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Assessment of the Impact of Spreading Raw and Neutral pH Olive Oil Mill Wastewater on Agricultural Land

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Abstract

The primary byproducts of the olive oil industry consist of pomace and Olive Mill Wastewaters (OMWs). Pomace can be reclaimed in several areas without adverse environmental effects, however, OMWs pose a significant waste challenge, necessitating specialized and costly treatment. Numerous studies have examined the mineral and organic composition of OMWs, substantiating

their potential for soil enrichment, antioxidant production, and antimicrobial applications. Our study aims to evaluate soil fertilization by applying untreated Olive Mill Wastewater at a neutral pH in incremental doses (50 m3/ha, 80 m3/ha, and 100 m3/ha) on land cultivated with olive trees, aromatic plants, and medicinal herbs. We employed a conventional method for monitoring and applying varying OMW doses. Field trials revealed that optimal fertilization outcomes were achieved with neutral pH OMW. Doses of 50 m^3/ha and 80 m^3/ha exhibited favorable yields within the initial months of cultivation, whereas the 100 m^3/ha dose only germinated after the fifth month of cultivation. Notably, crops treated with raw OMW germinated only after ten months. OMWs hold potential as organic fertilizers for alkaline soil following neutralization treatment, thus mitigating potential phytotoxicity associated with acidity and polyphenols.

Keywords: Spreading, Richness of Olive Mill Wastewaters, Valorization, Soil Fertilization.

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1. Introduction

The production of olive oil encompasses both traditional and modern methods, yielding a liquid byproduct known as Olive Mill Wastewater (OMWW) or vegetation water. Typically, these byproducts are directly discharged into natural environments, resulting in adverse environmental impacts. Due to their inherent acidity and phenolic content, they remain non-biodegradable and are typically stored in evaporation ponds [1-2]. These phenolic compounds, while acting as antimicrobials [3], hinder the natural degradation of these effluents. Moreover, the richness of OMWW in potassium, mineral salts, organic matter, sodium, and magnesium [4] underscores their potential value as a nutrient source for soil enrichment [5]. This can be achieved through either direct or indirect application, such as composting on a carbonaceous substrate. Utilizing effluents from the olive oil extraction industry in agriculture offers numerous advantages. It promotes water conservation and sustainable development, unlike untreated wastewater, as it poses no harm to agriculture or human health, being free of harmful pathogens (indicators of fecal pollution) [6–9]. OMWW is also employed as antibacterial and biopesticides [3,10]. Analyses conducted by the French Chamber of Agriculture indicate that OMWW holds promise as an organic soil amendment [11], considering its chemical composition, both in terms of mineral and organic content [12]. According to [13], applying OMWW at a rate of 100 m3/ha/year contributes on average 400 to 1800 kg/ha of organic matter (OM), 50 to 200 kg/ha of total nitrogen, 65 to 200 kg/ha of phosphorus, 350 to 1100 kg/ha of potash, and 15 to 200 kg/ha of calcium [14]. Additionally, OMWW consists of approximately 83.2% water, 15% organic matter, and 1.8% mineral matter. [15] argued that annual OMWW application to olive groves enhances the physicochemical properties and biological activity of the soil, provided good spreading practices, such as appropriate doses and timing, are observed.

Research by [16–20] demonstrated that OMWW application to olive trees increased olive production per tree.

Similarly, OMWW application to plots cultivated with corn and grapes improved agricultural yields, particularly for doses exceeding 30 m3/ha [21–23]. Moreover, [24] conducted a study on the effects of OMWW application to soils cultivated with olive trees, finding that a dose of 100 m3/ha had no toxic effects on soil microflora, whereas a dose of 400 m3/ha led to a 50% decrease in yield. [25] suggested that land spreading of OMWW at rates of 50 - 100 m3/ha could be considered a means of waste disposal with potential fertilization value, recommending application rates of 30 and 100 m^3/ha.

In this study, we conducted trials applying OMWW on an organically certified farm cultivated with olive trees, aromatic, and medicinal plants. Field trials and monitoring enabled us to identify best practices and determine the optimal, environmentally acceptable dose of OMWW for application to fine-textured silty-clay-sandy soils, based on vegetation yield.

