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Detection and Risk Assessment of Heavy Metal Contamination in the Meats Local Goat Breeds

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Abstract | This study was conducted to assess the levels of contamination of selected heavy metals in the muscles, liver and kidney meat of domestic goats in the east of Salahalddin governorate in the spring season of both male and female sexes. Samples were randomly collected from animals (aged 4-10 months) from three areas in the east of Salah al-Din governorate (Dour, Tuz Khurmatu and Amerli). Analysis indicted that lead concentration had a significant effect ($p \leq 0.05$) on the location (muscles, liver, kidneys) and on gender where the liver recorded the highest concentration (8.479 part per million) for male goats, and the lowest concentration (0.580 part per million) in the female kidney. As for cadmium, there was a significant difference ($p \leq 0.05$), and the highest concentration level was recorded in the liver (4.970 part per million) for females and the lowest concentration (1.743 part per million) recorded in the liver for males. As for copper, there was a significant difference ($p \leq 0.05$), where the male liver recorded the highest results (19.970 part per million), while the female liver recorded lower values (2.003 part per million). For cobalt, the highest concentration of cobalt was recorded in the liver (8.167 part per million) for male goats, while the lowest concentration was recorded in the muscles for females for the half of the city and was (0.753 part per million). Totally forms of minerals recorded ranks above internationally conventional confines. Taken together, the results identify the presence of substantial metallic remains in the animal's meat of local black goats and underscore the potential health risks associated with the consumption of contaminated meat.

Keywords | Goat, Muscle, Liver, Kidney, Heavy metals

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INTRODUCTION

Meat with its by-products is considered very imperative in the diet because they provide most of the human needs and their important nutrients, including trace elements (Demirezen and Uruc, 2006). Meat shows an identical imperative role in diet by providing the body with high-quality proteins, essential minerals and

trace elements, a complex of vitamins (Ponnambalam *et al.*, 2001). Consumers today are health-conscious and demand high-quality food products (Dransfield, 2001). The consumer pays great attention to the cleanliness and health of meat to reduce the health risks that are associated with animal health (Abass *et al.*, 2019).

There is an increase in the population of Iraq and

because of this increase, there is a growing demand for meat production. The main sources of heavy metals in environmental include factory, cars, industrial waste (Al-Perkhdri *et al.*, 2020). Biological age is one of the factors by which heavy elements or materials can be identified, in addition to the biodegradability of these elements in the environment and human cells (Salim *et al.*, 2023). Heavy metals pose a potential risk to human health. Heavy metals enter food animals through several routes, including inhalation of wind containing metal-laden dust, oral intake of water and feed contaminated with toxic metals (Palani *et al.*, 2019; Abd-Elghany *et al.*, 2020).

One of the animals that have a role in human nutrition is goats, which is considered one of the important farm animals in Iraq, and goat meat is one of the meats consumed in many regions of Iraq, especially in central and northern Iraq (Romidi and Alper, 2010). Animal meat is affected by many factors, including heavy metals and even radiation emitted by pollution sources, and there is a significant effect of radiation in together rate of explosive nitrous centers Vin plus peroxide morals, where in the quantity of Vin in animal substance has bigger (Al-Perkhdri, 2022).

There is contamination with heavy metals in field crops, which are transmitted to the food chain by feeding and eating meat and milk of animals (Ashfaq *et al.*, 2022; Ahmad *et al.*, 2022; Castro-González *et al.*, 2017). Heavy metal contamination in meat and other edible tissues is a major concern for food safety and hominoid condition (Santhi *et al.*, 2008). Several scientists found the presence of cases of heavy weight metallic contamination in substance foodstuffs through treating (Brito *et al.*, 2005). People consume most plants as natural foods without any concern, especially since most of them do not cause side effects (Al-Saadi *et al.*, 2023). The use of clean, magnetically treated water resulted in an increase in the body weight of ewes and the average weight gain of animals (Al-Mufarji and Al-Saadi, 2023). The results of a study by Al-Dawudi *et al.* (2019) on the effect of ginger extract processing and cold storage on some chemical, sensory, and bacteriological properties of Al-Karady lamb meat showed significant differences between different treatments in total bacterial counts, and a decrease in total bacterial counts.

