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Genotoxic Effects Of Lead (Pb) On Seedlings Of Bread Wheat Triticum Aestivum

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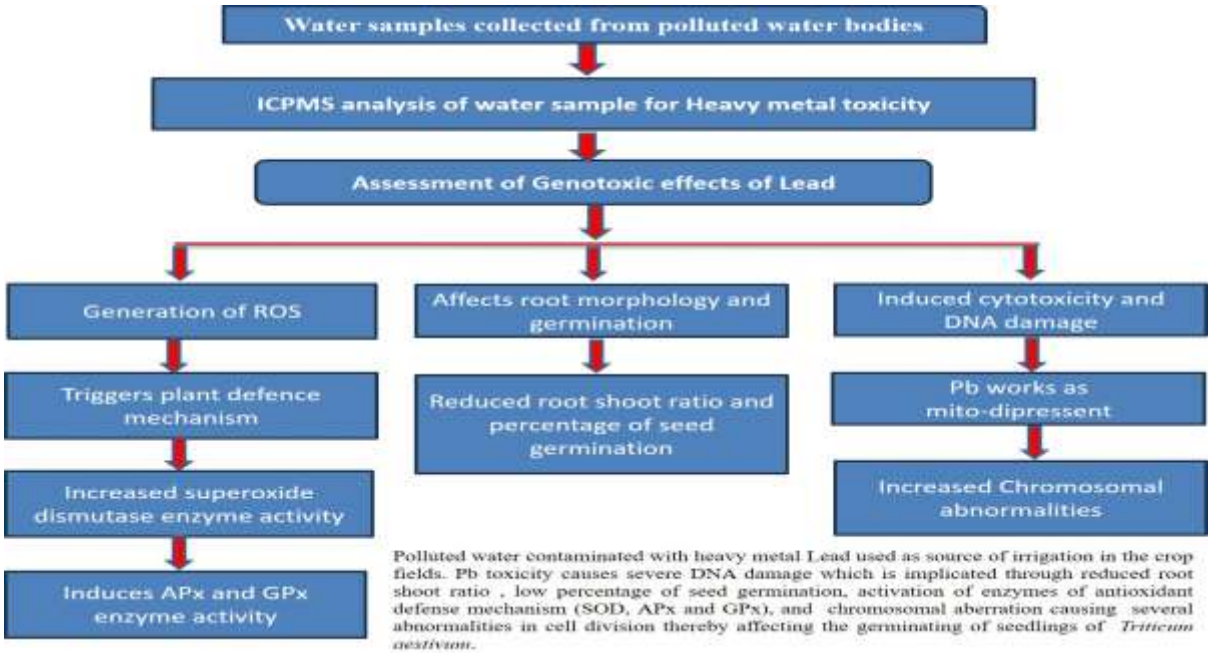
Abstract: *The genotoxic effects of heavy metal pollution are rapidly increasing due to anthropogenic activities. Rivers near major cities are contaminated with heavy metals such as Pb, As, and Cd, posing a significant risk to crops in the surrounding areas. The rising level of heavy metals in Ganga water is leading to a rapid increase in reactive oxygen species (ROS), which pose a serious threat to the protein, lipid, and DNA content in plants. Plants possess a natural defense system that protects them from reactive oxygen species (ROS). Catalase and peroxidase are among the most common antioxidant enzymes in plants and are responsible for converting reactive oxygen species (ROS) into water and oxygen. Triticum aestivum is a globally important cereal crop and primary source of bread. This study aimed to examine the cytological and physiological effects of lead (Pb) pollution in Ganga water. The impact of Pb toxicity on the growth and development of Triticum aestivum seedlings was assessed through seed germination, cytotoxicity analysis, enzymatic assays, and Pb bioaccumulation in the seedlings. A significant increase in APx and GPx enzyme activities was observed, along with chromosomal abnormalities such as reduced condensation of chromatin, chromosomal laggards, C-mitosis, anaphase bridges, micronuclei formation, and vagrant metaphase, as a result of high Pb accumulation in the mitotic cells of Triticum aestivum.*
Keywords: *Genotoxicity, Free radicals; Oxidative stress; Heavy metals, Lead, Triticum aestivum*

Graphical Abstract
Genotoxic effects of Lead (Pb) on seedlings of bread wheat *Triticum aestivum*



1. INTRODUCTION

River Ganga is life line of North Indian population passing through 19 big cities covering an area of 2525 km. It is the major source of irrigation in the Indo-Gangetic plains, providing life substance to the environment and affecting the ecology of the area. Anthropogenic activities have made rivers vulnerable to heavy metal pollution.

