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Studying the effect of heavy metals and the preservative at the cellular level using Allium Cepa. L as a bioindicator

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***Abstract*---**Environmental pollution is a matter of concern for living organisms and the environment, so bioindicators have been used to detect the harmful effect of chemicals. In this study, *Allium cepa L.* plant was used as a bioindicator to detect the toxicity of heavy metals that causes chromosomal abnormalities in the growing points of meristematic cells of onion plants; the metals were: cadmium, zinc, copper, lead, polluted water and the preservative (sodium benzoate). It has been observed that heavy metals and the preservative cause various types of chromosomal abnormalities, which include chromosomal dispersal, sticky chromosome, ring chromosome, chromosomal fragments, lagging chromosome, multipolar chromosome, and c-mitosis, star chromosome, chromosome bridge, and centromere break. Depending on the mitotic index, its decrease is an indication of a decline in the division average, indicating a disorder in the cell division pathway, from the most effective to the least effective: sodium benzoate > copper > lead > polluted water > zinc > cadmium. The study aimed to estimate the percentage of damage in living cells resulting from the effect of heavy metals and the preservative used in food products that are directly related to human life.

***Keywords*---**heavy metals, chromosomal abnormalities, Allium cepa. L.

Introduction

Environmental pollution with heavy metals has become a global problem because of the increasing industries. Metals and minerals pose detrimental effects on ecosystems and affect human health due to their toxicity, non-degradable nature, and ease of bioaccumulation. Thus, they can be a potential threat to food security (Sabeen et al.,2020). Heavy metals can cause oxidative damage to macromolecules and the photosynthetic system in the cell resulting in physiological and/or biochemical irregularities with decreased membrane stability, photosynthesis, nutrient imbalance, and inhibition of cell division, and DNA replication, and gene expression. Although plants have evolved complex regulatory mechanisms to adapt to heavy metals stress, however, under extreme conditions, it may severely affect plant health which results in cell death (Jeena et al.,2021). Exposure to toxic substances leads to a group of negative symptoms that occur in the organism, which are cellular genetic symptoms that are naturally reflected in the activities and functions of different organisms. Cell division is considered a breeding ground for discovering many cytogenetic abnormalities and changes (Fadel,2015).

As a result of the rapid increase in the use of chemicals such as food preservatives, it has become essential to know whether these chemicals have adverse effects on the genetic makeup of living organisms (Dosay,2020). Sodium Benzoate SB is widely used as a food preservative and in a wide range of cosmetic products. Sodium benzoate has been reported to have genotoxic and carcinogenic effects. The studies that have been conducted on the living body indicated the efficacy of sodium benzoate in causing anxiety and causing oxidative stress, and many genotoxic factors (Qari,2017).

Because toxicants in humans are of great concern, various plant, animal, and microbial bioindicators have been developed to assess toxicity and contamination. The onion test reveals *Allium Cepa L.*, which is a member of the family Liliaceae. Still, according to modern taxonomic charts, the genus *Allium* belongs to the family Amaryllidaceae, which is the most common botanical experiment about chromosomal damages in the meristematic cells of A. cepa root caused by the genotoxicity of dangerous chemicals, *Allium* test is suitable for being cheap and straightforward, with few chromosomes and a large size (Marrelli et al.,2019; Çavuşoğlu et al.,2022).

Material and Methods

Preparation of the sample

Allium Cepa L. plant sources

The plant *Allium cepa L.* with a diameter of (1-2.5 cm) of the crystal cultivar was obtained from the local markets of the city of Mosul.

Treating The Roots of Onion with Heavy Metals

Standard solutions of the four metals: cadmium, zinc, copper, and lead, were prepared for the following concentrations (5, 10, 20, and 25 ppm) (Abubacker et al.,2017). Also, a sample of polluted water was taken and prepared with two concentrations, diluted with a concentration of 50 ppm and a concentrated sample. Also, standard solutions of the preservative sodium benzoate were ready for the following concentrations (50, 100, 200, and 500 ppm) (Kostadinova et al.,2021).

