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Theoretical docking analysis for a multi-scale experimental investigation of biochar filtration of olive mill effluents: Unraveling genotoxicity and polyphenol-DNA interactions

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ABSTRACT

Olive mill wastewater (OMWW) is a major by-product of olive oil production and a serious environmental pollutant due to its high organic and phenolic content, which can harm soil, water, and biodiversity. In this study, we aimed to evaluate the effectiveness of olive-stone-derived biochar as a sustainable filtration method to reduce both phytotoxicity and genotoxicity of OMWW. The raw effluent (pH = 4.8, COD = 65.15 g/L, total phenolics = 6.8 g/L) was filtered using biochar pyrolyzed at 585°C for 90 min. To evaluate the effect of the treatment, a micronucleus assay was performed on Vicia faba root cells to assess genotoxicity, complemented by phytotoxicity tests using radish and lettuce seeds. Five concentrations of treated OMWW (25–100 % v/v), raw OMWW, distilled water (negative control), and maleic hydrazide (positive control) were tested in triplicate. The biochar treatment significantly reduced phytotoxicity, with germination rates exceeding 80 % and germination indices above 100 % in treated samples, indicating improved seed germination and seedling vigor compared to untreated OMWW. Genotoxicity was significantly decreased, with micronucleus frequency dropping from 15.2 % in raw OMWW to below 2 % after biochar filtration. Molecular docking revealed strong binding of key OMWW polyphenols to the DNA double helix (binding energies ranging from −4.39 to −8.96 kcal/mol), with quercetin exhibiting the highest affinity. These interactions likely induce structural alterations in DNA, providing a molecular basis for the genotoxicity observed experimentally. The reduction in genotoxic and phytotoxic effects observed after treatment indicates that biochar likely adsorbs bioactive pollutants, reducing their availability and capacity to induce harmful biological responses. To the best of our knowledge, this is one of the first studies to combine phytotoxicity, genotoxicity, and molecular modeling approaches to assess the detoxification of olive mill wastewater (OMWW). These findings highlight the potential of olive-stone-derived biochar as a low-cost, eco-friendly solution for the sustainable management of olive mill effluents. Future large-scale applications should focus on optimizing the biochar dosage and ensuring the sustainability of the treatment system.

1. Introduction

Olive oil production in Mediterranean countries accounts for over 96 % of the global olive oil supply, making it the principal agricultural activity in these regions (Kovačević et al., 2022). The olive oil production industry generates a significant amount of by-products, including olive mill wastewater (OMWW), olive pomace, olive stones, olive

sludge, and olive leaves (Boutafda et al., 2020). OMWW, representing the liquid phase, is considered one of the most challenging pollutants and the primary environmental issue in the Mediterranean countries (Haddad et al., 2017; El-Fels et al., 2019). The greatest volume of OMWW is estimated at 5.4 million m³, which is produced over a short period, from October to March (Hodaifa et al., 2019; Fakhfakh et al., 2024).

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In Morocco, the annual generation of olive mill wastewater (OMWW) is estimated to reach approximately 180,000 m³ during the olive oil production season (El Moudden et al., 2022). Currently, increasing concern is being raised regarding the management of this waste (S'habou et al., 2009). Typically, OMWW is pumped and stored into evaporation ponds, leaving solid residues behind, or it is either directly discharged into rivers or spread on soil (Dehmani et al., 2020). OMWW represents a hardly treated effluent containing high levels of toxic organic chemical substances (Boutafda et al., 2019, 2023). Its composition is mainly characterized by elevated concentrations of polyphenolic compounds (1–80 g/L) and a marked acidity (pH < 5). The inorganic fraction is dominated by substantial amounts of mineral salts, particularly magnesium, potassium, and phosphates (Morillo et al., 2009). In addition, OMWW contains a heterogeneous organic fraction composed of sugars, nitrogenous compounds such as proteins and amino acids, as well as low-molecular-weight organic acids, including acetic, citric, malic, and oxalic acids (Stasinakis et al., 2008), which together contribute to its high organic load. As a result, chemical oxygen demand (COD) values typically range from 50 to 150 g O₂/L, while biological oxygen demand (BOD₅) values fall between 10 and 60 g O₂/L (McNamara et al., 2008; Lissaneddine et al., 2021). Moreover, the physicochemical composition of OMWW is highly variable and influenced by multiple factors, including geographic and climatic conditions, the olive cultivar, the degree of fruit ripeness, the extraction technique employed (traditional or modern), and the duration and conditions of storage (Regni et al., 2017; Hachicha et al., 2009). The disposal of OMWW poses significant environmental risks impacting the soil's physical and chemical properties, and potentially leading to soil degradation (Karaouzas et al., 2011), water pollution (Aggelis et al., 2003), disruption of microbial life due to its high organic content (El-Abbassi et al., 2017), and inhibiting plant seed germination because of the phytotoxic activity of phenolic compounds. Paixao et al. (1999) confirm that many OMWW constituents have been shown to cause physiological alteration at both the organism and cellular levels.

These considerations have prompted several researchers to develop treatment processes for OMWW, aiming to reduce its toxicity and limit its secondary effects (Danellakis et al., 2011; Gwenzi et al., 2017), particularly when large amounts of OMWW need to be managed. Various pretreatment techniques have been developed, many of which use microorganisms as part of a biological process (Boutafda et al., 2023a). Chemical methods have also been explored; for example, Demirci et al. (2024) used amine-functionalised halloysite nanotubes to remove over 70 % of hydroxytyrosol, tyrosol, and oleuropein from OMWW samples. Physicochemical treatment systems, such as those described by Enaime et al. (2020a), have also been employed to mitigate the environmental impact of OMWW (Micoli et al., 2023). Research conducted on the treatment of OMWW has investigated the treatment of OMWW by physicochemical processes such as ozonation (Martins et al., 2015), distillation, ultrafiltration using different membrane types (Raza et al., 2019), advanced oxidation processes (AOPs), especially heterogeneous photocatalysis, which have thus gained attention for their ability to degrade recalcitrant pollutants such as chlorophenols and pathogenic bacteria. For instance, Li-doped Zn_{0.8}Ni_{0.2}Fe₂O₄ (Li/ZNF) photocatalyst synthesised via co-precipitation has been shown to effectively degrade 2,4,5-trichlorophenol (TCP) and exhibit notable antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* when exposed to UV light (Mustafa et al., 2022; Martins et al., 2021). A hybrid CuCr₂O₄/CaFe-LDO-based photocatalytic process activated by sunlight and combined with an oxidant (K₂S₂O₈) was developed by Oladipo et al. (2021) to effectively remove organic compounds and phenols from highly concentrated olive mill effluents. In addition, Elayadi et al. (2021) recently demonstrated the effective use of activated carbon derived from olive pomace, encapsulated in alginate beads, for phenolic compound removal from OMWW. Their system exhibited a high adsorption capacity (68 mg g⁻¹) and good regeneration efficiency (>40 % desorption). Although these methods yielded promising results,

their application is limited by the high cost of solvents and energy (Ali et al., 2012; De Gisi et al., 2016; Girish and Ramachandra Murty, 2012). Biological treatment is considered more environmentally friendly and cost-effective than physico-chemical methods (Borja et al., 1997; McNamara et al., 2008). For example, Micoli et al. (2023) have successfully implemented an anaerobic digestion process on OMWW to produce biogas. Nevertheless, the biological solution is often hindered by the presence of inhibitors or toxic chemicals, such as polyphenols and fatty acids, which impact the activity of microorganisms and make this treatment difficult to achieve (Borja et al., 1997; Hamdi, 1992; Morillo et al., 2009). To reduce the toxicity of OMWW and lower polyphenol content, the use of biochar could be a sustainable and economical alternative (Hasnain et al., 2023). Waste biomass such as rice straw, bagasse, bamboo, poplar wood, and olive stone (Li et al., 2022; Rabichi et al., 2024) was pyrolyzed under limited oxygen levels (Cantrell et al., 2012). Biochar can adsorb a wide range of contaminants, including organic pollutants, through various mechanisms such as electrostatic interactions and van der Waals forces, and hydrogen bonding (Gwenzi et al., 2017). The sorption capacity of biochar depends on raw materials, pyrolysis temperature, and residence time (Li et al., 2020). This versatility enables biochar to effectively remove pollutant compounds from water (Achak et al., 2009; Senol et al., 2017). Kizito et al. (2019) demonstrated that biochar serves as an efficient catechol sorbent, while Yakout et al. (2015) reported that biochar produced from rice straw at various pyrolysis temperatures effectively removed phenols from industrial effluents. Micoli et al. (2023) found that adding biochar before or during the anaerobic digestion process reduced the phenolic compounds, COD, and phytotoxicity of OMWW. Moreover, Oladipo and Ifebajo (2018) demonstrated that magnetic chicken bone biochar efficiently removed tetracycline and dyes, supporting its potential as a reusable filtration material. The biochar-based treatment of OMWW converts the effluent into a beneficial organic amendment that contributes to the improvement of soil physical, chemical, and biological properties, ultimately enhancing soil fertility (Gwenzi et al., 2017). While this approach is promising, assessing its safety and environmental impact through genotoxicity testing remains essential. To reach this objective *Vicia faba* has been used evaluating chromosomal aberrations, this plant species is a valuable bioindicator in genetic studies due to its large chromosomes and sensitivity to various environmental stressors (El Fels et al., 2015), in addition the detection of micronucleus in *Vicia faba* root tips has been more recently developed to assess the genotoxicity in some different matrices as soil, water, waste-water and industrial effluent (El Hajjouji et al., 2007; Zegzouti et al., 2020), currently There are no studies that evaluate the genotoxicity or phytotoxicity of treated (OMWW) when biochar is used as an adsorbent. In the present paper, lettuce and radish were selected as sensitive species to assess the effects of various pollutants and evaluate their potential phytotoxicity. The effect of raw and treated OMWW. Molecular docking studies were also conducted to assess the probable DNA damage caused by the different phenolic compounds present in OMWW, and the *Vicia faba* root micronucleus was used to assess genotoxicity induced by different concentrations of raw and treated OMWW to investigate the efficacy of the biochar filtration system in removing pollutant compounds.

