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FIGHT AGAINST PERSISTENT ORGANOCHLORINATED POLLUTANTS: DISAPPEARANCE IN PRESENCE OF MICROORGANISMS

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Abstract

The bioremediation represents an ongoing challenge, especially in the case of organochlorinated compounds, due to the difficulties in their degradation that causes persistence in the environment. Herein we report a study on the ability of a mixture of microorganisms (MOM) to interact with organochlorinated compounds belonging to different chemical classes, i.e. DDT, PCB, tetrachlorobenzene, and lindane. Experiments in vitro showed the disappearance, partially reversible of these compounds in mixtures containing microorganisms, with a trend dependent on the kind of used pollutant. Unexpected 'complexation' by some components of molasses used as growth nutrient for microorganisms was found. Experiments carried out in the presence of soil showed that also in this case the participation of MOM to hide pollutants cannot be excluded. The obtained results may be an interesting starting point for further investigations on the bioremediation of organic pollutants using biological and not expensive method.

Key words: bioremediation, microorganisms, organochlorinated, pollutants

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1. Introduction

The degradation of persistent organic pollutants into not-toxic derivatives, both with chemical and/or biochemical methods, is one of the main challenges in the contemporaneous fight against environmental disasters produced by humanity. Among the more persistent pollutants there are organic chlorine-containing substances such as polychlorinated biphenyls (PCBs), polycyclic aromatic carbons (PAHs), and hydrocarbon derivatives (Liu et al., 2017; Liu et al., 2018; Savic et al., 2016). From a lot of scientific reports (Kruger et al., 2008; Ritter et al., 1995), as well as from media information, it is well known that organic chlorine-containing substances, which are largely used as pesticides or in industrial technical devices, are very persistent under natural conditions and they are considered severe dangerous pollutants. This is the case of the DDT (2,4-dichlorodiphenyltrichloroethane, listed in class 2A of IARC (IARC, 2013), as probably carcinogenic to humans) which is banned in several nations by exception of those infested with mosquitos to eliminate malaria epidemics. Polychlorobiphenyl (PCB) class involves a large number (209) of chlorine-containing biphenyl derivatives which mixtures were used in industrial processes and discharged in cities

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and countryside by several fortuitous accidents: in Italy, large amount of PCB [generally included in Group 1 of IARC, (IARC, 2015)] are present in the territory of Brescia (Italy). Lindane is also listed in class 1 of IARC. Thus, the bioremediation of soils polluted with many and different organic compounds is of great and growing interest, as documented by many literature reports (*e.g.* Cheng et al., 2016; Megharaj and Naidu, 2017, 2018; Muñiz et al., 2017).

Recently we studied the possibility to carry out experiments to make bioremediation of aldehydes, in particular formic aldehyde, by a mixture of microorganisms (Boga et al., 2014). Our searches indicate that aldehydes are wholly eliminated by simple treatment with microorganism mixture.

Now we are trying to obtain elimination of the chlorine-containing pollutants reported in Fig. 1. In a preliminary way, we checked the possibility to have bioremediation also on a polycyclic aromatic hydrocarbon, fluorene, which is also reported in Fig. 1.

2. Material and methods

2.1. Materials

Gas chromatographic analyses were carried out with a Hewlett-Packard (HP) 5890 gas chromatograph directly interfaced with an Agilent 5970 mass selective detector. Injection temperature was 250 °C (split injection mode split ratio 50/1) (HP-5MS column, 30m, 0.25mm, 0.25μm film thickness). The oven temperature was programmed as follows: 60 °C for 2 min, increased up to 260 °C at the rate of 20°C/min, followed by 260 °C for 20 min. In the case of experiments in soil with PCB 15 the above parameters were as follows: 60 °C for 2 min, increased up to 160 °C at the rate of 20°C/min, 160 °C for 5 min, increased up to 200 °C at the rate of 3°C/min, 200 °C for 5 min, increased up to 260 °C at the rate of 20 °C/min, 260 °C for 2 min. The carrier gas was helium, used at a flow rate of 1 mL/min; the transfer line temperature was 280 °C; the ionization was obtained by electron impact (EI), acquisition range was 50–500 m/z. ^{1}H NMR spectral data were recorded on a Varian Inova 600 spectrometer at 600 MHz in $CDCl_3$. Chemical shifts were measured in parts per million (ppm) and referenced to the solvent (7.26 ppm). EM-1® and sugar cane molasses were supplied by Punto EM S.r.l. (Sanremo, Italy). EM-1 contains five families, ten genera and more than 80 types of aerobic and anaerobic microbes including photosynthetic bacteria, lactic acid bacteria, yeast, actinomycetes, fungi (Ahn et al., 2014; Gaggia et al., 2013). San Benedetto mineral natural water from the spring in Scorzè (Venezia, Italy) was used for the activation of EM-1. The activation procedure was performed as previously reported (Boga, 2014) and the activated mixture will be hereafter indicating as MOM. All other reagents used were purchased by Sigma-Aldrich (Milano, Italy). Solvents were from VWR International PBI Srl (Milano, Italy). The soil sample was collected in the park of the Department of Industrial Chemistry, Viale del Risorgimento, 4, Alma Mater Studiorum - University of Bologna to a depth of about 30 cm. The soil was analyzed by the CSA Group S.p.A. laboratory (Rimini, Italy), and their main parameters are reported in Table 1.

