



Impact of Environmental Pollutants (Bisphenol A and Tebuconazole) Associated with a High-Fat Diet on Oxidative Disorders and Intestinal Microbiota in Wistar Rats

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ABSTRACT

Background: The purpose of this study is to investigate the effects of the interaction between environmental pollutants and a high-fat diet on oxidative balance and intestinal microbiota composition. The research focuses on chronic exposure to low doses, equivalent to the acceptable daily intake (ADI), of bisphenol A (BPA), or tebuconazole (TEB), both incorporated into a high-fat diet.

Methods: Thirty female *Wistar* rats were allocated into six groups: Groups 1 and 2, which served as control groups, were not exposed to pollutants and were fed a standard diet (STD) and a high-fat diet (HFD), respectively. Groups 3 and 4 were exposed to bisphenol A (BPA), with group 3 receiving a standard diet (BPA) and group 4 a high-fat diet (BPAHFD). Groups 5 and 6 were exposed to tebuconazole, with group 5 receiving a standard diet (TEB) and group 6 a high-fat diet (TEBHFD), oxidative parameters in plasma and liver and microbial analyses were conducted.

Result: After 16 weeks of experimentation, the findings revealed a negative impact on the oxidative balance of rats on HFD combined with BPA or TEB, as evidenced by an 89% increase in hepatic malondialdehyde (MDA) in the TEBHFD group compared to the BPAHFD group, along with a 30% reduction in plasma reducing power and a 47% reduction in liver reducing power. Furthermore, this treatment altered the intestinal flora composition of exposed rats, resulting in a 5% increase in enterobacteria. To this end, these data are sufficiently compelling to implicate TEB, even at tolerable doses, in the development of health issues and the exacerbation of oxidative stress and intestinal dysbiosis. The effect of TEB appears to be more pronounced than that of BPA.

Key words: Bisphenol A, Chronic toxicity, Intestinal microbiota, Oxidative stress, Tebuconazole, *Wistar* rat.

INTRODUCTION

The rising global population and improved living standards are compelling nations to increase their agricultural investments, primarily by expanding arable land and ensuring high crop productivity. This strategy aims to strengthen global food security and self-reliance (Bessaoud *et al.*, 2019). A key component of this approach is the extensive application of pesticides, which are substances designed to eradicate pests, including fungi, animals or plants, during the production, storage, or sale of agricultural products. This practice facilitates the production of aesthetically flawless plant goods and boosts overall yield.

Developing and developed countries are responsible for 30% and 70% of pesticide usage, respectively (Bajwa and Sandhu, 2014). The widespread and often unregulated use of these substances poses significant risks to human health and the environment (Sahoo *et al.*, 2024). Many pesticides have been linked to the onset of chronic and sometimes fatal diseases including cancer (Kumar Sathish *et al.*, 2024). According to the World Health Organization, there are approximately 3 million cases of pesticide poisoning annually, resulting in up to 220,000 deaths, predominantly in developing countries (WHO, 1991). Among the pesticides used globally, tebuconazole, a systemic fungicide from the triazole family, is recognised

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for its impact on ecosystems and human health. It affects the gonads, thyroid and adrenal glands, functioning as an endocrine disruptor (Ying *et al.*, 2021) and altering the

synthesis and regulation of steroid hormones, particularly androgens and oestrogens (Dong, 2024).

Endocrine disruptors, over time, lead to developmental and reproductive disorders and hormone-dependent cancers in individuals and/or their descendants (Desbiolles and Gaillot, 2019). These substances are prevalent in everyday life, with bisphenol A, parabens and phthalates being the most recognised. Already linked to reduced male fertility and foetal development, endocrine disruptors are suspected of contributing to various conditions associated with metabolic disorders (Cander and Yetkin, 2023). These environmental pollutants, along with traditional risk factors like increased calorie intake due to an unbalanced diet and a sedentary lifestyle, play a crucial role in the progression of obesity and type 2 diabetes, further contributing to their increasing prevalence worldwide (Chevalier *et al.*, 2017). They can affect weight homeostasis and disrupt the mechanisms controlling abiogenesis and energy balance (González-Casanova *et al.*, 2020). They may also induce oxidative stress and intestinal dysbiosis (Ma *et al.*, 2023). Numerous studies have highlighted the harmful effects of these disruptors, particularly tebuconazole, at high doses (Bühler and Waeber, 2024). However, it appears that even at low doses, which are presumed to be safe for human health, they can have detrimental long-term effects.

