OLIVE MILL WASTES MANAGEMENT: A NOVEL APPROACH WORKING AT MILLING LEVEL FOR RECYCLING IN AGRICULTURE ALL KINDS OF EFFLUENTS

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SUMMARY: The ISAFoM-CNR, in the context of the EU Life-TIRSAV project, developed a technology named M.A.T.Re.F.O. [Metodo ed Apparato per il Trattamento dei Reflui dei Frantoi Oleari, patent n. RM2004A000084] for recycling all kinds of raw Olive Mill Waste Effluents (OMWEs) in agriculture. The procedure usually occurs at milling level by mixing OMWEs, previously destoned, with raw hygroscopic bulking agents (straw, wool waste, sawdust, olive leaves, twigs and prunings) in order to obtain a dryer non percolating and non bad-smelling Olive Mill Waste Mixture (OMWM) which is directly packaged into 20-30 kg net bags, hence resulting easy to be managed. Chemical properties of OMWM, recorded after short aerobic storage trials, make it suitable for different agronomic applications as soil amendment/fertilizer or as peat substitute in making nursery growth media or for mushroom cultivation. By far, according to results achieved and preliminary cost/benefit evaluations, the OMWEs recycling system described appears particularly fitting for "two phases" olive oil extraction plants, resulting in added-value end products for agriculture, certifiable also in organic farming.

1. INTRODUCTION

Worldwide olive oil industry production is increasing and accounts for about $2.8x10^6$ tons per year, 76% of which comes from European Union countries (IOOC, 2006). As a consequence of such increasing trend, modern olive mills are facing severe environmental problems due to lack of feasible and/or cost-effective solutions to olive-mill waste management. The high organic load, mainly made by polyphenols as well as short and long-chain fatty acids, is believed to contribute to the phytotoxic nature (Cassa et al., 2003; Di Gioia et al 2002, Kistner et al., 2004; Isidori et al., 2005) and antimicrobial effect (González et al., 1990; Paixão et al., 1999; Fiorentino et al., 2003) of such waste materials.

Although several patented techniques exist for olive-mill waste management, they are often too costly, and therefore unfeasible for most of olive-oil entrepreneurs all over the world (Niaounakis and Halvadakis, 2006). Therefore, it often happens that olive mills illegally dispose of their waste in the environment, with risks of severe ground or surface water pollution.

Italy is the only olive-oil producing country with a special legislation for disposal of raw olive mill wastes in soil (waste water and/or wet husk coming from two-phase decanters) through controlled spreading. Italian Law n. 574/96 allows yearly soil spreading of up to 50 or 80 m^3 ha^{-1} olive mill wastewater generated respectively by press or continuous centrifugation systems.

However, taking into account the restrictive prescriptions of Law n. 574/96 and the mostly hilly landscape of Italian olive-growing regions, direct spreading in soil of olive-mill by-products often is not feasible and/or very costly.

Nevertheless, to recycle into soil such industrial waste, rich in organic matter and low on xenobiotic pollutants, must be considered a good tool for opposing soil desertification in most olive-growing regions (Alburquerque et al., 2007)

For this reason methods to transform organic wastes into organic fertilizers or amendments, have progressively received growing attention in modern sustainable agriculture (Felipó, 1996; Sequi, 1996).

Given the above scenarios, the olive-cropping section of ISAFoM-CNR has recently developed a new technology, named M.A.T.Re.F.O. (Metodo ed Apparato per il Trattamento dei Reflui fri Frantoi Oleari, patent n. RM2004A000084), that facilitates overcoming difficulties in recycling raw olive-mill effluents for agronomic purposes. (Fontanazza et al, 2000; TIRSAV project, 2001, Altieri et al., 2005). It results in Olive Mill Waste Mixtures (OMWMs) that can be usefully applied in agriculture, either in open field, as soil conditioner/fertilizer or in nursery, as peat surrogate or for mushroom cultivation.

The aim of this paper is to describe the M.A.T.Re.F.O. technology and presents the main results, collected during the last six years, concerning chemical and microbial characterization of OMWMs in storage trials and evaluation of OMWM applications in different agronomic trials.

2. DESCRIPTION OF THE M.A.T.Re.F.O. RECYCLING SYSTEM

By using a specific machinery the M.A.T.Re.F.O. technology is designed to recycle all kinds of raw Olive Mill Waste Effluents (OMWEs) like olive mill wastewater, “two” or “three” phases husks, with particular reference to those coming out from continuous extraction plants.

This end of pipe system, typically working at milling level, consists in four sequential “in batch “ steps: 1) de-stoning, 2) mixing, 3) packaging and 4) storage (Figure 1)

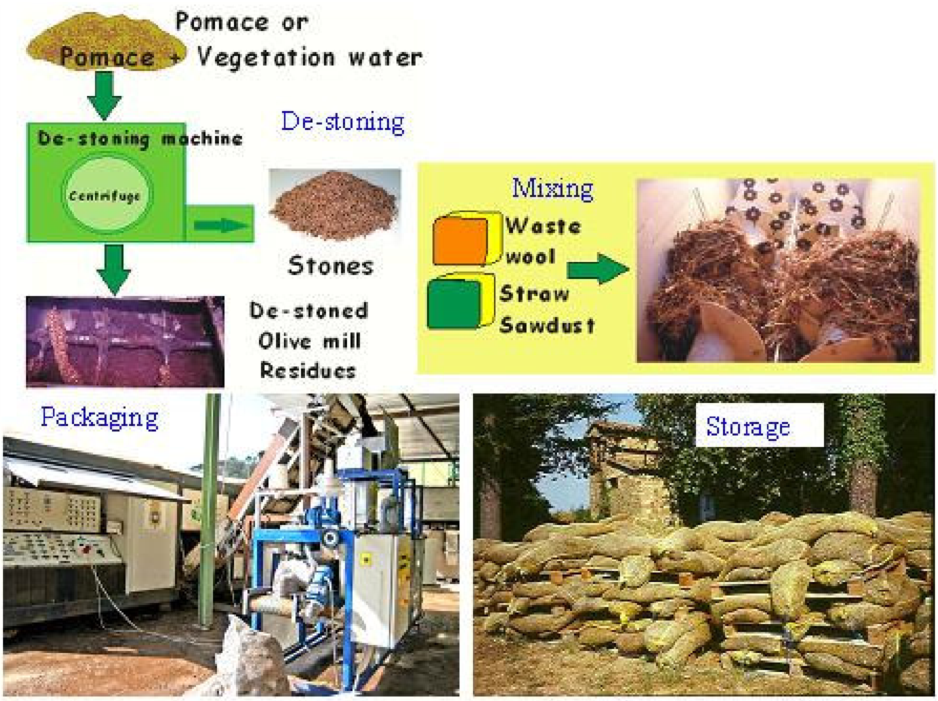


Figure 1. Diagram of the M.A.T.Re.F.O. procedure

In the first step the olive stones are separated from the semi-liquid waste coming out from the mill by a centrifugal equipment. The olive stones, characterised by low moisture (about 25%), are very appreciate by the local market as a fuel for heat production. However, in order to increase more the added-value of the olive stones, alternative industrial application can be hypothesized as the transformation into bio-fuel by pyrolysis, the use in sandblasting monuments and aircraft fuselages or as a growth media for hydroponic cultivation, or for production of insulating material, floor covering and active carbon. With this respect, more additional studies should be planned by researchers to find out possible convenient applications.

