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# Enhanced detoxification methods for the safe reuse of treated olive mill wastewater in irrigation

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**Abstract**

Olive Mill Wastewater (OMWW) is produced in large quantities and contains high levels of nutrients that can be reused for irrigation, reducing the demand for freshwater resources. However, OMWW is phytotoxic and expensive to treat, making it important to develop more cost-effective treatment methods. This study aims to investigate an integrated detoxification treatment sequence consisting of acid precipitation, Fenton oxidation, and electrical coagulation to safely reuse OMWW for barley germination. Raw, treated and diluted OMWW (25% and 50% OMWW) were tested. The results showed that raw and diluted OMWW suppressed seed germination at all concentrations, while diluted treated OMWW enhanced seed germination and plant growth. In addition, treated OMWW (acid precipitation treatment) at 25% dilution reported 0% phytotoxicity significantly improved plant growth, where plant fresh weight (FW) reached 123.33 mg. Moreover, α-amylase, lipase, and protease enzyme activity confirmed the superior enhancement of barley growth parameters, where the highest enzyme activity value recoded 0.870 mg maltose/g FW. The integrated treatments reduced detoxification by 97.90% for total phenolic, 98.37% for total flavonoids, and 99.18% for total tannins. Reductions of around 95.78%, 60.00%, and 78.90% in total organic carbon, electric conductivity, and total solids, respectively, were achieved. A significant decrease in heavy metals was observed with removal ratios 98.64%, 94.80%, 96.88%, and 95.72% for Fe, Cu, Mn, and Zn, respectively. Seedling Vigor Index as an indicator of crop productivity was successfully predicted using neural network modeling. Therefore, the applied method can be used as a fertilizer to support plant growth and reduce fertilization costs.

**Keywords** Olive mill wastewater, Acid precipitation, Fenton oxidation, Electro-coagulation, Irrigation, Barley germination

## Introduction

Water scarcity is a growing challenge for sustainable development [1]. As urbanization and industrialization increase, so does the demand for water, creating a gap between supply and demand and putting pressure on agriculture to reduce its use of freshwater and seek alternative sources [2]. Treated wastewater is a promising alternative for meeting agricultural water demand, freeing up freshwater for domestic and industrial use, and contributing to improved socio-economic conditions and sustainable development [1, 3]. However, the chemical composition of wastewater must be controlled due to

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the presence of pollutants such as heavy metals, organic compounds, and bacteria, which can affect crop quantity and quality [4–9]. As a result, interest has shifted towards agro-industrial wastewaters due to their large quantities and high nutrient content [10, 11].

Olive cultivation covers 10.8 million hectares worldwide [12], with an annual production of approximately 3.1 million tons of olive oil [13]. Olive mill waste, including olive mill wastewater (OMWW), wood, leaves, olive pomace, and stones [14], is generated in both liquid and solid forms. Olive oil extraction poses a serious environmental challenge due to the large amount of waste generated in a short period of time [12]. With an average of 0.2 ton of olive oil extracted per ton of processed olives and an average of 1.2 $m^3$ OMWW produced per ton of milled olives [15], the estimated annual worldwide production of OMWW is 18.6 million $m^3$ with a high chemical oxygen demand (COD) due to its considerable sugar content and high phosphorus content [13]. Olive oil-producing countries face environmental problems due to the lack of practical or reasonable solutions for disposing of OMWW [16], which has been shown to be hazardous to plants even after 100-fold dilution [17].

OMWW is an acidic liquid that is dark brown in color with a pH between 3 and 6 due to its high polluting load, making it one of the most polluting byproducts of the agro-food industry [18]. Olive mill black liquid wastewater contains oil emulsion, fruit pulp, crushed seeds, and a large amount of water (> 65%), as well as protein, carbohydrates, aromatic compounds, organic matter, polyphenols, tannins, polyalcohols, organic acids, and high mineral content with high biological oxygen demand (BOD) and COD [19]. OMWW is an ecologically harmful dark-colored pollutant that is phytotoxic and resistant to biodegradation due to its antibacterial phenolic substances [20]. Polyphenols in OMWW are phytotoxic and have been shown to hinder plant development as well as have antibacterial activities, increase soil salinity, and inhibit plant development [13, 21]. The dumping of OMWW in soil or rivers remains a critical concern due to its extreme phytotoxicity and antimicrobial capabilities, which can disrupt the equilibrium of biological systems and have long-term environmental consequences [12, 21].

OMWW must be treated before it can be reused or disposed of in the environment. Various treatment methods have been used in recent years, including evaporation, electrocoagulation, ozone oxidation, Fenton reagent oxidation, aerobic and anaerobic biological treatments, and spreading onto agricultural soil as an organic fertilizer [21, 22]. Electrocoagulation has advantages of cheap cost, ease of operation and automation, small footprint, and robustness to pollutant variability [23]. Moreover, Fenton's process is still one of the most interesting advanced oxidation processes due to its low investment cost, easiness of implementation, low toxicity of reagents, and capacity to mineralize a wide range of organic compounds [24].

Numerous studies have been conducted on OMWW itself as well as its management and disposal. Diluting OMWW with water has been reported to eliminate its phytotoxic effects on plant growth [25]. However, when diluted OMWW was used for sorghum irrigation, it was found to be ineffective in increasing growth yield and should be pretreated to reduce its organic loads and acidity before use [14]. In contrast, using OMWW on wheat grown in Vertisol soil resulted in a significant increase in germination rate, plant height, kernel number, and grain yield compared to the control [16]. Mixing treated industrial water with OMWW had a positive effect on vetch germination and early seedling growth [26]. Co-treating acid mine drainage and OMWW using sulphate-reducing bacteria-enriched bioreactors reduced some heavy metals and total phenol concentrations by 80–90%, although their final concentrations were still above the limit for wastewater discharges [13]. Biological treatments of OMWW, either aerobic or anaerobic, are not entirely effective due to the high toxicity of phenolic compounds even at low concentrations [7, 19, 27]. Several other techniques, such as ultrafiltration, reverse osmosis, photoxidation, photocatalytic, and wet air oxidation, are highly costly [28–30].

The Al-Jouf region is an important agricultural area in Saudi Arabia with approximately 460,000 ha of cultivated land [31]. The region is known for its orchards, particularly olive and date palm trees, as well as other field crops such as wheat, barley, alfalfa, sorghum, and watermelon [32, 33]. It is home to 5 million olive trees. The climatic condition of Al-Jouf is arid and the contribution of rainfall to agriculture is minimal in this region. Agriculture in Al-Jouf primarily depends on limited nonrenewable groundwater sources that may not survive for a long time. In addition, climate change may impose further stress on the availability of water and agricultural productions [34]. The reuse of treated OMWW can decrease groundwater requirements; moreover, OMWW is rich in organic matter and various mineral nutrients (high potassium and phosphorus content) that represent an important source for plants [35, 36].

Thus, the main objective of this study is determine the effect of different treatments and integrated technologies including acid precipitation, Fenton reaction process, and electro-coagulation, as well as dilution of treated OMWW on recovering phenolic compounds from OMWW and safe reuse of treated OMWW for plant growth. This will be achieved by: (a) assessing the efficacy of integrated systems based on acid precipitation, Fenton reaction process, and electrocoagulation as efficient, ecofriendly, and cost effective methods for recovering phenolic compounds from OMWW [23, 24]; (b) conducting chemical analysis of the treated OMWW enriched fractions; and (c) investigating the phytotoxicity properties of these treated fractions to propose a potential safe reused water. This research work contributes to open a window on the potential benefit from OMWW reuse and converting its consideration from an environmental problem to a valuable raw material to solve the issue of droughts, increase cereal production, and reduce the impact of pollution on the environment, therefore, contributing to a circular economy and sustainability.

## Materials and methods

### Reagents and samples

Chemicals, HPLC-grade solvents, and Folin–Ciocalteu reagents were obtained from Sigma-Aldrich. OMWW was freshly collected from two-phase olive oil mill processing systems located in the Al-Jouf region of Saudi Arabia.