Genotoxic analysis

The bulbs were treated with heavy metal solutions for four days (Abubacker et al.,2017), while the preservative SB was treated for three days with different exposure times (Kostadinova et al.,2021). after that, the roots were cut to (0.5-1.5 cm) and then placed in Clark's solution (ethanol: acetic acid 3:1) for 24 hours. After the end of the treatment period, the roots are set in 70% ethanol alcohol and kept at a temperature of 4 C until performing the test.

When conducting the test, the roots are placed in HCl 1N acid for a minute to soften the tissues. The roots are washed with water to remove the acid. 2-3 drops of Aceto carmine dye prepared by Solarbio company are added to the root, and the slice is exposed to fire. The duration of root dyeing is 20 minutes. Then the roots are washed with water three times to get rid of the excess dye, then the root is cut with a blade, and a drop of water is placed on the cut root for ease of the mashing process, then the slide cover is placed, taking into account that no bubbles occur. The slide is placed between the pages of a book and is pressed; then, nail polish is applied to the edge of the slide cover. All slides were analyzed using an oil loupe at 1000X magnification.

Mitotic index (MI) was determined by scoring approximately 23,000 cells (1,000 cells per slide) using the following formula:

Mitotic Index (%) = (Number of dividing cells/Number of total observed cells) ×100 (Yadav et al.,2019).

The decrease in the mitotic function indicates a reduction in the division average, indicative of a disorder in the cell division pathway (Fadel,2015).

Results and Discussion

The results indicated that the meristematic cells of the growing points of *Allium cepa. L* roots treated with different concentrations of heavy metals showed chromosomal abnormalities compared with the control treatment. The results were compared based on the mitotic index MI as shown in Table (1) and (2), which indicates the types of chromosomal abnormalities in the growing points of onion plants exposed to heavy metals. It was observed that cadmium metal at concentrations (5 and 10 ppm) had a higher percentage than the control treatment, with a value of 30.5%. In comparison, the concentration (10 and 20 ppm) was lower than the control treatment. The concentrations were compared

with each other. It was noted that the 25 ppm concentration showed a 24.6% decrease in the mitotic index. As for zinc, it was observed that the concentrations (5 and 10 ppm) showed a higher percentage than the control treatment, while the concentrations (20 and 25 ppm) showed a lower percentage than the control treatment. When comparing the concentrations with each other, it was found that the 25 ppm concentration showed a 17.2% decrease in the mitotic index. As for copper, the results showed that the percentage of the mitotic index for all concentrations was less than the control treatment. When comparing the concentrations with each other, it was noticed that the concentration of 20 ppm showed a significant decrease of 0.6% in the mitotic index. As for lead, the results showed that the concentrations (10 and 25 ppm) increased the mitotic index compared with the control treatment, while a decrease in the mitotic index was observed at the concentrations (5 and 20 ppm).

Table (1) Types of chromosomal abnormalities in the growing points of onion plants exposed to heavy metals