The residual de-stoned olive mill effluent, composed by de-oiled olive pulp, skin, vegetation and olive mill processing water, has a moisture content of about 75% that makes it fairly fluid; it is collected in a special tank and slightly stirred to prevent physical separation; then it is pumped into another larger weighting mixer tank where the second step occurs. The semi-liquid flow is mixed, in preset right weight to weight proportion, to hygroscopic organic waste additives in order to get an end product with a right carbon to nitrogen ratio (about 20) and a good natural aeration in the biomass.

According to local availability, the main organic hygroscopic wastes used are straw, wool waste, sawdust, leaves and twigs separated in the mill and pruning residues.

In the second "in batch" step, the organic additives are coarsely chopped (3-4 cm) and homogenously mixed with the destoned OMWE for about 15 minutes to ensure an internal macro-porosity of the biomass that naturally supports aerobic metabolisms. At the end of this step the mixture results in a non bad-smelling and non percolating organic product ready for the packaging.

In case of a higher nitrogen content desired, mineral N-rich fertilizers, like potassium or calcium nitrate, can be added to the mixture during the mixing phase. In this case the resulting end product will be classified as an organic-mineral fertiliser.

Figure 2. M.A.T.Re.F.O. prototype equipment

The third step provides an automatic packaging of the organic mixture in net sacks which are then submitted to the 4th final step of the procedure: the storage. The packaging makes the aerobic storage easier; it typically occurs in stack piles, protected against rain to avoid undesired percolation, turning out, shortly, in a partially dryer and stabilized end product easy to transport and manage at district level. The original weight of each sack generally ranges between 20-30 kg to assist possible handling.

A right aerobic storage determines a microbial-driven maturation of the biomass as a result of the action mainly of yeasts and bacteria and it can be extended without any inconvenient for several months, thus improving the chemical and physical properties of the end product.

Within the framework of the EU LIFE-TIRSAV project, experimental M.A.T.Re.FO. equipment were developed by Verdegiglio Macchine Agricole S.p.A. company, partner of the project, which installed two prototypes at two olive mills located in the Cilento and Vallo di Diano National Park. (Figure 2).

3. EXPERIMENTAL SECTION

3.1 Chemical and biological characterization of the olive mill waste mixture in storage trials

Firstly, each component added to the mixture was chemically characterized (Table 1).

With the aim to identify the best end product for agronomic use and to reduce the cost of the mixture by using lesser amount of hygroscopic additives as possible, different combination weight to weight of destoned OMWEs and bulking agents were tried. Non percolating and non-bad smelling mixtures were obtained by using approximately 72 % of destoned OMWEs + 28% of hygroscopic materials. The initial mixing trials led to identify the following best Olive Mill Waste Mixture (OMWM) for agronomic purposes: 72% OMWEs + 11% wool waste + 8.5% straw + 8.5% sawdust, whose chemical characteristics are reported in Table 1.

Table 1. Characterization of raw destoned Olive Mill Waste Effluents (OMWEs) coming out from a two phases decanter, organic hygroscopic additives (WW = Wool Wastes) and fresh Olive Mill Waste Mixture (OMWM)

		OMWEs	WW	Straw	Sawdust	OMWM
Moisture	%	76	15	33	17	67
pH		4.69	7.08	7.26	6.70	5.66
Electrical conductivity	$dS(m)^{-1}$	1.19	1.20	1.48	0.08	1.56
Ash	% dw	5.6	10.9	9.8	0.6	10.2
Total organic carbon	% dw	47.2	44.6	45.1	49.7	42.1
N	% dw	0.76	5.24	0.34	0.15	1.96
C/N		61.9	8.5	133.0	331.0	21.5
Fat	% dw	3.7	nd	nd	nd	4.9
Phenols	% dw	2.6	nd	0.1	nd	0.7
K	% dw	2.1	1.4	1.3	0.1	2.6
Ca	% dw	0.15	0.13	0.2	0.18	1.12
Mg	ppm dw	729	405	1600	427	2903
Fe	ppm dw	200	706	170	77	3809
Mn	ppm dw	7	37	45	< dl	88
Zn	ppm dw	21	85	< dl	< dl	104
Cu	ppm dw	24	8	3	< dl	28
GI	%	0	nd	nd	nd	51

nd = not detected, dl = detection limit, dw = dry weight, GI = germination index

Compared to destoned OMWEs the fresh mixture showed the following main changes: a higher nitrogen content (about 2%) a lower C/N (about 20), about 75% reduction of phenols and a remarkable K content (2.6%).

In order to determine the main chemical and microbial parameters evolution, packaged mixtures were submitted to two different aerobic storage trials: in “stack pile” and in “big bag” (1000 kg), both outdoor protected against rain (Figure 3a and 3b)

(a) (b)

Figure 3. Storage trials in stack pile (a) and in big bag (b)

Both trials showed at the beginning of storage a fast increase in temperature as result of a strong aerobic metabolism. The higher surface to mass ratio in stack pile can justify the following rapid decrease in temperature of the OMWM which reached the air value in less than a week. On the other hand as a result of a lower heat exchange, the temperature in the big bag kept higher than 40 °C over two months, with values over 50 °C for all along the first 20 days of the storage (figure 4).

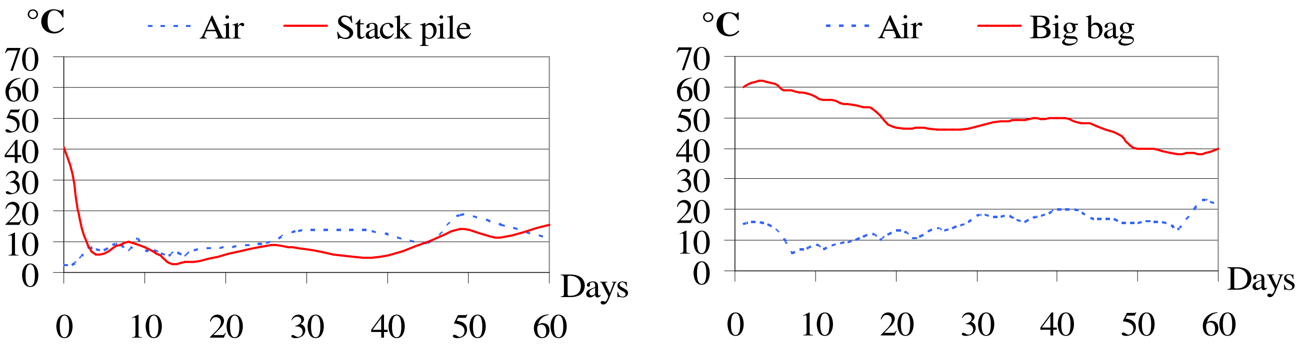


Figure 4. Temperature monitored in OMWM during the storage trials

The different performance of temperature during the storage trials resulted in different changes in some chemical and biological parameters of OMWM, as reported in Table 2.

Storage in big bag determined a considerable higher mineralization of OMWM, as proved by the increase in the ash content, reaching after 90 days a value 2.9 times higher than the initial one. Decrease of C/N also confirmed the intense aerobic metabolism occurred, without a significant nitrogen release in the environment.

