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Original research article

GENOMIC INSTABILITY IN ONION (ALLIUM CEPA L.) IRRIGATED WITH INDUSTRIAL WASTEWATER: A CYTOGENETIC STUDY

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Abstract: *Wastewater negatively affects the production of plants. The impact can vary depending on the wastewater content and the sensitivity of the plant type. The purpose of the current study was to look at the hazardous effects of wastewater from the steel and rubber industries on onions (Allium cepa). Twenty-four pots were filled with soil. Three replicates are used in this study to investigate the impact on Allium cepa. To evaluate the level of toxicity of the rubber and steel industries' waste waters, three concentrations of each wastewater and a control were selected. The concentrations for rubber industry effluent were chosen: 80% (S1), 90% (S2), and 100% (S3) and for steel industry 80% (R1), 90% (R2), and 100% (R3). Wastewater treatment inhibited root development when compared to control. Genotoxic research at different intensities showed chromosomal abnormalities. As the concentrations increased, the mitotic index fell and chromosomal abnormalities grew. Chromosome bridges, nuclear budding, sticky and coagulated anaphase and metaphase, and other chromosome abnormalities were seen. Overall, it was shown that the application of wastewater from the steel sector and wastewater from the rubber business had a detrimental effect on plant growth. However, when compared to rubber industry wastewater, steel sector wastewater has a higher level of toxicity. Compared to rubber sector waste water, steel industry wastewater has a higher concentration of heavy metals.*

Keywords: Heavy metals, toxicity, bridges, wastewater.

Introduction

Complex synthetic blends, including metallic and natural synthetic substances, might be available in modern effluents. Physico-synthetic boundary guideline limits relating to the arrival of modern wastewater in scene waters have been laid out in numerous countries (1). The metal substance and part that has to some degree extraordinary thickness and is risky or poisonous in little obsessions is implied to be a weighty metal. Because of their drawn-out determination in arrangements and troubles being changed into insoluble mixtures in surface waters, weighty metals rank among the most unsafe regular water impurities (2). At the point when the wastewater from the sugar manufacturing plant is delivered generally around the entire environment without being appropriately treated, it causes a hostile scent and air. Ranchers who have involved these effluents in the water system have seen diminished plant development boundaries, the organic product creation rate, and soil quality. The different metals which are chloride, and nitrate, are available in sugar plant effluents and are delivered by various organizations, and they are undesirable to check out, smell, and taste because of these elements. Such harmful water is perilous to individuals, creatures, and plants. Numerous specialists are keen on what different modern effluents mean for seed germination, development, and harvest yield (Road et al., 2007). Because of monetary development during the 1950s, which brought about modern control of land that encroached on ecological assurance zones, homegrown and modern effluents sullied water bodies. Moreover, streams and waterways near agrarian regions are sullied by pesticides (3). The material colours are incredibly harmful and might be cancer-causing, joined with a critical number of modern toxins (4). The animals in the biological system are affected by this horrible change in the climate. The expression "ecological contamination" alludes to what happens in the climate. Defilement of the climate is the most difficult issue (5). Fast industrialization endangers organic structures. Modern and homegrown outflows are the principal wellsprings of perilous mixtures (6). The logical name for the onion is (Allium cepa L). The Liliaceae family has more than 250 genera and it contains 3700 species (7). This is the earliest gasp to be prepared (8), the onion started in focal Asia (9) and it is currently developed around the entire world, particularly present in a couple of districts where atmospheric conditions are mild (10). The antibacterial, antithrombotic, anticancer, hostile to hyperlipidaemic, hostile to ligament, hostile to hyperglycemic, and anticarcinogenic impacts of A. cepa were likewise illustrated (11). A. cepa ingestion for a significant period defensively affects the improvement of waterfalls, neurodegenerative sicknesses, and vascular and heart

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infirmities (9). Plant frameworks have been demonstrated to be delicate, reasonable, and proficient among the bioassays made for the identification of mutagenicity, genotoxicity, cytotoxicity, and clastogenicity attributable to natural contaminations (12). The Allium test is one of the most famous tests for cytogenetic examination surveying cytogenotoxicity that antagonistically affects the climate because of their unreasonable and aimless delivery. Short application times, common sense, cost viability, great reproducibility of the ends, from immense to humble chromosome numbers (2n = 16), and serious areas of strength for the various bioassays are a portion of this technique's distinctive advantages (13). For natural checking of streams defiled with weighty metals and cyanides, the Allium cepa test has been demonstrated to be compelling (14). The objective of this study is to explore the major destructive impacts of soil contamination on all genotoxicity of plants. To test the toxicity of effluent from the rubber and steel industries at various concentrations and times. To ascertain the hazardous consequences of each industrial water dose rate (concentration).

