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Research article

Biorecovery of olive mill wastewater sludge from evaporation ponds

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ABSTRACT

Olive mill wastewater (OMW) resulting from the olive oil extraction process is usually disposed of in evaporation ponds where it concentrates generating a sludge that pollutes the ponds nearby area. In this study, four bio-treatments were applied for the *in-situ* bioremediation and valorization of OMW sludge: Landfarming, phytoremediation, composting and vermicomposting. In all cases, the OMW sludge was added with organic residues (mushroom compost, rabbit manure, and chicken manure). The bio-treatments were carried out in duplicate, inoculated and non-inoculated, to determine the effect of a specialized fungal consortium (*Aspergillus ochraceus* H2 and *Scedosporium apiospermum* H16) on the efficacy of the bio-treatments. The evaluation of chemical parameters, toxicity, and functional microbial biodiversity revealed that the four techniques depleted the toxicity and favored the stimulation of functional microbiota. Landfarming and phytoremediation allowed the decontamination and improvement of soils. Composting and vermicomposting also offered high-quality products of agronomic interest. Inoculation improved the bioremediation effectiveness. Biological treatments are effective for the safe recovery of contaminated OMW sludge into high-quality services and products.

1. Introduction

Olive oil is a traditionally essential product in the Mediterranean diet, which is also recognized and marketed worldwide for its nutritional and organoleptic properties. Although olive oil production varies depending on climatic factors, in the last decade total world production reached about 3 million tons per year (Lee et al., 2019). This agro-industrial activity generates a large amount of waste that poses a management challenge, being the liquid residue named olive mill wastewater (OMW), one of the principal environmental concerns, because of its polluting effects and large volume (El-Abbassi et al., 2012). Around 1.5 m^3 of OMW is generated per ton of olives (Lee et al., 2019; Pardo et al., 2017). It consists of 83–96% water, 3.5–15% organic constituents, and 0.5–2% mineral compounds (Lee et al., 2019). Thus, OMW has a high organic load, including recalcitrant and toxic substances, such as phenolic compounds, responsible for the characteristic blackish color, phytotoxic and antimicrobial effects, high biological and chemical oxygen demand (BOD and COD, respectively) and low biodegradability (Kovačević et al., 2022; Mekki et al., 2013). In addition, it usually presents acid pH and high electrical conductivity, derived from its content in organic acids and salts (Jeguirim et al., 2017).

The most widespread method for managing this waste has been its storage in artificial ponds for natural evaporation during the hot period of the year. This method does not eliminate the problem, but rather moves it, since most ponds are not waterproofed and often overflow polluting the surroundings through the lixiviates (Martínez-Gallardo et al., 2021; Kavvadias et al., 2017; S'habou et al., 2009). Additionally, OMW is transformed into a more polluting and recalcitrant semi-solid sludge in which toxic compounds are concentrated. Nonetheless, it has been reported that the OMW sludge from evaporation ponds harbors a remarkable microbial community, which can potentially respond to biostimulation (Martínez-Gallardo et al., 2021). That consists of adding nutrients such as organic residues and/or providing oxygen (aeration) to promote the activity of the microorganisms present in the material, allowing its transformation and the reduction of toxic compounds (Martínez-Gallardo et al., 2021). Also, the inoculation with microorganisms (bioaugmentation) having phenol-degrading activity helps to alleviate the toxic load of these residues and biotransform them (Martínez-Gallardo et al., 2020). Landfarming (soil aeration), phytoremediation (plant-mediated), composting, and vermicomposting are

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techniques that met bioremediation objectives by eliminating toxic compounds. They also biotransform the polluted matrix into a substrate for plant growth or as a product for agricultural applications (Bargougui et al., 2020; Sáez et al., 2020). The basis of these methods is the use of microbial communities to eliminate organic pollutants and transform organic matter. Phytoremediation and vermicomposting treatments use, in addition to microorganisms, plants and earthworms, respectively (Chatterjee et al., 2008). Besides, in composting and vermicomposting there is a more intense and accelerated degradation of the material due to the piling of the material and, therefore, the generation of heat (Juwarkar et al., 2010). There are very few studies, based on the application of composting and vermicomposting for biorecovering of OMW sludge (Hachicha et al., 2009; Sáez et al., 2020; Vivas et al., 2009). Most of them demonstrate the usefulness of these treatments to reduce or eliminate the toxicity of the OMW sludge, through the decrease of phenolic content or phytotoxicity, as well as, to generate a high-quality product (compost or vermicompost). However, to our knowledge, none so far has analyzed the effectiveness of other treatments such as landfarming and phytoremediation for bioremediation of OMW sludge. All these methods are based on the fundamental role of the microorganisms present in the material, which under suitable conditions can eliminate toxic compounds and stabilize the organic matter thanks to their wide range of metabolic activities (Ntougias et al., 2013). Microbial communities quickly adapt to changes that may occur in the environment, modifying the physiological and microbial profile of the treated waste (Feigl et al., 2017). Because each mentioned bio-treatment involves different conditions at diverse levels, the study of the functional diversity of the microbial community will offer information on the performance of each technique for the decontamination and safe recovery of OMW sludges. Thus, we hypothesize that several biological-based treatments can be applied to solve the problem of OMW sludge stored in evaporation ponds, whose effectiveness could be improved through inoculation with specialized microorganisms. Because of the key role of the microbial community in these treatments, the study of their activity is essential to determine the efficiency of each treatment to further optimize them.