To this end, this study aimed to compare the effect of chronic exposure to low doses, around the acceptable daily intake (ADI), of two environmental pollutants associated with a high-fat diet, namely bisphenol A (BPA) and tebuconazole (TEB), on oxidative balance and the composition of the intestinal microbiota in young female Wistar rats.

MATERIALS AND METHODS

Animal and experimental design

Young Wistar rats, aged 4 to 6 weeks and weighing between 90 and 115 g, were provided by Pasteur Institute Algiers (Algeria). After an acclimatisation period, the rats were divided into 6 groups (n=5), housed in a controlled environment at a constant temperature (25±2°C), while feed and water were provided *ad libitum*. All experiments were conducted in compliance with the guidelines of the Institutional Animal Protection Committee of the Algerian Higher Education and Scientific Research (agreement number 45/DGLPAG/DVA.SDA.14). For 16 weeks, the rats were fed either a standard diet (8.05% fat, 45.36% carbohydrate and 19.07% protein; 330.21 kcal/100g) or a high-fat diet (48% animal fat of ovine origin, 6.1% carbohydrate and 20% protein; 591.93 kcal/100g) and were exposed or not to two pollutants (BPA and TEB) at doses of 50 µg/kg/d and 30 µg/kg/d, respectively, administered orally and through drinking water provided in feeding bottles.

The six experimental groups were subject to the following dietary conditions:

- The first control group was provided with a standard diet (STD).

- The second group was administered a high-fat diet (HFD) along with drinking water.
- The third group received a standard diet and was exposed to bisphenol A (BPA) at a concentration of 50 µg/kg/d through drinking water.
- The fourth group (BPAHFD) was given a high-fat diet in combination with BPA exposure at 50 µg/kg/d.
- The fifth group (TEB) was subjected to a standard diet and exposed to TEB at 30 µg/kg/d.
- The sixth group (TEBHFD) received a high-fat diet and was exposed to TEB at 30 µg/kg/d.

It is important to note that throughout the 16 weeks of experimental period, daily assessments of food intake, water consumption and body weight were performed.

This study was conducted at the following laboratories:

- Laboratory for the improvement and valorization of Local Animal Productions, Ibn Khaldoun University- Tiaret, Algeria.
- Clinical Autopsy Laboratory, Institute of Veterinary Science, Ibn Khaldoun University- Tiaret, Algeria.

The study was initiated in January 2020 and the practical phase was completed in June 2022. The theoretical part and research writing commenced and were ultimately finalized in December 2024.

Blood collection

Following sacrifice, plasma was separated *via* centrifugation, aliquoted into tubes and stored at -20°C for subsequent analysis.

Preparation of tissue homogenate

The liver was excised, rinsed with a 0.9% NaCl solution and 0.2 g of the tissue homogenised in 2 ml of phosphate buffer at 24000 rpm using an Ultra-TURRAX electric grinder. The homogenate was then centrifuged at 5000 rpm for 15 min at 4°C and the supernatant was preserved at -20°C.

Oxidative parameter analysis in plasma and liver

Lipid peroxidation assessment

This analysis was conducted on both plasma and liver homogenate. A reaction mixture comprising thiobarbituric acid (0.375%), trichloroacetic acid (20%), 2,6-di-tert-butyl-4-methylphenol (0.01%) and hydrochloric acid (1 N) was incubated in a water bath at 100°C for 15 min, facilitating the release of malondialdehyde (MDA) functions. The MDA reacted with TBA, forming a pink-coloured complex. This reaction was halted by placing the tubes on ice and the complex was extracted with 1-butanol before centrifugation at 4000 rpm for 10 min at 4°C. The complex was measured at 532 nm with the MDA concentration determined using a calibration curve (Yagi, 1976).

Total antioxidant power determination using FRAP

The FRAP method, as developed by Benzie and Strain (1996), uses a solution prepared from three initial solutions: an acetate buffer at pH 3.6, 8 mM tripyridyl-s-triazine and 20 mM FeCl₆H₂O₆, all maintained at 37°C throughout the

analysis. A volume of 100 μL of diluted sample or blank was added to 900 μL of FRAP solution. Samples were analysed after a 30 min incubation by spectrometry with an absorption maximum at 593 nm.