2. Materials and methods

2.1. Study area and sampling

The olive mill wastewater (OMWW) used in this study was sourced from an olive oil production plant employing a three-phase system. This plant is located in Tagart village, Touama, Al Haouz province. The effluent samples were stored at -20 °C before analysis.

2.2. Biochar preparation and characterization

The biochar was produced from olive stones by pyrolysis, using a

Table 1
Physicochemical and surface structures of the biochar.

Parameters	Unit	Biochar
pH	-	8.8 ± 0.04
electrical conductivity	μS	1202.5 ± 0.5
Ash content	%	4.89
H	%	2.12
C	%	83.70
N	%	0.80
O	%	8.65
Fe	%	0.11
Cu	%	0.13
Zn	%	0.00
Na	%	0.06
Mg	%	0.26
P	%	0.53
Cl	%	0.37
K	%	1.82
Ca	%	1.31
Mn	%	2.26
BET surface area (m ² g ⁻¹)	m ² g ⁻¹	258.72
Micropore surface area (m ² g ⁻¹)	m ² g ⁻¹	115.58
Mesopore surface area (m ² g ⁻¹)	m ² g ⁻¹	143.14

Table 2
Physico-chemical characteristics of olive mill wastewater (OMWW) before and after biochar filtration.

Parameters	Unit	Raw OMWW	Treated OMWW
EC (mS/cm)	(mS/cm)	13.90 ± 1.2a	1.89 ± 0.9b
pH	-	4.80 ± 0.03a	7.05 ± 0.05b
TS	(g/L)	120.13 ± 0.80a	ND
TSS	(g/L)	12.98 ± 0.20a	ND
COD	(g/L)	65.15 ± 1.02a	4.7 ± 0.05b
TPC	(g/L)	6.80 ± 0.10a	0.77 ± 0.01b
Total organic matter	(g/L)	64 ± 0.75a	9.88 ± 0.05b

TSS: Total suspended solids; TS: total solids; EC: electrical conductivity; COD: Chemical Oxygen Demand; TPC: total phenol compounds; ND: not detected (LOD for TS = 4 mg/L, and TSS = 3 mg/L). Values with different letters in the same line are significantly different

heating rate of approximately 10 °C/min until 585 °C was reached and maintained for 90 min. pH was determined according to the method used by Hmid et al. (2014). Ash content was determined following the method described by Peng et al. (2011). Fourier transform infrared spectroscopy (FTIR) was employed to determine the functional groups present on the surface of the biochars were recorded in the 400–4000 cm⁻¹ range using a Bruker VERTEX 70 FTIR spectrophotometer. elemental analysis (C, H, N, O, and S) was performed using a Euro EA-CHNSO Elemental Analyzer, the morphology of the biochar was carefully examined using a field emission scanning electron microscope, in particular the VEGA3 LMU model (Tescan) / Energy Dispersive X-ray Spectroscopy (EDX) was performed to determine the elemental composition of the biochar samples. BET specific surface area, and pore size distribution of the biochar were measured by desorption experiments at −196 °C using a Micromeritics (Norcross, GA, USA) adsorption analyser model 3Flex, the T-plot method was applied to estimate the micropore external surface area.

2.3. OMWW physicochemical characterization

Raw and filtered OMWW samples were analyzed for the following parameters reported in Table 2. The pH and electrical conductivity were measured at ambient temperature using a multiparameter probe (Hanna HI 9829) and a pH/ORP meter (Model 920), following the methodology recommended by Rodier (1971). Total suspended solids (TSS) were determined by filtering a sample of OMWW through a Whatman nylon membrane filter (0.45 μm pore size, 47 mm diameter), followed by drying the retained residue at 105 °C until a constant weight was

achieved. Phenolic compounds were extracted according to the method of Macheix (1996), using 80 % methanol in the presence of phosphoric acid and ammonium sulfate. The phenols were measured with the Folin-Ciocalteu reagent (Vasquez Roncero et al., 1974). The chemical oxygen demand (COD) was established according to Rodier (1987). According to the APHA standard method, Total solids (TS) were determined by oven-drying 10 mL of the sample at 105 °C for 24 h. Total ash content was assessed by the incineration of TS at 600 °C for 6 h. Dry matter was measured after drying the OMWW at 105 °C for 24 h. Organic matter content was assessed by measuring the loss-on-ignition at 550 °C for 24 h.

2.4. Two-step biochar filtration process for olive mill wastewater treatment

The treatment of olive mill wastewater (OMWW) involves a two-step process utilizing biochar to maximize the removal of contaminants, with a particular focus on polyphenols. The adopted OMWW treatment process is illustrated in Fig. 1.

Biochar samples with two different particle size ranges were prepared through mechanical crushing and sieving. The first fraction consisted of particles ranging from 1 to 2 mm, while the second fraction included finer particles smaller than 0.5 mm.

Dynamic filtration: Initially, OMWW undergoes dynamic filtration as it continuously flows through a column packed with biochar. This step removes a portion of suspended solids and organic compounds, reducing the polyphenol content from 6.8 g/L in raw OMWW to 2 g/L in the pretreated OMWW. During the first filtration cycle, a working volume of 2 L of raw OMWW was passed through the olive stone biochar filter using a Pharmacia Biotech peristaltic pump P-1 at a flow rate of 1 mL/min. The partially treated effluent from this step is then subjected to a second phase.

Static filtration: The pretreated OMWW was subsequently mixed with fine biochar (particle size < 0.5 mm) at a concentration of 30 g/100 mL in a batch system, allowing extended contact time under continuous agitation. This dosage was selected based on a series of preliminary tests conducted with various biochar concentrations. Among the tested concentrations, this dosage demonstrated the most effective adsorption performance. The effluent was then centrifuged to recover only the liquid phase, which consists of the treated OMWW. This static phase targets the remaining polyphenols, achieving near-complete removal.

This combined approach leverages the efficiency of dynamic filtration for initial pollutant reduction and static filtration for thorough polyphenol elimination.

2.5. Germination test

A toxicity test was performed to assess the effect of irrigation with treated OMWW on the germination of radish and lettuce, following the ISO (1995) standard. The germination test conducted as follows: seeds of radish (*Raphanus sativus*) and lettuce (*Lactuca sativa*) were disinfected with 3 % sodium hypochlorite for 5 min then rinsed five times with distilled water, 20 seeds for each plant are placed on Whatman filter in a sterilized 9 cm Petri dish, then watered with 5 mL of effluents from the different treatments. The Petri dishes were incubated in darkness at 25 °C for 24 h to observe seed emergence and root growth. The number of germinated seeds was monitored for 72 h (Charles et al., 2011). Three replicates were made for each treatment. Seed germination and root length of plants moistened with deionized water were also measured and used as the control under the same conditions.

The phytotoxicity was assessed using germination rate (GR), which was calculated using Eq.1:

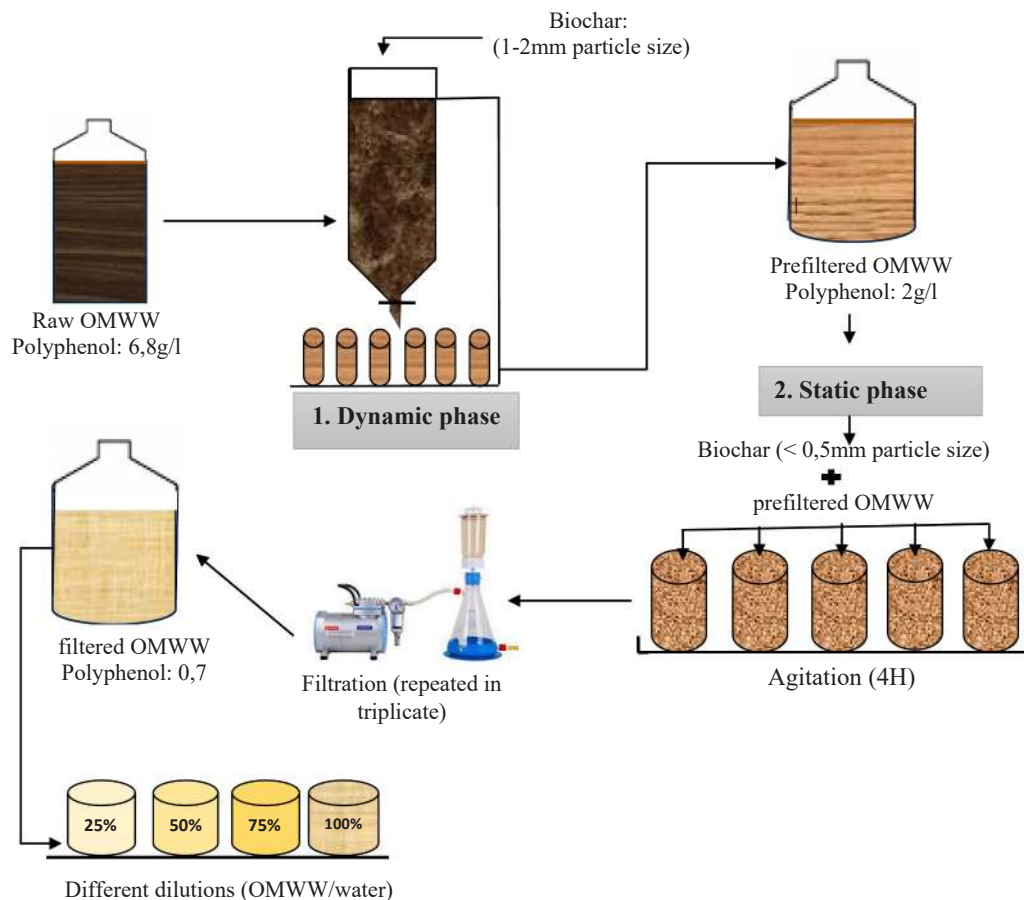


Fig. 1. Schematic diagram illustrating the Two-Step Biochar Filtration Process of OMWW.