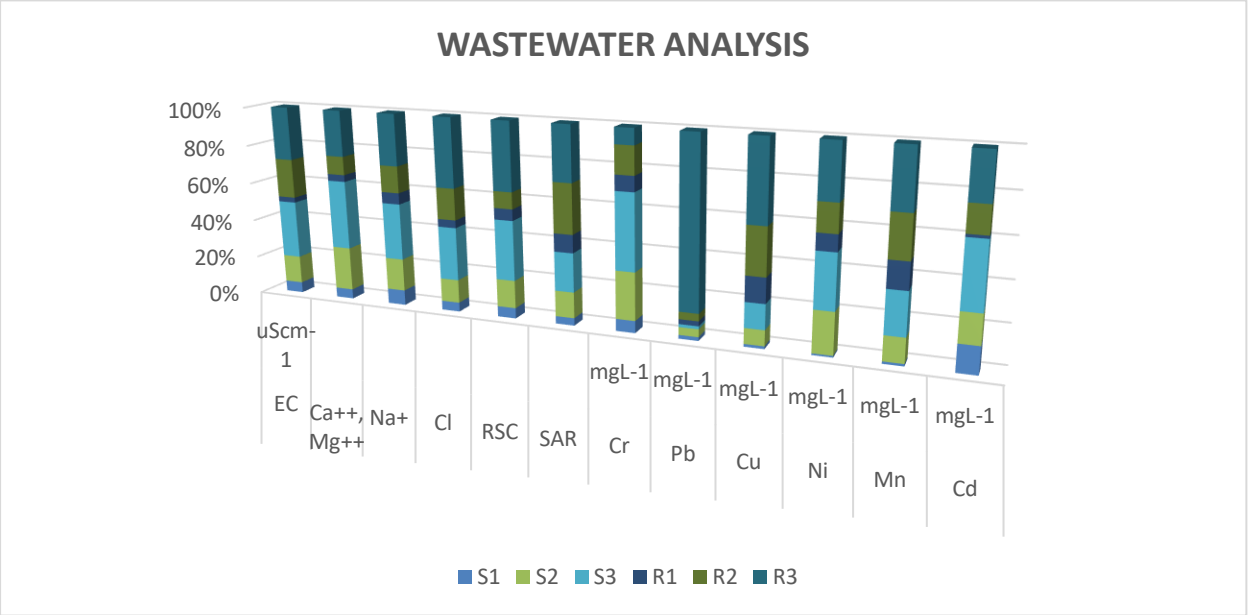

Figure 1: Wastewater analysis

Methodology

Experimental work

Wastewater collection

Water samples were gathered and kept in water bottles from the rubber industry, Kasur, and the steel industry, in Lahore, Pakistan.**Experiment Site:**

In the nursery close to the University of Education, Bank Road Campus in Lahore, the experiment was conducted.

Onion (Allium cepa L.) bulbs collection

The plant onion bulbs are bought from a nearby store and Cleaned with tap water to remove any dirt.

Soil Preparation

Each treatment had three duplicates made. The soil was poured into twenty-four pots. Hoeing the soil for three days made the surface permeable. Bulbs were planted in each planter. Bulb planting was carried out in August. The bulbs were planted such that they were a half inch below the soil surface. To ensure that bulbs had enough soil contact after planting, a little pressure was applied to the soil. The soil around newly planted bulbs was then thoroughly irrigated to close up any air spaces. Water was applied to bulbs once daily.

Table 2: Experimental treatment

S0	**Control (without stress)**
S1	Wastewater of rubber industry at 80% conc.
S2	Wastewater of rubber industry at 90% conc.
S3	Wastewater of rubber industry at 100% conc.
R1	Steel Industry wastewater at 80% conc.
R2	Steel Industry wastewater at 90% conc.
R3	Steel Industry wastewater at 100% conc.

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Assessment of Genotoxicity

Frequent Chromosome aberrations were seen under a microscope after seven days of stress. As a control, distilled water-treated onion bulbs were used.

Genotoxicity analysis

Onion (Allium cepa L.) bulbs are cleansed under flowing water and have their roots removed in preparation for cytological investigations. We just require the meristematic portion of the root for cytological research. The tips of the roots are next immersed in a fixative solution which contains three ratios of glacial acetic acid & ethanol for two hours. 15 minutes after hydrolysis in 1 N HCl Mitotic squash preparations were created at room temperature with the aid of 2% aceto-orcein and improved methods. Only 5 to 10 minutes of heating, followed by a 15 to 20 min wait time. Put the neatly trimmed root tip on the surface of the glass slide after this cut the root tip. By applying pressure with your thumb, squash the root tips. Slides should be placed under a microscope at 4x, 10x, 40x, or 100x magnification. There are various chromosomal aberrations visible in the cells. Wherever necessary, take photos. Calculations were made for the frequency of chromosomal aberrations (CA) and the mitotic index (M.I) (15).

