

Pleurotus Ostreatus and *Trametes Versicolor* Fungi to Decontaminate Soils Containing Organophosphates *Methamidophos* and Cadmium

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The intensive use of agricultural soils and the appearance of pests on crops make it necessary to use pesticides to achieve crop production. As a consequence of this, soils accumulate polluting elements that are incorporated into agricultural insecticides, one of them being *Methamidophos*, which is an insecticide belonging to the group of organophosphate pesticides. One of the methods to reduce this contaminant that have been tested recently is mycoremediation, which consists of the use of fungi. The investigation evaluated the use of the white rot fungi *Pleurotus ostreatus* and *Trametes versicolor* to decontaminate soil with *Methamidophos* and cadmium. The research design was experimental, with three treatments and three replicates: Treatment A using the *Pleurotus ostreatus* fungus, treatment B using the *Trametes versicolor* fungus, and treatment C using the two aforementioned fungi; It was also supplemented with eucalyptus sawdust plant substrate. The result was a decrease of *Methamidophos* 64 % to 73 % and Cadmium by 99.3 %. This method of remediation is a friendly alternative to the environment and low economic cost, easy to apply and good results are achieved.

1. Introduction

The presence of pests and viruses has been occurring more frequently in agricultural activities in recent years, leading to the use of various types of pesticides to control them and obtain greater production; However, this has resulted in soil degradation by contaminating it with residual toxic substances such as *Methamidophos* (O,S-dimethyl phosphoramidothioate), an organophosphate pesticide that, when released into the environment, causes contamination of soil, air, water bodies, birds, etc. As noted by the National Pesticide Information Center (NPIC) (2019). In other cases, these agrochemicals also contaminate soils with heavy metals such as cadmium (González B., Pazmiño D., 2016). *Methamidophos* is a foliar insecticide to control insects (aphids, leafhoppers, leaf-eating caterpillars) and also acaricidal side effects, its use is in citrus, corn, cotton, rice and grapevine crops among others (Baer and Marcel, 2014).

One way to minimize the presence of *Methamidophos* and Cadmium (Cd) in the soil is through the use of white rot fungi (they degrade plant lignin) (Quintero, et al., 2006). Among these fungi is *Trametes versicolor* widely used in biotechnology, such as the biosynthesis of cadmium sulfide quantum dots due to its adsorption power (Quin Z., 2018), another fungus is *Pleurotus ostreatus* which has been used for the extraction of trace heavy metals such as Cd and Pb in a bentonite column. (Kocaoba S. and Arisoy M., 2018). In an investigation, efficiency in the degradation of organophosphorus insecticides in contaminated soils wearing *Trichoderma harzianum* and *Pleurotus ostreatus* was the 66.8 % (Maldonado L., 2017); on the other hand, good results of biodegradation of a pyrotroid insecticide in potato crop soil wearing *Trichoderma harzianum* and *Pleurotus ostreatus* have also been obtained (Torres, 2017).

In Peru, agriculture uses pesticides in its activities and it is considered that pesticide contamination is important and especially in cultivation areas, this was reported in the research conducted by Castillo B., et al. (2020) in the agricultural area of Cañete. Because of this and other studies on the negative impact of some chemical elements of pesticides, since November 20, 2020, the Peruvian government through the National Agricultural Health Service (SENASA) of the Ministry of Agriculture, prohibited the marketing of pesticides for agricultural use that contain Methamidophos in their composition because of the health hazards they represent (R.D. No. 0022-2020-MINAGRI-SENASA-DIAIA); however, the soils where this chemical contaminant was used are still present to date, since Methamidophos is an organophosphate insecticide - acaricide with high residual power that has been widely used for agricultural soil. The objective of the research was to test the use of *Pleurotus ostreatus* and *Trametes versicolor* fungi in the decontamination of soils with the presence of the organophosphorus chemical compound Methamidophos and the heavy metal Cadmium.

2. Methodology

2.1 Experimental process

The experimental development of the research consisted of incorporating the fungi *Pleurotus ostreatus* and *Trametes versicolor* fungi into soil contaminated with the pesticide component Methamidophos and the heavy metal Cadmium, to establish whether the fungi exerted decontamination remediation activity of these contaminants. The steps are shown in Figure 1.

