

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

**Organo-clays and nanosponges for acquifer bioremediation: Adsorption and degradation of triclopyr**

**This is the author's manuscript**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/136093> since

*Published version:*

DOI:10.1080/03601234.2013.780943

*Terms of use:*

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)



# UNIVERSITÀ DEGLI STUDI DI TORINO

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12

***This is an author version of the contribution published on:***

*Questa è la versione dell'autore dell'opera:  
Journal of Environmental Science and Health, part B, 48,  
<http://dx.doi.org/10.1080/03601234.2013.7809-43>*

***The definitive version is available at:***

*La versione definitiva è disponibile alla URL:<http://www.tandfonline.com/loi/lesb20>*

1  
2 **Organo-clays and nanosponges for acquifer bioremediation: adsorption and**  
3 **degradation of triclopyr.**

4  
5 ANDREA BAGLIERI<sup>1\*</sup>, MICHÈLE NÈGRE<sup>2</sup>, FRANCESCO TROTTA<sup>3</sup>, PIERANGIOLA  
6 BRACCO<sup>3</sup>, MARA GENNARI<sup>1</sup>

7  
8 <sup>1</sup>*University of Catania, Dipartimento di Scienze delle Produzioni Agrarie e Alimentari, Sezione di*  
9 *Chimica agraria, via S. Sofia, 98, 95123 Catania.*

10 <sup>2</sup>*Univeristy of Turin, Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, via*  
11 *Leonardo da Vinci, 44, 10095 Grugliasco, Torino.*

12 <sup>3</sup>*University of Turin, Dipartimento di Chimica, via P. Giuria, 7, 10125 Torino.*

13  
14 \* Corresponding author. Tel.: +39 095 7580241; fax: +39 095 7141581. E-mail address:  
15 abaglie@unict.it

16  
17 **ABSTRACT**

18  
19 To avoid the problem of groundwater contamination, mitigation techniques have been proposed  
20 that consist in creating barriers made of suitable materials that can facilitate the adsorption and  
21 degradation of the pollutants.

22 The work aims at evaluating the capacity of two organo-clays (Dellite 67 G and Dellite 43 B)  
23 and one nanosponge to adsorb the herbicide, triclopyr. Triclopyr was chosen because it is a good  
24 example of a moderately mobile, leacheable molecule. The rate of degradation of the molecule in  
25 the soil, both with and without the presence of the materials under examination, was also  
26 determined.

1 Both the organo-clays adsorbed more than 90% of the herbicide. The nanosponge and the soil  
2 adsorbed less than 10% triclopyr. When the soil was added with the two organoclays, adsorption  
3 increased to 92%.

4 When added to the soil, the materials accelerated the degradation of triclopyr. The half life in  
5 soil was 30 days, whereas in soil with Dellite 67G and Dellite 43B it was 10 and 6 days  
6 respectively. The addition of the nanosponge to the soil decreased the half life by 50 %.

7 These results lead us to suggest that they be used in creating reactive barriers for the  
8 remediation of soils and aquifers.

9

10 **Keywords:** Triclopyr, organoclays, nanosponges, reactive barriers

11

## 12 INTRODUCTION

13

14 Considerable importance is now being given to resolving the environmental problems  
15 associated with the presence of pesticides in the soil and groundwater.

16 Many compounds are not retained by soil colloids and there is a tendency for infiltrating water  
17 to carry them into groundwater. Moreover, the low rate of degradation of the pesticides increases  
18 the possibility of water becoming contaminated. <sup>[1]</sup>

19 In order to avoid this problem, mitigation techniques have been proposed that consist in creating  
20 barriers made of suitable materials that can favour the adsorption and degradation of pollutants.  
21 Reactive barriers are a semi-permeable system placed along the flow path of infiltrating water. As  
22 the water passes through the barrier, the pollutant is adsorbed and degraded releasing only a small  
23 quantity of non-toxic compounds.

24 Barriers made from a mix of soil and phyllosilicates, such as bentonite, are widely used to limit  
25 the migration of mobile pollutants. All kinds of soil can be used and the quantity of bentonite can  
26 vary from 6% to 15%. <sup>[2]</sup>

1       Recent studies have suggested that organo-clays have a high water-decontamination potential  
2 because they can absorb considerable quantities of organic compounds, both apolar <sup>[3-4]</sup> and polar.  
3 <sup>[5-7]</sup> An evaluation on the effects of organoclays on the soil eubacterial community, showed that  
4 these materials can both inhibit and stimulate soil microorganisms. <sup>[8]</sup>

5       Rodriguez-Cruz et al. <sup>[3]</sup> demonstrated the efficiency of intercalating barriers made from a mix  
6 of organo-clay and soil in reducing the mobility of linuron, atrazine and metalaxyl by column (3  
7 cm i.d. and 20 cm long) testing with 500 mL of water.

8       Witthuhn et al. <sup>[9-10]</sup> showed that the association of organo-clays with some soil micro-organisms  
9 made it possible to absorb and degrade 2,4-dichlorophenol and chlorobenzene.

10       Cyclodextrins are a class of cyclic oligosaccharides synthesized from starch by enzymatic  
11 reaction. They have a characteristic toroidal shape which generates a frusto-conical internal cavity.  
12 This cavity measures about 8 Å in depth and 5-9 Å in diameter, according to the number of  
13 oligosaccharide rings. Cyclodextrins can include organic compounds inside these cavities. Inclusion  
14 is based mainly on an interaction between the guest molecule and the cavity which is particularly  
15 hydrophobic. This type of reaction between cyclodextrins and organic molecules can be used as the  
16 basis for the adsorption or separation of a number of organic agents. Nevertheless, cyclodextrins  
17 are soluble in water and in some organic solvents. Given their solubility in water, cyclodextrins  
18 cannot therefore be used directly for retaining organic substances. To overcome these problems of  
19 solubility, cyclodextrins have been used as basic building blocks in the preparation of cross-linked  
20 insoluble polymers. <sup>[11]</sup>

21       These cyclodextrin-based, highly cross-linked polymers are called nanosponges and have a 7-9  
22 Å cavity and a lower surface area (1-2 m<sup>2</sup> / g) than activated carbon (600 m<sup>2</sup> /g), but their organic  
23 molecule loading capacity is similar to that of activated carbon. This implies that the organic  
24 molecules are not only adsorbed on the polymer surface but also included within the nanocavity. <sup>[11]</sup>

25       Triclopyr is a frequently-used herbicide for post-emergence control of a wide range of annual  
26 and perennial broadleaf weeds and woody plant species. It is an auxinic type herbicide that is

1 adsorbed by roots and leaves, is translocated through the plant and accumulates in the meristematic  
2 tissues. The auxin-type response in plants is associated with triclopyr interfering with the normal  
3 growth processes. <sup>[12]</sup>

4 Symptoms of short-term exposure to triclopyr include lethargy and tremors. Anorexia and  
5 diarrhoea have also been observed in animals exposed to triclopyr. <sup>[13]</sup> In a long-term laboratory  
6 feeding study, rats fed triclopyr developed kidney damage more often than unexposed rats. In a  
7 long-term study using dogs, the animals which were fed triclopyr gained less weight, had less  
8 hemoglobin and red blood cells and more microscopic liver damage than did unexposed dogs.  
9 These symptoms were observed at doses of 25 mg/kg per day in the rat study and 20 mg/kg per day  
10 in the dog study. <sup>[14]</sup>

11 Photolysis is the principal degradation pathway for triclopyr in aqueous solution. Woodburn et  
12 al. <sup>[15]</sup> studied the photolysis of triclopyr in both buffered and natural waters under natural and  
13 artificial sunlight. Triclopyr in buffered water (pH 7) had an average photolysis half-life of 0.5  
14 days, while dissolving the compound in natural water resulted in a half-life of 1.3 days. No  
15 degradation or loss of triclopyr was observed in the dark control samples.