Protein oxidation: evaluation of thiol groups

The assessment of protein oxidation was conducted following the method described by Faure and Lafond (1995). A volume of 375 μL of phosphate buffer (0.05 M with 1 μM EDTA) was mixed with 250 μL of either the sample or a standard solution prepared from 1 mM N-acetyl cysteine. Following gentle agitation, 125 μL of Ellman's reagent was added. The mixture was then vortexed and incubated for 15 min at room temperature, in the dark. Absorbance was measured at 412 nm.

Histopathological analysis

Liver samples were collected immediately post-sacrifice, rinsed with 0.9% NaCl and fixed in 10% formalin for histological examination. All liver tissues underwent staining with haematoxylin and eosin, as per the protocol outlined by Bancroft *et al.* (2008).

Effects of pollutants on intestinal microbiota composition

The enumeration of certain bacteria from the intestinal microbiota was performed in the faecal matter of rats. This technique involves dissolving 1 g of faecal matter in 9 mL of 0.9% physiological saline. From this, ten-fold dilutions were then prepared for each dilution and surface spreads were performed on MacConkey, Chapman and de Man Rogosa Sharpe agar for the enumeration of Enterobacteria, Staphylococci and *Lactobacillus* sp. respectively. All the Petri dishes were incubated at 37°C for 24 h. However, the incubation of *Lactobacillus* was carried out under anaerobic conditions (Liong and Shah, 2006). The bacterial counts were expressed as the $\log_{10}$ colony forming units (CFU/g) of faecal matter.

Statistical analysis

Results are presented as mean $\pm$ SEM (Standard error of the mean). Data analysis was performed using STATISTICA software (version 8.0). The comparison of means among the six groups of rats was conducted using the one-way

analysis of variance (ANOVA) test, followed by the Tukey test for pairwise comparison of means. A value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Oxidative parameters in plasma

Plasma MDA levels were significantly lower in the STD group compared with the HFD group, with values of $3.16 \pm 0.40 \mu\text{mol/L}$ and $4.31 \pm 0.33 \mu\text{mol/L}$, respectively. Conversely, a highly significant increase was observed in the BPAHFD and TEBHFD groups, as well as in the BPA and TEB groups, compared with the HFD group. A notable difference was also observed between the TEB and TEBHFD groups, with an increase of 52% and 27%, respectively (Table 1).

Plasma-reducing power was significantly reduced in the BPAHFD, TEBHFD, BPA and TEB groups compared to the HFD group. The plasma-reducing power was significantly different between the TEB and TEBHFD groups, with values of $542.63 \pm 77.83 \mu\text{mol/L}$ versus $393.82 \pm 18.71 \mu\text{mol/L}$.

The evaluation of protein oxidation in plasma revealed that protein thiol levels in the BPAHFD, BPA, TEBHFD and TEB groups were significantly lower than those of the HFD group.

Hepatic oxidative parameters

The HFD group exhibited elevated MDA levels compared to the STD group, with measurements of $0.0056 \pm 0.0013 \mu\text{mol/g}$ versus $0.0021 \pm 0.00026 \mu\text{mol/g}$. The TEBHFD group showed a 40% increase in oxidised lipid levels, while the BPA and TEB groups demonstrated increases of 100% and 20% respectively, relative to the HFD group. Furthermore, pollutants and HFD exerted a significant effect on reducing power. The TEBHFD and TEB groups experienced reductions in hepatic reducing power by 47.7% and 24.47%, respectively, compared to the HFD group. A notable decrease in hepatic reducing power was observed with combined exposure to TEB and the HFD, as opposed to TEB alone ($6.96 \pm 0.94 \mu\text{mol/g}$ versus $10.06 \pm 0.74 \mu\text{mol/g}$).

Protein levels in the BPAHFD, BPA, TEBHFD and TEB groups were lower than those in the HFD group, with reductions of 5.39%, 7.55%, 25.21% and 10.33%, respectively. Additionally, in the TEBHFD group, protein concentrations

Table 1: Effect of exposure to pollutants on plasma oxidative parameters.

