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Use of Germination Test for the Evaluation of OMWW as A Fertilizerin Agricultural Lands

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Abstract : *Albaniaproduced a large amount of olive millwastewater. The direct discharges on soil or surface water maycreatesignificantenvironmentalproblems, becausethis have significantly high organicmatterload. Olive millwastewaterisbelieved to inducephytotoxiceffect on organismsincludingseed germination and plant growth. The aim of thisstudywas to evaluate the characteristics of the vegetable water (olive millwaste water), and to investigate the effects of olive millwastewater on seed germination of tomato (SolanumLycopersicum), and spinach (SpinaciaOleracea). A germination test wasconducted in an incubatorattemperaturebetween 20-30◦C. Water samplesweretaken in the exit of decanter and are analyzed the followingindicators: pH, temperature, COD, DBO5, CE, turbidity, density, NO3, oil and grease, total dissolvedsolids, salinity and total chlorine, colour, odour. In eachpetridish, a filterpaperwasmounted and 100 seedswereplaced on the filterpaper. Five milliliter of water wasadded to eachpetridish. The seed germination wasdetermined by counting the number of germinatedseeds to calculate the percentage of germination (G %). The results show that olive millwastewaterwasveryphytotoxic and completelyprohibitedseed germination.*

Keywords: *olive mill wastewater, soil, seed germination, seeds*

I. INTRODUCTION

One of the traditional industries in Mediterranean countries is the production of olive oil from the different systems. Eliminating or depuration of production in the industry, particularly wastewater (alpechin) constituting a problem that requires a solution for this sector (García Rodríguez, 1990;Rodrigo Román, 1990; Fiestas Ros de Ursinos y Bor Padilla, 1992; López y Cabrera, 1993) [15]. Its treatment is a major environmental problem in Mediterranean countries, where the generation rate is very high and concentrated in a short period of time (November- February). Alpechini (OMWW) contains 83-94% water, 4-16% organic matter and 0.4-2.5% mineral salts (Connenzana-Ramos, 1986). Organic matter of olive mill wastewater (alpechinit) it is composed of fats, sugars, nitrogenous compounds, organic acids, polyalcohols, pectins, tannins and polyphenols. Olive mill wastewater has a high ability of the pollution which comes as a result of organic matter, high content of soluble solids (CE 8-22 dS/m) and the suspension that exceed the allowable limits for waters that flow into rivers, lakes, lagoons, different terrains, etc. underground. The organic matter of the alpechin is measured by COD and BOD and that is many times higher than the effluent of other agro-food industries (Fuller y Warrick, 1985; CuadrosGarcía, 1989,F. Cabrera 1995) [15]. The composition of OMW is very variable and depends on olive variety, the ripeness of the fruit, and the extraction process (press or centrifuge) (Lopez & Ramos-Cormenzana, 1996) [6]. This industrial waste water causes many environmental problems, including the following: deterioration of air quality, endangerment of aquatic life, pollution of surface and ground waters, degradation in soilquality, especially soil enzymatic activities, phytotoxicity and odor nuisance.(DellaGreca, 2001)[20]. Olive Mill Wastewater is acidic, and contains high concentration of total suspended solids (TSS), total dissolved solids (TDS), and other organic matter. The organic content is characterized by high levels of chemical oxygen demand (COD), biochemical oxygen demand (BOD), and very high concentration of fat, oil, and grease [10].Many study report that OMW is a major pollutant to surface and ground water resources because it is one of the least biodegradable natural compounds due to its high phytotoxic component, high organic matter concentration, high chemical oxygen demand (COD), low pH, oil and grease (O&G) (Paredes et al, 1986, Saez et al, 1992, Al Khudari, et al, 2004, Mekki et al. 2007) [11].