16 Cleveland and Holbrook <sup>[16]</sup> studied the hydrolysis of triclopyr in buffered solutions with pH 5,  
17 7 and 9. After 1 month, triclopyr constituted 97.2% of the activity remaining in the samples,  
18 suggesting that simple hydrolysis is not an important degradation pathway for triclopyr in the  
19 environment.

20 Most of the laboratory and field studies carried out indicate that, under most conditions,  
21 triclopyr is a relatively mobile herbicide. Values of between 12 and 78 were found for the  
22 repartition coefficient  $K_{oc}$  of triclopyr in a study performed on 12 soils with different organic  
23 carbon content (between 0.081% and 21.7%), indicating that the herbicide is highly mobile under  
24 these conditions. <sup>[17]</sup> Another laboratory study on a sandy-loam soil with a low organic matter  
25 content (0.62%) observed that, after leaching through a 12 inch column, 75-80% of the triclopyr  
26 was recovered 11-15 days after treatment. <sup>[17]</sup>

1 During a field study, a concentration of 3 litres/hectare of Garlon 3A<sup>®</sup> was applied to six plots  
2 with different soil textures. Small quantities of triclopyr and its metabolites were found at depths of  
3 15-30 cm and 30-45 cm, 28 and 56 days after application, respectively. <sup>[18]</sup>

4 A study performed in Louisiana on the adsorption of 2,4 D, glyphosate and triclopyr to three  
5 soils, revealed a low retention of triclopyr (<13%). The adsorption of triclopyr was positively  
6 related to the percentage of soil organic matter. The low adsorption of triclopyr to the three soils  
7 studied, suggests that this compound has a high surface water contamination potential. <sup>[19]</sup>

8 An analysis of water samples collected from different agricultural and urban sites in the Lower  
9 Fraser Valley region of British Columbia, demonstrated that triclopyr was present at varying levels.  
10 <sup>[20]</sup>

11 In a study on the occurrence of various pesticides in vernal pools and adjacent streams in  
12 different American states, Battaglin et al. <sup>[21]</sup> detected triclopyr at two sites. They suggested that the  
13 compound originated from use within the park and was transported to the site by surface runoff or  
14 shallow groundwater flow.

15 The aim of this work is to evaluate the capacity of different organo-clays and nanosponges to  
16 adsorb triclopyr and also to determine the degradation rate of triclopyr in the soil, with and without  
17 these materials.

18 Triclopyr was selected as the model for a mobile, leachable herbicide, which is minimally  
19 adsorbed by the soil.

20

## 21 **MATERIALS AND METHODS**

22

### 23 **Chemicals**

24

1 The triclopyr (98% purity) was purchased from Sigma-Aldrich (Milan, Italy). All the other  
2 chemicals used were analytical reagents or LC grade. The  $\beta$ -cyclodextrin (Kleptose) was a gift  
3 from Roquette Freres, Lille, France.

4 The nanosponge was synthesized using a  $\beta$ -cyclodextrin which was left to react with  
5 carbonyldiimidazole in dimethylformamide at 90°C for 5 hours. When the reaction was complete,  
6 the solid was crushed in a mortar and then treated with ethanol in a Soxhlet extractor to remove the  
7 by-products of the reaction. The resulting nanosponge was composed of  $\beta$ -cyclodextrin blocks  
8 crosslinked with carbonate bonds. Nanosponges are insoluble in water and organic solvents and  
9 have high thermal and chemical stability. Haemolytic and cytotoxic tests demonstrate the low  
10 toxicity of the material. <sup>[22]</sup>

11 The Dellite 67 G and Dellite 43 B organo-clays were supplied by Laviosa Mineraria (Livorno,  
12 Italy). Dellite 67G is a material deriving from a natural montmorillonite especially purified and  
13 modified with a high level of quaternary ammonium salt (di-di (hydrogenated tallow) dimethyl  
14 ammonium). Dellite 43B is an organo-clay deriving from a natural montmorillonite especially  
15 purified and modified with a quaternary ammonium salt (tallow dimethyl benzyl ammonium).

16

## 17 **Soil**

18

19 The soil was sampled to a depth of 0.25 cm, dried to a 10% water content (w/w), sieved to  
20 obtain a < 2 mm fraction and stored at room temperature in black PVC bags. The soil contained  
21 6.2% clay and had an organic carbon content of 0.88%, its pH in water was 7.8 and its Water  
22 Holding Capacity (WHC) was 49% (on dry weight).

23

## 24 **Adsorption and desorption studies**

25



1 Adsorption isotherms were obtained using the batch equilibration method. Duplicate 50 mg  
2 samples of the organoclays and the nanosponge were weighed into 15 mL glass centrifuge tubes  
3 (with Teflon-lined caps), which were filled with 5 mL 0.01 N CaCl<sub>2</sub> solution of triclopyr with  
4 initial concentrations ranging from 10 to 35 µg mL<sup>-1</sup>.

5 In addition, adsorption isotherms were determined on the soil (5 g) and on the soil (5 g) added  
6 with organo-clays (0.1 g of Dellite 67G or Dellite 43B) and 25 ml of 0.01 N CaCl<sub>2</sub> solution of  
7 triclopyr at different concentrations (0.5 to 5 µg mL<sup>-1</sup>).

8 The suspensions were shaken mechanically at 30° ± 2°C for 16 hours. Previous kinetic studies  
9 showed that 16 h were enough to reach adsorption equilibrium. After equilibration, the suspensions  
10 were centrifuged at 3000 rpm for 30 min. Solutions shaken in tubes without adsorbent served as  
11 control and showed no loss of triclopyr.

12 The amount of pesticide adsorbed was considered to be the difference between that initially  
13 present in the solution and that remaining after equilibrium with the adsorbent.

14 The adsorption data were modelled using the Freundlich equation:

15 
$$x/m = KfCe^{1/n}$$

16 where Kf and 1/n are Freundlich parameters, characteristic of the sorbate-adsorbent system, x/m is  
17 the amount of triclopyr adsorbed and Ce is the triclopyr equilibrium concentration.