### OMWW characterization

Raw Olive Mill Wastewaters (OMWWs) obtained from a two-phase centrifugation process were supplied during the olive harvesting season and stored at 4 °C until use in laboratory experiments. The pH and electrical conductivity (EC) were measured using a pH-meter and conductivity meter, respectively. The total solid (TS) content was determined by weighing the samples before and after drying at 105 °C for 24 h. The total organic carbon (TOC) was determined by combusting the dried samples in a furnace at 550 °C for 4 h. Phenolic compounds were quantified using the Folin–Ciocalteau method with gallic acid as the standard [37]. Total flavonoid content was quantified using catechin as the standard [38]. The absorbance was measured at 760 nm. Tannin condensed content (TCC) was quantified according to Ozgen et al. [39]. These aromatic compounds were identified by HPLC after preparing the OMWW organic phase. Total carbohydrates were determined using the colorimetric method described by DuBois et al. [40]. Total nitrogen (TN) was determined according to Rice et al. [41]. Total phosphorus (TP) was measured calorimetrically. Ca, Mg, Na, K, Fe, Cu, Mn, and Zn were determined by atomic absorption (Fisher Scientific ice 3000). The oil fraction was determined after acidifying the sample according to the standardized method by Kiran et al. [42], then extracted by adding hexane.

### The integrated detoxification system

OMWW contains high-value compounds such as phenolics, recalcitrant, pectin, and important enzymes but also causes phytotoxicity due to its phenolic compounds [43]. Therefore, OMWW treatment is necessary. Various techniques have been investigated for treating OMWW and recovering or removing its phenolic compounds, which are toxic to microorganisms and plants. Physical techniques are used for solid removal and extraction purposes, while chemical methods are applied to reduce organic load. Physicochemical technologies such as flotation and settling, coagulation, oxidation using Fenton reagent, flocculation, filtration, sedimentation, and dilution are generally considered safe and inexpensive as they have been widely applied in various food industry and potable water sectors [44, 45]. Combining different physicochemical techniques (especially physical ones) can result in a high level of phenol recovery [43]. Detoxification methods aim to reduce the impact of pollution load on the recipient. Thus, the integrated detoxification system used in this study consisted of three stages: (A) OMWW acid precipitation; (B) Fenton oxidation reaction with zero-valent iron (ZVI); and (C) electric coagulation process. These stages are described in detail in the following subsections.

#### *Acid precipitation (T1)*

The initial pH of raw OMWW samples (about 4.5) was adjusted to 2.6 by adding sulphuric acid (95–98% purity) to achieve coagulation and precipitation of suspended solids according to Stokes law [46]. Coagulation is a physio-chemical process that facilities the destabilization of fine particles (colloids) from wastewater to form a floc that can be easily filtered. The phenolic compounds possess an aromatic ring with one or more substituted hydroxyl groups and a functional side-chain, which under acidic condition resulted in phenolic charge neutralization that represent less solubility properties in addition to the presence of various inorganic metals ($Fe^{+2}$ and $Fe^{+3}$), $Cu^{+2}$ and $Zn^{+2}$ that promote the formation of complex coagulant's metal ions into insoluble aggregates [47]. In addition, polyfunctional tannins with several dihydroxyphenyl functional groups in their molecule are good chelators that can form precipitates with metal ions. The OMWW samples were then allowed to sediment overnight and prefiltered through a nylon filter followed by filtration with a wire mesh filter of 30–40 µm (Fig. 1). The system operated according to a continuous acid concentration operation at normal bar pressure and a temperature of 25 ± 1 °C.

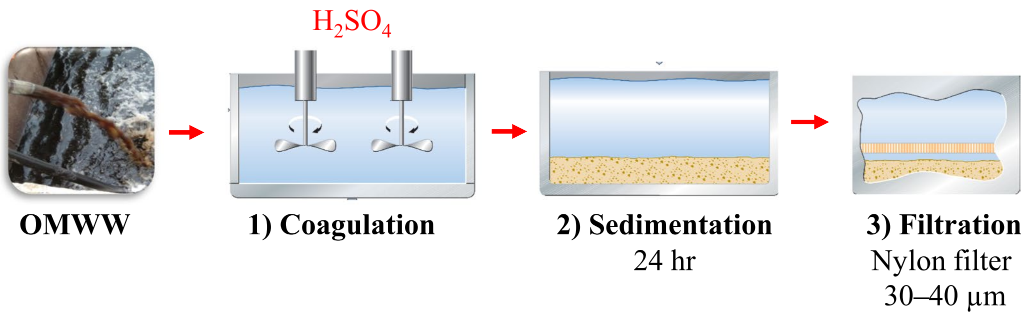


**Fig. 1** Simplified diagram of the OMWW acid precipitation process (T1)

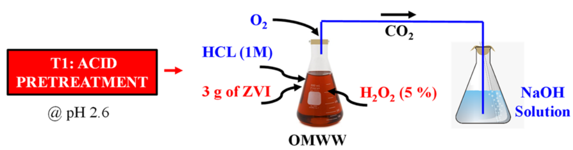


**Fig. 2** Simplified illustration of fenton reactions with ZVI oxidation reactor (T2)

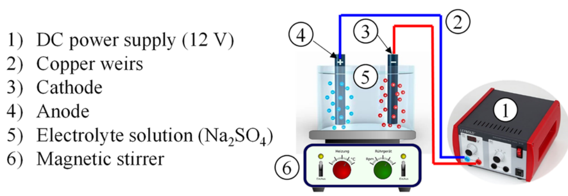


**Fig. 3** Simplified block diagram of the OMWW electric coagulation process (T3)

#### *Fenton oxidation (T2)*

The reactor is a 500 ml Pyrex flask equipped with inlets for sampling and oxygen gas bubbling and an outlet for carbon dioxide. A volume of 100 ml of filtered OMWW from the acid precipitation stage (T1) with an acidic pH (2.6) and without dilution was transferred into the reactor containing 3 g of ZVI powder. The pH of the solution was adjusted as needed to a pH of 2.6 by adding a certain volume of 0.1 M HCl and 5% $H_2O_2$ concentration. The mixture was then exposed to air gas bubbling into the reactor (Fig. 2). The carbon dioxide produced by the reaction was trapped in a sodium hydroxide solution (NaOH is used to verify the $CO_2$ produced by the mineralization of organic contents). This process was performed at room temperature [48].

#### *Electric coagulation process (T3)*

A laboratory-scale reactor was used for electrocoagulation, as shown in Fig. 3. The reactor is made of glass material with dimensions of 11cm x 11cm x 17cm. The working volume of the reactor was 2L.The reactor voltage was 12 V, with a range of direct current variations of 100, 250, and 500 mA cm $^{-2}$, and the coagulation process lasted for approximately 60 min. The temperature was maintained at a constant 25 ℃ during the experiments, and the electrodes were immersed in 1% HCl for 8 h. In electrochemical processes, the material of the electrodes is essential for reactions. The electrocoagulation unit consisted of two effective aluminum electrodes in plate shape. The electrodes' number = (8 Anode; 8 Cathode). The space between the two electrodes was 3 mm in all the experiments. All experiments were performed with solutions of a specific concentration of phenol and 0.25 g/L of electrolyte ($Na_2SO_4$). The solution was continuously stirred using a magnetic stirrer at a constant speed. The pH was adjusted using 0.1 N sodium hydroxide or 0.1 N sulfuric acid solutions [49].

### HPLC analysis of phenolic compounds

An Agilent 1260 series was used to perform HPLC analysis. The EclipseC18 column (4.6 × 250 mm, 5 µm) was used for separation. The mobile phase was composed of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) and flowed at a rate of 0.9 ml/min. The mobile phase was programmed in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5–8 min (60% A); 8–12 min (60% A); 12–15 min (82% A); 15–16 min (82% A) and 16–20 (82%A). The multi-wavelength detector was set to monitor at 280 nm. Each sample solution was injected with a volume of 5µl. The column temperature was kept at 40°C.

### Phytotoxicity test

The Zucconi test [50] was used to measure phytotoxicity by assessing grain germination. The experiment was conducted in triplicate, with each plate containing ten barley grains (Hordeum vulgare) collected from a farm in the Al-Jouf region of KSA. Only grains of similar size and weight were selected for the germination experiment. The grains were placed on filter paper in glass Petri dishes measuring 110 × 20 mm. Each dish was uniformly added with 10 ml of tap water (the control), OMWW and its dilutions, or treated OMWWs and their dilutions. The dishes were covered and the grains were germinated under continuous light in an incubator at 25 °C. To accelerate the germination process, all grains were soaked in tap water for 12 h before starting the germination experiments. The germination ratio (5 days after imbibition, DAI), shoot and root length (10 DAI), and seedling fresh weight (SFW) (10 DAI) were recorded and compared to the control [51]. Traditional phytotoxicity bioassays rely on measuring germination and root elongation [52]. Therefore, the phytotoxicity index (PI) was estimated by comparing the length of treated roots to control roots of barley seeds [44]. In addition, the seedling Vigor index (SVI) was calculated for each group of grains by multiplying the germination ratio and seedling length [53].

