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# Effect of heavy metals on mitotic chromosome behaviour of *Aloe vera*

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## Abstract

The present work was conducted with a view to explore the effects of heavy metals on mitotic chromosomes of *Aloe vera*. Heavy metals viz., Cu, Ca, Fe, Zn and Mn were prepared in 50 ppm and 100 ppm concentrations each. Mitotic index was found to be decreased in heavy metal treated root tip cells. MI values range from 10.11 to 37.20 in 50 ppm concentration of heavy metals, while at 100 ppm concentrations of the heavy metals, the MI values showed variation from 9.33 to 24.33. Interestingly, Fe at 100 ppm concentration, completely checked mitotic activities of the cells. Frequencies of the individual mitotic stages also varied under different heavy metal treatments. Heavy metal treatments also resulted in chromosomal abnormalities viz., laggards, anaphase bridge, multipolarity, chromosome fragmentation, clumping, stickiness etc. Chromosomal aberrations were more pronounced at 100 ppm of the heavy metal concentration. Total abnormality percentage varied from 0 to 9.32 and 0 to12.0 at 50 ppm and 100 ppm concentration of heavy metals respectively. No chromosomal anomaly was detected in case of Mn treated root tip cells, while combined effects of heavy metals at 50 ppm concentrations lead to toxicity of the mitotic cells.

**Keywords:** Heavy metal, *Aloe vera*, root tip, mitotic index, ppm, chromosome abnormality, laggards, bridge, multipolarity etc.

## Introduction

Now a days heavy metals are one of the major concerns for the biologist because of their detrimental effects on living beings. Heavy metals are found in excess concentration in the contaminated sides which cause toxicity in both plants and animals (Nagajyoti et. al, 2010).Heavy metals in the forms of Cd, Hg, Ni, Cr, Pb are mostly lethal to the biological organisms. Toxicity heavy metals is shown by visible injury, retardation of growth and morphological deformity in the plants and animals. They have detrimental effects on cells metabolism and overall physiological activities of different life forms. In most severe cases it has been found that

heavy metals even cause chromosome breakage and nucleolytic attack on DNA leading to severe DNA damage (El-Shahaby et al, 2003). Accordingly, chromosome behaviour is subject to change when expose to different heavy metals in the concentrations beyond the permissible limit. Anomaly in the chromosome behaviour in the plants could act as in indicator of heavy metal pollution in the soil. Chromosome clamping, formation of laggards and acentric chromosome fragment and also the multi polarity are the major outcome in the chromosomal study in presence of toxic concentration different heavy metals (Kwankura et al, 2012). In these contexts, change of mitotic index value could also be taken as a parameter to evaluate the effect of different heavy metals on the mitotic chromosome behaviour.

Heavy metals are genotoxic chemicals as they do not break down and persist in the environment for prolonged period. Plant metabolic pathways are the main route for transferring toxic elements from the soil to humans (Chen et al., 2017). The resistance to toxic heavy metals in plants depends largely on age, family, nutritional status physiology and translocation efficiency of the plants, particularly within growing regions (Lasat et al., 2005). Heavy metals cause damage genetic material during the cell cycle (Gebhart, 1984). The genotoxic potential of iron, cadmium, nickel and copper has made them particularly challenging pollutants in soil (George, 2000; Kumar and Tripathi, 2007; Pandey and Upadhyay, 2010). Since the water and soil in agricultural areas may be polluted with heavy metals, the plants cultivated in the polluted soils will certainly be affected.

Root tips of seedlings are frequently used for chromosome or DNA damage assessment in mitotic cells of higher plants. *Aloe vera* is a commonly available model plant for the chromosomal study because of its bimodal karyotype. It's a succulent which requires minimum maintenance and could grow well even in oligotrophic soil. The roots are profusely branched and grow as a faster rate. It grows in soils having minimum water without any fertilizer requirement. The root tips are very delicate thus the effect of heavy metals will be very much pronounced. The present study aimed to evaluate the vulnerability of mitotic chromosomes of *Aloe vera* to the genotoxic effect of individual heavy metals, viz., Cu, Ca, Fe, Zn, Mn and also their synergistic effects.

## Materials and Methods

### Preparation of heavy metal solution:

The following heavy metals viz., Cu, Ca, Fe, Zn and Mn are prepared in 100 ppm concentrations from their corresponding sulphate salts.

### Preparation of plant material after heavy metal inoculation:

*Aloe vera* plants are grown freshly in plastic and earthen pots. 50 ml of each of the heavy metals in 100 ppm concentrations were added to the soil. After few days of growth, the root tips were collected by uprooting the plant gently from the soil. The roots are washed in tap water and 0.5 to 1.0 cm root tips were excised using forceps and put into a vial containing appropriate pre-treating agents. A control was also set to compare the results.

### Preparation of pre-treating agent and pre-treatment:

Colchicine was weighed 100 mg and was dissolved in 100 ml sterile water to make 0.1 % colchicine solution which was used as pre-treating agent for the mitotic arrest of dividing cell. Colchicine dissolves the spindle fibre resulting in inhibition in the progress of mitosis. The freshly excised the root tips submerged in pre-treating agent was kept 4-8°C temperature for 1 to 2 hours.

### Fixation:

After incubation in the pre-treating agent for the desired period of time, the root tips were transferred to 1:2 acetic alcohol solution and was kept at 4°C temperature for overnight period. This led to permanent killing of the dividing cells.

### Prior staining:

After overnight fixation the root tips were incubated in 45% acetic acids for 10 minutes. As the stain was prepared using the solvent 45% glacial acetic acid.