18 Desorption experiments were conducted immediately after adsorption from the highest point of  
19 the adsorption isotherms by batch successive dilution method. Four mL of supernatant were  
20 removed for the adsorption analysis and were then replaced with 4 mL of 0.01 N CaCl<sub>2</sub> solution.  
21 After shaking at 30°C for 2 h, the suspensions were centrifuged and 4 mL supernatant removed for  
22 analysis. The desorption cycle was repeated three times for the desorption isotherm.

23

## 24 **Degradation tests**

25

1 In order to evaluate the effect of the organo-clays and nanosponges on the persistence of  
2 triclopyr in the soil, the degradation kinetics were measured. 40.0 mg of the materials under  
3 examination were added to 20 g of dry soil and 1 mL of triclopyr in aqueous solution (100 µg mL<sup>-1</sup>).  
4 After thorough mixing, the correct quantity of distilled water was added to bring the soil  
5 humidity level to 50% of the WHC.

6 The samples were then placed in a thermostated cell to incubate at 25° C for 56 days. During  
7 the incubation period, the aerobic conditions of the samples was assured by the partial closure of the  
8 screw-top on the container. At prefixed time intervals (0, 3, 7, 14, 28 and 56 days) the  
9 concentration of triclopyr remaining was determined by means of liquid chromatography. The  
10 active ingredient was extracted by adding 50 ml of ethyl acetate to the soil and shaking the mixture  
11 for 30 minutes. After centrifugation (3000 rpm for 30 min) the supernatant was collected in a  
12 vacuum flask. This operation was repeated twice using 25 ml of solvent and halving the shaking  
13 times. The three extracts were combined, filtered through anhydrous sodium sulfate, dried in a  
14 rotary evaporator and then hydrated with 10 ml of acetonitrile.

15

## 16 **Chromatography analyses**

17

18 The concentration of triclopyr was determined by means of liquid chromatography, using a  
19 Shimadzu LC10 ADvp instrument, fitted with a UV/vis set at 230 nm and a Supelcosil-LC ABZ  
20 column (15cm x 4.6cm x 5µm). The mobile phase was water acidified to pH 3 with phosphoric  
21 acid (30%) and acetonitrile (70%).

22

## 23 **RESULTS AND DISCUSSION**

24

### 25 **Adsorption studies**

1 Before determining the adsorption isotherms, a single concentration of the active ingredient was  
2 used to verify the extent of the interaction between triclopyr and the adsorbent. The results of this  
3 preliminary test are shown in Fig. 1.

4 The herbicide appeared to have little affinity either with the soil or the nanosponge while it  
5 interacted intensively with the two pure organo-clays and the soil-organo-clay mix.

6 Obenshain et al. <sup>[19]</sup> evaluated the adsorption of various pesticides including triclopyr on a soil  
7 with a 3.4% organic carbon content. The authors found that only 1.2% of the herbicide was  
8 adsorbed on the surface layer and 3.5% on the deeper layer. The same experiment showed that the  
9 small quantity of the product adsorbed was totally released during the 24 hours following  
10 adsorption. These results confirm the poor affinity of triclopyr with the solid soil phase, in  
11 particular, with the large quantity of organic matter content in the tested soil.

12 The lack of interaction between the triclopyr and the nanosponge was unexpected, given that  
13 cyclodextrin based nanosponges are known to have a high solubilizing power when forming  
14 inclusion complexes with numerous active molecules, such as camptothecin <sup>[23]</sup>, paclitaxel <sup>[24]</sup> and  
15 1-methylcyclopropene <sup>[25]</sup> to name only a few. In this case, it is probable that the structure of the  
16 triclopyr kept it from penetrating the crosslinked carbonate bonds to a significant extent.

17 A recent study by Singh et al. <sup>[26]</sup>, presents the results of an experiment regarding the adsorption  
18 and desorption of carbofuran on different soils, with and without  $\beta$ -cyclodextrin. In all the soils  
19 studied, the adsorption capacity of carbofuran was lower when  $\beta$ -cyclodextrin was present than  
20 when only water was present. According to the authors, this decrease could be due to the formation  
21 of inclusion complexes between the  $\beta$ -cyclodextrin and the carbofuran, characterized by a higher  
22 hydrosolubility than pure carbofuran. This condition leads to a reduced adsorption of the molecule  
23 to the soil. Using a  $\beta$ -cyclodextrin solution there was a more pronounced desorption of carbofuran  
24 than when it was treated with distilled water, indicating the formation of inclusion complexes that  
25 reduced the number of molecules interacting with the soil colloid surfaces.

1 Even only small proportions of organoclays added to the soil appeared to be very efficient in  
2 extracting triclopyr from water.

3 The poor adsorption of triclopyr to the soil and the nanosponge meant that it was not possible to  
4 determine the relative isotherms.

5 As regards the pure organoclays, the adsorption isotherms are well-described by the Freundlich  
6 equation ( $r^2 = 0.98$  and  $0.99$  for the Dellite 67 G and 43 B respectively). In both cases, the  $1/n$   
7 coefficient was higher than 1 placing the isotherms as type S in the Giles et al. Classification. [27]  
8 The  $K_f$  value was seen to be higher for the Dellite 43 B than for the Dellite 67 G, indicating that the  
9 former has a greater aptitude to retain the active ingredient (Figs. 2 and 3).

10 During the first phase, desorption from the surface was about 15%, reducing thereafter. In the  
11 case of the Dellite 43 B a larger quantity of the herbicide was desorbed than with the Dellite 67 G.  
12 It is possible that the organic cation in the latter creates more stable interactions with the molecule.

13 The  $1/n$  coefficient was also higher than 1 for the soil-organoclay mix. As regards the soil-  
14 Dellite 67 G mix, the value was calculated at 2.56; this indicates a great increase in adsorption as  
15 the concentration of the herbicide increases. This behaviour determined a high level of  $K_f$ , which  
16 indicates the entity of adsorption when the pesticide concentration is equal to 1.

17 The  $1/n$  value of the soil-Dellite 43 B mix was 1.81 and indicates the same behaviour as  
18 observed for the other organoclay but with a different response to increased concentrations. The  $K_f$   
19 value is, in fact, lower (Figs. 4 and 5).

20 Triclopyr was released in small quantities by the two mixtures.

21

## 22 **Degradation kinetics**

23

24 The degradation kinetics were all first order kinetics ( $r^2 > 0.94$ ). In the soil, the active ingredient  
25 had a half-life time of 30 days while in the soil mixed with Dellite 67 G and 43 B half-life times of  
26 10 and 6 days respectively were found (Fig. 6).