$$GR = GSs / GSc \times 100 \quad (Eq.1)$$

With: GSs: Number of germinated seeds in the OMWW
GSc: Number of germinated seeds in the control

Germination index (GI), which measures both seed germination and root development, was used to assess if a medium contains detrimental substances for seed germination or growth of the radicle (Rusan et al., 2015) based on the formula below Eq.2:

$$GI = (NSs \times LR_s) / (NSc \times LR_c) \times 100 \quad (Eq.2)$$

NSs, NSc are the number of germinated seeds in olive mill wastewater and the distilled water, respectively

LRs, LRc are the root length in the treated and untreated olive mill wastewater and the distilled water, respectively

2.6. OMWW genotoxicity analysis

2.6.1. *Vicia faba* micronucleus test

To assess the toxicity of OMWW on plant health, a micronucleus test was performed on *Vicia faba* seeds to reveal the genotoxic effects of both raw and treated OMWW as described in Fig. 2. The micronucleus frequency test was conducted according to (AFNOR, 2013; Knasmüller et al., 1998; Ma et al., 2014; El Fels et al., 2015; Boutafda et al., 2023). Seeds of *Vicia faba* stored at 4°C were used for the micronucleus test. The dry *Vicia faba* seeds were disinfected with calcium hypochlorite Ca (ClO)₂ 1 % for 10 min and rinsed five times, then soaked in tap water for 12 h, after which the seed coats were removed to promote germination. The seeds were allowed to germinate between two layers of moist cotton for 4 days at 25°C. Once the primary roots reached 3–5 cm in length, their tips were cut to promote the growth of secondary roots. These germinated seedlings were selected as the starting material for the micronucleus test. Five different concentrations of OMWW were tested

(25, 50, 75, 100 % v/v of treated OMWW as well as raw OMWW). Maleic hydrazide (MH) (10 – 5 M) and water were used as positive and negative controls, respectively.

Primary roots of germinated seedlings were exposed directly to the five different concentration of OMWW effluent (direct exposure), while the other Seeds were sown in sterilized standard soil (pH=7.55 ± 0.1; CE=417 ± 2; TOC=1.07 %±0.25; TNK=0.13 ± 0.008 %; PT=0.432 mg/kg±0.77) and soaked with five different concentrations of OMWW. Moisture content was consistently maintained at two-thirds of the water holding capacity (solid matrix). Five independent replicates were made in each treatment of both experimental groups, and samples were kept at 25°C ± 1.

2.6.2. Observation and quantification of micronuclei

Following all treatments, the secondary root tips were harvested and fixed in Carnoy's solution (glacial acetic acid/ethanol 1/3; v/v), at 4°C overnight, then washed thrice with deionized water, and transferred to 70 % ethanol for storage. Subsequently, the root tips underwent hydrolysis in 1 N hypochlorite acid at 58°C for 5 min (El Fels et al., 2015). The root tips were stained with 1 % aceto-orcin and then placed on a slide with a cover slip after being squashed to evenly spread the dividing cells. The frequency of micronuclei (MN) was expressed in ‰, using Eq. 3

$$MN (\text{‰}) = (\text{Number of micronucleated cells} / \text{Total number of observed cells}) \times 1000 \quad (Eq.3)$$

while the mitotic index (MI) was expressed as a percentage (%), by applying Eq.4

$$MI (\%) = (\text{Number of mitotic cells} / \text{Total number of cells}) \times 100 (Eq.4)$$

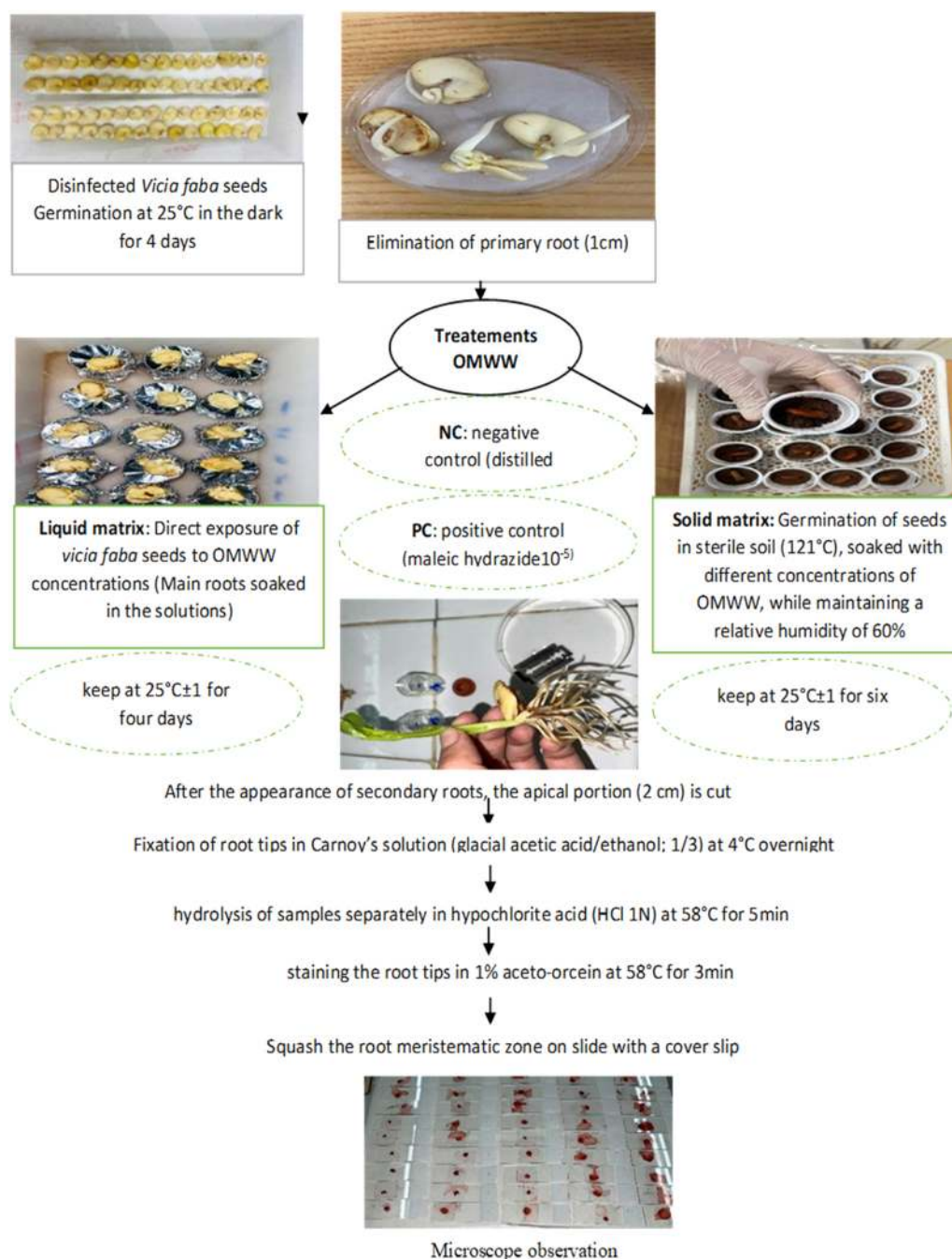


Fig. 2. Experimental design for micronuclei assessment in *Vicia faba* roots during liquid and solid exposure.

Both parameters were determined from a minimum of six thousand cells per treatment (Zegzouti et al., 2020). To avoid underestimation of micronuclei due to impaired cell proliferation rate, the micronuclei test was performed only in root tips with an MI greater than 2 % (AFNOR, 2003), and a significant response of the positive control was observed. The percentage reduction in micronucleus frequency was calculated by comparing the values before and after treatment applying Eq. 5

$$\text{MN removal (\%)} = \frac{[\text{MN}_{\text{raw}} - \text{MN}_{\text{treated}}]}{\text{MN}_{\text{raw}}} \times 100 \quad (\text{Eq. 5})$$

2.7. Molecular docking studies

Computational docking studies were carried out using AutoDock 4.2.

The X-ray crystallographic structure of the DNA double helix was obtained from the RCSB Protein Data Bank (RCSB PDB) with ID: 355D. The Target was prepared by deleting water molecules to ensure that the receptor was free of any ligands before docking. Polar hydrogens, Kollman charges, and Gasteiger charges were then added, and the protein was saved in the PDBQT format. The structures of the ligands were created separately using ChemDraw Ultra 12.0, the energy was minimized in Chem3D, and the torsional bonds of the ligands were set flexibly and saved in the PDBQT format. Next, the receptor was kept rigid, and the grid map was built (Grid parameters: X: 62, Y: 74, Z: 108 with 0.375 spacing and centered at X: 14.730, Y: 20.967, Z: 8.211). The best conformers were searched using the Lamarckian genetic algorithm (LGA), with a population size set to 150 and the maximum number of energy evaluations set to 25,000,000. Finally, the results were analyzed

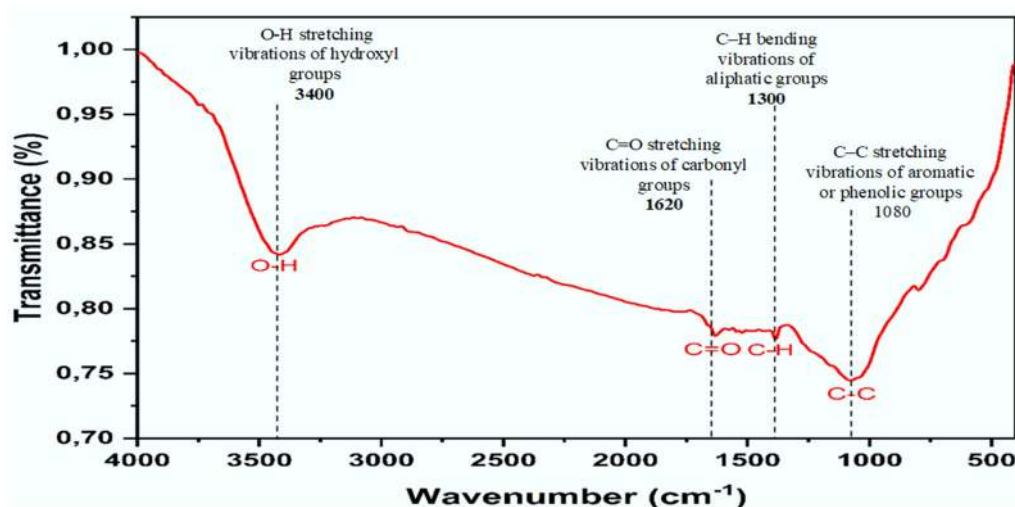


Fig. 3. FTIR spectrum of biochar.