In this work, we evaluate the effectiveness of four *in situ* bio-treatments (composting, vermicomposting, phytoremediation, and landfarming) for the decontamination and valorization of OMW sludge in evaporation ponds. Organic residues were supplemented in all the cases as biostimulants and the effect of inoculation with phenol-degrading microorganisms was assessed. Parameters that reveal the reduction of toxicity, such as phytotoxicity and ecotoxicity, and provide information on the quality of the final product, including humic substances, water-soluble carbon and carbon in the sludge were determined. In addition, the microbial activity on each treatment was also analyzed by determining the physiological profile at the microbial community level.

2. Material and methods

2.1. Study area and start-up

The study was carried out in a OMW evaporation pond located in Mora municipality (Toledo, Spain) (39°40′ 07.4″ N 3°49′ 40.2″ W), which has been in disuse since 2006 and has a total surface of 2400 m^2 (Supplementary material Fig. S1). The partially dried OMW sludge contained in the pond was extracted and mixed with spent-mushroom compost (SM), rabbit manure (RM) and chicken manure (CM) at the following w/w ratios 0.5:0.12:0.33:0.05 (OMW: SM: RM: CM). The selection of these wastes, as well as the respective proportions indicated above were chosen amongst fresh wastes available close to the site and to achieve a C/N ratio of the mixture around 25 (Supplementary material Table S1). At the same time, an important issue in choosing the organic wastes was the fact that they were generated in the surroundings, so as not to increase the carbon footprint. This material was incorporated into eight plots of 7.5 m × 7.5 m x 0.2 m built up in the pond, where four bio-treatments were applied: landfarming, phytoremediation, composting and vermicomposting (Supplementary material Fig. S1). Two plots were allocated for the development of each bio-treatment with the intention of studying the effect of bio-augmentation. At the beginning of the experiment, every second plot was inoculated with biomass from a microbial consortium consisting of the phenol-degrading fungi *Aspergillus ochraceus* H2 and *Scedosporium apiospermum* H16, both isolated from OMW sludge in a previous study (Martínez-Gallardo et al., 2020). The fungal biomass was evenly distributed on the surface of the landfarming and phytoremediation plots, and layer by layer, every 50 cm, of the initial mixture during the construction of the composting and vermicomposting piles as described below.

2.2. Development of bio-treatments and sampling

For the development of the landfarming bio-treatment, two plots were filled with the previously described initial mixture. This material was subjected to biweekly aeration during the first 4 months and monthly for the following 5 months by tilling with a rototiller. Daily sprinkler irrigation was applied by drippers (2 L/min) in both plots, resulting in a flow rate of 140 L/week.

Composting and vermicomposting biotreatments were performed as detailed by Sáez et al. (2020) and Martínez-Gallardo et al. (2020). Briefly, a composting process was first carried out in four plots where trapezoidal piles of 7.5 m × 3.75 m x 1.5 m were constructed with the initial mixture. Two of the piles were inoculated with microbial inoculum to study the effect of bioaugmentation. From composted material up to a stage corresponding to the end of the bio-oxidative phase (188 days), the piles destined for vermicomposting were placed in a trapezoidal trench of 7 m × 1.3 m x 0.4 m. They were moistened to 80% and added with earthworms from a mixture of the species *Eisenia foetida* and *Eisenia andrei* (at a population ratio of 90/10, respectively) at a density of 2500/m^3. On the other hand, the composted piles were considered finished after passing the cooling and maturing phase. Water was periodically added to the piles to maintain the moisture around 50–60% and during the bio-oxidative phase, the piles were periodically turned over using a loader according to the thermal record which was monitored every day using a hand probe. The bio-oxidative phase was considered finished when the pile temperature was close to that of the ambient and reheating did not occur after turning. The procedures were based on previous work.

The plant species selected for phytoremediation biotreatment were *Juncus acutus*, *Chrysopogon zizanioides* and *Claudium mariscus* because of their known bioremediation potential. They were planted, in seedling stage, in two plots which were divided into three 2.5 m × 7.5 m frames. Each specy was placed in its corresponding frame (sub-plot) with a density of 3.5 individuals/m^2. The irrigation system was the same as described above for landfarming.

The samplings were carried out at the beginning and every two months for a total period of 269 days. Composite samples from five equidistant points were taken in each plot destined for landfarming, composting and vermicomposting. In the case of phytoremediation, three composite samples were obtained from each sub-plot based on 5 points located in each frame of plant species included in the trial. In order to characterize the initial state of the material and, therefore, to set initial values that allow to study the efficacy of the bioremediation bio-treatments, a composite sample from the partially dried OMW sludge contained in the pond was collected before the aforementioned extraction and mixture. All samples were used for the analytical described below except OMW sludge samples, in which just phytotoxicity, ecotoxicity and functional biodiversity were analyzed.

