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CONTRIBUTION OF GREEN MANURE, RHIZOBIUM AND HUMIC + FULVIC ACID ON RECOVERING SOIL BIOLOGIC ACTIVITY OF OLIVE MILL WASTEWATER CONTAMINATED SOIL

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Abstract

Olive trees cultivation is one of the important agricultural product in especially Mediterranean, Aegean and Marmara regions of Turkey. There are about 750 million productive olive trees worldwide whereas 98% of them located in the Mediterranean region. Three major olive oil producers worldwide are Spain, Italy, and Greece, followed by Turkey. Olive Oil Mill Wastewater (OMWW) is one of the waste products of olive oil process that contains different harmful substances such as polyphenols and long-chain fatty acids which risky for soil and plants. These harmful materials are environmentally not safe, while they cause economic and ecological problems. Due to the considerable amount of OMWW occur every year, a number of approaches tested by the researchers to overcome this issue. In this study the effect of OMWW alone and combination with green manure, rhizobium and humic acid on soil microbial activity evaluated. Results gathered revealed that OMWW was reduced determined microbial activity parameters as soil respiration (CO_2 production), dehydrogenase enzyme activity and microbial biomass carbon content. All applications were effective on restoring CO_2 production that diminished due to OMWW; however, humic acid and green manure+humic acid applications yielded the highest benefit. Dehydrogenase activity did not differ strongly by OMWW applications. Microbial biomass carbon (MBC) values decreased rapidly by OMWW incorporation, but green manure and bacteria application together restored MBC, even higher values determined in that variant.

***Key words**: olive oil mill wastewater, soil microbial activity, bioremediation.*

INTRODUCTION

Olive Oil extraction facilities are producing large amount of wastewater, which contain undesired toxins. Three-phase and two-phase centrifugation systems are used in olive oil industry, more or less, both of them produce these toxins containing wastes. In general three-phase systems produce more wastewater or sludge comparing to two-phase systems. Thus, two-phase centrifugation systems were commonly referred by olive oil producers, recently. The environmental impact of olive oil production is considerable, mainly due to the utilization of large amounts of water and the production of large amounts of either wastewater or sludge. Pressure and three-phase centrifugation systems produce substantially more olive mill wastewater (OMWW) than two-phase centrifugation, which significantly reduces liquid waste yet produces large amounts of semi-solid or slurry waste commonly referred to as two-phase pomace or alperujo in Spanish (Alburquerque et al., 2004; Niaounakis and Halvadakis, 2006). But according to some researcher, the two-phase-system has not penetrated significantly into the other olive oil-producing countries, mainly due to difficulty in handling the sludge for this reason most countries continue to make use of the three phase centrifugation system (Kotronarou and Mendez, 2003).

OMWW has very high organic load and also contains high levels of phytotoxic and microbially inhibitory compounds, such as phenolics and long-chain fatty acids (Niaounakis and Halvadakis, 2006; Thomsom, 1964; Fountoulakis et al., 2002). Phenolic compounds are present in wastes from several industrial processes, likewise, olive oil mill wastewater (OMW) is an effluent containing many of these compounds which are responsible for its black color, and its phytotoxic and antimicrobial (mainly antibacterial) properties (Martinez et al., 1986; Martinez-Garcia, et al., 2007; Tsioulpas et al., 2002). This wastewater constitutes a serious problem with severe negative impact on soil and water quality, and thus on agriculture, environment and health (Parades et al., 1999; Khoufi et al., 2007).

Vast majority of olive oil production occurs in the Mediterranean region, utilizing a tremendous volume of water in an area of the world in which water resources are limited. Treatment and refuse of olive mill wastewater (OMWW) presents significant challenges both due to the nature of olive oil production and due to the characteristics of the wastewater (high chemical oxygen demand, high phenolic content, and dark color). Christopher et al. (2007) use bioremediation technic to solve the OMWW problem. They have tested number of different microorganisms (Archaea, Bacteria and fungi) and processes (aerobic or anaerobic bioreactors, composting) to treat OMWW. According to their results, biological processes provide some of the most viable options for the treatment of OMWW. Effective application of these techniques, yielding significant reductions in high chemical oxygen demand, phenols, and colour, will allow safe and economical disposal of OMWW.

