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Microorganisms isolated from polluted urban soils highly effectives in degrading recalcitrant pesticides

Microorganismos aislados de suelos urbanos contaminados altamente efectivos en la degradación de pesticidas recalcitrantes

Liliana Rocío Botero-Botero[1], Sergio Augusto Upegui-Sosa[2], Gustavo Antonio Peñuela-Mesa[3*]

[1]Grupo de investigación Biodiversidad, Biotecnologia y Bioingenieria (GRINBIO), Universidad de Medellín. Carrera 87 # 30-65. Medellín, Colombia.
[2]Grupo de Investigaciones y Mediciones Ambientales (GEMA), Universidad de Medellín. Carrera 87 # 30-65. Medellín, Colombia.
[3]Grupo de investigación Diagnóstico y Control de la Contaminación (GDCON), Facultad de Ingeniería, Universidad de Antioquia. Calle 67 # 53-108. A. A. 1226. Medellín, Colombia.

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ABSTRACT: Between 1972 and 1984 all types of solid waste from the city of Medellin were deposited in an area which had no technical specification as a landfill. Domestic, hospital and industrial waste was deposited, and accumulated to form a mountain of waste more than 10 meters high. To exacerbate the problem, when the site was closed for the deposit of solid waste, people remained living there to recycle materials. A study funded by the Valle de Aburrá Metropolitan Area and carried out by the GDCON Group at the Universidad de Antioquia between 2004 and 2005 found that leachate from the rubbish dump of Moravia contained heavy metals, phenols, sulphides, benzene, toluene, xylene, etc. In another study carried out by the GDCON and National University of Colombia (Medellín) between 2007 and 2009, it was found that plants and animals (mice, cockroaches etc.) in Moravia also contained these toxic pollutants. For this reason, the government of Medellin decided to move the people living in Moravia to another site in Medellin (between 2010 and 2014). Microbial consortia isolated from Moravia soils (MS) showed a high capacity to degrade chlorpyrifos, methyl parathion and malathion pesticides (20, 30 and 130 mg Kg^{-1}). To provide a point of comparison, the degradation of the 3 pesticides was also performed with isolated pools of immature compost. The MS microbial consortia showed higher degradation rates than CI microbial consortia when malathion, methyl parathion and chlorpyrifos were degraded.

RESUMEN: Entre 1972 y 1984 todo tipo de residuos sólidos de la ciudad de Medellin fueron depositados en un área ocupada que no tuvo especificaciones técnicas para que fuesen depositados aquellos residuos. Allí fueron depositados residuos domesticos, hospitalarios e industriales que se fueron acumulando hasta alcanzar una montaña de residuos de más de 10 metros de altura, con el agravante que cuando clausuraron el sitio para el depósito de residuos sólidos allí se quedaron viviendo las personas que hacían reciclaje. Un diagnóstico llevado a cabo por el Grupo GDCON de la Universidad de Antioquia entre el 2004 y 2005, y financiado por el Área Metropiltana del Valle de Aburrá, se comprobó que los lixiviados de la montaña de residuos de Moravia, y que atravesaban algunas casas, tenía metales pesados, fenoles, sulfuros, benceno, tolueno, xileno, entre otros. En otro estudio realizado por el GDCON y la Universidad Nacional-sede de Medellín entre 2007 y 2009, se comprobó que las plantas y animales (ratones, cucarachas) de Moravia también contenían dichos contaminantes tóxicos. Por esta razón, la alcaldía de Medellín decidió trasladar a las personas que vivian en la montaña de residuos de Moravia a otro sitio de Medellin (entre 2010 y 2014). Consorcios microbianos aislados de los suelos de la montaña de residuos de Moravia (MS) mostraron una alta capacidad para degradar los pesticidas clorpirifos, metil paratión y malatión en concentraciones de 20, 30 y 130 mg Kg^{-1} en un estudio realizado en el

* Corresponding author: Gustavo Antonio Peñuela Mesa
e-mail: gustavo.penuela@udea.edu.co
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laboratorio del GDCON. Como un punto de comparación, la degradación de los 3 pesticides fue también realizada por conconsorcios aislados de compost inmaduro (IC). El consorcio microbiano MS mostró mayor velocidad de degradación de clorpirifos, metil paratión y malatión que el consorcio microbiano IC.