2.3. Analytical

The total content of organic matter (OM) was determined by weight loss on ignition at 550 °C for 3.5 h. The pH and electrical conductivity were analyzed in a 1:10 (w/v) water extract.

Microbial biomass carbon (CBIO) was tested using the fumigation-extraction technique according to Vance et al. (1987). The fumigated and non-fumigated extracts were analyzed using a TOC-VCSN (Shimadzu Co., Japan). CBIO was calculated by subtracting the total organic carbon (TOC) of the non-fumigated samples from the fumigated ones.

The water-soluble carbon (WSC) was obtained following the protocol described by Goyal et al. (2005), and the organic carbon was analyzed in the aqueous extract using a TOC-VCSN (Shimadzu Co., Japan).

Humic acid (HA) and fulvic acid (FA) fractions were extracted as described by Ciavatta et al. (1991). TOC was measured in the extracts using an automatic analyzer for liquid samples (TOC-V CSN Analyser, Shimadzu Japan).

Functional biodiversity was determined by analyzing the physiological profile at the community level using Biolog® EcoPlate ™ microplates (Biolog, USA). These plates consist of 96 wells grouped into three identical blocks (as replicas) with 32 wells: 1 well contains water and the remaining 31 wells contain different carbon sources. The analysis was performed according to the protocol described by Martínez-Gallardo et al. (2021). Functional biodiversity was expressed as Shannon index (H′) = -Σpi (lnpi) and average well color development (metabolic intensity), AWCD = ΣODi/N; where ODi = proportional color development of each well, pi = ODi (proportional color development of each well) over total color development of all wells on a plate; and N = number of total substrates (31). In addition, the average absorbance values of each substrate category, SAWCD = ΣODi/N, were calculated, taking into account that the 31 substrates can be classified into 6 groups or classes of substrates, based on their chemical structure. in this case, ODi is the OD value of the substrates within each substrate category and N is the number of substrates in each category.

The phytotoxicity analysis was carried out by evaluating the germination of *Lepidium sativum* seeds, according to Zucconi et al. (1985) slightly modified by Martínez-Gallardo et al. (2020).

To determine ecotoxicity, the Aboatox kit (kit 1243-500 BioTox; Aboatox, Finland), based on *Aliivibrio fischeri* bioluminescence inhibition, was used according to Jarque et al. (2016). Luminescence was measured in Luminoskan Ascent luminometer (Thermo Fisher Scientific, USA). The concentration of sample causing a 50% reduction of the light emitted by the *A. fischeri* (EC50) was calculated using regression equations. Toxicity units (TU) were calculated as follows: TU = [1/(EC50)] × 100.

2.4. Data analysis

Analyses were performed at least in triplicate and data are presented as the mean. A one-way analysis of variance (ANOVA) and multiple comparison tests of Fisher's least significant difference (LSD) at a 95% confidence level were used to test for significant differences between factor levels. Normality and homogeneity of the variances were checked using the Shapiro–Wilk and Levene tests, respectively. Analyses were carried out using Statgraphics Centurion XVII version 17.1.1.

3. Results and discussion

3.1. Evolution of chemical fractions and biomass carbon

In this work, four bio-treatments were compared in order to evaluate the most efficient and sustainable treatment to manage OMW sludge in evaporation ponds. These polluting residues, together with other residual organic materials of agricultural and livestock origin, are intended to be exploited to obtain added-value products and services. For this purpose, the evolution of released water-soluble carbon (WSC), organic matter stabilized as humic compounds (TH) and microbial biomass carbon (CBIO) was evaluated over time in the four biotreatments (Fig. 1). The analysis of the initial mixture revealed a high content of microbial biomass carbon (CBIO) (2.77 ± 0.12 mg g^{-1}) because of the microbial load supplied by the organic residues (Pardo et al., 2017).

Landfarming degrades organic matter more slowly (Koul and Taak, 2018) than other techniques, such as composting, which is reflected in the continuous increase of WSC values through the process. The total humic (TH) content was higher in the initial mixture than at the final of the landfarming process reaching values of 1.92 and 1.97 g 100 g^{-1}, in the inoculated and non-inoculated plots, respectively (Fig. 1a). This phenomenon explained by the slight structural similarity between the humic substances and polyphenolic compounds can lead to incorrect detection of phenolic molecular complexes instead of TH content. According to these results, this process allows the degradation of the organic matter contained in OMW sludge. However, a more intensive degradative activity is necessary to reach a mature and stable product, as it happens during composting. The inoculum did not affect the parameters studied in landfarming.

