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Assessment of Organochlorine Pesticide Residues in Soil and Water in the Al-Jabal Al-Akhdar Region, Libya

Azzelden Khairallah Elgoul

Department of Chemistry, Faculty of Education, Tobruk University. Libya

Corresponding author E-mail : *Azzelden.elgoul@tu.edu.ly*

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A B S T R A C T

This study aimed to quantitatively assess the residues of seven organochlorine pesticides (OCPs) in soil and water samples from five major agricultural areas in Al-Jabal Al-Akhdar (Al-Wasita, Qarnada, Lamluda, Al-Hannaya, and Al-Faydiya) during March 2025. A total of 30 samples were collected, equally distributed between soil (n=15) and water (n=15). Extraction and cleanup procedures were performed following the Pesticide Analytical Manual (1978) [14], and quantitative analysis was conducted using High-Performance Liquid Chromatography (HPLC) with a UV detector. The results revealed the presence of three pesticides (Endrin, Heptachlor, and p,p'-DDT) in positive samples, with a total contamination rate of 33.3%. Soil samples recorded the highest mean concentration for Endrin (0.061 ppm), which was statistically significantly above the permissible limit (P=0.034). Pesticide concentrations in water were generally below permissible limits, except for Heptachlor in the Lamluda area, which recorded a concentration equal to the maximum permissible limit (0.037 ppm). Qualitative correlation analysis revealed matching pesticide types in both media, supporting the hypothesis of their transfer from soil to groundwater through leaching. The study recommends intensifying periodic environmental monitoring programs and investigating transfer through the food chain..

تقييم متبقيات المبيدات الكلورونية العضوية في التربة ومياه بمنطقة الجبل الأخضر– ليبيا.

عزالدين خيرالله الغول

قسم الكيمياء, جامعة طبرق , ليبيبا

المُخلص

هدفت هذه الدراسة إلى التقدير الكمي لمتبقيات سبعة مبيداتكلورونية عضوية في عينات التربة والمياه من خمس مناطق زراعية رئيسية في الجبل الأخضر (الوسيطة، قرنادة، لملودة، الحنية، الفايدية) خلال مارس 2025. تم جمع 30 عينة موزعة بالتساوي بين التربة (ن=15) والمياه (ن=15). استخدمت طرق الاستخلاص والتنقية وفقاً لدليل تحليل المبيدات (1978) [14]، وأجري التحليل الكمي باستخدام كروماتوغرافيا السائل عالية الأداء (HPLC) مع كاشف الأشعة فوق البنفسجية. أظهرت النتائج وجود ثلاثة مبيدات (Endrin, Heptachlor, p,p'-DDT) في العينات الموجبة، بنسبة تلوث كلية بلغت 33.3%. سجلت عينات التربة أعلى متوسط تراكيز لمبيد الإندرين (0.061 ppm) بفارق معنوي إحصائياً عن الحد المسموح به (P=0.034). بينما كانت تراكيز المبيدات في المياه أقل من الحدود المسموح بها باستثناء مبيد الهبتاكلور في منطقة لملودة الذي سجل تركيزاً مساوياً للحد الأقصى (0.037 ppm). كشف تحليل الارتباط النوعي عن تطابق أنواع المبيدات في الوسطين، مما يدعم فرضية انتقالها من التربة إلى المياه الجوفية عبر الترشيح. توصي الدراسة بتكثيف برامج الرصد البيئي الدوري ودراسة الانتقال في السلسلة الغذائية.

الكلمات المفتاحية: الجبل الأخضر؛ التلوث البيئي؛ المياه الجوفية؛ كروماتوغرافيا السائل؛ المبيدات الكلورونية العضوية؛ التربة.