M.I %= All the total number of cells in the dividing stage /overall number of cells observed X 100

Frequency of CA % = the number of abnormal cells counted/the overall total number of cells observed X 100

Statistical analysis

IBM SPSS statistics were used to analyse the experimental work data. ANOVA was applied to analyse the mean values of data of the wastewater from the rubber and steel industries. Duncan's multiple range tests were used to compare all data at a significant value of 5% level.

Results

Genotoxicity

The effects of rubber industry wastewater from Kasur on the roots of Allium cepa were investigated. The data analysis revealed differences in the mitotic index values and chromosomal aberrations, which illustrate the impact of effluent from the rubber industry. The greatest number of total cells, dividing cells, mitotic index, and a very small number of chromosome aberrations were detected under S1 at 80% concentrations of stress. Decreased number of total cells, dividing cells, mitotic index, and greatest chromosome aberrations were detected under S3 at 100% concentrations of stress. After administering R1, R2, and R3, at 80%, 90%, and 100% concentrations respectively the genotoxicity effects of Lahore steel industry effluent on the roots of Allium cepa were investigated. The data analysis revealed differences in the mitotic index values and chromosomal aberrations, which illustrate the impact of wastewater from the steel sector. The highest number of total cells, dividing cells, mitotic index, and lowest number of chromosome aberrations were detected under R1 at 80 % concentrations of wastewater stress. Decreased number of total cells, dividing cells, mitotic index, and most chromosome aberrations were seen under R3 stress at 100% concentrations of stress. A greater number of Mitotic bridges, anaphase bridges, and chromosome breaks were observed under 100% concentrations of stress. A very small number of cells were observed at 80 % concentrations of wastewater. The root length was highest at the control stage (without stress), whereas under stress conditions the length of roots was significantly decreased. Diagonal anaphase & formation of the bridge, Disturbed metaphase spindle, and Elongated cell with expanded nucleus were all observed under S1 stress at 100% wastewater concentrations. Chromosomes break, Binucleate cells and Diagonal anaphase are observed under S2 stress at 90% wastewater concentrations. Chromosome breakage, Multipolar anaphase, Disturbed metaphase & anaphase, Multiple bridge formation, Disturbed chromosome orientation, and Multiple bridges of anaphase & multiple polar anaphase were all observed under S3 stress at 100% wastewater concentrations. Nucleus bud formation, Notched nucleus, and Multipolar & diagonal anaphase were all observed in R1 stress at 80% concentrations. Stickiness of chromosomes, Multipolar anaphase & single bridge formation, and Multiple bridge formation were all seen on R2 stress at 90% concentrations. Laggards & spindle disturbance, Bridge formation, and Diagonal anaphase were all observed under R3 stress at 100% concentrations. It is observed that several abnormalities were higher at higher concentrations of wastewater.

Table 3: Values are Means ± standard error of Genotoxicity analysis

Treatment	Total Cells	Dividing Cells	Mitotic Index%	Chromosomes Abnormalities %
S0	246±0.45	237±0.34	97.13±0.28	0±0
S1	245±0.33	144±0.45	58.7±0.147	4.88±0.23
S2	231±0.43	119±0.99	51.51±0.619	5.14±0.23
S3	229±0.12	102±0.34	44.54±0.23	7.08±0.13
R1	244±0.1102	116±0.23	47.54±0.48	1.21±0.39
R2	239±0.83	94±0.27	39.33±0.92	5.41±0.55
R3	233±0.77	73±0.28	31.33±0.88	7.72±0.137