1. Introduction

The pesticides, including methyl parathion, malathion and chlorpyrifos, are widely used for pest control in agriculture and public health programs [1, 2]. However, they generate toxic wastes that contaminate cultivated soils and their surrounding environments [3, 4]. The search for alternatives to mitigate the impact of pollution by pesticides is a priority. Bioremediation is a strategy that presents important advantages for the treatment of contaminated soils due to its low cost and easy *in situ* application [5]. Compost has been proposed as an alternative for the treatment of soils contaminated by xenobiotics. This is because its nutrient contribution [6], that increases microbial populations [7] and augments the release of extracellular enzymes which depolymerize a wide variety of organic compounds [8-10]. Moreover, highly contaminated soils have also been used for the selection of microbial populations capable of metabolizing different pollutants by using enzymes [11]. Soil from Moravia provides these populations [12] because several types of wastes were deposited there, including those with a high chemical contamination level. The microbial ability of immature compost and soil from Moravia to degrade the pesticides chlorpyrifos, Malathion and methyl parathion was evaluated in this study.

2. Experimentation

2.1. Matrices

Degradation tests were carried out using two microbial matrices: 1) immature compost (CI) (with 5 days of activation conditions) produced by mixing organic waste (mainly from pineapple, banana, papaya and mango) and fine-grained sawdust at a 50/50 ratio (v/v); and soil from Moravia (MS) collected from the first horizon (first 10 cm), transported to the laboratory in plastic sterilized bags and immediately stored at 4 °C until the time laboratory tests were started. The matrices were dried at room temperature and passed through a 2 mm pore diameter sieve to determine their physicochemical properties: pH, humidity percentage, maximum moisture retention capacity -MMRC-, bulk density (BD), organic carbon content -OC- [13, 14], organic matter content -OM- [13, 14], cationic exchange capacity -CEC- and total available phosphorus content -TP- [15, 16].

2.2. Degradation assays

Degradation assays were performed during the solid phase. For this purpose, 50-mL glass containers were used and filled with 10.0 g of IC or MS. Each microcosm assay was contaminated with a mixture of the three pesticides, reaching initial concentrations of 130, 30 and 20 mg Kg^{-1} of chlorpyrifos, methyl parathion and malathion, respectively. For 30 days, the microcosm assays were kept in darkness, at room temperature (25 ± 3 ºC) and in controlled humidity conditions (50-60%). The evaluation of Pesticide degradation was performed 0, 1, 3, 5, 7, 15 and 30 days after starting the culture. Analyses were carried out in duplicate.

Pesticides were extracted from the matrices with 30 mL of ethyl acetate. The container-flasks were sealed and then ultrasonic extraction (57 Hz, 30 min.) and shaking extraction (300 rpm; 24 h) were carried out. The recovery percentage reached was over 85% for the three pesticides in both matrices. The residual concentration of pesticides was determined in a 6850 Agilent Technologies gas Chromatograph connected to a micro-capture electron detector (μ-ECD). The GC–μ-ECD system was equipped with an HP-1 fused methyl siloxane capillary column (with a film thickness of 30 m×320 μm ×0.25 μm), and helium was used as the carrier gas at a 2-mL min^{-1} flow rate.

2μL of the concentrated extracts were injected in splitless mode and the μ-ECD temperature was 300 ºC. The three pesticides were separated by means of a 13.7-min oven temperature program. The initial temperature was 100 ºC, which was increased at a rate of 40 ºC min^{-1} up to 180 ºC (during 2 min). The temperature was further increased at a rate of 10ºC min^{-1} up to 230 ºC (during 3 min) and finally increased at a rate of 40 ºC min^{-1} up to 290 ºC (and maintained for 1 min). The retention times for methyl parathion, malathion and chlorpyrifos were 6.41, 7.14 and 7.40 min, respectively. Sterile IC and MS matrices were used as controls of the degradation process.

2.3. Mineralization monitoring

Assessment of the biological activity of the microorganisms in the matrices, and the toxic effect of the pesticides on them, were determined through a mineralization respirometry test. This was in accordance with the method described by [17], but with several modifications. Thus, the daily CO_2 production in each microcosm assay was captured with 0.8 N NaOH, which was then titrated with 0.4 N HCl and phenolphthalein as an indicator. The CO_2 formed Na_2CO_3, which was precipitated with 10% $BaCl_2$. Microcosm assays were used without pesticide contamination as biological activity control tests. Later, mineralization was evaluated over a 30 day-period and tests were performed in duplicate.

2.4. Statistical data analysis

Kinetics degradation data were evaluated through simple regression analysis using the statistical software Statgraphics Plus Version 5.1. A statistical significance level (*p*-value) and a correlation coefficient (r^2) were reported for adjusted models of the first order. Microbial activity results were also evaluated through a simple regression analysis. Calibration models with the best statistical fit were described according to the statistical significance level between variables (*p*-value) and their correlation coefficients (r^2).