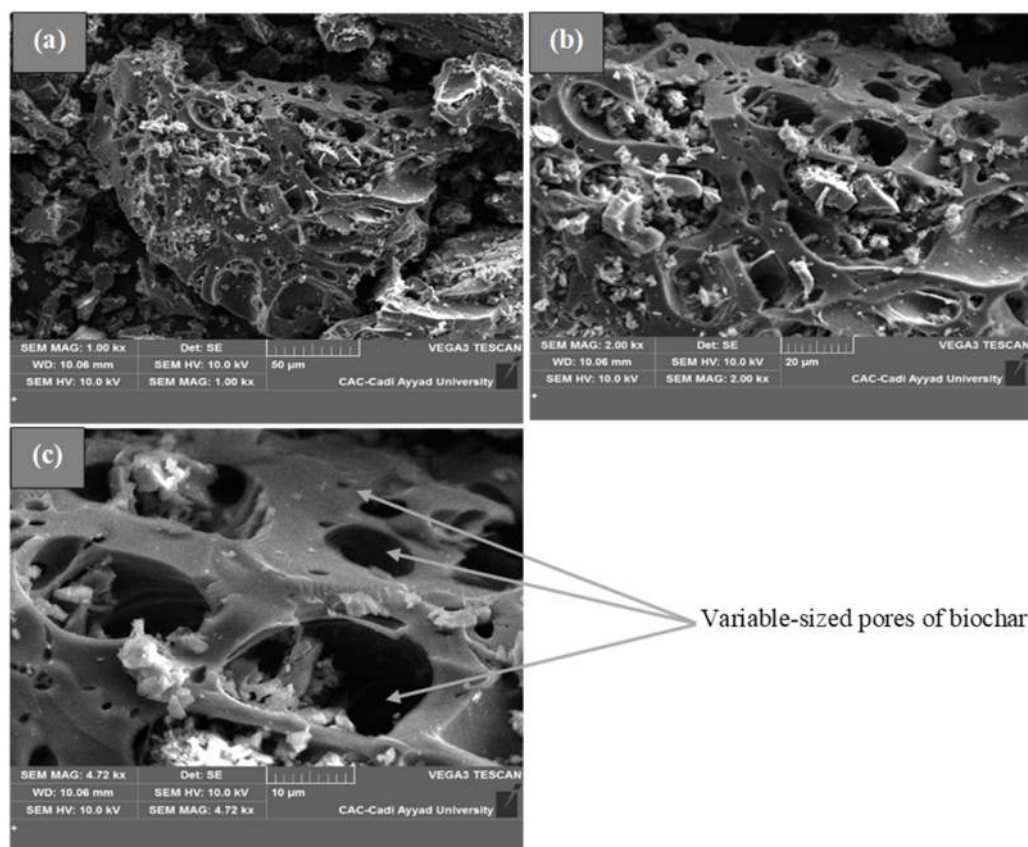


Fig. 4. Secondary electron SEM images of the biochar samples displayed in different magnifications (a) 50 μm , (b) 20 μm , and (c) 10 μm .

and visualized using Discovery Studio.

2.8. Statistical analysis

All the physicochemical analyses were presented by means $\pm$ S.E.M of three replicates. For the genotoxicity and phytotoxicity test, the comparison of the averages was made by the Tukey Test, the data are presented with standard deviation, and the values followed by the same letter are considered not statistically different at $P < 0.05$.

3. Results and discussion

3.1. Biochar characterization

The physicochemical and morphological properties of the biochar are highlighted in Figs. 3–5, and Table 1 below.

The biochar exhibited an alkaline pH of 8 and a relatively high electrical conductivity of 1202.5 $\mu\text{S}/\text{cm}$. It was characterized by a high carbon content (83.70 %) accompanied by low levels of hydrogen (2.12 %), oxygen (8.65 %), and nitrogen (0.80 %) (Table 1). The FTIR

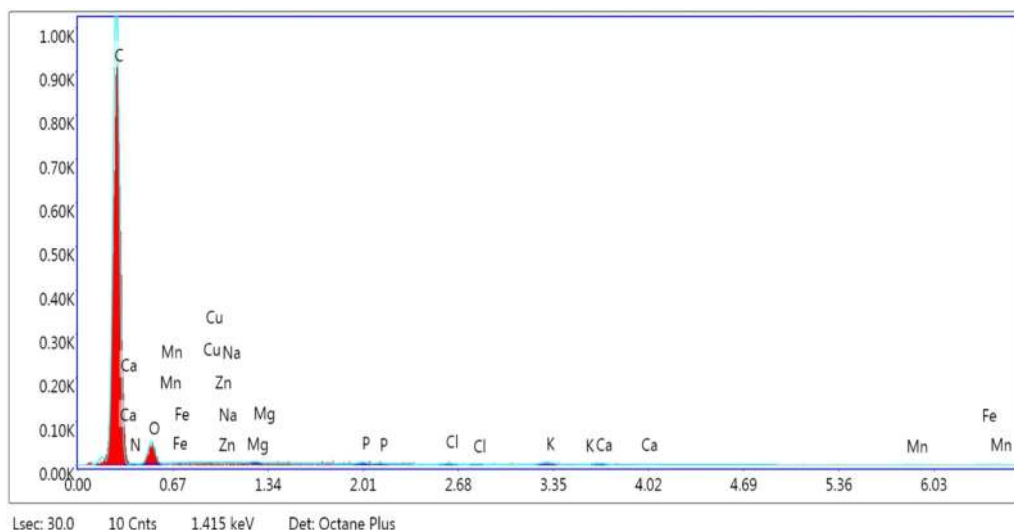


Fig. 5. EDX spectrum showing the elemental composition of the biochar.

spectrum of biochar reveals distinct functional groups indicative of its surface chemistry and adsorption potential (Fig. 3). The broad absorption band observed around 3400 cm^{-1} corresponds to the O-H stretching vibrations of hydroxyl groups, suggesting the presence of adsorbed water, alcohols, or phenolic compounds, confirming the hydrophilic character of biochar (Peer et al., 2023). The band at 1620 cm^{-1} is attributed to C=O stretching vibrations of carbonyl groups, commonly found in carboxylic acids or ketones, and is a typical product of biomass pyrolysis (Abid et al., 2022). A distinct peak at 1300 cm^{-1} is associated with C-H bending vibrations of aliphatic groups, while the band near 1000 cm^{-1} corresponds to C-C stretching vibrations, likely originating from aromatic or phenolic structures. The presence of these oxygenated and aromatic moieties plays a pivotal role in the adsorption of polar organic compounds through mechanisms such as hydrogen bonding, π - π interactions, and surface complexation (Dąbrowski et al., 2005). Overall, the FTIR analysis confirms that biochar contains a variety of chemically active sites that contribute to its effectiveness as an adsorbent in environmental applications (Jagadeesh and Sundaram, 2023).

The SEM micrographs of the synthesized biochar, as shown in Fig. 4, reveal a highly porous and heterogeneous surface morphology featuring an extensive network of irregular cavities and interconnected pores formed during pyrolysis. The surface exhibits a rough and fragmented texture, indicative of abundant active sites available for adsorbate binding (Abid et al., 2022). These morphological observations are strongly supported by BET surface area analysis (Table 1), which demonstrates a high specific surface area of $258.72\text{ m}^2/\text{g}$, substantially greater than the typical $201.7\text{ m}^2/\text{g}$ reported for carbonized olive stone biochar (Villardón et al., 2025). The Langmuir surface area ($278.48\text{ m}^2/\text{g}$) closely aligns with the BET value, suggesting a uniform distribution of adsorption sites and a predominant monolayer adsorption mechanism. Additionally, t-plot analysis reveals a micropore surface area of $115.58\text{ m}^2/\text{g}$ alongside an external (mesoporous) surface area of $143.14\text{ m}^2/\text{g}$, confirming the hierarchical pore structure. This combination of micro- and mesoporosity enhances selective adsorption of small molecular species while facilitating the efficient transport of larger molecules (Alshehri and Pugazhendhi, 2024). Collectively, the SEM and BET characterizations corroborate the biochar's well-developed porous framework, underpinning its superior adsorption capacity and suitability for various environmental remediation applications (Lehmann and Joseph, 2009).

3.2. Physico-chemical characteristics of OMWW

The physicochemical parameters of raw and treated OMWW are

shown in Table 2. The physicochemical characteristics present a significant improvement after the filtration of OMWW. The treated OMWW are characterized by an increase in pH, rising from 4.80 ± 0.03 in the raw OMWW to 7.05 ± 0.05 in the treated OMWW. These values fall within the recommended range of Moroccan standards for reuse and discharge (State Secretariat in charge of Water and the Environment, 2007; Delegated Ministry in charge of Water, 2014). The chemical composition of biochar is strongly influenced by the nature of the feedstock and the pyrolysis conditions (Yu et al., 2018). It typically contains mineral elements such as magnesium (Mg^{2+}) and potassium (K^+) (Song et al., 2022), which can be released during treatment, increasing the pH of the effluent (Lustosa Filho et al., 2020). Furthermore, basic functional groups, such as hydroxyl and carbonate, present on the biochar surface can react with hydrogen ions (H^+) in the solution (Van Nguyen et al., 2022), neutralizing acidity and further contributing to a rise in pH (Lustosa Filho et al., 2020).