Tsioulpas et al. (2002) studied at Phenolic removal in olive oil mill wastewater by strains of *Pleurotus spp*. in respect to their phenol oxidase (laccase) activity. They used several *Pleurotus spp*. strains to remove phenolic compounds from an olive oil mill wastewater. Martinez-Garcia et al. (2007) investigated Two-stage biological treatment of olive mill wastewater with whey as co-substrate. Their results show that fifty-four percent of the phenol was biodegraded during the aerobic treatment stage, and biogas with between 68% and 75% methane was produced during anaerobic digestion.

Roig et al. (2005) studied on overview on olive mill wastes and their valorization methods. According to these researchers olive mill wastes represent an important environmental problem in Mediterranean areas where they are generated in huge quantities in short periods of time. Their high phenol, lipid and organic acid concentrations turn them into phytotoxic materials, but these wastes also contain valuable resources such as a large proportion of organic matter and a wide range of nutrients that could be recycled.

In this research, the effects of OMWW on some biologic parameters of soil was evaluated while useful part of this substance considered as indicated Roig et al., (2005). To support microorganisms to denature toxic ingredient of OMWW, green manure, rhizobium and humic+fulvic acid were applied.

MATERIALS AND METHODS

An incubator experiment was carried out at Mustafa Kemal University, Faculty of Agriculture; Soil Sci. & Plant Nutrition Department. OMWW was obtained from any olive oil factory in Hatay, where located Southeast of Turkey, in Mediterranean region (Figure 1).

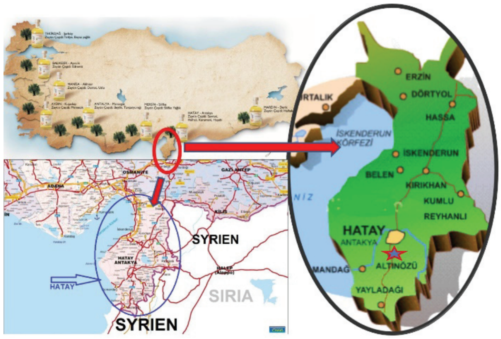

Figure 1. Geographic location of research Area

Olive cultivation's proportion of Hatay is around 10% of Turkey. There are 85 olive oil factories available that using two or three-phase centrifugation systems. The total processing capacity of these companies is approximately 22 thousand tons. Soils used in the experiment were collected from Mustafa Kemal University research and implementation area. Some soil properties are provided in Table 1. Explanation of abbreviation used in the tables provided in Table 2.

Table 1. Some soil properties of experimental soil

pH	Salt (%)	$CaCO_3$ (%)	Corg (%)	Texture class
8.35	0.22	17	1.15	CL

Sieved 200 g of soils was placed to open lid jar and alone or combination of the practices provided in Table 2 realized. Afterwards samples were incubated for one month at 28°C. Soil moisture controlled at regular interval and sustain at field capacity.

Table 2. Explanation of practices

Abbreviations	Explanations
BE	Before experiment – natural situation
OMWW	Olive Oil Mill Wastewater: 10 ml of OMWW homogenized to 200 g of soil.
GM	Green manure: Doses adjusted according to 20 tons of green manure to one hectare
HA	Humic and fulvic acid: Doses adjusted according to 50 liter to one hectare.
Br	Bacteria: Bacteria suspension containing 4 x 10^9 cells applied to 200 g of soil.

An organic matter content of humic+fulvic acid was 5% where pH was 11-13. At the end of the experiment samples removed out of the incubator and analyses started immediately. CO_2 analyses were done by titration method which CO_2 was captured by $Ba(OH)_2$ and remaining BaOH titrated by HCl (Isermayer, 1952). Dehydrogenase enzyme activity (DHA) was determined according to Thalman (1956). Microbial biomass carbon (MBC) content was determined by fumigation-extraction method (Ohlinger, 1993). Completely randomized design was used, obtained results were statistically analysed via MSTAT-C pocket software and ranged with Duncan multiple range test.

RESULTS AND DISCUSSIONS

The effect of OMWW, green manure, humic+fulvic acid, *rhizobium* bacteria and all of randomized combination of this application on CO_2 production presented in Figure 2 and Table 3. All applications significantly influenced CO_2 production of soils, whereas, OMWW application diminished CO_2 production rapidly. Only 0.75 mg CO_2-C from 100 gram of dry soil at 24 h which the highest value among the applications was 12 mg at both OMWW+HA and OMWW+GM+HA variants. These two was followed by OMWW+GM+HA+Br and OMWW+GM+Br with 11.50 and 10.75 mg CO_2, respectively. Considering overall results it seems to be Br and HA more effective to contribute soil on recovering harmful effects of toxic substances added by OMWW.