	STD	HFD	TEB	BPA	BPAHFD	TEBHFD
TBARS ($\mu\text{mol/L}$)	$3.16 \pm 0.40^{**}$	4.31 ± 0.33	$6.69 \pm 0.36^{***}$	$6.31 \pm 0.23^{***}$	$5.52 \pm 0.26^{**}$	$5.30 \pm 0.18^{**}$
FRAP ($\mu\text{mol/L}$)	$787.88 \pm 25.4^{**}$	674.57 ± 34.6	$542.6 \pm 77.8^{***}$	$533.3 \pm 27.2^{**}$	$566.54 \pm 36.32^{**}$	$393.82 \pm 18.7^{**}$
Thiol ($\mu\text{mol/L}$)	$949.26 \pm 66.2^{***}$	723.7 ± 11.24	$664.94 \pm 58.61^{**}$	$479.56 \pm 18.4^{***}$	$504.71 \pm 16.7^{***}$	$518.60 \pm 50.2^{***}$

The results are expressed as means $\pm$ SE (n=5). *** $P < 0.001^{**}$ $P < 0.01$ * $P < 0.05$ Significant difference compared to HFD.

Table 2: Effect of exposure to pollutants on liver tissue oxidative parameters.

	STD	HFD	TEB	BPA	BPAHFD	TEBHFD
TBARS ($\mu\text{mol/g}$)	$0.002 \pm 0.0002^{**}$	0.005 ± 0.001	0.006 ± 0.0009	$0.01 \pm 0.002^{***}$	0.003 ± 0.0007	$0.007 \pm 0.0007^{*}$
FRAP ($\mu\text{mol/g}$)	$19.07 \pm 3.6^{**}$	13.31 ± 5.55	10.06 ± 0.74	$18.67 \pm 3.24^{**}$	13.22 ± 3.03	$6.96 \pm 0.94^{*}$
Thiol ($\mu\text{mol/g}$)	$23.85 \pm 5.15^{**}$	$22.25 \pm 1.02^{*}$	19.95 ± 1.11	20.57 ± 2.6	21.05 ± 1.33	$16.64 \pm 2.81^{*}$

The results are expressed as means $\pm$ SE (n=5). ** $P < 0.01$ * $P < 0.05$ Significant difference compared to HFD.

of the thiol groups were lower than those of the BPAHFD group ($16.64 \pm 2.81 \mu\text{mol/g}$ versus $21.05 \pm 1.33 \mu\text{mol/g}$) (Table 2).

Histopathological study

Exposure to BPA or TEB resulted in vacuolated hepatocytes and degeneration, characterised by necrosis and sinusoidal dilation. Macrovesicular steatosis, accompanied by inflammatory cell infiltration around the vessels and an increased number of Kupffer cells, was observed in the BPAHFD and TEBHFD groups. Centrilobular hepatocyte necrosis was also noted in these groups (Fig 1).

Exposure to BPA or TEB resulted in centrilobular hepatocytic degeneration (characterized by vacuolation), necrosis, as well as sinusoidal dilation. Macrovesicular steatosis was observed in the BPAHFD and TEBHFD groups, accompanied by an increased number of Kupffer cells and inflammatory cell infiltrates. These groups also showed evidence of centrilobular hepatocyte necrosis (Fig 1).

Intestinal flora composition

The results reveal a significant increase in *Enterobacteria* numbers in rats exposed to the HFD combined with TEB or BPA, compared to the HFD group ($6.21 \pm 0.08 \text{ Log}_{10} \text{ CFU/g}$; $5.91 \pm 0.09 \text{ Log}_{10} \text{ CFU/g}$ versus $5.48 \pm 0.04 \text{ Log}_{10} \text{ CFU/g}$). A significant difference was also observed between the TEB and TEBHFD groups, with a 14% increase of *Enterobacteria* and between BPA and BPAHFD groups, with a 11%, respectively.

A notable disparity was detected between the TEB and TEBHFD groups with 14% rise in *Enterobacteria* and between the BPA and BPAHFD groups with an 11% increase.

The staphylococcal count results indicate that TEB exposure led to a significant increase, estimated at 17% in

the TEB group compared to the BPA group and 23% compared to the STD group (Fig 2).

On the other hand, a significant reduction in *Lactobacillus* numbers was observed in the BPAHFD, TEBHFD and TEB groups as compared with the HFD group. Furthermore, a marked difference was detected between the BPAHFD and TEBHFD groups (5.21 ± 0.13 versus $3.62 \pm 0.15 \text{ Log}_{10} \text{ CFU/g}$) (Fig 3).

During our study, female rats were preferentially selected as they represent a particularly sensitive target to endocrine disruptors, according to Robert Barouki, Director of Research at the French National Institute for Health and Medical Research (Inserm). The majority of endocrine disruptors can imitate oestrogens and thus attach to their receptors.

