Morphological and cytological analysis characteristics in *Withania somnifera* and *Achyranthes aspera* associated with effects of industrial effluents

Manendra Pratap Singh[1], Ashok Kumar Verma[1], Sandeep Kumar Barwal[2,3*]

[1]Department of Botany, MMH College Gaziabad, Chaudhary Charan Singh University, Meerut - 250004, India
[2]Department of Botany, NREC College, Khurja, Bulandshahr, Chaudhry Charan Singh University, Meerut - 250004, India
[3]Plant Physiology and Tissue Culture Laboratory, Department of Botany, Chaudhary Charan Singh University, Meerut- 250004, India

Corresponding author Email: barwal.sk18@gmail.com

ABSTRACT:

The objective of the study was to conduct a comparative analysis between plants grown in environments affected by pollution and those cultivated in unpolluted areas and to assess the influence of industrial wastewater on the plants subjected to polluted conditions. The APHA technique was used to conduct analyses of the industrial effluent. According to cytological formulas, the cytological variables were determined. The results showed that as the amount of the effluent discharge grew, the chemical and physical variables of the analyzed effluent were found to have greater values compared to standard values, while the morphological and anatomical characteristics were found to have a downward tendency in effluent treated plant samples. In effluent treatment sets of 100%, 75%, and 50%, cytological metrics, including the mitotic and amitotic index were reduced, while they were found to be raised in the 25% concentration. As the effluent concentration rose, so did the number of mitotic abnormalities. The results show that Industrial wastewater discharge significantly impacts shoot, root length, and weight.

Keywords: effluent discharge, industrial pollution, mitotic index, cytological studies

INTRODUCTION:

Ahmad *et al.* (1988) and Threshow (1984) were among the first to draw attention to the negative effects of pollution on vegetation. Ghaziabad is a big industrial region close to New Delhi, India's capital. In the area around these businesses, many medical plants are growing, and their shape and structure are changing, which was studied in this study. It is a widespread practice to use industrial effluents in agricultural production as a substitute for traditional waste recycling methods. When added to soil, textile effluents may improve soil fertility, soil organic matter, and nutrient content, all of which are important for plant development and yield (Jothimani *et al.,* 2002). Elevated levels of nitrates and sulphates may stimulate protein synthesis and the creation of other organic molecules like chlorophyll in effluents. Even if these nutrients are present, their diluted concentration may not be enough to promote seedling development (Yousaf *et al.,* 2010). However, it has been found that using undiluted effluents negatively impacts growth and development advancements in agricultural technology. According to the source or kind of pollutant (Niroula, 2003) and the degree of toxicity of its substance, crops may react negatively to increased levels of industrial effluents. For instance, increased concentrations of textile waste in the water used for irrigation have been shown to reduce the amount of sprouting in plants (Saravanamoorthy and Ranjitha-Kumari, 2007).

High industrial effluent concentration in irrigation water can lead to delayed fruiting and poor yield due to cytological and structural disruption of plants (Uaboi-Egbenni *et al.* 2009). Heavy metal toxicity, inhibited by digestive enzymes, is another adverse effect of higher concentrations of industrial effluents on crop germination and seedling growth (Yousaf *et al.,* 2010). Toxic heavy metals are often included in synthetic carbon-based organic products like dyes (Balakrishnan *et al.,* 2008), which may contain phytotoxic components in the effluents from the dyeing process (Kaushik *et al.,* 2005). Roots are the initial structures in higher plants to come into touch with the poisonous metal, and the tip of the root is a primary site of damage, resulting in suppression of root development, a truncated roots system, and lower yield due to decreased absorption of both nutrients and water (Becker, 2000). Soil and crops watered with dye-effluent pollution run the danger of becoming a storage depot for these pollutants and other harmful heavy metals. The vitamins, minerals, and antioxidant-rich fibres abundant in vegetables make them an essential element of the human diet. Vegetables cultivated in polluted soils may contain genotoxic chemicals that might harm humans and other animals (Mathur *et al.,* 2006). Industrialization has had a negative effect on the growth and health of medical plants. As a result, researchers in the district of Ghaziabad, Uttar Pradesh, and at the ALTT Centre in Ghaziabad set out to examine the effects of industrial pollution on the development of Achyranthes aspera Linn. a plant with medicinal properties that grows abundantly in both polluted and unpolluted areas. Among its many medicinal applications, this plant is used in the "Siddha" concoction known as "Naaga Parpam" (Tyagi *et al.* 2013).