The EC of treated OMWW showed a significant decrease from $13.90\text{ mS/cm} \pm 1.2$ – $1.89\text{ mS/cm} \pm 0.9$, which explains that the dissolved salt content in OMWW was removed efficiently after biochar filtration, these findings are in agreement with those of previous studies of; Cao et al., (2009) which revealed that the highly porous structure of biochar, combined with its extensive specific surface area, enable the adsorption of various ions dissolved in effluents (Ahmad et al., 2014), including cations such as Na^+ , Ca^{2+} as well as anions like chloride and sulfate (Abdelhafez and Li, 2016). This ion adsorption process effectively decreases the electrical conductivity of the effluent (El Barkaoui et al., 2023).

The treatment process effectively decreased the electrical conductivity of the effluent (El Barkaoui et al., 2023). The COD levels of raw OMWW were initially very high ($65.15\text{ g/L} \pm 1.02$), reflecting the substantial presence of organic pollutants, and were substantially reduced to $4.7\text{ g/L} \pm 0.05$ following filtration through biochar. A similar trend was observed for TSS and TS, which showed significant reductions after treatment, eventually reaching undetectable levels. This marked reduction in COD can be primarily attributed to the adsorption capacity of biochar (Enaïme et al., 2020), which efficiently binds a wide range of organic molecules, including polyphenols, sugars, and low-molecular-weight acids (Park et al., 2019). The high specific surface area and porous structure of biochar enhance its interaction with these organic compounds, facilitating their removal from the effluent, contributing to COD reduction (Abdelhafez and Li, 2016). Previous studies have reported similar trends; for instance, Gwenzi et al. (2017) highlighted that the efficiency of TSS and COD removal varies depending on the characteristics of the biochar and operational conditions.

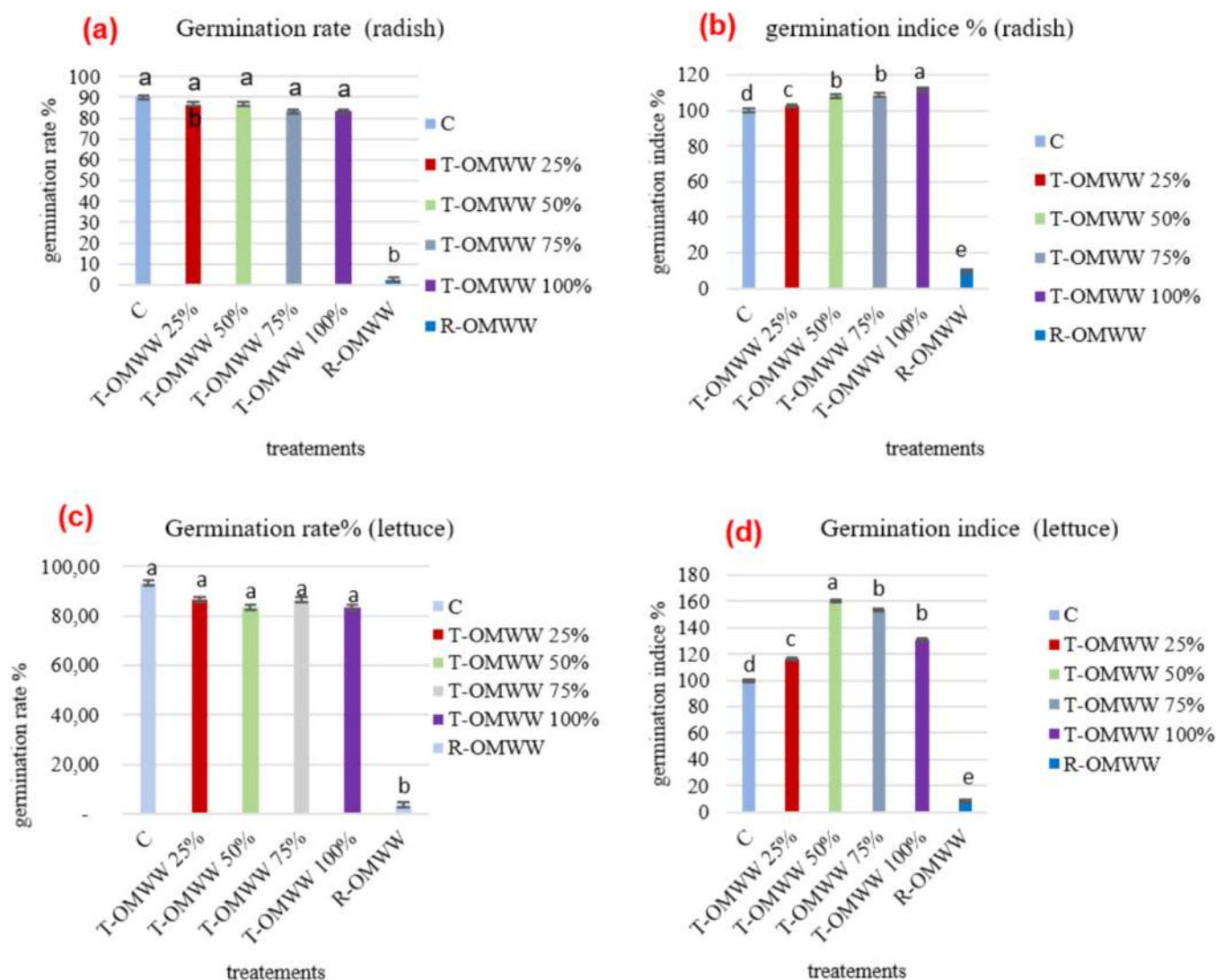


Fig. 6. Results of the phytotoxicity test of different OMWW concentrations (25, 50, 75, and 100 % Treated OMWW v/v; Raw OMWW) and distilled water as a control: C, on radish seeds: (a) germination rate; (b) germination index, and lettuce seeds (c) germination rate; (d) germination index. When the statistical difference was significant, a multiple mean comparison was performed using Tukey's multiple range test for all comparisons, differences were considered significant when the probability level was 5 %.

More generally, OMWW organic load can be mitigated by processes such as chemical reduction (Belaqziz et al., 2022), oxidation, microbial degradation (Boutafda et al., 2023), adsorption (El Hajjouji et al., 2014), and filtration (Chen et al., 2018). By employing optimization methods, several works have revealed the adsorption behavior of phenolic compounds from OMWW using biochar (Senol et al., 2017; Stasinakis et al., 2008). Our results are consistent with prior studies, where the removal rate of phenolic compounds after OMWW filtration using biochar reached up to 88.67 %.

Various studies have been presented encouraging results regarding the treatment of OMWW using other processes, Belaqziz et al., (2022) proved that the percolation of the OMWW via a mixture of soil and gravel filter shown a reduction of 52 % of fat content, 55 % of the phenolic content, 68 % of suspended solids and 69 % of the organic carbon. Additionally, Enaïme et al., (2019) have demonstrated that the use of olive stone filters leads to higher removal of fatty matter (73.8 %), total suspended solids (82.5 %), COD (23.2 %), and phenolic compounds (11.3 %). In this context, Abu-Dalo et al. (2023) developed a GAC/Cu-MOF composite capable of adsorbing up to 20 mg/g of phenolic compounds within 4 h under acidic conditions, demonstrating favorable adsorption kinetics and thermodynamics. Similarly, Chaari

et al. (2021) found that thermal activation of Tunisian natural clay significantly enhanced its adsorption efficiency, achieving up to 84.21 % phenolic removal. Moreover, Mustafa and Oladipo (2023) developed a dual-function Mg-doped $\text{Zn}_{0.5}\text{Ni}_{0.5}\text{Fe}_2\text{O}_4$ photocatalyst capable of achieving 83.8 % degradation of 2,4,5-trichlorophenol under optimized conditions, along with potent bactericidal effects against *E. coli* and *S. aureus* within 60 min, even in the absence of UV light. Additionally, titanium dioxide (TiO_2) nanoparticles have been reported to reduce chemical oxygen demand (COD) by up to 90 % within 2 h (Al Bsoul et al., 2019). However, this approach requires stringent operating conditions and incurs higher costs, which can limit its practical scalability. In contrast, natural adsorbents such as volcanic tuff, clay, and charcoal, examined by Azzam (2018), showed comparatively lower removal efficiencies, particularly volcanic tuff alone, with only 14 % COD and 21 % phenolics reduction, although synergistic effects were observed when combined with clay and charcoal.

These studies highlight the growing interest in natural and composite materials for OMWW treatment. In this regard, our study demonstrates that biochar-based filtration can achieve important phenolic removal (>90 %) and significant COD reduction under mild and cost-effective conditions. These results are consistent with findings by Iadipo et al.

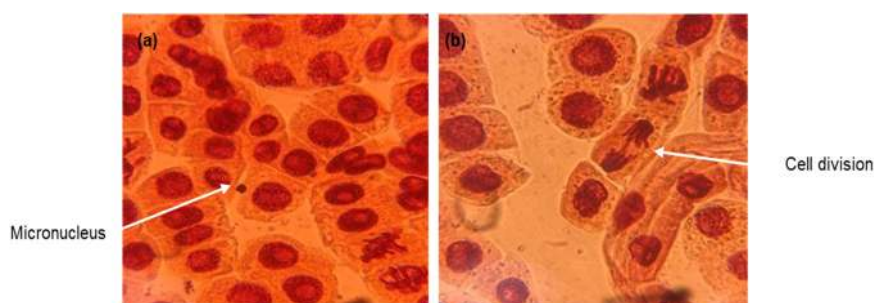


Fig. 7. Observation of *Vicia faba* root cells. (a) Micronucleus induced by hydrazide maleic at 10–5 in *Vicia faba* roots; (b) *Vicia faba* cells in anaphase; (magnification).