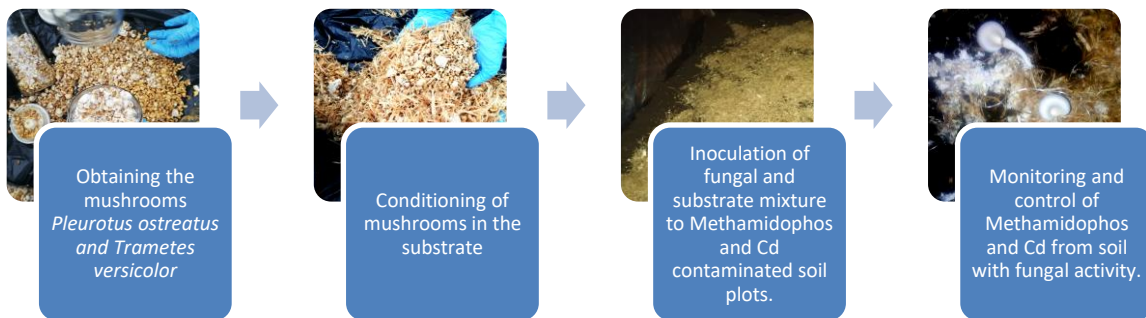


Figure 1: Experimental process

The research was carried out in 10 experimental units: 1 control plot and 9 soil plots where the fungi for the experiment were inoculated, 3 for treatment A (with *Pleurotus ostreatus* fungus), 3 for treatment B (with *Trametes versicolor* fungus) and 3 for Treatment C (for the combination of *Pleurotus ostreatus* and *Trametes versicolor* fungi). The dimensions of the soil plots were 3 m long and 1.5 m wide separated by 1 m from each other, as shown in Figure 2 for the first 4 plots. These plots conditioned for the research were located in the district of Ninabamba -Santa Cruz, Cajamarca (Latitude: -6.65, Longitude: -78.7894; 6° 39' 0" South, 78° 47' 22" West)

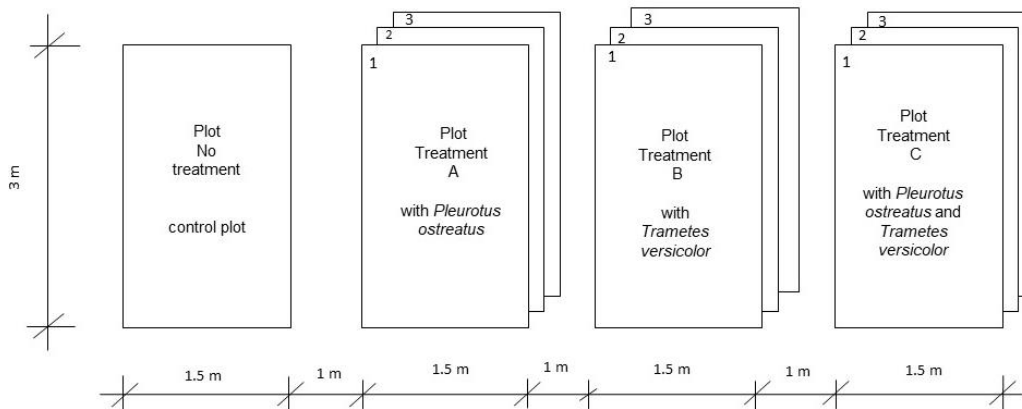


Figure 2: Experimental soil units

2.2 Experimental treatments

The substrate with the fungi *Pleurotus ostreatus* and *Trametes versicolor* were introduced into the experimental soil units as described in Figure 2. The pH, temperature, humidity, fungal growth and inhibition areas were monitored for four consecutive weeks. Methamidophos and Cadmium contaminants in the soil were analyzed at the beginning and end of treatment with the fungi, for which samples were taken from the plots following the Guide for soil sampling approved by D.S. N°002-2013-MINAM (MINAM, 2014).

These experimental soil units were conditioned in the form of a greenhouse to provide the climatic conditions of temperature and to protect the fungi from possible predators.

The pollutant analysis methods were:

Reference Standards EPA Method 3050-B, Rev. 02, 1996; EPA Method 200.7, Rev. 4.4., 1994. By: Acid Digestion of Sediments, Sludges and Soils. /Determination of Metals and Trace Elements in Water and Residues by Inductively Coupled Plasma – Atomic Emission Spectrometry ICPS – AES. Analysis carried out in the Envirotest Laboratory certified by INACAL.

