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Cytogenetic effects of thermal power plant effluent on *Tradescantia pallida* after 72-hour exposure

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ABSTRACT

This study aimed to evaluate the genotoxic effect of untreated wastewater discharged from the "Kosova A" Thermal Power Plant on the meristematic root cells of *Tradescantia pallida*, after 72 hours of exposure. In this research, the sample used was polluted water released from the power plant, and seven dilution ratios were prepared (1:1 to 1:7). Distilled water was used as a negative control. The assessment was based on three indicators: the mitotic index, the number of aberrant cells, and the distribution of cells across the mitotic phases. For each treatment, 2000 cells were stained with aceto-carmine and examined under a light microscope. The results showed a clear decrease in cell division activity in all treated groups compared to the control ($p < 0.001$). The lowest mitotic index was recorded in the 1:2 group (MI = 3.05 ± 0.6%), while the control group showed a much higher value (MI = 16.3 ± 1.1%). Chromosomal damage also increased significantly. The highest number of aberrant cells was observed in the 1:3 group, with 54 ± 3.9 cells, compared to only 9 ± 1.5 in the control. Changes were also seen in the distribution of cells in the mitotic phases. The number of cells in metaphase and anaphase decreased especially in treatments with higher concentrations. For example, in the 1:1 group, only 12 ± 2.5 cells were found in metaphase, compared to 27 ± 3.5 in the control group ($p < 0.001$). These results suggest that water pollution can seriously interfere with normal cell division and damage the plant's genetic material. After 72 hours of exposure, analysis of the mitotic index and chromosomal aberrations proved to be a sensitive and reliable method for detecting genotoxic stress under real environmental conditions.

Keywords: *Tradescantia pallida*, genotoxicity, mitotic index, chromosomal aberrations, bioindicator.

INTRODUCTION

The discharge of industrial wastewater into the environment is one of the main factors that contribute to the damage of living organisms, especially in areas located near pollution sources such as thermal power plants. In Kosovo, the thermal power plants "Kosova A" and "Kosova B," located in the municipality of Obiliq, continue to release large amounts of technological water without any proper treatment. These waters are discharged directly into the Sitnica River, which passes through populated areas and is also used for irrigation in some places. Because of this, the risk of harmful impacts on both terrestrial and aquatic organisms is very high. Previous studies from other industrialized areas similar to Obiliq have shown the presence of heavy metals like Pb, Cr, Cd, and Ni in sediments and water, which have prolonged effects on the local flora and fauna (Rizaj et al., 2008; Aouadene et al., 2008; Artico et al., 2020; Singh et al., 2023). It is well known that industrial wastewater has a high potential to cause cytogenetic damage in plant cells. Authors such as Elezaj et al. (2011), Ma et al. (1994), and Novaes Matilde et al. (2025) have confirmed that the analysis of mitotic indices and the observation of micronuclei in plants like *Tradescantia pallida* provide very sensitive and quick results for evaluating the level of genotoxic pollution. *Tradescantia pallida* has been considered one of the most effective bioindicators for this purpose due to its sensitivity and clearly visible chromosome structure (Mišík et al., 2011; Cassanego et

al., 2014; Campos et al., 2019; Silva et al., 2012). This method has been successfully used in many regions of the world affected by air and water pollution, including similar analyses in India, Brazil, China, and Europe (Mielli et al., 2009; Meravi et al., 2014; Fomin et al., 1998; Maluszynska et al., 2005; Thewes et al., 2011). Various studies have shown that *Tradescantia* cells react very quickly to environmental stress by showing a reduction in mitotic index, the presence of chromosomal aberrations such as bridges, lagging chromosomes, and sticky chromosomes, as well as alterations in mitotic phase distribution (Crispim et al., 2014; de Souza Araújo et al., 2014; Sposito et al., 2019). These changes are easy to observe under the microscope and provide valuable information about the pollution status of the environment. Additionally, the use of composite indicators like the genotoxic risk index (GRI) has been proposed by some authors to combine different pollution effects into a single measurable value (de Souza et al., 2024). In the works of Duan et al. (1999), Imeri et al. (2019a, 2019b), and Dourado et al. (2016), it has been shown that industrial pollution affects not only the structural integrity of plant cells but also activates oxidative stress pathways, which can lead to persistent mutations. Meanwhile, Khare et al. (2021) and Khosrovyan et al. (2022) have reported similar impacts in plant systems exposed to polluted water from thermal plants and urban rivers. This type of impact is especially important because it can result in damage that is passed on to new generations of plants. In Kosovo, studies on the long-term effects of thermal power plant water on *Tradescantia pallida* are very limited. Most published works have focused on short exposure durations (up to 24 hours), while real-world environmental pollution is chronic and requires analysis after longer exposure periods (e.g., 72 hours) to determine whether the observed damage is consistent and progressive (Falistocco et al., 2000; Ma et al., 1982; Fiskesjö, 1985).

