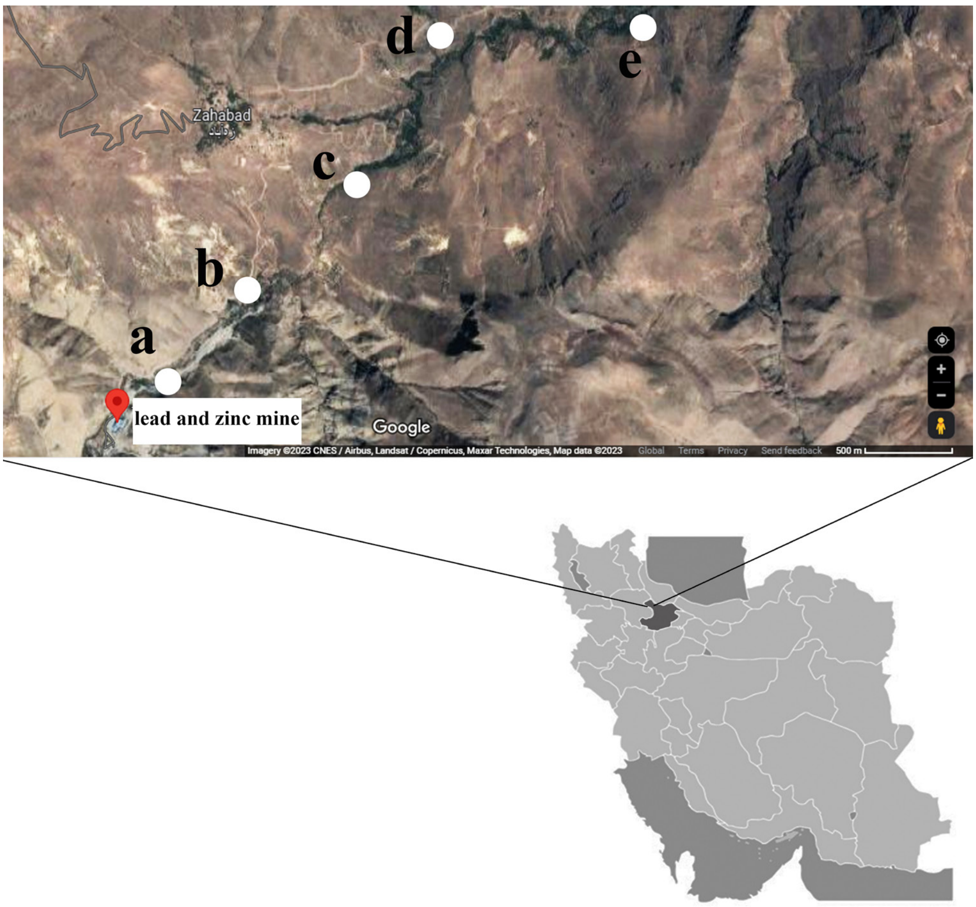

Figure 1. Sampling polluted areas

away from the mine at a higher elevation than the mine's mineral veins, tailings dam, and free of pollution.

Soil preparation

Soil samples were collected from five distinct areas near the growth site of the studied plant, adjacent to the Zehabad-e-Qazvin Lead and Zinc Mine in Zeh Abad village, with each area having three replicates. These samples were taken from the soil surface down to a depth of 60 cm using a stainless steel shovel. After removing any large rocks and extraneous materials, the samples were placed in plastic bags and transported to the laboratory. Upon arrival at the laboratory, the soil samples underwent a drying process at 80 °C for 24 hours. Subsequently, the samples were homogenized through a 2-mm sieve. For the measurement of HMs, including Pb and Zn, atomic absorption spectroscopy was utilized. About 5 g of the soil was mixed with a solution comprising nitric acid (65%), hydrochloric acid (70%), and hydrogen peroxide (30%) in a ratio of 6:4:1. This mixture was then heated to 200 °C for 3 hours and subsequently filtered through Whatman filter paper No. 24. Before analysis, the solution volume was adjusted to 50 mL by the addition of distilled water.[18,19]