2.Materials and methods

2.1. Sampling and characterization of Olive Mill Wastewaters (OMW).

Our Olive Mill Wastewaters are taken from a modern Oil mill with a continuous system by three-phase centrifugation, located near the city of Meknes Morocco (Organic farm), during the 2018-2019 olive growing campaign. The crunched olives are of the Moroccan Picholine variety. The samples were taken directly from the wastewater discharge line in sterile cans and quickly transported in a cooler to the laboratory for analysis, to avoid any change in their physicochemical and microbiological properties.

2.2. Physic-chemical parameters

Selected physic-chemical properties are mentioned in (table1), including: pH using pH-meter calibrated with buffer solutions of pH 4, 7 and 10 ; Salinity expressed in % and Conductivity in µs/cm are determined by (multi-parameter conductivity meter, Model CONSORT C831; Organic Matter is the difference in weight between dry matter and mineral matter obtained by calcinations at 550°C; Mineral Matter represents the mineral fraction obtained by complete calcinations of dry matter at 550°C for 2h; Total Nitrogen Kjeldhal we have proceeded to hot mineralization of the sample with concentrated sulfuric acid and catalyst (Cu) (6,25% as CuSO4, 5H2O). After adding soda the product of mineralization which releases nitrogen in the form of ammonia, it is entrained with steam and trapped in boric acid and then titrated with hydrochloric acid [26]; Total Sugars we are based on the method of dinitrosalicylic acid (DNS) [27], the OMW are diluted to 1/3, after preparation of the reagents and the samples, we carried out the reading by a spectrophotometer at an optical density of 530nm. The concentration of total sugars is determined from a calibration curve of a mixed solution of glucose and fructose; Total phenols: are determined by colorimetric technique of Folin-Ciocalteu described by [28] the concentration is deduced from the calibration curve at an optical density of 760nm give, by a spectrophotometer; Chemical Oxygen Demand is determined by the oxidation method at 105°C for 2 hours, using a strang oxidant (potassium dichromate) in a acidic medium (H2SO4) and in the presence of silver sulfate considered as a catalytic converter as well as mercury sulfate. As a complexing agent for chlorides. At the end of the oxidation, the COD of our samples is measured by a UV/ Visible Spectrophotometer at a wavelength of 620nm. And Biological Oxygen Demand, we are based on the respirometry method in chambers thermostatically controlled at 20°C, in the dark for 5 days (AFNOR, T90-103).

HPLC analysis of phenolic compounds

Liquid chromatography was used to analyze the OMW phenols using internal standard calibration method. 10 µL of the samples were injected into the HPLC system, which was equipped with a C18 column (4.6 mm × 250 mm) at 40°C and flow rate of 0.7 mL/min for a total running duration of 60 minutes using phosphoric acid (A) and Acetonitrile (B) as mobile phase. The phenols were analyzed at 280 nm, and identification was achieved by comparing the peak retention time and spectra to those of commercial standards.

2.3. Microbiological parameters

Preparation of dilutions:

We performed a cascade dilution series in sterile physiological water in test tubes from the10^{-1} dilution to the 10^{-6} dilution. We took 1ml of the sample undergoes homogenization, puts it in 9ml of sterile physiological water. 1ml of each dilution is deposited in three sterile Petri diches, and approximately 15ml to 20ml of sterile agar is poured. After homogenization of the boxes by manual string, they are incubated in an oven. The tests are repeated 3times, and the results are expressed in CFU (Colony-Forming Units). Microbiological parameters determined of OMW are: Total Aerobic Microbial Flora; we proceeded to count this load on a PCA medium (Plate Count Agar), incubated at 37°C for 48hours. Total and Fecal Coliforms; is carried out on an EMB medium (Eosin Methylene Blue), after incubation for 48hours at 37°C for total coliforms and at 44°C for fecal coliforms. Fecal Streptococci; is done on Litsky medium, followed by incubation at 37°C for 48 hours. Staphylococci; are carried out on Chapman medium after incubation at 37°C for 48 hours. Lactic Bacteria; The detection and counting of lactic acid bacteria are generally done on a MRS medium (Man Rogosa Sharpe, Difco, Detroit, USA); incubation at 30°C for 24 hours. Yeasts and Molds; The search for yeasts and molds is carried out on the medium (Sabouraud Chloramphenicol Agar), after incubation for 48 hours at 30°C for the Yeasts and 5-7 days for the molds. Pseudomonas: The count of pseudomonas is carried out on the pseudomonas agar medium, followed by incubation at 37°C for 24 hours.

