

Effects of raw and treated olive mill wastewater (OMW) by coagulation-flocculation, on the germination and the growth of three plant species (wheat, white beans, lettuce)

F. Elayadi^{*a,c}, C. El Adlouni^b, M. Achak^c, E. El Herradi^d, M. El Krati^e, S. Tahiri^e, M. Naman^f, F. Naman^a

^a Laboratory of Plant Biotechnology, Ecology and Valorization of Ecosystems, Sciences Faculty, Chouaib Doukkali University, El Jadida, Morocco.

^b Laboratory of Marine Biotechnologies and Environment, Sciences Faculty, Chouaib Doukkali University, El Jadida, Morocco.

^c Science Engineer Laboratory for Energy, National School of Applied Sciences, Chouaib Doukkali University, El Jadida, Morocco.

^d Laboratory of Physico-Chemistry of Inorganic and Organic Materials, Normal Superior School, Mohammed V University, Rabat, Morocco.

^e Laboratory of Water and Environment, Sciences Faculty, Chouaib Doukkali University, El Jadida, Morocco.

^f Microbiology Laboratory, Higher Institute of Nursing Professions and Health Techniques, Casablanca, Morocco.

Abstract

Olive mill wastewater (OMW) constitutes a grand environmental issue for olive oil producing countries. It produces a large amount of organic matter, which is difficult to degrade, in particular phenolic compounds which are responsible for the phytotoxic effect. The aim of this research is to determine the potential COD and phenolic compounds removal of OMW treated by coagulation-flocculation with lime as coagulant and to evaluate the effects of raw and treated OMW on the germination and the growth of three plant species (wheat (*Triticum turgidum* L. var *Durum*), lettuce (*Lactuca sativa varcrispa*) and White bean (*Phaseolus vulgaris* L.). This treatment induced a high removal at a dose of 30g/L of lime by 40% and 97% respectively. The raw OMW showed an inhibitory effect on the germination and the growth of the three species. However, the three OMW dilutions (50%, 25% and 12.5%) stimulated the germination and the growth of wheat and white bean. The germination tests with OMW treated with lime and diluted at different concentrations present the best medium for growth of the three plants and the germination rate can reach 100%.

* Corresponding author:

elamalfatima11@gmail.com

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1.Introduction

Olive oil is considered as the most important product that increases the economic and social value for many Mediterranean countries such as Spain, Italy, Greece, Turkey, Tunisia and Morocco [1]. In Morocco, the olive oil is currently becoming increasingly important in parallel with the increase in the cultivated area of the olive tree, which is reinforced by the green Morocco plan with 947000 hectares in 2014 [2]. However, this production generates important quantities of solid residue (pomace) and liquid wastes; In each production area, the quantity of Olive Mill Wastewaters (OMW) estimated to 3 million m³ per year during its harvest season between December-January [3]. Pomace is reused in agriculture as cattle feed, while the OMW are discharged into receiving waters without control and treatment lead to serious environmental problems. OMW is rich in sugars, lipids, proteins, acids, organic matter and phenolic compounds [4]. These compounds modify the stability of soil microorganisms, affect the quality of water where they are discharged and inhibit many plants such as chickpea, tomato, durum wheat and maize [5-7]. In order to reduce the phenolic content and the organic matter from OMW, several treatment processes have been proposed, such as biological treatment: infiltration-percolation, aerobic and anaerobic digestion [8-10], physico-chemical methods: electrocoagulation, coagulation-flocculation and adsorption [11-13]. Infiltration-percolation on a sand filter is one of the biological treatments bacterial cultures, which fixed fine supports. According to [14], the first infiltration by the upper layer of the soil remove the organic and inorganic components of OMW. However, the high mineral salt content of OMW, low pH and the presence of polyphenols compounds limit the application of this process [15]. Aerobic biological treatments have long been proposed for OMW treatment by using several microorganisms as *Pleurotus ostreatus*, *Bacillus pumilus*, *Phanerochaete chrysosporium*, *Aspergillus niger* [16-18]. While anaerobic digestion technology allows the treatment of wastewaters OMW, and produces biogas that can be used as a primary energy resource. Nevertheless, these two processes can cope up with the high organic load of OMW that need to be diluted several times prior to biological treatment. One of the most effective processes of advanced wastewater treatment is the adsorption. It reduces hazardous organic and inorganic wastes and remove toxic inorganic and organic compounds from contaminated ground water. Activated carbon considered the most popular and exceedingly used adsorbent material for treatment of OMW [19, 20]. However, for a best regeneration system and high initial the activated carbon cost make less economically and viable as excellent adsorbent [21, 22]. Although advanced oxidation processes is a suitable treatment method for removal or degradation of several emerging micropollutants are not able to complete treating highly concentrated OMW [23, 24]. Coagulation-flocculation process is exceedingly used, due to its simplicity and cost-effectiveness. Regardless of the overall applied treatment scheme; coagulation-flocculation and the nature of the treated sample, is usually included, either as pre- or as post-treatment step. Many coagulation-flocculation substances are used as aluminium sulphate, ferric chloride, ferric sulphate and lime. These compounds are relatively less expensive and smoothly available [25]. The use of lime concentration from 5 to 30 g/L was used to test the effectiveness of lime to remove polyphenols from raw OMW [12]. Other study evaluated the effect of lime pre-treatment on phenols elimination for many different olive mill effluents [26]. The irrigation of the plants with raw or pre-treated OMW was considered as easy relatively inexpensive. Several authors have tested the effects of OMW on seed germination and seedling growth, of many seeds, such as (*Vicia faba* L.), sulla (*Hedysarum coronarium* L.), chicory (*Cichorium intybus* L.), wheat (*Triticum durum* Desf.) and (*Lolium multiflorum* Lam) [27, 28]. The present study consists to determine the potential application of lime as coagulant to removal pollutant load from OMW and to evaluate the effects of undiluted and treated OMW on the germination and the growth of three plants species. For this, we had firstly characterized the olive mill wastewater, produced by a modern unit located in the Fkih Ben Salah region of Morocco and examined the efficiency of lime as a coagulant for the removal of phenolic compounds and COD from OMW. The effects of various operating parameters on coagulation-flocculation process, like coagulant dosage and initial pH, were tested and the optimal experimental conditions were determined. Then, we studied the inhibitory