Plant Preparation

To assess the health of plants that grow naturally near the lead and zinc mine, we used atomic absorption spectroscopy to measure the levels of these metals in *Verbacsum speciosum*, a medicinal plant. We first washed the plant samples with distilled water to remove any surface impurities. Then, we dried them at room temperature and further dried them at 105 °C for 24 hours until they reached a constant weight. We ground and homogenized the samples and weighed about 0.5 grams of each. We digested the samples with a solution of nitric acid (65%), hydrochloric acid (70%), and hydrogen peroxide (30%) in a 6:3:1 ratio. We heated the samples at 80 °C for 3 hours and filtered the resulting clear and colorless solution through Whatman filter paper No. 42. We diluted the solution to 50 mL with distilled water before analysis. We measured the concentration of HMs in the samples using atomic absorption spectroscopy.[18,19]

Transfer Factor

To determine the extent of the transfer of HMs from roots to stems, the transfer factor (TF) was calculated using the following equation[20]:

Concentration of elements in roots/Concentration of elements in aerial tissue

Cytogenetic Analysis

To assess the impact of Pb and Zn HMs on chromosomal behavior in *Verbacsum speciosum* plants, PMCs were utilized. Flower buds were immediately immersed in Carnoy's solution, which consisted of ethanol, chloroform, and propionic acid in a 6:3:2 ratio. After 24 hours, the buds were rinsed with distilled water and preserved in 70% alcohol at 4 °C for subsequent examination. Following this, the squash technique was employed to observe various stages of meiotic division and identify chromosomal abnormalities in the young flower buds. These samples were then stained using a 2% acetocarmine solution. PMCs were meticulously examined at distinct stages of meiotic division, and their images were captured using an Olympus light microscope.

Meiotic Index

To determine the meiotic index (MI), the number of normal tetrads observed was divided by the total number of observed tetrads, and the resulting quotient was multiplied by 100.[21]

Results and Discussion

Concentration of Pb and Zn in the Soil

The concentrations of HMs, specifically Pb and Zn, in soil samples collected from the vicinity of the mine were analyzed using an atomic absorption spectrometer. The results indicated a significantly elevated concentration of these metals in the soil around the mine compared to uncontaminated soil, as shown in Table 1. According to standards, the permissible limit for Pb in soil stands at 50 mg/kg. However, in the examined area, this concentration was nearly 70 times higher. Similarly, while the acceptable limit for Zn in soil is 200 mg/kg, the observed concentration in the soil sample was approximately 8 times above this standard threshold.[19] Area 'a' exhibited the highest HM levels, attributed to its proximity to the mine. As one moved farther away from the mining site, the concentration of these elements in the soil progressively decreased.

Human economic activities frequently result in irreversible environmental damage. Soil, being a primary environmental reservoir, significantly influences plant contamination. Among the many detrimental activities, mining stands out for its profound environmental impacts. The prolonged operations of the Zehabad-e-Qazvin Lead and Zinc Mine, combined with the discharge of effluents and tailings into the environment, have elevated the levels of Pb and Zn in the surrounding soil. Research on HM distribution and dispersion, focusing on particle size near mines in southwest Spain, revealed that particles larger

Table 1. The concentration of Pb and Zn in the Soil of the Contaminated and Control Area

Area		Heavy Metals (mg/kg)	
		Pb Mean ± SE	Zn Mean ± SE
Polluted area	a	4120 ± 0.12	2870 ± 0.07
	b	3548 ± 0.03	2184 ± 0.16
	c	2741 ± 0.17	1735 ± 0.12
	d	1046 ± 0.23	975 ± 0.07
	e	6182 ± 0.04	593 ± 0.13
Control		43 ± 0.31	162 ± 0.19
Standard		50	200

The data includes the mean ± standard error of 3 repetitions.

than 1 mm tend to remain closer to the source, intensifying pollution in the immediate vicinity of the mine. In contrast, smaller particles contribute to contamination at greater distances.[22] Similarly, a study examining HMs concentrations in the Asalouyeh region found that metal concentrations in the soil diminished as the distance from industrial zones increased.[23]