Elevated levels of heavy metals in water bodies are a potential threat to humans, animals, and agricultural crops. Certain heavy metals, such as Fe, Al, Cu, Ni, Mn, Mo, Mg, Zn, V, Co, and Cr, serve as essential major or minor nutrients in plants and play a crucial role in normal metabolic pathways. In contrast, metals such As, Hg, Ag, Sb, Cd, and Pb do not contribute to these pathways but can disrupt normal plant growth and physiology when present in excessive amounts. (36). Higher concentrations of heavy metals have detrimental effects on the cellular and physiological functions of plants, even though a certain amount of heavy metal is tolerated by the plant system (18). Harmful effects include the inhibition of enzymes, inactivation of biomolecules, and generation of ROS and oxidative stress in plant cells. The generation of ROS leads to oxidative stress, which consequently activates plant defense mechanisms. ROS also activate programmed cell death, DNA damage, unintended oxidation of proteins and membrane lipids, and enzyme inhibition (6, 37). Oxidative stress, commonly referred to as oxidative homeostasis, occurs when there is an imbalance between prooxidants and antioxidants. It is often triggered by free radicals and reactive oxygen species (ROS), which contain activated oxygen atoms.

The genotoxic effects of heavy metals are well known to affect seed germination, biomass production, and other metabolic activities, as well as chromosome morphology and mitotic index. Plants have innate strategies to deal with heavy metal intake at the primary, secondary, and tertiary levels. The primary defense mechanism involves restricting heavy metal entry by immobilizing them through mycorrhizal associations, metal sequestration, and complexation with organic compounds exuded from the roots. (2, 43). The secondary level of defense involves activating tolerance mechanisms for detoxification, which include metal sequestration and compartmentalization in different intracellular compartments, metal ion trafficking, and mycorrhizal association. If the metal still finds a way to enter the plant system, the tertiary defense mechanism is activated, triggering the antioxidant response to counteract oxidative stress caused by heavy metal overexposure while maintaining cellular homeostasis. (9, 12).

This study embodies the experimental procedures to reveal heavy metal toxicity in river bodies and its effects in terms of seed germination assay, cytotoxic assay, and generation of ROS leading to oxidative stress. Oxidative stress, in turn, triggers the plant defense machinery, thereby protecting the plants from the deleterious effects of ROS, which is implicated through enzymatic activity and other parameters, such as ROS generation. Activation of some of the defense-regulated enzymes, such as catalase and peroxidase, was observed and recorded in the study, revealing the significance of auto-generated plant defense mechanisms in converting reactive oxygen species into water and oxygen.

2. MATERIALS AND METHODS

2.1. Chemicals:

Mercuric chloride, ethanol, acetocarmine, tertiary butyl alcohol(TBA), glacial acetic acid, ethylenediamine tetraacetic acid(EDTA), ascorbate, sodium (acetate, carbonate, bicarbonate, hydrogen phosphate), Bradford's reagent, reduced nicotinamide adenine dinucleotide(NAD), reduced glutathione, 1-chloro-2,4-dinitrobenzene(DNCB), glycerol, sodium pyruvate, tris-base, sodium chloride, hydrogen peroxide(H_2O_2), and bovine serum albumin were purchased from Sisco Research Labs Pvt. (Mumbai, India). All additional compounds were of analytical grade.

2.2. Collection of water samples

Freshwater samples were collected from five different sites along a 15-20 km stretch of the River Ganges, covering both upstream and downstream areas of District Prayagraj, Uttar Pradesh, India. **(Table 1: Sample codes and their respective collection sites, as identified on Google Maps)**. The water samples were filtered with Whatman's filter paper before storage in sterilized containers and marked with codes given in **table 1**.