### Preparation of Stain:

2grams of orcein powder was weighed and mixed in 100ml of 45% glacial acetic acid in simmering temperature, after proper mixing by stirring the solution with glass rod. The solution was never heated at boiling temperature. After 20 minutes of heating the solution was filter using Whatman filter no. 1. The red coloured filtrate is used as the stain in the chromosome study.

### Staining:

The root tips were transferred to 9 drops of 2% aceto-orcein added with 1 drop of 1N (HCl) after decanting 45% glycerol acetic acid in a test tube vial. The test tube was gently heated over a flame for 3-4 seconds and was kept at room temperature 30-45 minutes for staining.

### Mitotic slide preparation

The slides were finally prepared through the squash preparation technique. The light microscope under oil immersion was used to calculate the mitotic index and chromosomal abnormalities in a different phase of mitosis. A minimum of 10 microscopic fields (70-100 cells/ field) were studied from each slide (Ranjbar et al., 2012). The mitotic index was determined by dividing the number of cells x 100 / total number of cells(Love, 1949). Total Abnormality Percentage (TAP) was also calculated by dividing the number of abnormal cells x 100 / total number of cells (Kumar and Bhardwaj, 2017). Also, the frequencies of the individual divisional stages were calculated.

## Results and Discussion

### Mitotic index in metal treated root tip cells:

Mitosis is looked upon as an important parameter of cells physiological activities. Observing mitotic cells under the microscope gives an impetus about cells normalcy. Chromosomes are discernible clearly under the microscopes and it is recommended as an efficient way to evaluate the effects of heavy metals on the behaviour of chromosomes. Chromosomal anomaly in mitosis is a reliable criterion for measuring the effect of physical mutagens (Ignacimuthu and Babu, 1989).

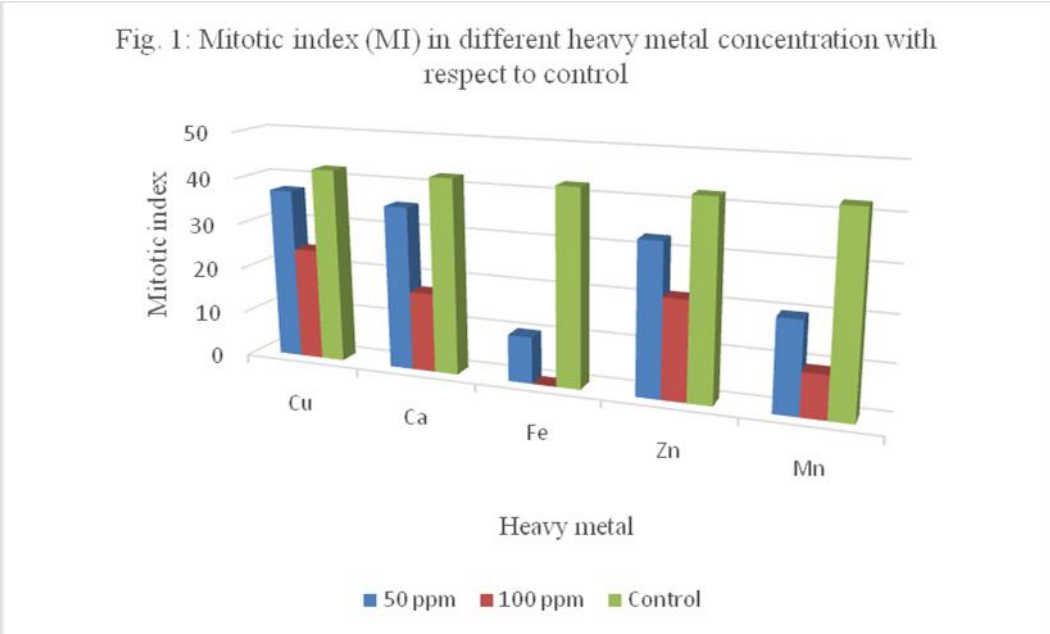
All studied mitotic cells of *Aloe vera*, were diploid and possessed a chromosome number of 2n=14. Mitosis was normal in the control set. However, an increase in mitotic abnormalities was observed in the meristematic root cells of *Aloe vera* in all Cu, Ca, Fe, Zn and Mn treatments at 100 ppm concentrations (Fig. 3). Though, aberrant chromosomes were also detected in treatments with heavy metals at 50 ppm concentrations, albeit in lower frequencies.