Concentration of HMs in the Roots and Shoots of *Verbacsum speciosum*

The primary objective of this research revolves around examining *Verbacsum speciosum* in its natural habitat, specifically within the mining area. Nonetheless, one significant challenge we faced was identifying suitable sampling locations within the designated zone. Utilizing the atomic absorption technique, we analyzed the concentrations of Pb and Zn within *Verbacsum speciosum*, as outlined in Table 2. Specimens of this plant were sourced from five distinct areas proximate to the Zehabad Pb and Zn mine in Qazvin. Across all sampled areas, our findings revealed that the accumulation of both elements in both the roots and above-ground parts of the plant surpassed standard limits. Notably, area "a" exhibited the highest concentrations for both Pb (204 ± 0.05 mg/kg) and Zn (570 ± 0.16 mg/kg) in the roots. Similarly, this area also recorded peak concentrations in the plant's aerial parts, with values of 312 ± 0.12 mg/kg for Pb and 638 ± 0.06 mg/kg for Zn. As one moved farther from the mine, there was a noticeable decline in the concentrations of Pb and Zn in both the roots and aerial sections of *Verbacsum speciosum*, with the lowest concentrations observed in area "e" (see Table 2).

Overall, our analysis of HMs in both the aerial parts and roots of the examined plant indicated consistently higher concentrations of HMs in the plant's above-ground tissues across all contaminated zones compared to its roots. As depicted in Table 2, Zn concentrations in the examined plant surpassed those of Pb. Various factors, including the solubility of HMs and the metabolic processes within plants, influence the absorption and subsequent accumulation of metals.

Consistent with soil trends, as one moves away from the mine, the concentration of these elements within the plants also diminishes. This observation aligns with studies conducted in other regions. For instance, research on HMs pollution around mines in southwestern Madrid, Spain, highlighted excessively toxic concentrations of cadmium (Cd), copper (Cu), and Zn in both the soil and plants.[24] Within our study, the aerial tissue of the examined plant demonstrated the highest absorption rates of Pb and Zn.

Similarly, investigations around the Khatoon Abad copper mine revealed an increase in Cu concentration with distance from the facility. This trend was attributed to the deposition of finer copper-laden particles that traveled farther from the source.[25] Chen et al[26] further underscored that the presence of HMs in the soil inevitably leads to contamination of plants within the mining vicinity.

Transfer Factor

The calculated TF values for the examined HMs reveal that *Verbacsum speciosum* predominantly accumulates higher concentrations of Pb and Zn in its aerial parts compared to its subterranean parts, as evidenced by TF coefficients exceeding one (Table 2). The TF serves as a pivotal metric to assess a plant's efficacy in translocating elements from its roots to its stems. A TF value surpassing one signifies the plant's heightened propensity to sequester the element in its above-ground portions, denoting a pronounced movement of the element within the plant's structures. This translocation is modulated by factors such as the element's solubility within plant tissues and the metabolic dynamics of the plant species.[27,28] Consistent with our findings, several studies underscore that plants tend to accumulate Pb and Zn more profusely in their aerial parts than in their root systems.[6,29] In a relevant study, Sadeghi et al[30] observed a TF value below one when examining the impact of Pb on grass, suggesting its potential utility for phytoremediation in areas contaminated with Pb. Our research underscores that given the elevated concentrations of Pb and Zn in the soil surrounding *Verbacsum speciosum*, this plant species emerges as a promising bio-indicator for the presence of such HMs in its environment. Notably, prior research has identified other species within this genus as effective biological indicators.[7,8]

Table 2. Concentrations of Pb and Zn measured in both contaminated and control samples of *Verbacsum speciosum*

Verbacsum speciosum		Heavy metals (mg/kg)					
		Pb Mean ± SE		TF	Zn Mean ± SE		TF
		Roots	Aerial Part		Roots	Aerial Part	
Polluted plant	a	204 ± 0.05	312 ± 0.12	1.52	570 ± 0.16	638 ± 0.06	1.11
	b	173 ± 0.14	216 ± 0.07	1.24	417 ± 0.3	519 ± 0.17	1.24
	c	83 ± 0.2	114 ± 0.17	1.37	324 ± 0.08	460 ± 0.08	1.51
	d	49 ± 0.07	83 ± 0.03	1.69	193 ± 0.15	317 ± 0.14	1.64
	e	15 ± 0.16	37 ± 0.4	2.46	95 ± 0.07	172 ± 0.09	1.81
Control plant		0.8 ± 0.23	1.7 ± 0.12	2.12	37 ± 0.15	57 ± 0.01	1.54
Standard		2			60		

The data provided includes the mean ± standard error obtained from three repetitions.