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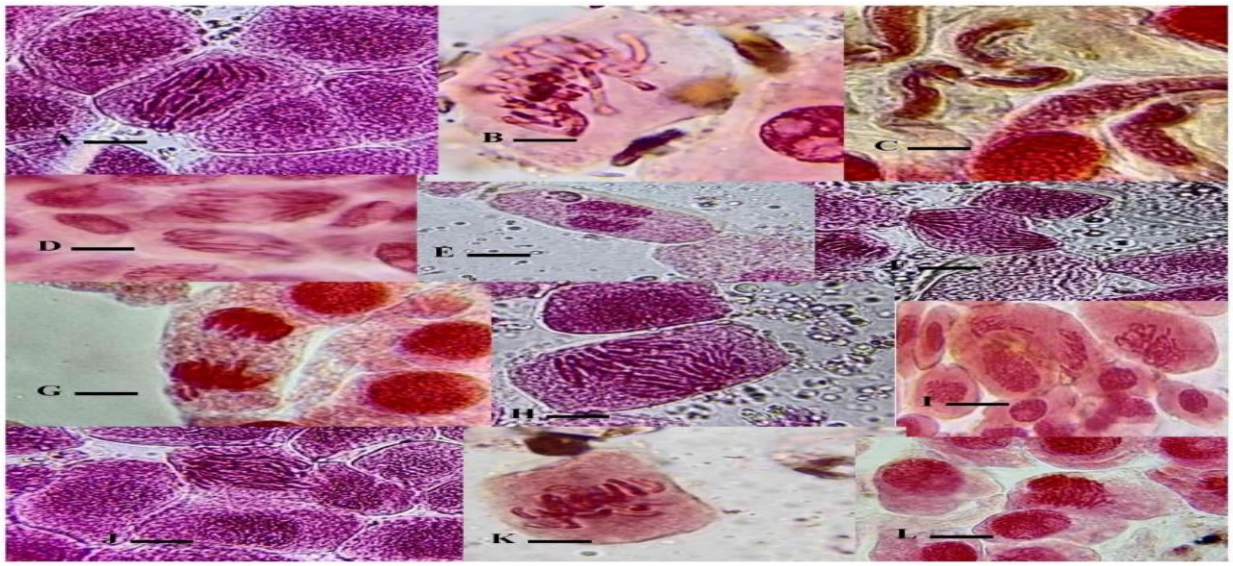

Figure 2: Some of the chromosome abnormalities of rubber and steel industry wastewater A. Diagonal anaphase & formation of bridge B. Disturbed metaphase spindle C. Elongated cell with expanded nucleus D. Chromosomes break E. Binucleate cells F. Diagonal anaphase G. Chromosome breakage H. Multipolar anaphase I. Disturbed metaphase & anaphase J. Multiple bridges formation K. Disturbed chromosomes orientation L. Multiple bridges of anaphase & multiple polar anaphase

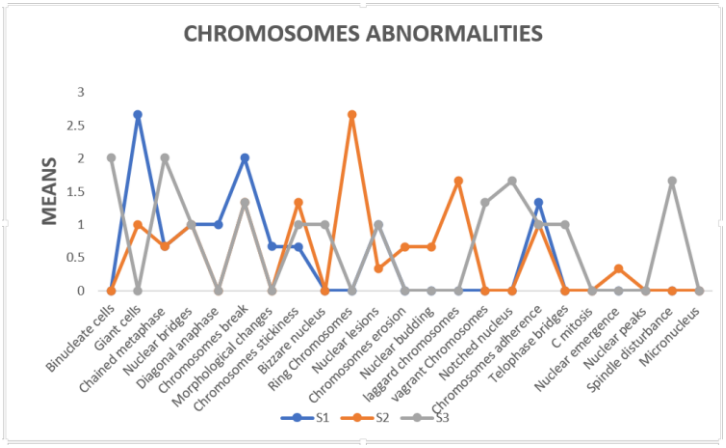

Figure 3: Graphical presentations of general chromosome abnormalities of rubber industry wastewater

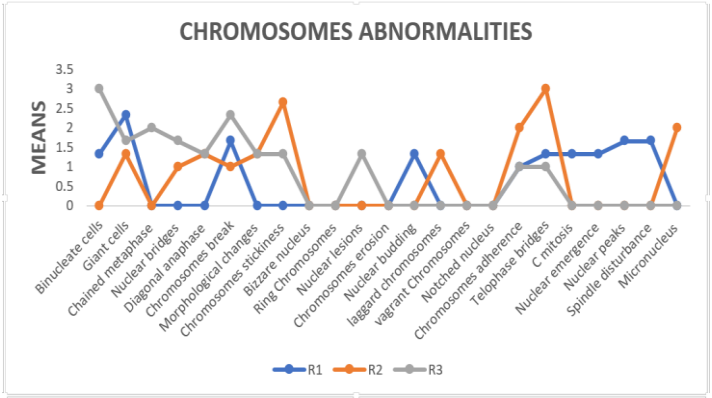

Figure 4: Graphical presentations of general chromosome abnormalities of steel industry wastewater

