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THE GENOTOXIC EFFECTS OF HEAVY METALS IN FLY ASH FROM THERMAL POWER PLANTS ON WHEAT, DEPENDING ON WIND DIRECTION

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Abstract

Plants, due to their fixed locations, are susceptible targets to environmental stress. Among these stresses, heavy metal stress has increased since the Industrial Revolution. Thermal power plants that utilize fossil fuels play a significant role in the release of heavy metals into the environment. This study investigates the role of air currents in the transport of fly ash from thermal power plants and its genotoxic effects on wheat using molecular methods. The mobility of retrotransposons affecting genomic stability was assessed through IRAP (Inter-Retrotransposon Amplified Polymorphism) analysis. The IRAP analysis results indicate a polymorphism rate of 54.6% in wheat seedlings located 1 km northwest of the thermal power plant. The lowest polymorphism rate, 29.66%, was observed in wheat samples 10 km east of the plant. Physical damage in the nucleus caused by heavy metals was evaluated using the comet assay, which measures tail length, tail DNA percentage, and tail moment. Similar to the IRAP analysis, significant damage was observed in the northwest, southwest, and west directions across all three parameters. The findings of this study underscore the impact of heavy metal pollution and emphasize the importance of air currents in the dispersion of these pollutants.

Key words: comet assay, heavy metals, IRAP, *Triticum aestivum* L.

Introduction. The use of coal for energy production in Türkiye increased from 24.5% to 28% over the 44-year period from 1973 to 2017 [1]. Thermal

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power plants consume coal during electricity production, leading to the release of various trace elements into the environment, some of which are toxic [2]. The primary environmental impacts of thermal power plants include soil, air, and water pollution [3]. Millions of tons of slag, ash, and various particulates are carried to high altitudes, contaminating agricultural lands and forests. In Türkiye, 34.6% of electricity production was derived from coal in 2022 [4]. Approximately 82% of the annually produced 7 million tons of lignite coal is used in thermal power plants for electricity generation. Consequently, 24.4 million tons of fly ash are released into the ecosystem as waste each year [5]. Studies have reported high concentrations of heavy metals such as Cd, Cu, and As in the fly ash emitted from smokestacks [6]. In recent years, there has been an increase in research on environmental heavy metal concentrations [7,8]. The accumulation of heavy metals (HMs) in soil, their non-biodegradable nature, and their toxicity have attracted significant interest from scientists worldwide [9,10]. Although heavy metals are naturally present in trace amounts in soil, they become toxic when their concentrations exceed normal limits, reducing soil fertility and damaging plant cells [11–13]. Heavy metals are considered a major concern for human health due to their carcinogenicity, cytotoxicity, and genotoxicity [14]. Crops grown in arable land contaminated with heavy metals pose a threat to food safety [15,16].

The aim of this study is to determine the role of air currents in the dispersion of coal ash from the Afшoin-Elbistan thermal power plant and its genotoxic effects on wheat.

Materials and methods. ***Plant material and experimental design.*** Equal-sized and sterilized *Triticum aestivum* L. seeds were planted in eight different directions (north, northwest, northeast, south, southwest, southeast, east, and west) at distances of 1 km, 5 km, and 10 km from the thermal power plant centre. The control group wheat seeds were germinated outside the influence zone of the thermal power plant. Fourteen days after germination, the seedlings were collected, cleaned of dirt, and stored at −80 °C.

DNA isolation and determination of concentrations solation of genomic DNA. Genomic DNA isolation was performed using a previously described procedure [17]. The extraction solution was prepared with 1 M TRIS-HCl, 5 M NaCl, 0.5 M EDTA, 2% CTAB, 0.2% β-mercaptoethanol, and 0.1% $Na_2S_2O_3$.