MATERIAL AND METHODS:

The effects of industrial contamination on *Withania somnifera* and *Achyranthes aspera* was studied by collecting samples from a location of effluent discharge close to a river basin in

Ghaziabad, UP, India. APHA, 1981 (APHA) analyzed industrial wastewater. Industrial effluents were applied to seedlings at three concentrations for cytological analysis: 25%, 50%, and 100%. After a thorough cleaning in distilled water, the root ends were placed in newly made Carnoy's fluid for 48 hours before being transferred to 70 per cent alcohol and placed in the fridge. Hydrolysis in a 2% acetocarmine solution followed by retention in the same solution allowed the root tips to be used for cytological analysis. Then, depending on the darkness of the stain, the root tips were crushed in acetic acid at a concentration of 45 per cent. The index of mitosis (MI) and amitotic index (A.M.I.) were used to track cytotoxicity, while the proportion of mitotic abnormalities (M.A.) was used to track cardiotoxicity.

Study of the concentration of the industrial effluents:

Researchers collected specimens from a river body near Ghaziabad where the species of *Withania somnifera* and *Achyranthes aspera* were growing. The samples were washed twice, first with regular water and then with distilled water, before being dried in an oven at 60 degrees Centigrade and carefully placed in clean, dry polythene bags. Each dried sample was then ground to a powder using an agate vibratory disc mill and sieved with a 1.3 mm stainless steel mesh. The material was subjected to an oxidative treatment to extract the desired components. One gram of plant material was placed in a porcelain crucible and ignited in a muffle furnace at an elevation not exceeding 500 degrees Celsius for 24 hours to create a dry ash. Before being broken down in a water bath for 20 minutes, the ashed sample was placed in a beaker with 10 mL of 20% extremely pure grade HCl. The final product was filtered via a 0.8 m pore-diameter ultrafilter membrane. After adding enough deionized water to bring the filter up to 100 mL, it was put through a Perkin Elmer A.A.S. under normal conditions for the quantitative measurement of Cr, Zn, Cu, Cd and Ni.

B.O.D. and C.O.D. values: The samples of the industrial waste effluents were analyzed after 5 days. The formulas used are as follows:

Formula used: Formula used for calculating BOD demand= $BOD_n = [D_1 – D_n] / P$

Where,

D_1 = initial sample dissolved-oxygen (D.O.) concentration,

D_n = sample D.O. after 5 days,

P = Dilution factor (decimal volumetric fraction of sample used),

BOD_n = n^{th} day biochemical oxygen demand.

The formula for calculating the C.O.D. demand values

= COD = [(A - B × N × 8 × 1000)] / Volume of sample taken

Where,

A = Volume of Ferrous Ammonium Sulphate for blank

B = Volume of Ferrous Ammonium Sulphate for sample

N = Normality of Ferrous Ammonium Sulphate

V = Volume of sample taken while conducting the experiment

These parameters were calculated with the help of the following formula:

$$(\mathbf{A})\ \text{Mitotic Index } \% = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} 100$$

$$(\mathbf{B})\ \text{Amitotic Index } \% = \frac{\text{Number of actively dividing cells}}{\text{Total number of cells}} 100$$

$$(\mathbf{C})\ \text{Mitotic Anomalies cells } \% = \frac{\text{Number of cells showing anomalies}}{\text{Number of cells in mitotic phase}} 100$$

RESULTS AND INTERPRETATION:

Table 1:

S. No.	**Parameters**	**Limit value of effluent discharge in the water sample being checked and investigated.**	**Maximum permissible limits**	**I.S.I. reference standards authentication.**
1.	Colour	Yellowish green	It should be colourless and transparent without odour.	ISO standards: 24512:2007.
2.	Odour	Pungent bad smell	It should be odourless.	ISO standards: 24512:2007
3.	pH	4.5	5.0-9.0	ISO standards: 24512:2007
4.	Total dissolved solids (mg/l)	850	2000	ISO standards: 24512:2007
5.	Total suspended solids (mg/l)	1500	600	ISO standards: 24512:2007
6.	B.O.D. value	21	30	ISO standards: 24512:2007
7.	C.O.D. value	210	250	ISO standards: 24512:2007
Values of different heavy metals present in the industrial effluent discharge				
8.	Chromium (mg/l)	6	Not applicable	ISO standards: 24512:2007
9.	Zinc (mg/l)	13	Not applicable	ISO standards: 24512:2007
10.	Nickel (mg/l)	16	Not applicable	ISO standards: 24512:2007
11.	Cadmium (mg/l)	5	Not applicable	ISO standards: 24512:2007
12.	Copper (mg/l)	6	Not applicable	ISO standards: 24512:2007

Table 1 represents the different parameters calculated for the industrial effluent discharged from the industries located near the river basin in Ghaziabad, Uttar Pradesh.