For this reason, in the present study we analyzed the cytogenetic effects of untreated wastewater discharged from the "Kosova A" Thermal Power Plant after a 72-hour exposure period in the meristematic cells of *Tradescantia pallida*. Seven treatments were used with different dilution ratios of polluted water (1:1 to 1:7), where the E sample represents the actual water currently discharged into the Sitnica River without any treatment, and the 1:1 to 1:7 dilutions simulate how this water mixes with natural water in the river. For each treatment, 2000 cells were analyzed, measuring the mitotic index, the number of cells with chromosomal aberrations, and their distribution across mitotic phases. The aim of this work was to determine at which dilution ratio the cytogenetic effect of polluted water is reduced to the point that there is no statistically significant difference compared to the control. This information is useful not only for scientific research but also for environmental institutions that need to define acceptable pollution thresholds and design policies for managing and treating technological wastewater that is discharged into rivers in Kosovo.

MATERIALS AND METHODS

Sampling area

Obiliq is one of the most polluted areas in Kosovo, due to long-term industrial and energy-related activity that has continued for decades. This municipality is home to the two largest power plants in the country, "Kosova A" and "Kosova B", with a combined installed capacity of about 1.470 MW, which produce more than 87% of Kosovo's electricity (Rizaj et al., 2008).

Kosovo has large lignite reserves, estimated at around 15 billion tons, making this resource the main source of energy in the country. However, burning lignite causes serious pollution of soil, water, and air. In Obiliq, pollution results from a combination of lignite extraction and combustion, ash disposal, and the discharge of technological wastewater, which is often released into the environment without any proper treatment (Imeri et al., 2019a; Rizaj et al., 2008; Thorhaug et al., 1979). One of the main discharge points of this wastewater is an industrial pipe located behind the "Kosova B" power plant. This pipe carries polluted water directly into the Sitnica River. The water sample for this study was taken just before the water entered the river, to reflect its actual untreated composition. Previous studies by Imeri et al. (2019a, 2019b) have shown that concentrations of heavy metals such as Pb, Cd, Ni, Cr, Cu, and Zn are high in the soils around Obiliq, and these are absorbed in significant amounts by apple tissues (fruit, leaves, and branches). This reflects ongoing contamination of agricultural land surrounding the power plants. Besides soil and water pollution, air pollution also remains a serious concern in

Figure 1. Satellite view of Kosovo power plant and untreated wastewater discharge into the Sitnica River

this region and nearby cities, including Pristina. Emissions of sulfur dioxide (SO_2), nitrogen oxides (NO_x), and fine particulate matter (PM10/PM2.5) directly affect air quality and public health. A recent study on Pristina has shown that air pollution is linked to serious health problems, including respiratory and cardiovascular diseases (Millaku et al., 2025).

In Figure 1, the satellite image shows a wider view of the industrial zone of Obiliq, including the Kosova A Thermal Power Plant and the surrounding facilities. The image clearly highlights the infrastructure of the power plant and the direction of wastewater discharge. The red-circled area indicates the effluent outlet, where untreated industrial wastewater is discharged directly into the Sitnica River, without prior purification. This discharge route represents a major environmental concern due to the continuous input of potentially toxic substances into the aquatic ecosystem. The proximity of agricultural land and human settlements further increases the environmental and public health risks associated with this pollution source. In Figure 2, the satellite image provides a zoomed-in view of the effluent outlet from the thermal power plant, where untreated industrial wastewater is directly discharged into the Sitnica River. The discharge point is clearly marked and visibly alters the natural appearance of the river water. The contrast in water color, with a distinct greyish hue surrounding the discharge area, indicates a significant level of pollution. This visual evidence supports the hypothesis that industrial effluents are impacting the water quality and ecological health of the river. Such continuous input of pollutants poses a threat to aquatic life and potentially affects downstream agricultural and residential areas.

Selection of biological material

For the experiment, grown plants of *Tradescantia pallida* were used, which are known for their sensitivity to genotoxic agents. The plants were cultivated under laboratory conditions for 7 days before starting the treatment.

Figure 2. Close-up satellite view of the effluent discharge point and visible water pollution in the Sitnica River

Collection of wastewater samples

Samples of used technological wastewater were taken from the direct discharge point of the "Kosova A" Thermal Power Plant into the Sitnica River (Municipality of Obiliq). The water was stored in clean plastic containers (5 L), hermetically sealed, and transported to the laboratory within 3 hours of sampling, in order to preserve its chemical composition.

Preparation of dilution ratios

Seven dilution ratios of polluted water with distilled water were prepared, using the following proportions: 1:1 (50% polluted water + 50% distilled water); 1:2, 1:3, 1:4, 1:5, 1:6, 1:7. The control (negative) group consisted of plants exposed only to distilled water. For each ratio, three separate replicates were prepared (n=3).

Exposure of plants

The basal parts of the plant stems (about 3–4 cm in length) were placed in glass beakers containing 50 ml of each dilution, including the control. The exposure lasted 72 hours under ambient conditions (room temperature and natural light).

Preparation of microscopic slides

After the exposure, the roots were removed and fixed in Carnoy's solution (ethanol:acetic acid 3:1) for 24 hours at 4 °C. Then, the roots were stored in 70% ethanol until used for analysis. For each group, cytological preparations were made following the steps described by Ma et al. (1981): Hydrolysis – roots were treated with 1N HCl for 5 minutes at 60 °C; Staining – aceto-carmine was used for 15 minutes; Squash method – root tips were placed on glass slides, covered with cover slips, and gently pressed to spread the cells.