2.4. Preparation and neutralization of Olive Mill Wastewaters

The samples used in soil fertilization have undergone another centrifugation to remove all residual oils, to ensure good aeration of the agricultural land, and avoid any clogging of the soil. The Olive Mill Wastewaters neutralization laboratory tests are carried out on test volume of 100ml of our samples, using three strong bases with a concentration of 10g/l: lime $Ca(OH)_2$, Soda NaOH and Sodium hyposulphite $Na_2S_2O_3$. During this operation, we followed the evolution of the pH of the Olive Mill Wastewaters, until a neutral to alkaline pH was reached, as well as the salinity and conductivity.

2.5. Soil study

The soil studied was taken from plots of the organic farm in the Meknes region, cultivated with olive trees and aromatic and medicinal plants. Samples were taken within 30 to 60cm of the depth of three plots. The soil analyzes are carried out by the LCA Maroc laboratory.

2.6. Spreading of Olive Mill Wastewaters and tracking of the effect of the doses.

The choice of doses was based on the results obtained by several previous studies [23,24,29] which recommend the use of from 20m^3/ha to 100 m^3/ha. According to the recommendations cited by [30], we have practiced spreading on flat agricultural land, to avoid any problem of runoff or infiltration of Olive Mill Wastewaters, the water table closest to the land is far from 30m. The spreading was warried out using a cittern with an adjustable flow rate then, we proceeded to the incorporation of Olive Mill Wastewaters into the soil by vibroculturing plowing, this technique aims to ensure the bonding of the clay-humic complex soil and Olive Mill Wastewaters.

- The dose of raw Olive Mill Wastewaters is 50m^3/ha spread in a plot cultivated with olive trees and plots prepared for the planting of aromatic and medicinal plants.
- After preparation of the Olive Mill Wastewaters and neutralization with lime (pH= 7,01), we spread the samples in three plots of the olive trees, the doses of which are respectively: 50m^3/ha, 80m^3/ha, and 100m^3/ha. The findings and observations of the effects of Olive Mill Wastewaters on agricultural yield mentioned in the results and discussion section.
- Spreading period: we chose to carry out the spreading operation in February, a month considered to be the period when conventional fertilization is most practiced.

3. Results and Discussion

Results of physico-chemical analyses of Olive Mill Wastewater are mentioned in the following Table 1. The physicochemical analyzes of Olive Mill Wastewaters justified the need for their enhancement in soil fertility or as an amendment in organic matter, minerals, and nitrogen. Our results are close to the results obtained by [31].

Phenolic compounds profile

Analysis of the HPLC chromatogram (Figure 1) shows that the major monomers present in the ethyl acetate extract of the raw olive mill wastewater are hydroxytyrosol and tyrosol respectively of 332.2 mg L^{-1} and 140 mg L^{-1} as indicated in Table 2. The sample also included small peaks corresponding to Luteolin7-O-glucoside (56.4mg L^{-1}), Elenolic acid (34.9 mg L^{-1}), Elenolic acid derivative (11.3 mg L^{-1}), Caffeic acid (5.7 mg L^{-1}) and Hydroxytyrosol glucoside (4.5 mg L^{-1}). These results are in agreement with those obtained in previous studies [1,2,32,37] and demonstrate that olive mill wastewater has an antibacterial property due to these phenolic compounds, making biological treatment of them challenging. The Concentrations of phenolic compounds of Raw Olive Mill Wastewater are presented in Table 2.