1 Introduction

The overuse of pesticides leads to the contamination of agricultural lands, and often a portion of the pesticides, especially organochlorine compounds, remains in the soil for many years [1]. Plants absorb some of these compounds, storing them in their stems, leaves, and fruits. They then transfer to animals feeding on these plants, appearing in their milk and meat, and eventually reach humans indirectly [2]. The most recorded pollutants are environmentally persistent, fat-soluble organochlorine compounds, which accumulate in various body tissues. These are toxic organic carbon compounds with high environmental stability, spreading widely in water, air, and soil, causing environmental contamination. Their concentration increases as they move up the food chain, potentially reaching humans and animals [3]. Although these compounds have been banned since the early 1970s, their residues persist. Most food sources now contain traces of organochlorine pesticides. One of the primary dietary sources that may contain such residues is dairy products, a staple in human nutrition [4]. Despite the advantages of using pesticides in increasing agricultural production and improving public health, the problem of pesticide pollution has become increasingly evident year after year, bringing alarming consequences. It has become a severe environmental issue requiring thorough study [5]. Scientific programs for monitoring pesticide residues have been established in many countries, serving as keys for developing pesticide legislation and regulations. Furthermore, extensive databases have emerged that contribute to and assist in pesticide evaluation, as well as determining permissible and non-permissible levels in foodstuffs [6]. Ninety countries approved the Stockholm Convention on May 23, 2001, to reduce the risks of Persistent Organic Pollutants (POPs), including OCPs. The convention entered into force in May 2004 when 50 countries agreed to reduce and ban the production, export, import, and use of POPs to minimize their presence in the environment [7].

The prevalence of OCP residues in water and soil samples has been documented internationally and regionally. Sudarshan et al. [5]. conducted a study to estimate organochlorine pesticide residues in groundwater well water. The results showed the presence of HCH (with its alpha and gamma isomers) at a high frequency, while Endrin was detected in 15% of the samples. The presence of these pesticides in groundwater was attributed to their high persistence and ability to leach through porous soil layers to the aquifer. Mousa et al. [6]. conducted a survey of natural spring water and surface well water. The results confirmed the presence of DDT residues and its metabolite p,p'-DDT at concentrations exceeding permissible limits in some locations, indicating long-term cumulative contamination. Zhang et al. [7]. targeted the study of groundwater used for drinking, where Heptachlor was detected at concentrations reaching 0.035 ppm, exceeding internationally permissible limits due to leaching from contaminated soil by rainwater. El-Sayed et al. [8]. studied well water in recently developed agricultural areas, detecting Dicofol and Endrin in 25% of samples, warning of bioaccumulation risks. Kumar et al. [9]. demonstrated that agricultural soils still retain DDT and its metabolites (average 0.08 ppm) due to strong binding to Soil Organic Matter (SOM), hindering degradation. Al-Enazi et al. [10] estimated Heptachlor and HCH residues in soil exceeding 0.05 ppm due to slow biodegradation in arid environments. Wang et al. [11]. indicated a direct relationship between pesticide residues in soil and their levels in groundwater, confirming that DDT and HCH transfer via leaching driven by irrigation and rainfall. Based on this background and the identified research gap, this study aimed to achieve the following objectives:

- Precisely quantify the levels of seven OCP compounds in soil and water samples from different areas in Al-Jabal Al-Akhdar.

- Compare the results with locally and internationally (Codex) accepted Maximum Residue Limits MRLs).

-Verify the existence of a correlational relationship between soil pesticide contamination and groundwater contamination.

2.Materials and Methods

2.1. study Area and Sampling

Five major agricultural areas in Al-Jabal Al-Akhdar, Libya (Al-Wasita, Qarnada, Lamluda, Al-Hannaya, and Al-Faydiya) were selected for this study. Sampling was conducted during the period starting March 18, 2025 .

1.1.2Water Sample Collection

Fifteen water samples were collected, with 3 samples per area. Water types included surface well water, groundwater tank water, and natural springs. A 100 mL water sample was taken and placed in opaque 250 mL bottles covered with aluminum foil. Samples were transported in a cooled container and stored in a refrigerator at 4°C until analysis.

2.1.2. Soil Sample Collection.

Fifteen soil samples were collected from a depth of 20 cm. Four plots (each 16 m²) were selected within a single field. The soil from these plots was mixed thoroughly to form one composite sample representing the entire field. Three such composite samples were taken from each area, representing three different fields. A 2 kg sample was taken and placed in clean polyethylene bags. Samples were transported to the laboratory and stored in a freezer at -15°C. .