(2019), who reported the high adsorption capacity of calcined magnetic biochar. Therefore, biochar appears to be a promising, sustainable, and environmentally friendly adsorbent for effective OMWW management.

3.3. Phytotoxicity test

The phytotoxicity test is an assay used to evaluate the phytotoxicity of raw and treated effluents on seed germination. The germination index is a sensitive parameter that refers to high toxicity affecting germination and low toxicity affecting root elongation (Zucconi et al., 1985). Fig. 6 presents the germination rate (GR) and germination index (GI) for lettuce and radish seeds exposed to raw and treated OMWW. The data demonstrate a significant reduction in germination capacity in the presence of raw OMWW, indicating a strong phytotoxic potential of the untreated effluent.

The phytotoxicity of OMWW, as expressed by GR, was approximately 86.61 %, 86.67 %, 84.33 %, 83.33 % and 2.5 % for radish variety (a) and 86.67 %, 83.33 %, 86.67 %, 83.33 % and 3.5 % for lettuce (c), for 25 %, 50 %, 75 %, 100 % treated OMWW, and for raw OMWW respectively; The results demonstrate that biochar-based treatment of OMWW improved the germination rate across all treated OMWW samples, with enhancements of approximately 33-fold for radish and 25-fold for lettuce. The improvement in seed germination observed with treated OMWW is primarily attributed to the effective removal of phytotoxic phenolic compounds (Mekki et al., 2007). These compounds are adsorbed onto the surface of biochar through hydrogen bonding and electrostatic interactions involving its –OH and –COOH functional

groups (El Barkaoui et al., 2023). By reducing the concentration of these toxic molecules, biochar treatment alleviates oxidative stress and enzyme inhibition in seeds, thereby restoring normal germination processes (El Hajjouji et al., 2014). Similar mechanisms have been reported by Elayadi et al. (2021), who showed that the adsorption of phenolic compounds onto sugarcane bagasse is largely governed by hydrogen bonding and electrostatic interactions between the functional groups of the adsorbent and phenolics. Numerous studies (Enaïme et al., 2020; Boutafda et al., 2023) have documented the inhibitory effects of OMWW on seed germination and early growth in various cultivated species, highlighting the phytotoxic potential of its organic load. The filtration process through biochar effectively removes these pollutant compounds, significantly reducing their concentration in the effluent. While germination rate (GR) is a commonly used and reliable indicator of seed germinability (Leather and Einhellig, 1988), additional parameters such as root length and early seedling growth can provide complementary and more sensitive insights into the alleviation of phytotoxic effects. The germination index (GI) was first introduced by Zucconi et al. (1981) to assess compost toxicity. GI integrates both GR and RRG, serving as a reliable indicator of phytotoxicity. (Wang et al., 2011; El Fels et al., 2014). For radish and lettuce, the results of the tested effluent samples are shown in Fig. 6, where a very high value of GI was recorded for treatments with treated olive mill wastewater. We noted that GI exceeded 100 % for all treatments with treated OMWW. However, a low GI of 9.6 %, 8.7 % was attributed to raw OMWW for radish and lettuce, respectively. The toxicity of olive mill wastewater (OMWW) is primarily attributed to its high content of organic pollutants, including phenolic

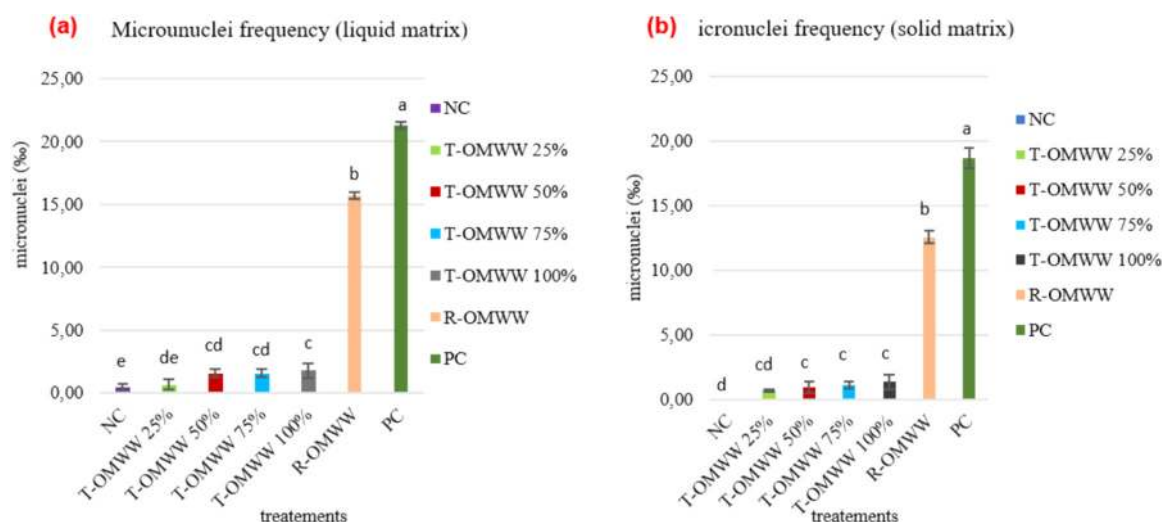


Fig. 8. Results of micronucleus frequency in the liquid matrix (a) and solid matrix (b) values in *Vicia faba* roots at the liquid and solid matrix exposed to different OMWW concentrations (25, 50, 75, and 100 % Treated OMWW v/v; Raw OMWW). PC: positive control (maleic hydrazine); NC: negative control (distilled water). When the statistical difference was significant, a multiple mean comparison was performed using Tukey's multiple range test for all comparisons. Differences were considered significant when the probability level was 5 %.

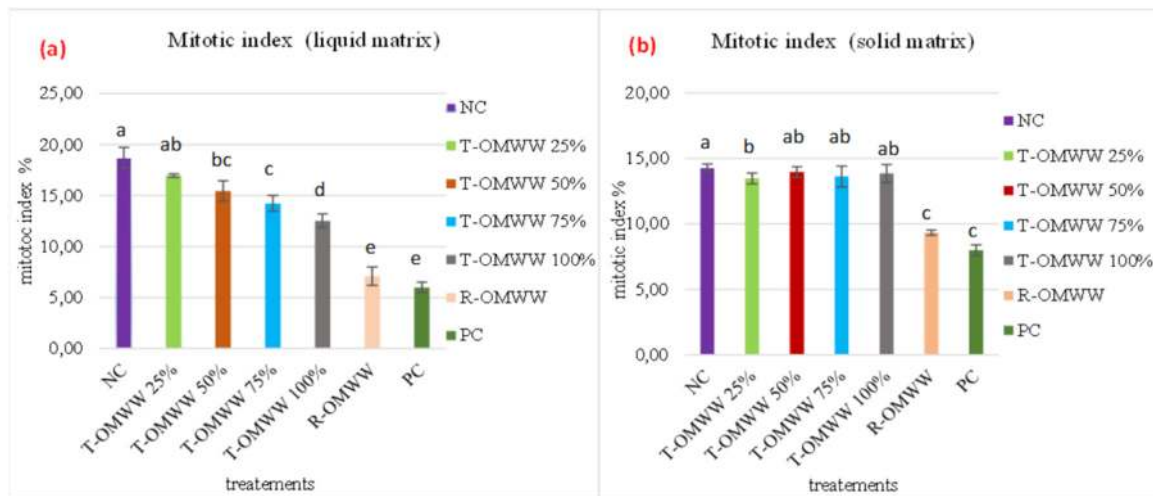


Fig. 9. Results of Mitotic index in the liquid matrix (a) and solid matrix (b) values in *Vicia faba* roots at the liquid and solid matrix exposed to different OMWW concentrations (25, 50, 75, and 100 % Treated OMWW v/v; Raw OMWW). PC: positive control (maleic hydrazine); NC: negative control (distilled water). When the statistical difference was significant, a multiple mean comparison was performed using Tukey's multiple range test for all comparisons; differences were considered significant.

compounds, fatty acids, tannins, and carbohydrates. The phytotoxic effects of these substances have been extensively documented (El Fels et al., 2014, 2015; Piotrowska et al., 2006), showing that exposure can inhibit seed germination and reduce root and shoot growth (Belaqziz et al., 2016). For example, Abid et al. (2022) reported a germination index of approximately 0 % for *Lepidium sativum* seeds exposed to OMWW containing 5 g/L of phenolic compounds. Similar inhibitory effects have been observed in other studies (Nouairi et al., 2019; Sharma et al., 2019; Boutafda et al., 2020; Enaïme et al., 2020b), highlighting the high phytotoxic potential of untreated OMWW.

In addition to its organic load, OMWW phytotoxicity is influenced by physicochemical factors such as pH and electrical conductivity. In the present study, raw OMWW exhibited a strongly acidic pH (4.8) and high electrical conductivity (13.9 mS/cm), which likely contributed to the lowest germinability observed. These conditions can induce osmotic stress and ion toxicity in seeds, further inhibiting germination and early growth (Rusan et al., 2015). Overall, the phytotoxicity of OMWW results from the synergistic effects of phenolic compounds, acidic pH, and salinity. Filtration of OMWW through biochar markedly improved seed germination, as reflected in the high germination index (GI) and germination rate (GR) observed for lettuce and radish. This improvement can be attributed to the effective removal of phytotoxic substances, including lipids, ammonia, and organic compounds (Enaïme et al., 2019). The highly porous structure of biochar allows it to act as an efficient adsorbent, trapping various contaminants present in OMWW (Stasinakis et al., 2008; Van Nguyen et al., 2022), thereby alleviating its phytotoxicity.