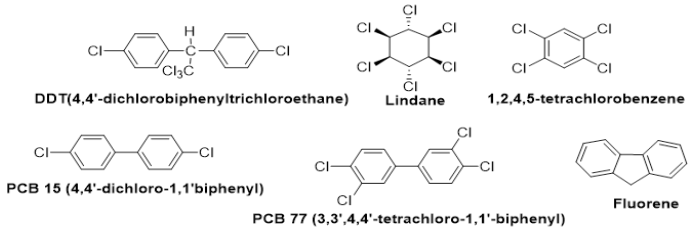


Fig. 1. Selected pollutants tested for bioremediation by microorganism's mixture

Table 1. Physical-chemical properties of the test soil

Parameter	***Measure Unit***	***Value***	***I.M.***
pH (in water)	pH units	7.18	± 1.08
Electric conducibility at 25 °C	μS/cm	772	± 116
Sand	%	59	± 9
Silt	%	31	± 5
Clay	%	10	± 2
Total limestone (calcium carbonate)	%	13.7	± 2.1
Cationic exchange capacity (CSC)	meq/100 g	26	± 4
Organic matter	%	3.97	± 0.60
Total nitrogen (as N)	%	0.23	± 0.03
C/N Ratio	-	10	± 2
Assimilable fosforus (as P)	mg/kg	7	± 1
Exchangeable Potassium	mg/kg	136	± 20
Sulfur	%	0.03	± 0.01
Assimilable iron	mg/kg	6.9	± 1.0
Magnesium	mg/kg	4069	± 610
Sodium	mg/kg	< 300	

2.2. 'In vitro' tests on organic pollutant/MOM mixture. General procedure.

The organic pollutant was dissolved in ethanol (in an ultrasound bath) and brought to volume with ethanol in a volumetric flask (DDT: 0.03170g in 20 mL; PCB 77: 0.00517g in 10 mL; PCB 15: 0.0027g in 10 mL; tetrachlorobenzene: 0.00772g in 10 mL, Lindane: 0.012g in 10 mL; Fluorene: 0.00732g in 10 mL). In some vials 0.5 mL of the above mother solution (DDT, PCB 77, lindane, or fluorene) and 5 mL of MOM were added; other vials with 0.5 mL of the mother solution and 5 mL of water were prepared. After opportune time (Figs. 2 and 4) the mixture was extracted with dichloromethane (4 x 5 mL) and the solvent was removed. The residue was dissolved in 1.0 mL of CH_2Cl_2 and added of 0.1 mL of a solution of hexamethylbenzene (0.008M in dichloromethane); 0.7µL of the solution were injected in the GC-MS spectrometer. In case of PCB 15 and 1,2,4,5-tetrachlorobenzene several vials with 0.5 mL of the mother solution and 9 mL of MOM and other vials with 0.5 mL of the mother solution and 9 mL of water were prepared. After opportune time (Fig. 3) the sample was extracted with dichloromethane (4 x 5 mL), the solvent was removed and the residue dissolved in 1.0 mL of $CHCl_3$ and added of 0.2 mL of a solution of phenanthrene (0.0603 g in 50 mL of $CHCl_3$); 0.7µL were injected in the GC-MS spectrometer.

2.3. 'In vitro' tests on soil samples. General procedure.

Mother solution of analyte in acetone (1.4 mL) was added to 50g of soil sample made homogeneous by sifting and poured in a glass vessel. Acetone was removed by evaporation at 60°C for 5 min. then 25 mL of the activated MOM mixture was added. An equal number of samples was prepared as above except for the substitution of MOM with an equal amount of water (control sample). After the time indicated in the corresponding Figures (Figs. 6 and 7) $CHCl_3$ (50 mL) was added to the mixture, the system was stirred and subjected to vacuum filtration over Celite (Sigma-Aldrich, Milano, Italia). The cake was transferred in vessel, extracted with $CHCl_3$ (20 mL) and filtered as above. The procedure was repeated a third time. The filtrate was introduced in a separatory funnel and the organic layer was dried over anhydrous $MgSO_4$. After filtration and solvent removal under vacuum, the residue was dissolved in 1.0 mL of $CHCl_3$ and after addition of 0.2 mL of standard solution (phenanthrene) the sample was analyzed by GC-MS. In most cases the solution was concentrated and dissolved in $CDCl_3$ to be analyzed by ^1H NMR.