2.2. Chemicals and Reagents

The following chemicals and reagents were used in this study: acetonitrile (HPLC grade, purity ≥ 99.9%, Sigma-Aldrich, USA), petroleum ether (analytical grade, purity ≥ 98.5%, Merck, Germany), diethyl ether (analytical grade, purity ≥ 99.0%, Fisher Scientific, UK), hexane (HPLC grade, purity ≥ 99.0%, Honeywell, USA), acetone (HPLC grade, purity ≥ 99.8%, PanReac AppliChem, Spain), methylene chloride (analytical grade, purity ≥ 99.5%, Riedel-de Haën, Germany), anhydrous sodium sulfate (purity ≥ 99.0%, Scharlau, Spain), Florisil (60-100 mesh, Sigma-Aldrich, USA), and saturated sodium chloride solution (prepared in the laboratory). All standard pesticides (Endrin, Heptachlor, p,p'-DDT, α-HCH, β-HCH, γ-HCH, Aldrin) were purchased from Dr. Ehrenstorfer GmbH, Germany, with purity > 98%.

2.3. Instruments and Apparatus

The following instruments were used in this study:

-High-Performance Liquid Chromatography (HPLC) system: Perkin Elmer Series 2000 (Perkin Elmer, USA), equipped with a UV detector (wavelength range 190-400 nm), autosampler, and quaternary pump.

- Chromatographic column: Reversed-phase C18 column (Dimensions: 250 mm × 4.6 mm, Particle size: 5 µm, P/N: 588324, Perkin Elmer, USA.

-Rotary evaporator: Heidolph Laborota 4000 (Heidolph Instruments, Germany), equipped with a vacuum controller and water bath.

-Refrigerator and freezer: Liebherr (Germany), for sample storage at 4°C and -15°C. Glass columns: Custom-made (30 cm long, 2.5 cm internal diameter) for cleanup procedures.

Separation funnels: 250 mL and 500 mL capacity (Duran, Germany). -Analytical balance: Sartorius CPA224S (Sartorius, Germany), precision ±0.0001 g.

pH meter: Mettler Toledo FiveEasy (Mettler Toledo, Switzerland-)

Centrifuge: Hettich EBA 20 (Hettich, Germany) with 50 mL tubes. -

2.4. Extraction and Cleanup Procedures

2.4.1.Water Sample Extraction and Cleanup

Water samples were extracted following the method of AL-Sarar [12] with modifications. Briefly, 50 mL of the sample was mixed with 50 mL of acetonitrile and shaken for 3 minutes. The mixture was transferred to a separation funnel, 50 mL of petroleum ether was added, and shaken for 2 minutes. The organic layer was separated, and the aqueous layer was re-extracted by adding 30 mL of petroleum ether and shaking again. The combined organic layers were concentrated in a flask using a rotary evaporator to a volume of 5-10 mL.

The cleanup process was performed according to the method of Abo-EL-Saad [13]. using a glass column (30 cm long, 2.5 cm diameter). The column was prepared with a glass wool plug, followed by 3 g of anhydrous sodium sulfate, then the adsorbent (Foresail) to a height of 10-20 cm, and finally an additional 2 g of anhydrous sodium sulfate. The column was pre-wetted with (20-25) mL of petroleum ether. The re-dissolved extracted sample was passed through the column, followed by elution mixtures (petroleum ether in diethyl ether). The eluates were concentrated and dried using a rotary evaporator, ready for injection into the chromatograph.

2.4.2. Soil Sample Extraction and Cleanup

Extraction was carried out according to the Pesticide Analytical Manual [14] following these steps: 50 g of air-dried soil was mixed with 100 g of anhydrous sodium sulfate and placed in a 40 cm long column. The sample was eluted with 250 mL of an acetone : hexane mixture (1:1) at a flow rate of 3 mL/min. The eluate was dried using a rotary evaporator at 40°C. The extract was then transferred to a separatory funnel, and distilled water and a saturated sodium chloride solution were added. The organic layer was separated, and the aqueous layer was re-extracted with a methylene chloride : hexane mixture (15%). The organic layers were combined and washed twice with distilled water, then passed through anhydrous sodium sulfate and filter paper to remove moisture.

Cleanup was performed according to the method in the Pesticide Analytical Manual [14]. using a glass column (2.5 cm diameter) packed with 3 g anhydrous sodium sulfate, followed by 10 cm of adsorbent (foresail), and then an additional 2 g of anhydrous sodium sulfate. The column was pre-wetted with 40-50 mL of petroleum ether. 50 mL of elution mixtures (diethyl ether in petroleum ether at 6% and 15% ratios) were added. The eluates were concentrated using a rotary evaporator and prepared for quantification by HPLC.