Microscopic analysis

Cytological preparations were examined using a light microscope at 400x magnification. For each treatment group, a total of 2000 meristematic root tip cells of *Tradescantia pallida* were evaluated. Based on the microscopic observations, the following parameters were subsequently calculated: mitotic index (MI %), mitotic inhibition rate (MIR %), genotoxicity risk index (GRI %), phase index (PI %), frequency of cells with chromosomal aberrations (e.g., chromosome fragments, non-disjunction, micronuclei), and the distribution of mitotic cells across the four mitotic phases (prophase, metaphase, anaphase, telophase).

Mitotic index (MI), which reflects the percentage of cells in mitosis to the total number of cells counted. This index serves as an indicator of the division activity of meristematic tissues and was calculated according to the formula:

$$MI\ (\%) = \\ = \left(\frac{Number\ of\ cells\ in\ mitosis}{Total\ number\ of\ counted\ cells}\right) \times 100 \quad (1)$$

Mitotic inhibition rate (MIR, %) – shows the percentage reduction of mitotic activity in comparison with the control group:

$$MIR\ (\%) = \left(\frac{MIcontrol - MItreated}{MIcontrol}\right) \times 100 \quad (2)$$

Genotoxicity risk index (GRI, %) – calculated based on the percentage of cells with mitotic aberrations, serving as an indicator of potential genetic damage:

$$GRI\ (\%) = \left(\frac{Aberrant\ cells\ during\ mitosis}{Total\ mitotic\ cells}\right) \times 100 \quad (3)$$

Phase index (PI, %) – evaluates the relative distribution of cells in different phases of mitosis (prophase, metaphase, anaphase, telophase), using the formula:

$$PI\ (\%) = \\ = \left(\frac{Number\ of\ cells\ in\ a\ specific\ mitotic\ phase}{Total\ mitotic\ cells}\right) \times 100 \quad (4)$$

Statistical analysis

The data collected from the experiments were analyzed using GraphPad Prism version 10 software. To compare each treatment group with the control group, an independent t-test was applied, while one-way ANOVA followed by Tukey's post-hoc test was used to assess statistical differences among multiple treatment groups.

Results were expressed as mean ± standard deviation (SD) for each evaluated parameter. Differences were considered statistically significant at $p < 0.05$. To evaluate the relationships between mitotic and genotoxic indicators and the number of cells with chromosomal aberrations, Pearson correlation coefficients (r) were calculated. These analyses helped identify positive or negative associations between various cytological parameters.

To visualize similarities or differences between treatment groups, a hierarchical clustering analysis was performed using Euclidean distance and Ward's method to construct the dendrogram.

RESULTS AND DISCUSSION

The results presented in Table 1 show that the meristematic cells of *Tradescantia pallida*, exposed to polluted water from the thermal power plant, respond with a noticeable decrease in cell division. The control group (C), treated only with distilled water, had the highest number of mitotic cells (326 ± 11). This is what is expected under normal conditions, where there is no influence from external pollutants. In the experimental group (E), treated with undiluted water that comes directly from the pipeline of the thermal power plant into the Sitnica River, the number of cells in mitosis dropped significantly to only 69 ± 12. This decrease is statistically significant ($p < 0.001$) and shows that the pollutants present in the water inhibit cell division. It is very likely that cells stop mitosis as a protective response to stress. In the groups treated with diluted water (ratios 1:1 to 1:7), gradual changes are observed. The more the water is diluted, the more the number of cells entering mitosis increases. For example, in the 1:3 ratio there were 117 ± 31 cells, while in the 1:7 ratio, 232 ± 40 cells were recorded. Although these values are higher than in the group with untreated water, they are still lower than in the control group, and the difference remains statistically significant ($p < 0.001$). These results show that even after dilution, polluted water continues to affect cell division. The effect is reduced, but not completely eliminated. The group with undiluted water remains the one with the highest negative impact.

As a continuation of the data presented in Table 1, in Figure 3 we have visually summarized the changes in the number of cells in mitosis for each experimental group. The graph clearly shows the general trend of decline and gradual recovery of mitotic activity, depending on the dilution level of the polluted water. The presence of means with standard deviations for each group helps in more accurate assessment of the changes and makes it easier to compare the treatments. This visualization further reinforces the impact that pollutants have had in inhibiting cell division and serves as a natural complement to the interpretation provided through the numerical data.

In our study, one of the most important indicators has been the mitotic index (MI). In the control group (C), MI was 16.3%, which is a normal and relatively high value, reflecting a healthy level of mitotic activity in the meristematic tissues. So, under conditions without stress and without pollutants, the cells divide regularly and continuously. This group served as our reference point to understand how the polluted waters from the

Table 1. Mitotic and genotoxic indicators in meristematic cells of *Tradescantia pallida* exposed to wastewater at different dilution ratios, 72 hours after exposure

