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Genetic Impact of Bottled Water Exposed to Different Temperatures on *Drosophila melanogaster*

Lamyaa Majeed Al-Amidi[1]* Forat Abd Al-Hamzah Hadi[2]
[1]Babylon Education Directorate, Ministry of Education, Iraq
[2]Department of Biology, College of Education, University of Al-Qadisiyah, Iraq
*Corresponding author email: edu.bio.posta119@qu.edu.iq

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Abstract This study was conducted to explore the genetic effects of bottled water stored at different temperature conditions (25°C, 30°C, 40°C, and 50°C) on Drosophila melanogaster. The water samples were first analyzed chemically for lead, cadmium, and ethylene concentrations, followed by their incorporation into the insect's culture medium. The experiment examined phenotypic mutations, specifically vestigial wing and ebony body color traits, as well as changes in the mitotic index of third instar larvae. Thus, exposure to bottled water stored at 50°C induced a higher incidence of wing deformities (25%) and ebony body mutations than that of the control group (25°C; no abnormalities) as shown. In addition, there was a clear increase in mitotic activity, from 65 in the control to 76.3 at 50°C, these results pointing to genotoxic effects induced possibly by migration of toxic plastic-derived compounds into the water. The findings of this study highlight the need for rigorous storage of bottled water, and suggest the need for stronger regulation and measurement at the molecular level of migration of chemicals from packaging materials.

Keywords: bottled water, Drosophila melanogaster, temperature stress, genetic mutation, mitotic index.

Introduction Everything needs water to live. Bottled water consumption has become increasingly popular as a main drinking water source in the last few years, especially in urban and disadvantaged areas that have continued to be worried about the safety of municipal water (1,2). The growing dependence on bottled water has been driven by both concerns about microbial and chemical contaminants as well as by the occurrence of waterborne illness (diarrhea, cholera) outbreaks (3). This trend is reflected in the high market share of local bottled water factories in Iraq, which exceeded 100 active ones, producing different brand names for bottled-water(4).

But even seemingly harmless bottled water isn't always safe. Environmental conditions, including both high temperatures and exposure to sunlight, have been reported to cause leaching of harmful substances from polyethylene terephthalate (PET) bottles into the water (5–7). These include lead, cadmium, bisphenol A (BPA), phthalates, antimony, and formaldehyde, among others, that have toxic, carcinogenic, or endocrine-disrupting effects (8–11). This process is commonly referred to as chemical migration, 72 which is affected by various aspects including the temperature and storage, the light exposure, and the chemical composition of the bottle material.72, 12, 13.

Temperature in the storage and transportation, however, can exceed 40 °C in the hot regions (which are widespread in Iraq) and cooling becomes problematic. At these concentrations, the structural and chemical characteristics of the plastic deteriorate (locally), which promotes a risk of migration of contaminants (14–16). These pollutants may not only cause damages in organ systems, but may also have direct interaction with genetic material, leading to oxidative stress, DNA mutations, and mitotic disturbances (17,18)

Because of its mutagen sensitivity and rapid life cycle, Drosophila melanogaster is well-established as a model organism in environmental and genetic toxicology. It is a useful model organism for evaluating phenotypic abnormalities and changes in the mitotic index induced by toxic agents (19,20).

Thus, the aim of the present study was to analyze the suspected genotoxic effects of bottled water (which were stored under different temperature conditions)

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on Drosophila melanogaster, based on the alterations observed in the shape of mutant spot and changes in mitotic index, with the intention of determining the genetic hazards created by unsafe storage.

Material and Methods

Ethical Approval.

All experimental procedures involving Drosophila melanogaster were conducted in accordance with ethical standards for the use of invertebrates in research, as approved by the Institutional Research Ethics Committee of the College of Education, University of Al-Qadisiyah, Iraq, in 2023 (Approval No. EDU/BIO/2023/018).

Temperature Stress for the Bottled Water

Bottled waters were kept in an incubator at temperatures (25, 30, 40 and 50)°C for a period of three weeks and change in lead and cadmium concentrations were determined and ethylene was detected in the exposed samples..