On the contrary, OMWM stored in stack pile did not change considerably neither in carbon content nor C/N ratio probably as a result of a lower metabolism occurred.

However, in both storage trials a relevant reduction in phenols were recorded, particularly marked in big bag trial where, as expected, a clear higher oxygen-driven degradation and flocculation took place.

OMWM phytotoxicity was assayed by the *Lepidium sativum* test, according to Zucconi et al. (1981) procedure. Both storage systems, at the end of trials, showed Germination Indexes (GI) values higher than 70%, thus indicative of a complete suppression of the original OMWEs phytotoxicity.

Table 2 Evolution of the main chemical parameters of OMWM during the storage trials; quantities are referred to dry matter base

		Stack pile			Big bag		
	Storage day	15	30	90	15	30	90
Moisture	%	63	65	64	77	72	70
pH		6.10	6.90	6.85	8.06	7.31	6.40
Electrical conductivity	dS m^{-1}	1.42	1.33	1.35	2.47	5.41	9.35
Ash	%	10.0	9.8	9.9	14.7	26.2	29.8
Nitrogen	%	2.25	2.50	2.17	2.68	3.60	4.25
C/N		22.2	20.0	23.1	17.7	10.8	9.7
Phenols	ppm	3500	2200	2700	560	615	367
GI*	%	70	74	71	44	32	77

* GI = Germination index

Additional samples collected in the stack pile storage trial were also microbiologically characterized and analyzed for humification parameters.

Bacteria, yeasts, and moulds found were tested in terms of Colonies Forming Units (CFU) at different storage times (at the moment of production, after 15 and 60 days of storage) with the aim to identify those able to metabolise phenolic compounds. The microbial performance is reported in Figure 5.

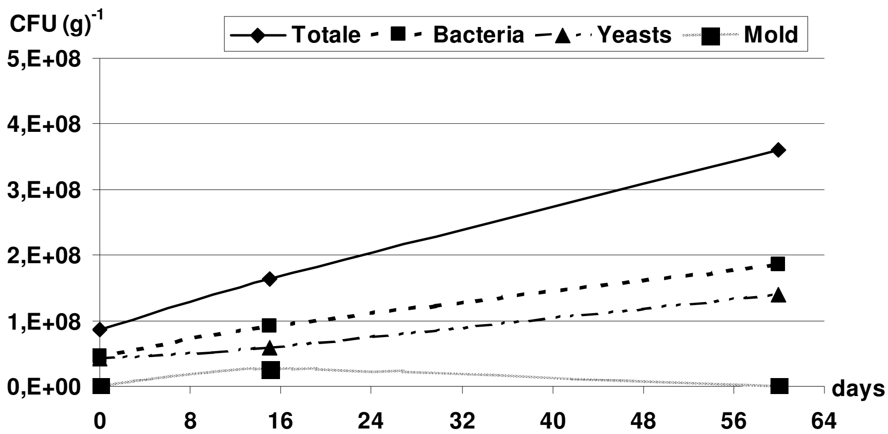


Figure 5. Microorganisms content on Olive Mill Wastes Mixture stored in stack piles; data are evaluated in TSA medium and referred to fresh matter base.

The microbial analyses confirmed the limited metabolism occurred in stack pile, as proved by the small increasing trend recorded, mainly for bacteria and yeasts.

With regard to the humification parameters, determined according to Ciavatta et al. 1990 procedure (data not showed), analyses stated a significant improvement of the OMWM organic carbon quality after storage, as both DH (Degree of Humification) and HI (Humification Rate) increased and HI (Humification Index) decreased.

A similar microbial and carbon quality study is still in progress on samples collected from the big bag storage trial.

3.2 Agronomic trials

3.2.1 Field experiment

In the present study are reported results dealing with the effects on soil organic carbon evolution of two OMWM amendment trials, both performed for five-year in an olive grove; plants growth and yield were also taken into account.

The two mixtures used were composed as follows: OMWM (type"a") containing 72% of de-stoned olive-mill waste, 11% wool waste, 8.5% straw and 8.5% sawdust; OMWM (type"b") containing 72% of destoned olive mill waste, 14% straw and 14% sawdust. Percentage are referred as weight to weight ratio. The mixtures were used as amendment after three months of stack pile aerobic storage.

Figure 6. Field experiment: intensive olive grove

The field experiment (Figure 6), was an intensive olive grove (600 plants per hectare located at Doglio, Montecastello di Vibio, Perugia, Italy, North latitude 42°50' N, East longitude 12° 21' at 400 m. u.t.s) characterized by 737 mm mean annual rainfall, with very poor precipitation during the summer, a sandy-clay-loam soil with pH of 7.9, specific electrical conductivity of 0.37 dS m^{-1}, total calcium carbonate of 17.4% (d.w.), total organic carbon 1.4% (d.w.) and cation-exchange capacity of 15.4 $cmol^{(+)}$ kg^{-1}. Plants were watered by means of a drip irrigation system and no tillage was performed, allowing the growth of natural cover grass, regularly mowed and left on top the soil as mulching. The experimental design consisted of 9 plots (15 trees per plot) according to a randomized block design with three replicates. The two experimental OMWMs were yearly spread in spring on soil surface at a rate of 9 tons (dry matter) per hectare. No additional fertilizers were provided for amended plots while standard mineral fertilization (100 kg N per hectare as urea) was yearly performed in the control plots.

Soil samples (0-20 cm depth) were collected from each plot every year before OMWMs spreading and analyzed for TOC and humification parameters according to standard procedures (Ciavatta et al. 1991)

Vegetative and productive data were yearly recorded randomly from 5 olive plants per plot; trunk diameter was measured at 30 cm above ground.

In Table 3 are reported some chemical characteristics of raw destoned OMWEs and OMWMs samples collected after 3 months of aerobic storage, before soil spreading.

Table 3. Chemical characterization of the raw Olive Mill Waste Effluents (OMWEs) and Olive Mill Waste Mixtures (OMWMs) after 3 months storage; values are referred to dry matter base.

		raw OMWEs	OMWM (a)	OMWM (b)
Moisture	%	76.50	27.90	22.12
pH		4.69	6.70	6.40
Electrical Conductivity	dS m^{-1}	1.19	2.74	3.42
Kjeldhal-nitrogen	%	0.76	2.22	0.95
Total phosphorus	%	0.15	0.16	0.14
Total potassium	%	2.10	2.38	2.63
Total magnesium	%	0.07	0.18	0.12
Total bio-phenols	%	2.60	0.22	0.30
TOC	%	53.68	39.43	43.81
TEC	%	27.54	26.96	20.48
HA + FA	%	14.93	17.40	13.07
DH	%	54.23	64.55	63.82
HR	%	27.82	44.05	29.83
HI		0.84	0.55	0.57

TOC = Total Organic Carbon TEC = Total Extractable Carbon; HA = Humic Acid; FA = Fulvic Acid; DH = Degree of Humification = (HA+FA)x100/TEC; HR = Humification Rate = (HA+FA)x100/TOC; HI = Humification Index = [TEC – (HA+FA)]/(HA+FA)

As to humification parameters, both OMWMs reached an acceptable degree of stabilization after storage since DH and HR increased and HI decreased (Sequi et al., 1986).