2.3. Experimental design

Healthy seeds of *Triticum aestivum* procured from the market were screened for uniform size, color, and weight. The seeds were surface sterilized with 1% (w/v) mercuric chloride solution for 2 min and thoroughly washed with tap water. The seeds were soaked in distilled water and kept overnight, after which they were allowed to germinate on moistened filter paper (35). Three independent replicates of each treatment were used in a randomized design. The control Petri plate was retained in distilled water, while the other Petri plates with seed samples were germinated on filter papers moistened with the test solution in three replicates each. The Petri plates were aerated daily to prevent root anoxia.

2.4. Seedling Germination assay

The Petri dishes were kept at ambient temperature (28 ± 2°C) under diffused sunlight. Data were recorded for the number of seeds germinated and the length of the root and shoot after 96 h of treatment (**table.3**). The root-to-shoot ratio was calculated, and the percentage seed germination was tabulated using the following formula:

$$Germination\ \%\ of\ seeds = \frac{Total\ number\ of\ Germinating\ Seeds}{Total\ number\ of\ Seeds} \times 100$$

2.5. Cytotoxic Assay:

Radicles were excised periodically from the germinated seedlings of *Triticum aestivum* growing in Petri plates moistened with test water samples **(Table.1).** Roots were excised periodically from the test samples and fixed in Carnoy's fixative (ethanol: glacial acetic acid, 3:1) for 24 h at room temperature and later stored in 70% alcohol for subsequent use. The fixed roots were later processed for microscopic studies to determine the effect of xenobiotics and its impact assessment, as per the procedure laid down by Levan and revised by Fiskesjo (11, 24). The roots were hydrolyzed in 1N HCl for 1 min at 60 °C in a hot air oven. Hydrolysis was followed by washing with distilled water three times for 15 min. The roots were then transferred to 45% acetic acid and then squashed on a clean slide in 2 % acetocarmine. Chromosome spreads were prepared by gently tapping and heating the slides. Observation for Mitotic index and chromosomal abnormalities were recorded from 10 different mitotic spreads from each sample with 10 readings from the microscopic field at 40 X magnification. The data were recorded for Mitotic Index and other chromosomal abnormalities and statistically tabulated in the mean value and standard error of the mean was tested at 0.05 level of significance (ANOVA and Duncan's multiple range test) where n=3 (table.3, 6, and 7) and n=10 (Table 4 and 5). Well-spread squash from each treatment was permanently fixed using the TBA-acetic acid schedule. Micrographs representing chromosomal abnormalities were captured using a Leica Microscope DM 3000 at 40x magnification (Figure.1).

The mitotic index was calculated using the following formula:

$$Mitotic\ Index = \frac{Number\ of\ dividing\ cells\ in\ a\ microscopic\ field}{Total\ number\ of\ cells\ observed\ in\ a\ microscopic\ field} \times 100$$

2.6. Detection of heavy metals by ICPMS

ICP-MS analysis was performed to detect the types of heavy metals present in the water samples. The concentration of heavy metal was calculated as per the following formula and the data is given in**(table.2)**

$$Concentration\ of\ metal\ (\mu g/mg) = \frac{Concentration\ (\mu g/ml)\ X\ Dilution\ factor}{Weight\ of\ plant\ material}$$

2.7. Antioxidant enzyme assay

Enzyme activity was determined in the treated seeds according to the following protocol. The activity was recorded in tables and correlated with the heavy metal stress response of the seedlings.

2.8a. APx assay

Antioxidant enzyme extraction was performed as specified by Zhang and Nan (47). Ascorbate peroxidase (APX; EC1.11.1.11) was measured according to the method described by Nakano and Asada (29). The assay mixture (2 mL) contained 0.2 mL of enzyme extract, 0.1 mM EDTA, 0.25 mM ascorbate, 1.0 mM H_2O_2, and 25 mM potassium phosphate buffer (pH 7.0). Using an extinction value of 2.8 mM^{-1} cm^{-1}, the absorbance was computed after measuring for 1 min at 290 nm. The amount of enzyme needed to oxidize 1 µM ascorbate min^{-1} was used to quantify the enzyme's specific activity, expressed as enzyme unit g-1FW.