### Quantitative assay of hydrolytic enzymes

The alpha amylase activity in germinated seeds was determined for the whole seedling at 10 DAI according to Muscolo et al. [54]. Germinated seeds weighing exactly 0.5 g were homogenized in a 1:4 w/v ratio with distilled water using a chilled pestle and mortar. After centrifuging the extract at 14,000 rpm for 30 min at 4 ℃, the supernatant filtered through muslin cloth was used for the quantitative assay of α-amylase activity. One unit of α-amylase activity represents the number of μmoles of reducing sugars (RS) formed per min per g of FW. It can be calculated using the following equation:

$$\alpha - \text{Amylase activity (U)} = \frac{\text{RS } (\mu\text{moles})}{\text{FW(g)} \times \text{time (min)}} \tag{1}$$

Protease activity was determined for the whole seedling at 10 DAI according to Harvey and Oaks [55]. Fresh seed samples weighing 1 g were homogenized in ice-cold acetone and mixed with a buffer containing 10 mM Tris–HCl at pH 8.0 and 2 M NaCl before being incubated on an orbital shaker for 3 h. After centrifuging the extract at 10,000 rpm for 10 min at 4 ℃, it was used for protease assay at 660 nm. The reaction mixture contained 1 ml of crude enzyme extract, 3 ml phosphate buffer, and casein as substrate at a concentration of 0.5%. In the control tube, distilled water was added instead of enzyme extract, and the tubes were incubated at 30 ℃ for 1 h. A standard graph using tyrosinase (0–100 μg) was used to calculate protease activity by measuring absorbance at 660 nm against a reagent blank using a tyrosine standard according to Harvey and Oaks [55]. One unit of protease is defined as the enzyme amount (EA) that releases one μg of tyrosine per ml per min under the standard conditions. It can be calculated using the following equation:

$$\text{Protease activity (U)} = \frac{\text{EA } (\mu\text{g tyrosine})}{\text{Volume (ml)} \times \text{ time (min)}} \tag{2}$$

#### *Lipase extraction and partial purification*

Lipases were extracted and purified from whole seedlings at 10 DAI. The seedlings were homogenized in chilled acetone at 4 °C, and the resulting suspension was centrifuged at 3000 rpm. The residue was dissolved in 100 mL of distilled water and centrifuged again at 7500 rpm. The supernatant was used as a source of crude enzyme.

#### *Lipase assay*

The titrimetric method described by Malik and Faubel [56] was used to determine lipase activity. An olive oil emulsion was prepared by mixing 180 mL of distilled water with 20 mL of olive oil, 0.4 g of sodium benzoate, and 1 g of Arabic gum. The assay mixture contained 5 mL of the olive oil emulsion, 5 mL of 0.1 M Tris buffer (pH 8), and 1 mL of crude enzyme, and was incubated at 35 °C for 10 min. The reaction was stopped by adding 10 mL of a mixture of acetone and methanol (1:1). Each sample was then titrated against 0.025 N NaOH using phenolphthalein as an indicator at a concentration of 1%. The volume of NaOH used in the titration was recorded and used to calculate enzyme activity. One unit of lipase is defined as the EA required to release one μmol of free fatty acid from olive oil per min under standard assay conditions. It can be calculated using the following equation:

$$\text{Lipase activity (U)} = \frac{\text{EA } (\mu\text{moles free fatty acid})}{\text{Time (min)}} \tag{3}$$

### Statistical and mathematical analysis

Statistical programs including IBM SPSS Ver. 27, GraphPad prism ver. 9.0.2, JMP pro 16, and MS-Excel ver. 365 were used to analyze and present experimental data and study the relationship among variables using methods such as analysis of variance (ANOVA), Duncan's post hoc test, factor analysis, two-dimensional cluster analysis, and neural modeling.

Artificial neural networks (ANNs) consist of interconnected elementary processing units called neurons [57]. These statistical modeling methods have a wide range of engineering applications, including prediction problems, and have been applied to model and predict various environmental problems [57–63]. ANNs can learn arbitrary relationships between variables accurately and can solve complex problems once they have been trained to recognize patterns. Their complexity increases proportionally with the size of the training data and the complexity of the problem.

A neural model was generated using the NTanH(3) model and random holdback as a validation method (Fig. 4) to predict SVI as an indicator for crop productivity in a simple way based on EC, Na, K, Ca, TS, TOC, total phenols, and total flavonoids.

## Results and discussion

### Physicochemical properties of raw OMWW

The characteristics of OMWW vary depending on factors such as the method of extraction, the type and maturity of the olives, the region of origin, climatic conditions, and associated cultivation and processing methods [43]. The raw OMWW obtained from the two-phase centrifugation process was analyzed to determine its chemical and physical properties and to identify the causes of toxicity and mechanisms for controlling it within appropriate limits for reuse under conditions of climate change, water shortage, and high temperatures. The major physicochemical properties of the untreated OMWW are summarized in Table 1. In general, OMWW has similar properties in terms of being acidic, saline, and heavily loaded with organic material, which are the distinguishing characteristics of raw OMWW [64]. The untreated OMWW used in the experiment was acidic (pH 4.37), saline with a high value for TS (102.67 g $L^{-1}$) and EC (8.15 dS/m), and had similar properties to those found in previous studies [13, 14, 16, 22, 65].

On the other hand, it had high contents of TOC (37.47 g $L^{-1}$), nitrogen (0.30 g $L^{-1}$), carbohydrates (16.24 g $L^{-1}$), and total lipids (8.53 g $L^{-1}$), reflecting the economic vision to define upcoming treatments for OMWW to partially detoxify it by reducing phenolic contents while conserving valorized organic compounds as biofertilizers for reuse in new crop agriculture. The total polyphenols were highly observed in the untreated OMWW with total phenolic (14.70 g $L^{-1}$), total flavonoids (9.83 g $L^{-1}$), and total tannins (4.06 g $L^{-1}$) values exceeding authorized standards limits [23]. Phosphorus, potassium, and calcium concentrations were also high in the untreated OMWW (0.25, 1.68, and 2.32 g $L^{-1}$, respectively), while sodium had the highest concentration at 3.31 g $L^{-1}$. Similar results have been reported by previous studies [11, 12, 26, 66]. Based on these data, partial OMWW detoxification treatments could be applied for irrigation and/or fertilization to improve agricultural crop productivity and maximize economic value through treatment of phenolic compounds as growth inhibitors at high concentrations while fulfilling sustainability and environmental preservation through OMWW valorization [12, 13, 23].

**Table 1** Physicochemical characteristics and elemental profile of raw OMWW

| Parameter | Mean | SD |
|---|---|---|
| pH | 4.37 | 0.09 |
| EC (dS.$m^{-1}$) | 8.15 | 0.23 |
| Density (g.cm-$^{3}$) | 1.11 | 0.01 |
| TS (g.$L^{-1}$) | 102.67 | 1.70 |
| TOC (g.$L^{-1}$) | 37.47 | 0.81 |
| Total Lipids (g.$L^{-1}$) | 8.53 | 0.34 |
| Total Carbohydrate (g.$L^{-1}$) | 16.24 | 0.84 |
| Total Proteins (g.$L^{-1}$) | 1.88 | 0.18 |
| Total phenols (g.$L^{-1}$) | 14.70 | 0.54 |
| Total flavonoids (g.$L^{-1}$) | 9.83 | 0.53 |
| Total tannins (g.$L^{-1}$) | 4.06 | 0.50 |
| TN (g.$L^{-1}$) | 0.30 | 0.03 |
| TP (g.$L^{-1}$) | 0.25 | 0.04 |
| K (g.$L^{-1}$) | 1.68 | 0.20 |
| Na (g.$L^{-1}$) | 3.31 | 0.16 |
| Ca (g.$L^{-1}$) | 2.32 | 0.23 |
| Mg (mg.$L^{-1}$) | 0.59 | 0.03 |
| Fe (mg.$L^{-1}$) | 0.66 | 0.03 |
| Cu (mg.$L^{-1}$) | 0.60 | 0.04 |
| Mn (mg.$L^{-1}$) | 0.16 | 0.01 |
| Zn (mg.$L^{-1}$) | 0.12 | 0.02 |