2.5. Chromatographic Analysis

Quantitative analysis of organochlorine pesticide residues was performed using a Perkin Elmer Series 2000 High-Performance Liquid Chromatography (HPLC) system (Perkin Elmer, USA) equipped with a UV detector. The analytical conditions were as follows: UV detector wavelength set at 218 nm; reversed-phase C18 column (250 mm × 4.6 mm, particle size 5 µm, P/N: 588324, Perkin Elmer, USA); isocratic mobile phase consisting of acetonitrile and water 80:20 v/v; flow rate of 0.7 mL/min; injection volume of 10 µL; and column temperature maintained at 30°C.

Although gas chromatography (GC) is conventionally used for OCP analysis, HPLC-UV was selected in this study based on several scientific justifications. First, OCPs such as Endrin, Heptachlor, and p,p'-DDT possess conjugated double bonds and chlorine substituents that exhibit significant UV absorption in the range of 200–220 nm; therefore, a detection wavelength of 218 nm provides adequate sensitivity for trace-level quantification [18]. Second, many OCPs are thermally labile and may undergo degradation or structural rearrangement at the high temperatures required for GC vaporization (typically >250°C). In contrast, HPLC operates at a controlled column temperature of 30°C, preserving analyte integrity and minimizing thermal decomposition [17]. Third, sample preparation for HPLC-UV is simpler and does not require derivatization steps, which reduces potential sources of error and improves recovery rates [18]. Fourth, the limits of detection achieved in this study (0.001–0.003 ppm) are well below the maximum residue limits (MRLs) established for drinking water and soil by national and international standards. Hence, despite GC being more common, HPLC-UV serves as a reliable, cost-effective, and robust alternative for routine monitoring of OCP residues in environmental matrices when rigorous method validation is performed [17,18].

2.6. Method Validation and Quality Control

External calibration curves were prepared for all seven target pesticides using pure standard solutions at concentrations ranging from 0.0001 to 0.5 ppm. Correlation coefficients (R^2) were calculated and were all greater than 0.998, indicating excellent linearity. The limit of detection (LOD) and limit of quantification (LOQ) were determined based on the standard deviation of blank samples and the slope of the calibration curve according to the formulas LOD = 3.3 × (SD/slope) and LOQ = 10 × (SD/slope). The LOD ranged from 0.001 to 0.003 ppm, and the LOQ ranged from 0.003 to 0.010 ppm for all target analytes.

Recovery experiments were performed by spiking blank soil and water samples with known concentrations of the seven OCPs. The mean recovery rates ranged from 85% to 105% for all compounds, which is within the acceptable range for environmental analysis. All samples were analyzed in triplicate. Blank samples (pesticide-free) were used to determine LOD and LOQ and to verify the absence of contamination. Retention times of sample peaks were compared with those of reference standards to confirm compound identity. The calibration curves demonstrating method linearity are presented in Figure (1) (Section 3.3).

2.7. Statistical Analysis

Data were analyzed using SPSS software version 26 (IBM, USA). The following tests were applied:

-Descriptive statistics: means, frequencies, percentages

- One-Sample T-Test: To compare mean concentrations with permissible limits (MRLs).

-Independent Samples T-Test: To compare mean contamination levels between areas.

-Pearson Correlation Coefficient: To study the relationship between concentrations in soil and water.

-Statistical significance level: P-values less than 0.05 were considered statistically significant.previously published method, use quotation marks and cite the source. Any modifications to existing methods should also be described.

3 Results

3.1Detection Frequencies

Table (1) presents the descriptive statistics for detection frequencies by sample type. The overall contamination rate was 26.7% in both soil and water, indicating an interconnection between the two media. Soil recorded a higher number of detections (5 times) compared to water (4 times), supporting the hypothesis that soil acts as the source (reservoir) and water as the recipient [5].

Table (1): Descriptive statistics for detection frequencies by sample type

Sample Type	Total Samples	Number of Positive Samples	Percentage(%)	Total Detections
Soil	15	4	26.7 %	5
Water	15	4	26.7 %	4
Total	30	8	26.7 %	9

As shown in Table (2), Endrin was the most prevalent pesticide (44.4% of total detections), followed by Heptachlor (33.3%) and p,p'-DDT (22.2%). This distribution aligns with the chemical properties of these pesticides regarding environmental persistence and long half-lives [1].