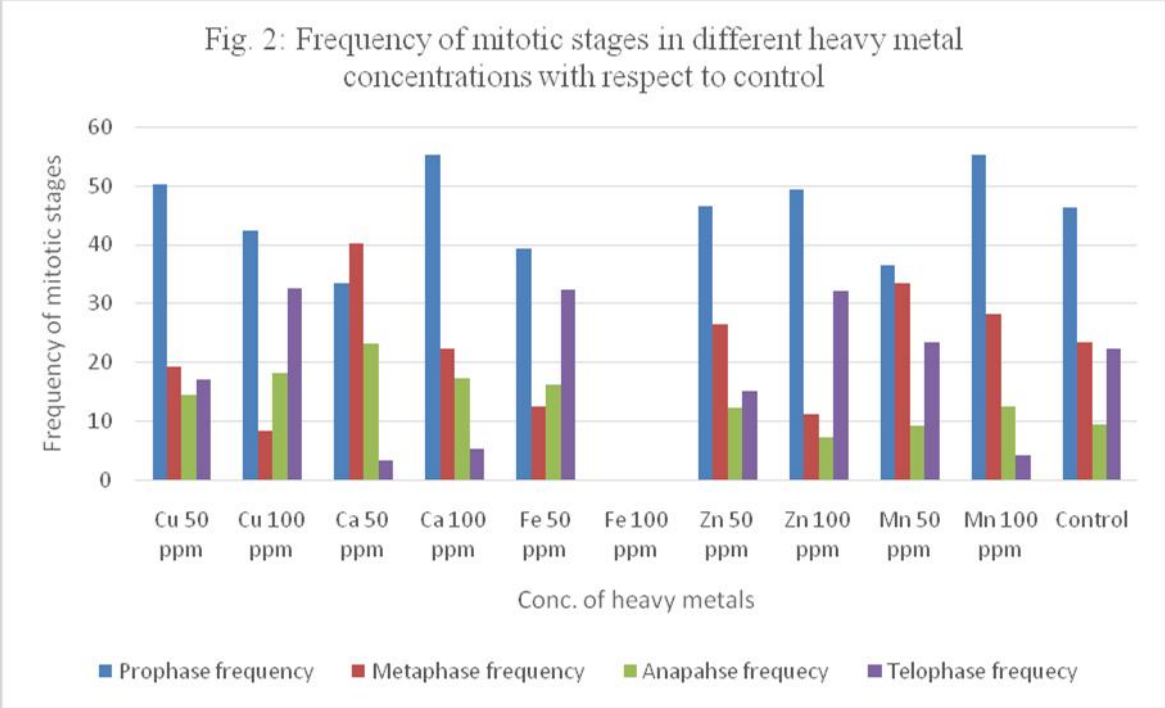
The mitotic index was used for the assessment of the genotoxicity of metals. The MI value in control was found maximum (42.40). In all the treated set up, MI value decreased (Table 1). The results were in agreement with the findings on effects of heavy metals on *Allium cepa* (Nafea& Khalil, 2022). The decline in MI value was more pronounced with treatments of heavy metals at 50 ppm concentrations. Minimum MI value was detected in case of Fe at 50 ppm concentration. The values of MI range from 10.11 to 37.20 at 50 ppm concentration of the respective heavy metals in the following order Cu (37.20)>Ca (35.54) >Zn (32.88) > Mn (19.75) > Fe (10.11). Likewise at 100 ppm concentrations of the heavy metals, MI values decreased even more and were in the range of 9.33 to 24.33. It is worth mentioning that Fe at 100 ppm concentration completely checked mitotic activity of the root tip cells of *Aloe vera* thus proving its genotoxicity (Fig. 1).

There was also considerable variation in the frequencies of the individual mitotic stages under different heavy metal treatments (Table 1). In most of the treatments, the frequencies of the mitotic stages were found to be decreased with increasing concentration of the respective heavy metals (Fig. 2).

Table 1: Mitotic index and frequencies of different mitotic stages in different heavy metal treated root tip cells of *Aloe vera*

| Name of heavy metals | Mitotic index in different conc. of heavy metals (ppm) | | Frequency of individual mitotic stages (%) in different conc. of heavy metals (ppm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 50 | 100 | Prophase | | Metaphase | | Anaphase | | Telophase | |
| | | | 50 ppm | 100 ppm | 50 ppm | 100 ppm | 50 ppm | 100 ppm | 50 ppm | 100 ppm |
| Cu | 37.20 | 24.33 | 50.25 | 42.24 | 19.30 | 8.25 | 14.35 | 18.11 | 17.10 | 32.40 |
| Ca | 35.54 | 17.22 | 33.33 | 55.10 | 40.20 | 22.24 | 23.23 | 17.33 | 3.24 | 05.33 |
| Fe | 10.11 | - | 39.20 | - | 12.33 | - | 16.22 | - | 32.25 | - |
| Zn | 32.88 | 21.50 | 46.45 | 49.33 | 26.30 | 11.25 | 12.15 | 07.24 | 15.10 | 32.18 |
| Mn | 19.75 | 09.33 | 36.44 | 55.19 | 33.33 | 28.11 | 9.19 | 12.50 | 23.04 | 4.20 |
| Control | 42.40 | | 46.33 | | 23.30 | | 09.32 | | 22.15 | |