Table 2:

S. No.	Industrial effluent under study	Mitotic Value (Mean ± Standard deviation)	Amitotic Value (Mean ± Standard deviation)	Mitotic abnormality analysis
1	Water control	15.24 ±0.82	5.23 ± 0.54	.021 ±0.002
2	25% concentration of discharge effluent	16.24 ± 1.014	6.24 ± 0.42	6.02 ± 0.21
3	50%	12.51 ± 0.52	5.02 ± 0.21	7.234 ± 0.978
4	70%	8.12 ± 1.05	5.05 ± 1.12	8.231 ± 0.56
5	100%	4.23 ± 0.57	3.23 ± 1.02	2.41 ± 0.23

Table 2 provides the effect of different industrial effluent to varying concentrations on cells undergoing mitosis in Withania somnifera plant growing near a river basin in Ghaziabad.

Figure 1:

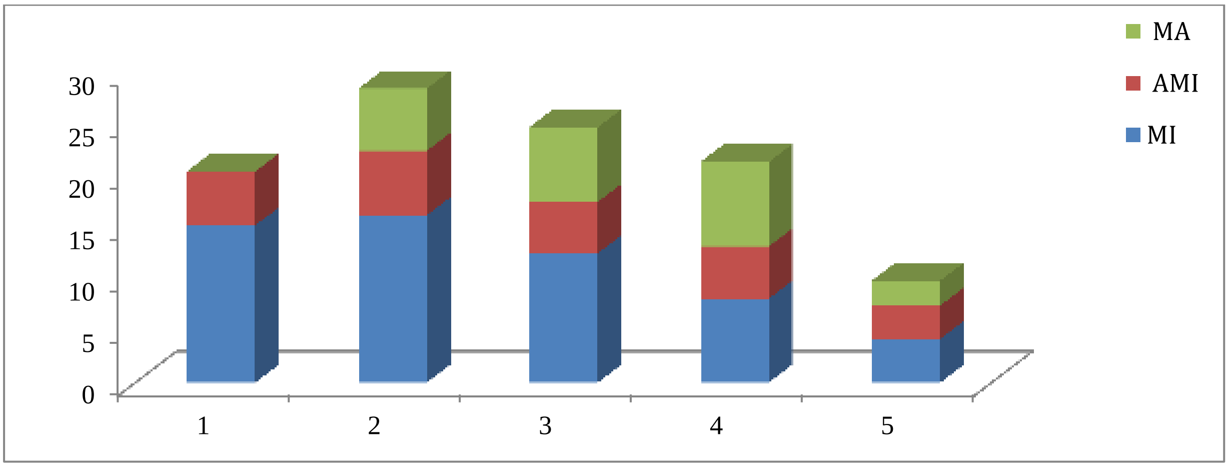

Fig 1. Shows the effect of different effluent concentrations on the MI, A.M.I. and M.A. in the cells of the *Withania somnifera* plant. X-axis shows the key for 1: water sample, 2:25% effluent treatment, 3: 50% effluent treatment, 4:70% effluent concentration, 5: 100% effluent concentration treatment on the *Withania somnifera* plant. The X-axis shows the key for treating the *Withania somnifera* plant with different concentrations of industrial effluents, and the Y-axis shows the MI, A.M.I. and M.A. values**.**

Table 3:

Effluent discharge	Effluent discharge concentration	Height of the plant (cm)	Width of the plant stem (cm)	Fresh weight of shoot (g per plant)	Fresh weight of root	The dry weight of the shoot	Dry weight of root
Sample 1	Control 0%	25.24	0.53	15.24	1.54	1.23	0.15
Sample 1	25%	24.12	0.49	14.21	1.02	0.87	0.09
Sample 1	50%	23.21	0.47	13.56	0.97	0.82	0.12
Sample 1	100%	NA	NA	NA	NA	NA	NA
Sample 2	Control 0%	32.45	0.67	25.27	2.32	1.76	0.21
Sample 2	25%	28.75	0.56	17.37	1.87	1.69	0.17
Sample 2	50%	25.74	0.53	14.26	1.54	1.58	0.11
Sample 2	100%	NA	NA	NA	NA	NA	NA
Sample 3	Control 0%	28.97	0.59	16.24	1.37	0.98	0.09
Sample 3	25%	24.78	0.53	11.37	1.32	0.87	0.08
Sample 3	50%	21.15	0.49	8.24	1.29	0.54	0.07
Sample 3	100%	NA	NA	NA	NA	NA	NA

Table 3: shows the effect of the 3 different samples of the industrial effluents collected from a river basin in Ghaziabad with the impact on the height of the plant, width of the stem and girth, weight of the shoot of the plant, root weight taken fresh along with the dry weight of shoot and root after observing the growth of the *Withania somnifera* plant starting from the first week to the sixth week.