Table 4. Carbon content and humification indexes in soil amended for five years with OMWMs. Data are referred to the mean value recorded in 2001-03 (T1) and 2004-05 (T2) periods.

	TOC	TEC	HA+FA	DH	HR	HI	TEC/TOC
			%				
Start of trial	1.42	0.74	0.42	57.20	29.85	0.75	0.52
				T1			
OMWM (a)	1.89b	1.11b	0.68b	61.26	35.96	0.63	0.59
OMWM (b)	1.80b	0.99ab	0.67b	67.68	37.22	0.48	0.55
Control	1.35a	0.76a	0.43a	56.58	31.85	0.77	0.56
				T2			
OMWM (a)	1.85b	1.02a	0.69b	67.65	37.30	0.48	0.55
OMWM (b)	1.61a	0.86a	0.52a	60.47	32.30	0.65	0.53
Control	1.47a	0.82a	0.51a	62.20	34.69	0.61	0.56

Data flanked by the same letters are not significantly different according to SNK test for $p<0.05$.
TOC = Total Organic Carbon; TEC = Total Extractable Carbon; HA = Humic Acid; FA = Fulvic Acid; DH = Degree of Humification = (HA+FA)x100/TEC; HR = Humification Rate = (HA+FA)x100/TOC; HI = Humification Index = [TEC – (HA+FA)]/(HA+FA).

In comparison with the control, OMWMs amended plots showed modification of soil organic carbon parameters mostly in the first period monitored (Table 4); OMWM (a) plots showed 40.0% and 25.8% TOC increase in 2001-03 (T1) and 2004-05 (T2) periods, respectively, while OMWM (b) plots 33.3% in T1 and 9.5% in T2.

As to TEC analyses, no statistical differences were found, except for OMWM (a) plots higher than control in T1; a higher content of humic substances (HA+FA) were found in both amended plots in T1 and in OMWM (a) in T2.

The analyses of the evolution of humification parameters evidenced the improvement of soil organic carbon quality in amended sites, with particular reference to OMWM type ”a” plots where both DH and HR were higher and HI was lower than control in both periods monitored. This can be explained by the lower C/N in the OMWM (a), as evidenced in Table 3, which probably supported more the humification reactions in soil.

Positive modification of soil humic parameters achieved in short time (five years) in the OMWMs amended plots suggests that OMWMs contain humus-like substances that rapidly incorporated into the soil stable organic fraction.

Vegetative and yield parameters did not show significant differences between amended and non-amended plots (Table 5) thus indicating that OMWMs amendment was able to support well olive plant growth and productivity as a standard chemical fertilizing. With this respect, a recent study (Pepi et al. 2006) showed a higher nitrate content in test-crops amended with OMWMs, indicating an improved growth of nitrifying bacteria that led in turn to an enhancement in soil nitrogen availability for plants.

Table 5. Cumulated mean (2001-05) of vegetative and productive parameters measured in intensive olive grove yearly amended with 9 tons ha^{-1} of two different Olive Mill Waste Mixtures (OMWMs).

	Yield	Pruning residues	Trunk diameter increase
	kg per plant		cm
OMWM (a)	8.0a	17.9a	5.37a
OMWM (b)	5.3ab	18.0a	5.59a
Control	7.2ab	20.8a	5.95a

Data flanked by the same letters are not significantly different according to SNK test for P<0.05.

3.2.2 Nursery trial

Three different nursery trials were carried out by using the OMWM type (a) (Table 3) after three months of storage.

The first trial concerned *Olea europaea* (Figure 7): rooted olive cuttings were transplanted into two liter pots containing a standard olive growing media (Fontanazza, G. 1993) added of different % of OMWM: OM25, OM50, OM75, OM100 (25%, 50%, 75% and 100%, respectively). Urea was added to the growth media only in the control plots. The trial was run in a cold tunnel, using a randomised block design (50 pots per block). The pots were watered as required and no additional fertilization was conducted during the trial. Plants height and percentage of failure were periodically recorded. The experiment lasted ten months. At the end of the trial, five plants per plot were measured for wet and dry weight of roots, stems and leaves.

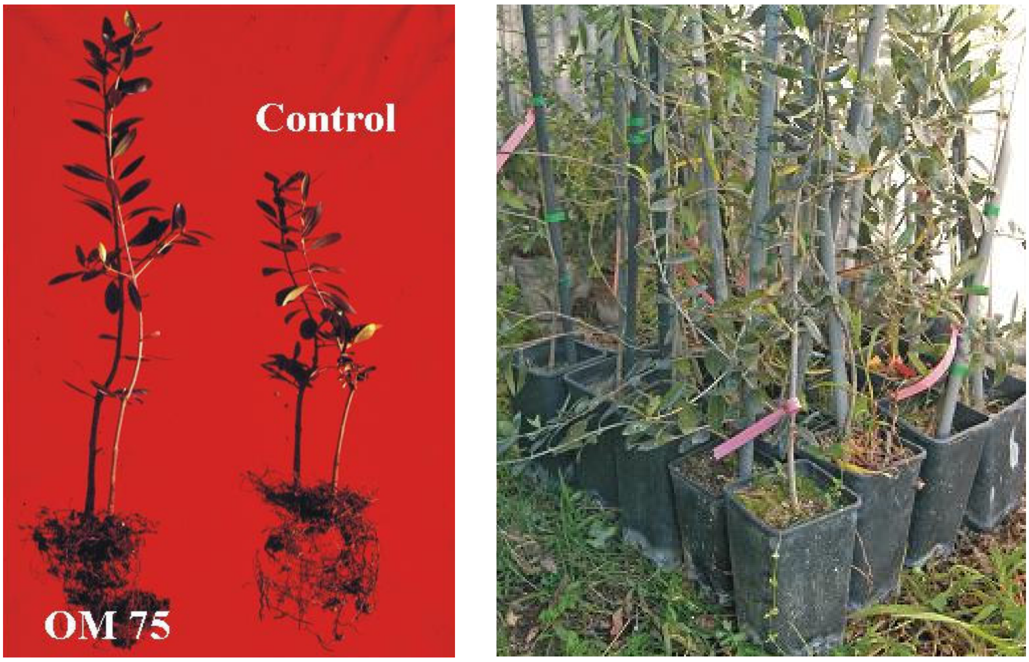


Figure 7. *Olea europaea* cuttings in two liter pots

The second trial concerned soil-less strawberry cultivation (Figure 8): *Sphagnum* peat (blond) was mixed to 25%, 50%, and 75% of OMWM (SOM25, SOM50, SOM75, respectively). The plantlets were grown in a cold tunnel in plastic trays (50x30x10 cm) at a density of 6 plants/tray, in triplicate. The trays were fertigated as required with a standard nutritive solution (Lieten, P., 1995). The trial was replicated without fertigation, using citric acid to adjuste irrigation water pH at the same value of nutritive solution. The plots with 100% of blond peat were used as control. Macro and micro nutrients content on foliar samples, collected at the end of the trial, were determined. Yield per plant was also recorded (data not showed).