*n* = 3 and *SD* standard deviation

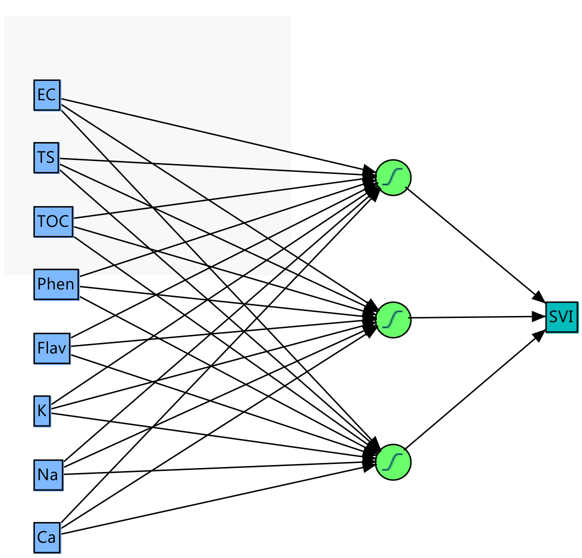


**Fig. 4** Structural diagram of NTanH(3) model of the neural network with 3 hidden factors predicting SVI from values of EC, TS, TOC, total phenols, total flavonoids, K, Na, and Ca

### Acid precipitation (T1)

In the raw OMWW, total polyphenols, total flavonoids, and total tannins had the highest percentages (14.70, 9.83, and 4.06 g $L^{-1}$, respectively). The acid precipitation significantly decreased the concentration of total phenols and flavonoids with decreasing pH value from 4.3 to 3.6, 3, and 2.6, while it showed significant difference in total tannin concentration at pH values 3.0 and 2.6 compared to that of raw value (4.3). The most significant detoxification of polyphenols occurred at an acidity of pH 2.6 (9.38, 5.23, and 2.98 g $L^{-1}$, respectively), as shown in Fig. 5. Therefore, acid precipitation at pH 2.6 is the most efficient and recommended pre-treatment for raw OMWW detoxification, with reductions in phenolic compounds, total flavonoids, and total tannins reaching 36.19%, 46.79%, and 26.42%, respectively, compared to other treatments (Fig. 6).

#### Fenton oxidation (T2)

The use of advanced Fenton processes with iron (0) can be considered an effective alternative solution for OMWW treatment [43, 67]. In our study, the optimal experimental conditions were as follows: a concentration of $H_2O_2$ at 10%, metallic iron in spiral form at a concentration of 16 g $L^{-1}$, a natural pH, and no dilution according to a previous study [67]. As illustrated in Fig. 5, the time-dependent oxidation reaction of polyphenols

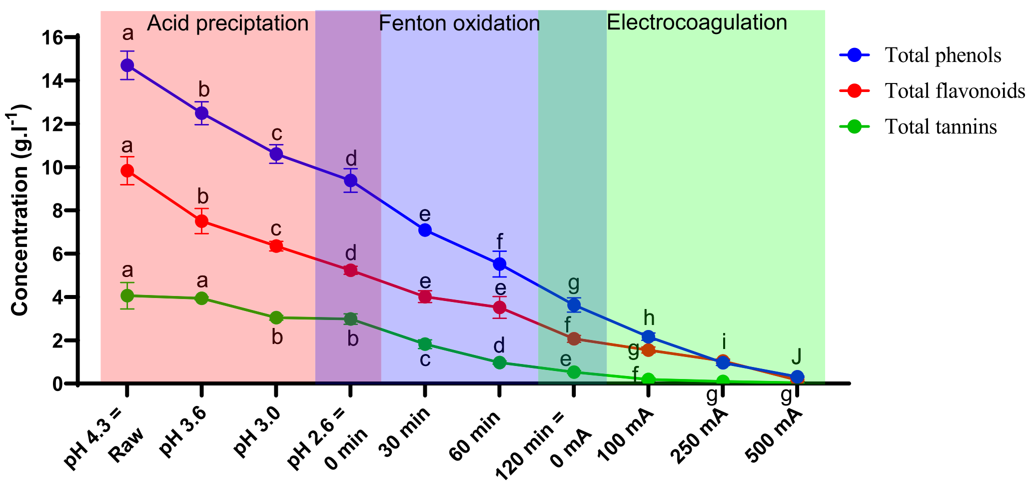


**Fig. 5** Effect of the different treatments on the concentration of total phenols, total flavonoids, and total tannins in OMWW. $n=3$ and mean ± standard deviation. The means with same letters in the same line show insignificant difference at $p \leq 0.05$

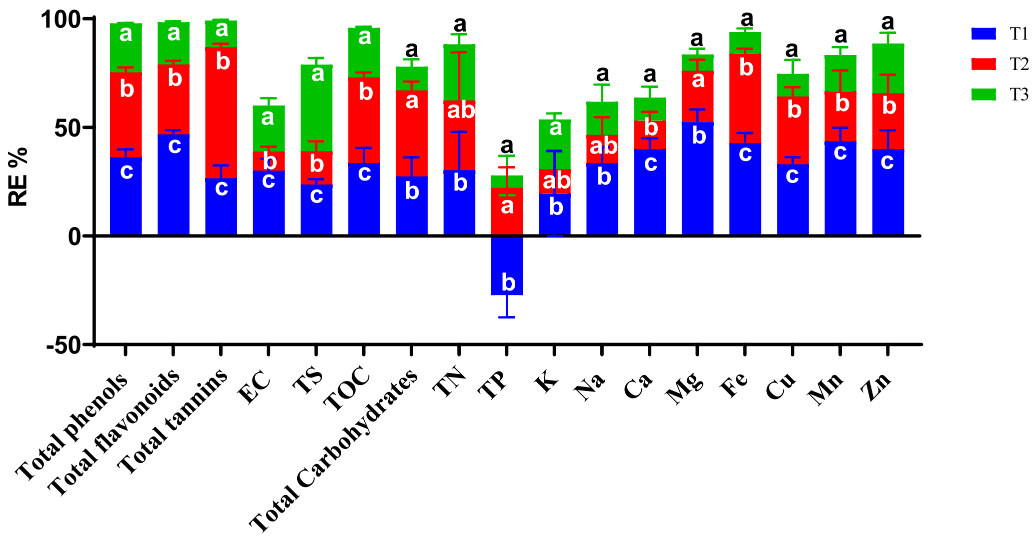


**Fig. 6** Removal efficiency (RE, %) of different parameters by T1 (acid precipitation at pH = 2.6), T2 (fenton oxidation for 120 min), and T3 (electrocoagulation by 500 mA). EC, TS, TOC, TN, and TP. Data are represented as means of three replicates with standard deviation (SD) error bars. The same letter in the same parameter indicates no significant difference at $p \leq 0.05$

showed a significant decrease in the total phenols and tannins with increasing oxidation reaction time from 0 to 30, 60, and 120 min, while no significant difference was detected between reaction time 30 and 60 min on the concentration of total flavonoids. The total phenols, flavonoids, and tannins had the lowest values after a Fenton reaction of 120 min compared to other treatments, with reduction percentages of 40.7%, 39.65%, and 17.41%, respectively. Therefore, the Fenton reaction detoxification process had the most significant effect at 120 min and is recommended for treating OMWW pre-treated with acid at pH 2.6 by improving destruction of phenolic compounds by 75.33% for total phenols, 78.98% for total flavonoids, and 86.85% for total tannins (Fig. 6) compared to raw OMWW, in agreement with results obtained by several authors [24].

### Electrocoagulation (T3)

Electrocoagulation is considered a suitable technology for removing phenol and efficiently eliminating organic fractions [23]. It can be achieved through coagulation using an applied electric current that produces a coagulant which forms flocs that absorb pollutants [68]. Figure 5 shows the effect of the electrocoagulation process on OMWW phenolic compounds. The total polyphenols and flavonoids significantly decreased with increasing current density (CD) to 100, 250, and 500 mA cm$^{-2}$, while no significant difference was observed between 250 and 500 mA cm$^{-2}$ on total tannins concentration. As observed, total polyphenols, total flavonoids, and total tannins had the highest OMWW detoxification percentage at a CD of 500 mA cm$^{-2}$ with the lowest contents of phenolic compounds (0.31 g L$^{-1}$), flavonoids (0.16 g L$^{-1}$), and tannins (0.03 g L$^{-1}$) compared to other treatments. Therefore, the most recommended detoxification treatment is at a current density of 500 mA cm$^{-2}$ for up to 120 min which detoxified phenolic compounds by 97.87% for total phenols, 98.37% for total flavonoids, and 99.18% for total tannins compared to raw OMWW, as shown in Fig. 6. Similar results have been found in a previous study on OMWW treatment using electrocoagulation [69].