## Chromosome abnormalities in metal treated root tip cells:

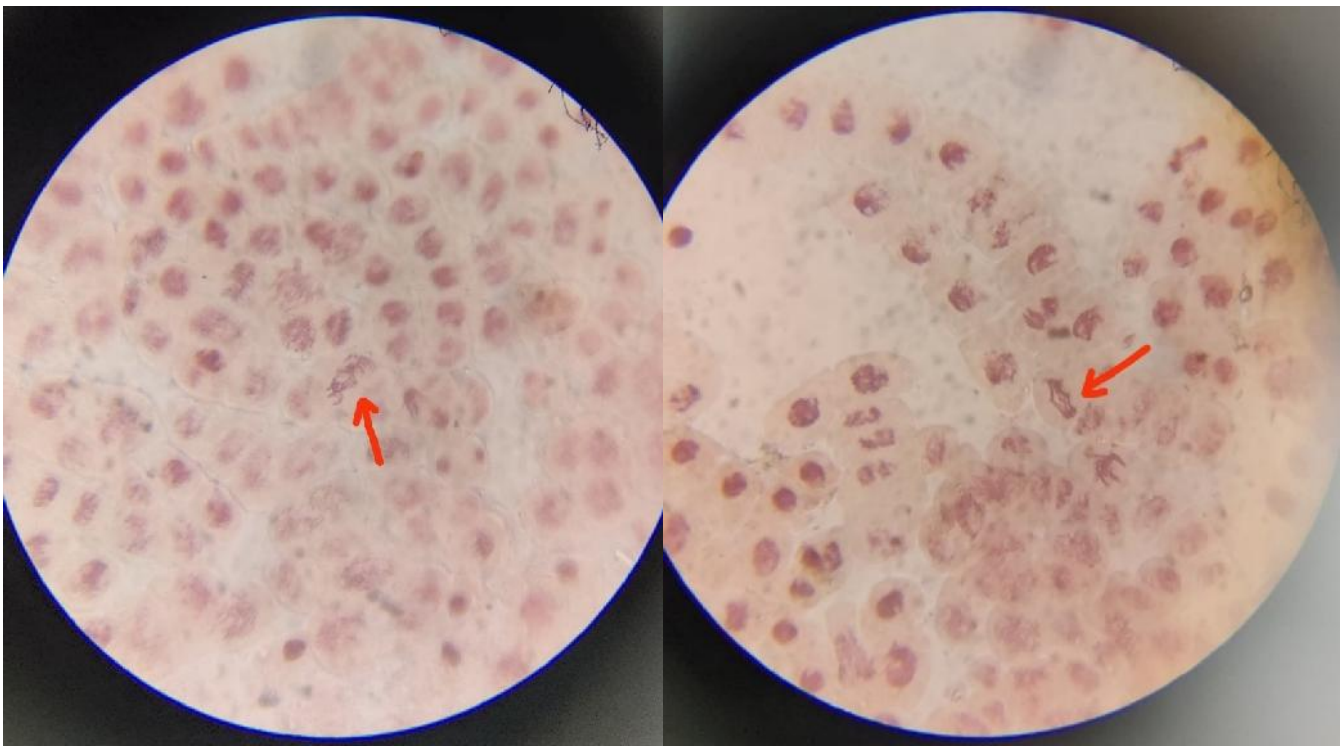
Heavy metal**s** viz.,Cu, Ca, Fe, Zn and Mn exhibited their toxic effects on chromosome behaviour of mitotic cells of *Aloe vera* in variable extent. This abnormality can be attributed to environmental factors (Ranjbar et al., 2012). A number of chromosomal anomalies were observed under the microscope in both 50 ppm and 100 ppm concentrations of the respective heavy metals. But chromosomal aberrations were more frequently found in 100 ppm of the heavy metal concentrations. Chromosome laggards, fragments, sticky bridge in anaphase, clumping of chromosomes, multipolarity were discernible under the microscope while observing metal treated mitotic cells of *Aloe vera*. Maximum chromosome abnormalities were detected in Cu treated root tip cells at 100 ppm concentration (Fig. 3a, 3b, 3c) with clumping, sticky bridge and multipolarity with anaphase bridge being the most common. Chromosome stickiness indicates highly toxicity of physical mutagen, which may lead to death (Turkoglu, 2009). The metal itself may react with the histone proteins and make them sticky (Ritambhara and Kumar, 2010). Chromosome stickiness in different plants can be attributed to genetic and environmental factors (Nirmala and Rao, 1996). More than one bridge and lagging chromosomes were observed in some mitotic cells treated with Cu. Chromosome bridges indicate a full chromosome structure breakdown, which leads to the formation of long and thin chromatin threads. Chromosome bridges are because of sticky chromosomes and subsequent free anaphase separation failure (Gomurgen, 2000). The single bridge is induced from the sister chromatid fusion at a common breakage point (Fig. 3 c).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.

Ca treatment result in chromosome fragmentation in both 50 ppm and 100 ppm concentrations (Fig. 3d, 3e). Gilli (1941) suggested that the acentric chromosome formation from lagging chromosomes. Fe at 50 ppm concentration led to chromosome clumping (Fig. 3. j) but 100 ppm concentration of the heavy metal exhibited toxic effects. Zn at 50 ppm generated normal

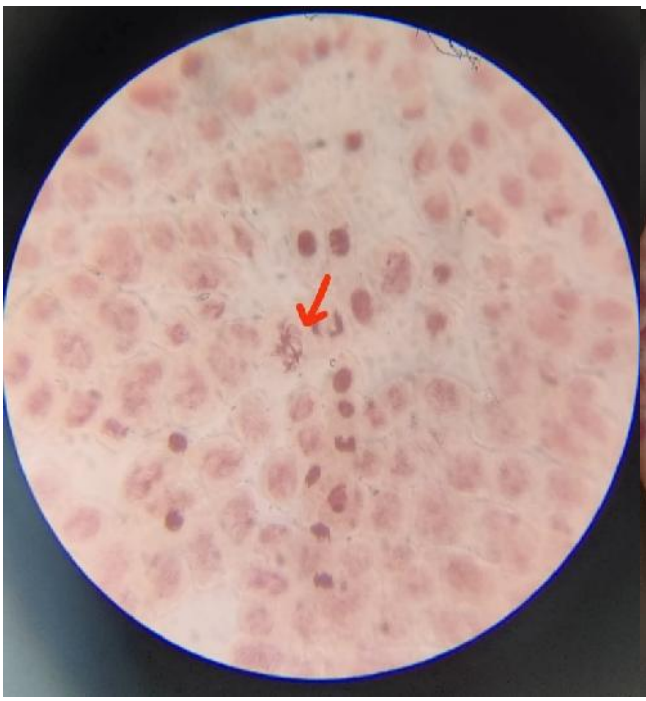
chromosome fragments (Fig. 3f) but at 100 ppm concentration excessive fragmentation of chromosome was found (Fig. 3g: pulverization) in addition to formation of chromosome laggards which proved its genotoxicity on the root tip cells of *Aloe vera*. The reason for the occurrence of lagging chromosomes 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 chromosomes breaks where there is a loss of chromosome integrity. Chromosome fragmentation can range from partial disintegration to complete disintegration of the chromosome called chromosome crushing, (William, 1978). Interestingly, no chromosome anomalies were discernible in Mn treated root tip cells. Genotoxity of the heavy metals were clearly evident when all the heavy metals were applied to root tip cells at 50 ppm concentrations each (Fig. 3k). The frequency of the abnormalities in different metal treatments is as follows: clumping >laggards >anaphase bridge > multipolarity > stickiness. The results were in accordance with the findings of genotoxic effects of heavy metals on mitotic chromosomes of *Trigonella foenum-graecum* (Hajmoradi & Kakaei, 2021)