2.8b. GPx assay

Guaiacol peroxidase (EC 1.11.1.7) assays were conducted following Hameda and Klein (13). The reaction mixture (2 ml) contained 0.2 mL enzyme extract, 1.0 mM H_2O_2, 25 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, and 0.05% guaiacol. At 470 nm, the increase in absorbance caused by guaiacol oxidation was monitored. With an extinction coefficient of 26.6 mM^{-1} cm^{-1}, the enzyme activity was estimated and reported as enzyme unit g-1FW.

2.8c. Superoxide radicals and superoxide dismutase activity

The formation of superoxide radicals (O_2) in treated and untreated *Triticum aestivum* roots was measured using the technique developed by Elstner and Heupel (8).

2.9. Statistical analyses

ANOVA was performed using SPSS v16. Data represent the means and standard errors of three independent biological replicates. Duncan's multiple range tests were used as a post hoc test to ascertain the significance between treatments at a 95% confidence level.

3. RESULTS AND DISCUSSIONS

3.1. ICP-MS analysis

Water samples were collected from different locations on the banks of the Ganga River going downstream from Chatnag ghat (S1), Sangam ghat (S2), Shivkuti ghat (S3), and Rasoolabad ghat (S4). ICP-MS analysis was conducted to detect the presence of heavy metals in water samples. The presence of heavy metals usually affects

the germination percentage, seedling growth, and root shoot ratio of *Triticum* seedlings as reported by Sheikh et al, (38), where the increased concentration of heavy metals (such as Cr, Cd, Mn, and Zn) induced dose-dependent inhibition of germination capability and seedling growth. ICP-MS analysis revealed that lead was invariably present in all the test samples, with a range of 0.015 -0.115 ppm. The concentration of lead increased downstream river banks with a maximum concentration detected at Chhatnag ghat (0.115 ppm), followed by Sangam (0.095)

3.2. Impact of lead toxicity on seedling germination

High lead concentrations cause oxidative stress by causing plants to produce more ROS (34). Therefore, Pb has been reported to be the most toxic element, altering the normal growth and physiology of plants, even at early stages. Seed germination is one of the earliest physiological responses affected by the presence of heavy metals. The present study indicated that the seed germination percentage and root morphology were adversely affected by the presence of lead in the test water samples; however, the response was variable. Water samples were detected with the presence of lead (**Table.2**) with decreasing concentration downstream ranging from S1-0.115, S2-0.095, S3-0.046, S4-0.015 in the order of S1>S2> S3>S4. The water samples with higher concentrations of lead, that is, S1-Chhatnagghat and S2-Sangam area, evidently showed lower seed germination. The range of seedling germination varied from 30% downstream to 85% in the control (Table 2). Heavy metals significantly inhibited seed germination at the 0.01 probability level. As reported earlier, this could be due to the interference of lead in the activity of certain enzymes, as reported earlier in *Spartiana alterniflora* (28). The findings were consistent with those made earlier in other plants, including soya bean (14); rice (26); barley (40); tomato, eggplant (20); and certain legumes (41); where lead toxicity affected seedling germination.

Root morphology was adversely affected in test samples containing higher lead values, showing a change in the color of the radicles from white to yellow, coupled with dark brown tips. Root shortening, accompanied by an increased root-shoot ratio, indicated a poor root system in Pb-exposed seedlings. The data on root morphology are tabulated in table.3 indicating the minimum root length in samples with higher lead values. Heavy metals impose oxidative stress on plants, which in turn increases the process of lignification, thereby shortening the root and shoot length, affecting the overall physiology of the plant (15).

3.3. Impact of lead on Chromosomes:

Lead has a strong inhibitory effect on the mitotic activity of *Triticum aestivum.* The experiments performed following the protocol laid down by Levan 1934 (24) and revised protocols by Feizko 1981 (11) revealed the mito depressive activity of lead accompanied with different chromosomal abnormalities in the root tip cells of *Triticum aestivum*. The most common chromosomal abnormalities observed during mitosis were chromosomal breakage, disturbed metaphase, stickiness, vagrant chromosomes, chromosomal bridges, micronuclei, chromosomal laggards, and tripolar nuclei. Most of the abnormalities scored were at metaphase, and the least at prophase, anaphase, and telophase. The mitotic index was decreased with increasing lead concentrations in the test samples. The percentage frequency of different chromosomal abnormalities, such as chromosomal laggards, fragmented anaphase, sticky metaphase, anaphase bridge, tripolar anaphase, vagrant in metaphase, c-mitosis, micronuclei, and vacuolated nucleus, were observed in the mitotic spreads under a Leica DM 3000 microscope at 40 x magnification.