Salihi and Saadi (2024) have indicated that animals treated with an aqueous solution of Spirulina powder with or without folic acid showed significant differences ($P \leq 0.05$) in kidney meat weight gain compared to the control group. The results of the study conducted by Safar and Al-Saad (2024) showed that the use of Spirulina led to an improvement in body meat weight gain and growth rate in sheep, there are large differences between the treatments in the total bacterial count, and there was a decrease in the total bacterial count when use ginger extracts treatment in

refrigeration storage (Al-Daoudi *et al.*, 2019). The amount of precipitation of heavy elements varies depending on the tissue, place and time of sampling (Mohammed and Alperkhdri, 2021). The researchers were interested in studying and analyzing food pollutants, the most important of these pollutants are heavy elements, including lead, cadmium and cobalt, and these pollutants reach food through the air, soil or air (Oehm, 1989). Researchers have found that feeding animals on contaminated feed and raising animals near contaminated industrial areas cause contamination of meat with heavy metals (Saber *et al.*, 2003; Miranda *et al.*, 2005).

Heavy metal pollution often has toxic effects (Bokori *et al.*, 1996; Mariam *et al.*, 2004). Heavy elements are deposited in the city center more than in the districts and districts, where the center of Kirkuk recorded an increase in the deposition of heavy elements in the liver, lungs and meat more than in other areas (Al-Perkhdri, 2021). There is a difficulty in accurately classifying heavy metals and identifying toxic ones, however, these elements, if taken in a large amount, are toxic Khurshid and Qureshi (1984). The element lead, for example, may enter the atmosphere during mining, oil refining and manufacturing operations (Benneth, 1981). The frequent use of copper alloys has a danger due to the corrosion of these alloys and the particles of these alloys enter the water stream and cause many problems, and the copper content of the average human adult is 50-120 MG, but above 15 mg causes nausea, vomiting, diarrhea, intestinal pain (Greenwood and Earnshaw, 1986).

Copper content in goat kidneys is reported as 12.43-14.5 part per million which is below the toxicity level and lead is 29 parts per million in goat meat from Chennai City, India, which is at the toxic level. In Sofia the copper content in the fresh liver of goats was reported in the range of 2.6-10.3 mg (Tomov, 1960). There are several studies to determine the health risks of heavy metals, including copper, by consuming contaminated food crops (Ilechukwu *et al.*, 2021). Heavy metals are not biodegradable and at the same time absorbed by plants, for this reason, many studies have been conducted to warn of the accumulation of these heavy metals in plants (Bonnet *et al.*, 2020; Buat-Menard, 1979). Food is one of the principle ecological sources of cadmium (Bykov *et al.*, 1996). Serious effects are found in cadmium through injuries of the digestive and respiratory systems, as well as cardiac injuries and hypertension (John and Jane, 1994), and to induce significant changes in enzymes that would act on detoxification in the liver and kidneys (Reddy-Willama, 1996). Cadmium is a dangerous and toxic metal that enters the ecosystem through the soil and vegetation through the Sewerage of cities and through inattention to the sewage system (Ebrahimi *et al.*, 2020). Cadmium is a water-soluble element that is easily absorbed by plants and is a serious environmental pollutant

in agroecosystems (Duan *et al.*, 2020). The persistence and toxicity of substantial metals in H₂O, topsoil, air with plants made them most dangerous of the many pollutants found in the soil (Duan *et al.*, 2020; Akhter *et al.*, 2022).

The aim of this study was to determine the concentrations of selected heavy metals (lead, cadmium, copper, and cobalt) in the muscle, liver, and kidney tissues of local black goats from different regions in eastern Salah Al-Din Governorate, Iraq, and to assess the potential health risks associated with their consumption.

MATERIALS AND METHODS

MEAT REIGN

This study used kidney, muscle, liver, and meat from goats (male and female). All animals used in this study were local black goat (Iraqi breeds) and were distributed in three areas east of the Salah al-Din region: Dur, Tuz Khurmatu, and Amarli. Samples were collected from goats aged 4 to 10 months. Meat samples (muscle and viscera) were randomly sampled in three replicates from three areas of the Salah al-Din site in March and April (spring). The meat sample was calf muscle, and viscera samples included liver and kidney. After slaughter, samples were hung at the point of sale between 6:00 AM and 4:00 PM.