The Results of Microbiological parameters are mentioned in the following Table 3. The neutralization tests of 100ml of Olive Mill Wastewaters, were carried out with three strong bases with concentration of 10 g/l. These tests allowed us to vary the pH of the Olive Mill Wastewaters from acidity to neutral and alkalinity. To obtain a neutral pH of our samples (pH=7,01), it was necessary to add 50ml of lime (Figure 2), 30ml of Soda (Figure 3), and 10ml of Sodium hyposulphite (Figure 4), in volume. This neutralization induced an increase in the salinity of Olive Mill Wastewaters, which is a parameter of toxicity that leads to saline stress for the plant [38]. Neutralization tests using Soda and Sodium hyposulphite, generated an increase in the salinity of Olive Mill Wastewaters at 10% even if we have reached the neutrality of the samples by adding small volumes (30 ml, 10 ml), with formation of foam. The salinity is mainly linked to Sodium ions, contained in Soda and Sodium hyposulphite. These drawbacks forced us to choose neutralization by lime which is less expensive, easy to use, and almost does not modify the salinity of the samples, in fact to neutralize 100ml of Olive Mill Wastewaters, only 50ml of lime must be added. Particle size and physicochemical analyzes of the soil are necessary before proceeding with the spreading of Olive Mill Wastewaters, so as not to degrade the quality and the biological activity of the soil. The grain size and texture of our soil, on the contrary, does not pose any problem, the advantage of storing water and fertilizing elements is higher for a loamy- clay-sandy soil with a finer texture [30]. The physicochemical analysis of the soil has shown that our soil has a basic pH of around 8.7, low salinity and conductivity, and low reserves of organic nitrogen, potash, and phosphorus. This deficit justified the inters in introducing organic amendments and hemic fertilizers such as Olive Mill Wastewaters, organic compost, or manure. The particle size and physicochemical parameters of the soil are mentioned in the following tables (4,5): After spreading we followed our land spread by Raw Olive Mill Wastewaters (ROMW), and we noticed a total absence of vegetation during the first 10 months in the plots of olive trees and aromatic and medicinal plants, this can be explained by the high acidity of Olive Mill Wastewaters (4,7), and total phenols disrupting the normal life of the soil microflora, which plays an important role in the transformation and exchange of hemic elements in the soil. The phenolic elements of Olive Mill Wastewaters behave like antimicrobials, which subsequently block the development of soil microflora, these findings join those obtained by [39].

Spreading Olive Mill Wastewaters at neutral pH (NOMW) increases the yield of the vegetation from the 2nd month of the campaign for the 50m^3/ha dose, the 3rd month for the 80m^3/ha dose, and the 5th month for the 100m^3/ha; this is due to compliance with the biological conditions of soil microorganisms, responsible for the degradation of organic matter in Olive Mill Wastewaters at neutral pH, and in a presence of oxygen, by transforming it into elements that can be assimilated by plants [39,40], and the exchange of minerals in the soil, these results are consistent with [39,41], which showed that the increase in the pH of neutrality favors the transformation of phenols into phenates by the soil microflora, with the formation of $C_6H_50^-$ ions which are retained by the cations in the soil.

This biodegradation gives rise to humic substances and ammonium ions, necessary for soil fertility. The neutralization of the Olive Mill Wastewaters allowed the reduction of the phenolic elements, the COD, and the DOB_5. This reduction is made by precipitation in the presence of lime, which absorbs the matter dissolved in the flocs [41]. The results obtained are presented in the following figure 5. The yield obtained with doses of 50 m3/ha and 80 m^3/ha, accompanied by high vegetation density, is primarily attributed to soil enrichment through the nitrogen, organic, and mineral content present in the applied Olive Mill Wastewater. This observation aligns with findings from a previous study [24]. The five-month delay in germination of grains and plants in plots treated with a dose of 100 m3/ha can be attributed to the antimicrobial properties of the polyphenols within the Olive Mill Wastewater, inhibiting soil microflora activity during the initial months. These results are consistent with prior research [25,30], which substantiates that doses of 100 m3/ha and 200 m3/ha induce a significant delay in barley grain germination compared to both the control group and the 50 m^3/ha dose. Our tests involving the use of neutralized margins, regardless of the applied dose, have consistently resulted in improved vegetation yields when compared to the application of untreated margins. These findings are consistent with various studies, notably one conducted by [42], which demonstrated that treating margins, whether through a simple lime neutralization or an advanced method like catalytic oxidation, led to increased plant yields and improved chemical and microbiological soil properties as compared to untreated margins.

Additionally, research conducted by [43] on the valorization of electrocoagulated margins, which raises the pH, has shown highly favorable effects on the germination of tomato seeds at various dilution rates. This is in contrast to the use of untreated margins, which inhibit germination due to their high acidity and phenolic content. Furthermore, a study by [44] revealed that employing neutral pH digest obtained through methane fermentation of margins enhances soil fertilization efficiency compared to the use of untreated margins. Tracking the effects of spreading both raw Olive Mill Wastewaters and neutralized Olive Mill Wastewaters on soil vegetation yield, by monitoring plant density and height (Figure 6), has enabled us to draw several important conclusions and recommend best practices for the application of these Olive Mill Wastewaters:

-When utilizing raw Olive Mill Wastewaters, it is advisable to plant crops only after a resting period of 10 months or even up to a year, especially if the primary goal is soil treatment or disinfection against insects or unwanted plants.