Three series of tests have been carried out on soil samples contaminated with PCB 15 or lindane. The first series of experiments was carried out in summer 2015 with 30°C average room temperature. Three mother solutions were prepared dissolving 0.1257g of lindane in 100 mL of acetone, 0.0508g of PCB 15 in 50 mL of acetone, and 0.1275 g phenanthrene (standard) in 100 mL of $CHCl_3$. After the time indicated in Figs. 6 and 7 the mixture was treated and analyzed as above indicated in general procedure. The second series was carried out in November-December 2015 with 15°C average room temperature and the following mother solutions were prepared: 0.0324g of DDT in 50 mL of acetone, 0.1068g of PCB 15 in 100 mL of acetone, and 0.0613 g of phenanthrene (standard) in 50 mL of $CHCl_3$. All the experiments (including the corresponding control mixture) were monitored through the above general procedure.

The third series of experiments was run between January and March 2016 and the following mother solutions were prepared: 0.0408 g of DDT in 50 mL acetone, 0.0531 g of PCB 15 in 50 mL of acetone, 0.0498 g of lindane in 50 mL acetone, 0.0664 g phenanthrene (standard) in 50 mL of acetone. In these cases, the analyte solution was added to the soil a week prior the addition of MOM. All the vessels were kept in a thermostatic bath (28±2°C) and monitored using the above procedure. Moreover, two samples of every analyte (immediately after the addition of MOM and at the end of the experiment) were subjected to Soxhlet extraction with $CHCl_3$.

3. Results and discussion

This study was carried out in two steps: a preliminary *in vitro* approach focused on the monitoring of the variations with time of the amount of the considered pollutant in the presence of the microorganism mixture (MOM) and a second phase in which the method was applied to a soil sample.

3.1. 'In vitro' experiments on the effect of MOM on analytes

Fig. 2 reports data obtained from a preliminar experiment *in vitro* about the variation of the amount of DDT (reported as DDT/standard ratio) in the presence of the microorganism mixture (MOM). The first part of the plot shows a regular decrease of DDT amount until about 10 days: the DDT is apparently eliminated for more than 50% of the initial amount, but, unexpectedly, after longer times the DDT amount recovered was more than that detected at zero reaction time. This behaviour was observed also by using PCB 77, PCB 15, 1,2,4,5-tetrachlorobenzene, lindane, and fluorene. The relative rapid decrease of the analyte appears to be not its definitive and irreversible transformation in other compounds, but it resembles a reversible (and apparent) concealment by MOM-containing mixture. The fast decrease of chlorine-containing organic compounds was observed by other authors (Derbalah et al., 2013) and it was considered an indication of the ability of some microorganisms to resolve the pollution arising from the presence of chlorine containing compounds.

The behaviour showed in Figs. 2–4 cannot be immediately explained but it is a clear indication of the ability of the mixture containing MOM to hide the chlorine-containing compound monitored and quantified by extraction methods using chloroform or dichloromethane as generally indicated by official protocols. The trend reported in plots of Figs. 1–4 is not the same for all analytes considered: this fact cannot be surprising, owing the different chemical classes of analytes considered.

This trend resulted to be reproducible being it observed in other experiments carried out in our research group (Strazzera, 2015); as a matter of fact, in all cases, about 1 week, the inversion of the decreasing trend of the analyte/standard ratio was observed.

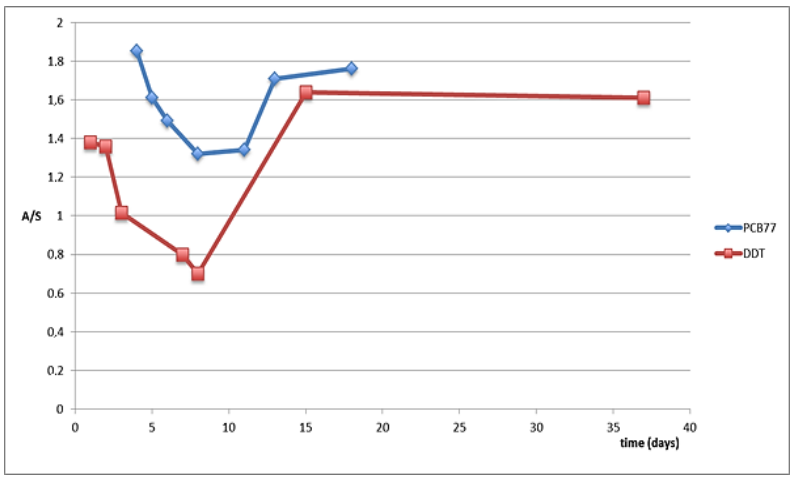


Fig. 2. Variation of amount of the analyte/standard ratio caused by addition of MOM