Samples were collected from the gastrocnemius muscle of all animals tested, regardless of region. Samples were then placed in polyethylene bags and stored in specialized containers and boxes, which were refrigerated until delivery to the laboratory. Meat and kidney samples were cut into pieces, placed in polyethylene bags, and then placed in specially prepared and numbered plastic containers.

Liver samples were cut into pieces, placed in plastic bags, and then placed in plastic containers. All samples were stored at a frozen temperature (-18 °C) until the proportions of their major components could be determined. A total of 54 goat samples (both male and female) were collected during one season.

HEAVY METAL ASSAY

Heavy metal concentrations were determined using the analytical procedure described by Ropme (1983). Briefly, tissue samples were first homogenized and then subjected to wet digestion using a mixture of concentrated nitric acid and perchloric acid under controlled heating conditions until complete mineralization was achieved. The digested solutions were filtered, diluted to a defined final volume with deionized water, and subsequently analyzed for lead (Pb), cadmium (Cd), copper (Cu), and cobalt (Co) using atomic absorption spectrophotometry (AAS). All measurements were performed in triplicate to ensure accuracy, and quality

control was maintained by analyzing reagent blanks and certified reference materials alongside the samples.

STATISTICAL ANALYSIS

In a completely randomized controlled trial (CRD), the effects of meat type, sex (year), and production area on various traits were investigated. Differences between means were compared using the multivariate Duncan test according to the following mathematical model. Statistical analysis was performed using the xlstat program:

$$Y_{ijklm} = m + A_i + B_j + C_k + ABC(ijk) + e_{ijklm}$$

As: Y_{ijklm}: Displayed values for each attribute. M: Overall mean, A_i: Effect of meat type (muscle, liver, and kidney), B_j: Effect of sex (male and female), C_k: Effect of production area (Tuzkhormatu-Amarli-Dour), ABC (ijk): Effect of the interaction between meat type, sex, and production area, e_{ijklm}: Random error, normally distributed with mean zero and variance S² e.

RESULTS AND DISCUSSION

As shown in the Table 1, the triple interaction between location and sex ($p \leq 0.05$) significantly affected lead concentrations in muscle, liver, and kidney of goats of different sexes (male and female) in the three regions of Salahuddin Governorate. In spring, male goats had the highest liver lead concentration (8.479 ppm), while female goats had the lowest liver lead concentration (0.811 ppm).

The effects of these factors on lead concentrations were significantly different across locations ($p \leq 0.05$) (Table 1). Tuz Khurmatu Town had the highest lead concentration (5.279 ppm), while Amerli Town had the lowest (1.246 ppm). When comparing the effects of meat type on muscle, liver, meat, and kidney, we compared the liver model, which had the highest dose (2.795 ppm), with the kidney model, which had the lowest dose (2.411 ppm).

Analysis indicated that the effect of lead exposure on sex wasn't significant in both male or female sex ($p \leq 0.05$). Males recorded the highest concentration in the liver (4.034 ppm), while females recorded the lowest concentration in meat (1.425 ppm).

Table 2 shows the effects of the triple interaction of meat type (muscle, liver, kidney), location, and sex on cadmium concentrations in muscle, liver, and kidney of goats of different sexes (male and female) from three districts of Salahuddin Governorate. Liver significantly contributed to the highest cadmium concentration in Tuz Khurmatu Township ($p \leq 0.05$). Females recorded cadmium concentrations of 4970 ppm, while males recorded the lowest concentration in the liver (1743 ppm).

Table 1: Lead contamination in goat samples from three areas in east of Salahalddin province from Male and Female (mean $\pm$ standard error).