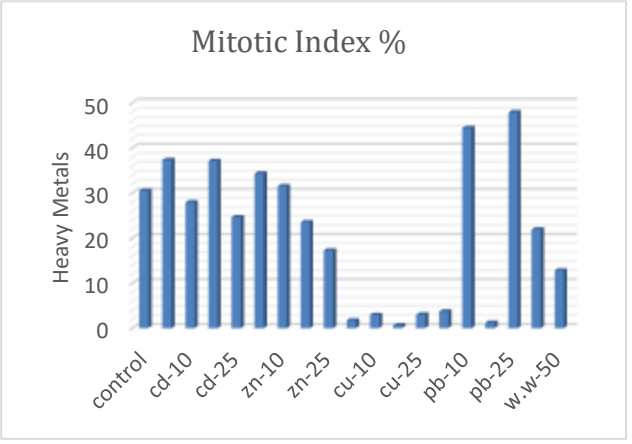
Treatments		Concentration ppm ppm	The number of examined cells	The number of dividing cells	The number of non-dividing cells	Types of chromosomal abnormalities										
						Prophase				Anaphase						
						Disturbed	Stickiness	ring chromosome	star chromosome	Disturbed	Stickiness	ring	fragments	lagging chromosome	c-mitosis	multipolar
The control treatment		0	1000	305	695	0	0	0	0	0	0	0	0	0	0	0
Heavy metals	Cd	5	1000	373	627	19	10	0	0	0	0	0	13	16	0	37
		10	1000	279	721	8	4	0	0	15	0	0	22	6	0	15
		20	1000	371	629	15	11	0	0	7	0	0	9	17	0	23
		25	1000	246	754	10	5	0	0	0	0	0	7	3	0	20
	Zn	5	1000	343	657	9	2	0	0	13	0	0	6	0	51	5
		10	1000	315	685	12	6	0	0	17	0	0	12	0	86	0
		20	1000	235	765	22	13	0	0	13	0	0	9	0	38	0
		25	1000	172	828	3	7	0	0	2	0	0	5	0	15	1
	Cu	5	1000	17	983	0	3	0	0	0	0	0	0	0	4	0
		10	1000	29	972	0	0	0	0	0	0	0	0	0	0	0
		20	1000	6	994	0	0	0	0	0	0	0	0	0	0	0
		25	1000	30	970	0	0	0	0	5	0	0	0	0	0	0
	Pb	5	1000	37	963	0	0	2	0	2	0	0	0	0	3	0
		10	1000	444	556	42	50	13	0	16	0	0	0	0	101	0
		20	1000	12	988	0	3	0	0	0	0	0	0	0	4	0
		25	1000	479	521	21	4	1	2	38	21	1	8	4	2	26
	w.w	Concentrated	1000	219	781	22	0	3	0	26	21	0	0	0	1	0
		Diluted	1000	128	872	3	0	1	2	13	7	0	0	0	0	1

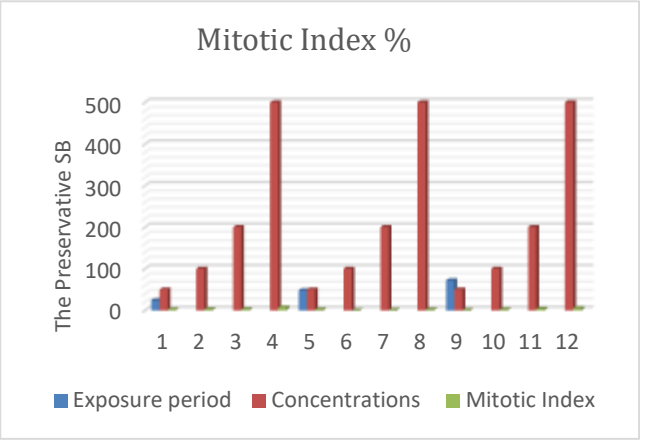
w.w: wastewater

Table (2) Types of chromosomal abnormalities in the developing points of onion plant roots treated with different concentrations of heavy metal

Treatment		Concentration PPm	Types of chromosomal abnormalities											Mitotic index %
			Anaphase								Telophase			
			Disturbed	lagging chromosom e	ring chromosome	chromosome fragments	lagging chromosom e	c-mitosis	multipolar	chromosome bridges	Stickiness	bridges	lagging chromosome	
control		0	0	0	0	0	0	0	0	0	0	0	0	30.5
Heavy metals	Cd	5	0	0	0	7	21	0	14	10	35	1	0	37.3
		10	0	0	0	2	10	0	9	8	15	0	0	27.9
		20	0	0	0	3	8	0	2	5	23	0	2	27.1
		25	0	0	0	1	7	0	1	5	28	0	2	24.6
	Zn	5	0	0	0	0	13	56	1	8	9	1	0	34.3
		10	0	0	2	0	0	68	0	0	0	0	0	31.5
		20	0	0	1	0	1	43	0	13	8	0	0	23.5
		25	6	0	0	1	1	4	0	2	13	0	0	17.2
	Cu	5	0	0	0	0	0	2	0	0	2	0	0	1.7
		10	0	0	0	0	1	1	0	0	11	0	0	2.9
		20	0	0	0	0	0	0	0	0	0	0	0	0.6
		25	0	0	0	0	0	0	0	0	11	0	0	3
	Pb	5	1	0	0	0	0	7	0	0	6	0	0	3.7
		10	4	0	0	0	0	44	0	0	7	0	0	44.4
		20	0	0	0	0	0	3	0	0	1	0	0	1.2
		25	6	0	0	6	18	6	0	14	41	0	0	47.9
	w.w	Concentrated	3	0	0	1	5	4	0	6	21	0	0	21.9
		Diluted	5	0	0	4	10	1	1	2	10	0	0	12.8