The chromosomal abnormalities due to metal accumulation has been reported by many workers (7,19,21,22, 25, 27,46,). Impaired spindle function leads to anomalies, such as lagging and vagrant chromosomes (39). If laggards are unable to reach the poles in time for their inclusion in the main nucleus, they may create micronuclei (23). Micronuclei were previously reported by Shylaja (39). The micronuclei observed in *Triticum aestivum* could be due to the treatment of a water sample containing a high concentration of lead. These micronuclei developed from lead-induced acentric chromatids and chromosomal breaks. The development of micronuclei after irradiating *Vicia faba* roots was investigated by Evans (10). According to Evans et al., heavy metals such as lead cause acentric chromatid or chromosomal fragments to develop, and in mitosis, acentric fragments would result from chromatid breaks, chromosomal breaks, isochromatic breaks, asymmetrical exchanges, and incomplete symmetric exchanges. These pieces are commonly excluded from the two daughter nuclei at a later stage of mitosis, and in the ensuing interphase, they manifest as micronuclei in one or both daughter cells. Schmidt said that the chromatin lagging at anaphase is the source of micronuclei and proposed the micronucleus test as an in vitro cytogenetic screening method for the detection of recently introduced structural chromosomal abnormalities and for identifying chromosome loss caused by partial spindle apparatus impairment (44). During telophase, this material is incorporated into one or both daughter cells, where it has the potential to merge with the other daughter cell, the main nucleus, or one or more subsidiary nuclei.

According to Evans, partial dissociation of nucleoproteins and altered chromosome arrangement are the two causes of stickiness. Another cause is the disruption of phytochemically balanced processes (16). The most likely scenario is that heavy metals trigger gene mutations that result in erroneous coding for some non-histone proteins essential for chromosome organization. These proteins cause chromosomal stickiness when affected. Severe defects such as stickiness, unequal distribution, multipolar anaphase, chromosomal bridges, transient chromosomal bridges, and lagging chromosomes have been reported in the present study and might have resulted from the suppression of spindle formation (1,3).

3.4. Impact of Lead on ROS and antioxidant activity

ROS are byproducts formed in cells as a result of metabolic activity in different cell organelles and are removed by enzymatic and non-enzymatic antioxidants. Biotic and abiotic stresses disrupt the equilibrium between ROS production and their degradation. To protect themselves against these toxic oxygen intermediates, plant cells and organelles, such as chloroplasts, mitochondria, and peroxisomes, employ antioxidant defense systems. Metal induces the production of reactive oxygen species in the vicinity of DNA, which principally generates the pro-mutagenic adduct 8-0xoG (7,8-dihydro-8-oxoguanine) that can mispair with adenine in the absence of DNA repair mechanisms, resulting in C to T transition mutations (5). According to earlier reports, heavy metals induce oxidative damage in senescing oat leaf cells, primary leaves of mung bean, and wheat leaves (30,45). ROS produced in leaf cells are removed by complex enzymes, including *catalase* (CAT), *ascorbate peroxidase* (APX), *glutathione peroxidase* (GPX), *superoxide dismutase* (SOD), and *glutathione reductase* (GR)) of antioxidant systems. In the present study, the activity of ROS-scavenging enzymes was enhanced in wheat seedlings in response to all treatments. APX and GPx work counteractively to each other. All treatments had a significant ($p<0.05$) effect on APX and GPx activity. APX activity was elevated over the control, and the maximum 510% stimulation of APX and GPx activity over the control was observed in S1 samples.

3.5. Impact of Lead on enzymes of plant defense machinery

3.5 a Superoxide dismutase activity

Lead is known to cause oxidative damage in several plants, either directly or indirectly, by triggering an increased level of production of reactive oxygen species (ROS). These reactive oxygen species include superoxide radicals, hydroxyl radicals, and hydrogen peroxide (H_2O_2), which cause damage to several biomacromolecules, such as lipids, proteins, and DNA. As the concentration of lead increased, the production of superoxide radicals also increased. Plants have inbuilt antioxidant defense mechanisms to combat these problems. There are enzymes, such as *superoxide dismutase* (SOD), which neutralize, remove, and scavenge superoxide radicals. SOD activity increased as the lead concentration increased in different treatment plants. SOD activity was high in the S1 treatment plant due to the high lead concentration and was lowest in the control. This was evident in the experimental seedlings, and the data are presented in **Table 5**.