### Integrated treatment technology (T1, T2, and T3)

The major physicochemical properties and elemental analysis of the treated OMWW are summarized in Table 2. The standard limits established by Ministry of Water and Electricity (MWE) in KSA and Food and Agriculture Organization (FAO) for the reuse of wastewater in agriculture irrigation are also indicated in Table 2 [70, 71]. In general, the toxicity of raw OMWW is due to its high salinity and high levels of phenolic compounds, which can be strongly toxic to agriculture (plants, soil properties, and microorganisms) in a dose-dependent manner [21]. Previous studies have shown

**Table 2** Physicochemical properties, some organic components, elemental profile of raw and treated OMWW and national and international standard limits

| Parameter | Raw OMWW | | T1 (pH 2.6) | | T2 (ZVIF 120 min) | | T3 (EC 500 mA.cm$^{-2}$) | | Standard limits | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | MWE [70] | FAO [71] |
| EC (dS.m$^{-1}$) | 8.15$^{a}$ | 0.23 | 5.71$^{b}$ | 0.36 | 4.99$^{c}$ | 0.16 | 3.26$^{d}$ | 0.22 | – | 3.00 |
| TS (g.L$^{-1}$) | 102.67$^{a}$ | 1.70 | 78.33$^{b}$ | 2.05 | 62.67$^{c}$ | 3.86 | 21.67$^{d}$ | 2.49 | 2.54 | 2.00 |
| TOC (g.L$^{-1}$) | 37.47$^{a}$ | 0.81 | 24.88$^{b}$ | 2.11 | 10.11$^{c}$ | 0.68 | 1.58$^{d}$ | 0.17 | – | – |
| Total Carbohydrates (g.L$^{-1}$) | 16.07$^{a}$ | 0.90 | 11.66$^{b}$ | 1.14 | 5.31$^{c}$ | 0.53 | 3.55$^{c}$ | 0.45 | – | – |
| Total Phenols (g.L$^{-1}$) | 14.70$^{a}$ | 0.54 | 9.38$^{b}$ | 0.44 | 3.63$^{c}$ | 0.27 | 0.31$^{d}$ | 0.02 | – | – |
| Total Flavonoids (g.L$^{-1}$) | 9.83$^{a}$ | 0.53 | 5.23$^{b}$ | 0.15 | 2.07$^{c}$ | 0.13 | 0.16$^{d}$ | 0.04 | – | – |
| Total tannins (g.L$^{-1}$) | 4.06$^{a}$ | 0.50 | 2.98$^{b}$ | 0.20 | 0.53$^{c}$ | 0.06 | 0.03$^{c}$ | 0.01 | – | – |
| TN (g.L$^{-1}$) | 0.301$^{a}$ | 0.029 | 0.210$^{b}$ | 0.043 | 0.113$^{c}$ | 0.054 | 0.035$^{c}$ | 0.011 | 0.015 | 0.030 |
| TP (g.L$^{-1}$) | 0.254$^{b}$ | 0.043 | 0.323$^{a}$ | 0.021 | 0.198$^{bc}$ | 0.020 | 0.183$^{c}$ | 0.019 | – | – |
| K (g.L$^{-1}$) | 1.677$^{a}$ | 0.198 | 1.350$^{ab}$ | 0.268 | 1.162$^{bc}$ | 0.119 | 0.778$^{c}$ | 0.039 | – | – |
| Na (g.L$^{-1}$) | 3.307$^{a}$ | 0.164 | 2.200$^{b}$ | 0.198 | 1.770$^{b}$ | 0.222 | 1.263$^{c}$ | 0.212 | – | – |
| Ca (g.L$^{-1}$) | 2.320$^{a}$ | 0.226 | 1.393$^{b}$ | 0.095 | 1.090$^{bc}$ | 0.078 | 0.843$^{c}$ | 0.095 | – | 0.40 |
| Mg (mg.L$^{-1}$) | 0.587$^{a}$ | 0.034 | 0.279$^{b}$ | 0.028 | 0.141$^{c}$ | 0.025 | 0.097$^{c}$ | 0.012 | – | 60 |
| Fe (mg.L$^{-1}$) | 0.663$^{a}$ | 0.033 | 0.379$^{b}$ | 0.025 | 0.107$^{c}$ | 0.012 | 0.040$^{d}$ | 0.009 | 5.00 | 5.00 |
| Cu (mg.L$^{-1}$) | 0.597$^{a}$ | 0.045 | 0.400$^{b}$ | 0.016 | 0.213$^{c}$ | 0.021 | 0.151$^{c}$ | 0.031 | 0.40 | 0.20 |
| Mn (mg.L$^{-1}$) | 0.160$^{a}$ | 0.008 | 0.090$^{b}$ | 0.008 | 0.053$^{c}$ | 0.012 | 0.027$^{d}$ | 0.005 | 0.20 | 0.20 |
| Zn (mg.L$^{-1}$) | 0.117$^{a}$ | 0.017 | 0.070$^{b}$ | 0.008 | 0.040$^{c}$ | 0.008 | 0.013$^{d}$ | 0.005 | 4.00 | 2.00 |

$n = 3$ and *SD* standard deviation. The means with same letters in the same column show insignificant difference at $p \leq 0.05$

the positive impact of integrating different processes such as Fenton oxidation, coagulation, and electrocoagulation on the degradation of organic matter and detoxification of phenols [72–74]. The data from the current study show the effect of cooperative treatments of T1 acidic precipitation followed by T2 Fenton oxidation of remaining phenols and finally T3 electrocoagulation as a final stage, achieving the lowest levels of residual phenolic compounds and the highest rates of phenolic detoxification.

As observed in Fig. 6, the cooperative treatments T1 followed by T2, and T3 showed significant effect on the removal ratio of the different parameters, while no significant difference between the removal ratio of T2 and T3 for the parameters, total carbohydrates, TN, TP, K, Na, and Mg. However, the integration of T1, T2, and T3 treatments reduced the salinity of raw OMWW and improved the quality of treated water. Reductions of around 95.78%, 60%, and 78.90% in TOC, EC, and TS, respectively, were achieved (Fig. 6). Detoxification reached 97.90% for total phenolic, 98.37% for total flavonoids, and 99.18% for total tannins (Fig. 6). Similar results were found in a previous study that showed a 92% reduction in phenolic content by electrocoagulation process for OMWW [74]. Another study using Fenton oxidation process showed reductions of 96.90% and 47.50% in total phenolic compounds and TOC, respectively [24]. A reduction of 96.40% for total phenols was also achieved by a combined Fenton oxidation and coagulation process as found by previous research [72]. Therefore, the current study showed higher detoxification efficiency compared to previous studies.

On the other hand, phosphorus, potassium, and calcium concentrations were highest in raw OMWW at 0.25, 1.68, and 2.32 g $L^{-1}$, respectively, and decreased to 0.18, 0.78, and 0.10 g $L^{-1}$, respectively, after treatment. The acid precipitation stage increased total phosphorus by 27.23% due to phosphorus impurities in sulfuric acid; however, T2 and T3 reduced it by 22.10% and 27.82%, respectively. As shown in Fig. 6, the triphasic detoxification technology reduced total nitrogen (after T1 = 30.32%, T2 = 62.39%, and T3 = 88.27%), potassium (after T1 = 19.47%, T2 = 30.69%, and T3 = 53.63%), calcium (after T1 = 39.94%, T2 = 53.02%, and T3 = 63.65%), and magnesium (after T1 = 52.44%, T2 = 76.02%, and T3 = 83.52%). A significant decrease in sodium and heavy metals was also observed by the integrated treatments with removal ratios reaching 61.79%, 93.92%, 74.64%, 83.33%, and 88.57% for Na, Fe, Cu, Mn, and Zn, respectively (Fig. 6). The results of heavy metals after the applied treatments showed lower values than the maximum allowable contaminant levels for both MWE and FAO standards, see Table 2.