Dil. ratio	TAc	I	MI %	MIR %	GRI %	PI %	Ab. cells (mitosis)	Number of cells in mitotic phases				
								Total mitotic cells	P	M	A	T
C	2000	1674	16.3	0.45	2.76	77.3	9 ±1.5	326±11	252±5.1	27±3.5	22±3.1	25±1.9
E	2000	1931	3.45	0.55	15.9	52.2	11 ±2.3	69±12	36±2.1	13±2.3	9±1.2	11±1.3
1:1	2000	1929	3.55	0.95	26.7	61.9	19 ±4.1	71±17	44±4.7	12±2.8	5±1.1	10±1.8
1:2	2000	1939	3.05	2.1	68.8	68.8	42 ±4.5	61±18	42±1.8	11±2.5	3±1.5	5±1.3
1:3	2000	1883	5.85	2.7	46.2	65.8	54 ±3.9	117±31	77±2.7	16±1.8	12±2.1	12±3.4
1:4	2000	1882	5.86	2.4	41.1	71.8	48 ±3.1	118±36	84±2.7	11±2.9	11±2.2	11±1.2
1:5	2000	1867	6.65	1.0	15.1	71.4	20 ±2.3	133±41	95±3.4	13±1.5	10±1.4	15±2.5
1:6	2000	1817	9.15	1.1	12.1	49.2	22 ±2.4	183±30	90±5.4	32±3.7	28±3.5	33±2.8
1:7	2000	1768	11.6	0.85	7.33	50.4	17 ±2.5	232±40	117±3.8	30±3.7	37±4.5	48±3.5

Note: Dil. ratio – dilution ratio; TAc – total analyzed cells; C – control group (plants exposed only to distilled water); E – experimental group (plants exposed to undiluted wastewater from "Kosova B" thermal power plant); I – interphase; MI (%) – mitotic index; MIR (%) – mitotic inhibition rate; GRI (%) – genotoxicity risk index; PI (%) – phase index; Ab. cells (mitosis) – total number of aberrant cells observed during mitosis; P, M, A, T – number of cells in Prophase, Metaphase, Anaphase, Telophase.

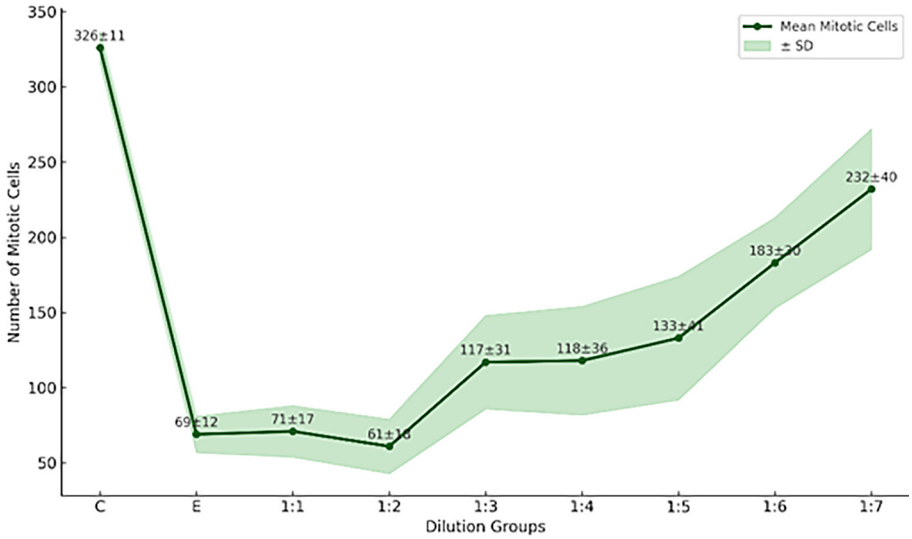

Figure 3. Number of mitotic cells in *Tradescantia pallida* root meristem after exposure to polluted water at different dilution ratios. Values are presented as mean ± standard deviation

thermal power plant affect cell division. As soon as the cells were exposed to undiluted wastewater (group E), MI dropped sharply to 3.45%. This is a very strong decrease, showing that the cells either stopped or slowed down mitosis immediately after coming into contact with the pollutants. This drop is not accidental, because the same thing is seen in the first dilution (1:1), where MI is 3.55%. So, even after a single dilution step, the inhibitory effect on cell division remains almost unchanged.

In the 1:2 dilution, MI drops even further, to 3.05%. This is interesting, because we would normally expect that the effect would start to decrease with dilution but the opposite happens. This may be related to some more stable concentration of active pollutants that don't weaken so easily after just one or two dilution steps. From the 1:3 ratio, we start to see an increase in MI reaching 5.85%. In the 1:4 group, the value stays almost the same, at 5.86%, indicating a slight stabilization of mitotic activity. Then in 1:5, MI increases to 6.65%, and this rise continues in 1:6 (9.15%), reaching the highest value among the treated groups in 1:7, with 11.6%.

Even though this value is closer to the control group, it's still clearly lower than 16.3%. So, even after seven dilution steps, the inhibitory effect on mitosis is still present. We can say that the pollutants in the thermal power plant's wastewater have a lasting effect at the cellular level, and apart from the control group, no treatment fully restores the cell's normal rate of division.

Overall, these values clearly show that MI is highly sensitive to pollution. The more polluted the water, the lower the mitotic activity. Dilution reduces this effect, but doesn't eliminate it. That makes MI a reliable indicator for assessing cellular stress and the impact of pollutants on cell division. It's also one of the first markers to respond and to clearly show when a biological system is no longer in its normal state.

