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IMPACT OF WATER POLLUTION ON PLANT CELL DIVISION: A CASE STUDY OF THE KALI RIVER OF DISTRICT MUZAFFARNAGAR

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ABSTRACT
Rivers play a vital role in ecosystems however, water pollution has become a significant global issue, impacting millions of people and ecosystems. This study focuses on the cytotoxic effects of water samples from the Kali River of district Muzaffarnagar on *Hordeum vulgare*, a key plant species. The research involved growing *Hordeum vulgare* seeds in both control (RO water) and polluted water samples collected, followed by cytological analysis of root tip samples. Various mitotic anomalies were observed in the treated seedlings, including chromosome erosion, clumped metaphase, C-metaphase, and grouping of chromosomes. The study highlights the severe impact of water pollutants, particularly heavy metals, on plant cell division.

Keywords: *Kali River, Water Pollutants, Hordeum vulgare, Mitotic Anomalies, Heavy Metals*

INTRODUCTION
Rivers are crucial to ecosystems, shaping landscapes, supporting biodiversity, and sustaining human communities. As sources of freshwater, rivers offer significant ecological, social, and economic benefits (Yang H. 2024). Rivers in India are under severe threat due to pollution from industrial (factory) discharge (including various hazardous heavy metals lead, mercury and arsenic etc.) and municipal (urban) waste, agricultural runoff (Pesticides, herbicides, and fertilizers run into water bodies via irrigation and rainfall). This pollution not only affects aquatic ecosystems but also poses major health risks to millions of living beings who depend on rivers for drinking water, agriculture, and daily use. Water pollution is a major global problem impacting millions of people. Chemicals, waste, plastic, and other pollutants contaminate rivers, lakes, reservoirs, and seas, making water unsafe for plants as well as for all the living beings. Plants are a key component of ecosystems, serving as sources of food, shelter, and habitat for various organisms. When water pollution impacts plants, it can lead to changes throughout the ecosystem. Pollutants can influence the functioning of plant cells by impacting physiological processes such as respiration, transpiration, and cell division. These effects may lead to changes in growth and development patterns. The present study aimed to analyze the cytotoxic effects of water samples of Kali River on *Hordeum vulgare* once a blessing for the local population and now has turned into a curse due to its lethal water quality (Khurana *et. al*., 2022).

The Kali River (also known as Kali Nadi) originates in the Upper Sivaliks and flows through Saharanpur, Muzaffarnagar, and Baghpat districts before joining the Hindon River at Barnava, Baghpat. The Hindon then merges with the Yamuna near Delhi, which subsequently joins the Ganga and eventually flows into the Bay of Bengal. The Kali River is 150 km long and is named after the Hindu goddess Kali (Zahoor Ali 1987).

MATERIALS AND METHODS
For the purpose of cytological examination, *Hordeum vulgare* seeds were cultivated in both a control environment using RO water and a test environment using water sourced from a river. The seeds underwent surface sterilization with 1% mercuric chloride, followed by thorough rinsing with distilled water to eliminate any remaining mercuric chloride. A minimum of 50 seeds were then placed in sterilized Petri dishes for growth. Root tip samples were collected from both the treated and control groups 48 hours after the experiment commenced. These root tips were preserved in acetic alcohol,

composed of three parts absolute ethanol and one-part glacial acetic acid, for at least 48 hours. Subsequently, the root tip samples were stored in 70% ethanol in a refrigerator. After the fixation process, the root tips were boiled in 1N HCl, then smeared and squashed in 1% aceto-orcein to assess the toxic impact of polluted water on the cytology of root meristem cells.

RESULTS AND DISCUSSION

Different types of mitotic anomalies were present in the treated seedlings of *Hordeum vulgare*.

a. Chromosome erosion,
b. Clumped metaphase,
c. C-metaphase,
d. Enlarged nucleolus,
e. Grouping of chromosomes,
f. Precocious disjunction at metaphase.
g. Chromatin bridge.
h. Late movement of chromosome for metaphase alignment.