effect of rawdiluted and treated OMW on the germination and growth of three plants species durum wheat (*Triticum turgidum* L. var. *Durum*), lettuce (*Lactuca sativa* var. *crispa*) and white bean (*Phaseolus vulgaris* L.).

2. Experimental and analytical

2.1. OMW and coagulant materials

In this study, the OMW treated, was obtained from a three-phase olive mill extraction plant from Fkih Ben Salah region of Morocco. The sampling period was from december to january. The OMW collected was separated into smaller vessels and then stored at 25 °C in order to prevent any alteration of its characteristics. The analysis of various parameters of the OMW was carried out as described by standard methods into the laboratory. The lime was taken from local supplier.

2.2. Experimental procedure

The coagulation-flocculation experiments were carried out in a jar-test apparatus consisting by six beakers of 500 L volume at a room temperature. All of OMW samples were treated with increasing concentrations of lime from 5 to 75 g/L. One beaker of them was kepted as control. The solutions were subdue a rapid stirring for 3 min at 200 rpm, to waste homogenization, then the slow stirring for 12 min at 50 rpm to allow agglomeration of the formed flocs. After settling the supernatant was separated from the precipitate for analysis.

2.3. Physical–chemical analysis of OMW

The pH of the samples was measured continuously using a pH meter WTW, 521 instruments pH/mV/°C. The total suspended solid was determined after filtration of sample by means of a filter (0.45 µm) by drying the obtained filtrate at 105 °C to 24 hours. The COD was measured by the NF T 90-101 method. It was based on the oxidation of closed reflux dichromate (148 °C for 2 h). The model of the COD apparatus was a CR 2200 WTW thermal reactor. Electrical conductivity was measured using the conductivity meter (EUTECH CON 510). Polyphenols were extensively present in the OMW. They were assayed by the colorimetric method of Folin-ciocalteu using Gallic acid as standard at 760 nm. The total nitrogen (NTK) was determined by the mineralization of the organic nitrogen of the samples in sulfuric acid in the presence of a catalyst (K₂SO₄, selenium) according to the standard (AFNOR T90-110). The total phosphorus was determined by a spectrophotometer method of (AFNOR T 90-023) at 880 nm. The chloride ions were determined according to the standard (AFNOR T90-014). The measurement of the color was carried out using UV-visible spectrometry at 280nm.

2.4. Physical-chemical analysis of lime

The X-ray diffraction analysis of lime was carried out using a Philips X-ray generator type PW 3710 with an anticathode of copper at the wavelength $\lambda=1.5406$. The acquisition was made for 2θ between 0 and 80°C. Their crystal phases are identified by comparison with databases using the PANalyticalX'Pert High Score Plus software. The analysis by Fourier transformed infrared spectroscopy of lime was performed using a Perkin-Elmer 1720-x spectrometer. The spectra was carried out by scanning between 4000 and 400 cm⁻¹ with a resolution of 2.00 cm⁻¹ on samples pelletized by means of a press in potassium bromide in the proportion of 1 mg of product per 100 mg of KBr. The elemental chemical analysis of the lime were carried out using an "Axion" X-ray fluorescence spectrometer with 1kw wavelength dispersion at the UATRS division of the National Center for Scientific Research.

2.5. Seed germination tests

For germination tests, 20 seeds of durum wheat (*Triticum turgidum* L. var *Durum*), lettuce (*Lactuca sativa varcrispa*) and White bean (*Phaseolus vulgaris* L.) were scattered on Whatman's filters papers in each petri dish (9 cm). These papers were firstly sozzled with raw OMW, diluted OMW or treated OMW by lime, pH adjusted at 7.5 at different OMW concentrations (50%, 75% and 87.5%). Each analysis was run in three replicates. Once the radicle projection of about 2 mm was observed, the seeds were marked as germinated. Germination was evaluated 24 hours apart for 8 days. For measurements of root length, sprout the seeds of each Petri dish 8 days after the start of germination. The fresh biomass was weighed immediately after harvest. While dry biomass was determined after drying in oven at 70 °C for 48 hours. Statistical analyzes of the germination were released out using SPSS (Statistical Package for Social Sciences) software with a level of significance $\alpha=0.05$.