-Opt for the use of neutralized or treated Olive Mill Wastewaters to raise their pH levels. This results in a higher fertilizing value when compared to raw Olive Mill Wastewaters.

- Consider using lime as the neutralizing solution for Olive Mill Wastewaters instead of alternative products. This choice helps limit the increase in salinity, contributing to more favorable soil conditions for cultivation.

Table 1: Physicochemical parameters

Parameter	pH	Sl%	C µS/cm	O.M g/l	M.M g/l	NTK g/l	T.S g/l	T.P g/l	COD g/l	BOD_5 g/l
Results	4,6	4	16,7	8,5	16,4	1,8	4,2	7,8	363,24	87

Table 2: Concentration of phenolic compounds of Raw Olive Mill Wastewater

Phenolic compounds	Concentration (mg L^{-1})	References
Hydroxytyrosol glucoside	4.5	
Hydroxytyrosol	332.2	
Tyrosol	140.6	
Caffeic acid	5.7	1-8
Elenolic acid derivative	11.3	
Elenolic acid	34.9	
Luteolin 7-O-glucoside	56.4	

Table 3: Microbiological parameters

Parameter	TAMF	TC	FC	FS	St	LB	Y&M	Ps
Number of germs in CFU/ml	309	0	0	0	0	302	0	0

Table 4: Particle size characteristics of the soil

Depth	%Clay <2µm	%Fine stringers 2-20µm	%Coarse silts 20-50µm	%Fine sands 50-200µm	%Coarse sands 0,2-2 mm
0-30 cm	27,9	40,8	13,9	7,4	7,6
30-60 cm	20	42,8	11,1	10,5	12,9

Table 5: Physicochemical characteristics of the soil

Depth	pH	%OM	%Nitrogen	%Potash	%Phosphorus	%Salinity	Conductivity µS/cm
0-30 cm	8,7	2,52	0,17	241	13	0,55	20
30-60 cm	8,6	2,75	0,17	514	89	0,54	18

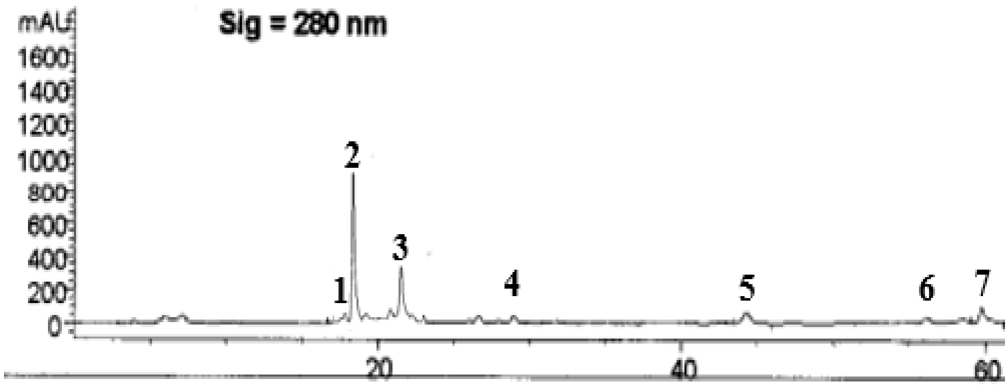

Figure 1: Chromatographic profile of phenolic extract of Raw Olive Mill Wastewater at 280 nm, (1) Hydroxytyrosol glucoside, (2) Hydroxytyrosol, (3) Tyrosol, (4) Caffeic acid, (5) Elenolic acid derivative, (6) Elenolic acid, (7) Luteolin 7-O-glucoside