When comparing the concentrations with each other, it was found that the 20 ppm concentration showed a 1.2% decrease in the mitotic index. Figure (1) shows the percentage of chromosomal abnormalities in the roots of onion plants treated with heavy metals and the preservative. Polluted water showed that both concentrated and diluted in the concentration of 50 ppm treatments showed a decrease in MI compared to the control treatment, and 50 ppm showed a more significant reduction in the mitotic index than concentrated polluted water. This result agrees with the study (Paul *et al.*,2018).





a- heavy metals b- preservative (sodium benzoate)

Figure (1) Percentage of chromosomal abnormalities in the developing cells of onion plant roots treated with heavy metals and the preservative

Heavy metal exposure prevented plant cells from entering the cell division stages, which decreased the mitotic index. Also, exposure of spindle filaments to heavy metals enhanced chromosomal abnormalities during cell division. The decrease in the mitotic index of cells treated with heavy metals is due to disturbances in the cell cycle or chromatin abnormalities caused by the interaction of metals with DNA (Abubacker et al.,2017).

The roots of the onion plant were also exposed to the preservative sodium benzoate SB for different periods, as the preservative caused chromosomal abnormalities in the meristematic cells of the roots of *Allium cepa L.* Table (3) shows the types of chromosomal abnormalities of onion plants caused by exposure to the preservative. The results showed that the mitotic index for all exposure periods (24, 48, and 72 h) and at concentrations (50, 100, 200, and 500 ppm) decreased compared to the control treatment. The concentrations were compared with each other at different exposure periods. It was noted that the mitotic index decreased at the concentration of 50 ppm and for the duration of exposure of 24 and 72 h. As for the 48h exposure period, the lowest percentage was at a concentration of 100 ppm, in which no cell division occurred. Where the results are consistent with the study of (Kostadinova *et al.*, 2021) that the preservative SB has a significant effect on reducing cell division, it was also mentioned that when the cell is exposed to adverse conditions such as exposure to chemicals, i.e.,,, under a toxic effect, this can disrupt cell division and cause abnormal division.

Table (3) Types of chromosomal abnormalities in the developing tops of onion roots treated with the preservative sodium benzoate

Exposure period (hour)	Concentration ppm	The number of examined cells	The number of dividing cells	The number of non-dividing cells	Types of chromosomal abnormalities													Mitotic index %
					Prophase			Metaphase				Anaphase					Telophase	
					Disturbed	Stickiness	ring	Disturbed	Stickiness	c-mitosis	break	Disturbed	Stickiness	fragment	c-mitosis	bridge	Disturbed	
24	50	1000	20	980	2	2	2	2	8	0	0	1	0	0	3	0	1	2
	100	1000	24	976	1	0	0	4	2	0	0	6	0	0	0	0	1	2.4
	200	1000	26	974	0	0	0	2	2	10	0	0	0	0	3	1	0	2.6
	500	1000	67	933	3	8	0	1	16	7	0	0	4	0	7	0	7	6.7
48	50	1000	18	982	0	4	0	3	1	0	0	1		1	1	0	3	1.8
	100	1000	0	1000	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	200	1000	8	992	0	1	0	0	0	3	1	0	2	0	1	0	1	0.8
	500	1000	17	983	3	1	0	0	4	2	0	0	0	0	0	0	1	1.7
72	50	1000	6	994	0	4	0	0	0	0	0	0	0	0	0	0	2	0.6
	100	1000	18	982	0	0	0	0	6	1	0	0	0	0	0	0	2	1.8
	200	1000	35	965	2	3	0	3	7	4	0	0	1	0	4	0	0	3.5
	500	1000	49	951	3	7	0	0	9	10	0	0	1	0	3	0	1	4.9