1 The literature reports relative values of half-life times for triclopyr as varying from 8 to 69 days  
2 according to the type of soil. Under anaerobic conditions the rate of degradation appears very low  
3 (half-life = 1300 days). This data indicates that the aerobic microbial population affects the  
4 degradation process. Biodegradation is the main path for the dissipation of triclopyr in the soil.  
5 Under aerobic conditions, the main metabolite is 3,5,6-trichloropyridinol (TCP), which undergoes a  
6 further transformation in carbon dioxide, water and organic acids. TCP is more stable than triclopyr.  
7 [28]

8 The increase in the rate of degradation obtained in the presence of the two organo-clays could be  
9 ascribed to a catalytic reaction by the inorganic fraction with which the triclopyr has interacted.  
10 The catalytic activity of clay minerals in the degradation of pesticides is well documented.

11 Yaron et al. [29] studied the interaction of different phosphorated pesticides and three mineral  
12 clays (montmorillonite, kaolinite and attapulgite). The pesticides adsorbed by the clay degraded by  
13 means of hydrolysis to become nitrophenol and phosphate ions. The rate of hydrolysis was  
14 influenced by the type of clay, the type of exchangeable cation and the state of hydration. Kaolinite  
15 was the most efficient, determining a 93% decrease in parathion in 40 days.

16 An experiment on the chemical hydrolysis of atrazine showed the formation of formamide near  
17 the surface of a Na-montmorillonite. Infrared analyses showed that the formamide, which formed  
18 as a consequence of the interaction between the herbicide and the Na-montmorillonite, was  
19 collocated in the crystal interspace. [30]

20 Nevertheless, the degradation process can also have been influenced by the organic cation in the  
21 organo-clay.

22 Groisman et al. [31] proposed using bifunctional organoclays as adsorbents and as detoxifying  
23 agents for pesticides. Their experiment involved a Na-montmorillonite intercalated with  
24 decyldimethyl-2-aminoethyl ammonium and the pesticides used were methyl parathion and  
25 tetrachlovinphos. The aminoethyl functional group exercised a detoxifying effect by catalyzing the  
26 hydrolysis of the adsorbed compounds. When bifunctional organoclays were present, the products

1 under examination hydrolyzed at a rate 12 times higher than when there was a spontaneous reaction.  
2 It was hypothesized that the catalytic effect can be attributed to a nucleophilic attack on the  
3 organophosphate ester by the unprotonated ethylamino group in the organic cation.

4 In a later work, Rav-Acha et al. <sup>[32]</sup> demonstrated that the methyl parathion was degraded on the  
5 surface of a clay modified with a bifunctional organic cation containing an alkylamino group. They  
6 identified three possible catalytic paths: a) a nucleophilic attack on the centre of reaction of the  
7 phosphate ester by the organic cation amino group; b) a similar mechanism mediated by water  
8 molecules; c) a methylation of the amino group followed by a catalytic attack on the  
9 organophosphorous compound.

10 Studies are available in the literature demonstrating that the desorption of pollutants is not a pre-  
11 requisite for degradation and that the phase adsorbed by the substrate can be degraded. Park et al.  
12 <sup>[33]</sup> developed an experimental mathematical approach to evaluate whether desorption and  
13 degradation are strictly sequential or independent processes. In their experiment, adsorption was  
14 effected using 2,4 -D and sepiolite, while *Flavobacterium* sp. was the degrading microorganism. It  
15 was shown that the degradation of 2,4-D was not a sequential process that depended exclusively on  
16 the concentration in the liquid phase. In fact, in all the experiments, degradation occurred more  
17 quickly than would be expected considering a single concentration in liquid phase.

18 A work by Sigh et al. <sup>[34]</sup> shows that fenamiphos adsorbed by an organo-clay can be degraded by  
19 *Brevibacterium* sp in quantities 77% higher than can be desorbed under abiotic conditions.

20 Neither is it possible to eliminate the hypothesis that the development of soil bacteria  
21 populations can stimulate an acceleration in degradation. An experiment carried out to verify the  
22 effect of Dellite 67 G and 43 B on soil microorganisms showed that some microbial populations in  
23 the soil increase when the two organoclays are present. This information was obtained by analyzing  
24 DNA extracted from a soil with and without the materials under examination. The results, that do  
25 not form part of this paper, have not yet been published.

1 This hypothesis may also be supported by the knowledge that soil micro-organisms play an  
2 important role in the degradation of triclopyr. Newton et al. <sup>[35]</sup> maintain that hot, humid climate  
3 conditions and high levels of organic matter factors that favour the development of soil micro-  
4 organisms, generally determine an increase in the rate at which the pesticide disappears.

5 This hypothesis must be confirmed by further experimentation including a molecular analysis of  
6 microbial populations treated with the materials under examination.

7 The rate of degradation of the active ingredient also increased when nanosponges were present  
8 (half-life = 15 days) (Fig. 6). There is no proof that nanosponges can catalyse degradation  
9 phenomena but information is available in the literature regarding the capacity of cyclodextrins to  
10 favour the degradation process of organic compounds.

11 Fava and Ciccotosto <sup>[36]</sup> studied the biodegradation of polychlorinated biphenyls (PCBs) in the  
12 soil in the presence of methylated –  $\beta$  – cyclodextrins (RAMEB). They found that the presence of  
13 RAMEB led to an increase in the biodegradation of the PCBs because water-soluble inclusion  
14 complexes formed which supported the co-metabolic processes of the microorganisms in the soil.  
15 Similar results were obtained by Wang et al. <sup>[37]</sup> who evaluated the efficiency of hydroxypropil- $\beta$ -  
16 cyclodextrin in the solubilisation and biodegradation of phenantrene.

17 On the basis of the undeniable fact that nanosponges are insoluble in water and have a limited  
18 capacity to include the triclopyr molecule, their strong influence on degradation kinetics is probably  
19 due to their great capacity to adhere to the cell membranes, as has been widely shown and reported  
20 in the literature regarding a number of anti-cancer drugs.<sup>[38]</sup> Similar behaviour could lead the  
21 nanosponges to adhere to the walls of the micro-organisms thus facilitating the degradation process.

22 The results of this work suggest that it is possible to use the Dellite 67 G and 43 B organo-clays  
23 to create barriers that limit the contamination of deep and surface water bodies.

24 Nanosponges also appear to be suitable for use in limiting the risks of water pollution as they  
25 favour the degradation process of the molecule in the contaminated soil.

1   **REFERENCES**

2

3   [1] Baglieri, A.; Gennari, M.; Arena, M. Abbate, C. The adsorption and degradation of  
4 chlorpyrifos-methyl, pendimethalin and metalaxyl in solid urban waste compost, *J. Environ. Sci.*  
5 *and Health B.* **2011**, *46*, 454-460.

6

7   [2] Kumar, S.; Yong, W.L. Effect of bentonite on compacted clay landfill barriers, *Soil Sediment*  
8 *Contam.* **2002**, *11*, 71-89.