IRAP amplification. Six LTR (Long terminal repeat) primers were used for IRAP reactions. Twenty microlitres reaction mixture containing 50 ng DNA, 1×PCR buffer, 2.5 mM $MgCl_2$, 0.25 µM dNTP, 1.5 U Taq DNA polymerase, and 0.25 mM primer was used to perform PCR amplification. The profile of amplification consisted of one cycle at 95 °C, 5 min; followed by 42 cycles at 94 °C; 1 min, in diversified structure temperature for every one primer; 1 min, 72 °C; 2 min, next to last elongation 72 °C; 15 min.

IRAP PCR electrophoresis protocol. Genomic DNA products obtained by IRAP-PCR were run with electric current on agarose gel for imaging. For

this, 0.7 g agarose was purified to 100 ml with distilled water by adding 10 ml of TBE (Tris/Borate/EDTA) pH 8, heated in a microwave oven and 2 µl of ethidium bromide was added. The samples were run for 105 min in electrophoresis at 90 V and viewed with a UV camera.

IRAP analysis and determination of GTS. To determine the sizes of DNA fragments obtained using IRAP primers through PCR, the PCR products were electrophoresed on an agarose gel under an electric current. The bands formed as a result of the DNA movement were visualized using a UV camera. The sizes of the obtained bands were determined by referencing a marker and analyzed using Total Lab TL120 software. Genomic template stability of plants (%); $100 \times 1 - a/n$ determined by the formula. Here a is the IRAP polymorphic band determined for each application sample, and n is the total number of DNA bands obtained in the corresponding primary negative control group. The sum of genomic template stability and polymorphism value was evaluated as 100%.

Comet assay. Three slides per group were prepared according to the method described by Mukherjee and Gichner [18]. The extent of DNA damage was scored as the percentage of tail DNA (% tail DNA) by DNA fragments. Fifty nuclei (total of 150 nuclei) from each of the three slides per treatment group were analyzed using Comet 5.5 software, and the median values of % tail DNA were represented for statistical analysis. Statistical analyses were performed using GraphPad Prism 5 (for Windows). The Kruskal–Wallis H test, a non-parametric test, was used to determine whether DNA damage differed statistically between groups. Differences in DNA damage were analyzed using the Dunn test. The results were compared with the negative control, and the significance of the positive control versus the negative control was assessed using the Mann–Whitney U test. The obtained results were compared with the negative control, and the significance of the positive control compared to the negative control was determined using the Mann–Whitney U test.

Results and discussion. ***Findings from the IRAP analysis.*** Retrotransposon activity was detected using all six primers in the IRAP analysis. The stress caused by the existing heavy metals resulted in the appearance of new bands or the disappearance of existing ones in the wheat genetic profile, indicating polymorphisms. The highest polymorphism rate, at 54.60%, was observed in seedlings planted in the northwest area, 1 km away from the plant, compared to the control group. This was followed by seedlings planted 1 km southwest of the plant with a polymorphism rate of 53.14%, and those planted 1 km west of the plant with a rate of 52.26%. The lowest polymorphism rate was found in seedlings planted 10 km east of the plant, at 29.66%, followed by 34.08% in the southeast samples at 10 km, and 35.48% in the east samples at 5 km.

Regarding GTS values, the lowest GTS value of 45.40% was recorded in samples from 1 km northwest of the plant. This was followed by 46.86% in the southwest samples at 1 km and 47.74% in the west samples at 1 km. The highest

Table 1

Polymorphism and GTS values obtained according to direction and distance

1 km	Polymorphism rate	50.12	54.6	46.46	52.26	53.14	48.36	43.22	40.84
	GTS value	49.88	45.4	53.54	47.74	46.86	51.64	56.78	59.16
5 km	Polymorphism rate	47.54	52.24	42.64	49.44	50.2	44.72	38.54	35.48
	GTS value	52.46	47.76	57.36	50.56	49.8	55.28	61.46	64.52
10 km	Polymorphism rate	41.04	47.28	36.22	41.26	45.18	38.52	34.08	29.66
	GTS value	58.96	52.72	63.78	58.74	54.82	61.48	65.92	70.34

GTS value, at 70.34%, was found in samples from 10 km east of the plant. The detailed IRAP analysis results are presented in Table 1.