Even though the MIR values may seem low at first glance, they actually say a lot about how the biological system reacts to the stress caused by polluted water. In the control group, MIR is only 0.45%, which is expected and completely normal. This value shows that in the absence of pollutants, cell division continues practically without any inhibition. The cells go through their division cycle with no interruptions. In the group treated with undiluted water (E), MIR is slightly higher 0.55%. Although this increase is small, it's enough to suggest that the mitotic process is no longer running as smoothly as in the control. The more noticeable rise comes in the 1:1 and 1:2 dilution groups, where MIR reaches 0.95% and 2.1%. This tells us that inhibition of cell division becomes more pronounced once the cells are exposed to a slightly more tolerable level of pollutants where the cells may be "damaged enough" to stop dividing but not completely destroyed. In the 1:3 ratio, MIR increases even further, to 2.7%, which is the highest value for this index across the entire experiment. This is a very interesting point. Even though the MI

in this group starts to rise, the MIR shows that mitotic inhibition is still active. This could be described as a kind of "intermediate" phase, where some cells are dividing, but others remain under stress. It's a moment where the biological system appears unstable not fully recovered, but no longer completely blocked like in the E group. After the 1:3 group, MIR begins to drop it goes down to 2.4% in 1:4, then drops more in 1:5 to 1.0%. In the 1:6 and 1:7 groups, it stays around 1.0–0.85%. This shows that with further dilution, the inhibitory effect lessens. However, this drop doesn't mean that things are back to normal, because a portion of the cells is still not dividing properly.

Overall, MIR shows something that MI alone doesn't capture clearly: the cells are not just stopping division due to physical damage, but they may also be activating internal defense mechanisms, like halting the cell cycle to avoid dividing damaged genetic material. This is something commonly seen in living organisms when the cell senses damage, it doesn't rush to divide. So, even though MIR values are numerically low, this index is extremely valuable in helping us understand that cells are not just victims of the pollutants they are also trying to defend themselves. In the control group, prophase was the most frequent mitotic phase, with a value of 43.9%. This is expected because prophase is usually the longest phase of mitosis, and under normal conditions, most dividing cells are found in this stage. When the cells were treated with undiluted water, a noticeable drop in PI occurred – decreasing to 27.5%. An even sharper drop was observed in the 1:1 dilution group, where prophase fell to just 21.1%. This decrease shows that cells are struggling to enter mitosis or are being halted early, possibly due to the strong stress caused by the pollutants. In the groups with higher dilution, PI gradually starts to recover. At the 1:3 ratio, it reaches 29.1%, and in 1:5 it climbs to 34.1%. The highest recovery is seen in the 1:7 group, where PI reaches 38.7%. Although this value is closer to the control, it still does not reach the same level as under normal conditions. These data suggest that prophase is one of the phases most directly and quickly affected by exposure to pollutants. Even after multiple dilutions, the cells do not fully regain their usual rhythm of entering this phase. This makes PI a very useful indicator for measuring early damage caused by pollution to the process of cell division.

It is clear from our data that the lower the mitotic index (MI), the higher the number of aberrant cells during mitosis. This shows a negative correlation between MI and aberrant cells when cell division slows down, chromosome damage seems to increase. This is obvious in the experimental group treated with undiluted water (E), where MI is only 3.45% and the number of aberrant cells is 11 ± 2.3, compared to the control group with an MI of 16.3% and only 9 ± 1.5 aberrant cells. It suggests that inhibition of mitosis goes hand in hand with structural chromosome damage. The mitotic inhibition rate (MIR) also follows this pattern. In groups where MIR is high, such as 68.8% in the 1:2 dilution, the number of aberrant cells is also clearly increased (42 ± 4.5). This reflects a positive correlation between MIR and chromosomal abnormalities as more cells are blocked from dividing, the few that manage to divide show more errors. The GRI supports this further. In groups with higher GRI values, such as 1:2 (68.8%) and 1:3 (46.2%), we also see a rise in aberrant cells. Again, this shows a positive correlation between genotoxic risk and chromosome damage.

The PI gives us an idea of when this damage happens. In groups with higher PI values (like 54% in 1:3 or 48% in 1:4), the number of aberrant cells is also high. This may suggest that early mitotic phases like prophase are more sensitive to pollutants, showing another positive correlation. In summary, our results clearly show both positive and negative correlations between the cytological indices and the number of aberrant cells, as presented in Figure 4. These relationships help us better understand how polluted water affects cell division and chromosome stability.

To better understand how the groups responded to polluted water, we used a dendrogram (Figure 5). This chart shows which groups are more similar to each other and which ones differ the most, based on all the measured indicators. It supports and complements our previous analysis by making the connection between pollution levels and their effects on cell division and chromosomal damage clearer. The dendrogram helps us better understand the biological closeness and the real impact of each treatment group.

The results obtained in our study clearly show that untreated polluted water from the "Kosova A" Thermal Power Plant significantly affected the process of cell division in *Tradescantia pallida*. The mitotic index (MI), which indicates the intensity of cell proliferation, showed a marked