3.5b. APx GPx activity

APx is involved in the scavenging of H_2O_2 in water and the ASH-GSH cycles and utilizes ASH as the electron donor. In all the samples, the amount of lead varied, with the maximum amount in the S1 water sample, followed by S2 and S5. In fact, the accumulation of lead in germinating wheat seedlings also showed a similar trend. Recent reports have also shown that lead accumulation occurs in the shoots of *Brassica rapa* ((4). In many plant species, heavy metals have been reported to cause oxidative damage due to the production of excess ROS, and increased APx activity was observed in the present study when exposed to heavy metals (31). GPx activity possibly functions as an efficient quencher of reactive oxygen species and peroxy radicals, the production of which is stimulated by increased heavy metal doses in plant cells (32). GPx consumes H_2O_2 to produce phenoxy compounds, which are usually polymerized to produce cell wall components, such as lignin. High levels of GPx may also reduce the length of seedlings due to lignification (15). The present results are in agreement with other reports showing the positive effects of heavy metal treatment on antioxidative defense systems (17, 31).

The accumulation of heavy metals induces stress in germinating seedlings, increasing ROS production. Numerous studies have established that the induction of cellular antioxidant machinery is important for protection against various stresses. The direct interaction of heavy metals with cellular components can initiate a variety of metabolic responses, ultimately leading to a shift in plant development. For metal toxicity, this stress point is reached at the toxic threshold level of the metal in the tissue. Above this level, the physiological state of the cell irreversibly changes. This change is reflected by an increase in the activity of certain enzymes. Heavy metal ions accumulate in different parts of plants after being absorbed by the root system, resulting in the retardation of plant growth. This could be due to their interference with the activities of several enzymes essential for normal metabolism and

developmental processes (42). Antioxidative enzymes protect the cell structure against ROS generated under stress conditions (33).

Ascorbate peroxidase (APx) is thought to play an essential role in scavenging ROS and protecting cells in higher plants, algae, euglena, and other organisms. APx is involved in the scavenging of H_2O_2 in water and the ASH-GSH cycles and utilizes ASH as the electron donor. The APx family consists of at least five different isoforms, including thylakoid (tAPx), glyoxisome membrane (gmAPX), chloroplast stromal soluble (sAPx), and cytosolic (cAPx) forms. GP is a large family of diverse isozymes that use GSH to reduce H_2O_2 and organic and lipid hydroperoxides, thereby helping plant cells to withstand oxidative stress.

Antioxidant enzymes such as APx and GPx activity were analyzed with the help of a spectrophotometer, and the readings were recorded in **table.5**. APx and GPx activities increased with increasing Pb concentration. The lead concentration was high in the Chhatnag ghat and Sangam water samples. GPx activity was higher in seeds grown in Chhatnag ghat water (S1) and lower in the control medium. APx activity was increased in the S1 water samples of wheat seedlings. This indicates that Pb exposure induces ROS production, which in turn results in the production of antioxidant enzymes. The stress response was observed in all the seedlings, but it was found to be maximum in *Triticum* grown in Chhatnag ghat water.

4. CONCLUSION

Heavy metals, especially lead, are genotoxic to plants in terms of their normal growth and development. Lead has deleterious effects on seedling growth in *Triticum aestivum*. A considerable decrease in the percentage of seed germination was observed with increased lead accumulation in the seedlings. The root-to-shoot ratio increased in wheat seedlings growing at high Pb concentrations, indicating that Pb affects the water absorption system in seedlings, thereby affecting the overall growth and survival of plants. The abiotic stress developed due to lead toxicity increased GPx activity, which pronouncedly increased the process of lignification, causing shortening of the root length in seeds germinated in water samples contaminated with lead. Exposure to heavy metal like lead caused abiotic stress response in *Triticum* seedlings leading to ROS generation which was consummated by increased activity of ROS scavenging enzymes like superoxide dismutase, APx and GPx indicating triggering of plant defense machinery. Lead has been shown to be mito-depressive, and a low MI and higher percentage of chromosomal abnormalities, such as the formation of chromosomal laggards, micronuclei, tripolarity, and stickiness in chromosomes at the metaphase and anaphase levels, were observed in the *Triticum* seedlings.