Table 4:

Effluent discharge	Effluent discharge concentration	Height of the plant (cm)	Width of the plant stem (cm)	Fresh weight of shoot (g per plant)	Fresh weight of root	The dry weight of the shoot	Dry weight of root
Sample 1	Control 0%	26.34	0.63	16.24	1.34	1.03	0.09
Sample 1	25%	25.12	0.51	15.13	1.12	0.97	0.05
Sample 1	50%	22.21	0.31	14.56	0.91	0.79	0.14
Sample 1	100%	NA	NA	NA	NA	NA	NA
Sample 2	Control 0%	33.65	0.76	26.31	2.12	1.65	0.25
Sample 2	25%	29.31	0.61	17.29	1.79	1.61	0.11
Sample 2	50%	24.86	0.59	14.31	1.51	1.56	0.12
Sample 2	100%	NA	NA	NA	NA	NA	NA
Sample 3	Control 0%	29.91	0.51	16.12	1.30	0.91	0.08
Sample 3	25%	23.56	0.54	12.12	1.29	0.82	0.07
Sample 3	50%	20.24	0.45	8.16	1.28	0.49	0.06
Sample 3	100%	NA	NA	NA	NA	NA	NA

Table 4 shows the effect of the 3 different samples of the industrial effluents collected from a river basin in Ghaziabad with the impact on the height of the plant, width of the stem and girth, weight of the shoot of the plant, root weight taken fresh along with the dry weight of

shoot and root after observing the growth of the *Achyranthes aspera* plant starting from the first week to the sixth week.

Table 1: Results indicate that the colour of the industrial waste effluent is yellowish-green in colour with a pungent odour. The total dissolved contents, total suspended contents, and C.O.D. and B.O.D. values levels in the effluent discharge collected from a river source are less than the permissible levels (Table 1). **Table 2** provides the effect of different industrial effluent to varying concentrations on the root meristem cells undergoing mitosis on Withania somnifera plant growing near a river basin in Ghaziabad.

Table 3: shows the effect of different concentrations of industrial discharge at a concentration of 25%, 50% and 100% on the MI (mitotic index), A.M.I. (amitotic index) and abnormal mitosis index (AM) in the cells of *Withania somnifera* plant (Table 3). **Table 4:** shows the effect of different concentrations of industrial discharge at a concentration of 25%, 50% and 100% on the MI (mitotic index), A.M.I. (amitotic index) and abnormal mitosis index (AM) in the cells of *Achyranthes aspera* plant (Table 4). Table 4 indicates that the levels of the mitotic index and the mitotic division decrease as the concentration of the industrial effluent discharge increases from 25% to 50% and finally to 100%. **Fig 1.** Shows the effect of different effluent concentrations on the MI, A.M.I. and M.A. in the cells of the *Withania somnifera* plant. X-axis shows the key for 1: water sample, 2:25% effluent treatment, 3: 50% effluent treatment, 4:70% effluent concentration, 5: 100% effluent concentration treatment on the *Withania somnifera* plant. The X-axis shows the key for treating the *Withania somnifera* plant with different concentrations of industrial effluents, and the Y-axis shows the MI, A.M.I. and M.A. values**.** The index of mitosis (MI) and amitotic index (A.M.I.) were used to track cytotoxicity, while the proportion of mitotic abnormalities (M.A.) was used to track cardiotoxicity.

Fig 2. Shows the changes in the different stages of mitosis in the plant *Achyranthes aspera* after treating the cells with industrial effluents. **Fig 3.** Shows the effect of industrial effluents on the different stages of meiosis in the plant *Withania somnifera* from panel a to l.