Average oil production per year is around 6-8 thousand tones. According the data from Ministry of Agriculture, Albania has about 40000 ha of land with 6 million plants. In Albania from the statistic of 2006 result around 4.497 000 olive trees, from which the output is about 3,603,000 root, with an average production

14 kg per tree, or over 40 thousand tones average production. The total installed capacity is about 88 tons / hour of work [7] [8]. During the olive oil production a large volumes of the waste are generated that vary in what type of technology is used and what cultivar is used. Many Mediterranean countries are characterized by the presence of many olive plantations with over 10 million trees [12].

Table 1: Characteristics and composition of OMW (Skerratt&Ammar, 1999; Lopez, 1992, Hashwa 2010) [11]

OMW characteristic	Value
Color	Intensive violet-dark brown up to black
Odor	Strong specific olive oil smell, foetid smell
pH	4.5 - 6.0
Water Content	83 - 92 %
Organic and Volatile Material	7 - 15 %
Mineral Solids	1 - 2 %
Residual Oil	0.3 - 10.0 %
Total Sugars	2 - 8 %
Reducing Sugars	1 - 8 %
Polyalcohols	1.0 - 1.5 %
Protein	0.5 - 7.5 %
Pectins and Tannins	1.0 - 1.5 %
Phenols	17 %
Suspended Solids	5 - 35 g/l
BOD_5	65 - 70 g/l
COD	40 - 200 g/l

The OMW show highly variable properties but they are generally characterized by acidic pH (4.5–5.5) and a high organic load, with average biological and chemical oxygen demand values of 60 and 100 g L1, respectively. The organic matter of OMW mainly consists of sugars, tannins, lipids, aromatic acids and phenolic compounds, with the latter being suggested as the main cause of its reported antibacterial and phytotoxic effects (Karpouzas, 2009) [3].The direct discharges on soil or surface water may create significant environmental problems. Indeed, soil application of OMW results in an increase in organic matter content and in available inorganic nutrients, mainly K, P, Mg and Fe (Karpouzas, 2009) [3].

II. MATERIAL AND METHODS

2.1. Olive mill wastewater

For the realization of the experiment we took vegetable water at the exit of the decanter 3 phase system. The physicochemical analyses were conducted according Standard Methods for the Examination of Water and Wastewater 20th Edition [22]. Water samples are analyzed the following indicators: pH, temperature, COD, DBO5, CE, turbidity, density, NO3, oil and grease, total dissolved solids, salinity [9].

a. Seed germination test

Seeds were obtained from Central laboratory testing seeds in Tirana. The experiment were carried out in petri dishes using four seeds of different crops such as tomato *(Solanumlycopersicum*), and spinach *(Spinaciaoleracea).* In each petri dish, a filter paper was mounted and 100 seeds were placed on the filter paper. Five milliliter of water was added to each petri dish [13]. Germination was performed with olive mill wastewater and normal water. For each crop are taken five replicates with different varieties. The germination test was realized from International Rules for Seed Testing 1996, in a laboratory testing seeds. Seeds were considered to be germinated when its root was visible and measurable. The seed germination was determined by counting the number of germinated seeds to calculate the percentage of germination (G %) [4].

$$G\,\% = \frac{SG}{ST}\ x\ 100$$

Where SG is the number of seeds germinated and ST is the total number of seeds.

A seed was considered grown when its root length exceeded 5 mm. For root length less than 5 mm, the root length was taken equal to 0 and the seed was not considered grown [2].

III. RESULTS AND DISCUSSION

3.1. Physicochemical composition of Olive Mill Wastewater

Results (Table 2) have demonstrated the physical characteristics of OMWWfrom the three-phase system similar to those described by other authors [11] [19] with dark color, heavy aroma and turbulence up.The pH has resulted acidic and out of the permissible emission standards for the waters used by the Albanian Standards Catalog of 2011.The OMWW organic load expressed in the BOD and COD contents has resulted to be very high, outside the limits and very high compared to the effluents coming from other agro-industrial industries (García, 1989) [16]. High values compared to standards have been observed for TDS, N-NO3 and P-PO4.These high values of OMWW on the surface water quality are translated into increasing concentrations of organic and inorganic wastes of K, P. At the same time, the utilized water of the olive processing industry results in a drastic reduction of dissolved oxygen resulting in the development of deadly microbes, asphyxia and deaths of aquatic fauna (Cabrera 1984) [17].