Figure 8. Soil-less strawberry cultivation trial

The third nursery trial interested 2 liter potted seedlings of *Laurus nobilis* and *Cupressus spp*. (Figure 9). Peat, usually added to the standard growth media at rate of 30%, was substituted with OMWM at rate of 50% (LOM15, COM15, for *Laurus nobilis and Cupressus spp.,* respectively), and 100% (LOM30, COM30, for *Laurus nobilis and Cupressus spp.,* respectively). Each trial was run both in fertilised and non fertilised growing media using Multicote fertilizer (NPK:18-6-12) at a rate of 1.5 kg m^{-3}. In order to collect and analyse percolate, pots were weekly watered over the water-holding capacity in a one month trial, with 200 ml of water per irrigation. Height of plants were also monitored.

(a) (b)

Figure 9. Potted seedlings of *Cupressus spp*. (a) and *Laurus nobilis* (b)

In Table 6 are reported data on height and failure of *Olea europaea.* Plants in pots containing OMWM exhibited a faster growth than control, recovering in less than 1 month the lower height evidenced at transplanting. Percentage of failure was low in all treatments, tolerable for a nursery production. No significant differences were also found between all plots in wet and dry weight of roots, stems and leaves (data not reported).

Table 6. Height (cm) and failure of *Olea europaea* cuttings cultivated in pots amended with different % of OMWM. Data flanked by the same letter are not significantly different according to Student-Newman-Keuls test for $p < 0.05$.

	At transplanting	1 month later	3 month later	10 month later	Failure
OM25	17.4 B	36.4 A	48.4 A	50.7 A	2%
OM50	19.1 B	33.7 AB	44.8 AB	50.4 A	0%
OM75	18.9 B	30.8 B	41.0 BC	49.0 A	4%
OM100	16.8 B	25.1 C	37.3 C	45.4 AB	4%
Control	23.1 A	29.7 B	38.5 C	42.5 B	2%

As to strawberry trial, the leaf analyses showed a higher N and K content in both fertigated and no fertigated OMWM amended plots (Table 7), evidencing a good nutrient supply provided to plants

Table 7. Main nutrients in strawberry leaves collected at the end of the trial. Data are referred to dry matter base

	N	K	Na	Ca	Mg	Fe	Mn	Zn
Non fertigated			%				mg kg^{-1}	
SOM25	1.10	1.83	1.63	1.33	0.41	7.2	12.4	10.3
SOM50	1.55	2.81	1.58	1.39	0.44	37.0	6.8	6.1
SOM75	1.57	3.03	1.50	1.34	0.37	19.9	3.7	18.0
Control	0.88	0.51	0.40	1.31	0.45	18.0	14.1	5.4
Fertigated			%				mg kg^{-1}	
SOM25	2.45	2.09	0.92	0.97	0.41	8.0	12.8	8.4
SOM50	2.44	2.74	1.07	0.97	0.43	23.7	15.6	6.2
SOM75	2.60	4.16	1.01	0.89	0.33	9.7	7.8	5.6
Control	2.24	1.31	0.91	0.83	0.40	11.1	10.0	9.0

As regards *Laurus nobilis* and *Cupressus spp.* trials, no significant differences in plant height was recorded in all plots (data not showed). Analysis of percolate, reported in Table 8, showed a considerable higher nitrate content in OMWM amended plots. This could be explained as a consequence of a stronger nitrification process due to a higher organic nitrogen content in the growth media. In particular, non fertilized OM15 *Cupressus spp* plot showed 672 ppm nitrate, value comparable to that found in fertilized control plot, demonstrating that the use of OMWM can effectively surrogate low-release nitrogen fertilizers, reducing by far the requirement of additional chemicals during cultivation. Also a worrying higher chlorine content was detected in almost all OMWM amended plots; however, even in the higher (551 ppm in non fertilized OM30 *Laurus nobilis* plot) no adverse effect in plant growth was showed up. As regards to sulphate, the occurrence of this anion in the percolate was clearly correlated to the other components of the growth media.

Table 8. Mean values of anions detected in percolate collected from pots containing 15% and 30% of OMWM after four controlled irrigation in *Laurus nobilis and Cupressus spp.,* both in fertilized and non fertilized growing trials.

	Laurus nobilis				*Cupressus spp*			
	Cl^-	NO_2^-	NO_3^-	SO_4^-	Cl^-	NO_2^-	NO_3^-	SO_4^-
Non fertilized	mg kg^{-1}				mg kg^{-1}			
OM 15	158	ldl	81	169	153	9	672	395
OM 30	551	ldl	280	233	174	135	215	502
Control	65	ldl	37	318	79	ldl	ldl	384
Fertilized								
OM 15	53	90	513	206	129	ldl	3844	371
OM 30	246	ldl	1497	225	177	65	3900	463
Control	63	ldl	643	297	89	ldl	826	511

ldl= lower detection limit

3.2.3 Mushroom cultivation

The present investigation was aiming at exploring valuable application of OMWMs in mushroom cultivations. A preliminary cultivation trial of *Pleurotus ostreatus* was performed on growing media containing wheat straw, previously soaked overnight in tap water, cut in long pieces (5-10 cm) and added with different percentage of experimental OMWM type (a), as reported in Table 9.

Table 9. Composition of experimental organic mixtures used in the *Pleurotus ostreatus* growing trial; percentages are referred to fresh weight basis.

Experimental mixture	Wet wheat straw %	OMWM type (b) %
10P	10	90
50P	50	50
100P = Control	100	0

Culture media and conditions adopted in the trial were those widely reported in literature as standard procedures (Ferri, 1985). Ten polypropylene bags were filled with 1.5 kg of experimental substrates, previously soaked up to 70 % of moisture; they were sterilized once for 60 min at 121 °C and for several hours allowed to cool down to the desired inoculation temperature in a dark room. All substrates were then inoculated, under aseptic conditions, with 5% (w/w) of wheat spawn (Figure 10). The closed bags were then incubated and maintained in a cultivation room at 25 ± 1 °C for 40 days (Figure 11). During the mycelial growth phase the bags were neither aerated nor illuminated. After the substrate was fully colonised (Figure 12), the bags were removed from the substrate which was then incubated at 16 ± 2 °C, relative humidity 85 ± 5% and a light intensity of 600 lux m^{-2} for 12 h day^{-1} by fluorescent lamps (Figure 13). After primordium formation, the CO_2 level was maintained around 1000 ppm by aeration.

Figure 10 Spawn on wheat of *Pleurotus ostreatus* used in the cultivation trial

Figure 11. Incubation at 25°C of inoculated mixtures with *P. ostreatus* in 1.5 kg plastic bags

Figure 12. Experimental mixture after 40 days incubation at 25°C, colonized by *P. ostreatus* and ready for inducing fruiting.

Figure 13. First flush of *P. ostreatus* grown on experimental mixtures.

In order to evaluate experimental mixture performances and production quality, a number of parameters were recorded during the trial: earliness, number of flushes, flush yields, biological efficiency on total yield, carpophores dry matter and bio-phenols contents (Table 10).