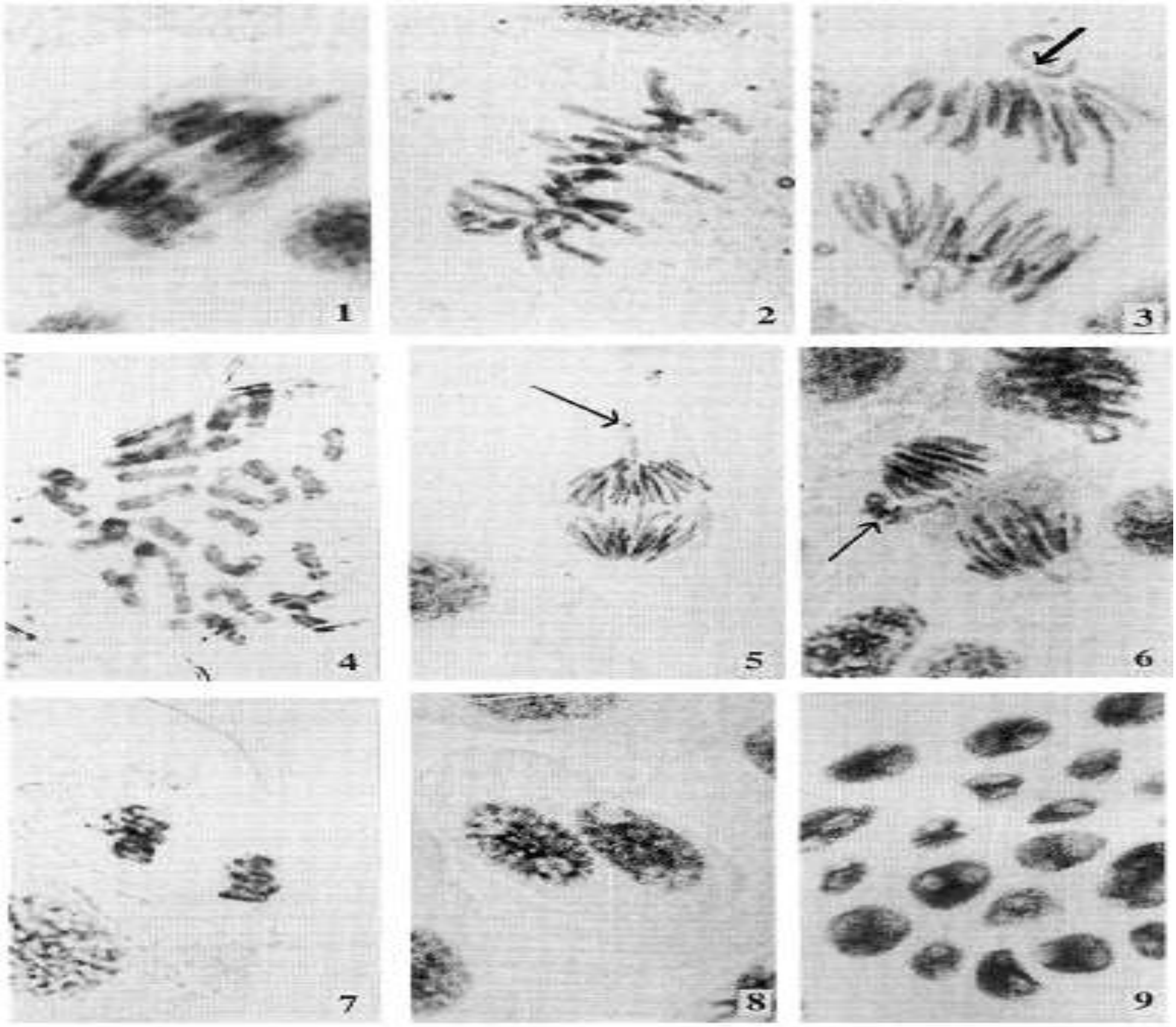

Fig: 2 Shows the changes in the different stages of mitosis in the plant *Achyranthes aspera* after treating the cells with industrial effluents.

Figure 2 shows the changes in the structure of chromosomes with chromosomal abnormalities during the different stages of mitosis as evident in the mitosis images. The picture shows the chromosome breaks due to the effect of heavy industrial metal pollutants present in the industrial sewage waste discharge from industries as indicated in the slides from number 1-9.