Table 2. Characteristic of olive mill wastewater used on seed germination

Values	Units	Results	Discharge rates by VKM Nr 177 Albania		Italian discharges [18]
pH		5.39	6-9		5.5-9.5
Temperature	˚C	15	3		
CE	ms/cm	3.2			
Salinity		1.3			
Apparent density	g/ml	1.031			
COD	g/l	30.25	0.25		0.16-0.5
BOD	g/l	16.32	0.05		0.04-0.25
TDS	g/l	87.14	50		
Oil & grease	g/l	3.42	10		0.02-0.04
$N\text{-}NO_3$	mg/l	89	10		20-30
$P\text{-}PO_4$	mg/l	102			10
Total chlorine	mg/l	732.2			

3.2. The effects of olive mill wastewater on seed germination

Tomato cultivars treated with normal water have been resulted in their percentage of germination according to this order: (89%, 89%, 88%, 87%, 86%), while the cultivars treated with OMWW have been resulted in their percentage of germination according: (10%, 10%, 10%, 10%, 9.5%). From the results it is easy to see the toxic effects of the use of this water for tomato seed germination in all cultivars studied. The opposite has happened with spinach's culture, this crop seems to be much more resistant from the toxic effect of OMWW. The results have been demonstrated that there is not a big difference between the impact of normal water and OMWW in germination index. The results achieved were very close in both cases. Even in the case of spinachan increase in seed germination percentage has been observed, over the time use of this water, making it possible to increase the percentage of 5-10% by cultivars. The highest percentage of germination has resulted in the fourth cultivar with 96.5%, while the lowest was observed in the fifth cultivar with 93.5%. In both cases our results coincided with other authors but in the culture of Raphanus Sativa (Garcia et al. 1994).

Over time, from the first use of OMWW coming out of the decanter, in 210 days was observed the reduction of the phytotoxicity in the OMWW which is demonstrated with a higher percentage of seed germination in both cultures. The percentage was ranged from 20-30% to tomato and 5-10% to spinach, demonstrating that the change of OMWW characteristics is positive over time. Improving the characteristics of OMWW, decrease of BOD and COD make it possible to use them after a time of storage and pre-treatment.

OMWW (olive mill wastewater) treatments were less phytotoxic to spinach plants than tomato plants (Ouzounidou et al 2008)

It is proposed that dilutions of OMW could be considered as a treatment allowing the reduction of OMWW toxicity (Mekki et al. 2006; Ouzounidou et al. 2008;Chartzoulakis et al. 2010) [1].

Fig.1. Photo from the experiments of tomato *(Solanumlycopersicum)*, spinach*(Spinaciaoleracea)*