Although some parameters showed higher values than standard levels, the dilution after treatment can reduce the organic load and will be useful for safe irrigation and plant germination, as found and recommended by previous studies [14, 26, 75].On the other hand, bioactive constituents and organic matter in the treated OMWW contains useful compounds, namely, polysaccharides, lipids, and proteins, in addition to substantial potassium, nitrogen, phosphorus, and other elements, which could be applied as natural pesticides as an alternative to harmful agrochemicals [75]. Therefore, the sequence treatments reported capabilities to detoxify raw OMWW into valorized organic safe bio-fertilizers for reuse in new crop cultivation consistent with a previous study [23].

### Polyphenols analyzed by HPLC profile

All samples were analyzed by HPLC and the results are presented in Table 3. As expected, raw OMWW had the highest phenolic content, with Chlorogenic acid having the highest content (1967.68 mg $L^{-1}$) followed by Gallic acid (302.82 mg $L^{-1}$), Querectin (195.10 mg $L^{-1}$), and Pyro catechol (150.17 mg $L^{-1}$), which were many times higher than the other detoxified treatments. Other compounds were detected, especially in T1, such as Coumaric acid (0.53 mg $L^{-1}$), Vanillin (11.77 mg $L^{-1}$), and Ferulic acid (11.55 mg $L^{-1}$). The T3 (500 mA cm−2)

**Table 3** HPLC analysis of raw and treated OMWW

| Compounds (mg.$L^{-1}$) | Treated OMWW | | | |
|---|---|---|---|---|
| | Raw | T1 (2.6 pH) | T2 (120 min) | T3 (500 mA $cm^{-2}$) |
| Gallic acid | 302.82 | 251.01 | 64.13 | 6.62 |
| Chlorogenic acid | 1967.68 | 831.77 | 54.96 | 1.57 |
| Catechin | ND | 5.95 | ND | ND |
| Methyl gallate | 15.98 | 65.22 | 11.80 | 1.99 |
| Coffeic acid | 13.65 | 9.07 | 0.75 | 0.50 |
| Syringic acid | 30.62 | 8.12 | 2.19 | 0.72 |
| Pyro catechol | 150.17 | 54.07 | ND | ND |
| Rutin | 18.33 | 2.08 | ND | ND |
| Ellagic acid | 5.26 | 6.15 | ND | ND |
| Coumaric acid | ND | 0.53 | ND | 0.03 |
| Vanillin | ND | 11.77 | 5.32 | ND |
| Ferulic acid | ND | 11.55 | 6.55 | 2.58 |
| Naringenin | ND | ND | ND | 0.05 |
| Daidzein | 4.03 | ND | 25.71 | ND |
| Querectin | 195.10 | 63.36 | 1.18 | ND |
| Cinnamic acid | 4.77 | 0.26 | ND | ND |
| Apigenin | 4.73 | 1.32 | 0.73 | ND |
| Kaempferol | ND | ND | ND | ND |
| Hesperetin | 7.37 | ND | ND | ND |

*ND* Not detected

sample showed the lowest identified phenolic compounds compared to other treatments, with the presence of Chlorogenic acid (1.57 mg $L^{-1}$), Gallic acid (6.62 mg L-1), Querectin (0.00 mg L-1), and Pyro catechol (0.00 mg $L^{-1}$). Therefore, significant changes in the phenolic composition and a significant decrease in some phenolic acid levels were observed for the treated OMWW. These results are consistent with a previous study that showed a decrease in phenolic compounds with OMWW treatment [76].

### OMWW phytotoxicity

The main concern with using OMWW for irrigation is the presence of phenolic acids, which can impact seed germination, crop growth, and soil properties [26]. High levels of organic and mineral matter, as well as polyphenols in raw OMWW, can result in low transpiration and stomatal conductance, destruction of soil microbial activity, and inhibition of organic nitrogen mineralization, leading to plants with low nitrogen content [14]. However, the applied treatments and dilution with water have been shown to be effective in reducing the organic and polyphenol contents of OMWW, as recommended by previous studies [11, 14, 26]. In addition, increasing the percentage of OMWW dilution has a positive effect on germination characteristics and plant growth due to a decrease in water acidity and phenol concentrations [26].

Therefore, physicochemical treatment (T1, T2, and T3) and dilution at factors (0%, 25%, and 50% of OMWW) can improve the quality of OMWW. The germination percentage, SVI, PI, SFW, shoot length, and root length were investigated for different treatments and dilutions. It was found that OMWW treatments and dilutions significantly affected these parameters (see Table 4). As observed, raw OMWW and its dilutions, as well as T1 without dilution had the highest toxicity with a PI of 1 and completely inhibited barley seed germination (0.0% germination). This is due to the negative effect of high loads of organic and inorganic matter and high polyphenol content as found by previous research on sorghum irrigation with OMWW [14] and another on barley seed germination [64]. Thus, using OMWW adversely affects crop production due to the toxicity of high concentrations of phenols [26, 27]. The highest germination percentage and SVI were found at 25% T1 followed by 25% T2 and control (tap water), which all showed no toxicity with a PI of zero. On the other hand, T2 without dilution showed the lowest germination (40.67%) and SVI (346.17).

The highest root and shoot length were found at 25% T1 followed by control and 25% T2. In addition, FW recorded the highest value at 25% T1 (123.33 mg). Thus, treatment at 25% T1 showed the best results according to different parameters. Therefore, it can be considered the most appropriate treatment for barley irrigation as it is less expensive compared to other treatments. These results are consistent with previous studies [14, 22, 26, 64].

### Biological enzymes activities

Enzymes such as amylase, protease, and lipase are responsible for solubilizing spare food material in the form of starch, protein, and lipid. Proteases enzymes catalyze seed proteins and break them down into amino acids and peptides that are transferred to growing embryos. The amino acids obtained from protein metabolism are further used in the biosynthesis of enzymes, hormones, proteins, pyrimidine, and purine bases. Alpha

**Table 4** Effects of treated and untreated OMWW and their dilutions on the germination ratio, shoot and root length, SFW, PI, and SVI

| OMWW | Germination (%) | | Shoot length (cm) | | Root length (cm) | | SFW (mg) | | PI | | SVI | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Raw | 0.00$^{f}$ | 0.000 | 0.00$^{g}$ | 0.000 | 0.00$^{f}$ | 0.000 | 0.00$^{f}$ | 0.000 | 1.00$^{a}$ | 0.000 | 0.00$^{h}$ | 0.000 |
| T1 | 0.00$^{f}$ | 0.000 | 0.00$^{g}$ | 0.000 | 0.00$^{f}$ | 0.000 | 0.00$^{f}$ | 0.000 | 1.00$^{a}$ | 0.000 | 0.00$^{h}$ | 0.000 |
| T2 | 40.67$^{e}$ | 1.247 | 6.33$^{f}$ | 0.236 | 2.17$^{e}$ | 0.236 | 73.00$^{e}$ | 0.000 | 0.64$^{b}$ | 0.050 | 346.17$^{g}$ | 33.060 |
| T3 | 52.33$^{d}$ | 2.055 | 7.17$^{e}$ | 0.236 | 2.50$^{de}$ | 0.408 | 83.00$^{d}$ | 0.002 | 0.58$^{bc}$ | 0.090 | 505.17$^{f}$ | 32.210 |
| 0.50 Raw | 0.00$^{f}$ | 0.000 | 0.00$^{g}$ | 0.000 | 0.00$^{f}$ | 0.000 | 0.00$^{f}$ | 0.000 | 1.00$^{a}$ | 0.000 | 0.00$^{h}$ | 0.000 |
| 0.50 T1 | 62.33$^{c}$ | 1.247 | 7.33$^{e}$ | 0.236 | 3.17$^{d}$ | 0.236 | 90.67$^{c}$ | 0.001 | 0.47$^{c}$ | 0.050 | 655.00$^{e}$ | 47.130 |
| 0.50 T2 | 85.33$^{b}$ | 1.247 | 11.50$^{d}$ | 0.408 | 3.17$^{d}$ | 0.236 | 106.00$^{b}$ | 0.010 | 0.47$^{c}$ | 0.050 | 1251.50$^{d}$ | 29.790 |
| 0.25 Raw | 0.00$^{f}$ | 0.000 | 0.00$^{g}$ | 0.000 | 0.00$^{f}$ | 0.000 | 0.00$^{f}$ | 0.000 | 1.00$^{a}$ | 0.000 | 0.00$^{h}$ | 0.000 |
| 0.25 T1 | 91.33$^{a}$ | 2.494 | 15.50$^{a}$ | 0.408 | 7.33$^{a}$ | 0.471 | 123.33$^{a}$ | 0.005 | 0.00$^{e}$ | 0.000 | 2083.67$^{a}$ | 51.690 |
| 0.25 T2 | 90.67$^{a}$ | 0.943 | 13.33$^{c}$ | 0.471 | 4.33$^{c}$ | 0.236 | 90.67$^{c}$ | 0.001 | 0.28$^{d}$ | 0.050 | 1602.33$^{c}$ | 89.200 |
| Tap water | 90.33$^{a}$ | 1.247 | 14.83$^{b}$ | 0.624 | 6.00$^{b}$ | 0.816 | 95.67$^{c}$ | 0.001 | 0.00$^{e}$ | 0.140 | 1881.67$^{b}$ | 9.104 |