3. Results and discussion

3.1. Characterization of OMW

Physical-chemical characteristics of different OMW according to its origin and olive oil extraction method are indicate in table1.

Table 1: Physical-chemical characteristics of many OMW

REFERENCES	OUR STUDY	[29]	[30]	[25]	[28]	[12]
OMW ORIGIN	Morocco	Morocco	Morocco	Morocco	Italy	MOROCCO
EXTRACTION PROCESS	3-phase	3-phase	3-phase	3-phase	3-phase	3-PHASE
PH	4.80	5.00	4.48	4.50	4.00	4.55
EC (MS.CM⁻¹)	6.30	16.00	13.18	8.40	7.30	8.40
TOTAL PHENOLS (G/L)	4.79	8.00	1.28	1.40	2.97	1.42
DRY MATTER (G/L)	10.06	n.d.	5.70	56.70	6.60	56.75
COD (G/L)	76.80	320.00	329.68	55.20	50.00	71.56
TKN (G/L)	0.06	n.d.	0.68	1.40	0.62	1.40
PHOSPHORUS (G/L)	0.02	n.d.	0.82	0.06	n.d.	0.06
CHLORIDE (G/L)	1.75	6.00	1.05	6.00	N.D.	6.15

As indicated in Table 1, the all OMW samples show an acidic pH (4-5) due to the presence of compounds acids such as phenolic acids and fatty acids. At these low pH values, the biological treatment of OMW is very difficult. According to [31], the high acidity could be due to self-oxidation reactions of phenolic compounds. The EC of our all OMW samples (6.3 ms.cm⁻¹) is very low to those obtained (16 ms.cm⁻¹) [29, 30]. For these authors, the high EC value can be mainly due to the natural richness of olives in mineral salts and added of salt in order to preserve olives before trituration. All OMW samples contain a high content of organic matter (50-330 g/L). This large organic load makes the biological purification very difficult to achieve. The high polyphenol content of OMW samples (1.28 to 8.00 g/L) makes limited natural biodegradation. Indeed, the OMW phenolic content in most cases is above the standard limits (less than 0.5 mg/L) established for its release into the aquatic medium. The analysis of the mineral fraction of all OMW samples shown a high amount of chlorides (1.05-6.15 g/L). This high level is due to the use of salt to preserve the olives before being crushed. All OMW samples contained a high level of TKN (0.06-1.40 g/L) and total Phosphorus (0.02- 0.82 g/L). They may be due to salting of the olives. The visible UV absorbance of OMW showed the existence of absorbance peak at around 280 nm which is specific to polyphenols compounds (Figure 1).

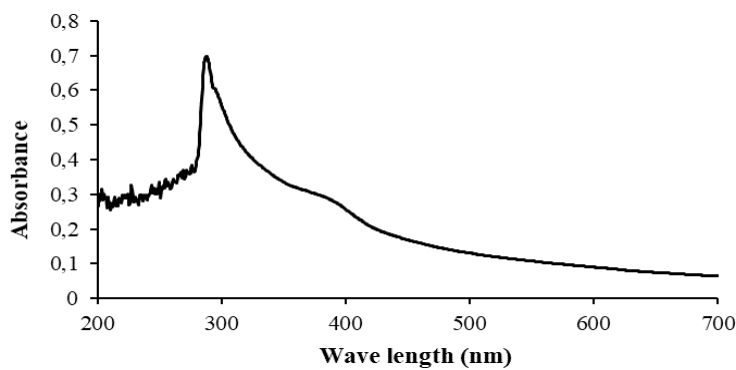


Figure 1. Visible UV of raw OMW spectrum

3.2. Characterization of lime

The Figure 2 gives the results of the X-ray diffraction analysis of the lime. The analysis of the diffractogram revealed the predominance of calcium oxide (CaO), periclase (MgO) and the presence of calcite (CaCO_3), dolomite ($\text{CaMg}(\text{CO}_3)_2$) and portlandite ($\text{Ca}(\text{OH})_2$). The amount of lime chemical elements and its fire loss value, are indicated in table 2

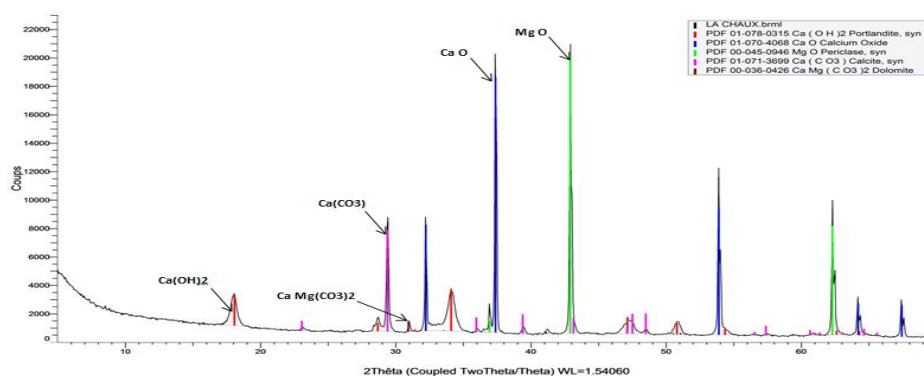


Figure 2. Lime X-ray Diffraction Diagrams

Table 2. Amount of lime chemical elements

SiO_2	Al_2O_3	Fe_2O_3	CaO	K_2O	Na_2O	MgO	SO_3	P_2O_5	Fire Loss
2.51%	1.1%	0.43%	45.5%	0.05%	0.43%	40.2%	0.08%	0.08%	9.48%

The infrared lime spectrum showed two intense and well resolved C-O elongation vibration bands at 1413 cm^{-1} and 873 cm^{-1} (Figure 3).