The results reveal that the exposure to BPA or TEB, in conjunction with a HFD, leads to oxidative imbalance in plasma and liver tissues. Research has implicated cytochrome P450 in the bioactivation of these pollutants, particularly TEB, through increased radical production (Dong *et al.*, 2023). In a murine study, Ma *et al.* (2023) demonstrated that TEB exposure caused lipid peroxidation, evidenced by elevated hepatic MDA levels. Ku *et al.* (2021) corroborated these findings in C57BL/6 mice, highlighting TEB's hepatotoxicity, notably through oxidative stress induction and a decrease in hepatic antioxidant defence enzymes, such as SOD, catalase and GSH. The study concludes that the accumulation of reactive oxygen species (ROS) activates pro-inflammatory cytokines (TNF- α , IL-6) and disrupts hepatic metabolism by affecting several processes: β -oxidation of fatty acids, promoting hepatic steatosis; glycolysis and gluconeogenesis, disrupting the

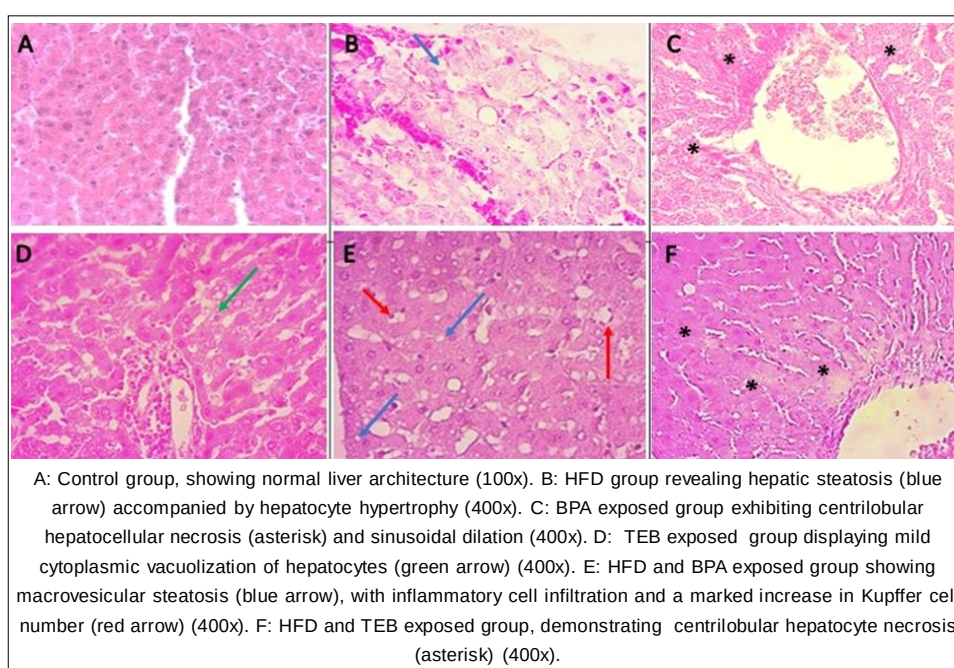


Fig 1: Photomicrograph of female rat liver (H and E).

liver's energy balance and lipid metabolism, leading to excessive lipid accumulation (Goudarzi *et al.*, 2022).

Othmène *et al.* (2021) reported that TEB induces excessive ROS production in H9C2 cardiac cells, contributing to DNA damage, genotoxicity and apoptosis in a dose-dependent manner. This process is mediated by the activation of a caspase cascade, particularly caspases 3 and 9 and by altering the Bax/Bcl-2 ratio. The accumulation of ROS results in a reduction in mitochondrial membrane potential, thereby disrupting the cell cycle. BPA appears to operate through a similar mechanism, as observed in TM3 cells (Zhang *et al.*, 2023). Kamel *et al.* (2018) found that rats administered a high dose of BPA exhibited steatosis with inflammatory cell infiltration, sinusoid dilatation and increased Kupffer cells. Alyasiri *et al.* (2025) conclude that in male rats, BPA impairs hepatic function.

Conversely, exposure of rats to TEB or BPA, either alone or in combination with HFD, alters the intestinal microbiota composition. Specifically, there was a notable increase in enterobacteria (proteobacteria) in the exposed groups compared to the control. Liu *et al.* (2021) demonstrated

that TEB exposure in rats disrupts the intestinal barrier, following an increase in enterobacteria and lipopolysaccharides (LPS). As noted by Shi *et al.* (2019), low-grade inflammation is caused by significant translocation of LPS into the bloodstream; a phenomenon considered a key factor in the amplification of several chronic pathologies, including cardiovascular diseases and metabolic disorders.