In the case of the composting, the CBIO and TH content increased at the final of the process showing values of 1.99 and 1.88 mg g^{-1}, 10.49 and 7.59 g 100 g^{-1}, in inoculated and non-inoculated pile, respectively (Fig. 1b). These results were expected due to the degradation of organic matter that takes place in the composting process (Bustamante et al., 2008). The organic matter, which includes contaminants such as phenolic compounds, is degraded and transformed into humic substances by the microbial communities in the composting process (Mondini et al., 2006). The final values of WSC were 0.76 and 1.10 mg g^{-1} in the inoculated and non-inoculated piles, respectively. Those values are well below 4 mg g^{-1} which is the threshold established to consider a stable and mature compost (Zmora-Nahum et al., 2005). The effect of bioaugmentation was noticeable since the fungal consortium seems to improve the humification process due to its high capacity to phenolic compounds degradation and the subsequent monomers incorporation to humic complex (Hachicha et al., 2009).

The evolution of vermicomposting is very similar to composting in the maturity parameters studied, such as CBIO, WSC and TH (Fig. 1c). However, it was remarkable that the values of TH content (11.45 and 15.87 g 100 g^{-1} in inoculated and non-inoculated piles, respectively) were higher at the end of the process than those obtained in the other bio-treatments tested. This phenomenon can be explained by the positive effect of the earthworm in improving the stabilization of organic matter by transforming and incorporating the most recalcitrant compounds, that may remain in the pre-composted material, into the humic matrix (Rékási et al., 2019).

The final results about WSC obtained from phytoremediation biotreatment showed values above 5 mg g^{-1} indicating that this process, like landfarming, is slower than composting and vermicomposting (Fig. 1d). On the other hand, it is well-known the high potential of plants in order to phytodegrade recalcitrant organic substances (Cunningham et al., 1996) which could result in the release of water-soluble phenols. This might explain the high increase in humic substances content, which results, as before mentioned, from an incorrect detection of phenolic substances in their place.

The overall results showed that composting and vermicomposting successfully decontaminated the OMW sludge from evaporation ponds as well as transforming it together with the organic waste from nearby sources into a mature and stable product. The high temperatures reached during these processes accelerate the biodegradation by microorganisms of pollutants present in OMW that are highly resistant to degradation, such as phenolic and lingo-cellulose compounds. The landfarming technique was not as efficient in the biorecovery and stabilization of the material since the conditions of this process did not favor such an intense microbial activity as in the case of composting. On the other hand, phytoremediation is an adequate bio-treatment to eliminate pollutants in soils being more effective when plants are

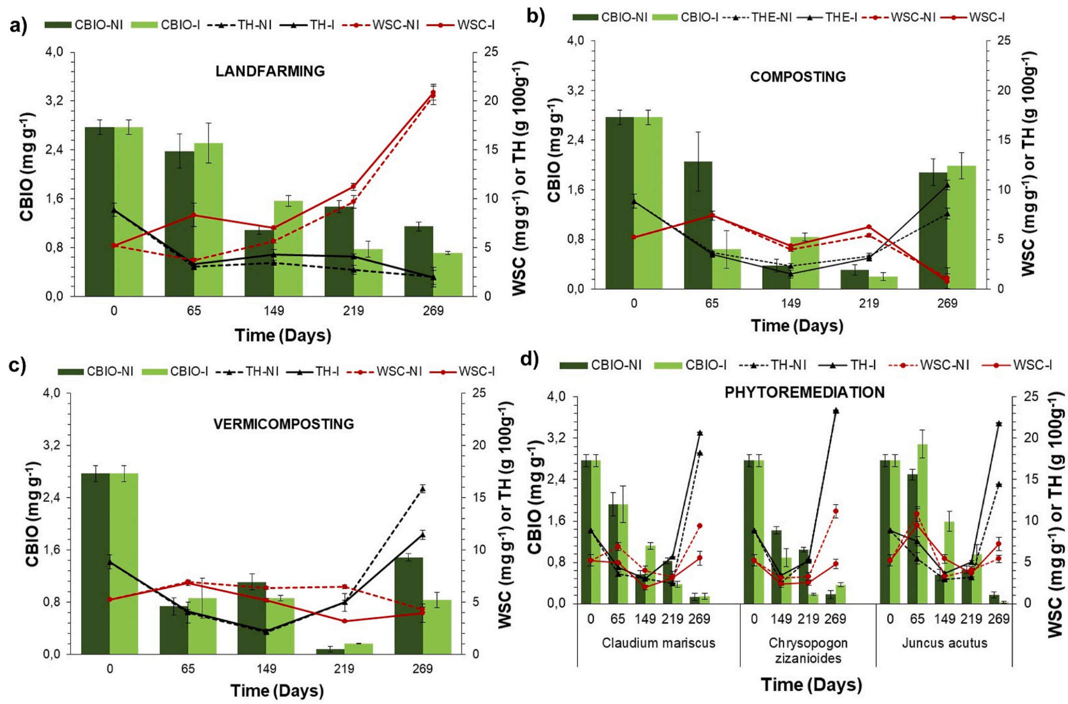

Fig. 1. Evolution of microbial biomass carbon (CBIO) (principal axis), total humic (TH) and water-soluble carbon (WSC) fractions (secondary axis) in the non-inoculated (NI) and inoculated (I) landfarming (a), composting (b), vermicomposting (c) and phytoremediation (d). Results are the mean of three replicates. Errors bars represent the LSD Fisher interval ($p < 0.05$).