9

10   [3] Rodriguez-Cruz, M.S.; Sánchez-Martín, M.J.; Andrades, M.S.; Sánchez-Camazano, M.  
11 Modification of clay barriers with a cationic surfactant to improve the retention of pesticides in  
12 soils, *J. Hazard. Mater. B*, **2007**, *139*, 363-372.

13

14   [4] Goisman, L.; Rav-Acha, C.; Gerstl, Z.; Mingelgrin, U. Sorption of organic compounds of  
15 varying hydrophobicities from water and industrial wastewater by long- and short- chain  
16 organoclays, *Appl. Clay Sci.* **2004**, *24*, 159-166.

17

18   [5] Carrizosa, M.J.; Calderon, M.J.; Hermosin, M.C.; Cornejo, J. Organoclay adsorbent and carrier  
19 of the herbicide bentazone, *Sci. Total Environ.* **2000**, *247*, 285-293.

20

21   [6] Hermosin, M.C.; Celis, R.; Facenda, G.; Carrizosa, M.J.; Ortega-Calvo, J.J.; Cornejo, J.  
22 Bioavailability of the herbicide 2,4 D formulated with organoclays, *Soil Biol. Biochem.* **2006**, *38*,  
23 2117-2124.

24



- 1 [7] Baglieri, A.; Borzì, D.; Abbate, C.; Nègre, M.; Gennari, M. Removal of fenhexamid and  
2 pyrimethanil from aqueous solutions by clays and organoclays, *J. Environ. Sci. and Heal. B* **2009**,  
3 *44*, 220-225.
- 4
- 5 [8] Abbate, C.; Arena, M.; Baglieri, A.; Gennari, M. Effects of organoclays on soil eubacterial  
6 community assessed by molecular approaches, *J. Hazard. Mater.* **2009**, *168*, 466-472.
- 7
- 8 [9] Witthuhn, B.; Klauth, P.; Klumpp, E.; Narres, H.D.; Martinius, H. Sorption and biodegradation  
9 of 2,4-dichlorophenol in the presence of organoclays, *Appl. Clay Sci.* **2005**, *28*, 55-66.
- 10
- 11 [10] Witthuhn, B.; Klauth, P.; Pernyeszi, T.; Vereecken, H.; Klumpp, E. Organoclays for aquifer  
12 bioremediation: adsorption of chlorobenzene on organoclays and its degradation by *Rhodococcus*  
13 B528, *Water Air Soil Poll.* **2006**, *6*, 317-329.
- 14
- 15 [11] Li, D.Q.; Ma, M. Nanosponges for water purification, *Clean Products and Processes* **2000**, *2*,  
16 112-116.
- 17
- 18 [12] Petty, K.D.; Getsinger, K.B.; Woodburn, A. Review of the aquatic environmental fate of  
19 triclopyr and its major metabolites, *J. Aquat. Plant Manage.* **2003**, *41*, 69-75.
- 20
- 21 [13] U.S. Dept. Of Justice. Drug Enforcement Administration, Cannabis eradication in the  
22 contiguous United States and Hawaii, Supplement of the environmental impact statements.  
23 Washington D.C. **1998**, 145-146.
- 24
- 25 [14] U.S. EPA. Prevention, Pesticides and Toxic Substances. Reregistration eligibility decision  
26 (RED): Triclopyr. Washington D.C. **1998**, 2-5.

1

2 [15] Woodburn, K.B.; Batzer, F.R.; White, F.H.; Schultz, M.R. The aqueous photolysis of triclopyr,  
3 Environmental Toxicology and Chemistry **1993**, *12*, 43-55.

4

5 [16] Cleveland, C.B.; Holbrook, D.L. A hydrolysis study of triclopyr. GH-C 2491R. Dow Agro  
6 Sciences, Indianapolis, IN. **1992**.

7

8 [17] GEIR Generic Environmental Impact Report: Control of vegetation of utilities and Railroad  
9 Rights of Way Pub. by Harrison Biotech, Cambridge, MA **1985**.

10

11 [18] USDA Forest Service Agriculture Handbook. Pesticide Background Statements 1 **1984**.

12

13 [19] Obenshain, K. R.; Metcalf, M.C.; Abdelghani, A.A.; Regens, J.L.; Hodges, D.G.; Swalm, C.M.  
14 Spatial analysis of herbicide decay rates in Louisiana, Environ. Monit. Assess. **1997**, *48*, 307-316.

15

16 [20] Woudneh, M.B.; Sekela, M.; Tuominen, T.; Gledhill, M. Acidic herbicides in surface waters of  
17 lower Fraser valley, British Columbia, Canada, J. chromatogr. **2007**, *1139*, 121-129.

18

19 [21] Battaglin, W.A.; Rice, K.C.; Focazio, M.J.; Salmons, S.; Barry, R.X. The occurrence of  
20 glyphosate, atrazine and other pesticides in vernal pools and adjacent streams in Washington, DC,  
21 Maryland, Iowa, and Wyoming, 2005-2006, Environ. Monit. Assess. **2009**, *155*, 281-307.

22

23 [22] F. Trotta, W. Tumiatti, Pat. WO 03/085002, **2003**.

24

- 1 [23] Swaminathan, S.; Pastero, L.; Serpe, L.; Trotta, F.; Vavia, P.; Aquilano, D.; Trotta, M.; Zara,  
2 G.; Cavalli, R. Cyclodextrin based nanosponges encapsulating camptothecin: physicochemical  
3 characterization, stability and cytotoxicity, *Eur. J. Pharm. Biopharm.* **2010**, *74*, 193-201.  
4
- 5 [24] Ansari, K.; Torne, S.J.; Vavia, P.; Trotta, F.; Cavalli, R. Paclitaxel loaded nanosponges: in  
6 vitro characterization and cytotoxicity study on MCF-7 cell line culture, *Curr. Drug Delivery* **2011**,  
7 *8*, 194-202.  
8
- 9 [25] Seglie, L.; Martina, K.; Devecchi, M.; Roggero, C.; Trotta, F.; Scariot, V.  $\beta$ -cyclodextrin based  
10 nanosponges as carriers for 1-MCP in extending the postharvest longevity of carnation cut flowers:  
11 an evaluation of different degrees of cross-linking, *Plant Growth Regul.* **2011**, *65*, 505-511.  
12
- 13 [26] Singh, R.P.; Khan, N.U.; Srivastava, G. Effect of  $\beta$ -cyclodextrin on adsorption and desorption  
14 of carbofuran in soils of different texture, *Toxicol. Environ. Chem.* **2011**, *93*, 1613-1622.  
15
- 16 [27] Giles, C.H.; McEwan, T.H.; Nakhwa, S.N.; Smith, D. Studies in adsorption. Part XI. A system  
17 of classification of solution adsorption isotherms, and its use in diagnosis of adsorption mechanisms  
18 and measurement of specific surface areas of solids, *J. Chem. Soc.* **1960**, *4*, 3973-3993.  
19
- 20 [28] Marin Municipal Water District Vegetation Management Plan, Chapter 4 - Triclopyr, DRAFT-  
21 8/27/08                      Herbicide                      Risk                      Assessment                      (2008),  
22 [http://www.marinwater.org/documents/Chap4\\_Triclopyr\\_8\\_27\\_08.pdf](http://www.marinwater.org/documents/Chap4_Triclopyr_8_27_08.pdf)  
23
- 24 [29] Yaron, B. Some aspects of surface interactions of clays with organophosphorous pesticides,  
25 *Soil Sci.* **1978**, *125*, 210-216.  
26