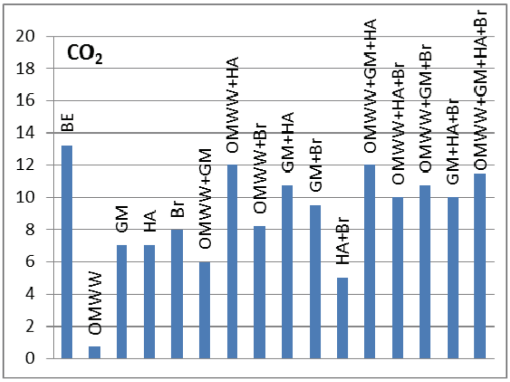

Figure 2. CO_2 production (mg CO_2-C 100 gds^{-1} 24 h^{-1})

Determined dehydrogenase (DHA) values are presented in Figure 3 and Table 3. There was no similarity between CO_2 production and DHA activity, which DHA was not effected from OMWW application considerably. Even so-called safe substrates reduced dehydrogenase activity more than OMWW. The lower DHA values were determined in OMWW, OMWW+HA, GM+HA, HA+Br and OMWW+HA+Br as 307, 262, 236, 276, 259 g TPF at 10 gram of dry.soil for 24h, respectively.

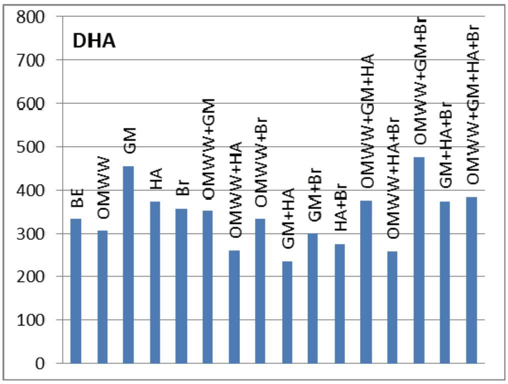

Figure 3. DHA (g TPF 10 gds^{-1} 24 h^{-1})

The highest DHA values determined in OMWW+GM+Br variants as 478 g TPF 10 gds $^{-1}$ 24h^{-1} which approximately 55% higher than OMWW applied soils.

Microbial biomass carbon values which effected from OMWW, green manure, humic+fulvic acid, *rhizobium* and their combination are presented in both Figure 4 and Table 3.

The lowest MBC values are determined in OMWW+Br and OMWW+GM as 2.1 and 2.5 µg MBC in one gram of soil. The negative effect of OMWW become more dramatic when applied with GM or Br in term of MBC. However, surprisingly when GM and Br incorporated together with OMWW, the highest MBC value was achieved as 95.4 µg C g dry soil^{-1}.

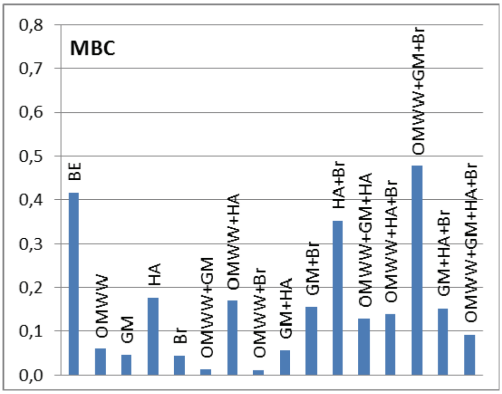

Figure 4. MBC Content (mg MBC gds^{-1})