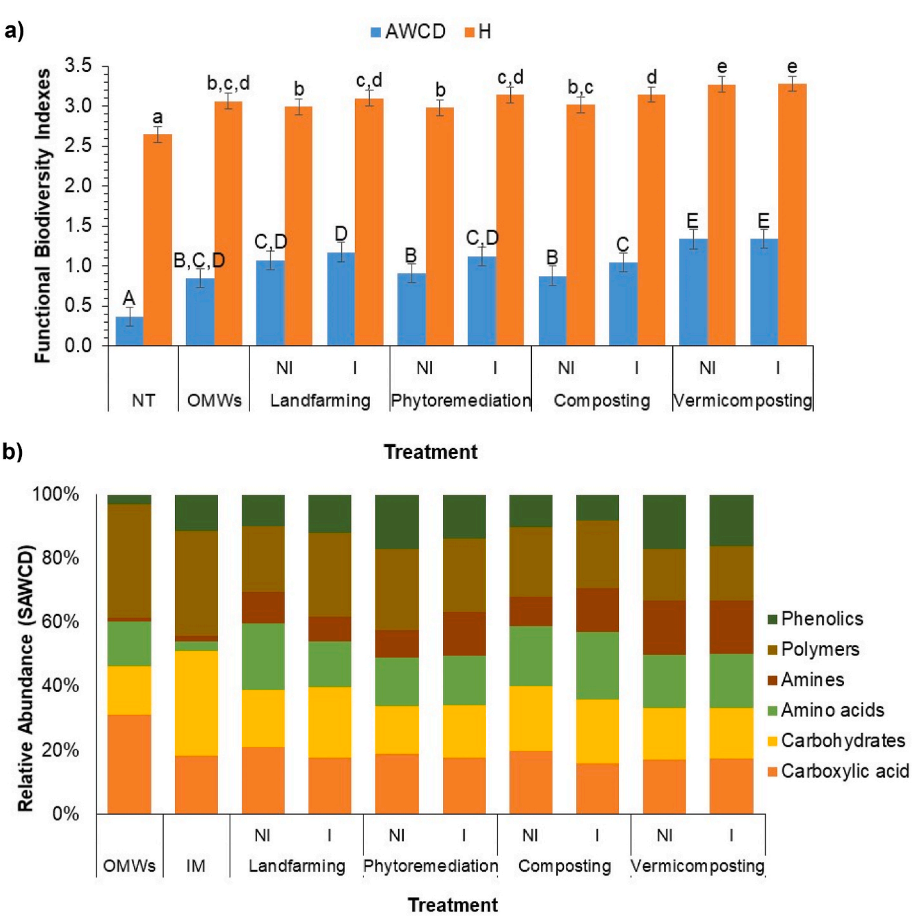

Fig. 2. Mean values of functional biodiversity indexes (a), such as Shannon Index (H′) and average well color development (AWCD), and metabolic profile (b) in the OMW sludge (non-treated material, NT), the initial mixture (IM) and the final inoculated (I) or non-inoculated (NI) material from landfarming, phytoremediation, composting, and vermicomposting treatments, expressed as a percentage of utilization of each group of substrates. The values are the average of three replicates. Error bars represent LSD interval, the bars bearing different letters were significantly different according to Fisher's LSD test ($p < 0.05$) (lower case letters for AWCD and uppercase letters for H′).

established in a fertile substrate. According to the results, the combination of several bioremediation techniques, such as composting or vermicomposting, followed by phytoremediation, could be the most suitable and sustainable solution in OMW contaminated soils, recovering and recycling them into a high-quality amendment.

3.2. Study and functionality of microbial community in each bio-treatment

The metabolic analysis of microbial communities reflects the interactions between the microorganisms and the environment. In this study, this assay was performed using the Biolog Ecoplate technique that is a culture-dependent approach, which reveals the most active populations within the microbial community and thus, the community's physiological patterns (Feigl et al., 2017). The abundance metabolic activity of the microbial community (average well color development, AWCD) present in the OMW sludge from evaporation pond, the initial mixture and the final materials from each bio-treatment, in addition to its physiological diversity (Shannon Index, H) are shown in Fig. 2a. The AWCD and H, as functional biodiversity indexes, analyzed from OMW sludge were the lowest values (0.368 and 2.643, respectively). This low microbial activity and its diversity are mainly due to the toxic effect derived from the high level of phenolic compounds which alter the microbial community (Ntougias et al., 2013), as well as the OMW sludge recalcitrance and its resistance to biodegradation (Vivas et al., 2009). The addition of organic residues to the OMW sludge significantly increased the microbial functionality reaching values of 0.847 and 3.060 in AWCD and H′, respectively. This potential metabolic increase is very evident as nutrients incorporation stimulates indigenous microbiota activity and growth (Mekki et al., 2017). This microbiota is capable of detoxifying OMW, allowing greater colonization and transformation of residual organic matter by microorganisms that were previously unable to inhabit there (Ntougias et al., 2013). In comparison to the initial metabolic status of the material (OMW sludge), all bio-treatments favored the increase of metabolic activity in the microbial community. Similar results showed previously by other authors, such as Pardo et al. (2014), who demonstrated that organic matter incorporation to the soil resulted in functional diversity increase. In most of the biotreatments, the results related to metabolic abundance, expressed as AWCD, as well as functional biodiversity (H′), were significantly higher than that obtained from IM. However, the results of AWCD from vermicomposting bio-treatment were the highest significantly, showing values of 1.336 and 1.339, in the piles without consortium and inoculated, respectively. The microbial consortium significantly improved the diversity and abundance in most of the treatments. These results are in agreement with other research such as Vivas et al. (2009) where it was proved that vermicomposting is an effective bio-treatment to activate functional microbial abundance and diversity of a pollutant residue such as OMW. They also affirmed that *E. foetida* probably favors the metabolization of more recalcitrant substances and, consequently, the release of smaller molecules easier to use by microorganisms and, thus, the enhancement of microbial activity.