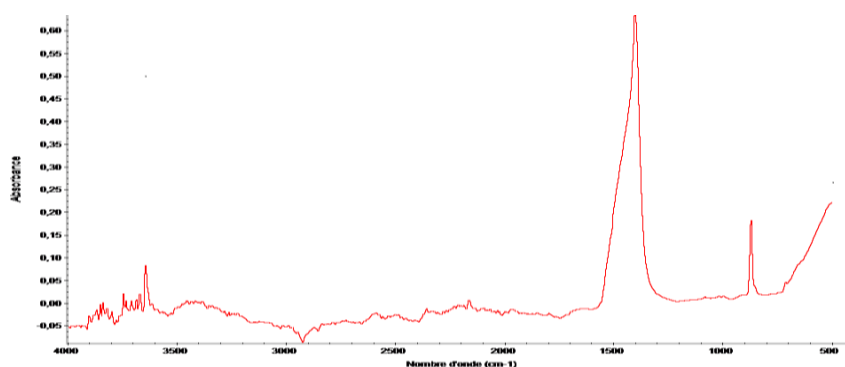


Figure 3. Lime Infrared Spectrum

3.3. OMW treatment by coagulation-flocculation with lime

3.3.1. Evolution of pH

The pH is the main factor influencing the coagulation-flocculation process. It plays an important role in determining the quality of wastewater effluents. As shown in Figure 4, the addition of the lime in the range between 5 and 75 g/L to raw OMW increased its acidic pH (4.8) to a basic pH (12). The basic pH eliminates some toxic and difficult-to-degrade constituents of the OMW [32]. The lime is often used for wastewater treatment for its effectiveness, in odor's elimination and its availability at low prices in countries as Morocco [33]. However the elimination of the other pollutants need a biological treatment.

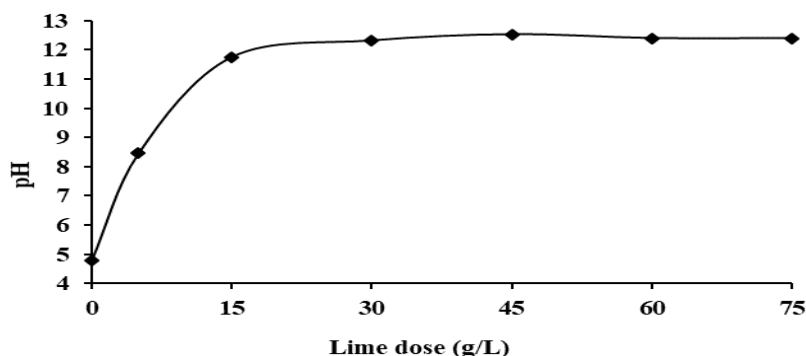


Figure 4. Effect of lime dose on pH of OMW

3.3.2. Removal of COD

The chemical oxygen demand (COD) value indicate toxic condition and presence of no-biodegradable substances. It is an important parameter to control the quality of waster treated. In our case, the COD value in OMW observed was very high (76.8 g/L) than consent limit allowed (6 g/L) [34]. It can plainly be seen that the increase in COD removal is proportional with lime doses until the maximum values 30 g/L for 40% COD removal (Figure 5). This finding was in accordance with results obtained by several authors. For [26] the lime treatment reduces COD values in wastewater by 42-46%. For [12] the high removal of COD (43%) from OMW was obtained with 20g/L of lime. These authors concluded that the entrapment of suspended and colloidal particles by lime is carried out through the sweep coagulation mechanism. On the other hand, the calcium carbonate formed at basic pH acts as a weighting agent by increasing the density of the settle able particles, thereby enhancing their settlement.