Comet test analysis for determining physical damage at the nuclear level in wheat seedlings. The extent of single- and double-strand breaks in DNA was evaluated using parameters such as tail length, tail DNA percentage, and tail moment. Comet assay images of the control group and samples grown at various distances and directions are presented in Fig. 1.

Tail lengths. Tail lengths of nuclei, indicative of DNA strand breaks caused by heavy metals in ash, were assessed to determine the extent of nuclear damage. Compared to the control group, tail lengths were found to decrease with increasing distance from the power plant. The longest tail length, 69.71 µm, was observed in samples located 1 km northwest of the plant. This was followed by 67.44 µm in samples 1 km west and 66.24 µm in samples 1 km southwest.

The shortest tail lengths were recorded at 10 km east (29.14 µm), 10 km southeast (37.54 µm), and 10 km northeast (40.08 µm). Tail lengths were observed to decrease as the distance from the power plant increased. For instance, tail lengths in samples 1 km northwest of the plant were 11.65% shorter at 5 km in the same direction and 26.10% shorter at 10 km. The greatest reduction in tail length occurred in samples planted west of the plant, with a 34.63% decrease between 1 km and 10 km. The smallest reduction, 11.00%, was observed between 1 km and 5 km north of the plant.

Tail DNA % values. In addition to tail length, tail DNA percentage values were also examined to assess physical damage in cells. Wheat seedlings exposed to ash had higher tail DNA percentages compared to the control group. Data revealed an inverse relationship between tail DNA percentage and distance from the ash source.

The highest tail DNA percentage, 54.16%, was observed in samples 1 km northwest of the plant, followed by 52.48% at 1 km west and 51.24% at 1 km north. The lowest percentages were recorded at 10 km east (23.89%), 10 km