The microbial community in soils is commonly regulated by the availability of organic matter content, which is considered as a key factor (Martínez-Gallardo et al., 2021). Therefore, the functional aspects related to the utilization of organic carbon can provide relevant information on the effect of the different biological treatments applied in this work for the recovery and reuse of OMW sludge. The relative abundance of the metabolic activities grouped according to the chemical family of the substrates (SAWCD) analyzed in samples from OMW sludge, initial mixture and the final material of inoculated and non-inoculated bio--treatments is shown in Fig. 2b. The pattern of relative abundance of functionalities in OMW sludge was dominated by the metabolic activities related to the utilization of polymers (35.3%) and carboxylic acid (31.3%) followed by carbohydrates and amino acids, both with average values of 14%, and amines and phenolic compounds which were the less represented with an average relative abundance of 2%. The predominance of these substrate chemical groups provides information on the availability of carbon in OMW sludges. In this case it makes sense since the predominant activities are related to the utilization of polymers and carboxylic acid which mainly constitute the recalcitrant compounds. After incorporating organic residues to OMW sludge, the activities related to carbohydrate utilization increased to 33%. This behavior reflects the effect of biostimulation of indigenous microbial community present in OMW sludge. Therefore, OMW sludge can be considered to have adequate metabolic potential to metabolize recalcitrance substances, but are limited by toxicity as well as the lack of rapidly useable carbon resources (Martínez-Gallardo et al., 2020; Ntougias et al., 2013). In this sense, other noticeable change in the pattern of relative abundance related to the organic matter addition was the increase of phenolic compounds utilization (11%). Similar results were shown by Federici et al. (2011) who demonstrated that bacterial community naturally present in OMW are specialized in organic matter decomposition, specifically phenolic compounds. Nevertheless, as previously mentioned, it must be stimulated to optimize its biodegradable potential. Thus, these results suggest that OMW sludge from evaporation ponds are naturally enriched in microorganisms potentially capable of degrading phenolic compounds. The functional analysis of the final product obtained from each bio-treatment revealed a very similar metabolic profile showing the following values ranges of substrates utilization: 14–22% in carbohydrates, 20–26% in polymers, 13–22% in amino acids, 11–21% in carboxylic acids, 8–17% in phenolic compounds and 8–17%. According to these results, it is highly remarkable such a well-balanced pattern analyzed in the final vermicompost, where the utilization of each tested substrate supposes around 16.5%. These results have shown that the biotreatments applied are optimal processes to stimulate the microbial community present in the OMW matrices to biodegrade the organic matter. Among these biotreatments, vermicomposting stands out, since it produces the most stable and functionally balanced product (Ntougias., 2013; Federici., 2011; Vivas et al., 2009). Some authors affirm that the joint action of the autochthonous microbiota from the composting pile and that present in the intestine of the earthworms under controlled conditions favors the accelerated bio-oxidation of organic matter which characterizes the final product obtained (Vivas et al., 2009).

3.3. Characterization of final material after biological treatments

To reflect the effectiveness of each bio-treatment here applied for biorecovery of OMW sludge stored in evaporation ponds, the values according to toxicity (phytotoxicity and ecotoxicity) from OMW sludge,

Table 1
Phytotoxicity (Germination Index, % GI) and ecotoxicity (Toxicity Units, TUs) values in OMW sediments, initial mixture and final material from inoculated and non-inoculated bioremediation treatments. Values are the mean of three replicates. Values with same letter in a column are not significantly different (LSD, $p < 0.05$).