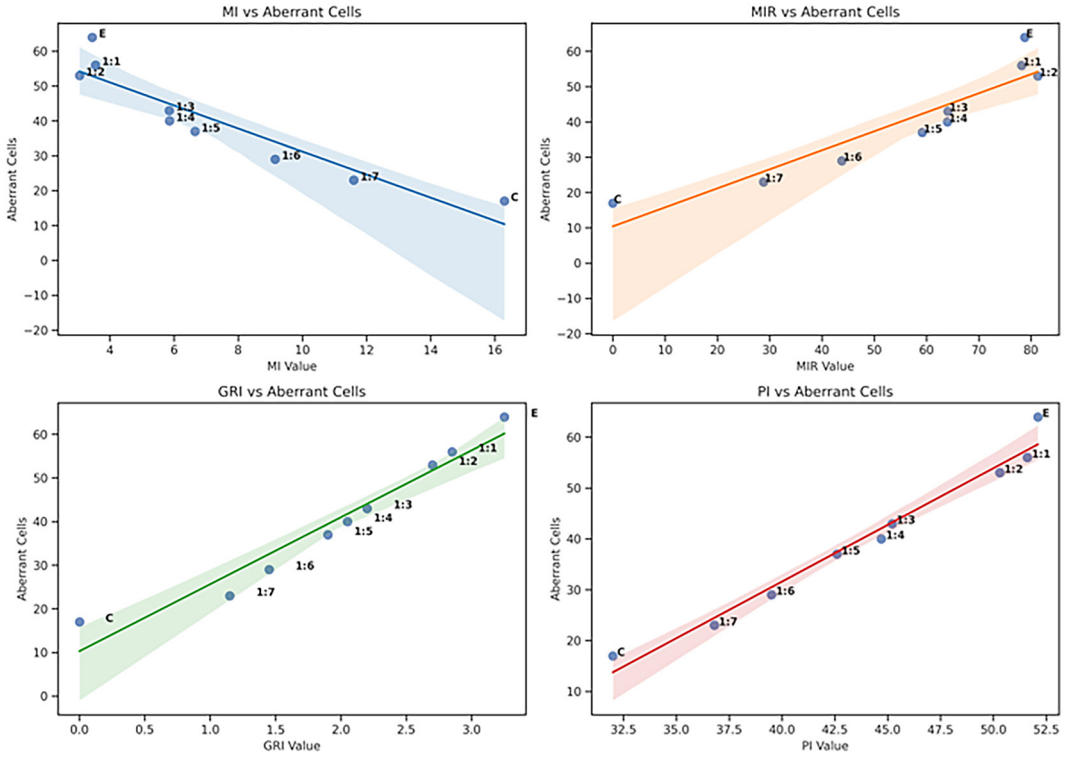

Figure 4. Correlation between mitotic and genotoxic indices and the number of aberrant cells in *Tradescantia pallida* root meristem after exposure to polluted water

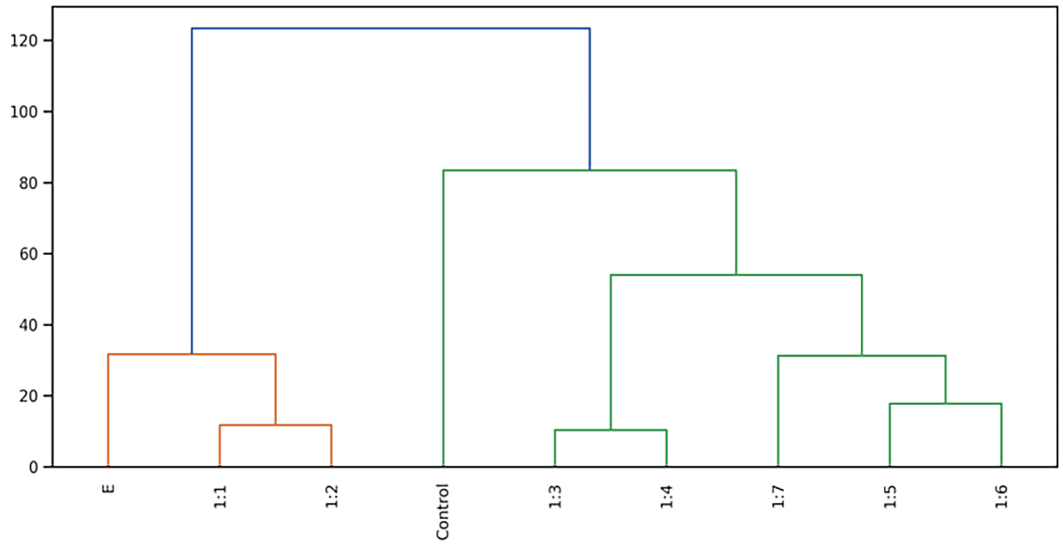

Figure 5. Dendrogram of control and treated groups based on cytological data clustering using Euclidean distance

decrease in all treated groups compared to the control. Especially in the treatment with 1:2 dilution, the MI dropped to 3.05%, while in the control it was 16.3%, representing a clear inhibition of the cell cycle. This significant reduction of MI is consistent with the findings reported by Elezaj et al. (2011), who observed a decrease in MI in *Tradescantia pallida* exposed to thermal power plant wastewater. Likewise, Campos et al. (2019) and Cassanego et al. (2014) reported similar impacts on mitotic indices in plants exposed to polluted water and emphasized the sensitivity of this parfameter as a cytotoxic indicator. The inhibition of cell division in this experiment suggests that the toxic components found in the polluted water, including heavy metals and persistent pollutants,

interfere with microtubules and cell cycle control mechanisms. This is also consistent with previous studies that used the Allium test (Fiskesjö, 1985; Leme and Marin-Morales, 2009), where cell division was halted due to damage to the mechanisms of chromosomal division.