Furthermore, treated OMWW retains valuable macro-elements such as nitrogen and phosphorus, as well as essential micro-elements including calcium and magnesium, which are beneficial for plant growth (Mekki et al., 2013). Consequently, biochar-treated OMWW represents a promising resource for ferti-irrigation, improving soil fertility while promoting healthy plant development (Abid et al., 2022; Gwenz et al., 2017).

3.4. Genotoxicity assessment of olive mill wastewater and molecular docking analysis

3.4.1. Micronucleus frequency

The genotoxic effect of different dilutions of treated OMWW (25 %, 50 %, 75 %, 100 % v/v) and raw OMWW assessed by the *Vicia faba* micronucleus (MN) test is shown in Figs. 7–9. The findings indicated

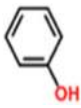
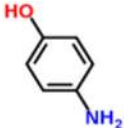
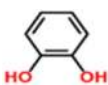
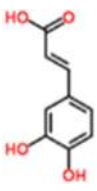
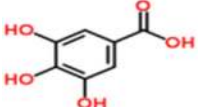
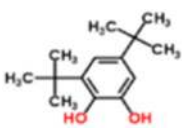
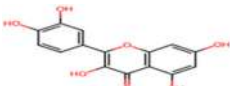
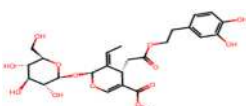
that the micronucleus frequency in raw OMWW is notably high for both solid (Fig. 8b) and liquid (Fig. 8a) matrix, measuring 12.56 % and 15.67 %, respectively, with no significant difference compared to the positive control (maleic hydrazide) = 18.7 % and 21.30 % in the solid and liquid matrix, respectively. The different dilutions of treated OMWW demonstrated a significant decrease in MN frequency, measuring 94.66 %, 92.91 %, 91.16 %, 89.4 % in the solid matrix (b); and 96 %, 90.04 %, 90.05 %, and 88.6 % in the soluble matrix (a) for the 25 %, 50 %, 75 %, and 100 % treated OMWW (v/v), respectively. Consequently, the genotoxicity of 100 % treated OMWW is considered 22-fold lower than of the raw OMWW. The micronucleus analysis values found support the findings of the phytotoxicity tests conducted on radish and lettuce seedlings. Halliwell and Gutteridge (1990) supposed that the increase in micronucleus frequency results from chromosomal fragmentation or mitotic irregularities (Fig. 7). Aybeke et al. (2008) demonstrated that OMWW toxicity disrupted protein synthesis in *Triticum aestivum* root tips and inhibited DNA synthesis. Similarly, Do Céu Silva et al. (2003) confirmed that DNA damage could be attributed to oxidative stress caused by phenolic compounds present in raw OMWW. These findings support the results of our molecular docking analysis and may be related to the presence of specific polyphenols. Compounds such as quercetin exhibit strong interactions with DNA, which could help explain the high frequency of micronuclei observed in this study. Overall, the genotoxic potential of raw OMWW was lower in the solid matrix (MN = 12.56 %) compared to the liquid matrix (MN = 21.30 %). This reduction can be attributed to the sorptive capacity of soil organic matter, which likely decreases the bioavailability of genotoxic compounds, thereby mitigating their toxic effects. Therefore, the solid matrix could serve as a chelating environment, capable of complexing several pollutants (Shahid et al., 2011; El Fels et al., 2015).

3.4.2. Mitotic index

The mitotic Index is often used as a parameter for tracking the cytotoxicity of tested industrial effluent substances. Conversely, a decline in this parameter relative to the negative control suggests an enhancement in toxicity (Raklami et al., 2021).

For our case, the mitotic index (MI) demonstrates a significant decrease in raw OMWW, with 7.10 % in the liquid matrix (Fig. 9a) and 9.34 % in the solid matrix (Fig. 9b), compared to their negative control, which showed values of 18.72 % and 14 % respectively. However, the filtration-based biochar of OMWW enhanced the mitotic index by 43.4 %, 49.5 %, 45.8 %, and 48.2 % in the solid matrix and 139.4 %,

Table 3
physicochemical properties of phenolic molecules tested in docking molecular studies (Haynes, 2017; Miramont, 2021; Elayadi et al., 2021).

Phenolic Compounds	CAS Number	Log P	Solubility (g/L at 25°C)	pKa	Chemical structure
Phenol (ChemSpider ID971)	108–95–2	1.46	76.04	9.99	
4-aminophenol (ChemSpider ID392)	123–30–8	0.04	6.5	10.30	
Catechol (ChemSpider ID13837760)	120–80–9	0.88	430	9.48	
Hydroquinone (ChemSpider ID764)	123–31–9	0.59	59	9.96	
Caffeic acid (ChemSpider ID600426)	331–39–5	1.15	1.8 g/L	4.62	
Gallic acid (ChemSpider ID361)	149–91–7	0.7	12	4.5	
3,5-Di-tert-butylcatechol (ChemSpider ID59488)	1020–31–1	3.7	0.5 g/L	9.5	
Quercetin (ChemSpider ID4444051)	117–39–5	2.16	0.06	6.95	
Oleuropein (ChemSpider ID4444876)	32619–42–4	0.0006	0.73 g/L	4.94–9.70	

117.6 %, 100.4 %, and 76.4 % in the soluble matrix for the 25 %, 50 %, 75 %, and 100 % treated OMWW (v/v) respectively. This could explain the high efficiency of the OMWW filtration and the capacity of biochar to adsorb pollutant compounds, particularly phenolic molecules, which are known for their genotoxic potential. The significant decrease in the mitotic index (MI) suggests that phenolic compounds may interfere with the synthesis of key metabolites such as nucleotides, proteins, or enzymes that are essential for the mitotic process. This disruption can hinder cell division and may result either from inhibition of DNA synthesis or a blockage of the G2 phase of the cell cycle (Mahoney et al., 2006). In particular, the pronounced reduction in MI observed in cells exposed to raw OMWW could be linked to the presence of specific polyphenols, such as quercetin, which are capable of binding to DNA and altering the normal progression of mitosis, as supported by the molecular docking results presented in the following section. These results are supported by Fiorentino et al. (2003), who demonstrated that raw OMWW is toxic to both plants and microorganisms, and the high

concentration of phenolic compounds, like nitrogenous compounds, polyphenols, volatile acids, and polyalcohols, can also contribute to the high toxicity of OMWW (Danellakis et al., 2011). Vicia faba seeds exposed to OMWW effluent exhibited a severe inhibition of mitotic activity, exceeding 78 %, which led to lethal effects on the entire root system of the tested seeds (Antosiewicz, 2005). Boutafda et al. (2023) showed that a fungal strain using *Penicillium chrysogenum* as a biological treatment reduced the micronucleus frequency by 95.66 % for the treated OMWW (10 %). Moreover, El Hajjouji et al. (2007) revealed that for all the phenols tested individually, the micronucleus frequency ranged between 3 and 24 ‰ for a 10 % dilution of raw OMWW, and root growth inhibition is caused by the chromosome abnormalities occurring and a reduction in the root length. In our study, the reduction in micronuclei (MN) frequency was calculated by comparing the values obtained before and after the biochar-based treatment of OMWW. This method revealed a notable decrease of approximately 96 %, demonstrating the potential effectiveness of the treatment. This method has

Table 4
Binding affinities and potential molecular interactions of phenolic compounds with the DNA double helix.

Compound	Phenol	4-aminophenol	Catechol	Hydroquinone	Caffeic acid	Gallic acid
E (Kcal/mol)	−4.62	−4.39	−4.48	−4.64	−5.50	−4.94
Number of Hydrogen bonds	1	3	1	3	3	5

Table 5
Binding affinities and potential molecular interactions of phenolic compounds with DNA double helix.

Compound	Diterbutylcatechol	Quercetin	Oleuropein	Tyrosol	Hydroxytyrosol
E (Kcal/mol)	−6.49	−8.96	−5.91	−5.64	−5.29
Number of Hydrogen bonds	2	9	5	1	4

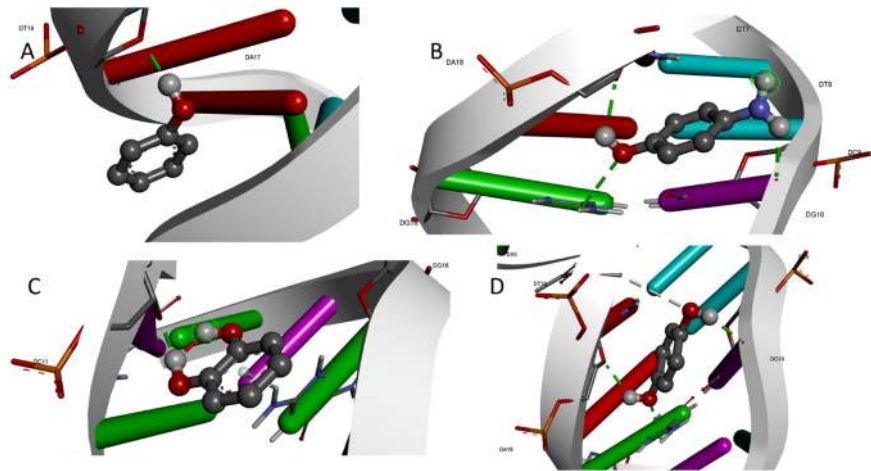


Fig. 10. Three-dimensional interactions of phenol (A), 4-aminophenol (B), catechol (C), and hydroquinone (D) with the nucleotides of DNA double helix.

been previously applied in similar genotoxicity assessment studies on OMWW, such as [Boutafda et al. \(2023\)](#). The capacity of biochar to remove multiple chemical and microbiological contaminants in aqueous systems has also been demonstrated by [Micoli et al. \(2023\)](#). Research has optimized biochar production parameters, including temperature and activation methods, to improve its adsorption efficiency ([Van Nguyen et al., 2022](#)). [Ahmad et al. \(2018\)](#), [Enaime et al. \(2020\)](#), and