Percentage of chromosomal abnormality showed a constant variation among the metal treated root tip cells. Percentage of anomaly increased in 100 ppm concentrations of heavy metal though significant percentage of chromosomal anomaly was also detected in 50 ppm concentrations of the heavy metals. Among the metals, Fe at 50 ppm and Cu at 100 ppm concentrations exhibited maximum chromosomal aberrations with the percentage values of 9.32 and 12.0 respectively. (Table. 2, Fig. 4). Similar results were also found while observing the genotoxic effects of heavy metals Cr, Cu, Pb and Zn on *Allium cepa* root meristematic cells (Abubacker & Sathya, 2017).

Heavy metal exposure prevented root meristematic cells from entering into the cell division stages, which decreased the mitotic index values considerably. Also, exposure to heavy metals enhanced chromosomal abnormalities during divisional phase. The decrease inthe mitotic index of the treated cells with heavy metals is due to disturbances in the cell cycle and chromatin abnormalities were caused by the interaction of metals with DNA (Abubacker et al., 2017)

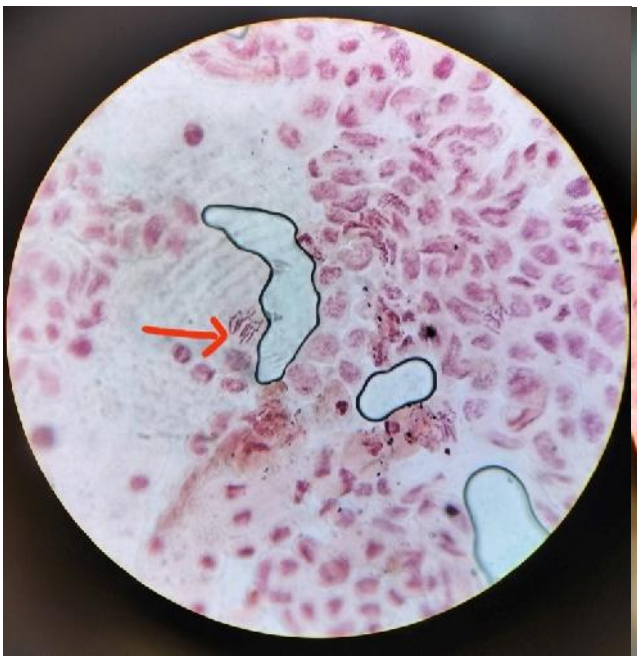


3.a: Treatment with Cu (50 ppm): Clumping3. b: Treatment with Cu (100 ppm): Sticky bridge