Author Contributions:

The manuscript was designed by Prof. Sarita Srivastava, and the sample collection along with physiology experiments were done by Mr. Suresh Kumar and Enzyme activity and its analysis by Dr Nimisha Amist. Ms. Surbhi Tiwari, Mr. Govind Bhushan Singh and Ms. Poonam Pandey have recorded data and done the statistical tabulation. The cytological work was done by Ms. Utkarsha Gupta and morphological and germination assay was done by Ms. Neeta Kushwaha under the guidance of Prof Manju Srivastava.

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Figure 1:
Table: 1: Sample codes and their collection sites as located on google map

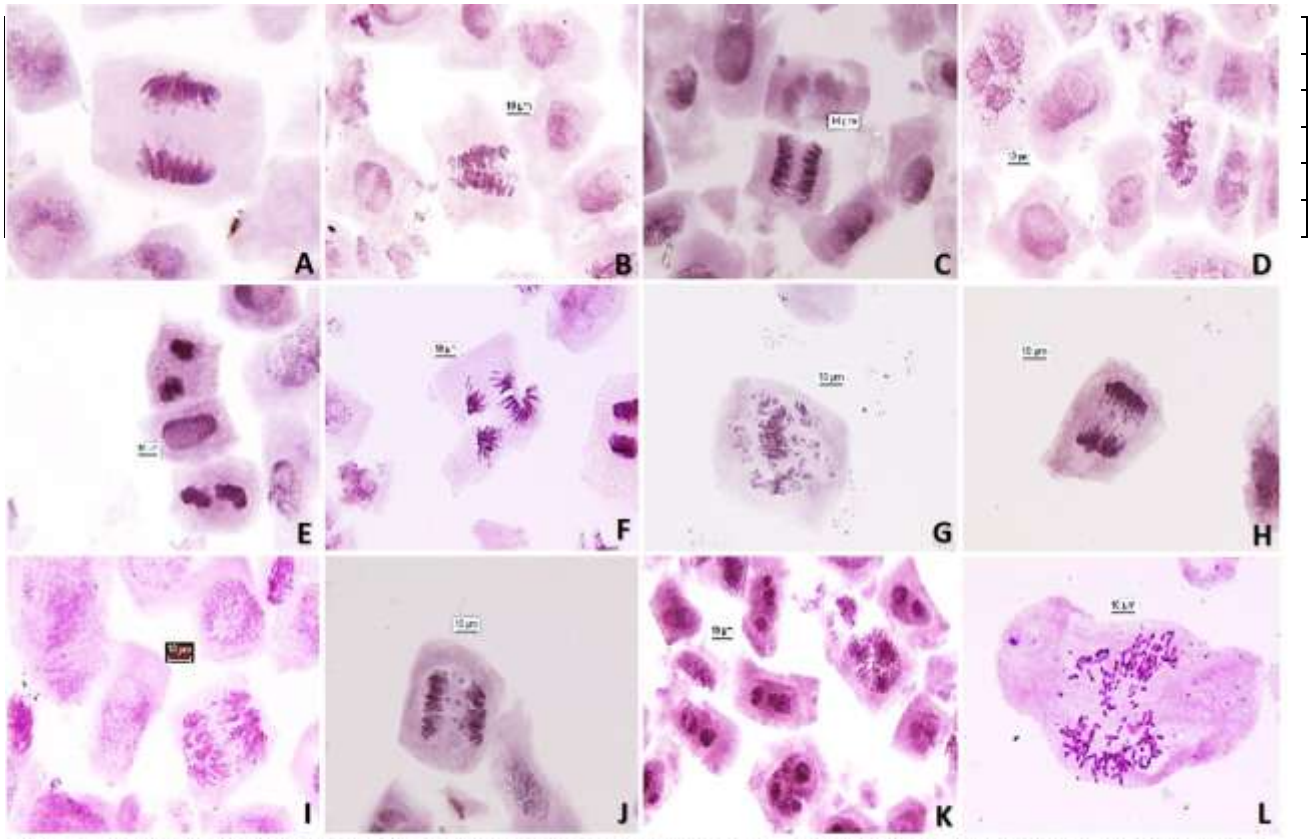

Figure 1: Chromosomal/mitotic abnormalities observed in somatic cells of root tips of *Triticum aestivum* treated with different water sample collected from the river Ganga as observed under Leica 300DM microscope at (40x): A. Normal anaphase ; B. sticky metaphase; C. Anaphase bridges; D. decondensed metaphase and tripolarity at anaphase; E. micronuclei formation due to laggards; F-Multipolarity G. Fragmented chromosomes H. Multiple laggards at Anaphase; I. Fragmented Anaphase; J. Chromosomal Laggards; K. micronuclei; L. Endopolyploid cell

Table 2: Concentration of Lead as detected by ICP-MS*

Sample	**control** µg/ml.	**S1** µg/ml.	**S2** µg/ml.	**S3** µg/ml.	**S4** µg/ml.
Pb	**BDL**	**0.115**	**0.095**	**0.046**	**0.015**

Table 3: Effects of Ganga water treatment on seed germination and root length in the seedlings of *Triticum aestivum*

Treatment	Seed germination parameters studied					
	Total number of seeds	Number of germinated seeds	% Germination	Shoot length (cm)	Root length (cm)	Root/Shoot ratio
C	100	85.0±4.04a	85	7.25±0.04a	2.17±0.02a	0.299±0.002 b
S1	100	30.0±3.61e	30	3.81±0.03d	0.35±0.02e	0.092±0.006 e
S2	100	40.0±1.53cd	40	3.93±0.02d	0.39±0.03e	0.099±0.007 e
S3	100	70.0±2.51b	70	6.03±0.08b	1.10±0.03c	0.182±0.001 c