Table 1 Physico-chemical characterization of matrices

Matrix	pH	Hum. (%)	BD (g cm^{-3})	MMRC (%)	O.M. (%)	O.C. (%)	CEC (meq $100g^{-1}$)	TP (mg Kg^{-1})
Soil	7.50	39.5	0.93	77.7	8.2	3.5	24.9	76.5
Compost	8.31	47.4	0.20	82.6	17.6	7.8	53.0	60.4

Hum. = Humidity, **BD** = Bulk density, **MMRC** = Maximum moisture retention capacity, **O.M.** Organic matter, **O.C.** = Organic Carbon, **CEC** = Cation Exchange Capacity, **TP** = Total Availabre phosphorus content.

3. Resultsand discussion

3.1. Matrices characterization

Both the IC and MS matrices presented slightly basic pH values. One of the most influential factors affecting the microbial community in soils is pH. The moisture corresponded to 50.8% and 57.3% of the field capacity in the MS and the IC respectively. Soil moisture refers to the volume of water in a given soil. Water availability is related with diffusion of soluble nutrients into and out of microbial cells and, therefore is necessary for microbial growth and other microbial metabolic activities as the degradation of pollutant compounds. However, a saturated soil with moisture excess, reduces the amount of available oxygen for aerobic respiration, becoming anaerobic respiration the predominant process, which produces less energy for microorganisms (than aerobic respiration) and slows the rate of biodegradation. Soil moisture content "between 45 and 85% of the water-holding capacity (field capacity) of the soil or about 12% to 30% by weight" is optimal for petroleum hydrocarbon degradation [18]. A summary of the matrices' physicochemical characterization results is presented in Table 1. Regarding the availability of organic matter, and hence organic carbon, both were higher in the IC (17.6%) than in the MS (8.2%). This indicates that the IC has a greater diversity and nutritional availability for microbial growth, which has been widely reported by [19-21].

The total availability of phosphorus, which is greatly important to microbiological growth, was higher in the MS (76.54 mg kg^{-1}) than in the IC (60.45 mg kg^{-1}). This is probably because of the matrix pH, since the optimal pH for phosphorus availability in soil is 6.5, as has been proved by [22]. The CEC of the MS (24.86 meq $100g^{-1}$) was lower than that of the IC (53.0 meq $100g^{-1}$). It is feasible that these values are related to the organic matter found in the soils, since there is a direct relationship between both parameters according to [23]. In the case of the MS, the CEC value found is within the range normally presented in soils.

Finally, the IC BD value (0.20 g cm^{-3}) was lower than the BD value of the MS (0.93 g cm^{-3}), indicating the presence of macro-pores. In turn, this indicates more aeration in the IC than in the MS. Soil bulk density refers to the weight of solid material in a given volume of soil. Typically, moist soil compacts more than dry or wet soil, and thus white clover is more tolerant of treading in summer than in spring [24].

3.2. Malathion, methyl parathion and chlorpyrifos degradation

The ability of the microorganisms of the IC and MS matrices to degrade the three pesticides was similar. The highest rate of degradation was seen for malathion, followed by methyl parathion and finally chlorpyrifos (Figures 1 and 2). This greater persistence of chlorpyrifos has been reported previously [25]. Pesticide degradation models in both the IC and the MS matrices, as well as their correlation coefficients (r^2), are shown in Table 2.

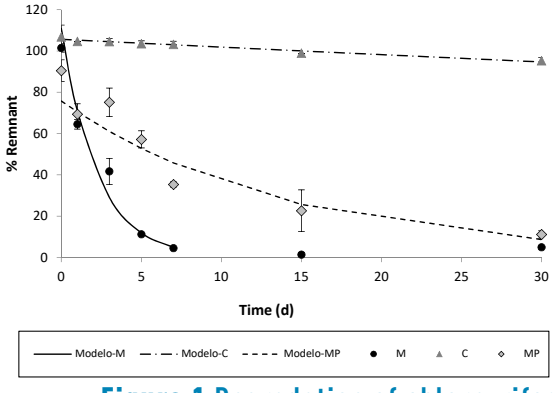

Figure 1 Degradation of chlorpyrifos(C), malathion (M) and methyl parathion (MP) by Immature Compost microorganisms