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Discussion

Results from our genotoxicity are steady with data tracked down in distributed examinations. All surveyed highlights and standard mistakes (complete cells, isolating cells, mitotic file, and chromosomal anomalies) all vary fundamentally from the control. As the convergence of modern wastewater rose, the Mitotic record fell. As the convergence of modern wastewater rose, Chromosome anomalies additionally rose. The coordinated effort of higher centralizations of metals continues with various exceptionally muddled mixes of manufactured intensifies inside the wastewater gushing may most likely be the justification for the quantifiably colossal decreases in mitotic documents provoked by all of the nine undiluted effluents, which show a concede in cell division in the root meristematic cells (16). Significant metals and other natural defilements have been associated with decreased mitotic records in the meristematic locales of A. cepa (17), Hemachandra and Pathiratne, (16). Ecological poison's influence on the natural framework of DNA/protein union has been embroiled in the bringing down of the mitotic file (18). Fabricated materials inside the wastewater effluents might potentially cause the passing of cells, it tends to be observable as a drop in the mitotic rundown. As per Pathiratne et al., (16), the development of micronucleus cells in the root locale where the tip is available is an indication of transformation because of tainting, especially inside the effluents from material organizations. As indicated by Leme and Marin-Spirits (2009), micronucleus cells are comprised of either complete chromosomes that don't move during anaphase because of shaft disappointment or little chromatin segments that structure given chromosomal breakage. Atomic irregularities were assessed present in the root zone region where the tip is available and introduced waste effluents were in the ongoing examination, including little gather cores, development of buds, and binucleate cells development (16). The examination of test expert exercises in relationship with their ramifications for the DNA of revealed natural substances has been demonstrated to be altogether more precise when atomic irregularity assessment is joined with chromosomal anomaly investigation (Leme and Marin-Spirits, 2009). As indicated by Fernandes et al., (19), Atomic buds are fostered by the course of the dispose of extra hereditary material from the course of the polyploidization process. Relatively to the core in the regular interphase cells, the cores of the cells with consolidated cores were more modest, obscurely hued, and would in general be situated close to the cell's fringe. These cells seem, by all accounts, to be necrotic because their cytoplasm has disintegrated. In meristematic cells under pressure conditions, nuclear chromatin development, which is in like manner seen as a sort of apoptotic marker, might be prompted, bringing about dense cores (20). As per Pathiratne et al., (16), Chromosome irregularities are considered in the root tissue locale which introduced different breaks in chromosome districts despite the presence of chromosome bonds (tenacity). As per Leme and Marin-Spirits (2009), upset metaphase and sporadic chromosomes happen from shaft disappointment in the mitotic axle, which are indications of aneugenic activity. As indicated by Leme and Marin-Spirits (2009), and Masood and Malik (2013), Chromosomal breaks welcomed on by clastogenic impacts are shown by different irregularities. Chromosomes can bring about the isolation of a disproportionate number of chromosomes, which thus can bring about the age-estimated or irregularly formed centres at interphase (21). A viable bioassay for deciding the overall poisonousness of substances is the A. cepa root improvement concealment test (Leme and Marin-Spirits, 2009). In Onion, the effluents produce Tacky chromosomes and scaffolds and make up the anomalies rate. There were additionally observable quantities of divided and axle scattered chromosomes. With expanding wastewater focuses, A. cepa mitotic list diminished and its mitotic hindrance expanded, which is characteristic of cytotoxicity (22). These discoveries are predictable with research led by us previously (23, 24) and by different analysts (25). As indicated by the review discoveries, Chironomus travalensis Kieffer's particular poisonous reactions to the modern effluents that were recently considered and the cytological qualities obtained from those tests firmly corresponded (26). With rising paint and material modern profluent focuses, the mitotic file (MI) fell. Two modern wastewater water systems caused chromosomal irregularities, with tacky, spanned, and transient chromosomes being the most frequently seen (25). As per Samuel et al., (25), EC50 should be within reach before being released into the water propensity.

Conclusion

The review discoveries support the possibility that different modern wastewaters, for example, those delivered by the rubber and steel areas, which incorporate weighty metals, can affect plants. Lead, cadmium, chromium, mercury, and arsenic are instances of weighty metals that are poisonous to the two plants and creatures. Their collection in soil and water can likewise affect plant development after some time. It is reasoned that emanating fixations from the elastic and steel area adversely affect the genomic design of Allium cepa. Generally speaking, the outcomes show that wastewater from the rubber business area and steel adversely affects plant improvement. Future farming examinations might profit from plants presented to gushing from the elastic and steel enterprises.

Declarations

Data Availability statement
All data generated or analyzed during the study are included in the manuscript.
Ethics approval and consent to participate.
Approved by the department concerned.
Consent for publication
Approved
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Not applicable

Conflict of interest

The authors declared an absence of conflict of interest.

Authors Contribution

AFSHAN AFZAL

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