Table 10. Weight and bio-phenols content of harvested carpophores from different flushes and BE* of *Pleurotus ostreatus* grown on experimental mixtures containing different % of OMWM

Experimental mixture		10P	50P	100P = Control
OMWM concentration	%	90	50	0
Cropping period	days	25	25	25
N° flushes		3	3	3
1 flush yield	g	1990	1630	2790
2 flush yield	g	380	360	1140
3 flush yield	g	175	54	140
Total yield (fresh)	g	2545	2044	4070
difference respect to control (fresh weight)	%	- 37	- 50	
carpophores dry matter (1 flush)	%	13.5	10.7	7.7
carpophores dry matter (2 and 3 flushes)	%	12.8	11.6	8.5
Total yield (dry weight)	g	340	222	324
difference respect to control (dry weight)	%	+ 5	- 31	
BE* on total yield	%	62,8	50.5	100.5
bio-phenols in carpophores (1 flush)	mg kg^{-1} dm.	5831	5206	1530
bio-phenols in carpophores (2 and 3 flushes)	mg kg^{-1} dm	4090	4285	3078

* BE = Biological Efficiency = Fresh mushrooms (g)/dry substrate (g) × 100.; dm = dry matter

No differences were found between experimental mixtures in terms of earliness and numbers of flushes as well as organoleptic characteristics of basidiocarps evaluated in taste trials. Total fresh yields and Biological Efficiencies of 10P and 50P were lower than control by 37% and 50%, respectively. On the contrary, taking into account the dry weight of the total yield, 10P showed the best performance, resulting 5% higher than control.

As regards bio-phenols content, 10P and 50P showed considerably higher values than control (up to almost 3 times), with particular reference to the first flush yield. Taking into account the well-known anti-oxidant nutritional properties of bio-phenols, further investigation should be carried out to clarify why higher dry matter and bio-phenols were found in the mushrooms grown on mixtures containing olive mill waste effluents, for possible positive impacts on nutritional value of mushrooms.

With this respect, two larger cultivation trials were performed in 2007, involving one of the main Italian mushroom grower company: Azienda Agricola Valfungo.

The first concerned again the cultivation of *Pleurotus ostreatus* (Figure 14) in a larger scale trial by using OMWM tipe (a) in the same way reported above.

The second test, even more significant, dealt with mushroom composting for A*garicus bisporus* (Figure 15), using OMWM type (a) as an alternative ingredient to chicken manure in the preparation of the mushroom compost. Starting from about 180 tons of organic mixture submitted to composting, the whole growing area interested by *Agaricus bisporus* trial was 2500 m^2, control plots included.

The very interesting results achieved in these industrial scale trials will be presented in the next future within the framework of the project *Soluzioni alternative allo spandimento in campo dei sottoprodotti dei frantoi* supported by the ARSIA Toscana Agency.

Figure 14. Plastic bags (15 kg) containing different % of OMWM type (a); *Pleurotus ostreatus* cultivation trial performed at Valfungo company, Gricicnano, Arezzo, Italy.

Figure 15. Inoculation of pasteurized compost, containing OMWM, with spawn of *Agaricus bisporus*; cultivation trial performed at Valfungo company, Gricicnano, Arezzo, Italy.

4. EVALUATION OF THE M.A.T.Re.F.O. SYSTEM ECONOMIC SUSTAINABILITY

Peculiarity that makes M.A.T.Re.F.O. an interesting olive mill waste management system are simplicity and flexibility in recycling in agriculture all kinds of OMWEs, thus reducing disposal problems and environmental impact.

However, concrete application of this technology could be exploited only if net revenues occur. With this respect, taking into accounts the technical and economical information available and the fact that there are no actual markets for the outcomes of the innovative systems, different costs/revenues evaluation attempts have been carried out in order to assess the best scenarios for potential convenient use of the M.A.T.Re.F.O. system.

Even though non exhaustive of all possible situation, the analyses reported in this study represents a primary raw evaluation on economic sustainability of this recycling system.

In table 11 are reported the main technical and economic parameters adopted in the estimation of costs and revenues, hypothesizing an equipment characterized by a working capacity of 2,8 tons per hour of OMWEs recycled.

Table 11. Main technical and economic parameters assumed for estimation of costs and revenues generated by the M.A.T.Re.F.O. technology in different hypothesized applications

Type of olive mill		2 phases	3 phases	Press system
Husk production	% olive milled	82	50	35
Olive mill wastewater production	% olive milled	10	75	50
Olive stones production	% husk	15	25	35
Loss of revenue for husk selling	€ / t	0	5	25
Cost saving for OMWEs soil spreading	€ / m^3	15	15	17
Straw used	% of mixture	13	15	13
Wool waste used	% of mixture	15	17	15

Revenues will be generated by selling the M.A.T.Re.F.O. outcomes (OMWM as amendment and stones for heat production) and by the cost saving for disposal OMWEs by controlled soil spreading, according to the Italian Law 574/96.

Costs will consist of fixed and variable categories and include also the loss of revenue for husk selling (with particular reference to the press mill one).

In tables 12, 13 and 14 are reported the estimations carried out, followed by the elaboration of data (Figure 16) in order to identify the minimum size of olive mills able to generate positive economic balances.

The results clearly show that M.A.T.Re.F.O. system is particularly fitting for two phases continuous extraction mills, producing profits in case of olive mill working capacity higher than 1316 t/year of olives milled.

Three phases olive mills, as expected, require a higher working capacity for getting returns (roughly double).

The fairly negative economic balances in press systems basically depend upon the high quality of husk produced, well valued by husk oil extraction plants because quite dry. However, the use of press systems have been rapidly abandoned in the last years by olive millers, for automatic continuous extraction plants, passing in Italy by 45% to 16% of total olive milled in the 1999-2003 period (elaboration Agecontrol data 2002/03); the reasons of such decreasing trend are essentially linked to the high costs of manpower required by this discontinuous system and often low quality of oil produced (husk oil included, extracted by chemicals).

Table 12. Evaluation of costs and revenues simulated in the application of M.A.T.Re.F.O. technology as result of different olive mill working capacity in two phases continuous extraction olive mills.