- 1 [30] Nguyen, T.T. Infrared spectroscopic study of the formamide Na-montmorillonite complex.  
2 Conversion of s-triazine to formamide, *Clays Clay Miner.* **1986**, *34*, 521-528.  
3
- 4 [31] Goisman, L.; Rav-Acha, C.; Gerstl, Z.; Mingerlgrin, U. Sorption and detossification of toxic  
5 compounds by bifunctional organoclays, *J. Environ. Qual.* **2004**, *33*, 1939-1936.  
6
- 7 [32] Rav-Acha, G.; Goisman, L.; Mingelgrin, Z.; Sasson, Y.; Gerstl, Z. A mechanistic study of  
8 methyl parathion hydrolysis by a bifunctional organoclay, *Environ. Sci. Technol.* **2007**, *41*, 106-111.  
9
- 10 [33] Park, J.H.; Kay, D.; Zhao, X.D.; Boyd, S.A.; Voice, T.C. Kinetic modeling of bioavailability  
11 for sorbed-phase 2,4-dihlorophenoxyacetic acid, *J. Environ. Qual.* **2001**, *30*, 1523-1527.  
12
- 13 [34] Singh, N.; Megharaj, M.; Gates, W.P.; Churchman, G.J.; Anderson, J.; Kookana, R.S.; Naidu,  
14 R.; Chen, Z.; Slade, P.G.; Sethunathan, N. Bioavailability of an organophosphorus pesticide,  
15 fenamiphos, sorbed on an organoclay. *J.Agric.Food Chem.* **2003**, *51*, 2653-2658.  
16
- 17 [35] Newton, M.F.; Roberts, A.; Allen, B.; Kelpsas, D.; White C., Boyd, P. Deposition and  
18 dissipation of three herbicides in foliage, litter, and soil of brush fields of southwest Oregon, *J.*  
19 *Agric. Food Chem.* **1990**, *38*, 574-583.  
20
- 21 [36] Fava, F.; Ciccotosto, V.F. Effects of randomly methylated  $\beta$ cyclodextrin (RAMED) on the  
22 bioavalilability and aerobic biodegradation of polychlorinated biphenyls in three pristine soils  
23 spiked with transformer oil, *Appl. Microbiol. Biotechnol.* **2002**, *58*, 393-399.
- 24 [37] Wang, J.M.; Marlowe, E.M.; Miller-Maier, R.M.; Brusseau, M.L. Cyclodextrin-enhanced  
25 biodegradation of phenanthrene, *Environ. Sci. Technol.* **1998**, *32*, 1907-1912.
- 26 [38] Mognetti, B.; Barberis, A.; Marino, S.; Berta, G.; De Francia, S.; Trotta, F.; Cavalli, R. In vitro

1 enhancement of anticancer activity of paclitaxel by chremophorfree cyclodextrin based nanosponge  
2 formulation, J. Incl. Phenomen. Macrocucl. Chem. **2012**, 74, 201-210.