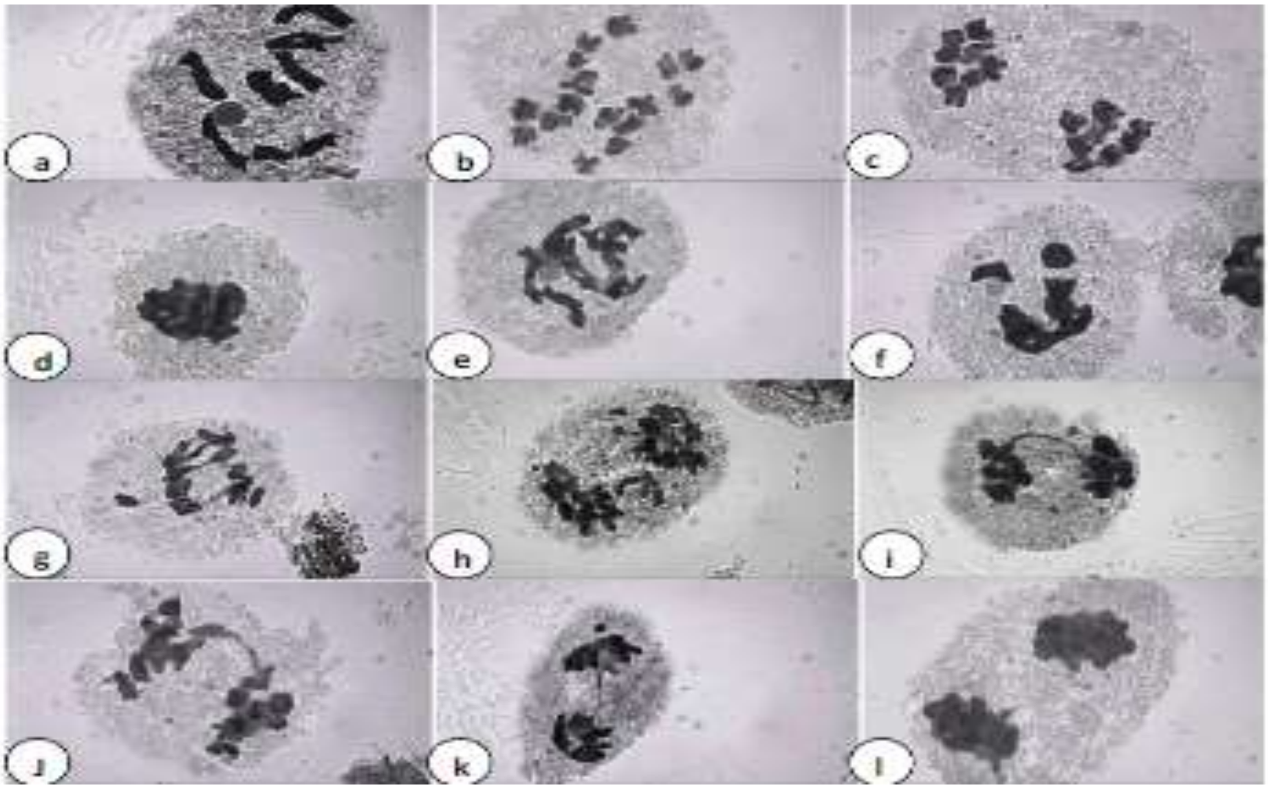

Fig: 3 Shows the effect of industrial effluents on the different stages of meiosis in the plant *Withania somnifera* from panel a to l.

ANALYSIS AND INTERPRETATION:

Analysis of the mitotic abnormality results in the *Achyranthes aspera* plant:

Figure 2: The panel 1 picture shows the anaphase bridge fragmentations formed due to the heavy metal pollutants found in the effluent discharge. The panel 2 picture shows the changes in the chromosome distortions during the metaphase stage. The panel 3 picture shows the spaces and gaps between the chromosomes in the anaphase stage leading to the segregation of the chromosomes. The panel 4 picture shows the abnormal banding pattern after treating the cells with industrial effluents. The panel 5 picture shows the distorted movement of the chromosomes during the anaphase stage. The panel 6 picture shows the change in the morphology and conformation of the chromosome present towards the side as shown with an arrow in the figure. The panel 7 picture shows the adhering and sticking of the chromosomes together during the telophase stage before the cell division or the cytokinesis. The panel 8 picture shows the bifurcated shaped nucleus with 2-split appearance due to the addition of industrial waste effluents. The panel 9 shows the presence of large spacious vacuoles due to the toxic effect of the industrial effluents.