In this study, the toxic effects of cadmium, zinc, copper, lead, polluted water, and the preservative led to many distortions. It was noted that cadmium metal caused chromosomal dispersal, sticky chromosome, chromosomal fragments, lagging chromosomes, multipolar chromosomes, and chromosomal bridges. As for copper, it caused sticky chromosomes, chromosomal dispersion, and c-mitosis. As for zinc, lead, and polluted water caused chromosomal dispersal, sticky chromosome, ring chromosome, chromosomal fragments, lagging chromosome, multipolar chromosome, and c-mitosis, except for lead, which caused a star chromosome polluted water caused a chromosome bridge.

As for the preservative is caused at the 24-hour exposure, chromosomal dispersal, sticky chromosome, ring chromosome, C-mitosis, and chromosomal bridge. As for the 48-hour exposure period, chromosomal dispersal, sticky chromosomes, C-mitosis, centromere break, and chromosomal fragments were observed. A 72-hour exposure period was observed for chromosomal dispersion, sticky chromosomes, and C-mitosis.

The reason for the occurrence of lagging chromosomes is due to the failure of the chromosomes to attach to the spindle filaments and move to either pole (Khanna et al.,2013) . Chromosome fragments result from multiple chromosome breaks where there is a loss of chromosome integrity. Chromosome fragmentation can range from partial disintegration to complete disintegration of the chromosome (chromosome crushing) (William,1978). The ring chromosomes result from a break in the ends of the two arms of the chromosome, followed by the fusion of the broken ends, or from the union of a broken end in the chromosome with the corresponding telomere region, resulting in the loss of genetic material. Alternatively, they can be formed by fusion of sub telomeric sequences or fusion of telomeres without deletion, resulting in complete ring chromosomes (Guilherme et al.,2011). Bridges are created by the breaking and fusion of chromosomes and chromatids. Such chromosomal bridges may occur as a result of exposure to chemicals, or chromosomal bridges may also be formed due to chromosomal adhesion and/or unsuccessful separation of chromosomes in anaphase phase;

otherwise, it may be attributed to unequal translocation or inversion of chromosome segments (Obidi et al.,2017).

C-mitosis occurs because one or more separate chromatids cannot move towards the poles of the cell (Lee et al.,2019). Figure (2) shows the types of chromosomal abnormalities in onion roots exposed to heavy metals and the preservative.