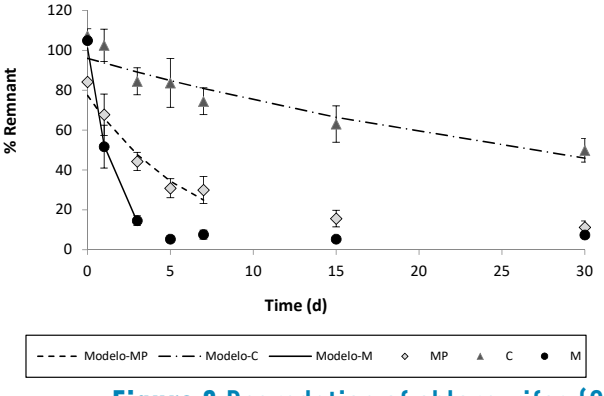

Figure 2 Degradation of chlorpyrifos (C), malathion (M) and methyl parathion (MP) by Moravia soil microorganisms

Table 2 Degradation models of pesticides in Immature Compost (IC) and Moravia Soil (MS)

Pesticide	Model* (r^2)	
	Immature Compost	Moravia Soil
Malathion	$\%R = 110.94e^{-0.445*t}$ (0.97)	$\%R = 101.19e^{-0.662*t}$ (0.93)
Methyl Parathion	$\%R = 75.79e^{-0.0720*t}$ (0.78)	$\%R = 77.40e^{-0.162*t}$ (0.76)
Chlorpyrifos	$\%R = 105.64e^{-0.00365*t}$ (0.96)	$\%R = 95.97e^{-0.0245*t}$ (0.67)

*Pesticide degradation was fitted to first-order models in all treatments. ($C_t=C_0*e^{-k*t}$).

%R = Remnant pesticide percentage. t = time (d)

3.3. Malathion degradation

The degradation rate of malathion was greater in the MS matrix than in the IC matrix, with degradation rate constants *(k)* of 0.662 d^{-1} and 0.445 d^{-1} respectively (p <0.005), and a half-life ($t_{1/2}$) of 1.04 d (p <0.05, r^2 = 0.67) for the MS and 1.9 d for the IC (Figures 1 and 2). The final degradation percentages were 96.4% for the IC and 95% for the MS. Degradation kinetics were fitted to the first-order models using the degradation values for the first 7 days in the IC (r^2 = 0.97) and for the first 3 days in the MS (r^2 = 0.93) because the highest degradation rates were observed during these periods of time.

3.4. Methyl parathion degradation

The methyl parathion degradation rate was also higher in the MS than the IC. The degradation rate values *(k)* were 0.162 d^{-1} for the MS and 0.0720 d^{-1} for the IC (p <0.005), and the $t_{½}$ values were 2.8 d and 6.4 d for MS and IC respectively. The final degradation percentages were 92.5% for the MS and 89% for the IC (Figures 1 and 2). As with malathion, degradation kinetics were fitted to first-order models for the degradation data of the first 7 days in the MS (r^2 = 0.76), since again it was when the highest degradation rate in this matrix was observed. The IC degradation kinetics were fitted to the first order model (r^2 = 0.78) using the degradation values obtained during a 30-day monitoring period.

3.5. Chlorpyrifos degradation

A higher degradation rate of chlorpyrifos was also observed in the MS (k = 0.0245 d^{-1}, p<0.005) than in the IC (k = 0.00365 d^{-1}, p<0.005), with half-life periods ($t_{1/2}$) of 28.3 d and 189.9 d for MS and IC, respectively. In this study, the microbial consortia were shown to be highly capable at degrading the malathion, methyl parathion and chlorpyrifos pesticides for both matrices. This degradation can occur by either metabolic or co-metabolic pathways. In the case of the latter, the microbial consortia do not necessarily use the pesticides. Results showed that the MS microbial consortia are more efficient at degrading the three pesticides (in comparison to the IC microorganisms). Such efficiency is related to the formation of a complex with organic molecules, and thus, with organic carbon. This factor determines the difference between the pesticide degradation rates, and is consistent with what [26], have reported. It was proven that the MS microbial consortia were better adapted to the degradation of malathion, chlorpyrifos and methyl parathion. These organisms were able to survive in an environment with a high content of toxic and persistent substances where there was a limited presence of nutrients. It has been observed that microorganism adaptation capacity favors contaminant degradation processes and makes such degradation faster [12].

Likewise, the enzymatic abilities of microorganisms are associated to the type of nutrients available in the soil (or solid matrices) [27, 28]. As has been stated by [29], microorganisms that have adapted to limited nutrients and varying environmental conditions develop characteristics that make them suitable for being used in bioremediation programs of environments contaminated with the aforementioned kinds of toxic compounds. As was observed in the MS microorganisms, the ability to degrade all three of the organophosphate pesticides can be largely attributed to the chemical similarity between these pesticides. They all contain certain similarities in their molecular structures, such as the phosphorus diester functional group (PO) and the phosphorus-sulfur bond (PS). This was reported by [30-32], who observed that both synthetic (including pesticides) and natural compounds with similar chemical structures were simultaneously degraded by microorganisms in environmental conditions. This phenomenon has been observed in several pesticides, including chlorpyrifos [33-35], malathion [35] and methyl parathion [35, 36].