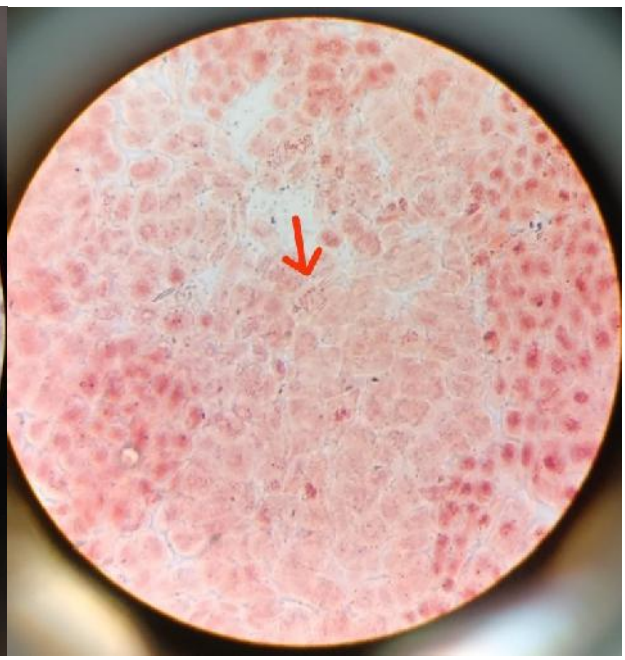


3.c: Treatment with Cu (100 ppm): Anaphase bridge with multipolarity

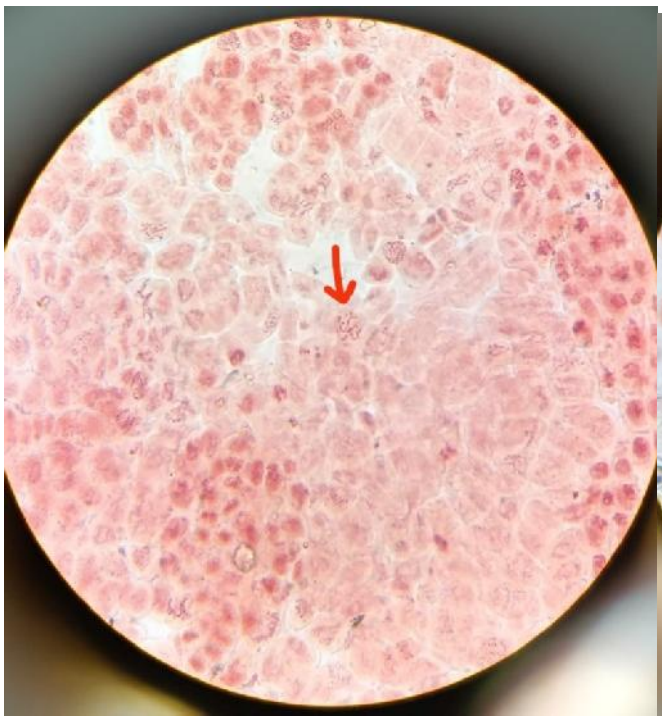
3.d: Treatment with Ca (50 ppm): chromosome fragments



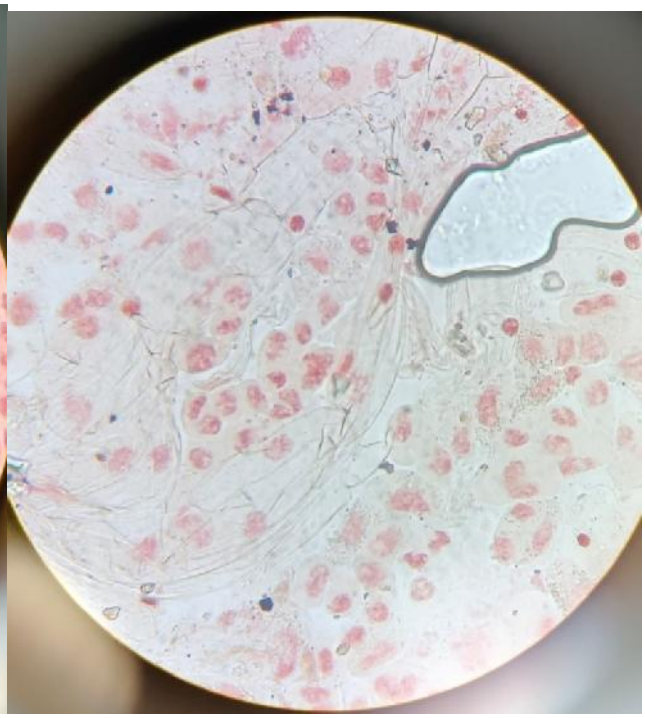
3.e: Treatment with Ca (100ppm): Chromosome fragmentsin excess