Analysis and interpretation of the meiotic abnormality results in the *Withania somnifera* plant:

Figure 3: Panel a diagram in fig 3 shows the unaligned chromosomes during the metaphase-I stage of meiosis. Panel b shows the abnormal condensation of the chromosomes during the anaphase-I stage of meiosis. Panel c shows the abnormal localization and condensation of the chromosomes towards one corner in the telophase-I stage. Panel d diagram shows the stickiness and aggregation of the chromosomes in the metaphase-I stage due to the effect of the addition of the industrial effluents. Panel e shows the abnormal condensation of the chromosomes during the telophase-I stage. Panel f shows the aggregations of the chromosomes at the metaphase-I stage. Panel g shows the formation of the abnormal bridges during the anaphase-I stage of meiosis. Panel h shows the lagging of the chromosomes in the telophase-I stage of meiosis. Panel i shows the formation of abnormal bridges between the chromosomes during the telophase-I stage. Panel j shows the telophase-I stage chromosomes with abnormal adhering of the chromosomes. Panel k shows the aggregated micronucleus in the telophase-I stage of the cell after addition of industrial effluents. Panel l shows the abnormal condensation of the chromosomes towards the poles due to the addition of industrial effluents.

DISCUSSION:

The physicochemical parameters measured in the effluent samples all had readings that were over the thresholds set by the Indian Standard Institute (I.S.I.). Both Sujatha and Gupta (1996) and Singh *et al*. (1996) found the same thing. A close examination of the data shows that the physical and anatomical properties of plants growing in contaminated areas were severely diminished compared to plants growing in control sites and that the number of metrics analyzed decreased significantly. Plants gathered from contaminated areas exhibited a decline in morphological features such as leaf surface, petiole size, amount of leaves per plant, and lamina size. Palaniswamy *et al.* (1995) had their observations added together and compared them to the total number of comments.

Similar results were found by Trivedi and Singh (1990) in microscopic investigations of the leaf architecture of plants gathered from polluted locations, where the size and occurrence of openings and epidermal cells were significantly reduced. Plants have diverse reactions to various contaminants and even varying concentrations of the same pollutant. Onion leaves produced by Hg showed structural stomatal abnormalities similar to those described here, as Srivastava and Bansikar (1996) noted. The morphological and anatomical features of medicinal plants are also highly crucial for establishing their quality in terms of genuineness or authenticity. Microscopical approaches are significant for identifying and differentiating the realism of the plant medication since anatomical information may be used to uniquely identify each plant. This assist demonstrates the affinities of hypothetical genera and gives evidence for the relationships of groupings like families. Consistent structural features across plant species include stomatal and epidermal cell densities, vein-islet and vein termination densities, palisade ratio, stomatal index, etc.

Additionally, the presence of various stomata, crystals, fibres, trichomes, etc., in the powdered medication aids in identifying plants or differentiation when comparing the same plant harvested from the industrial region. A high level of environmental contamination was linked to trichomes that were both longer and more numerous. Low stomatal frequency is seen in plants cultivated in contaminated environments as a possible response to the restricted and regulated entrance of dangerous gaseous contaminants into the plant tissues. Research on the root meristem revealed that MI and A.M.I. are reduced in cycle industrial effluent treatment sets until at 25% concentration when they are increased. As effluent concentration rose, so did the frequency of mitotic abnormalities. Several other researchers have come to similar conclusions (Kaushik *et al.* 1997). The result is cellular abnormalities. The findings were compared to those of Sahu *et al*. (1987) and Thangapandian *et al*. (1995). Heavy metals in the effluent may be to blame for these shifts.

Conclusion: The conclusion is that industrial effluent discharge has a tremendous effect on the shoot, root length and weight of the plants *Withania somnifera* and *Achyranthes aspera* growing in the water body region near Ghaziabad.

Future scope: Development of Bioindicators: Establish these plants as bioindicators for monitoring environmental pollution and its impact on ecosystems.

Biotechnological Interventions: Investigate the potential for genetic and biotechnological interventions to enhance the tolerance of these plants to industrial pollutants.

Acknowledgement:

Authors are grateful to Department of Botany, MMH College Gaziabad, Chaudhary Charan Singh University, Meerut and the water body authorities for providing me the opportunity to collect the samples.

Author Contribution

Conceptualization, methodology, design and technical assistance, MPS and AKV, Formal analysis review and editing AKV and SKB. All authors have read and agreed to submit the final version of the manuscript.

Conflicts of interest: All authors in this context have no conflict of interest with the other authors.

References

Ahmad K J, Yunus M, Singh S N, Shrivastava K, Singh N, Kulshreshtha K 1988 "Survey of Indian plants in relation to atmospheric pollution: A Research Report" In: Perspectives in Environmental Botany, Vol. 2. Rao. D.N., K.J. Ahamad, M. Yunus and S.N. Singh. Today and Tomorrow's Printer and Publishers, New Delhi, India, pp. 283-306.