Treatment	Phytotoxicity (GI, %)	Ecotoxicity (TUs)
OMW sludge	71 c,d	26.50 a
Initial Mixture	49 a	5.38 b
Landfarming Non-Inoculated	85 e	0.52 e
Landfarming Inoculated	94 f	0.54 e
PhytR[a] *J. acutus* Non-Inoculated	56 b	0.14 i
PhytR *J. acutus* Inoculated	58 b	0.29 g,h
PhytR *C. mariscus* Non-Inoculated	53 a,b	0.14 i
PhytR *C. mariscus* Inoculated	56 b	0.25 h
PhytR *C. zizanoides* Non-Inoculated	74 d	0.36 f,g
PhytR *C. zizanoides* Inoculated	66 c	0.37 f
Composting Non-Inoculated	118 h	0.82 d
Composting Inoculated	129 i	0.96 c
Vermicomposting Non-Inoculated	100 g	0.14 i
Vermicomposting Inoculated	101 g	0.13 i

[a] PhytR: Phytoremediation.

initial mixture and final materials were tested and shown in Table 1. Phytotoxicity was expressed as Germination Index (GI) indicating high phytotoxicity when values are below 50%, moderate phytotoxicity when values are between 50 and 80%, absence of phytotoxicity when GI is above 80%, and phytostimulant effect when values are above 100% (Zucconi et al., 1985; Emino and Warman, 2004). The ecotoxicity values were expressed as toxicity units (TUs). Persoone et al. (2003) established the following levels of ecotoxicity: very high acute toxicity when the values are higher than 100 TUs; high acute toxicity when the values are higher than 10 TUs but lower than 100 TUs; acute toxicity when the values are higher than 1 TU but lower than 10 TUs; slight acute toxicity when values are higher than 0.4 TUs but lower than 1 TU; and no acute toxicity when values are lower than 0.4 TUs. As other authors have previously described, OMW sludge is composed of a high load of organic substances such as phenolic compounds that provokes high ecotoxicity and presents a potent antibacterial effect (Kovačević et al., 2022; Hentati et al., 2016; Babi'c et al., 2019). Thereby, OMW sludge showed high acute toxicity (26.50 TUs) and moderate phytotoxicity (70.77% GI). These results are in concordance with other studies such as Martínez-Gallardo et al. (2021) where the toxicity of OMW sludges from seven evaporation ponds was analyzed. The value of phytotoxicity in most sludges was around 50–80% GI, and in the case of ecotoxicity they reached values above 10 TUs. However, when organic matter was added to the OMW sludge, there was a reduction of ecotoxicity (5.38 TUs). This phenomenon might be explained by the dilution of recalcitrant compounds which, as previously mentioned, cause ecotoxicity. On the other hand, phytotoxicity increased with the incorporation of organic waste matter (49.27% GI) likely due to the presence of phytopathogenic microorganisms in the raw materials, which supports the need to apply a biological treatment to detoxify the residual organic matter (Hachicha et al., 2009). Most biological treatments enhanced the reduction of toxicity by transforming OMW sludge together with agricultural and livestock waste organic matter into a material with ecotoxicity and phytotoxicity values below 1 TU and above 80% GI, respectively, which is considered mild or no toxicity. Values of TUs below 0.4 meaning absence of ecotoxicity were tested in final material from phytoremediation and vermicomposting, the latter being the best bio-treatment in eliminating ecotoxicity. Nevertheless, despite phytoremediation bio-treatment achieves the ecotoxicity disappearance, the final material obtained was moderately phytotoxic. Some authors have demonstrated the capacity of certain plant species to eliminate polluting compounds present in the soil through various mechanisms, including the assimilation of toxic compounds in their structures through a process of phytoextraction, as well as the development of rivalry mechanisms against surrounding plants through the release of phytotoxic molecules (Cunningham et al., 1996). In this way, it is easily explainable by the low values of GI present in these final materials. On the other hand, the higher GI values were found in the final material from composting, exceeding 100% GI in this case (117.88 and 129.24% GI in the non-inoculated and inoculated piles, respectively), which means the material presents phytostimulant properties. On the contrary, Soto et al. (2022) found that phytotoxicity was lower in vermicompost than in compost because of its higher humus content. This discrepancy could be explained by differences on raw materials, operational processes and maturity of the final compost and vermicompost.

In summary, vermicomposting proved to be an efficient biotreatment to reduce the toxicity of OMW sludge and supported the results mentioned above, based on the ability of earthworms to stimulate the functional biodiversity of OMW sludge, which favors the depletion of potential toxic compounds, such as phenolic compounds. However, composting bio-treatment ensures the final compost has phytostimulant capacity, which makes it an organic amendment of agronomic interest (Martínez-Gallardo et al., 2020; Sáez et al., 2020). On the other hand, inoculation has no effect on the reduction of ecotoxicity, but it does have an enhancing effect on the effectiveness of landfarming and composting biotreatments in reducing phytotoxicity and even, in the latter case, in improving the phytostimulant effect.

According to the previously discussed results about maturity and toxicity, as well as functional study, the final material from composting and vermicomposting are adequate and suitable substrate for agricultural purposes. Moreover, the physicochemical parameters related to the quality of inoculated and non-inoculated compost and vermicompost are shown in Table 2, and they are contrasted with those reported in the literature as optimal or with threshold limits from legislation. In general, all the final materials obtained from composting and vermicomposting with and without bioaugmentation were within the optimal range of each parameter necessary to consider the material as suitable for agricultural use. In that respect, it should be taken into account that OMW sludge is a difficult raw material to homogenize due to the hardness and the stone-shaped structure that form in the evaporation ponds (Martínez-Gallardo et al., 2021). This aspect of the raw material can influence the efficiency of the degradation process and, therefore, the stabilization of the final material (Sáez et al., 2021; Abid and Sayadi, 2006). This may also explain why some OM and C/N values are outside the optimal range.