Preparation of the Suitable Medium for Insect Rearing

The medium used to rear Drosophila melanogaster was prepared following the method of Shaffer et al. [21], using a medium consisting of 2 g agar, 10 g sugar, 10 g baker's yeast, 10 g yellow corn flour, and 100 mL distilled water. The above materials were thoroughly mixed and boiled for 5–10 min to sterilize. This mixture was poured into culture tubes that had been washed with soap and water to remove medium residues, and sterilized using a Gallenkamp oven at 200°C for 30 minutes. The openings of the tubes were then covered with cotton and left to cool and solidify. The tubes containing the medium were then sterilized using an autoclave. After completing the sterilization, a live yeast suspension prepared by dissolving 10 g of yeast in 100 mL of distilled water was added to the medium drop by drop, and the medium was left for 48 hours before introducing the insects.

Phenotype Mutations

The wing shape and body color were studied. The vestigial wing mutation was investigated for the first trait, and the ebony body color mutation for the second trait, as examples of phenotypic mutations in *Drosophila melanogaster*. Three temperature treatments were prepared and added to the rearing medium, in addition to the control at 25°C, and the phenotypic mutation rates were recorded.

Mitotic Index (MI) Count

According to the method of El Agoze et al. (22), third instar larvae were used. Pre-treatment with 0.1% colchicine was performed by placing a single larva in a drop of the solution for 90 minutes before dissection, as mentioned by Lachance and Whitten (23).

Experimental Design

The experiment included adult *Drosophila*, divided into four groups, with each group consisting of 20 insects, as follows:

T1: It is a control group, with bottled water at 25°C added to the insect rearing medium.

T2: The medium was added with bottled water at 30°C.

T3: The medium was added with bottled water at 40°C.

T4: The medium was added with bottled water at 50°C.

Results and Discussion

Lead Concentration

We note in Table 1 that the samples were free of lead in the control group, and then began to increase with the other temperatures, reaching (0.0425–0.0539–0.0718) mg/L respectively, showing a significant difference from 25°C at the 5% significance level. This increase exceeded the limits allowed by the Iraqi Health Organization (0.010 mg/L). The cause may be attributed to lead entering the water due to its possible leakage during production stages or pipelines, which corresponds to studies (6,7).

Table 1: Lead ion concentration (mg/L)

Temperature	Lead concentration ± SD	Significance
25°C	0 ± 0	D
30°C	0.0425 ± 0.0004	C
40°C	0.0539 ± 0.0008	B
50°C	0.0718 ± 0.0001	A
LSD	0.00072	

Note: Capital letters indicate vertical comparisons. Different letters between any two values indicate a significant difference at $p < 0.05$.

Cadmium Concentration

Table 2 refers to the concentration of cadmium in bottled water samples. A value of 0.0086 mg/L was recorded at 25°C, which did not differ significantly from the value at 40°C (0.0124 mg/L), while at 30°C and 50°C, the concentrations reached (0.0116 and 0.0226) mg/L respectively. A significant difference was recorded at the 5% significance level. At 50°C, the increase exceeded the permissible limit according to the Iraqi standard for drinking water (0.003 mg/L), which corresponds to studies [8,9] indicating high Cd concentrations in bottled water stored for 10–15 days at up to 50°C.

Table 2: Cadmium concentration (mg/L)

Temperature	**Cadmium concentration ± SD**	**Significance**

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25°C	0.0086 ± 0.0008	C
30°C	0.0116 ± 0.0004	B
40°C	0.0124 ± 0.0001	B
50°C	0.0226 ± 0.0006	A
LSD	0.00039	

Detection of Ethylene in Bottled Water

Table 3 refers to ethylene concentration in water samples at different temperatures: at 25°C, ethylene reached 3.34 mg/L. In the other treatments, it was 4.28, 7.70, and 9.31 mg/L respectively. An increase in ethylene values was observed with significant differences from 25°C, but all values remained within WHO's permissible limit of 1–10 mg/L. This increase may be due to the chemical migration of plastic elements from the packaging into the water when temperatures rise, during long-term storage, or exposure to inappropriate conditions like sunlight, consistent with studies (10,11). It may also result from inefficiencies in the treatment process, leading to microplastic transfer from the bottle into the water (12).

Table 3: Ethylene concentration (mean ± SD)

Temperature	Ethylene ± SD	Significance
25°C	3.34 ± 0.03	d
30°C	4.28 ± 0.04	c
40°C	7.70 ± 0.06	b
50°C	9.31 ± 0.12	a
LSD	0.137	

Genetic Testing

Vestigial Wing Mutation

The wing trait can be used as a sensitive indicator of oxidative stress in the organism. It was observed that the appearance of certain oxidative stress-sensitive mutations—including the phenotypic form of curled or incompletely extended wings—is due to a deficiency or depletion in antioxidant levels such as SOD and GSH [24].