Chromosomal Study of Verbacsum speciosum

The plants, both contaminated and control variants, were diploid, with a reported base chromosome number of 15 (2n = 30). Meiotic abnormalities in the control plants were minimal, as illustrated in Figure 2 and detailed in Table 3. Conversely, contaminated plants exhibited a concentration-dependent escalation in meiotic irregularities. Notably, the frequency of observed abnormalities diminished with increasing distance from the mining site. Some prominent abnormalities observed included chromosome stickiness, cytomixis, laggards, bridges, non-synchronous segregation, micronuclei, as well as tri- and pentapolar anomalies. Of all the abnormalities induced by Pb and Zn exposure, chromosome stickiness was consistently predominant across all examined locations. A comprehensive analysis involved 1969 cells in diakinesis/metaphase I (D/MI), 1,239 cells in anaphase I/telophase I (AI/TI), 690 cells in metaphase II (MII), and 1679 cells in anaphase II/telophase II (AII/TII) across both contaminated and control *Verbacsum speciosum* populations.

In terms of chromosome stickiness, chromosomes were observed as clusters, and individual chromosomes were not present. This abnormality was predominant during diakinesis, early metaphase I, and metaphase II in the polluted populations (Figure 2B). The highest percentage of abnormalities was observed in the locality near the mine (locality a). This abnormality was less abundant in the control plants compared to the polluted plants. Both environmental and genetic factors could be responsible for inducing chromosome stickiness.[31] Cytomixis, another anomaly, was observed at a relatively high frequency in different stages of meiotic division in polluted plants, except for prophase/telophase I and II of localities 'd' and 'e' (Figure 2F and 2H). The control plant exhibited this anomaly only in 0.9 ± 0.04% of the diakinesis/metaphase I stage. Lagging or unoriented univalents in the equatorial plane of cells, known as laggards, were observed in all contaminated plants during the first and second meiosis, except in the second meiosis of locality 'e'. The percentage of this abnormality was higher in locality 'a' compared to the others (Figure 2E). In the control plant, only 0.6 ± 0.2% of PMCs showed laggards in meiosis II. Chromosomal bridges were another anomaly observed more frequently in polluted plants. This anomaly was observed in all polluted plants during meiosis I. PMCs at the anaphase II stage exhibited chromosomal bridges in localities a, b, and c (Figure 2C). The bridge abnormality was observed only at a very low frequency (1.4 ± 0.1%) in control PMCs during anaphase I. The control plant did not exhibit this anomaly at the meiosis II stages. Non-synchronous segregation of univalents is one of the meiotic abnormalities that may occur early or late. This abnormality was observed in anaphase I in all polluted localities 'a' to 'e', and in localities 'a', 'b', and 'c' during anaphase II (Figure 2D). The control plant did not show any asynchronous separation anomaly during any of the meiotic stages. The highest percentage of this abnormality was reported in locality 'a'. Chromosomes that undergo unsynchronized movement may Pb to the formation of micronuclei in the final stages of meiosis. This anomaly was observed in all contaminated localities during the telophase II stage. The highest percentage of this anomaly was reported in locality 'a'. None of the PMCs