3. Results and discussion

3.1 Initial level of soil contamination

The soil plots where the research was carried out initially presented the level of contaminants shown in Table 1. The values were 0.244, 0.276, and 0.205 mg/kg on average for the plots labeled as A, B, and C respectively; and, as for the cadmium concentration for all the plots, it was 6 mg/kg on average. The level of Methamidophos and Cadmium is outside the standards of environmental quality of soil normed for Peru that indicates a maximum value of 1.4 mg/kg for the case of Cd (MINAM, 2017), and for Methamidophos should be 0 mg/kg (SENASA, 2020).

Table 1: The concentration of Methamidophos and Cd in the soil

Treatment Plot	Methamidophos concentration (mg/kg)	Cadmium concentration (mg/kg)
Plot A	0.244	6
Plot B	0.276	6
Plot C	0.205	6

For Methamidophos, it has a mean of 0.24167, standard error of mean of 0.20529 and a standard deviation of 0.035557; For cadmium, the standard deviation is zero.

3.2 Control of physicochemical parameters of contaminated soil

The physicochemical properties pH, temperature, humidity, and conductivity were monitored in the soil of the agricultural plots, from the first month until the end of the fourth month of treatment with the fungi *Pleurotus ostreatus* and *Trametes versicolor*, with the results shown in Table 2, (2014) found that at acid pH (4-5) for the case of an aqueous solution and using fungal mass they achieved higher absorption of Pb and Cd from water (92.4 % and 80 %); something similar was found in the present investigation where the removal efficiency was 73 % for the insecticide and 99 % for Cd at pH close to neutral. It is mentioned that the insecticide Methamidophos is stable at sterile acidic pH (pH 5) and fast hydrolyzing in alkaline environments (Baer and Marcel, 2014).

Table 2: Control of physicochemical parameters of agricultural soil with the presence of fungi.

Treatment Plot	pH		Temperature (°C)		Humidity (%)		Conductivity (µS/cm)	
	Month 1	Month 4	Month 1	Month 4	Month 1	Month 4	Month 1	Month 4
Plot A	6.8	6.0	24.1	20.7	80	82	200	190
Plot B	7.2	6.4	24.4	20.5	79	80	220	200
Plot C	6.9	6.6	24.4	20.0	80	85	245	230
Standard deviation	0.2081	0.3055	0.1732	0.3605	0.5773	2.5166	22.5462	20.8166

3.3 Growth control of the fungi *Pleurotus ostreatus* and *Trametes versicolor*

The soil-inoculated fungi were planted under greenhouse conditions to provide them with a favorable growth environment. At the end of each month for 4 months, growth was monitored with the results presented in Table 5. At the end of month 4, the highest fungal growth (7.1 cm) corresponded to the treatment of plots A (with *Pleurotus ostreatus*) and C (with *Pleurotus ostreatus* and *Trametes versicolor*), slightly higher than the treatment of plot B (with *Trametes versicolor*) which was 6.2 cm; this is probably due to the lack of good homogenization and to the type of substrate for the fungi used which was eucalyptus wood sawdust and which can be improved by trying with another type of substrate such as vegetable rubber sawdust which has been shown to be a good substrate for growth in the cultivation of the *Pleurotus ostreatus* fungus (Zakil, 2021); however also with a substrate based on oil palm leaves a good growth result has also been found (Ali N. et al., 2018); it is important to maintain the environmental factors conducive to fungal growth in order to preserve the bioremediation potential.

Table 3: Growth control of *Pleurotus ostreatus* and *Trametes versicolor* fungi in the soil during treatment

Treatment Plot	Mushroom growth (cm)			
	Month 1	Month 2	Month 3	Month 4
Treatment plot A	1.3	1.6	3.8	7.1
Treatment plot B	1.1	1.4	3.4	6.2
Treatment plot C	1.4	1.9	3.6	7.1
Standard deviation	0.152	0.251	0.200	0.519

Area of inhibition of *Pleurotus ostreatus* and *Trametes versicolor* fungi in the soil during treatment

Fungi by their mycelial growth are adapted to the colonization of the soil ecosystem through the production of extracellular enzymes such as laccases and ligninases; it is indicated that water stress and water potential have an impact on the degradation of pesticide mixture by not producing the number of extracellular enzymes in bioremediating fungi (Gouma et al., 2014). Table 4 shows the mycelial growth of *Pleurotus ostreatus* and *Trametes versicolor* fungi, and that for the three cases, there is a very similar average growth, standing out for plots A and B with 9 cm in diameter.