Table 3. Statistically results of values

Applications	CO_2 (mg CO_2-C 100 gds^{-1} 24 h^{-1})		DHA (µg TPF 10 gds^{-1} 24 h^{-1})		MBC (mg MBC in gds)	
BE	13.25		335		0.0833	
OMWW	0.75	h	307	ef	0.0118	e
GM	7.00	ef	456	a	0.0089	e
HA	7.00	ef	374	bc	0.0354	b
Br	8.00	cd	358	b-d	0.0086	e
OMWW+GM	6.00	b	353	b-d	0.0025	f
OMWW+HA	12.00	a	262	gh	0.0339	b
OMWW+Br	8.25	cd	334	de	0.0021	f
GM+HA	10.75	b	236	h	0.0111	e
GM+Br	9.50	bc	301	ef	0.0311	bc
HA+Br	5.00	g	276	fg	0.0700	cd
OMWW+GM+HA	12.00	a	376	b	0.0254	d
OMWW+HA+Br	10.00	b	259	gh	0.0275	cd
OMWW+GM+Br	10.75	b	478	a	0.0954	a
GM+HA+Br	10.00	b	374	bc	0.0300	b-d
OMWW+GM+HA+Br	11.50	ab	383	ab	0.0182	de

gds: gram dry soil, data obtained before experiment conducted was not included to statistical analyses.

CONCLUSIONS

Olive Oil Mill Wastewater (OMWW) is a huge pollution problem for some countries which produce oil olive especially in cities around Mediterranean Sea. Because of the oil extraction process has both two-phase and three-phase centrifugation systems which are unfortunately producing plenty amount of water that containing several toxic waste matters. These toxics are mainly consisted on phenolic matters and long-chain fatty acids that are strong phytotoxic and microbial inhibitory compounds. Addition to such as phenolic and long-chain fatty acids OMWW also contains very high organic load (Halvadakis, 2006; Christopher et al., 2008). OMWW is known as black water in olive grown region in Turkey.

OMWW is growing concern for Turkey, yet no feasible, economic and uncomplicated solution developed; although a number of researches carried out already. According to Martinez-Garcia et al., (2007) several OMWW purification systems and approaches have been reported including evaporation basins (Saez et al.,1992), physico-chemical treatments (Flouri et al., 1996), filtration processes (Almirante and Carlo, 1991), aerobic treatment (Benitez et al., 1997) and anaerobic digestion (Rozzi et al., 1989). However, recommendation must be done considering environmental concern and utilization opportunities. The aim of this study was determining the effects of some organic and ecological material such as green manure, *rhizobium* and humic+fulvic acid on recovering soils from hazardous effects of OMWW. It is well known, contamination of so-called "black water" diminishes microbial activity parameters such as CO_2 production, DHA activity and MBC contents that evaluated once more in this this research. CO_2 production values in all variants were determined higher than solely OMWW applied soil, whereas the lowest CO_2 production value is observed in OMWW soil as 0.75 mg CO_2-C 100 g.dry.soil^{-1} 24 h^{-1}. Other values are changed between 6-12 mg CO_2-C 100 g.dry.soil^{-1} 24 h^{-1}. Soil respiration consists of CO_2 produced from biochemical processes associated with root activities (autotrophic respiration), and microbial organic matter decomposition (heterotrophic respiration) (Saiz et al. 2006; Boone et al., 1998; Buchmann, 2000).

DHA value of OMWW soil was 307 μg TPF 10 g dry soil^{-1} 24 h^{-1} and the other values are changed between 236-478 μg TPF 10 g dry soil^{-1} 24 h^{-1}. According to DHA results treatments and their combinations improved DHA activity, but the differences between the values were not as evident in CO_2. Moreover, humic+fulvic acid and OMWW combination was caused decrease on determined DHA values. According to several researchers, soil quality defined as "the capacity to function within an ecosystem and sustain biological productivity, maintain environmental quality and promote plant, animal and human health". In natural conditions, soils tend towards maintaining an equilibrium between pedogenetic properties and the natural vegetation (Parr and Papendick, 1997; Pascual, 2000).

MBC value in OMWW soils is find as 0.0118 mg. dry. soil^{-1}. Some of application Some MBC values were higher than OMWW whereas some others were higher. Conversely DHA activity results, microbial biomass carbon (MBC) content increased in OMWW+humic+fulvic acid applied variant. The highest value of MBC is determined in OMWW+GM+Br soils as 0.03 mg MBC in gram of dry soil.

Generation of OMWW in the Mediterranean region has a significant environmental impact. Overall, the incorporation of biological processes provides some of the most viable options for the treatment of OMWW (Christopher et al., 2008). Based on the results gathered, OMWW applications decreased to soil microbial activity. However if some ecological material such as green manure, soil organisms and humic+fulvic acid use with this waste, it can reduce to toxic effect of OMWW.

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