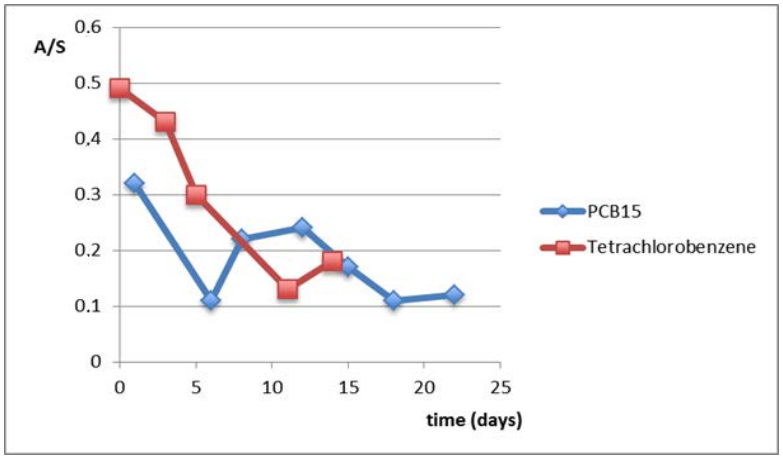


Fig. 3. Variation of amount of the analyte/standard ratio caused by addition of MOM

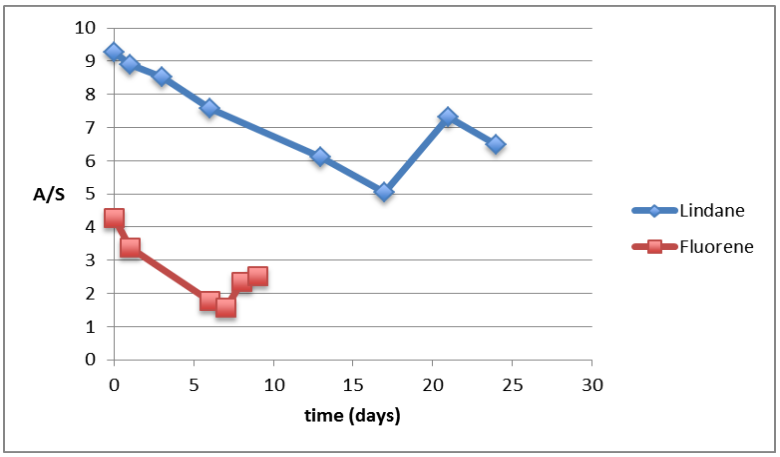


Fig. 4. Variation of amount of analyte/standard ratio caused by addition of MOM. For fluorene, the monitoring was stopped after 10 days

This might be ascribed to the presence, even contemporary, of several phenomena as follows:

1. A fast and partially reversible seizure of the analyte, almost immediate in the case of DDT, by "inert" substances added together with microorganisms, or by microorganisms itself.

2. The second phenomenon concerns the actual transformation of the analyte in unknown metabolites.

3. In the case of a fast interaction between analyte and microorganisms, a third process may be the decrease of the amount of active microorganisms (under the used experimental conditions) due to some nutritional shortages, with the consequence of the release from the microorganisms of the amount of the analyte not yet transformed.

With the purpose to investigate the anomalous behaviour of plots above reported, we performed parallel experiments adding MOM or only water (control) to a solution of analyte and working up in the same manner the mixture immediately after the addition. The values of the analyte/standard ratio obtained in both cases by GC-MS analysis are reported in Table 2.

Table 2 clearly shows that some chlorinated compounds (*i.e.* DDT and lindane) present an instantaneous decrease: for instance, the 75% amount of DDT is quickly (and apparently) depressed with respect to the control test carried out in the presence of only water. It has to be point out that data of Table 2 are obtained after usual extraction of the reaction mixtures (see experimental). On the contrary, after extraction with more drastic methods, *i.e.* by extracting under ultrasound irradiation or by heating the mixture in the presence of sulfuric acid, the analyte/standard ratio of the residue coming from treatment with MOM becomes very near to that obtained in the presence of water only. From these data, it is possible to evince that some chlorinated compounds are strongly complexed after the addition of MOM. This suggested us to check the effect of the addition of sugar cane molasses, the main component of the microorganism's culture broth added as nutrient for microorganisms, to a solution of DDT, without presence of microorganisms. Plot of Fig. 5 reports the results obtained after addition to the DDT solution of increasing amount of molasses and immediate work-up and analysis. Data of Fig. 5 show a noticeably decrease of the amount of DDT recovered by increasing the amount of molasses until to reach an almost constant DDT/Standard value independent from the amount of molasses thus suggesting the occurrence of a saturation phenomenon.

After this result we tried to investigate which component of molasses might be responsible for the behaviour observed. The composition of molasses depends mainly from the plant used as source, from the climate of the growing area and from the production process (http://www.feedipedia.org/node/561).