S4	100	75.0±5.03ab	75	6.16±0.03b	1.95±0.01b	0.316±0.003 a

Mean ± SE values followed by same letters within each column are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) n = 3. C = control (Untreated seedling cells), S1 =, S2=, S3 = and S4.

Table 4: Cytotoxic effects of lead (Pb) on seedlings of *Triticum aestivum

Mean ± SE values followed by same letters within each column are not significantly different at 0.05 (ANOVA and

Treatments	Total no of mitotic cells observed	Number of dividing cells	MI %	Number of cells with abnormalities	% abnormality
Control	1000±1.686e	928±1.032a	92.8	00±0.00f	0.0
S1	1122±1.159c	408±1.054f	36.36	184±0.943a	**16.39**
S2	1085±1.069de	430±0.845e	39.63	136±0.879b	12.53
S3	1160±0.924a	650±0.840c	56.03	77±0.774d	6.63
S4	1088±0.840d	880±1.689b	80.88	37±1.019e	3.40

Duncan's multiple range test) n = 10. C = control (Untreated seedling cells), S1 =, S2=, S3 = and S4.

Table 5: Antioxidant enzyme SOR and SOD activities observed in *Triticum aestivum* after treatment with different water samples

Samples	SOR content (nmol g^{-1} fresh weight)	SOD activity (U/mg Protein)
C	244.76±0.33f	0.85±0.27f
S1	843.23±1.43a	3.21±0.88a
S2	524.82±0.38b	2.78±0.22b
S3	431.13±1.34d	1.48±0.41d
S4	365.76±0.52e	1.10±0.32e

* Mean ± SE values followed by same letters within each column are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) n = 3. C = control (Untreated seedling cells), S1 =, S2=, S3 = and S4.

Table 6: Effect of Pb (Lead) on the activities of enzyme involved in antioxidant system*

Samples	APX (Enzyme unit/gm fresh wt)	GPX (Enzyme unit/gm fresh wt)
C	0.357± 0.057b	3.703±0.115b

S1	2.178± 0.350a	7.037±0.703a
S2	1.964± 0.474 a	6.124±0.054a
S3	0.607± 0.226b	4.075±0.368b
S4	0.382± 0.067b	3.985±0.373b

* Mean ± SE values followed by same letters within each column are not significantly different at 0.05 (ANOVA and Duncan's multiple range test) n = 3. C = control (Untreated seedling cells), S1 =, S2=, S3 = and S4.