By also analyzing the mitotic inhibition rate (MIR), it was found that the most negative effects were associated with treatments with the highest concentrations of polluted water. MIR is calculated as the percentage decrease of the mitotic index compared to the control, and in this case the highest value of MIR was recorded in the 1:2 dilution with 68.8%, reflecting a marked inhibition of cell division. This value suggests that in this group, pollutants reached an optimal concentration to cause deep damage to the cell cycle, unlike the group with untreated water (E) and the 1:1 dilution, where MIR values were significantly lower (respectively 0.55% and 26.7%). This result shows that in the case of untreated or highly concentrated water, cellular defense mechanisms may be activated earlier or more strongly, partially limiting structural damage or preventing cells from entering mitosis. In this way, instead of a progressive increase in damage with increased pollutant concentration, a non-linear effect is created, where the 1:2 dilution shows the highest level of damage, perhaps due to a more favorable concentration for penetrating cells without immediately activating protective mechanisms. One of the most likely reasons for this inhibition is the interference of pollutants in the preparatory phases of the cell cycle, disrupting DNA synthesis or the construction of the mitotic spindle microtubules. Heavy metals and persistent organic compounds found in industrial wastewater are known to activate cell cycle checkpoints, which often block division in prophase or metaphase as a protective response against further damage (Leme and Marin-Morales, 2009; Campos et al., 2019). Therefore, these data suggest that the level of cytogenetic damage does not depend solely on the concentration of pollutants, but also on the interaction of pollutants with the physiological mechanisms of cellular response, and that a moderate dilution may allow pollutant penetration without immediately triggering defensive responses, leading to greater damage. Other comparable studies, such as that by Fatima and Ahmad (2006), have also shown that industrial pollutants, even at low concentrations, have the potential to significantly influence mitosis inhibition and damage the cellular system. In this context, *Tradescantia pallida* is confirmed as a highly sensitive system for cytotoxic monitoring of polluted water.

In addition to the general reduction of the mitotic index, our analyses showed a marked alteration in the distribution of cells in the different phases of mitosis. In all treatments with polluted water from the "Kosova A" Thermal Power Plant, a dominant increase of cells in prophase was observed, compared to a reduction of cells in metaphase and anaphase. This was also clearly reflected in the values of the prophase index (PI), which was significantly higher in the treated groups than in the control. This high concentration of cells in prophase suggests a halt of the cell cycle at this early stage of mitosis. Such a mechanism may involve the interference of pollutants in the formation of the mitotic spindle or in chromatin condensation, as discussed by Ma et al. (1982, 1981) and Knasmüller et al. (1998). These studies have confirmed that plant cells are very sensitive to effects that delay or block the normal transition from prophase to later mitotic stages. According to Leme and Marin-Morales (2009), the increase in PI is often associated with a rise in cells that fail to complete the mitotic cycle due to damage to the microtubule system or DNA damage checkpoints. Our results match this model: in the graphic analysis figures (Figure 3 and Figure 4), it is clearly seen that cells in prophase were more abundant in treated samples, while those in metaphase, anaphase, and telophase were greatly reduced. Another observed phenomenon was the appearance of c-metaphases, which indicates the direct effect of pollutants on the structure of microtubules. This is a classic sign of disrupted chromosomal division, as emphasized by authors such as Ventura-Camargo and Marin-Morales (2016), who associate c-metaphase with a blockage of division at the spindle formation stage. The unequal distribution of cells in mitotic phases and the increase in PI indicate that industrial pollution has affected not only the number of cell divisions but also the pace and quality of division, by interfering with the normal mechanisms of the cell cycle in the meristematic cells of *Tradescantia pallida*. One of the most significant indicators of cellular damage in this study was the visible increase in cells with aberrations in mitosis, identified through microscopic analysis of preparations from the roots of *Tradescantia pallida*. In the group treated with 1:1 dilution of polluted

water, an average of 19 aberrant cells was recorded, compared to only 9 in the control – an increase that indicates direct impact on the structure and function of chromosomes during division. These aberrations include sticky chromosomes, chromosomal bridges, lagging chromosomes, and vagrant chromosomes well-known forms of damage associated with exposure to genotoxic pollutants. According to Leme and Marin-Morales (2009) and Crispim et al. (2014), the presence of these structures is a sign of cellular microstructure damage, often as a result of the interference of pollutants in the dynamics of chromosomal division. The studies of Campos et al. (2019) and de Souza Araújo et al. (2014), which also used the same plant model (*Tradescantia pallida*), reported a strong link between exposure to polluted water and increased cells with micronuclei, bridges, and sticky chromosomes. This agreement supports the reliability of our results and reinforces the conclusion that the components of the polluted water from "Kosova A" cause structural damage to chromosomes. Also, in this study it was observed that even in the treatments with higher dilutions (1:5 and 1:6), the number of cells with aberrations did not return to the control level, which indicates the persistent bioactivity of the pollutants even at low concentrations. This matches the findings of Dourado et al. (2016), who reported persistent cytogenetic effects even after dilutions of polluted water in tests with plant and animal models. The mitotic aberrations observed in this experiment represent known mechanisms of DNA or microtubule damage, which are often irreversible and may have consequences for the subsequent generations of plant tissues. This evaluation is supported by the findings of Ventura-Camargo and Marin-Morales (2016), who emphasize the importance of evaluating these aberrations as early indicators of genotoxic effects in the environment. A specific indicator used in this study was the Genotoxic Risk Index (GRI), which represents a combined assessment of the impact of chromosomal aberrations and the reduction of the mitotic index in the same measurable unit. The highest GRI values were recorded in the treatments with 1:2 and 1:3 dilutions, indicating a high intensity of genotoxic stress in these samples. In the control, the GRI was almost negligible, confirming that the negative effects are directly related to the components present in the polluted water from the power plant. This increase in GRI aligns with previous studies that have suggested the use of composite indicators to assess pollution at complex levels. For example, Cassanego et al. (2014) used similar indices to evaluate genotoxic impacts in *Tradescantia pallida* and considered it an effective approach for differentiating treatments by risk level.