In the literature (Browne, 1919; Hashizume et al., 1966;) it has been reported that molasses is a complex mixture containing sucrose as main constituent, followed by D-glucose and D-fructose derived from the hydrolysis of sucrose during the production process, ash, aminoacids, inorganic salts, gums and pectins. The addition of a solution of DDT to an aqueous solution of sucrose did not produce effect on the amount of DDT recovered, as well as the addition of a mixture of D-glucose and D-fructose. On the contrary, pectin, separately added to a solution of DDT, produced remarkable decrease (27%) of the amount of DDT recovered with respect to the control experiment (DDT solution dispersed in water and worked-up as described in experimental section).

Table 2. Analyte/standard ratio in the presence of microorganism's mixture (MOM) or in the presence of water only (H_2O), immediately after mixing

→ Analyte ↓ Medium	***DDT***	***Lindane***	***PCB 15***	***Tetrachlorobenzene***	***Fluorene***
H_2O	2.1	3.3	0.4	0.5	2.9
MOM	0.5	2.7	0.15	0.5	3.0

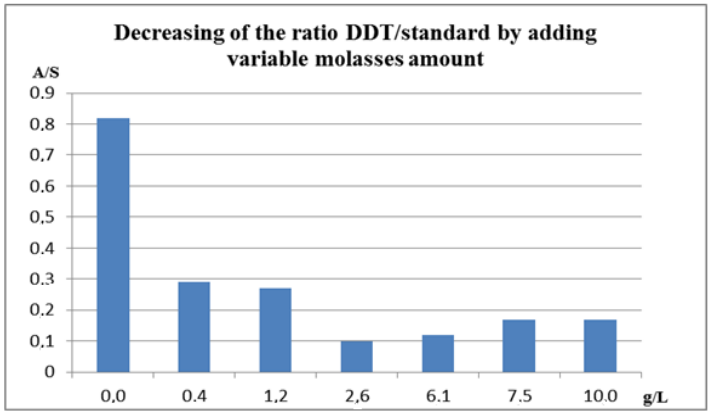


Fig. 5. Decrease of the DDT/Standard ratio after addition of variable amount of molasses

3.2. 'In vitro' experiments on soil contaminated with organochlorinated compounds

In order to investigate the possibility to use MOM in experimental conditions near to those presumably existing in natural conditions, we performed a series of test, carried out by adding a mixture of analyte (A) and MOM (or analyte and water) to a soil sample and monitoring with time the amount of analyte recovered that was quantified both through ^{1}H NMR and GCMS analyses.

The experiments were carried out by using PCB 15 and lindane as soil contaminants, and the results are summarized in plots of Figs 6 and 7, respectively. A not contaminated soil specimen (see experimental) was collected in the park of the Department of Industrial Chemistry, Viale del Risorgimento, 4, Alma Mater Studiorum–University of Bologna in a not anthropic hilly area. Plots A and B of Fig. 6 are obtained using soil as is stand. Plots C and D are obtained by soil "sterilized" as reported in the experimental part. Plots B and D of Fig.7 are obtained in soil as is stand. Plots A and C are obtained by soil "sterilized" as reported in the experimental part.

Apparently, the soil by us used (see experimental) presents very similar effect on the disappearance of the analyte considered in the presence and in the absence of MOM: in both cases there is a significant decrease of the amount of analyte. It is interesting to note that in the case of PCB 15, the A/S ratio found extracting the mixture immediately after the addition of the soil to MOM (plot A and C at t=0) was minor for about 40% of that found for the case of addition of soil to water (plots B and D, Fig. 6).

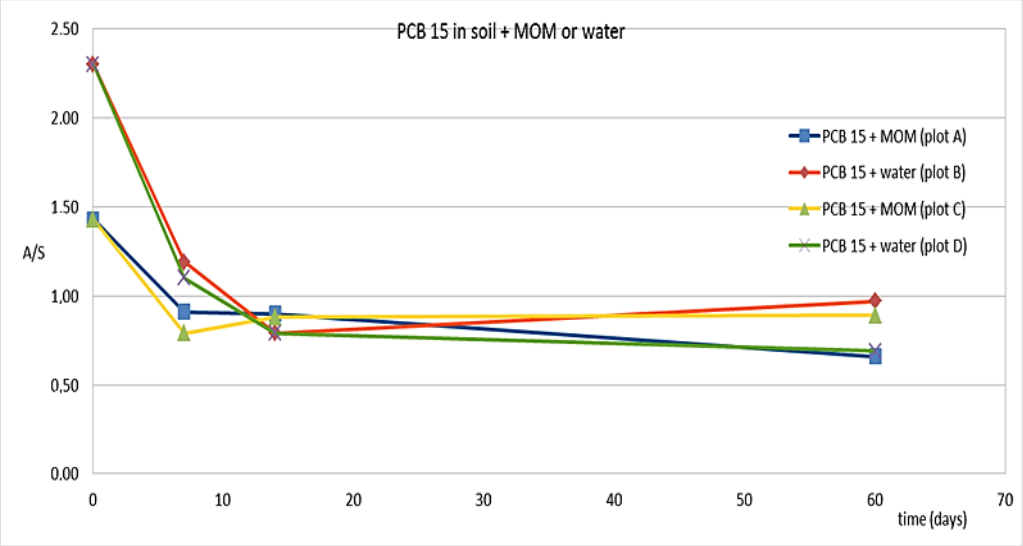


Fig. 6. A is PCB15. plot A: PCB15 + MOM; plot B: PCB15 + water, plot C: PCB15 + MOM in soil pre-treated in autoclave, plot D: PCB15 + water in soil pre-treated in autoclave