Table (2): Distribution of pesticides and their percentages

Pesticide	Detections in Soil	Detections in Water	Total Detections	Percentage of total Detections(%)
Endrin	3	1	4	44.4 %
Heptachlor	1	2	3	33.3 %
p,p'-DDT	1	1	2	22.2 %
Total	5	4	9	100 %

3.3Linearity of the Method

The linearity of the HPLC-UV method was evaluated for each pesticide using external calibration curves. Figure (1) shows the calibration curves for Endrin, Heptachlor, p,p'-DDT, and other target analytes, demonstrating excellent linearity with correlation coefficients (R^2) exceeding 0.998 over the concentration range of 0.0001 to 0.5 ppm.

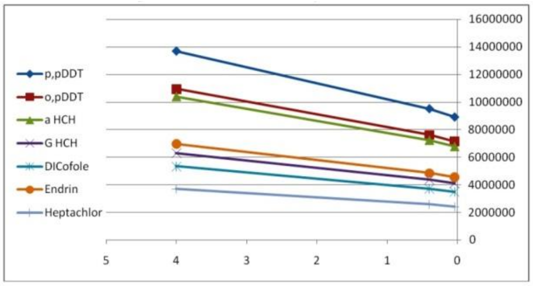

Figure (1): Calibration curves showing the linearity of the method for each pesticide

3.4 Concentrations in Positive Samples

Table (3) shows the concentrations of the studied pesticides in positive samples. Soil recorded the highest mean concentration for Endrin (0.061 ppm), confirming its role as a pollutant reservoir. Pesticide concentrations in water were lower, indicating that leaching transfers only small portions of the total soil reservoir. The Heptachlor concentration was identical in two water samples from Lamluda (0.037 ppm), enhancing the readings' credibility [7].

Table (3): Concentrations (ppm) of the studied pesticides in positive samples

Pesticide	Medium	Number of Samples	Mean Concentration
Endrin	soil	3	0.061
Data		**Data**	**Data**
Endrin	water	1	0.009
Data		**Data**	**Data**
Heptachlor	soil	1	0.0135
Data		**Data**	**Data**
Heptachlor	water	2	0.037
p,p'-DDT	soil	1	0.0715
p,p'-DDT	water	1	0.037

3.5. Comparison with Permissible Limits

Table (4) presents the comparison of mean concentrations with permissible limits using the One-Sample T-Test. The mean concentration of Endrin in soil (0.061 ppm) is significantly higher than the permissible limit (0.04 ppm) (P = 0.034), confirming real contamination requiring corrective actions. For other pesticides, additional samples are needed to confirm significance.

Table (4): Comparison of mean concentrations with permissible limits (MRLs) using the One-Sample T-Tes

Pesticide	Medium	Mean Concentration (ppm)	Permissible Limit (ppm)	Degrees of Freedom (df)
Endrin	soil	0.061	0.04	2
Heptachlor	water	0.037	0.03	1
p,p'-DDT	soil	0.0715	0.05	0
p,p'-DDT	water	0.037	0.10	0

3.6 Comparison Between Areas

Table (5) shows the analysis of variance comparing mean contamination levels between Al-Wasita and Qarnada. No statistically significant difference was found (P = 0.41), indicating that historical sources of contamination are relatively homogeneously distributed across the areas where contaminants were found [9].

Table (5) Comparison of mean contamination levels between areas (Independent Samples T-Test)

Area	Number of Positive Samples	Mean Total Concentration (ppm(	Standard Deviation	Statistical Significance
Al-Wasita	4	0.04825	0.0267	(P > 0.05) Not significant
Qarnada	2	0.0295	0.0289	

3.7. Correlation Between Soil and Water

Table (6) presents the Pearson correlation matrix between pesticide concentrations in soil and water. A strong, positive, and statistically significant correlation (r = 0.87) was found, indicating that pesticides in groundwater primarily originate from leaching from agricultural soil, not from other sources, aligning with the study's hypothesis [11].