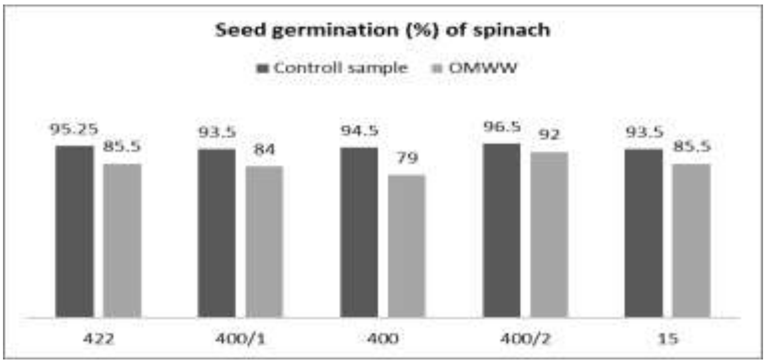

Fig.2. Photo from the seed germination (%) of spinach

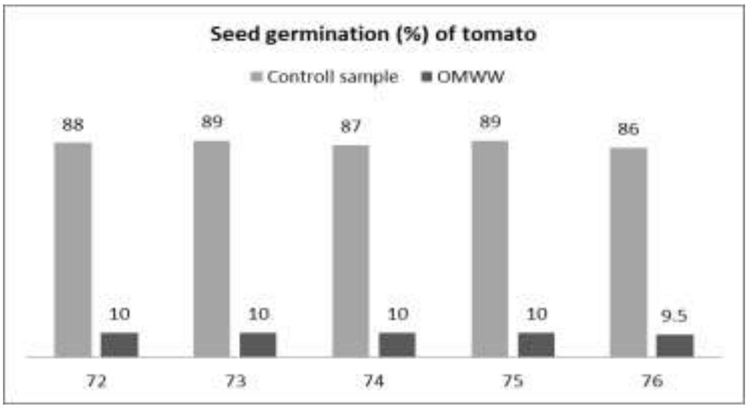

Fig.3. Photo from the seed germination (%) of tomato

The first five varieties were treated with normal water while 5 others with vegetable water. Results show decrease of seed germination when seeds species were cultivated with olive mill wastewater. The first five replicates treated with normal water have germinated over 80%. The second five replicates are treated with 100% olive mill wastewater, from the graphic we see spinach is more tolerant than tomato. Tomato is more sensitive from the olive mill wastewater [5]. The uncontrolled disposals of Olive oil Mill Wastewater in the environment represent a serious environmental problem. The antimicrobial activity (Capasso et al., 1995; Paixao et al., 1999), the inhibition of seed germination (Bonari et al., 1993; Perez et al., 1986), the phytotoxicity to herbage crops (Capasso et al., 1992; Tomati and Galli, 1992), and the production of stale odor by OMW have been demonstrated. More researcher from around the world have come to the results that the vegetable waters before being used should be treated, because it was established that these waters are too toxic. A significant degree of this phytotoxicity is attributed to the high phenol content (1.2-50 g/L) (Sáiz-Jiménez el al., 1987), in the untreated wastewater [5] [11]. It is evident from the present research and from the relevant bibliography that high phenol content (low dilutions of OMW) is universally phytotoxic but also those different species exhibit different sensitivities to OMW [2]. The phenolic compounds gives to alpechin three characteristics (ability) the bactericidal effect, toxic effects and color (González el al, 1990; Pérez el al., 1992).The organic fraction contains large amounts of proteins, lipids and polysaccharides, but unfortunately OMWW also contains phytotoxic components that inhibit microbial growth (Capasso et al. 1995; Ramos-Cormenzana et al. 1996), as well as the germination and vegetative growth of plants (Linares et al. 2003) (Morillo2009)[14].

IV. CONCLUSION

Analyzing the values of the parameters in this study does not recommend to use directly OMWW as a fertilizer without any prior treatment, a fact that is due to their acidity (5:39), to their high electrical conductivity (CE> 3.2) and high concentrations of polyphenols (Gonzales et al., 1990); responsible for non-life activity and phytotoxicity of OMWW [21].

Different methodologies have been studied for the elimination and treatment of waterthat derives from olive oil production, which due to low efficiency and high costs have led the olive oil producer companies not to accept them.Between economic solutions could be proposed the evaporation ponds, and fertilization treatments on the ground, each with its own advantages and disadvantages.

If we take in consider the evolution of OMWW parameters in the evaporation pond, which after a while have approximate values to those of the soil control (Zharra et al., 2015) and at the same time maintain high levels of organic matter and potassium needed to improve poor soil, this method can be accepted as an alternative to treat these water.

Based on the high germination indicators in the OMWW samples after a period of biodegradation, it would be considered interesting on all the evolution of organic matter content and potash, what would be a very important behavior for poor lands, because the olive needs the rich soil with this element.In all cases, olive oil productions must accept the fact that purification of OMWW involves an additional cost of the extraction process.A valid alternative to the discharge of OMW into waterbodies is the spreading on agricultural land, provided thatphytotoxic effects are neutralized (Casa et al. 2003).

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