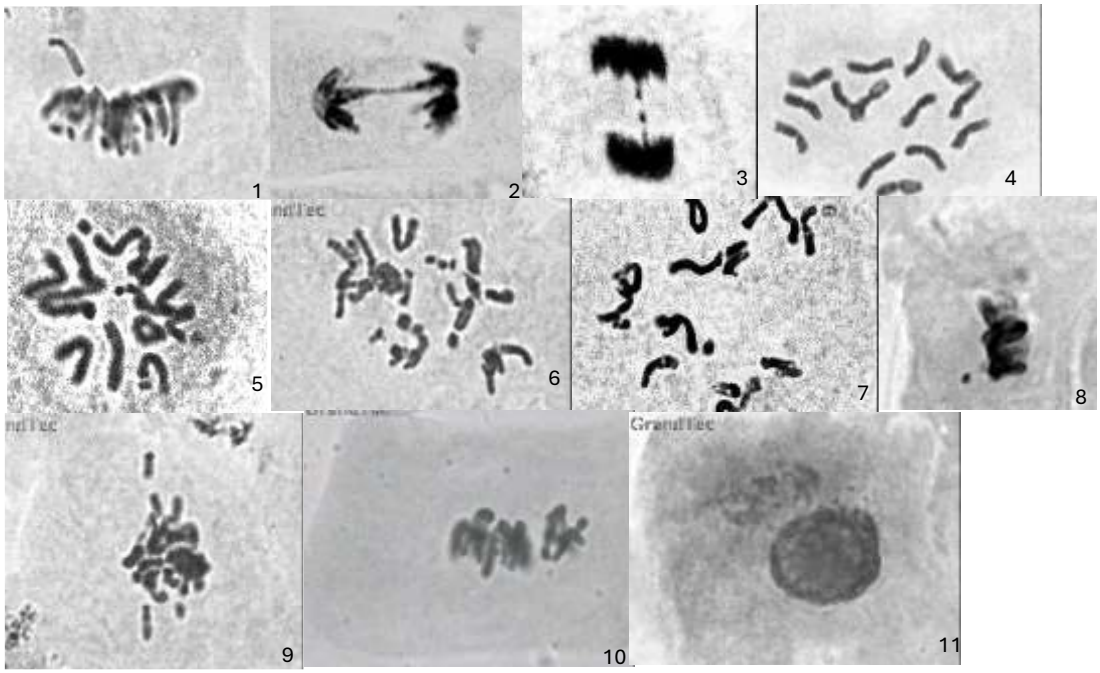

Figure: 1: Precocious disjunction at metaphase 2,3: Chromatin bridge 4,5: C-metaphase 6,7: Chromosome erosion 8: Clumped metaphase 9: Late movement of chromosome for metaphase alignment10: Grouping of chromosomes 11: Enlarged nucleolus.

The cell cycle produces two identical daughter cells from a single mother cell. The basic strategies followed during cell cycle are replication of genetic material and its accurate distribution between daughter cells. The events responsible for the former takes place during interphase, while the latter takes place during M-phase. The events taking place during the cell cycle follow a definite order and definite speed, suggesting the presence of precise biochemical control systems being executed in a temporal order. Reports about the mitotoxic effects of water pollutants and heavy metals were provided by several workers (Olorunfemi *et.al*., 2015, Patil *et.al*., 2019, da Cunha Neto 2023, Sass, 1937; MacFarlane *et. al*., 1951; Fiskesjo, 1969; Banerjee and Sharma, 1971; Levan, 1971; Ramel, 1972, 1974; Mukherji and Maitre, 1976; Singh and Sharma, 1980, 1982; Somashekhar and Arekal, 1983; Giri *et al.*, 1984; Sathaiah *et al.*, 1984; Massey and Raghuvanshi, 1985, 1986, 1989; Mishra and Sinha, 1985; Ahmed and Sinha, 1986; Mohapatra *et al.*, 1986; Pronoti and Raghuvanshi, 1986; Rangaswamy *et al.*, 1986; Ray and Banerjee, 1986; Grover and Dhingra, 1987; Kumar and Sinha, 1989; Roy *et al.*, 1989;

Vivekanandan and Boopathy, 1990; Singh, 1991; Lerda, 1992, Liu *et al.*, 1995, Anis *et al*., 1998; Rank and Nielsen, 1998; George, 2000; Shetty and Somashekar, 2000; Agrawal and Ansari, 2001; Sharma, 2001;Tomar and Srivastava, 2002,).