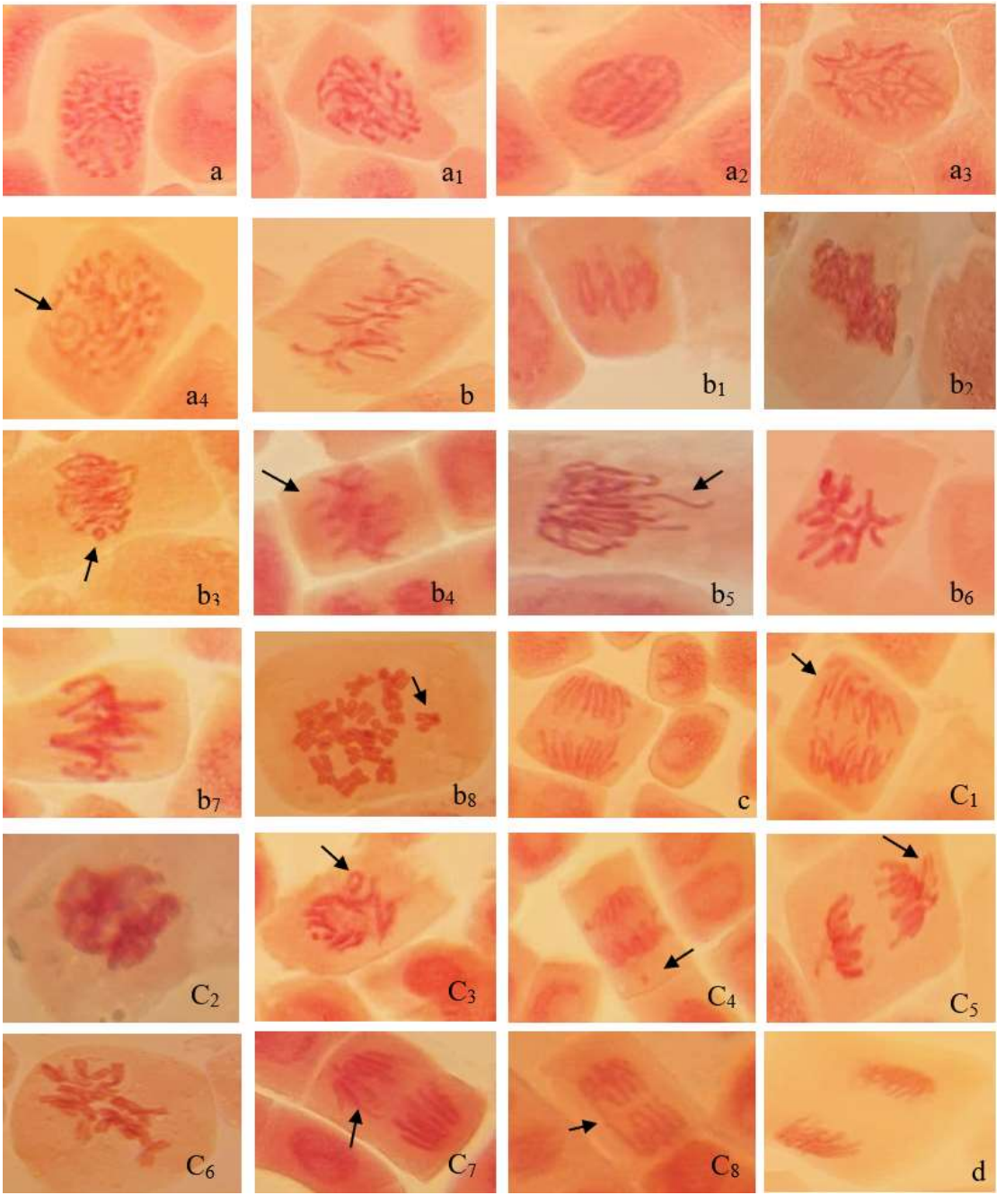


Figure (2) Types of chromosomal abnormalities in the growing points of *Allium cepa* roots treated with different concentrations of heavy metals and preservatives at 1000X magnification

a- Normal prophase	a_1- prophase-Disturbed	a_2- prophase- sticky
a_3- prophase- ring chromosome	a_4- prophase-star	b- natural Metaphase
b_1- Metaphase -Disturbed	b_2- Metaphase - sticky	b_3- Metaphase - ring chromosome
b_4- Metaphase - chromosomal fragments	b_5- Metaphase -lagging	b_6- Metaphase - c-mitosis
b_7- Metaphase - multipolar chromosome	b_8- Metaphase - break in centromere	C - normal Anaphase
C_1 - Anaphase -Disturbed	C_2 - Anaphase - sticky	C_3 - Anaphase - ring chromosome
C_4 - Anaphase - chromosomal fragments	C_5 - Anaphase - lagging chromosome	C_6 - Anaphase – c-mitosis
C_7 - Anaphase – multipolar	C_8 - Anaphase - Chromosome bridge	d - normal Telophase
d_1 - Telophase - sticky	d_2 - Telophase - lagging chromosomes	d_3 - Telophase - chromosomal bridges

Conclusion

The current study provides valuable research information about the toxic effects of heavy metals and the preservative by evaluating chromosomal abnormalities in meristematic cells of *Allium cepa L.* roots that are incurred by the discharge of heavy metals, whether from natural processes or human practices into the environment, as well as the preservative that is added to food. The A. cepa bioindicator is an integrated tool for detecting chemical toxicity and identifying cytogenetic abnormalities.

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