TWO PHASES OLIVE MILL

OLIVES MILLED	t	**915**	**1220**	**1829**	**2439**	**3049**	**3659**	**4878**
Husk production	t	750	1000	1500	2000	2500	3000	4000
Destoned husk production	t	638	850	1275	1700	2125	2550	3400
Waste water production	t	91	122	183	244	305	366	488
Olive Mill Waste Effluents to recycle	**t**	**729**	**972**	**1458**	**1944**	**2430**	**2916**	**3888**
Equipment working capacity (2.8 t/h)								
Seasonal working period of equipment (8 hour/day)	days	33	45	67	89	112	134	179
Fixed costs								
Equipment cost (V0 = 150,000.00 €)								
Estimated residual value of equipment (Vn = 30,000.00 €)								
Equipment amortization ((V0-Vn)/10)	€	12,000.00	12,000.00	12,000.00	12,000.00	12,000.00	12,000.00	12,000.00
Interest, rent and repair, taxes and insurance (5 % V0)	€	7,500.00	7,500.00	7,500.00	7,500.00	7,500.00	7,500.00	7,500.00
Variable costs								
Straw	€	6,159.74	8,212.99	12,319.48	16,425.98	20,532.47	24,638.96	32,851.95
Wool waste	€	2,186.89	2,915.85	4,373.78	5,831.71	7,289.63	8,747.56	11,663.41
Plastic net sacks (0.20 €/sack)	€	6,158.28	8,211.04	12,316.57	16,422.09	20,527.61	24,633.13	32,844.18
Manpower (10 €/h)	€	5,206.88	6,942.51	10,413.76	13,885.02	17,356.27	20,827.53	27,770.03
Energy (0.17 € KWh)	€	1,549.05	2,065.40	3,098.09	4,130.79	5,163.49	6,196.19	8,261.59
Loss of revenue for husk selling (0 €/t)		0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total cost	**€**	**40,760.84**	**47,847.79**	**62,021.69**	**76,195.58**	**90,369.48**	**104,543.37**	**132,891.16**
Cost per ton	€/t	43.68	38.46	33.24	30.62	29.06	28.01	26.70
OMWM production	**t**	**933**	**1,244**	**1,866**	**2,488**	**3,110**	**3,732**	**4,976**
Stones production	**t**	**113**	**150**	**225**	**300**	**375**	**450**	**600**
Revenues								
OMWM selling (15 €/t)	€	13,996.10	18,661.46	27,992.20	37,322.93	46,653.66	55,984.39	74,645.85
Stones selling (100 €/t)	€	11,250.00	15,000.00	22,500.00	30,000.00	37,500.00	45,000.00	60,000.00
Cost saving for olive mill wastewater/husk soil spreading (15 €/t)	€	9,562.50	12,750.00	19,125.00	25,500.00	31,875.00	38,250.00	51,000.00
Total reveneu	**€**	**34,808.60**	**46,411.46**	**69,617.20**	**92,822.93**	**116,028.66**	**139,234.39**	**185,645.85**
Revenue per ton	€/t	37.31	37.31	37.31	37.31	37.31	37.31	37.31
Net revenue	**€**	**-5,952.25**	**-1,436.33**	**7,595.51**	**16,627.35**	**25,659.18**	**34,691.02**	**52,754.69**
Net revenue per ton	€/t	-6.38	-1.15	4.07	6.68	8.25	9.29	10.60

Table 13. Evaluation of costs and revenues simulated in the application of M.A.T.Re.F.O. technology as result of different olive mill working capacities in three phases continuous extraction olive mills.

THREE PHASES OLIVE MILL

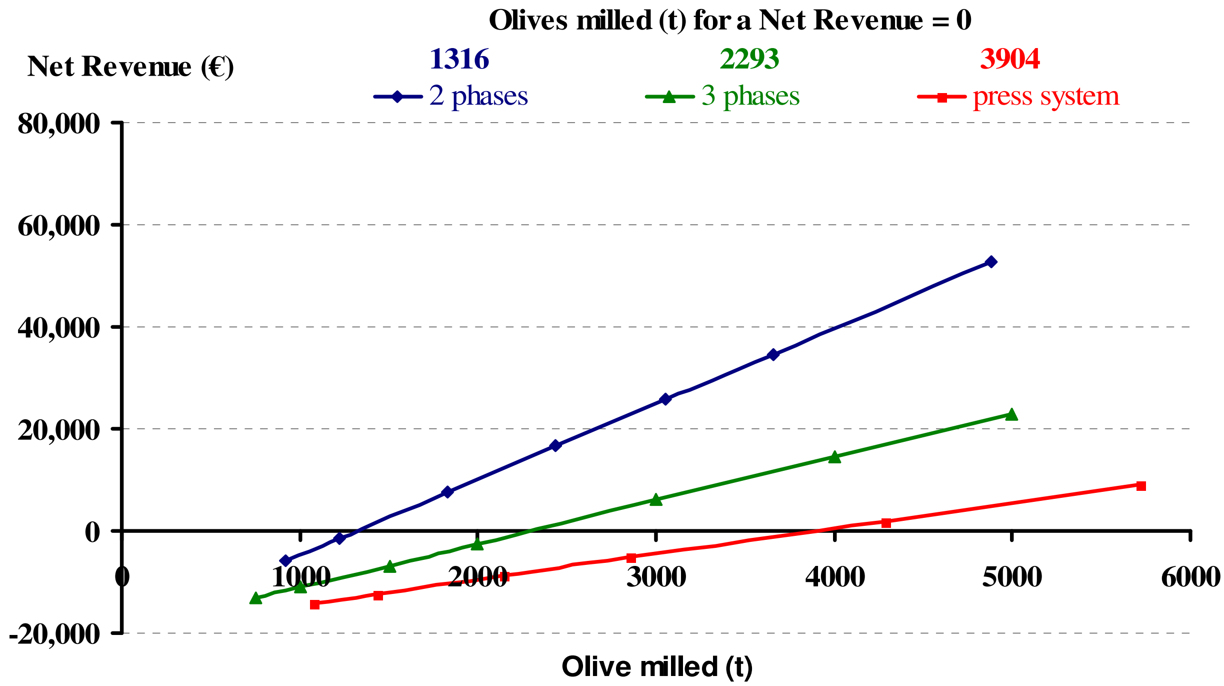
OLIVES MILLED	t	**750**	**1000**	**1500**	**2000**	**3000**	**4000**	**5000**
Husk production	t	375	500	750	1000	1500	2000	2500
Destoned husk production	t	281	375	563	750	1125	1500	1875
Waste water production	t	563	750	1125	1500	2250	3000	3750
Olive Mill Waste Effluents to recycle	**t**	**844**	**1125**	**1688**	**2250**	**3375**	**4500**	**5625**
Equipment working capacity (2.8 t/h)								
Seasonal working period of equipment (8 hour/day)	days	38	50	75	100	151	201	251
Fixed costs								
Equipment cost (V0 = 150,000.00 €)								
Estimated residual value of equipment (Vn = 30,000.00 €)								
Equipment amortization ((V0-Vn)/10)	€	12,000.00	12,000.00	12,000.00	12,000.00	12,000.00	12,000.00	12,000.00
Interest, rent and repair, taxes and insurance (5 % V0)	€	7,500.00	7,500.00	7,500.00	7,500.00	7,500.00	7,500.00	7,500.00
Variable costs								
Straw	€	8,226.56	10,968.75	16,453.13	21,937.50	32,906.25	43,875.00	54,843.75
Wool waste	€	2,868.75	3,825.00	5,737.50	7,650.00	11,475.00	15,300.00	19,125.00
Plastic net sacks (0.20 €/sack)	€	7,350.75	9,801.00	14,701.50	19,602.00	29,403.00	39,204.00	49,005.00
Manpower (10 €/h)	€	6,026.79	8,035.71	12,053.57	16,071.43	24,107.14	32,142.86	40,178.57
Energy (0.17 € KWh)	€	1,792.97	2,390.63	3,585.94	4,781.25	7,171.88	9,562.50	11,953.13
Loss of revenue for husk selling (5 €/t)		1,875.00	2,500.00	3,750.00	5,000.00	7,500.00	10,000.00	12,500.00
Total cost	**€**	**47,640.82**	**57,021.09**	**75,781.63**	**94,542.18**	**132,063.27**	**169,584.36**	**207,105.45**
Cost per ton	€/t	42.78	38.40	34.02	31.83	29.64	28.55	27.89
OMWM production	**t**	**1,114**	**1,485**	**2,228**	**2,970**	**4,455**	**5,940**	**7,425**
Stones production	**t**	**94**	**125**	**188**	**250**	**375**	**500**	**625**
Revenues								
OMWM selling (15 €/t)	€	16,706.25	22,275.00	33,412.50	44,550.00	66,825.00	89,100.00	111,375.00
Stones selling (100 €/t)	€	9,375.00	12,500.00	18,750.00	25,000.00	37,500.00	50,000.00	62,500.00
Cost saving for olive mill wastewater/husk soil spreading (15 €/t)	€	8,437.50	11,250.00	16,875.00	22,500.00	33,750.00	45,000.00	56,250.00
Total reveneu	**€**	**34,518.75**	**46,025.00**	**69,037.50**	**92,050.00**	**138,075.00**	**184,100.00**	**230,125.00**
Revenue per ton	€/t	30.99	30.99	30.99	30.99	30.99	30.99	30.99
Net revenue	**€**	**-13,122.07**	**-10,996.09**	**-6,744.13**	**-2,492.18**	**6,011.73**	**14,515.64**	**23,019.55**
Net revenue per ton	**€/t**	**-11.78**	**-7.40**	**-3.03**	**-0.84**	**1.35**	**2.44**	**3.10**