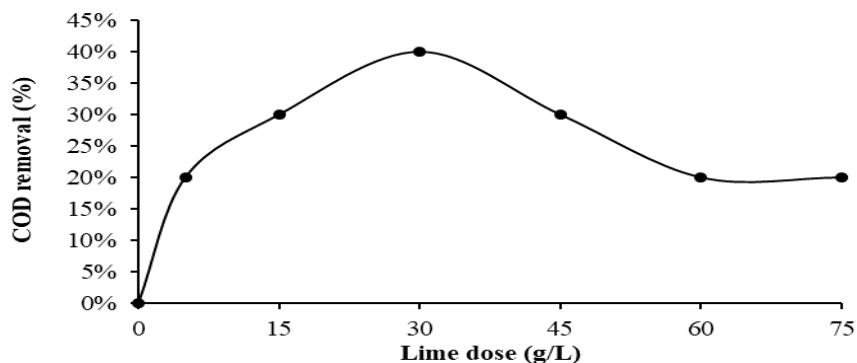
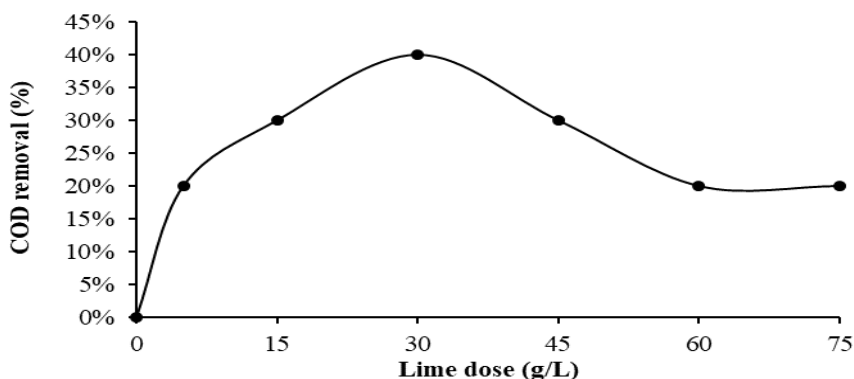


Figure 5. Percent removal of COD versus lime dose

3.3.3. Influence of coagulant dose on removal of phenolic compounds

Figure 6 shows the influence of lime doses (5 to 75 g/L) on the removal of polyphenols from OMW. The lime application efficiency in removing phenolic compounds increased from 94.6% (5 g/L) to 97.07 % (30 g/L). Between these two doses, the solution pH increases from 4.8 to 12. At this basic pH, the calcium hydroxide $\text{Ca}(\text{OH})_2$ dissociates and thus allows the release of the adsorbed polyphenols. However, in the range from 30 to 75 g/L, the removal efficiency of polyphenols showed a slight decrease were increase in pH values exceeding 12. For [26, 35], the use of lime as coagulant agent at 20 g/L and 60 g/L remove phenolic compounds from OMW by 75% and 67%, respectively. They reported this significant removal to the presence of the $\text{Ca}(\text{OH})_2$ precipitate which traps and



adsorbs the dissolved matter on the flocs. According to [36], the addition of CaO (7.5 g/L) increases the pH value from 4.6 to 10. This basic pH allows a significant elimination of polyphenols (68%).

Figure 6. Percent removal of polyphenols versus lime dose

3.3.4. Effect of coagulant dose on evolution of visible UV spectra

Figure 7 shows the UV-visible absorption spectra of OMW treated with increasing doses of lime. The intensity of the peak is inversely proportional to the dose of the lime applied in interval of dose (5-75 g/L). The absorption peak of the OMW located around the wavelength of 280 nm. This finding assigned to the polyphenols present in OMW.

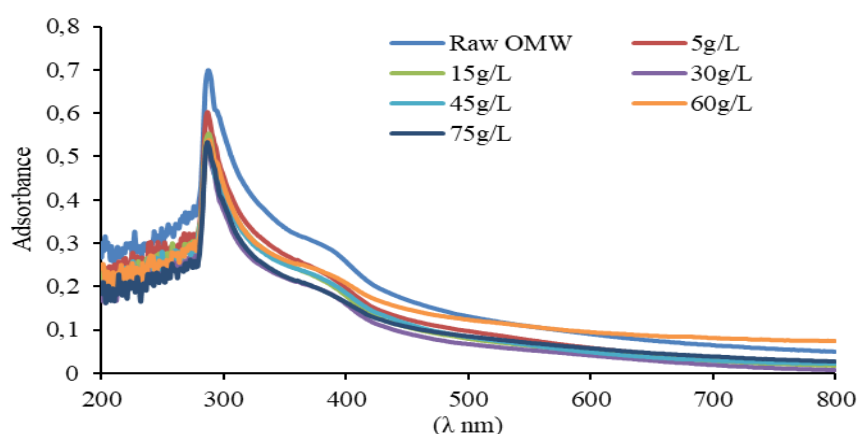


Figure 7. Effect of coagulant dose on the intensity of the visible UV

3.4. Effect of raw and treated OMW on seed germination

Figure 8 indicate a percentage of germination of the three examined plant species wheat (*Triticum turgidum* L. var *Durum*), lettuce (*Lactuca sativa varcrispa*) and White bean (*Phaseolus vulgaris* L.) sizzled to the raw and diluted OMW solution. The raw OMW is strongly phytotoxic for the three plant species and completely hinders plant growth. According to several studies, the high phytotoxicity of OMW is mainly owing to the existence of phenolic compounds, volatile fatty acids, aldehydes and alcohols [30, 37-40]. For [39] a significant reduction in seed

germination especially for tomato and wheat sozzled of OMW solution or their related soluble phenolic extracts was observed. They confer that predominantly inhibition effect of seed germination to OMW phenolic compounds. In the case of lettuce, the three dilution concentrations of OMW induced a complete inhibition of seed germination. This high phytotoxicity is also obtained by [35]. Wheat showed a complete inhibition at 50% diluted OMW and slight germination rate at 25% diluted OMW. However, the increase in seed germination rate of white bean with decreasing diluted OMW concentrations was observed. The phytotoxicity is very high and completely inhibited seed germination for all dilutions of OMW for lettuce. For [41] OMW- water dilution of 1:8 (v/v) is still phytotoxic. Its can origin an inhibition of 55% 60% and 98% in the germination index for radish, cucumber and lettuce species, respectively. However, the dilution of OMW by a factor of 10 was able of elimination totally phytotoxocity to tomato seeds and almost absolutely to chicory seeds [42].