According to the results obtained, the vestigial wing trait did not appear in the control (25°C), then began to appear at the other temperatures until it reached 25% at 50°C, and this represented a significant increase over the control at a probability level of ($p < 0.05$).

Figure (1) a-Vestigial Wing Mutation b-nature wing

Humphreys et al. (25) showed that the visible wing deformities observed in several strains carrying mutant alleles of various genes may also be the result of an elevated oxidative stress condition.

Kauts et al. (26) also indicated in their study that micro- and nanoplastics cause damage, oxidative stress, inflammation, genotoxicity, genetic changes, and programmed cell death in *Drosophila melanogaster*.

Moreover, heavy metals such as cobalt, iron, manganese, and nickel may stimulate genetic deviations and genomic instability (27).

Table 4: Rate of Vestigial Wing Mutation

Treatment	Mutation Rate ± SD	Significance
25°C	0 ± 0	D
30°C	5 ± 0	C
40°C	15 ± 1	B
50°C	25 ± 0	A
LSD	1.96	

Ebony Body Color Mutation

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According to the obtained results, the ebony body color trait did not appear at temperatures 25°C and 30°C. However, it appeared at 40°C and 50°C, reaching rates of 10.6% and 20% respectively, and showed a significant difference at the probability level ($p < 0.05$).

The variation in pigmentation within and among *Drosophila* species may be attributed to genes responsible for the melanin biosynthetic pathway, which results from dopamine metabolism. Most underlying genetic changes are responsible for pigmentation differences, particularly variations in the expression levels of structural genes in *D. melanogaster* (28).

The genes responsible for body color include:

ebony (e), which encodes the enzyme *N-β-alanyl dopamine synthetase (NBAD)*, converting dopamine into NBAD.

tan (t), which encodes the protein that catalyzes the reverse reaction, converting NBAD back into dopamine.

yellow (y), which is responsible for producing a protein that contributes to black melanin production [29].

There are two well-known mutations that significantly alter body color in *D. melanogaster*:

yellow (y): mutations in this gene eliminate all black pigments, indicating its critical role in black melanin production.

ebony (e): mutations in this gene produce the opposite phenotype to yellow, where loss of function in **e** leads to increased black pigmentation (30).

Table 5: Rate of Ebony Body Color Mutation

Treatment	Mutation Rate ± SD	Significance
25°C	0 ± 0	C
30°C	0 ± 0	C
40°C	10.6 ± 0.57	B
50°C	20 ± 0	A
LSD	0.76	

Figure (2) Ebony Body Color Mutation

Mitotic Index of Somatic Cells

Based on the results, an increase in the rate of dividing cells was observed in the larvae of *Drosophila melanogaster*, showing a significant difference at ($p < 0.05$). The mitotic index rose from 65 at 25°C to 76.3 at 50°C.

This increase in the mitotic index may be attributed to the migration of compounds such as phthalates and BPA, which may abnormally stimulate cell division through their effects on cell cycle-regulating genes such as CDKs and Cyclins. These genotoxic carcinogenic compounds are released during the storage of mineral water in PET bottles or may leak from the pipelines that supply water to bottling plants (31).

Yang et al. (32) also indicated that these compounds have hormone-like effects and may influence cell cycle regulation, resulting in abnormal stimulation of mitosis. In addition, the leakage of certain chemicals may lead to the generation of reactive oxygen species (ROS), which cause DNA damage. As a response, cells activate repair mechanisms to ensure increased division rates to compensate for the damaged cells (33).

Table 6: Mitotic Index Rate in *Drosophila* Larvae

Treatment	Mitotic Index ± SD	Significance
25°C	65 ± 1	D
30°C	67.16 ± 0.28	C
40°C	71.5 ± 0.5	B
50°C	76.3 ± 0.57	A
LSD	1.21	