3.f: Treatment with Zn (50 ppm): Chrmosome fragments

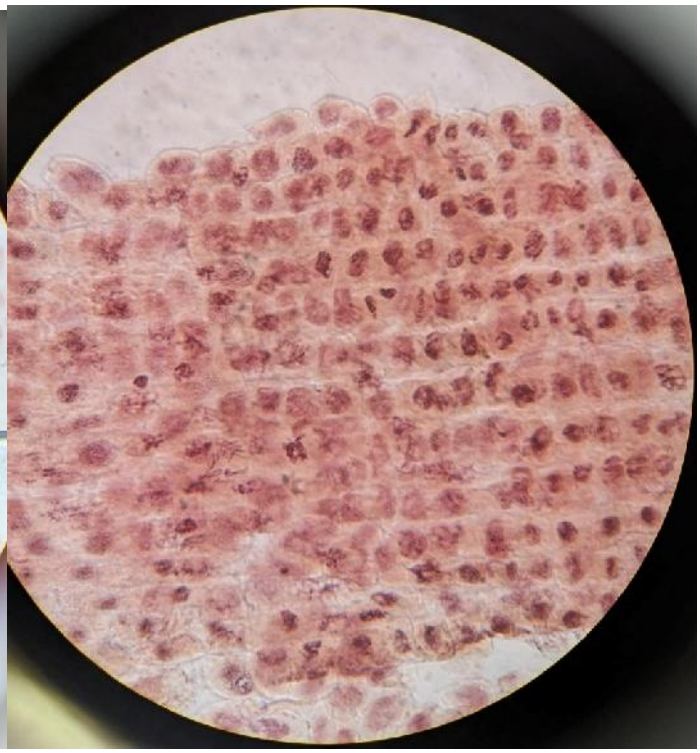


3.g: Treatment with Zn (100 ppm): pulverization

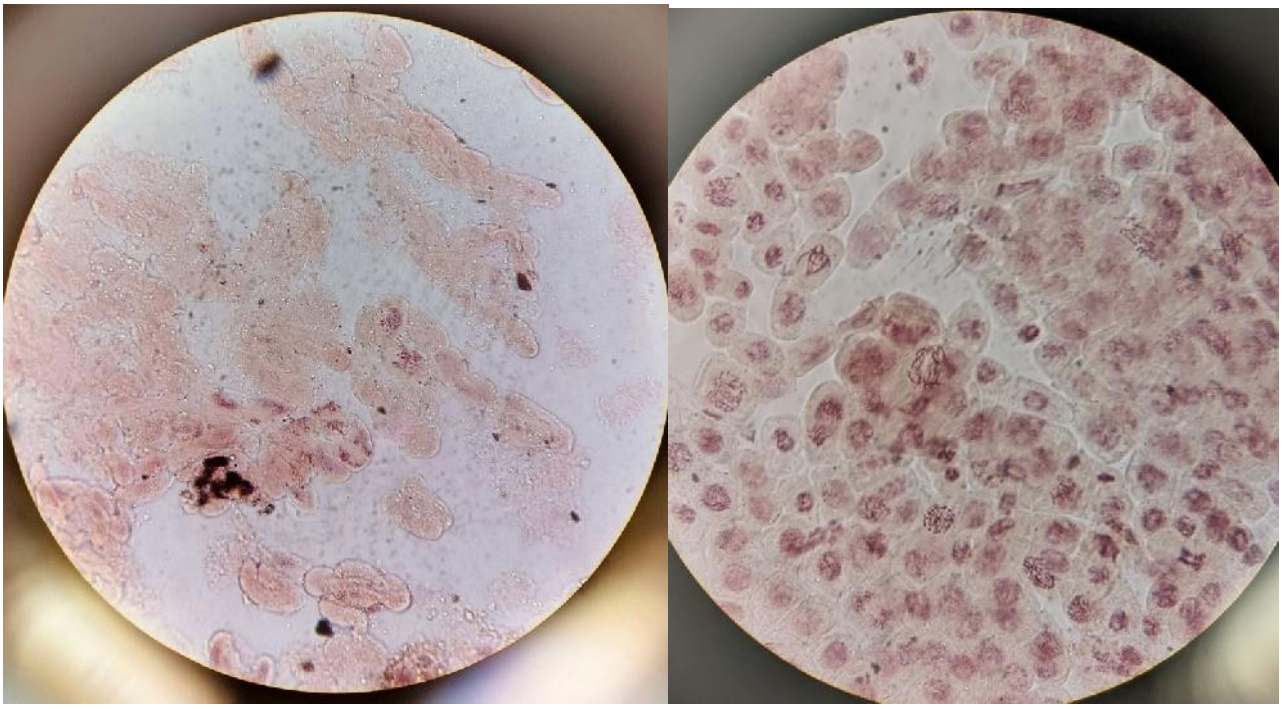


3.h: Treatment with Mn (50 ppm): no anomaly

3.i: Treatment with Mn (100 ppm): no anomaly



3.j: Treatment with Fe (50 ppm): chromosome clumping

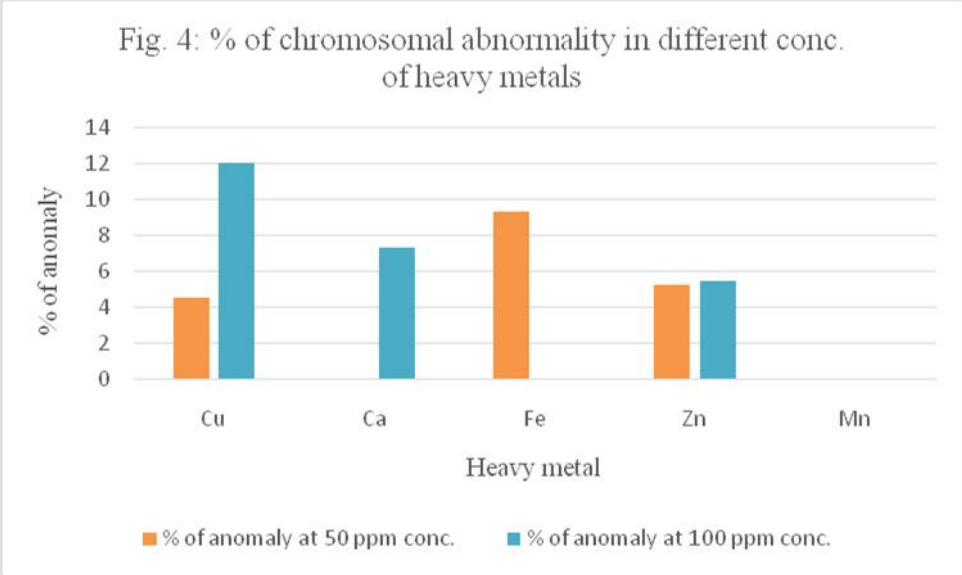


3.k: Treatment withheavy metals (50 ppm each): toxic effects 3.l: Control

Fig. 3: Effects of different heavy metals on mitotic chromosomes of *Aloe vera*

Table 2: Chromosomal anomaly in different heavy metal treatments

| Heavy metals | Concentration in ppm | Chromosomal Abnormality found | % of chromosomal abnormality at 50 pm concentrations of heavy metals | % of chromosomal abnormality at 100 pm concentrations of heavy metals |
|---|---|---|---|---|
| Cu | 50 | Chromosome clumping | 4.50 | 12.0 |
| | 100 | Sticky bridge, Multipolarity with anaphase bridge | | |
| Ca | 50 | Chromosome fragments | Nil | 7.28% |
| | 100 | Chromosome fragmentation | | |
| Fe | 50 | Chromosome clumping, stickiness | 9.32 | Checked mitotic activity |
| | 100 | - | | |
| Zn | 50 | Chromosome fragmentation | 5.22 | 5.40% |
| | 100 | Chromosome laggard, excessive fragmentation (pulverization) | | |
| Mn | 50 | Nil | Nil | Nil |
| | 100 | Nil | | |




## Conclusion

The results showed that Cu, Ca, Fe and Zn caused abnormality in mitotic cells of *Aloe vera* considerably. Moreover, it was evident that they had detrimental effects on chromosomes inducing stickiness, laggard, anaphase bridge formation and multipolarity. The present study showed an increase of chromosomal aberrations in mitotic cells of *Aloe vera* treated with heavy metals. The present study further confirms that heavy metals can have genotoxic effects on mitotic chromosomes. Experimental findings also revealed that, the toxicity of Cu and Fe at 100 ppm concentration was far more than the rest of the heavy metals tested. The concentration of heavy metals in the range of 50-100 ppm inhibited the cell division of root tip cells, as was evident in the decreased mitotic index values in the different treatments. Thus, the permissible range of the respective heavy metal concentrations within which the plant can exhibit its normal growth potential could be evaluated.