Overall, the results provided clear corroborating evidence that the supplementation of OMW sludge with organic wastes, combined with bioaugmentation by a specialized fungal consortium, increased the efficiency of biotreatments and allowed the recovery of polluting waste and the obtaining of valuable products within the framework of the circular economy. In this sense, landfarming and phytoremediation favored the decontamination of OMW sludge, resulting in a clean substrate, while composting and vermicomposting offered the ideal conditions to transform sludge into a stable material suitable for agriculture. Composting and vermicomposting have proven to be efficient in the elimination of toxic compounds from OMW sludge and, in addition, in the transformation of these wastes into products with high agronomic interest (Sáez et al., 2020; Jeguirim et al., 2017; Vlyssides et al., 1999). Landfarming and phytoremediation also reduced the toxicity of the OMW sludge, but the biotransformation of organic matter was not as efficient as in composting and vermicomposting. As for the effect of the specialized inoculum, its main benefits were the acceleration of decontamination in vermicomposting and the production of compost highly phytostimulant. Finally, our results confirm the suitability of the proposed treatments for OWM sludge bioremediation and valorization. However, the final choice of the best treatment to apply will depend on the physicochemical and chemical characteristics of the OMW sludge, which are known to be very variable (Martínez-Gallardo et al., 2021).

Table 2
Physicochemical characteristics of the final material from composting and vermicomposting.

Parameters*	Optimal values**	Compost		Vermicompost	
		Inoculated	Non-Inoculated	Inoculated	Non-Inoculated
OM (%)	>20 [b]	21	18	24	20
C/N	<15–20[b]	13	12	23	15
pH	6.5–8.5 [b]	8.0	8.5	7.5	8.2
EC (dS/m)	<4[c]	3.7	2.8	0.7	0.8
C_{HA}/C_{FA} ratio	>1 [b]	2.3	2.0	2.2	2.1
Cd (ppm)	<0.7	0.10	0.11	0.13	0.13
Cu (ppm)	<70	27	33	29	38
Ní (ppm)	<25	21.7	31.4	18.4	24.1
Pb (ppm)	<45	11.0	20.3	10.0	21.0
Zn (ppm)	<200	104	103	119	122
Cr (ppm)	<70	77	78	68	61

*Abbreviations: OM: organic matter; EC: electrical conductivity; C/N: carbon-nitrogen ratio; C_{HA}/C_{FA}: humification ratio.
**The optimal values have been reported in the following literature: (a) Bernal et al. (2009); (b) Bernal et al. (2017); (c) Jara-Samaniego et al. (2017). Heavy metal contents of compost and vermicompost are within Class A according to Real Decreto 999/2017.

Therefore, for OMW sludges with high organic matter content, composting and vermicomposting are the more suitable treatments; whereas landfarming and phytoremediation are the choices for materials with a low organic load. As an additional advantage, these treatments can be applied on the same OMW evaporation ponds (*in situ*), as well as, for the treatment of other recalcitrant wastes of similar nature or characteristics.

4. Conclusion

Supplementation of OMW sludges with organic residues combined with bioaugmentation by a specialized fungal consortium increases the efficiency of biotreatments for the revalorization of the waste into products of commercial interest in the framework of the circular economy. Landfarming and phytoremediation favor the decontamination of OMW sludge, resulting in clean soil. Composting and vermicomposting also offer ideal conditions for transforming sludge into a stable material suitable for agricultural purposes. The addition of earthworms to the composting process enhances the stimulation of functional biodiversity by achieving a well-balanced metabolic profile of the microbial community present in the final compost. Additionally, based on the results, it is possible to recommend the application of biotreatments that offer high degradation, such as composting and vermicomposting, for waste with a higher organic load. On the other hand, biotreatments such as landfarming and phytoremediation, could be recommended as a complementary treatment to the product of the previous mentioned treatments or to treat waste with a lower organic load.

Credit roles

María R Martínez-Gallardo: Conceptualization, Investigation, Methodology, Data curation, Writing- Original draft preparation. **Macarena M Jurado:** Investigation, Methodology, Writing- Reviewing and Editing. **Juan A. López-González:** Investigation, Methodology. **A. Toribio:** Investigation, Methodology. **F. Suárez-Estrella:** Investigation, Methodology. **José A. Sáez:** Investigation, Methodology, Data curation. **F.J. Andreu-Rodríguez:** Investigation, Methodology. **R. Moral:** Conceptualization, Supervision, Writing- Reviewing and Editing. **Joaquín Moreno:** Conceptualization, Supervision, Visualization, Formal analysis, Writing- Reviewing and Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2022.115647.

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