Tissue	Location	Female (ppm)	Male (ppm)	Overall mean	Significance
Liver	Dour	1.862 $\pm$ 0.238	0.811 $\pm$ 0.141	2.913 $\pm$ 0.041	e
Liver	Tuz Khurmatu	5.279 $\pm$ 0.365	2.080 $\pm$ 0.083	8.479 $\pm$ 0.115	a
Liver	Amerli	1.246 $\pm$ 0.125	1.791 $\pm$ 0.026	0.702 $\pm$ 0.023	g
Liver mean	—	2.795 $\pm$ 0.204	1.560 $\pm$ 0.108	4.031 $\pm$ 0.359	a
Muscle	Dour	1.730 $\pm$ 0.155	1.080 $\pm$ 0.051	2.381 $\pm$ 0.071	f
Muscle	Tuz Khurmatu	3.574 $\pm$ 0.394	1.764 $\pm$ 0.027	5.384 $\pm$ 0.135	c
Muscle	Amerli	2.758 $\pm$ 0.503	5.121 $\pm$ 0.073	0.396 $\pm$ 0.023	k
Muscle mean	—	2.687 $\pm$ 0.234	2.655 $\pm$ 0.299	2.720 $\pm$ 0.371	c
Kidney	Dour	2.266 $\pm$ 0.230	1.218 $\pm$ 0.089	3.315 $\pm$ 0.035	d
Kidney	Tuz Khurmatu	3.304 $\pm$ 0.596	0.580 $\pm$ 0.040	6.028 $\pm$ 0.169	b
Kidney	Amerli	1.664 $\pm$ 0.238	2.477 $\pm$ 0.305	0.852 $\pm$ 0.058	i
Kidney mean	—	2.411 $\pm$ 0.237	1.425 $\pm$ 0.164	3.398 $\pm$ 0.386	b

Means with the same letter do not differ significantly ($p \leq 0.05$).

Table 2: Cadmium contamination in goat samples (male and female) from three regions in eastern Salahardin Province (mean $\pm$ SE).

Tissue	Location	Female	Male	Overall mean	Significance
Liver	Dour	1.947 $\pm$ 0.066	2.151 $\pm$ 0.083	1.743 $\pm$ 0.043	fg
Liver	Tuz Khurmatu	3.677 $\pm$ 0.066	4.970 $\pm$ 0.020	2.385 $\pm$ 0.033	a
Liver	Amerli	2.141 $\pm$ 0.073	2.490 $\pm$ 0.019	1.792 $\pm$ 0.038	fg
Liver mean	—	2.588 $\pm$ 0.049	3.203 $\pm$ 0.058	1.973 $\pm$ 0.046	d
Muscle	Dour	2.274 $\pm$ 0.071	2.572 $\pm$ 0.037	1.977 $\pm$ 0.060	c
Muscle	Tuz Khurmatu	2.527 $\pm$ 0.037	2.661 $\pm$ 0.025	2.393 $\pm$ 0.034	d
Muscle	Amerli	2.344 $\pm$ 0.085	2.747 $\pm$ 0.034	1.942 $\pm$ 0.037	ef
Muscle mean	—	2.381 $\pm$ 0.039	2.660 $\pm$ 0.021	2.104 $\pm$ 0.038	c
Kidney	Dour	1.823 $\pm$ 0.034	1.746 $\pm$ 0.038	1.900 $\pm$ 0.054	fg
Kidney	Tuz Khurmatu	2.569 $\pm$ 0.058	2.778 $\pm$ 0.061	2.360 $\pm$ 0.023	d
Kidney	Amerli	2.158 $\pm$ 0.066	2.429 $\pm$ 0.062	1.887 $\pm$ 0.049	ef
Kidney mean	—	2.183 $\pm$ 0.047	2.317 $\pm$ 0.077	2.049 $\pm$ 0.043	d

Means with the same letter do not differ significantly ($p \leq 0.05$).

Table 3: Copper contamination in goat samples (male and female) from three regions in eastern Salahardin Province (mean $\pm$ SE).

Tissue	Location	Female	Male	Overall Mean	Significance
Liver	Dour	4.920 $\pm$ 0.321	3.874 $\pm$ 0.356	5.967 $\pm$ 0.324	c
Liver	Tuz Khurmatu	10.986 $\pm$ 2.436	2.003 $\pm$ 0.090	19.970 $\pm$ 1.268	a
Liver	Amerli	6.471 $\pm$ 0.428	7.376 $\pm$ 0.594	5.566 $\pm$ 0.423	fgh
Liver mean	—	7.459 $\pm$ 0.914	4.417 $\pm$ 0.452	10.501 $\pm$ 1.554	a
Muscle	Dour	4.915 $\pm$ 0.313	3.701 $\pm$ 0.136	6.130 $\pm$ 0.400	defg
Muscle	Tuz Khurmatu	7.095 $\pm$ 0.279	7.514 $\pm$ 0.474	6.677 $\pm$ 0.236	cde
Muscle	Amerli	5.948 $\pm$ 0.155	6.522 $\pm$ 0.231	5.375 $\pm$ 0.120	gh
Muscle mean	—	5.986 $\pm$ 0.194	5.912 $\pm$ 0.336	6.060 $\pm$ 0.205	bc
Kidney	Dour	6.043 $\pm$ 0.337	4.634 $\pm$ 0.247	7.453 $\pm$ 0.079	c
Kidney	Tuz Khurmatu	7.650 $\pm$ 0.236	6.606 $\pm$ 0.124	8.694 $\pm$ 0.055	b
Kidney	Amerli	4.828 $\pm$ 0.337	3.138 $\pm$ 0.569	5.779 $\pm$ 0.217	fgh
Kidney mean	—	6.173 $\pm$ 0.273	5.515 $\pm$ 0.292	7.308 $\pm$ 0.232	b