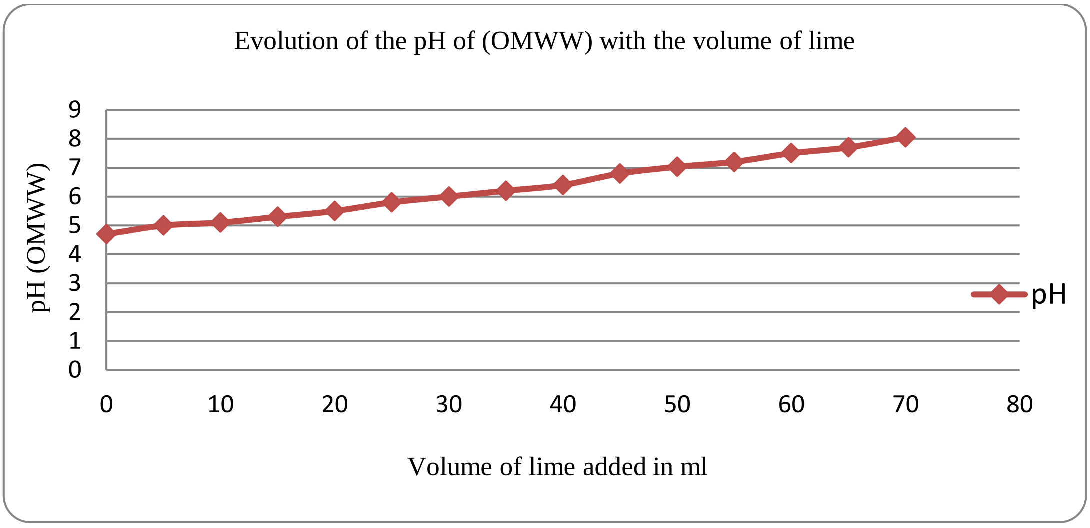

Figure 2: Evolution of the pH of (OMWW) with the volume of the lime added

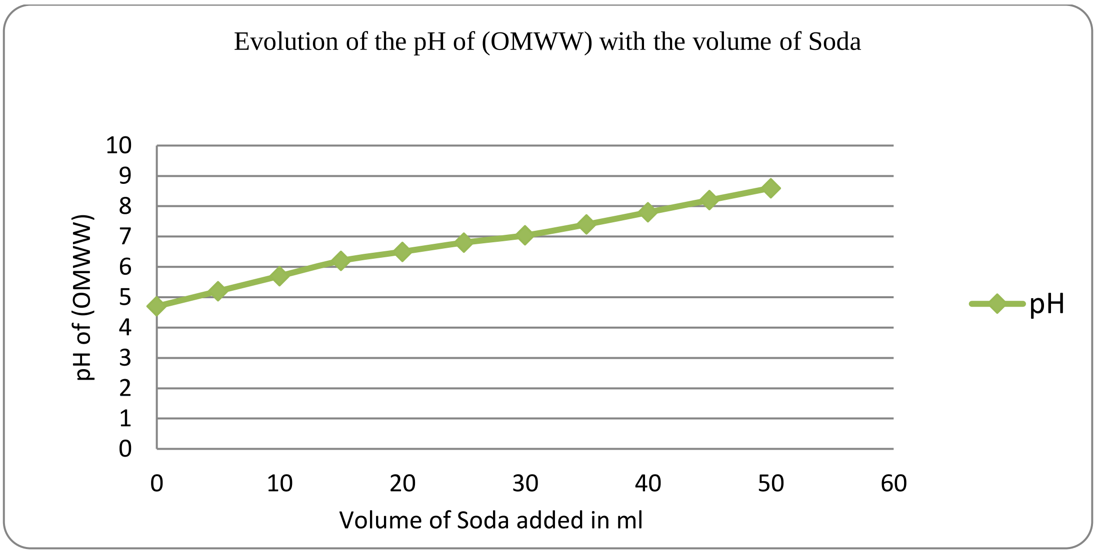

Figure 3: Evolution of the pH of (OMWW) with the volume of the Soda added

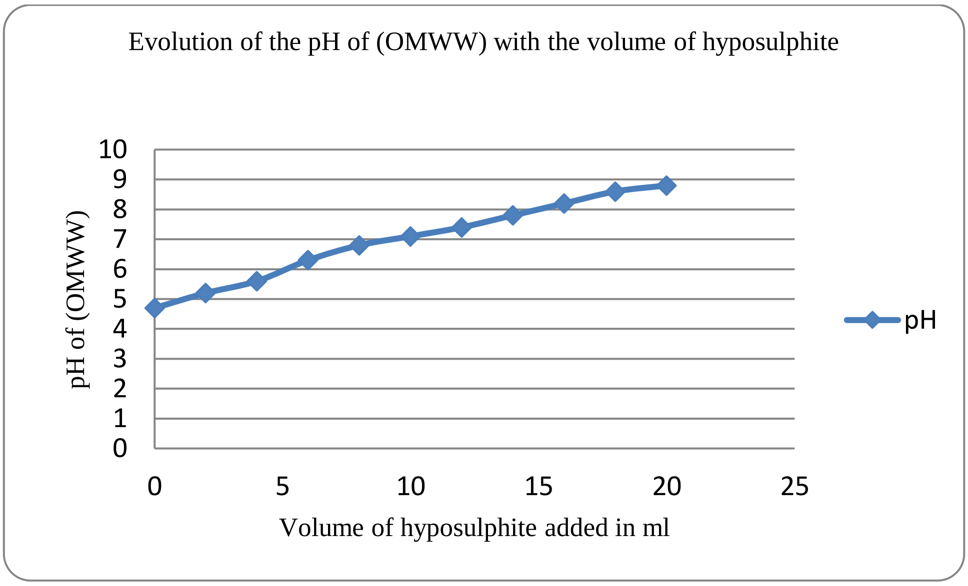

Figure 4: Evolution of the pH of (OMWW) with the volume of the hyposulphite added