The analysis of the variance indicates that the seed germination of the three plant species studied is significantly affected at $P < 0.001$ by the concentration of OMW.

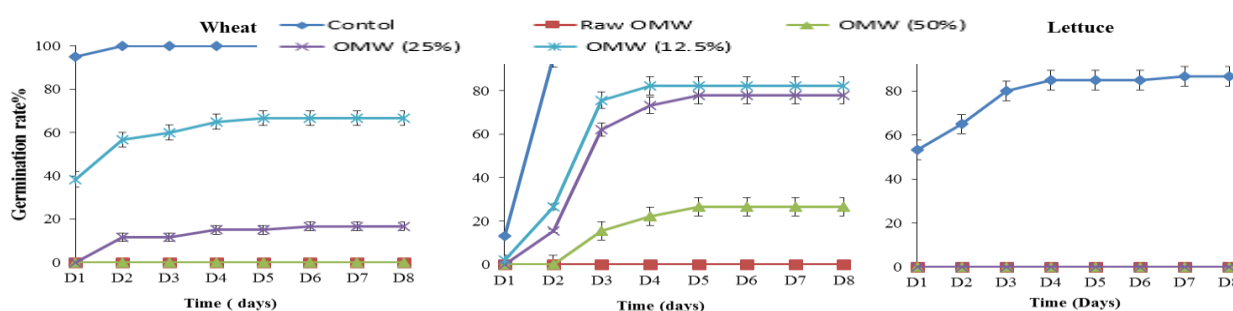


Figure 8. Effect of undiluted and diluted raw and different concentrations of OMW on seed germination .

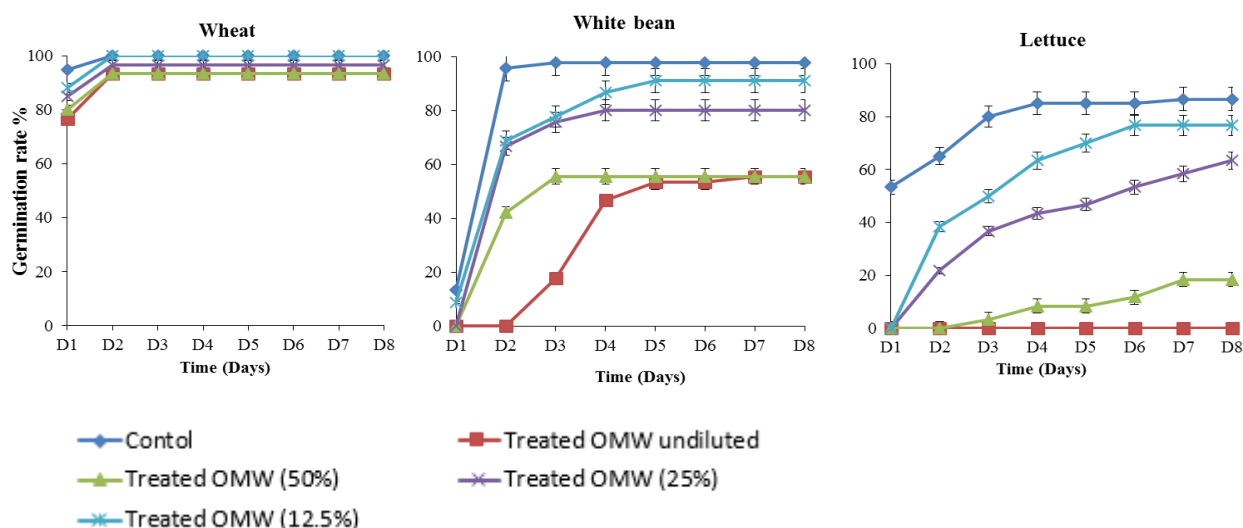


Figure 9. Effect of undiluted and diluted OMW treated by lime on seed germination of the three species

Figure 9 indicates the percentage of germination of the examined plant species after their irrigation by undiluted and deleted treated OMW. For wheat, high germination rate (93%) was observed for treated OMW by lime. The germination rate achieved 95%, 95% and 100% for 50%, 25% and 12.5% dilutions of treated OMW respectively at pH 7.5. This phytotoxicity behavior also obtained by [35]. These authors attributed the decrease of phytotoxicity after lime treatment to the elimination of phenols and other phytotoxic compounds in the liquid phase. In this study, phenolic compounds load pass from 0.20 g/L (95.82%) to 0.14 g/L (97.07 %). To detoxify OMW [43], used the

sodium polyacrylate polymers as an absorbent. This latter was able of shouldering the phenolics fractions within the polymeric chains, thereby reducing the phytotoxicity of residual liquid. As regards white bean and lettuce, the OMW dilution concentration 25% and 12.5% substantially reduce OMW phytotoxicity. Conversely, treated undiluted and diluted OMW at 50% are not sufficient to reduce the OMW phytotoxicity and the sensitivity of the used seeds. The coagulation-flocculation process using lime still contains substances while inhibit the germination and growth of three seed species. It is important to highlight that the seed germination depends on solution pH [42, 44], the dissimilarity in phytotoxicity levels between the OMW treated with lime (pH7,5) and the OMW untreated (pH 4,8) could be owing to their different pH values