Table 4: Control of the area of inhibition of *Pleurotus ostreatus* and *Trametes versicolor* fungi during treatment

Treatment Plot	Inhibition area diameter (cm)			
	Month 1	Month 2	Month 3	Month 4
Treatment plot A	1	3	6	9
Treatment plot B	1	4	5	8
Treatment plot C	1	4	6	9
Half	1.0000	3.6667	5.6667	8.6667
Standard deviation	0.00000	0.57735	0.57735	0.57735

3.4 Final concentrations of contaminants in agricultural soils

The results indicate that the degradation of the pollutant Methamidophos was between 64% and 73%, this is due to the affinity of the fungi with substances containing carbon, nitrogen, sulfur, iron and other minerals essential for their growth and enzyme production, carbon being the most important (Hernández-Mansilla, 2005); but also in the research, it was found that in the case of cadmium concentration, the reduction was 99% probably due to the ion exchange capacity of the mass of fungi with metals. See Table 5 and Table 6.

The reduction of the level of Methamidophos and Cadmium with the treatment leaves values below the quality standards and environmental regulations of Peru (SENASA, 2020 and MINAM, 2017).

The degradation of Methamidophos and Cd by the action of enzymes produced by fungi *Pleurotus ostreatus* and *Trametes versicolor* is important, similar to the degradation of recalcitrant compounds and xenobiotics because these enzymes are mainly related to the mineralization of lignin, oxidizing aromatic rings, as well as substituents present in the polymer (Fernandez, 2009). The action of fungi has also been effective in the degradation of dyes, pesticides and polyaromatic hydrocarbons with a chemical structure similar to lignin

phenylpropane, which leads to thinking that biodegradation also occurs with a mechanism similar to that experienced by lignin (Gouma et al., 2014).

The final evaluation was made at the end of 4 months as part of the first stage of an investigation to determine the applicability of fungi in the reduction of Methamidophos and Cadmium in agricultural soil; therefore, the second stage will continue with the objective of progressively evaluating the efficiency of these species in the reduction of the mentioned pollutants and others.

Table 5: The final concentration of the contaminant Methamidophos in agricultural soil

Treatment Plot	Initial concentration of Methamidophos in soil (mg/kg)	Final concentration of Methamidophos (mg/kg)	Methamidophos reduction (%)
Treatment plot A	0.244	<0.073	70
Treatment plot B	0.276	<0.073	73
Treatment plot C	0.205	<0.073	64

Table 6: The final concentration of the contaminant Cadmium in agricultural soil

Treatment Plot	Initial concentration of cadmium in soil (mg/kg)	Final cadmium concentration (mg/kg)	Cadmium reduction (%)
Treatment plot A	6	<0.04	99 %
Treatment plot B	6	<0.04	99 %
Treatment plot C	6	<0.04	99 %

The degradation of the insecticide by fungi follows the metabolic phases with intracellular mechanisms that transform contaminants with reductive and oxidative processes, a characteristic that makes it tolerant and suitable for bioremediation processes, but in the case of some resistant ones, extracellular oxidative processes also occur where ligninolytic enzymes allow decontamination to be carried out (Quinteros, 2011);

4. Conclusions

It was established that the fungi *Pleurotus ostreatus* and *Trametes versicolor* allowed reducing the presence of Methamidophos and Cadmium in agricultural soils. After the treatment it was possible to reduce 73% of the organophosphate insecticide and 99% for Cadmium; this means that it is possible to use fungi of this type to recover contaminated soils in an environmentally sustainable way and of easy application, requiring only basic training, with the perspective of using the method in areas of agricultural soils with intensive use of pesticides in order to reduce their contamination.

Acknowledgments

The authors would like to thank "Investiga UCV" of the Universidad César Vallejo for financial support for the publication of this research.

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