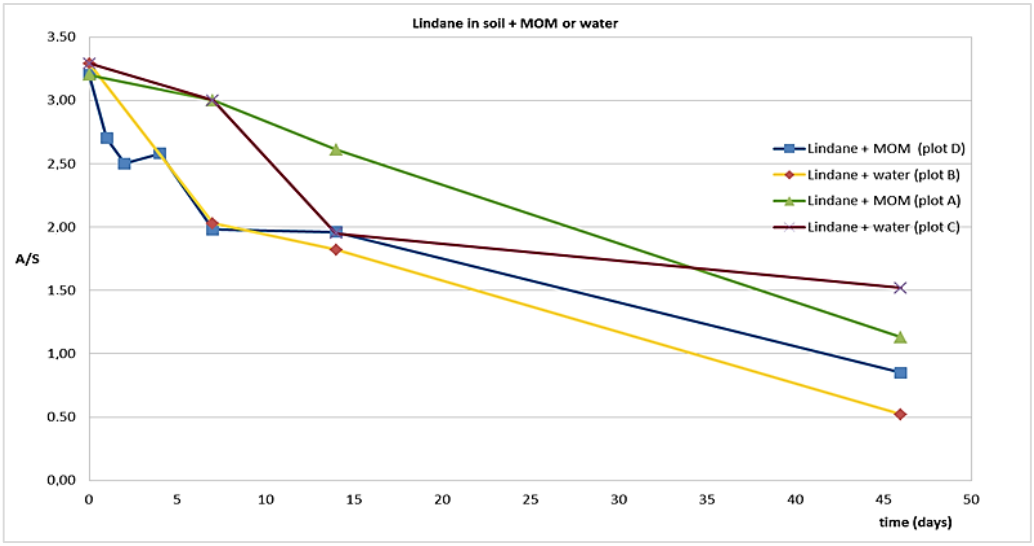


Fig. 7. A is lindane and S is phenanthrene used as standard. plot A: lindane + MOM in soil pre-treated in autoclave; plot B: lindane + water, plot C: lindane + water in soil pre-treated in autoclave, plot D: lindane + MOM

This difference is almost the same of that observed in the case of 'in vitro' experiments reported in Table 2; this finding provides a further confirmation of the occurrence of an immediate complexation phenomenon due to the presence of molasses in the culture broth. Data shown in Fig. 7 are also in line with those reported in Table 2: in this case no significant decrease of the amount of the analyte was observed after addition of MOM both on a solution of lindane (Table 2) and to a soil sample contaminated with lindane (Fig. 7, all plots at t=0) thus supporting the above deduction that lindane is not subjected to complexation phenomenon by molasses.

The general trend observed in Fig. 6 is the decrease with time of the A/S ratio within the first 15 days; the very close values found in the case of sterilized and not-sterilized soil suggest a not significant role by indigenous microorganisms of the soil while almost the same value reached after the following weeks for all plots might indicates the occurrence of a sequestering action by the soil: this might hinder the bioavailability of the analyte to microorganisms. Similar deductions can be applied to the trends observed in Fig. 7 for the case of lindane.

In order to gain support to the above hypothesis of a complexation phenomenon involving the soil components we planned to carry out further experiments using the Soxhlet extraction method. Table 3 reports the results obtained using this drastic extraction method. Both lindane and PCB 15 shows a strong decrease after long time, also in the presence of water only (without MOM): probably, the soil is able to hide, in some way, these chlorine-containing pollutants.

These findings can be considered an indication that the disappearance of the analyte may be hardly ascribed to a simple complexation (obviously, in a reversible process) and suggest the occurrence of another not-reversible process which causes the decrease the analyte (after long reaction time) probably by forming other different compounds which might be metabolites of the starting materials. We tried to have more information of the presence of metabolites (see section 3.3).

A particular behaviour was observed when analyte was DDT in presence of soil, as reported by plot of Fig. 8: the test carried out in the presence of MOM shows an apparent complete disappearance of DDT, while experiments in water show an initially noticeable decrease, but after the amount of DDT remains constant with time. Even if this experiment cannot be conclusive, we consider this behaviour as an indication of the formation of a complex between soil and DDT, while the possibility of a more important complexation or metabolization appears a reasonable explanation of the complete disappearance of DDT in the presence of MOM as indicated by plot B. However, we don't have the possibility to completely discriminate between the complexation or metabolization of analyte.