The averages with identical letters are not significantly different ($p \leq 0.05$) between them

The effects of the above factors on cadmium concentrations varied significantly ($p \leq 0.05$) across different body sites (Table 2). The highest cadmium concentration was measured in Tuz Khurmatu (3677 ppm), while the lowest was measured in Dour (1823 ppm). When comparing the effects of meat type on liver, muscle, and kidney, liver had the highest cadmium concentration (2588 ppm) and kidney had the lowest (2183 ppm). Table 2 also show that when examining the effect of sex on cadmium exposure, no significant differences in cadmium concentrations were found between males and females ($p \leq 0.05$). Females had the highest and lowest concentrations in their livers (3203 ppm) compared to males.

The effects of the triple interaction of meat type (muscle, liver, kidney), location, and sex on the copper concentrations in muscle, liver, and kidney of goats of different sexes (male and female) in spring from three districts of Salahuddin Governorate (Table 3). All factors showed significant interactions ($p \leq 0.05$). Liver contributed significantly to the highest copper concentration in Tuz Khurmatu city ($p \leq 0.05$): male goats reached 19,970 ppm, while the lowest concentration was found in the liver of female goats (2,003 ppm). The different locations had a significant effect on the copper concentrations of the above factors ($p \leq 0.05$): the highest copper concentration was measured in Tuz Khurmatu town (10,986 ppm), and the lowest copper concentration was measured in Amarli (4,828 ppm). Table 3 shows the effects of copper on muscle, liver, and kidney. All meat types showed significant effects ($p \leq 0.05$). Cobalt concentrations were highest in the liver (7.459 ppm) and lowest in the kidney (5.986 ppm).

Table 3 show that sex (male and female) significantly affected copper concentrations when examining the effect of sex on copper exposure ($P \leq 0.05$). Male goats had the

highest cobalt concentration in their livers (10.501 ppm), while female goats had the lowest concentration in their livers (4.417 ppm). The effects of muscle, liver, kidney, production area, and sex-sex interaction in goats from three regions of Salahuddin Province. Male goats from Tuz Khurmatou had the highest cobalt concentration in their livers (8.167 ppm), while male goats from Tuz Khurmatovo had the lowest cobalt concentration in their muscle (0.753 ppm), compared to meat type, sex, and production area.

Significant effects of cobalt concentration were observed in some locations ($P \leq 0.05$), but not in others. The highest and most significant cobalt concentrations were detected in the liver of Tuz Khurmatu (6.522 ppm), while the lowest concentrations were detected in the muscle of Dhule (1.438 ppm) (Table 4).

The effect of sex on cobalt concentrations. Results show that male animals had the highest and most significant cobalt concentrations in the liver (3.643 ppm). Female animals had the lowest cobalt concentrations in muscle (1.925 ppm).

The effect of meat type on cobalt concentrations. Significant effects were observed in meat, liver, and kidney ($P \leq 0.05$). The highest cobalt concentrations were found in the liver (4.576 ppm), the lowest in muscle (2.221 ppm), and the lowest in kidney (3.077 ppm) (Table 4).

All results in our study were above internationally permitted limits. The high concentrations of all heavy metals may be due to heavy metal contamination of drinking water sources unsuitable for drinking, agriculture, or animal feed, where concentrations exceed permitted limits (Ibrahim, 2013). These results are consistent with those of Al-Pirkhdri (2018), who found significant effects of location and

Table 4: Cobalt contamination in Goat samples from three areas in east of Salahalddin province from (Male and Female) (mean $\pm$ standard error).