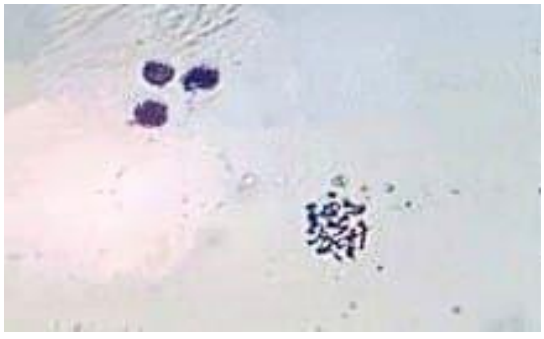

Figure (3) Undivided nucleus and divided nucleus

Conclusion

This study demonstrated that exposing bottled water to elevated temperatures (30°C, 40°C, and 50°C) for three weeks leads to significant chemical and genetic changes in *Drosophila melanogaster*. Concentrations

of lead, cadmium, and ethylene increased with rising temperature, in some cases exceeding permissible health limits. This was accompanied by a significant rise in phenotypic mutations such as vestigial wings and ebony body color, as well as an increase in the mitotic index in somatic cells.

These findings indicate that improper storage of bottled water especially under high temperatures—can result in the migration of harmful compounds from plastic packaging into the water. Such exposure may pose genotoxic risks to living organisms, warranting tighter control over storage conditions and encouraging further molecular studies on microplastic and plasticizer toxicity.

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Conflict of interest

No conflict of interest is disclosed by the authors.

References

1. Mandowa Wilfred. Rapid assessment of Malawi rural water supply and sanitation. Final Draft Report for African Development Bank (ADB). 2005.
2. Abbas Majeed Jasim, Al-Zubaidi Ali Hussein, Alwan Waleed Khalid. Microbiological and chemical evaluation of bottled water in Baghdad city. Iraqi Journal of Market Research and Consumer Protection. 2010;2(3):75–103.
3. Rizooqi Sadiq Mohammed, Al-Rawi Munther Abed. Evaluation of the quality of drinking water distributed in some areas of Baghdad. Iraqi Journal of Market Research and Consumer Protection. 2010;2(3):104–115.
4. Abdulabbas Mohammed Jawad, Al-Taie Faiza Jassim, Mahdi Fawzia Hadi. Evaluation of microbial contamination in some local bottled water in Babylon Province. University of Babylon Journal of Pure and Applied Sciences. 2015;23(1):193–203.
5. El-Salam Amal, El-Gamal Maged, Abdel Hady Hoda. Chemical and microbial contamination in some bottled water samples. Egyptian Journal of Botany. 2017;57(3):495–506.
6. Leber Andre P. Contaminants migration from plastic bottles into water. Chemico-Biological Interactions. 2001;135:215–220.
7. Ashby Joseph. Migration of plastic additives into water: an assessment. Food Additives and Contaminants. 1988;5(S1):485–492.
8. Wagner Martin, Oehlmann Jörg. Endocrine disruptors in bottled mineral water: total estrogenic burden and migration from plastic bottles. Environmental Science and Pollution Research. 2009;16(3):278–286.
9. Fasano Eva, Bono Luigi, Digenis George, Nobile Maria Angela, Cirillo Teresa, Montagnaro Fabio, et al. Migration of phthalates and heavy metals from PET bottles into mineral water: Analytical methods and health risk assessment. Food Control. 2012;27(1):132–138.
10. Bach Christian, Dauchy Xavier, Severin Isabelle, Munoz Jérôme. Effect of temperature on the release of organic compounds from PET bottles into water. Water Research. 2012;46(3):571–583.
11. den Braver-Sewradj Simone Patricia, Dollé Martine Elisabeth Theodora, Herremans Jos, de Boer Joop, Peijnenburg Ad A.C.M. Exposure and health risk assessment of chemical migrants in plastic food contact materials: A review. Critical Reviews in Toxicology. 2020;50(2):128–147.
12. Molaee Aghaee Elaheh, Velayati Shadi, Naseri Mohammad Taghi. Migration of hazardous compounds from plastic bottles into mineral water stored at various temperatures. Journal of Environmental Health Science and Engineering. 2014;12:1–7.
13. Re Depaolini Adriano, Russo Davide, Della Bella Lucia, Castrignanò Erika, Galli Giulia, Pomati Francesco. Environmental impact of bottled water: evidence from microplastic release and chemical migration. Environmental Science and Pollution Research. 2020;27:456–468.
14. Ahmed Maged Bassyouni, Zhou Jiabin, Ngo Huu Hao, Guo Wenshan, Chen Zhiqiang, Nguyen Dinh Duc. Unveiling potential microplastic release and chemical leaching from plastic bottled water under thermal exposure. Science of the Total Environment. 2021;775:145793.
15. Wegelin Markus, Canonica Silvio, Mechsner Kerstin, Fleischmann Thomas, Pesaro Fritz, Metzler Armin. Solar water disinfection: scope of the process and analysis of radiation parameters. Water Research. 2001;35(12):2794–2800.
16. Danopoulos Evangelos, Twiddy Margaret, Rotchell Jehan-Hervé. Microplastic contamination in bottled water: current knowledge and research needs. PLOS ONE. 2020;15(7):e0236838.
17. Mortelmans Kristien, Zeiger Errol. The Ames Salmonella/microsome mutagenicity assay. Mutation Research. 2000;455(1–2):29–60.