To strengthen the classification of effects according to the dose of pollutants, dendrogram analysis was also used in this study based on MI, GRI, PI, and the number of cells with aberrations. The dendrogram constructed on the basis of numerical similarity between treatments clearly showed that the groups with higher dilutions (1:2 and 1:3) were clustered together in a separate branch, far from the control and treatments with greater dilutions (1:5, 1:6). This visual separation confirms that the effects of pollutants are dose-dependent, with higher concentrations causing greater effects. A similar use of dendrograms for pollution assessment was reported by Goldoni et al. (2014), who built classifications for treatments with municipal wastewater using cytogenetic and chemical parameters. Likewise, Campos et al. (2019) emphasize that dendrograms help identify treatments with comparable impacts and assist in separating pollution effects according to concentration level. Our data show a strong consistency between the increase in GRI and the distance of groups from the control in the dendrogram, reinforcing the validity of both approaches. This shows that not only is genotoxic impact present, but it also increases progressively with increasing concentration of pollutants in the treatment. Therefore, the parallel use of GRI and the dendrogram provides a clear framework for interpreting the classification of effects by dose and helps identify critical concentrations that pose real risk to the genetic integrity of the plant cells used as indicators. The results obtained from this study clearly confirm that exposure for 72 hours to untreated polluted water from the "Kosova A" Thermal Power Plant causes pronounced cytogenetic effects in the meristematic cells of *Tradescantia pallida*. The reduction of the mitotic index, the noticeable increase of cells with aberrations in mitosis, the unbalanced distribution in the phases of the cell cycle, as well as the high values of GRI – all together form a clear model of genotoxic stress that affects the chromosomal stability of the plant. In accordance with our results, Crispim et al. (2014) and Sposito et al. (2019) emphasize that changes in mitotic and structural indicators

in plant cells are among the most sensitive ways to detect genotoxic pollution in aquatic environments. These models provide a direct and practical assessment of cellular damage as a result of pollutants of industrial origin.

It is important to emphasize that *Tradescantia pallida*, due to its sensitivity and ease of cytogenetic analysis, is increasingly being valued as a powerful bioindicator in ecological testing, which is also consistent with the findings of de Souza Araújo et al. (2014), Artico et al. (2020), and Falistocco et al. (2000). Furthermore, the fact that even the diluted treatments (1:5 and 1:6) did not reduce the damage to normal levels clearly shows that the pollutants in this water have biologically persistent character and long-term effects, as also emphasized by Dourado et al. (2016) and Fatima and Ahmad (2006). The presence of these pollutants, especially heavy metals and resistant organic substances, poses an ongoing risk not only for plants but also for other organisms in the ecosystem. Considering that the Obiliq area is one of the most polluted in Kosovo due to the thermal power plants, and considering the fact that the water used in this experiment did not undergo any filtering or treatment process, our findings are a clear signal for environmental institutions to take concrete measures for the purification and management of technological waters discharged into nature. In conclusion, our study confirms that the cytogenetic observation method using the *Tradescantia pallida* model is an effective instrument for monitoring industrial pollution and offers reliable data for the assessment of environmental quality. This paper contributes with concrete evidence to the existing literature and strengthens the view that the pollution of technological waters poses a direct threat to the genetic stability of organisms and the health of the ecosystem as a whole.

CONCLUSIONS

The data from this study clearly show that the untreated wastewater discharged from the "Kosova A" Thermal Power Plant into the Sitnica River has a significant impact on the meristematic root cells of the plant *Tradescantia pallida*. In all treated samples, there was a clear decrease in cell division activity (MI), especially in the 1:2 and 1:3 dilutions, where the percentage of dividing cells dropped well below the control. At the same time, the number of cells with chromosomal aberrations increased noticeably, indicating damage at the genetic level. Even after dilution, the water continued to have negative effects. Indicators like the GRI and the number of aberrant cells remained high compared to the control. This means that the pollutants were still active, even at lower concentrations. The dendrogram built from our data grouped the samples according to pollution levels: the more contaminated treatments were clustered together and clearly separated from the control group, showing that the effects depend on dose. The results show that pollution doesn't just slow down cell division it directly affects the structure of chromosomes. This is especially concerning if such water is used for irrigation or comes into contact with other living organisms, because the damage occurs at the cellular level. On the other hand, this study also delivers a strong message: wastewater from thermal power plants cannot be allowed to flow into the environment untreated. Our findings provide solid evidence that this water poses a real risk to plant life and to genetic stability in the environment. Furthermore, these results can serve as a starting point for future research aimed at identifying specific pollutants in this water and understanding the biological mechanisms behind the observed damage. That would help environmental institutions take more targeted action to monitor and reduce pollution especially from major sources like thermal power plants.

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