Consistent with the findings presented, extensive research has verified the influence of TEB and BPA on the composition of the intestinal microbiota and related inflammatory processes. Meng *et al.* (2022) illustrated that TEB exposure in mice led to modifications in the intestinal microbiota, characterised by structural damage and infiltration of inflammatory cells in colon tissue. Likewise, Ma *et al.* (2023) found that BPA exposure disrupts the intestinal microbiota, leading to an increase in proteobacteria and LPS levels, triggering the TLR4 pathway, inducing intestinal inflammation. These outcomes align with those of Lai *et al.* (2016), who observed that mice exposed to BPA and fed a high-fat diet exhibited changes in the intestinal microbiota composition. Further research by Moreno *et al.*

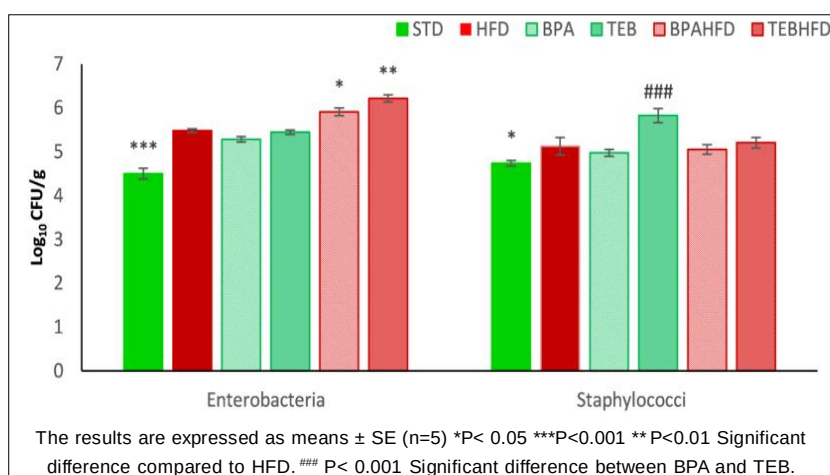


Fig 2 : Effect of exposure to pollutants on the number of Enterobacteria and Staphylococci.

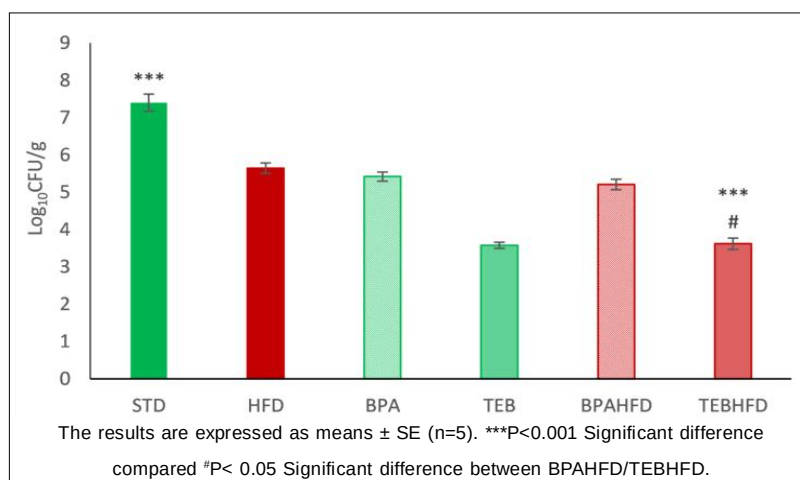


Fig 3: Effect of exposure to pollutants on the number of *Lactobacillus* sp.

(2024) identified a predominance of *Staphylococcus*, *Escherichia* and *Clostridium* in the intestinal microbiota of obese children exposed to BPA. These observations are consistent with the current study, which detected *Staphylococcus* and *Escherichia* in the presence of BPA.

CONCLUSION

The outcome of this study indicates that TEB, whether administered alone or in conjunction with a HFD, exerted a more pronounced effect on oxidative parameters and the composition of the intestinal microbiota, compared to BPA. The results underscore that the intensity of the observed effects is related to the type of pollutant, its propensity to accumulate in adipose tissue and the associated dietary conditions.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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