3

#### 4 **FIGURE CAPTIONS**

5

6 Figure 1. Percentage of triclopyr adsorption to the materials examined. Means of four replications.

7 SD < 10%

8 Figure 2. Triclopyr adsorption and desorption isotherms obtained with Dellite 67 G. Means of four

9 replications. SD < 10%

10 Figure 3. Triclopyr adsorption and desorption isotherms obtained with Dellite 43 B. Means of four

11 replications. SD < 10%

12 Figure 4. Adsorption and desorption isotherms obtained with the soil-Dellite 67 G mix. Means of

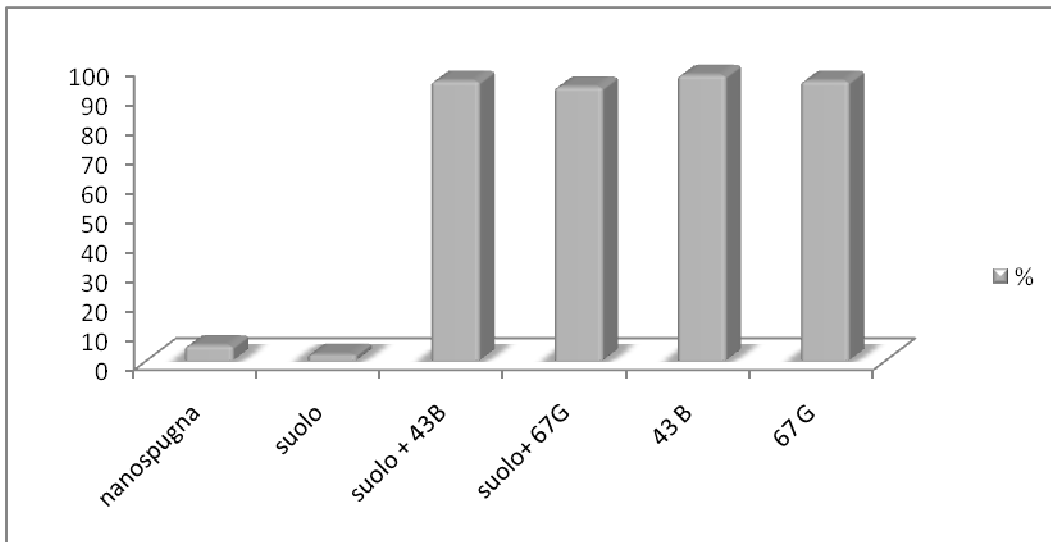
13 four replications. SD < 10%

14 Figure 5. Adsorption and desorption isotherms obtained with the soil-Dellite 43 B mix. Means of

15 four replications. SD < 10%

16 Figure 6. Triclopyr degradation kinetics in the soil, the soil-Dellite 67 G mix, the soil Dellite 43

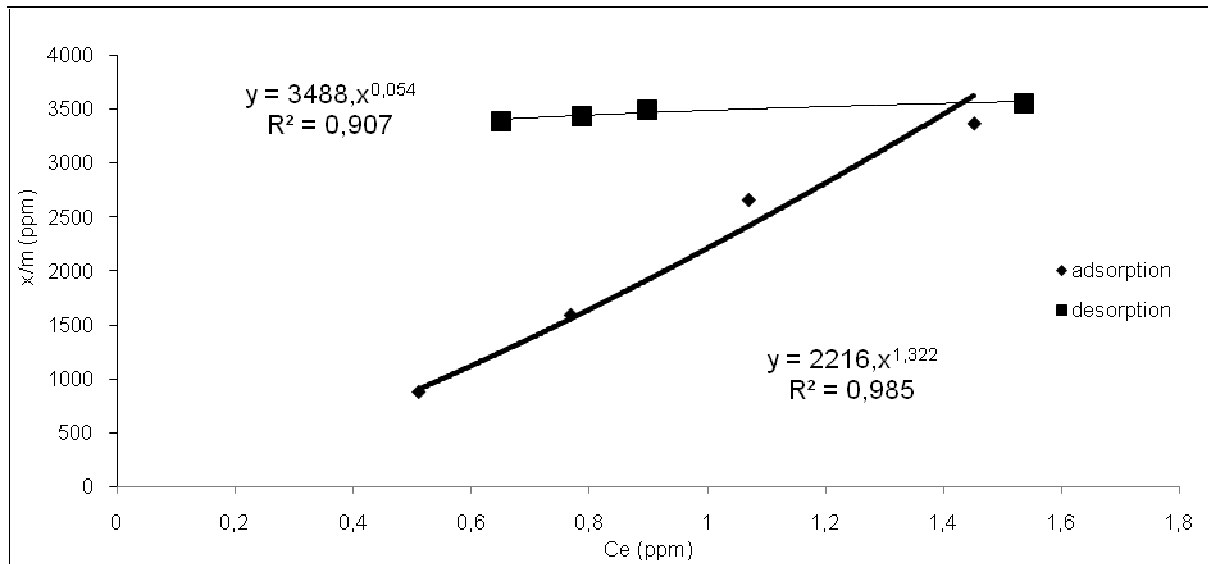
17 mix, and the soil-nanosponge mix. Means of six replications. SD < 10%



1

2

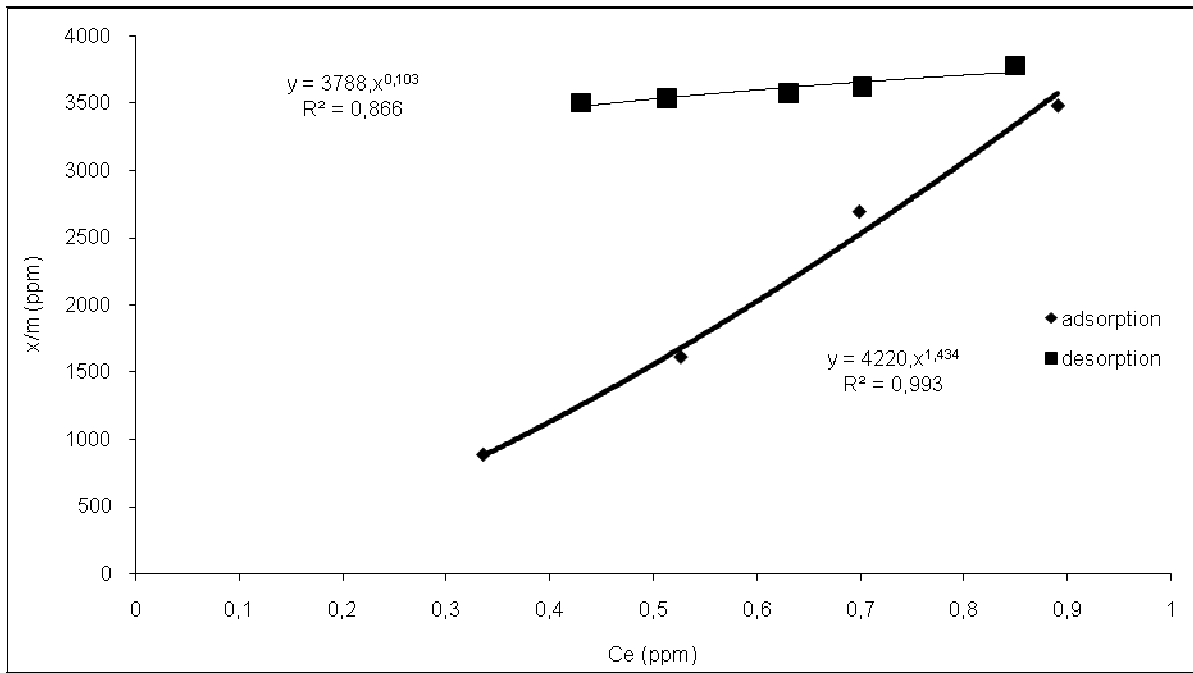
3 Fig.1.