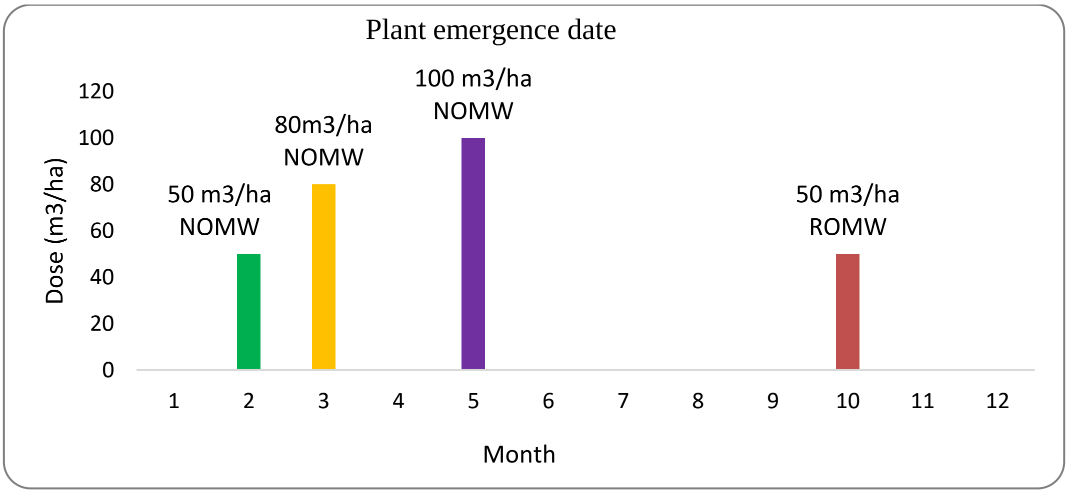

Figure 5: Plant emergence date

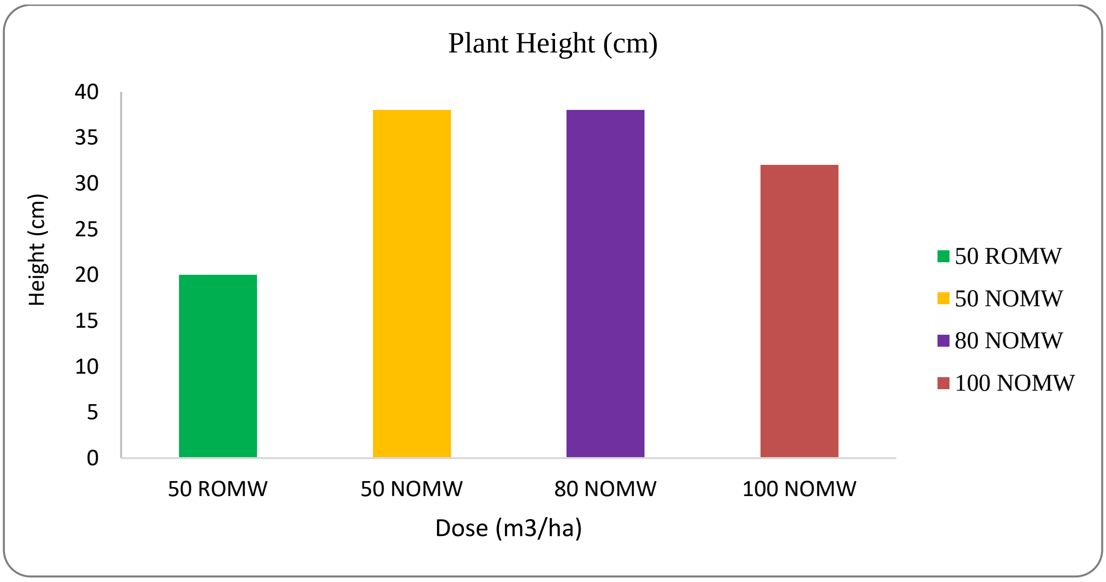

Figure 6: Average height of plants following the dose

This biodegradation gives rise to humic substances and ammonium ions, necessary for soil fertility. The neutralization of the Olive Mill Wastewaters allowed the reduction of the phenolic elements, the COD, and the DOB_5. This reduction is made by precipitation in the presence of lime, which absorbs the matter dissolved in the flocs [41]. The results obtained are presented in the following figure 5. The yield obtained with doses of 50 m3/ha and 80 m^3/ha, accompanied by high vegetation density, is primarily attributed to soil enrichment through the nitrogen, organic, and mineral content present in the applied Olive Mill Wastewater. This observation aligns with findings from a previous study [24]. The five-month delay in germination of grains and plants in plots treated with a dose of 100 m3/ha can be attributed to the antimicrobial properties of the polyphenols within the Olive Mill Wastewater, inhibiting soil microflora activity during the initial months. These results are consistent with prior research [25,30], which substantiates that doses of 100 m3/ha and 200 m3/ha induce a significant delay in barley grain germination compared to both the control group and the 50 m^3/ha dose. Our tests involving the use of neutralized margins, regardless of the applied dose, have consistently resulted in improved vegetation yields when compared to the application of untreated margins. These findings are consistent with various studies, notably one conducted by [42], which demonstrated that treating margins, whether through a simple lime neutralization or an advanced method like catalytic oxidation, led to increased plant yields and improved chemical and microbiological soil properties as compared to untreated margins.

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-Opt for the use of neutralized or treated Olive Mill Wastewaters to raise their pH levels. This results in a higher fertilizing value when compared to raw Olive Mill Wastewaters.