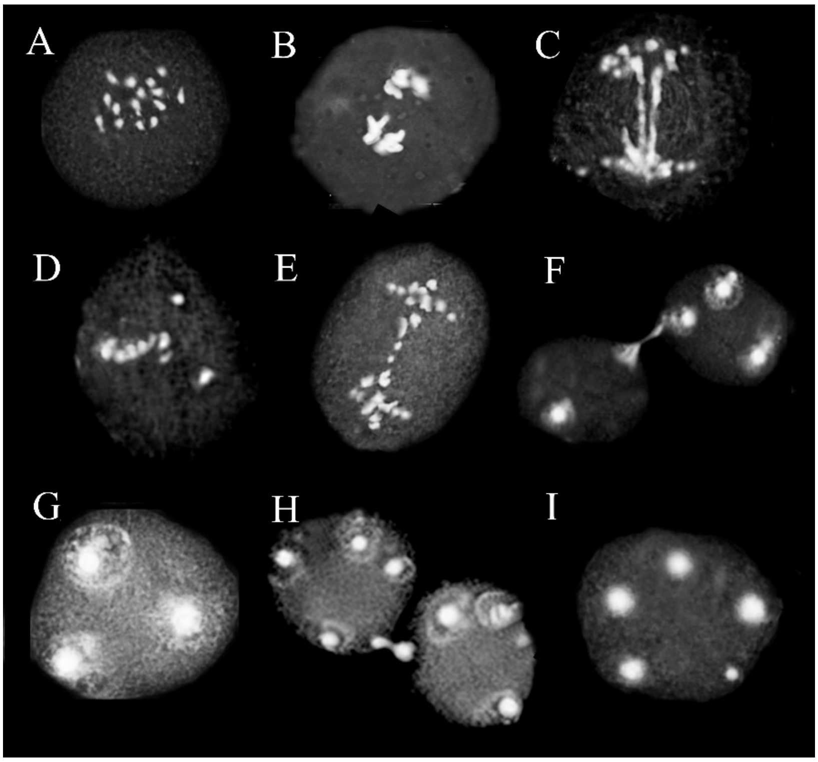

Figure 2. Different Abnormality in the Polluted Populations of *Verbacsum speciosum* induced by HMs: A, diakinesis showing 2n = 2x = 30 chromosome; B, Sticky chromosome; C, Bridge in anaphase I; D, non-synchronous segregation; E, Laggard in anaphase I; F, cytomixis between two cells in telophase I; G, tripolar cell; H, cytomixis between two cells in telophase II; I, Micronucleus in telophase II

Table 3. Percentage of PMCs in different stages, percentage of PMCs Exhibiting Meiotic Abnormalities

Meiosis Abnormalities	Polluted Plants (Mean ± SE)					Control
	a	b	c	d	e	
Total cell number	918	881	924	979	941	925
D/MI	345	293	336	355	315	323
% D/MI	37.5	33.2	36.3	36.2	33.4	35.1
% Sticky chromosome	35.2 ± 0.2	28.3 ± 0.1	19.5 ± 0.4	12.8 ± 0.01	7.6 ± 0.5	3.3 ± 0.2
% Cytomixis	17.6 ± 0.6	14.2 ± 0.3	10.2 ± 0.12	6.8 ± 0.9	3.2 ± 0.3	0.9 ± 0.04
AI/TI	187	223	215	197	207	210
% AI/TI	20.3	25.3	23.2	20.1	21.9	22.7
% Cytomixis	11.7 ± 0.1	6.3 ± 0.4	2.1 ± 0.2	-	-	-
% Bridge	10.3 ± 0.7	8.5 ± 0.2	5.7 ± 0.8	4.1 ± 0.1	3.3 ± 0.4	1.4 ± 0.1
% Laggard	17.8 ± 0.8	11.3 ± 0.2	9.6 ± 0.4	7.5 ± 0.2	3.6 ± 0.5	-
% Non-synchronous segregation	15.4 ± 0.3	10.7 ± 0.7	7.3 ± 0.8	5.8 ± 0.2	2.3 ± 0.6	-
MII	123	112	94	133	121	107
% MII	13.3	12.7	10.1	13.5	12.8	11.7
% Cytomixis	19.3 ± 0.5	15.6 ± 0.6	10.9 ± 0.8	5.8 ± 0.2	2.3 ± 0.6	0.9 ± 0.02
% Sticky chromosome	24.2 ± 0.3	18.7 ± 0.7	10.8 ± 0.6	6.5 ± 0.5	3.8 ± 0.2	2.8 ± 0.01
AII/TII	263	253	279	294	298	283
% AII/TII	28.6	28.7	30.1	30	31.6	30.5
% Laggard	14.6 ± 0.3	11.2 ± 0.8	7.3 ± 0.1	4.2 ± 0.4	2.1 ± 0.5	0.6 ± 0.2
% Bridge	7.3 ± 0.2	5.8 ± 0.6	2.4 ± 0.2	0.5 ± 0.02	-	-
% Cytomixis	7.5 ± 0.4	2.1 ± 0.2	1.8 ± 0.8	-	-	-
% Non-synchronous segregation	10.7 ± 0.2	3.2 ± 0.9	1.2 ± 0.3	-	-	-
% Micronucleus	8.3 ± 0.6	6.2 ± 0.2	4.7 ± 0.5	3.2 ± 0.3	2.1 ± 0.2	-
% Tripolar	4.3 ± 0.1	2.1 ± 0.4	-	-	-	-
% Pentapolar	28.5 ± 0.9	23.7 ± 0.2	16.1 ± 0.3	11.5 ± 0.5	7.4 ± 0.6	1.5 ± 0.2
% Meiotic Index	62.5	70	77.5	83	92	98.5