18. Speit Gunter, Merk Otto. Evaluation of mutagenic effects in mammalian cell lines. Mutagenesis. 2002;17(1):27–34.
19. Parkes Trevor L., Elia Anthony J., Dickinson Darlene, Hilliker Arthur J., Phillips John P., Boulianne Gabrielle L. Extension of Drosophila lifespan by overexpression of human SOD1 in motorneurons. Nature Genetics. 1993;6(4):363–369.
20. Humphreys John M., Waddington Conrad H., Lewis Edward B. Analysis of wing defects and genetic interactions in Drosophila. Genome. 1993;36(1):162–165.
21. Shaffer Christopher D., Burg Mary G., Saleem Saba, Bui Christine, Lin Di-An, Schade Kristen L., et al. Investigations in Molecular Cell Biology Using Drosophila melanogaster. Genetics. 1994;136(2):613–619.
22. El Agoze, M., Lemeunier, F., & Periquet, G. (1992). Mitotic and salivary
23. gland chromosome analyses in the Musca domestica L.(house fly)(Diptera: Muscidae). Heredity, 69(1), 57-64.
24. Lachance Louis E., Whitten Michael J. Genetics of insect vectors of disease. Amsterdam: Elsevier; 1986.
25. Parkes Trevor L., Elia Anthony J., Dickinson Darlene, Hilliker Arthur J., Phillips John P., Boulianne Gabrielle L. Overexpression of human SOD1 in Drosophila motorneurons extends lifespan. Nature Genetics. 1993;6(4):363–369.
26. Humphreys John M., Waddington Conrad H., Lewis Edward B. Genetic analysis of wing development in Drosophila melanogaster. Genome. 1993;36(1):162–165.
27. Kauts Mandeep L., Kumar Manoj, Kukreti Rishi. Microplastic and nanoplastic toxicity in Drosophila melanogaster: oxidative stress, inflammation, apoptosis and genotoxic effects. Environmental Pollution. 2024;331:121841.
28. Cappucci Ugo, Napoletano Francesco, Botti Giulia, Scala Francesco, Romano Massimo R., Di Lelio Ida, et al. Metal-induced genotoxicity in Drosophila melanogaster: evaluation of oxidative stress and mitotic abnormalities. Mutation Research. 2024;835:503917.
29. Takahashi Aya, Takano-Shimizu Tanja. Molecular basis of pigmentation variation in Drosophila melanogaster. Genetics. 2013;194(2):263–273.
30. Massey Jennifer H., Chung Dayeon, Siwanowicz Iwona, Stern David L., Wittkopp Patricia J. The yellow gene influences Drosophila male mating success through sex comb melanization. eLife. 2019;8:e49388.
31. Wittkopp Patricia J., True John R., Carroll Sean B. Reciprocal functions of the Drosophila yellow and ebony proteins in the development and evolution of pigment patterns. Development. 2002;129(8):1849–1858.
32. Biscardi Daniela, Carraro Emilio, Comino Anna, Romano Manuela, Giordano Mario, Gilli Giacomo. Chemical contamination of mineral waters commercialized in Italy. Journal of Preventive Medicine and Hygiene. 2003;44(3):120–125.
33. Yang Xiaojun, Li Wenjun, Shi Hui, Wang Xiaomei, Lu Wei. Endocrine-disrupting effects of bisphenol A on cell proliferation and apoptosis in model organisms. Toxicology Reports. 2022; 9:989–998.
34. Jomova Katarina, Vondrakova Daniela, Lawson Misty, Valko Marian. Metals, oxidative stress and neurodegenerative disorders. Molecular and Cellular Biochemistry. 2023;476(1):189–206.