Table 14. Evaluation of costs and revenues simulated in the application of M.A.T.Re.F.O. technology as result of different olive mill working capacity in discontinuous traditional press olive mills.

OLIVE PRESS MILL

OLIVES MILLED	t	**1071**	**1429**	**2143**	**2857**	**4286**	**5714**
Husk production	t	375	500	750	1000	1500	2000
Destoned husk production	t	244	325	488	650	975	1300
Waste water production	t	536	714	1071	1429	2143	2857
Olive Mill Waste Effluents to recycle	**t**	**779**	**1039**	**1559**	**2079**	**3118**	**4157**
Equipment working capacity (2.8 t/h)							
Seasonal working period of equipment (8 hour/day)	days	35	46	70	93	139	186
Fixed costs							
Equipment cost (V0 = 150,000.00 €)							
Estimated residual value of equipment (Vn = 30,000.00 €)							
Equipment amortization ((V0-Vn)/10)	€	12,000.00	12,000.00	12,000.00	12,000.00	12,000.00	12,000.00
Interest, rent and repair, taxes and insurance (5 % V0)	€	7,500.00	7,500.00	7,500.00	7,500.00	7,500.00	7,500.00
Variable costs							
Straw	€	6,586.47	8,781.96	13,172.95	17,563.93	26,345.89	35,127.86
Wool waste	€	2,338.39	3,117.86	4,676.79	6,235.71	9,353.57	12,471.43
Plastic net sacks (0.20 €/sack)	€	6,790.69	9,054.26	13,581.39	18,108.51	27,162.77	36,217.03
Manpower (10 €/h)	€	5,567.60	7,423.47	11,135.20	14,846.94	22,270.41	29,693.88
Energy (0.17 € KWh)	€	1,656.36	2,208.48	3,312.72	4,416.96	6,625.45	8,833.93
Loss of revenue for husk selling (25 €/t)		9,375.00	12,500.00	18,750.00	25,000.00	37,500.00	50,000.00
Total cost	**€**	**51,814.52**	**62,586.03**	**84,129.05**	**105,672.06**	**148,758.09**	**191,844.12**
Cost per ton	€/t	50.36	45.62	40.88	38.51	36.15	34.96
OMWM production	**t**	**1,029**	**1,372**	**2,058**	**2,744**	**4,116**	**5,487**
Stones production	**t**	**131**	**175**	**263**	**350**	**525**	**700**
Revenues							
OMWM selling (15 €/t)	€	15,433.39	20,577.86	30,866.79	41,155.71	61,733.57	82,311.43
Stones selling (100 €/t)	€	13,125.00	17,500.00	26,250.00	35,000.00	52,500.00	70,000.00
Cost saving for olive mill wastewater/husk soil spreading (17 €/t)	€	9,107.14	12,142.86	18,214.29	24,285.71	36,428.57	48,571.43
Total reveneu	**€**	**37,665.54**	**50,220.71**	**75,331.07**	**100,441.43**	**150,662.14**	**200,882.86**
Revenue per ton	€/t	36.61	36.61	36.61	36.61	36.61	36.61
Net revenue	**€**	**-14,148.99**	**-12,365.32**	**-8,797.97**	**-5,230.63**	**1,904.05**	**9,038.74**
Net revenue per ton	€/t	**-13.75**	**-9.01**	**-4.28**	**-1.91**	**0.46**	**1.65**

Figure 16. Simulated Net Revenue trend in the application of M.A.T.Re.F.O. technology as result of different olive mill working capacity in two, three phases continuous extraction olive mills and in discontinuous press olive mills.



5. CONCLUSIONS

Rate of 9 tons ha^{-1} of OMWM, annually spread for five years in an intensive olive orchard, determined significant increases in TOC (up to 40%) and humic-C (up to 58%) in soil, without negative interferences on plant growth and productivity, showing to be comparable to a standard mineral fertilization. OMWM amendments could therefore reasonably lead to a reduction in extra fertilizer needs for plants, providing an adequate nutrient supply, mainly K and N.

OMWM addition to the nursery growing media showed, for all species tested, satisfactory growth, fully compared to controls, without any apparent detrimental effects for plants, providing nutrients, mainly K, N and microelements and acting as a slow release organic fertilizer. Hence, OMWM confirmed to be an effective alternative to the very expensive *Sphagnum* peat, leading to a realistic cost reduction in nursery also for the lower mineral fertilizer requirements.

As well the use of OMWM in mushroom cultivation showed optimistic results which, however, need to be confirmed by additional larger scale trials (still in progress).

The simulated economic evaluations of M.A.T.Re.F.O. recycling system provided good-looking results on potential application in different scenarios, with particular reference to two phases olive mill extraction plants. Diffusion of two phase decanters, at present widely in Spain, has also been widening in Italy in the last years essentially because of reduced energy and water requirements and in consideration that they eliminate waste water production, improving olive oil quality as well. However, they produce a wet husk difficult to manage by available ready to use disposal/recycling techniques (i.e. controlled soil spreading, composting or oil extraction by chemicals after drying) which sensibly hinders a larger diffusion. With this respect, M.A.T.Re.F.O. technology could further enhance the diffusion of the two phases decanters because it is capable to take care quite easily of the two phases husk, according to adequate costs to revenues ratio.

Diffusion of M.A.T.Re.F.O. recycling systems could also generate benefits for environment because it reasonably incentives the application to soil of large amounts of “clean” amendment/fertilizer particularly useful for organic and sustainable farming, rich in nutrients and humus–like substances, able to preserve and enhance soil fertility, reduce CO_2 emissions thus contrasting mineralization process more and more in progress in the Mediterranean regions, where olive mills are largely diffused as well.

In addition, M.A.T.Re.F.O. system could also be wisely applied in consortium of small olive mills which, however, must be close each others for minimizing transportation costs, mainly where OMWEs direct spreading is not practical.

Policy of local public agency that promotes, by financial supports, the purchase of M.A.T.Re.F.O. equipments could sensibly reduce costs, (i. e. amortization); moreover, further advances of economic balances, decisive for a rapid diffusion of such recycling system, could be achieved by “high added-value” applications of OMWM, as investigated in this study.

The olive mill waste management system described in this work can be considered a worthily example of converting high polluting wastes into valuable resources through an environmental friendly way.

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