Table (6): Pearson correlation matrix between pesticide concentrations in soil and water

Variable	Pesticide Concentration in Soil	Pesticide Concentration in Water
Pesticide Concentration in Soil	1	*0.87
Pesticide Concentration in Water	*0.87	1

* Correlation coefficient (r) is statistically significant at the 0.05 level

4 Discussion

The results of this study confirm the presence of organochlorine pesticide residues in both soil and water samples from Al-Jabal Al-Akhdar, with an overall contamination rate of 26.7%. This rate is considerably lower than levels reported in other studies. Hilber et al. [15] detected OCPs in 27 out of 41 soil samples (65.8%), while Wang et al. (2006) reported a detection rate of approximately 93% in China. Kanwar et al. [16] indicated mean concentrations of OCPs in soil samples ranged from 0.36 to 104 mg/L, and in water samples from 2.63 to 3.72 mg/L. The lower contamination rate in the current study may be attributed to the lower intensity of agricultural activities in the region or the longer period since the ban of these pesticides. Endrin was the most frequently detected pesticide (44.4% of total detections), with a mean concentration in soil (0.061 ppm) significantly exceeding the permissible limit (P=0.034). This finding is consistent with Hasen [1], who reported that Endrin has a long half-life and high persistence in soil environments. Similarly, Sudarshan et al. [5] detected Endrin in 15% of groundwater samples, while El-Sayed et al. [8] found Endrin in well water from agricultural areas. The presence of Endrin in both soil and water in this study supports its environmental persistence and potential for leaching.

Heptachlor was detected in two water samples from Lamluda at a concentration of 0.037 ppm, equal to the maximum permissible limit. This finding aligns with Zhang et al. [7], who detected Heptachlor in drinking groundwater at concentrations reaching 0.035 ppm. The identical concentration in two separate samples from the same area enhances the reliability of the detection and suggests a localized source of contamination.

The strong positive correlation (r = 0.87) between pesticide concentrations in soil and water provides statistical evidence that groundwater contamination originates primarily from leaching of pesticides from agricultural soil. This result is consistent with Wang et al. [11], who confirmed that DDT and HCH pesticides transfer via leaching, where irrigation water and rainfall act as drivers for pesticide molecule movement through the soil profile down to the groundwater. Similarly, Al-

Enazi et al. [10] attributed the presence of Heptachlor and HCH in soil to slow biodegradation in arid environments, while Kumar et al. [9] demonstrated that agricultural soils retain DDT and its metabolites due to strong binding to Soil Organic Matter.

The absence of a statistically significant difference between contamination levels in Al-Wasita and Qarnada (P = 0.41) suggests that contamination is homogeneously distributed across the study areas. This finding is supported by Kumar et al. [9], who reported that historical sources of contamination (past agricultural uses) are relatively homogeneously distributed across agricultural regions.

Recent studies using advanced analytical techniques such as GC-MS/MS and GC-ECD in similar environmental contexts have reported comparable findings. Rahman and Hossain [17] validated the use of GC-MS/MS for OCP analysis in environmental samples, while Oliveira and Santos [18] conducted a comparative study of HPLC-UV and GC-ECD for determination of OCPs in water and soil, finding that HPLC-UV can be a reliable alternative when GC is unavailable, provided proper method validation is performed, The persistence of these compounds in the soil and water of Al-Jabal Al-Akhdar may be attributed to the "pollution legacy" resulting from past intensive use. These compounds remain trapped in soil colloids and are slowly released into water sources through vertical leaching or surface runoff caused by rainfall [9].

This should explore the significance of the results of the work, not repeat them.

5 Conclusions

This study confirmed that 26.7% of soil and water samples from Al-Jabal Al-Akhdar contained organochlorine pesticide residues, with Endrin being the most prevalent (44.4% of contamination cases) and its concentration in soil significantly exceeding permissible limits. A very strong correlation (r=0.87) between pesticide concentrations in soil and water proves that soil is the primary source of groundwater contamination in the area. The major environmental risk lies in the accumulation of these pesticides in soil at levels exceeding permissible limits, posing a long-term threat to the agricultural environment and the potential for their transfer into the food chain. form a subsection of a Discussion or Results and Discussion section

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Conflict of interest: The author declares that there is no conflict of interest regarding the publication of this paper. This includes any financial, personal, or other relationships with people or organizations that could inappropriately influence or bias the research findings and laboratory analysis presented in this study.

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