*n* = 3 and *SD* standard deviation. The means with same letters in the same column show insignificant difference at $p \leq 0.05$

amylase catalyzes starch to provide the energy required for embryo growth and development. Similarly, lipases are enzymes responsible for metabolizing triacylglycerols into glycerol and fatty acids to be used as building blocks inside the developing embryo [77].

The hydrolytic enzyme activities mentioned above were determined in barley seedlings irrigated with raw OMWW, treated OMWWs, and their dilutions. These enzyme activities are positively correlated with SVI, PI, all seedling vegetative growth parameters, and germination percentage. Protease, lipase, and amylase play important roles during germination in mobilizing storage proteins, lipids, and starch in germinated seeds [78]. Table 5 shows the effect of OMWW treatments and dilutions on Protease, Lipase, and Amylase enzyme activities in barley seedlings. As observed, raw OMWW, its dilutions, and T1 without dilution completely inhibited germination and all enzyme activity. However, 25% T1 had the highest enzyme activity compared to control and all treatments. Protease and lipase had the highest activity at 25% T1 followed by control and 25% T2. In the α-amylase activity test, the highest enzyme activity was reported with 25% T1 followed by 25% T2 and control at values of 0.870 mg maltose/g FW, 0.807 mg maltose/g FW, and 0.770 mg maltose/g FW, respectively. Meanwhile, the lowest activities were recorded by T2 without dilution.

This indicates that the highest enzyme activities, significant germination, and lowest PI were achieved with 25% T1 compared to all treatments. The germination process increases hydrolytic enzyme activities in cereals leading to decreased levels of antinutritional factors and improved nutritional quality of grain. Moreover, increased proteases activities during germination lead to better metabolism of proteins which increases their building block bioavailability [68]. The contents of essential amino acids (lysine, methionine, leucine, isoleucine, threonine, phenylalanine, and valine) also increase during germination resulting in improved nutritional quality of proteins in barley seeds [79].

## Factor and cluster analysis followed by neural modelling

Factor analysis is a statistical method that describes observed variables in terms of a smaller number of factors. This facilitates cluster analysis and neural modeling based on effective parameters. In the analysis you mentioned, the principal axis was used as the factoring method, with prior communality (principal components, diagonal = 1), Varimax as the rotation method, and the two highest factors with Eigenvalues of 32.63 and 13.21 (Fig. 7).

By suppressing absolute loading values less than 0.5, only two parameters, Daidzein and Naringenin, were neglected for the subsequent analyses. Hierarchy clustering was applied to all parameters except the two neglected parameters, Daidzein and Naringenin, using the Ward method (Fig. 8).

Figure 8 shows a two-dimensional clustering dendrogram and heatmap that summarizes the similarity among ten types of irrigating water: raw OMWW, its dilutions (50% and 25%), treated OMWWs (T1, T2, and T3), and their dilutions (50% T1 and T2; 25% T1 and T2). Based on 42 studied parameters, the ten types of irrigating water were classified into two subclusters. The first subcluster, the high phytotoxic types, includes

**Table 5** Effects of treated and untreated OMWW and their dilutions on the Protease, Lipase and α-amylase enzyme's activities in Barley plantlets

| OMWW | Proteases Activity (U) | | Lipases Activity (U) | | α-Amylase activity (U) | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| Raw | ND | ND | ND | ND | ND | ND |
| T1 | ND | ND | ND | ND | ND | ND |
| T2 | 82.70[d] | 1.700 | 25.70[e] | 3.700 | 0.137[e] | 0.025 |
| T3 | 72.70[e] | 3.900 | 103.30[d] | 2.500 | 0.610[d] | 0.045 |
| 0.5Raw | ND | ND | ND | ND | ND | ND |
| 0.5T1 | 151.30[c] | 12.400 | 112.70[c] | 6.500 | 0.610[d] | 0.029 |
| 0.5T2 | 160.00[c] | 5.100 | 131.70[b] | 3.900 | 0.737[c] | 0.033 |
| 0.25Raw | ND | ND | ND | ND | ND | ND |
| 0.25T1 | 211.30[a] | 4.000 | 151.30[a] | 2.600 | 0.870 [a] | 0.016 |
| 0.25T2 | 187.30[b] | 2.500 | 145.70[a] | 1.700 | 0.807[b] | 0.033 |
| Tap water | 203.30 [a] | 4.000 | 148.30[a] | 2.500 | 0.770 [bc] | 0.051 |

*n* = 3 and *SD* standard deviation. The means with same letters in the same column show insignificant difference at $p \leq 0.05$

*ND* Not detected

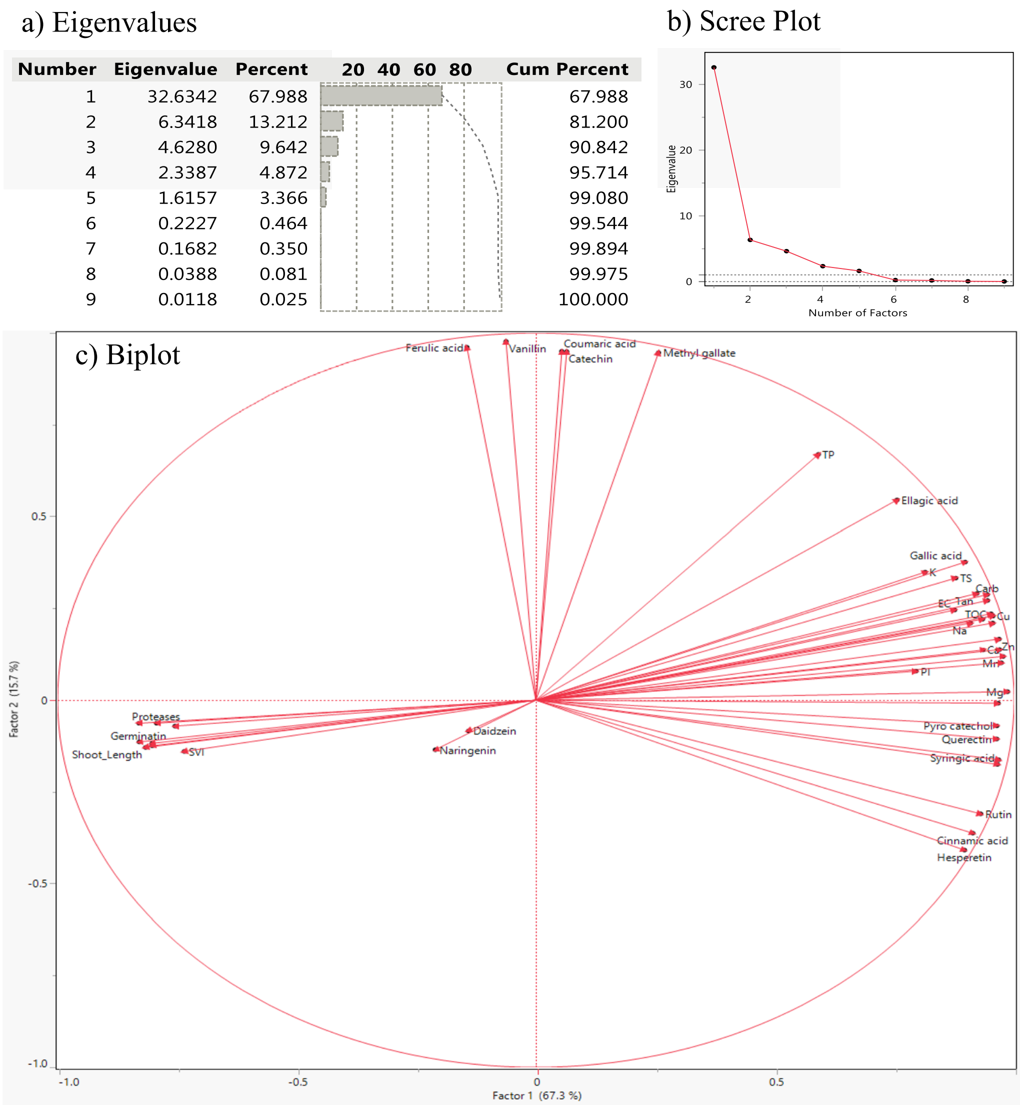


**Fig. 7** Factor analysis **a** Eigenvalues, **b** Scree plot and **c** Biplot

raw OMWW, T1, 0.5 raw, and 0.25 raw. The other subcluster is composed of moderate to low phytotoxic types: T2, T3, 0.5T1, 0.5T2, 0.25T2, and 0.25T1.