3.6. Effect of pesticides on microbial activity

The IC mineralization (mg CO_2 g^{-1} matrix) showed linear behavior, with a CO_2 increase of 3.36 (r^2 = 0.99, P <0.005) and 2.65 mg g^{-1} d^{-1} (r^2 = 0.99, p <0.005) for the tests with the pesticides and the tests with the control, respectively. Maximum mineralization in the IC reached a value of 105 mg CO_2 g^{-1} in the pesticide-spread test, and 80 mg CO2 g^{-1} in the control test. However, MS mineralization values did not exceed 25 mg CO_2 g^{-1} regardless of whether the pesticides were applied or not. In this matrix, the mineralization rate values were 0.718 (r^2 = 0.99, p < 0.005) and 0.714 mg CO_2 g^{-1} d^{-1} (r^2 = 0.99, p<0.005) for the pesticide-spread test and the control test, respectively (Figure 3).

These results reflect a greater richness and diversity of the microbial populations in the IC in comparison to the MS. The high respiration rates found in the IC are directly related to the nutritional quality and high population density of the microorganisms that inhabit it. This is consistent with the results reported by [37-39], who claim that compost is a matrix that has much greater microbial populations than soil (even if it is a fertile soil), and even more so in the case of soils found in highly contaminated conditions.

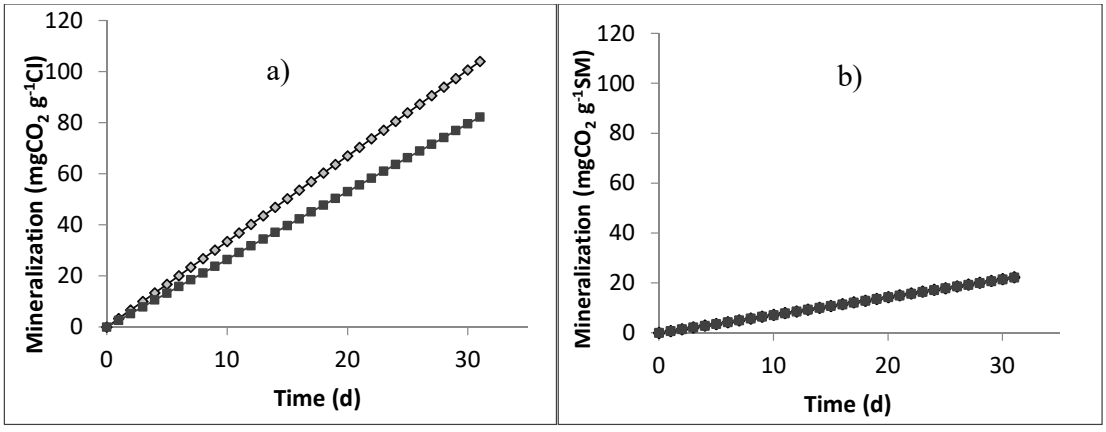

Figure 3 Microbial activity of a) the IC and b) the MS evaluated in both the contaminated test (♦) and the control test (■)

The differences in times and maximum mineralization values found for the MS and IC can be explained by the differences in nutrient availability in each matrix, as described by [40]. These authors also reported that the number of microorganisms in the soil is directly proportional to the amount of carbon that can be metabolized, since this is the most abundant element in cell biomass. In this study, the ability to degrade contaminants such as organophosphate pesticides were not directly related to the microbial activity of the matrices. This is demonstrated by the fact that the IC showed a lesser ability to degrade malathion, methyl parathion and chlorpyrifos than the MS matrix, despite being the most active matrix in microbiological terms. These results coincide with those reported by [29], who established that only a fraction of the soil microbiota is able to metabolize a specific compound, and that the vast majority of organisms use other carbon sources found in the matrix.

4. Conclusions

The MS microbial consortia showed higher degradation rates than IC microbial consortia when degrading malathion, methyl parathion and chlorpyrifos. This proves that the MS matrix has microbial consortia capable of degrading the above compounds. It can therefore be concluded that the MS microbial consortia is a good choice for the production of enzymes or active microbial communities which may be applied in bioremediation programs for environments contaminated with chlorpyrifos, malathion and methyl parathion.

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