Table 3. Analyte/standard ratios in soil additioned with MOM or water. Data obtained after extraction with Soxhlet apparatus

Analyte	***Time = 0***	***Time =40 days***
Lindane/water	3.0	0.4
Lindane/MOM	2.1	0.6
PCB15/water	0.4	0.1
PCB15/MOM	0.4	0.2

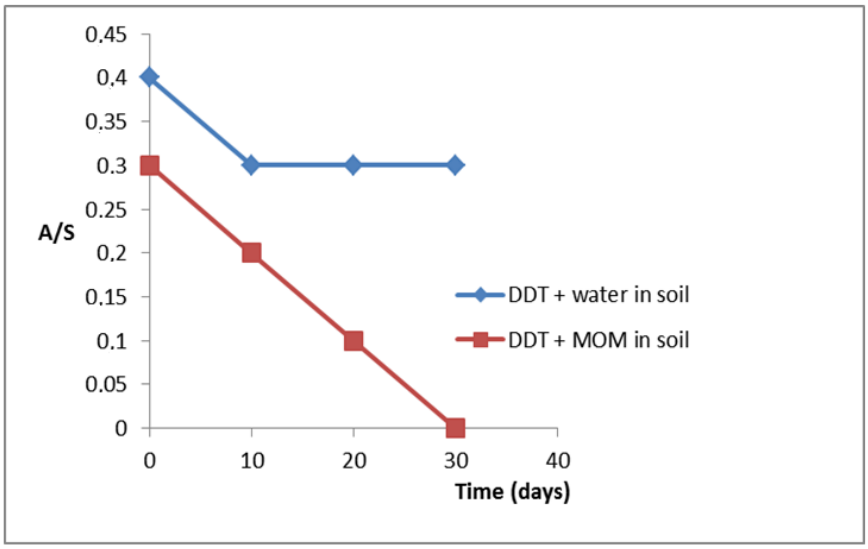
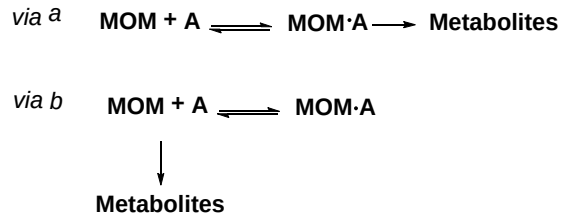


Fig. 8. A is DDT and S is phenanthrene used as standard. Plot A: DDT + water in soil; plot B: DDT + MOM in soil

The following schemes of interaction between chlorine-containing pollutants and MOM may be an explanation about the disappearance of these pollutants and they might be a starting point of further and more detailed investigations since the discrimination between the two schemes is a difficult problem, also from a chemical point of view Eq. (1).



(1)

were: A is the used analyte and MOM is the whole mixture containing microorganisms and culture medium. MOM.A indicates the interaction between MOM and the analyte producing the apparent decrease of the amount of analyte: this interaction may be an essential step in obtaining metabolites, *i.e.* the destruction/transformation of the analyte (Scheme 1, *via* a) or it may be a simple "cul de sac" which is non-producing metabolites (Scheme 1, *via* b).

A further parameter that can play an important role in determining the decrease of the rate of the amount of analyte is the temperature. Table 4 collects some data about this effect applied to mixtures containing DDT or PCB 15: at 30°C the mixture analyte/soil shows a regular decrease with time, while at 15 °C the amount of the analyte presents a trend to increase this amount: probably, at low temperature after an initial formation of a non-covalent interaction between analyte and soil or microorganism mixture (perhaps by the molasses components), complexes are destroyed and the analyte is prone to be revealed by usual analytic procedures.

However, the decrease with time of the analyte amount in soil observed when the mixture was kept at 30 °C cannot be ascribed to the only complexes formation through non-covalent bonds because usually this phenomenon is depressed by temperature increasing due to a dissociation of the complex. The fact that at higher temperature the amount of analyte recovered decreases, even if in slight amount, might indicate a certain activity of the MOM that is favoured at 30 °C.

3.3. Presence of 4-methyl-phenol in experiments carried out with chlorine-containing pollutants in soil by addition of microorganism mixture

During the first series of experiments carried out with PCB 15 in soil, after 60 days from the addition of EM and extraction of mixture with chloroform, we observed the presence, in the ^{1}H NMR spectrum, of two intense doublets at 6.73 e 7.03 ppm, coupled one with the other with J = 8.3 Hz and of one singlet at 2.27 ppm. These signals suggested the presence of an aromatic ring with *para*-substituents and of a methyl group, and we hypothesized the unexpected compound was 4-methyl-phenol. This was confirmed by addition in the NMR tube of an amount of authentic sample of 4-methyl-phenol, which enriched the above signals. These signals were not present in the ^{1}H NMR spectrum of PCB 15 in soil immediately after the addition of EM (zero reaction time) and were absent also in experiments carried out *in vitro* without soil. The presence of *p*-cresol was also confirmed by analysis by GC-MS apparatus. The absence of contamination of soil was ascertained by extraction of a soil sample both with chloroform and n-hexane; ^{1}H NMR analysis of the residue did not showed signals belonging to *p*-cresol.