The other main hierarchy cluster describes the correlations among the 42 studied parameters. This cluster is divided into two subclusters. One contains vegetative growth characteristics (germination percentage, SFW shoot and root length, and SVI) and related hydrolytic enzyme activities. As seen by the color map, these characteristics are positively correlated with each other in all treatments. Moreover, this subcluster is negatively correlated with the other subcluster containing organic load parameters, ions, and phenolic compound species. Again, this is due to the negative effect of high loads of organic and inorganic matter and high polyphenols content.

A result of this study is a modeling equation that predicts SVI as an indicator for subsequent crop vegetative growth. This equation depends on three factors in the hidden layer of the neural network: H1_1, H1_2, and H1_3. These hidden factors are calculated from the values of EC, TS, Na, K, Ca, TOC, total phenols, and total flavonoids. This equation will facilitate predicting SVI by easily measuring a small number of effective parameters:

$$H1\_1 = TanH\ ((-\ 0.39) + 0.37 \times Ca + -\ 0.017 \times EC + -0.016 \times Flav + -1.24 \times K + 0.23 \times Na + -\ 0.00031 \times Phen + 0.019 \times TOC + 0.004 \times TS);$$

$$H1\_2 = TanH\ (0.031 + 0.15 \times Ca + 0.19 \times EC + -\ 0.199 \times Flav + 0.24 \times K + -0.83 \times Na + 0.14 \times Phen + -0.0081 \times TOC + 0.0056 \times TS);$$

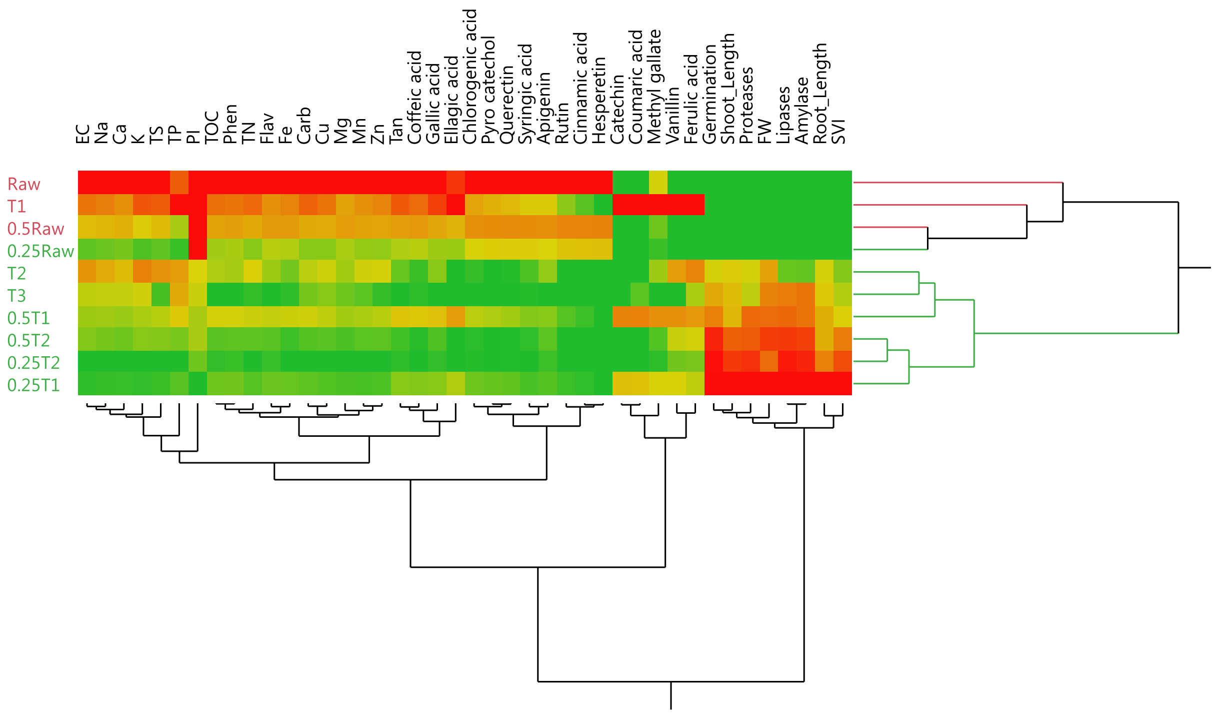


**Fig. 8** Two-dimensional dendrogram and a heatmap describing, in hierarchy clustering shape, the relationships among raw OMWW, treated OMWW and their dilutions from dimension and among all effective studied parameters in the other dimension. Colors' gradient goes from red to yellow to green, from the highest values to middle to the lowest values

$H1\_3 = TanH\ ((-\ 0.87) + 0.77 \times Ca + 0.099 \times EC + 0.080 \times Flav + -0.023 \times K + -0.29 \times Na + -0.025 \times Phen + 0.074 \times TOC + -0.0029 \times TS)$;

Predicted $SVI\_1 = 2283.31 + 3894.75 \times H1\_1 + 3973.23 \times H1\_2 + -21 \times H1\_3)$;

## Conclusion

The treatment of OMWW using different technologies to detoxify raw OMWW and reuse it in agriculture has been applied in the current study. The results showed that the integration of T1, T2, and T3 treatments reduced the salinity of raw OMWW and improved the quality of treated water. Reductions of around 60% in EC, 95.90% in TOC and 78.90% in TS were achieved. Detoxification reached 97.90% for total phenolic compounds, 98.37% for total flavonoids, and 99.18% for total tannins. A significant decrease in heavy metals was also observed with removal ratios reaching 98.64%, 94.80%, 96.88%, and 95.72% for Fe, Cu, Mn, and Zn, respectively.

Phenolic compounds have health benefits due to their antioxidant, anti-inflammatory, anticancer, cardio-protective, and hypoglycemic properties. Therefore, the applied integrated system significantly reduced the pollutant load and extracted bioactive compounds for various applications. Moreover, diluted treated OMWW enhanced seed germination and plant growth, where acid precipitation treatment of OMWW at 25% dilution (0.25 T1) reported 0% phytotoxicity and significantly improved plant growth. As 0.25 T1 is a simple and low-cost method, its potential application at a higher scale can detoxify raw OMWW into valorized organic safe bio-fertilizers, which could be applied as natural pesticides as an alternative to harmful agrochemicals, in addition it approved to have a significant impact on barely germination and growth. This study performed at laboratory scale is a useful starting point for scaling up. Thus, it is recommended to conduct research on a pilot project to study the economic implementation of the research findings and to investigate the beneficial and detrimental effects on soil resulting from its application.

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**Author contributions**

RA, RY, OA, MM, MMA, NS: conceptualization, methodology, MM: software (Statistical and ANN), RY, OA, MMA, NS: data curation, RY, OA, NS: writing—original draft preparation. RA, RY, OA, MM, MMA, NS: visualization, investigation, MMA: supervision. RA, RY, OA, MM, MMA, NS: writing—reviewing and editing. All authors read and approved the manuscript.

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## Declarations

**Ethics approval and consent to participate**
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**Consent for publication**
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**Competing interests**
The authors declare that they have no conflicts of interest.

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