3.6. Effect of raw and treated OMW on the length of roots and stems of the plant species

3.6.1. Effect of raw OMW on the length of roots and stems of the plant species

Figure 10 shows the effects of the three concentration of diluted OMW (50%, 25%, 12.5%) on the germination of the three plant species. In the case of wheat, OMW diluted to 12.5% and 25% are favorable for growth of stems and roots. Their length achieved 2.84cm and 4.17cm respectively. For white bean, all OMW diluted concentrations are appropriate for the growth of stems and roots. The highest growth were reached for OMW diluted at 12.5% and 25%, which are 6.76 cm and 6 cm respectively. However, for lettuce, all OMW dilutions are inappropriate for the germination and growth. According to [45], the length of root for *Solanum lycopersicum* are 3.7 cm and 2 cm for OMW diluted at 25% and 50%, respectively. For *Lepidium sativum* the length of root are 3 cm and 1 cm for OMW diluted at 25% and 50%, respectively.

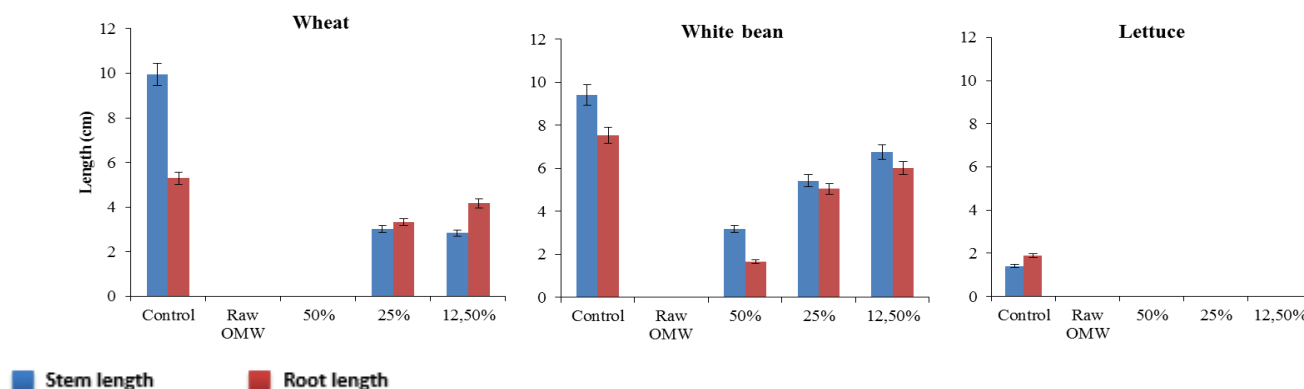


Figure 10. Effect of undiluted and diluted raw OMW on root and stem length of the three species

3.6.2. Effect of treated OMW on the length of roots and stems of the plant species

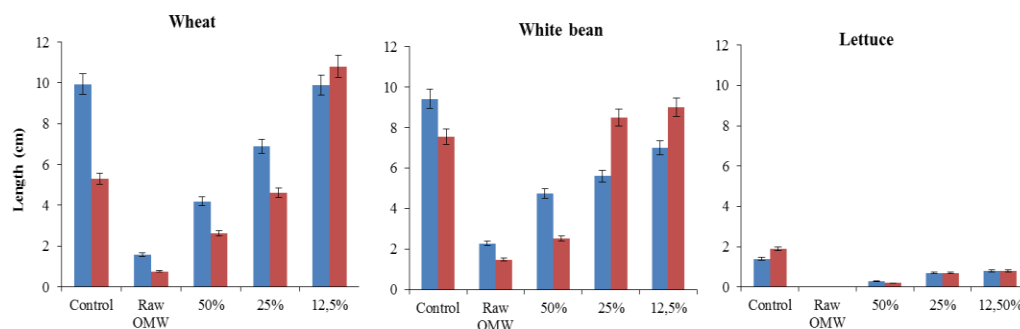


Figure 11. Effect of undiluted and diluted OMW treated on the length of roots and stems of the three species

The effects of three concentrations of OMW treated by lime (50%, 25%, 12.5%) on the seed germination of three plant species are showed in Figure11. For three plant species, the length of roots is greater than stems one. At higher dilution factors, the length of roots and stems drastically decreased. The highest length were obtained for OMW concentration of 12.5 % with 7.9cm and 9.5 cm for roots and stems, respectively. Significant evolution of stem and root length for treated and diluted OMW at different concentrations in all three species. untreated OMW still toxic for the lettuce

3.8. Effect of raw and treated OMW on the fresh weight and dry weight

Figure 12 and 13 show the effect of undiluted and diluted raw and treated OMW on the fresh and dry weight of three plant species. Although a slight evolution of fresh and dry weight of white bean were observed, the raw OMW and diluted OMW (50%) completely inhibited the germinability of wheat. In the case of lettuce, only traces of fresh and dry weight were found. This may be due to the small natural size of this plant. The statistical analysis reveals a very highly significant effect of the OMW concentrations on fresh and dry weight of the three plant species. The raw and treated OMW present the same evolution behavior in weight percentage except white bean, who showed a significant growth of fresh weight.