From the KEGG PATHWAY Database (http://www.genome.jp/kegg/pathway.html) data bank emerged that the first steps of biodegradation pathways of some aromatic chlorine-containing compounds involve the functionalization of the aromatic ring with formation of phenols. 4-Methyl-phenol was also indicated (Natarajan et al., 1999) to arise, as transient intermediate, from the biodegradation of diphenyl derivatives produced in a reductive dichlorination process of PCB derivatives. This suggested, as first hypothesis, that in our case *p*-cresol was produced from the biodegradation of PCB 15 but it was confuted by the finding of 4-methyl-phenol also in mixtures containing the other considered organochlorine analytes in experiments carried out in soil, both after addition of MOM and in some cases also in the presence of only water. In Table 5 are collected the 4-methyl-phenol/standard ratios calculated from ^{1}H NMR spectra of the residues obtained after work-up of the systems mixture MOM/soil/analyte and H_2O/soil/analyte monitored with time. The fact to have found *p*-cresol not only in mixtures containing PCB15 but also DDT and lindane agrees with the hypothesis that *p*-cresol can originate from microorganisms or from their action on some soil constituents (Dawson et al., 2011; Mathus et al., 1995). From data of Table 4 emerges that *p*-cresol is detected after 30 days (20 days in case of DDT-containing mixtures) and in major extent when MOM is present in the mixture. In the literature, *p*-cresol was indicated to be easily destroyed in aerobic situation (Boyd and King, 1984) the decrease (at 40 days reported in Table 5) may be ascribed to a similar phenomenon.

Based on the above, it is reasonable to admit that *p*-cresol is arising from some unknown reactions involving soil and microorganisms, or from the simple endogenous soil microorganisms. Even if this preliminary conclusion deserves more accurate investigation, in our opinion, in here reported experiments, it appears reasonable to state that is very poorly probable to ascribe the presence of p-cresol as metabolite of anyone chloro-containing pollutants, including PCB.

Table 4. Effect of variation of temperature on the apparent decrease of DDT and of PCB 15 amount (measured by the ratio analyte/standard) in the presence of soil and MOM

Days	***15°C DDT + MOM***	***30°C DDT + MOM***	***15°C PCB + MOM***	***30°C PCB + MOM***
0	0.4	0.4	1.8	1.4
7	0.4	0.2	1.9	0.9
14	0.4	0.1	2.9	0.9
60	0.8	0.0	3.2	0.7

Table 5. Presence of 4-methyl-phenol, at different times, of soil samples contaminated with organochlorine-containing pollutants

Time (days)	***Para-cresol / standard ratio from PCB15/MOM mixture***	***Para-cresol / standard ratio from PCB15/H_2O***	***Para-cresol / standard ratio from Lindane/MOM mixture***	***Para-cresol / standard ratio from Lindane/H_2O***	***Para-cresol / standard ratio from DDT/MOM mixture***	***Para-cresol / standard ratiofrom DDT/H_2O***
10	-	-	-	-	-	-
20	-	-	-	-	0.2	< 0.1
30	0.5	-	0.9	0.2	0.8	0.4
40	0.1	-	0.3	N.D.	0.3	0.6

4. Conclusions

The problem herein discussed shows severe complications arising from his natural complexity, however the data obtained and the consequent observations are exciting in biologically solving a pollution situation very diffused in large countries in the world.

The main conclusions from the current study can be summarized as follows:

- Experiments *in vitro* showed the disappearance of organochlorine-containing pollutants in mixtures containing microorganisms. The decrease of amount of pollutants is (in some cases) partially reversible and cannot have immediate explanation.
- In agreement with the fact that herein considered chlorine-containing pollutants belong to different chemical classes, the trend of the observed decreasing amount is dependent on the kind of used pollutants.
- Our experiences (even if they are part of a preliminary study) support the statement that the used microorganism mixture is able to depress the amount of chlorinated compounds.
- Some analytes (in particular DDT) are quickly hidden by mixtures containing microorganisms by some component of molasses, probably pectine.
- Experiences carried out in the presence of soil and in the presence/absence of microorganism mixture, suggest the same conclusion of the above point. The participation of microorganisms to hide pollutants cannot be excluded.
- Owing the fact that, in the natural environment, the chlorine-containing pollutants are very persistent), why the soil is able to hide (complexed or destroyed) some chlorine-containing compounds (such as DDT) is yet an unresolved problem. However, the formation of reversible non-covalent complexes can explain our findings as well as the results of other literature reports.
- Overall, the obtained results may be an interesting starting point for further investigations on the bioremediation of organic pollutants using biological and not expensive methods.

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