Treshow M 1984 *Environment and Plant Responses. New York: Mc. Graw Hill*. ISBN-0070651345 3.

APHA, AWWA, WPCP. Standard Methods for the Experimental of Water and Wastewater. 14th ed. New York: Amer, Publication Health Assoc. 1981.

Metcalfe C R 1980 *Anatomy of Dicotyledonous*. Oxford: Claredon Press.

Sujatha P, Gupta A 1996 "Tannery Effluents Characteristics and Its Effect on Agriculture." *J. Ecotoxiol Environ Alonit* 6, 1: 45-48.

Singh R S, Marwaha S S, Khanna P K 1996 Characteristics of pulp and paper mill effluents. *J. Ind. Poll. Cont.* 12, 163-172.

Palaniswamy M, Gunamani T, Swaminathan K 1995 Effect of air pollution caused by automobile exhaust gases on crop plants. *Proc. Acd Environ. Biol*, 4(2), 255-260.

Trivedi M L, Singh R S 1990 Effect of air pollution on epidermal structure of Croton Bonplandianum Baill. New Botanist, 17(3-4), 225-229.

Srivastava K, Bansikar V 1996 Mercury induced structural anomalies in the stomatal complexes of onion leaves. *J. Ind. Bot. Soc.*75: 73.e77.

Kaushik G, Yadav C S, Sharma H C 1997 "Cytological Effect of Sugar Mill Effluent on Meristematic Cells of" *Allium Cepa. J. Environ. Biol.* 18, 305-311.

Bera, A K, Saha A 1999 "Genotoxic Effect of Tannery Effluent on Mitosis of *Vicia faba* Root Tip Cells." *Legume Research* 20, 113-116.

Sahu R K, Acharya N N, Mishra R N 1987. "Cytomorphological Effects of Some Dyes Released with Paper Mill Effluents on *Allium cepa* Root Meristems. *Ind. Bot. Rept.* 6, no. 2: 73.e76.

Thangapandian V M, Swaminathan S K 1995 "Cytological Effect of Tannery Effluents on Root Meristems of *Allium cepa* L. Test System." *J. Environ. Biol.* 16, no. 1: 67-70.

Saravanamoorthy M D, Kumari B R 2007 Effect of textile waste water on morphophysiology and yield on two varieties of peanut (*Arachis hypogaea* L.). *J. Agri. Tech.* 3, 335-343.

Saravanamoorthy M D, Kumari B R 2005 Effect of cotton yarn dye effluent on physiological and biochemical contents of peanut (*Arachis hypogaea* L. cv. TMV-10) and green gram (*Phaseolus radiatus* L. cv. K1)1: 113–117.

Jothimani P J, Prabakaran A, Bhaskaran 2002 "Characterization and Impact of Dyeing Factory Effluent on Germination and Growth of Maize (CO-l) and Cowpea (CO-4)". Madras. *Agri. J.* 89: 568–71.

Kaushik P, Garg V K, Singh B 2005 "Effects of Textile Effluents on Growth Performance of Wheat Cultivars. *Biores. Tech*" 96: 1189–93.

Imrana Y, Ali M S, Yasmin A 2010 "Germination and early growth response of glycine max varieties in textile and paper industry effluents." Pak. J. Bot 42 (6): 3857–63.

Uaboi-Egbenni, PO, Okolie PN, Adejuyitan OE, Sobande A O, Akinyemi O 2009 "Effects of Industrial Effluents on the Growth and Anatomical Structure of *Abelmoschus esculentus* (Okra)." *Afr. J. Biotech.* 8: 3251–60.

Balakrishnan M, Antony S A, Gunasekaran S, Natarajan R N 2008 "Impact of Dyeing Industrial Effluents on the Groundwater Quality in Kancheepuram, India." *Ind. J. Sci. Tech.* 1: 1–8.

Nupur M, Bhatnagar P, Verma H 2006 "Genotoxicity of Vegetables Irrigated by Industrial Wastewater." *J. Environ. Sci.* 18, no. 5: 964–8. https://doi.org/10.1016/s1001-0742(06)60022-3.

Becker H 2000 "Phytoremediation Using Plants to Clean up Soil." *Agri. Res.* 23: 1151–7.

Tyagi K, Sharma S, Kumar S, Ayub S 2013 "Cytological, Morphological and Anatomical Studies of *Ricinus Communis* L. Grown under the Influence of Industrial Effluent – a Comparative Study." *J. Pharma. Res.* 7, 454-58.