Tissue	Location	Female	Male	Overall Mean	Significance
Liver	Dour	1.762 $\pm$ 0.056	1.514 $\pm$ 0.064	2.010 $\pm$ 0.087	fg
Liver	Tuz Khurmatu	6.522 $\pm$ 0.258	4.877 $\pm$ 0.184	8.167 $\pm$ 0.399	a
Liver	Amerli	5.446 $\pm$ 0.195	3.179 $\pm$ 0.139	7.713 $\pm$ 0.199	a
Liver mean	—	4.576 $\pm$ 0.179	3.190 $\pm$ 0.250	5.963 $\pm$ 0.264	a
Muscle	Dour	1.438 $\pm$ 0.125	0.877 $\pm$ 0.083	2.000 $\pm$ 0.109	fg
Muscle	Tuz Khurmatu	2.314 $\pm$ 0.691	3.875 $\pm$ 0.296	0.753 $\pm$ 0.082	h
Muscle	Amerli	2.913 $\pm$ 0.234	1.025 $\pm$ 0.065	4.801 $\pm$ 0.252	d
Muscle mean	—	2.221 $\pm$ 0.272	1.925 $\pm$ 0.474	2.518 $\pm$ 0.245	d
Kidney	Dour	2.786 $\pm$ 0.180	2.283 $\pm$ 0.192	3.290 $\pm$ 0.221	e
Kidney	Tuz Khurmatu	3.348 $\pm$ 0.627	5.935 $\pm$ 0.370	0.761 $\pm$ 0.040	h
Kidney	Amerli	3.098 $\pm$ 0.213	2.281 $\pm$ 0.157	3.916 $\pm$ 0.210	c
Kidney mean	—	3.077 $\pm$ 0.225	3.499 $\pm$ 0.361	2.655 $\pm$ 0.259	c

The averages with identical letters are not significantly different ($p \leq 0.05$) between them.

meat type on heavy metal concentrations. Similar results were reported by Palani *et al.* (2020) regarding the influence of mineral elements on animal health and carcass quality. These observations support previous findings by Palani and Omer (2025), who also emphasized the physiological impacts of mineral and hormonal imbalances in livestock. Regarding the effect of meat type, liver had higher concentrations of lead, cadmium, copper, and cadmium than muscle. As our results suggest, the location effect also varied significantly across locations. These results contradict those of Oladipo and Okareh (2015), who found lead concentrations in Nigerian goat meat within the internationally acceptable range, with a lead concentration of 0.1 ppm.

These results are also consistent with those of Okoye and Ugwu (2010), who studied copper concentrations in muscle, liver, kidney, and heart of Nigerian goats. This study is also consistent with the findings of Akan *et al.* (2010), who found higher cobalt concentrations in liver and kidney than in muscle of Nigerian goats.

CONCLUSION

The present study highlights the presence of substantial metallic remains in the animal's meat of local black goats in the east of Salah al-Din Governorate, Iraq. The findings underscore the potential health risks associated with the consumption of contaminated meat, which could pose substantial apprehensions used for public condition also food safety in region. The study emphasizes the necessity for regular monitoring and strict regulation of environmental pollutants, particularly those that contribute to heavy metal contamination in livestock. Furthermore, these results call for heightened awareness among stakeholders, including local farmers, policymakers, and consumers, to mitigate the exposure of animals to contaminated feed, water, and grazing areas.

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NOVELTY STATEMENT

This study provides new insights into heavy metal concentrations in the meat of different livestock species in Iraq and assesses their potential health effects. The findings highlight regional variations and underscore

the importance of ongoing monitoring and food safety measures.

AUTHOR'S CONTRIBUTION

Bakshan Mo designed the study, collected and analyzed the samples, and wrote the manuscript. Co-authors also made additional contributions to data interpretation, statistical analysis, and critical review of the manuscript, as listed in the authors list. All authors reviewed and approved the final manuscript.

STATEMENT ON GENERATIVE ARTIFICIAL INTELLIGENCE AND ITS SUPPORTING TECHNOLOGIES

AI analysis of all parts of this manuscript revealed that the text was free of plagiarism, receiving a zero percent originality score. AI tools were used solely to assist with linguistic editing and formatting, without affecting the scientific content or the study's conclusions.

CONFLICT OF INTEREST

The authors have declared no conflict of interest regarding the publication of this manuscript.

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