## References

Abubacker MN, Sathya C. 2017. Genotoxic effect of heavy metals Cr, Cu, Pb and Zn using *Allium cepa* L. Biosci Biotech Res Asia 14(3): 1181-1186.

Chen Q, Zhang X, Liu Y, Wei J, Shen W, Shen Z, Cui J. 2017. Hemin-mediated alleviation of zinc, lead and chromium toxicity is associated with elevated photosynthesis, antioxidative capacity, suppressed metal uptake, and oxidative stress in rice seedlings. Plant Growth Regul 81: 253-264.

El-Shahaby AO, Abdel Migid HM, Soliman MI, Mashaly IA. 2003. Genotoxicity screening of industrial wastewater using *Allium cepa.* chromosome aberration assay. Pak J Biol Sci 6: 23-28.

Gebhart E. 1984. Chromosome damage in individuals exposed to heavy metals. Toxicol Environl Chem 8 (4): 253-265.

George NM. 2000. Evaluation on mutagenic effects of three heavy metals on *Vicia faba* plants. Cytologia 65: 75-82.

Gilli W. 1941. Chromosome behaviour in mitosis in triploid tradescantia hybrids. Bull Torrey Bot 68: 207-221.

Gomurgen AN. 2000. Cytological effect of herbicide 2,4-D isooctylester 48% on root mitosis of *Allium cepa*. Cytologia 65: 383-388.

Hajmoradi F, Taleb beydokhti A. 2019a. Chromosomal study of yellow sweet-clover (*Melilotus officinalis* (L.) Lam.) under heavy metals pollution of lead and zinc mine of Zeh Abad, Qazvin. J Nat Environ 72 (2): 199-212.

Hajmoradi F, Taleb beydokhti A. 2019b. Effect of heavy metals on meiosis cell division in *Stachys inflata* Benth. Casp J Environ Sci 17 (3): 363-373.

Hajmoradi F & Kakaei M. 2021. Genotoxic Effects of Heavy Metals on Mitotic Chromosomes of *Trigonella foenum-graecum.* Genet Resour 2021; 7(2): 265-271

Ignacimuthu S, Babu CR. 1989. Induced chromosomal bb normality and pollen sterility in wild and cultivated urd and mungbean. Cytologia 54: 159-167.

Khanna, N., & Sharma, S. (2013). *Allium cepa* root chromosomal aberration assay: a review. Indian journal of pharmaceutical and biological research,1(03), 105-119. DOI: 10.30750/ijpbr.1.3.15

Kumar G, Bhardwaj M. 2017. Comparative genotoxicity of heavy metals in root meristems of *Cuminum cyminum* L. Chromosome Botany 12: 7356-62

Kumar G, Srivastava A. 2015. Clastogenic and mito-inhibitory effect of heavy metals in root meristems of *Vicia faba*. Chromosome Botany 10 (1): 23-29.

Kumar G, Tripathi R. 2007. Lead induced cytotoxicity and mutagenicity in grass pea. Turk J Biol 32: 73-78.

Kwankura, W., Sengsni, S., Muangphra, P. and Euawong, N., Screening of plants sensitive to heavy metals using cytotoxic and genotoxic biomarkers. Kasetsart, J. Natural Science, 2012,46, 10-23.

Lasat MM, Pence NS, Curvin DF, Ebbs SD, Kochian IV. 2005. Molecular physiology of Zn transport in pyper accumulation *Thlaspica erulescens*. J Exp Bot 51: 71-79.

Love RM. 1949. EstudoscitológicosPreliminares de trigos rio-grandenses. Secretaria da Agricultura, Porto Alegre.

Nagajyoti, P., Lee, K. D. and Sreekanth, T. V.,Heavy metals, occurrence and toxicity for plants. Environ. Chem. Lett., 2010, 8: 199-216.

Nafea, E. K., & Khalil, M. I. (2022). Studying the effect of heavy metals and the preservative at the cellular level using *Allium cepa*. L as a bioindicator. International Journal of Health Sciences, 6(S2), 11634–11643.

Nirmala A, Rao PN. 1996. Genetics of chromosome numerical mosaism in higher plants. Nucleus 39: 151-175. Pagliarini MS. 2000. Meiotic Behavior of Economically Important Plant Species: The Relationship between Fertility and Male Sterility. Genet Mol Biol 23: 997-1002

Pandey RM, Upadhyay SK. 2010. Cytological effect of heavy metals on root meristem cells of Vicia faba L. Toxicol Environ Chem 92 (1): 89-96.

Ranjbar M, Hajmoradi F, Karamian R. 2012. An overview on cytogenetics of the genus Onobrychis (Fabaceae) with special reference to O. sect. Hymenobrychis from Iran. Caryologia 65: 187-198.

Ritambhara T, Kumar G. 2010. Genetic loss through heavymetal induced chromosomal stickiness in grass pea. Caryologia 63: 223- 228.

Turkoglu S. 2009. Genotoxic effects of mono-, di-, and trisodium phosphate on mitotic activity, DNA content, and nuclear volume in *Allium cepa* L. Caryologia 62: 171-179.

William F. Grant (1978). Chromosome Aberrations in Plants as a Monitoring System. Environmental Health Perspectives, (27), 37–43doi:10.1289/ehp.782737

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