The meiosis index was assessed in both the polluted and control populations of *Verbacsum speciosum*. All values are expressed as mean ± SE (standard error). All values are expressed as mean ± SE (standard error).

in the control plant showed micronuclei (Figure 2I). In the tetrad stage of contaminated plants, PMCs with three and five poles were observed. Penta-polar anomalies were observed in all polluted localities, while tri-polar anomalies were only seen in localities ‘a’ and ‘b’ (Figure 2G). Only 1.5 ± 0.2% of PMCs showed penta-polar anomalies in the control plant, and no tri-polar anomalies were observed in this locality.

Numerous studies have documented various abnormalities resulting from the impacts of HMs.[12,13,32] Hajmoradi and Moghadami[28] demonstrated that Pb can cause abnormalities such as cytomixis. In a study by Kumar and Tripathi,[11] it was shown that environmental factors such as temperature, HMs (lead), and water can increase cytomixis and other chromosomal abnormalities. Another study investigating the effect of HM mercury (Hg) on the plant *Iberis amara* observed a high frequency of chromosome fragmentation and bridges among meiotic and mitotic abnormalities[12]. Furthermore, a study examining the potential genotoxicity of Cd and Hg in soybean plants observed a wide range of chromosomal abnormalities, including a high frequency of non-synchronous chromosome separation. The study stated that Hg has more genotoxic effects compared to Cd.[32]

Meiotic Index

As the distance from the mine increased, there was an observed increase in the MI, calculated by dividing the number of normal tetrads by the total number of observed tetrads. Among the contaminated plants, locality ‘a’ exhibited the lowest MI, while locality ‘e’ showed the highest. The MI is influenced by an increase in chromosomal abnormalities, leading to a decreasing trend, which can potentially impact the fertility of the plant. Previous studies have also reported a correlation between a decrease in the MI and an increase in chromosomal abnormalities.[13,21,33]

Conclusion

The accumulation of HMs in *Verbacsum speciosum* poses a potential risk to individuals who harvest these medicinal plants from their natural habitats. Moreover, *Verbacsum speciosum* serves a valuable role as a bio-indicator for monitoring specific HMs in the environment. Plants that exhibit high tolerance to HMs are well-suited for phytoremediation, effectively cleaning and removing

HMs from the soil. Utilizing *Verbacsum speciosum* for environmental purification and remediation can be an economical and cost-effective approach. Furthermore, considering the increased occurrence of meiotic chromosomal abnormalities at higher concentrations of Pb and Zn, these abnormalities can serve as suitable indicators for assessing the presence of mutagenic substance pollution.

Authors' Contribution

Conceptualization: Fatemeh Hajmoradi, Foozieh Moghadami.
Data curation: Fatemeh Hajmoradi.
Formal analysis: Fatemeh Hajmoradi, Foozieh Moghadami.
Funding acquisition: There was no funding for this work.
Investigation: Fatemeh Hajmoradi.
Methodology: Fatemeh Hajmoradi, Foozieh Moghadami.
Project administration: Fatemeh Hajmoradi.
Resources: Fatemeh Hajmoradi, Foozieh Moghadami.
Software: Fatemeh Hajmoradi.
Supervision: Fatemeh Hajmoradi.
Validation: Fatemeh Hajmoradi.
Visualization: Fatemeh Hajmoradi, Foozieh Moghadami.
Writing–original draft: Fatemeh Hajmoradi.
Writing–review & editing: Fatemeh Hajmoradi.

Competing Interests

The authors declared no conflict of interest.

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