During present study mitotic anomalies related to chromatin agglutination, spindle anomalies, chromosome condensation and chromosome erosion were observed. Chromatin agglutination produced clumped metaphase and chromatin bridges. Treatments with polluted water induced significantly higher amount of chromatin agglutination. Induction of chromatin agglutination was first reported by Beadle (1932) in *Zea mays*. In this plant a gene mutation was found inducing this effect. Since then, several chemicals including heavy metals were found inducing this. Reports about the induction of clumping during mitosis by water pollutants and other heavy metals are also available (Giri *et al.* 1984, Somashekhar *et al.* 1985, Chaurasia and Sinha 1989, Rangaswamy *et al.* 1986, Bakale and Srinivasu 1989, Datta and Biswas 1989, Anis *et al*. 1998). Similar observations were recorded by El-Shazly and El-Sheikh 2000. The biochemical mechanism for the chromatin agglutination is probably not known. It is quite probable that positively charged heavy metals may interact with basic histone proteins. This interaction may create structural changes in chromatin resulting into stickiness. Chromosome stickiness observed at metaphase may also cause a failure of spindle mechanism (Panneerselvam et al., 2012). Sharma and Mukherjee (1955) are of the view that stickiness can be due to depolymerization of nucleic acid. C-metaphase was another common mitotic anomaly induced by pollutants either due to inhibition of spindle organization in some cells or due to disorganization of spindle after its organization. The term C-metaphase was coined by Levan (1938) for a modified form of mitosis induced by partial or complete inactivation of the spindle mechanisms after treatment with colchicine, where chromosome movement was completely disturbed. Several workers have observed the induction of C-metaphase by water pollutants and heavy metals (Panneerselvam et al., 2012, Fiskesjo, 1988, Giri *et al.* 1984, Kostoff 1939, 1940; Levan, 1945; MacFarlane, 1953; MacFarlane and Schmoch, 1948; Nandi, 1985; Sathaiah *et al.*, 1984; Sharma, 2001; Shetty and Somashekar, 2000,). Murray (1991), however, found that phosphorylation by NIMA (a protein kinase) plays an important function in organizing the spindle. Therefore, it is quite probable that pollutants/heavy metals may be inactivating this protein kinase with consequent induction of C-metaphase. Chromosome erosion was first reported by Levan (1938) and Levan and Tjio (1948). Several other workers, like Grover and Dhingra (1987), MacFarlane (1951), Roy *et al.* (1989), Somashekhar and Arekal (1983) etc. also reported its induction by various water pollutants. Although definite reasons for this anomaly are not known, it was established by Pool *et al.* (1989) that several physical factors, carcinogens and mutagens in the environment may induce amplification of DNA sequences which can be located on the chromosome on non-staining regions. Whether the eroded portions of the chromosomes presently observed and these non-staining regions were parallel structures can only be inferred after proper biochemical and DNA sequence analysis.

Pollutants could induce functional anomalies in spindle apparatus became evident from the presence of retarded movement of chromosome for metaphase alignment and lagging of chromosome. Retarded movement for metaphase alignment and lagging of chromosome during anaphase apparently appears parallel phenomenon in the sense that system responsible for displaying chromosome from a particular place has been partially inactivated. Gomez-Arroyo and Villiobos-Pietrini (1983) while studying chromosomal alternations induced by some chromium salts, used expression “chromosomes with inactivated centromere” collectively for both these anomalies. Retarded movement of chromosome for metaphase alignment and lagging of chromosome cannot be considered parallel phenomenon the two mitotic anomalies have different cause behind them can be further resolving the controversy of attachment of kinetochore with microtubule. Late or no movement of chromosome towards poles, therefore, appears due to hindrance in capture of microtubule by kinetochore, while out of several reasons responsible for lagging of chromosome during anaphase, one reason might be inactivation of motor proteins responsible for anaphase A movement. The occurrence of lagging of chromosome may also be explained on the basis of abnormal spindle formation and failure of chromosomal breakage by binding to DNA regions rich in GC pairs causing these to become unstable (Lawley and Brooks, 1963). In all the treated sets pollutants caused significant number of mitotic anomalies. This effect suggested that even if pollutants could not obstruct mitosis, it was potent enough to distort the course of mitosis.

Further, this can be used to infer that in those treated sets where mitotic index was decreased, (Smail et. al., 2024) the expected frequency of the cells with cytological anomalies must be higher than the observed frequency, because many cells having the anomalies might have not been allowed to enter mitosis. This assumption cannot be presently defended/abandoned enumerating works of the other people, due to lack of this type of cytogenetic analysis.

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