[Yuan et al. \(2021\)](#) showed the effectiveness of biochar for pollutant removal. The adsorption of organic pollutants typically occurs through several mechanisms ([Yao et al., 2020](#)), including complexation, electron acceptor-donor interactions, pore filling ([Enaime et al., 2020a](#)), electrostatic attraction, hydrophobic interactions ([El Barkaoui et al., 2023](#)), and hydrogen bonding ([Teixidó et al., 2011](#)). In this regard, A study by [Elayadi et al. \(2021\)](#) demonstrated that the adsorption of phenolic

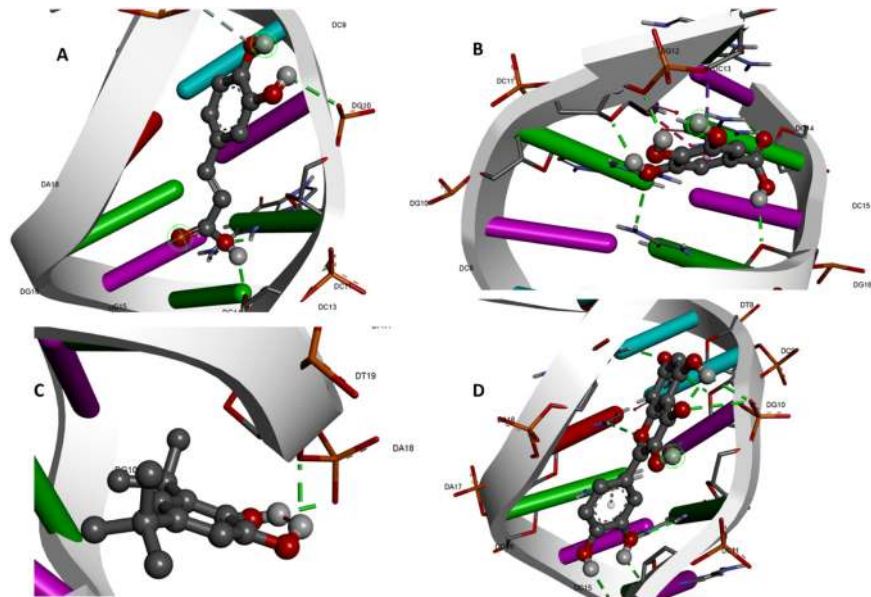


Fig. 11. Three-dimensional interactions of caffeic acid (A), gallic acid (B), diterbutylcatechol (C), and quercetin (D) with the nucleotides of DNA double helix.

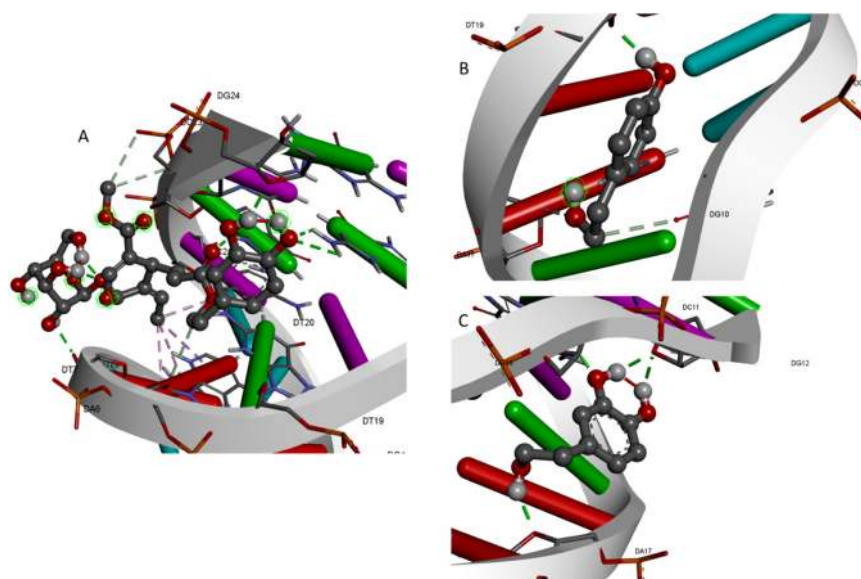


Fig. 12. Three-dimensional interactions of oleuropein (A), tyrosol (B), and hydroxytyrosol (C) with the nucleotides of DNA double helix.

compounds onto sugarcane bagasse is mainly governed by chemical interactions, particularly hydrogen bonding and electrostatic attraction between the $-OH$ and $-COOH$ groups of SCB and the functional groups of phenolics. Oladipo et al. (2020) demonstrated the efficiency of a CuO-coated olive cake nanocomposite for the removal of phenolic compounds and discoloration of high-strength OMWW, achieving up to 95 % removal within 6 h. However, despite their performance, such engineered materials can be costly and less accessible. Another study conducted by Jedynak and Charnas, (2024) demonstrated that the chemical activation of biochar prepared from olive mill solid waste, enhance biochar's surface area and porosity, and increase its efficiency to remove phenolic compounds in OMWW, in most case activated biochar has a higher specific area ($>200\text{m}^2/\text{g}$), providing an important number of adsorbing sites (Gotore et al., 2024; Zhang et al., 2021). Carbon with developed porosity, especially mesopore, favors phenol adsorption, and carbon with microporosity favors total organics adsorption, for the complex system of OMWW (Galiatsatou et al., 2002), (Daifullah and Girgis, 1998) demonstrates that smaller particles enhance the van der Waals forces, facilitating the penetration of the adsorbate into the adsorbent, while the large particles can cause a blockage of the sorption site. It concluded that biochar has a high potential as an eco-friendly solution for OMWW treatment and reducing the toxicity of OMWW.

3.4.3. Molecular docking analysis

The selected phenolic compounds exhibit a wide range of physico-chemical properties, reflecting their structural diversity and potential to interact with biomolecules such as DNA (Table 3). Molecular docking was examined to gain insight into the mechanism of DNA damage mode of action at the molecular level. A subset of polyphenols detected in olive mill wastewater was selected for docking into the DNA double helix (PDB ID: 355D). The docking results revealed that the phenolic compounds exhibited interesting binding energies ranging from -4.39 to -8.96 kcal/mol (Tables 4–5). Specifically, quercetin strongly binds to the nucleotides of the DNA double helix, with an excellent estimated binding energy of -8.96 kcal/mol. The DNA–ligand interaction studies (Figs. 10–11) showed that all polyphenols form hydrogen bonds with the target. Interestingly, quercetin forms nine hydrogen bonds with DG A:10, DC A:11, DA B:17, DC A:9, DT A:8, and DA B:18, which supports its high binding affinity to the DNA double helix. We observed that polyphenols form different hydrogen bonds with various constituents of nucleotides, including ribose, the phosphate group, and the nucleobase.

Moreover, other interactions were observed, as van der Waals, carbon-hydrogen bonds, Pi-donor hydrogen bonds, Pi-sigma, Pi-Pi stacking, and Pi-alkyl interactions. Based on the results of the molecular docking studies, it can be proposed that polyphenols induce DNA damage through their interactions with all structural components of the nucleotide namely the ribose, phosphate group, and nucleobase primarily via strong hydrogen bonding. Among the tested compounds, quercetin and other polyphenols commonly found in OMWW exhibited high binding affinities toward DNA, suggesting a capacity to interfere with its structural integrity and function. These interactions include not only hydrogen bonding but also potential intercalative binding modes, which may disrupt normal DNA replication and transcription. These docking-based results offer a mechanistic explanation for the genotoxic effects observed in vitro, such as the increased frequency of micronuclei and the reduction in mitotic index, particularly in cells exposed to raw OMWW. The significant decrease in genotoxicity following treatment may thus be attributed, at least in part, to the removal or transformation of these high-affinity polyphenolic compounds. Together, these results support the hypothesis that specific polyphenols are key contributors to the genotoxic potential of OMWW. (Fig. 12)

4. Conclusion

Olive mill wastewater (OMWW), rich in organic and chemical compounds, poses a significant threat to aquatic and terrestrial ecosystems when improperly disposed of. This study highlights the effectiveness of biochar, produced from olive stones through pyrolysis at 585°C , in reducing the phytotoxicity and genotoxicity of OMWW. Treatment with biochar significantly improved pollutant removal, achieving a 92.78 % reduction in COD and an 88.67 % reduction in phenolic compounds. Phytotoxicity tests revealed that untreated OMWW was 25 times more toxic to lettuce seeds and 33 times more toxic to radish seeds compared to treated OMWW. Additionally, genotoxicity assays demonstrated that biochar-based filtration of OMWW was highly effective, achieving a reduction of nearly 96 % in genotoxic effects. Molecular docking studies further supported these findings by suggesting that certain polyphenols present in OMWW may induce DNA damage through the formation of strong hydrogen bonds with the DNA double helix. The observed reduction in genotoxicity after treatment indicates that biochar effectively removes or neutralizes these harmful compounds.

These results demonstrate the potential of biochar-based filtration as

a sustainable and eco-friendly solution to mitigate OMWW pollution. This approach enables the reuse of treated effluent in agriculture, enhancing soil fertility, providing essential nutrients, and contributing to sustainable agricultural practices. However, further in situ and pilot-scale studies are needed to assess operational feasibility, economic viability, optimize the amount of biochar used, and explore biochar desorption mechanisms that enable its regeneration and reuse across various applications.

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CRediT authorship contribution statement

Khalid Oufdou: Visualization, Validation, Investigation. **Hassan Laouane:** Software. **record eric:** Visualization, Validation, Formal analysis. **El Fels Ioubna:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Asma Dahani:** Writing – original draft, Methodology, Formal analysis, Data curation. **Imad Rabichi:** Software, Methodology, Investigation. **Kawtar Ezzahi:** Software, Methodology, Investigation. **El Mansouri Az-eddine:** Software, Resources. **Elmostapha Outamamat:** Visualization, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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