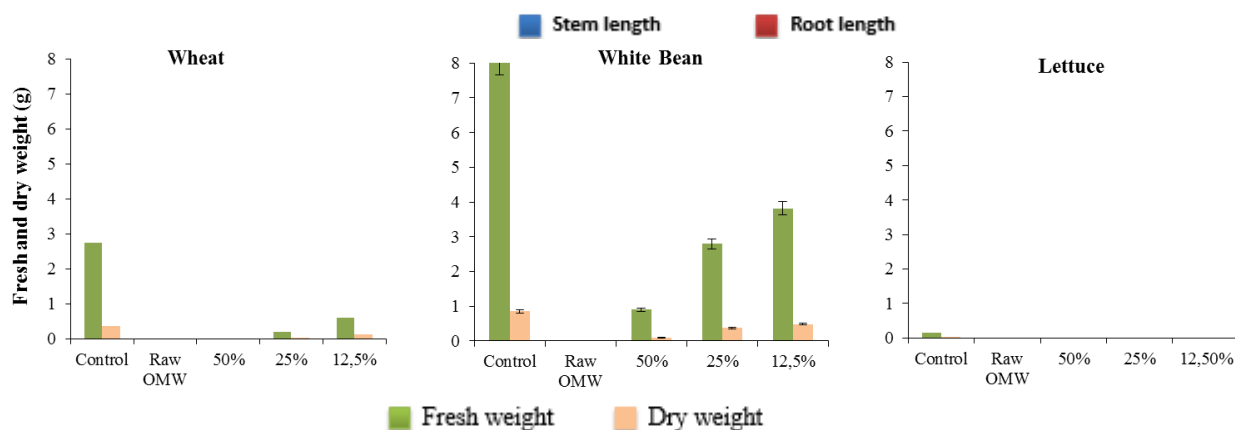


Figure 12. Effect of undiluted and diluted raw OMW on the fresh and dry weights of the three species

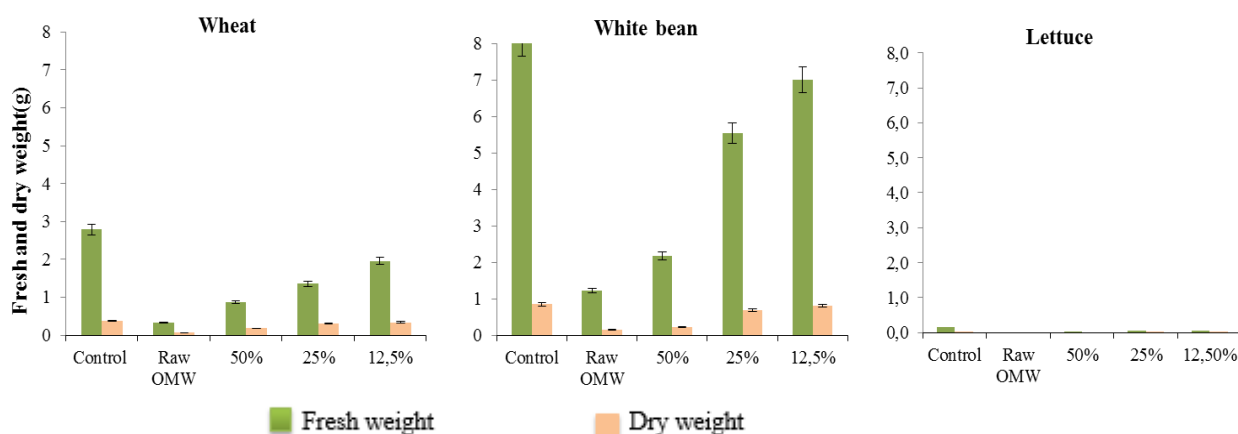


Figure 13. Effect of undiluted and diluted OMW treated on the fresh and dry weights of the three plants species

Conclusion

The OMW is considered as problematic and polluting effluent due to its load content of organic matter and phenolic compounds. Before discharging the OMW into freshwater streams or into environmental compartments must be

characterized and then treated. Coagulation- flocculation technique with lime is one of the perfect solution for the purification of OMW resulting in considerable organic load removal. It allowed a considerable elimination of COD (40%) and high reduction of phenolic compounds (97%) at 30 g/L dose of lime. We are looking for the effects of undiluted and diluted (12.5 %, 25 %, 50%) raw OMW and treated OMW by lime of the three different plant species (wheat, white bean, lettuce). The raw OMW inhibit seed germination, root and stem length of three plant species. The diluted raw OMW at 50% inhibit seed germination root and stem length of wheat and lettuce. Whereas, the two dilutions (12.5%, 25%) of untreated and treated OMW by lime seems able of achieve a reduction of the OMW toxicity for wheat and white bean seeds.

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