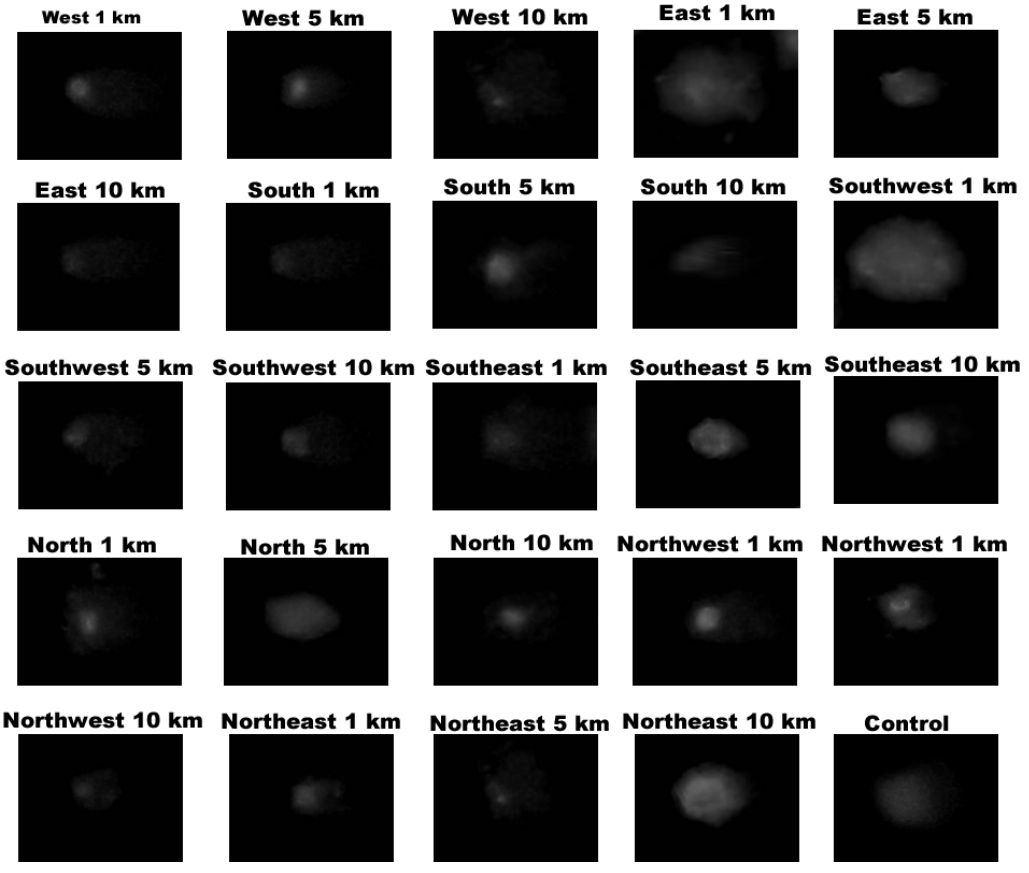

Fig. 1. The images of nuclear damage obtained based on direction and distance

southeast (28.04%), and 10 km northeast (31.07%). The largest change in tail DNA percentage occurred between samples 1 km and 10 km east, with a 26.76% difference. The smallest change, 4.95%, was observed between samples 1 km and 5'km southeast of the plant.

Tail moments of cells. Tail moments, calculated as the product of tail DNA percentage and tail length, were also evaluated in this study. Results showed that tail moments increased proportionally with proximity to the ash source. The highest tail moment was observed in samples 1 km northwest of the plant. Tail moment values varied across directions and distances, decreasing with increasing distance from the ash source. The observed parameters, including polymorphism, genomic template stability (GTS), tail length, tail DNA percentage, and tail moment values, are summarized in Fig. 2.

Wheat is a valuable source of protein, minerals, dietary fibre, and vitamins [19], making it an essential nutrient for living organisms. However, its production is susceptible to pollutants such as heavy metals in the soil. Fossil

Fig. 2. Polymorphism, GTS, tail length, tail % DNA value, and tail moment values according to distances and directions

fuel combustion, traffic emissions, industrial discharges, and particulate matter are recognized as primary sources of airborne heavy metals [20].

This study evaluated wheat samples grown near a thermal power plant for physical damage to nuclei caused by DNA strand breaks using the comet assay. Genomic changes due to retrotransposon activity were assessed via IRAP analysis. Alterations and damage in the DNA strands caused by the activity of LTR retrotransposons, including LTR 6150, Sukkula, 3LTR-5, LTR 6149-5, 5LTR1, and Nikita E2647, were identified as polymorphisms. Wheat samples grown near the thermal power plant, a source of heavy metals, exhibited higher levels of damage and associated polymorphism values.

Retrotransposon activity negatively impacted genomic stability, with the lowest GTS values observed in samples closest to the power plant. Significant levels of polymorphism were detected in wheat samples grown in eight directions, particularly northwest, west, and southwest. Samples located 5 km and 10 km northwest and southwest of the plant exhibited polymorphism values comparable to or exceeding those of samples located 1 km away. Similar findings were obtained from comet assay analyses, with wheat samples from the west of the plant showing higher tail lengths, tail DNA percentages, and tail moments compared to samples from other directions. These differences highlight the critical role of air currents in heavy metal dispersal.

Conclusion. The unfiltered release of flue gas from coal combustion and improper storage of ash contribute to the spread of heavy metals, affecting large areas via air currents. This study provides an example of how air currents influence the distance and extent of heavy metal pollution. Heavy metal contamination poses a serious threat to ecosystems. To mitigate the spread and impact of heavy metals, proper filtration and storage practices are essential. Additionally, regional air currents should be considered in the planning of cultivation areas.

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