- Consider using lime as the neutralizing solution for Olive Mill Wastewaters instead of alternative products. This choice helps limit the increase in salinity, contributing to more favorable soil conditions for cultivation.

4. Conclusions

In conclusion, our study focused on the application of raw Olive Mill Wastewaters and those neutralized to a pH level, shedding light on their practical utility in managing these effluents derived from three-phase milling processes. We observed that the application of raw Olive Mill Wastewaters resulted in a complete absence of vegetation across the plots for a duration of 10 months. This phenomenon underscores the potent antimicrobial properties of Olive Mill Wastewaters, attributable to their acidity (pH of 4.7) and the rich chemical composition characterized by high phenolic content.

To preserve the vitality of soil microorganisms, it is imperative to neutralize Olive Mill Wastewaters before their application in agricultural fields. Our findings demonstrate that doses of 50 m3/ha, 80 m3/ha, and 100 m3/ha at a neutral pH have proven to be beneficial for vegetation growth. These doses contribute essential Nitrogen, organic matter, and mineral components that are indispensable for plant development.

In summary, our research reaffirms that Olive Mill Wastewaters can indeed serve as valuable organic fertilizers for alkaline soils, provided they undergo neutralization treatment to mitigate the phytotoxic effects stemming from acidity and polyphenolic compounds. Implementing these practices offers a sustainable and environmentally responsible approach to managing Olive Mill Wastewaters in agriculture.

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Conflict of Interest

The authors declare that there is not any conflict of interests regarding the publication of this manuscript.

Life Science Reporting

No life science threat was practiced in this research

Data availability statement

No data availability

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