1

2

3 Fig.2.

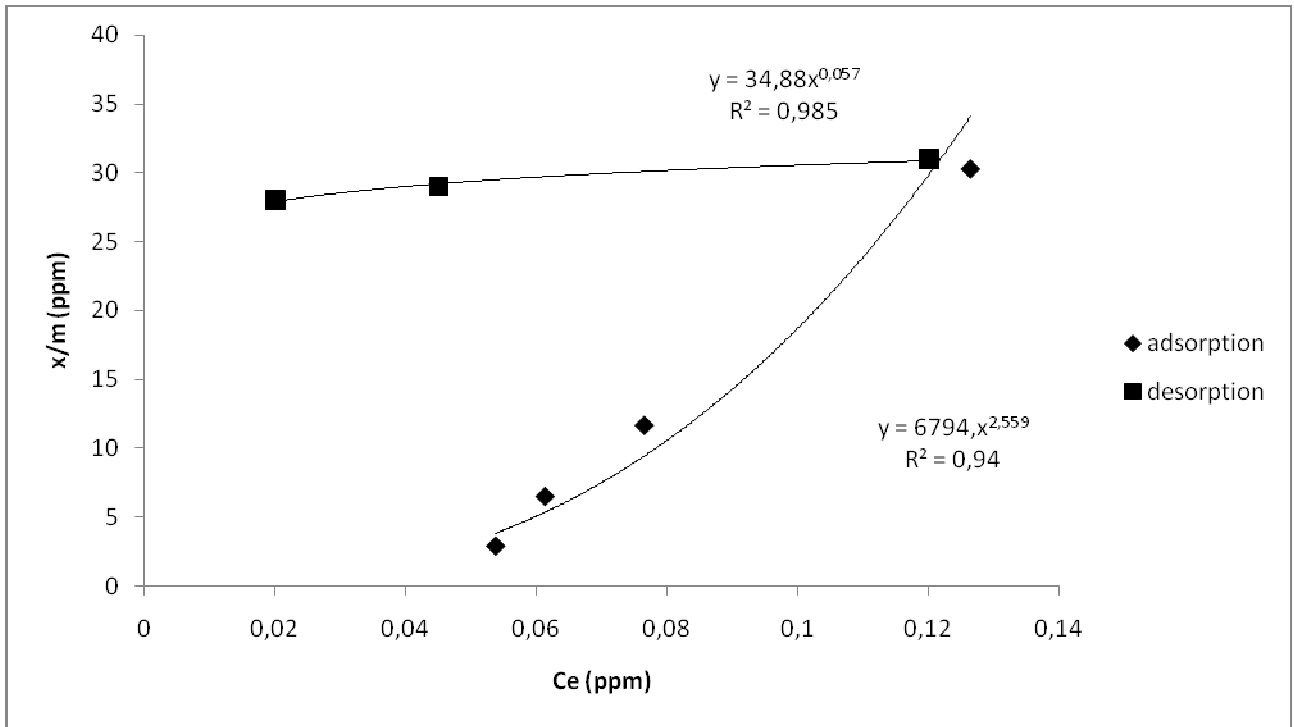


1

2

3 Fig.3.

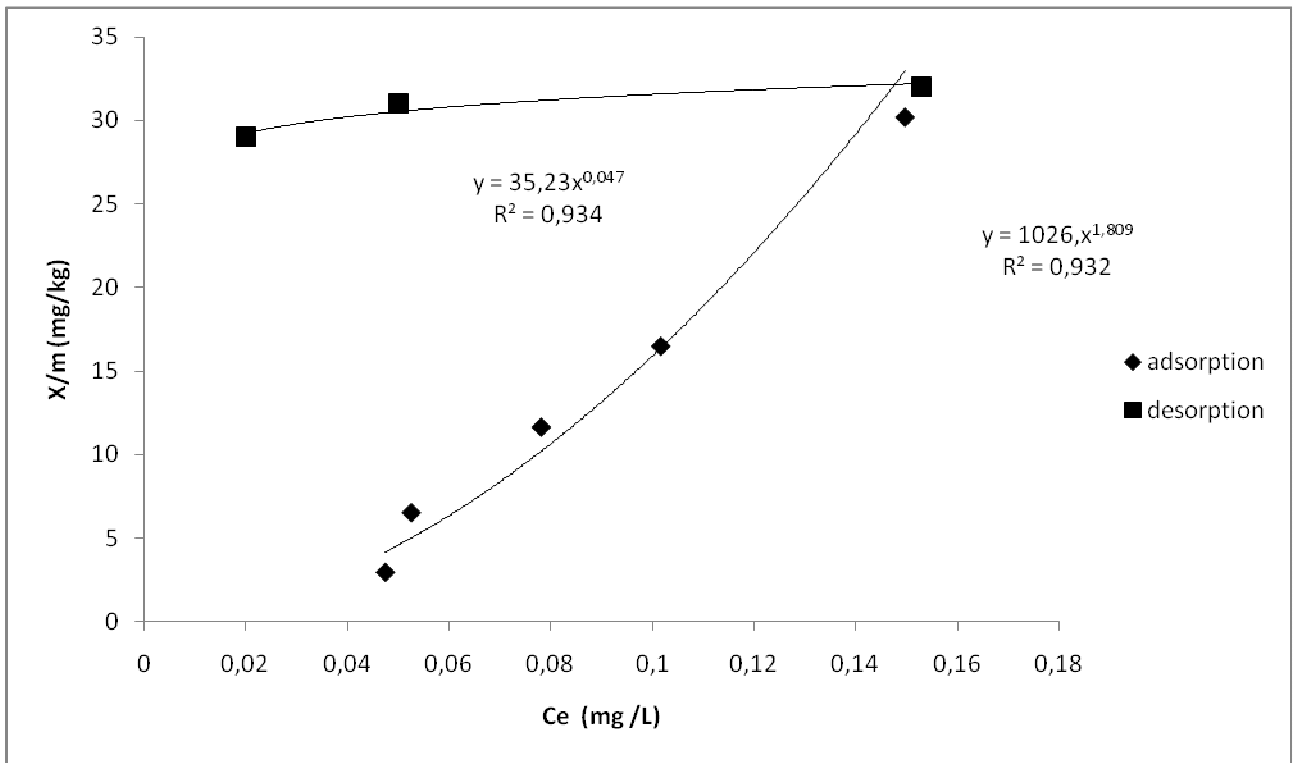




1

2

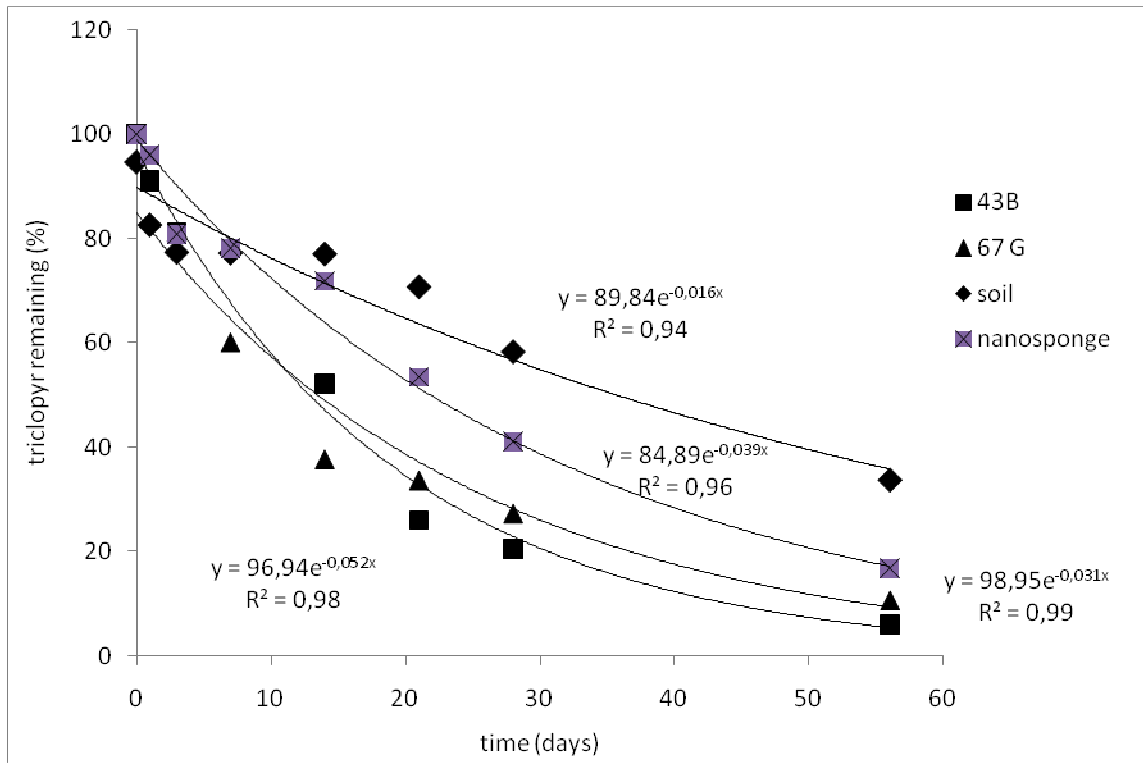
3 Fig.4.



1

2

3 Fig. 5.



1

2

3 Fig. 6.