Research paper

Adsorption/desorption of fungicides in natural clays from Southeastern Spain

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ABSTRACT

Nine natural clay samples, collected in the eastern Andalusian region (South of Spain), were evaluated for the retention of two widely used non-ionic fungicides, metalaxyl and fludioxonil. The clays showed different mineralogical composition and granulometry, had high Ca content, and presented medium–low cation exchange capacity and specific surface area and low organic carbon (OC) content (≤0.8%). Various equations were used for the fitting of kinetic and adsorption data. The pseudo-second-order kinetic model was successfully fitted to the experimental kinetic data. The Freundlich equation was better suited for the fitting of metalaxyl adsorption, while both Freundlich and Langmuir equations could be used to explain the retention of fludioxonil. Adsorption experiments, carried out using the batch approach, indicated that metalaxyl, a more polar fungicide, was always retained to a greater extent than fludioxonil, probably through electrostatic interactions. The retention of both fungicides was related with the OC content of the clays and their granulometry. The removal of clay OC led to a greater decrease in fludioxonil adsorption, probably due to its higher hydrophobicity. The retention extent of metalaxyl on selected clays, at 10 and 20 °C, was maintained up to 6 months, reflecting the high stability of pesticide adsorption on the selected adsorbents. A preliminary study at two pesticide concentrations showed that pilloring onion seeds with metalaxyl or fludioxonil may cause phytotoxicity to seedling growth when compared with control seeds.

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1. Introduction

Natural clays have been proposed for the remediation of environmental waters contaminated with organic pollutants, like pesticides (Sidhoum et al., 2013; Ouali et al., 2015; Azarkan et al., 2016), because they can be used as cheap and readily available retention barriers. The presence of pesticides in environmental waters is highly dependent on pesticide properties, mainly on their solubility and persistence (Gustafson, 1989). For instance, metalaxyl, a highly soluble and moderately persistent fungicide, has been detected in monitoring programs in different countries (Bermúdez-Couso et al., 2013; Triantafyllidis et al., 2013; Allinson et al., 2015), while no record has been found in the literature about the presence in water of the hydrophobic fludioxonil. On the contrary, residues of both fungicides have been detected in various crops (Dedola et al., 2014; Wanwimolruk et al., 2015).

On the other hand, natural clays have been also considered as carriers of seed treatments (Nisar et al., 2009), which could release to the seeds, in the early stages of development, pesticides, nutrients, or mycorrhizal fungi, among others (Taylor and Salanenka, 2012; Colla et al., 2015; Adhikari et al., 2016). Seed treatment, apart from providing

added value to the seeds, is directed to the optimization of plant sowing. In this sense, the inclusion of pesticides in the clays would result in (a) a reduction in seed quality deterioration during storage due to pests or diseases; (b) a decrease of pesticide application doses, depending on planting density; (c) a decline in environmental risks, including surface water, groundwater, soil, or air pollution; (d) a minimization of the destruction of beneficial fauna and (e) an enhancement of safety conditions for seed handlers, regarding traditional treatments.

The incorporation of pesticides in the clay minerals, in comparison with conventional pesticide applications, has the advantage of retaining them in the clay matrix, enabling accurate and uniform pesticide application, and providing controlled availability of the active ingredient to the seedlings in the initial development. Therefore, pesticide availability for the seeds in their immediate surrounding would be maintained for longer periods of time and the released pesticide concentration would be kept to a safe value, reducing the potential pesticide leaching and its availability for non-target organisms. At the same time, when adsorbed on the clays in the seed coating, pesticides are protected from dissipation processes, such as photodegradation or volatilization (El-Nahhal et al., 2001; Si et al., 2004).

Clay minerals are negatively charged and hydrophyllic, therefore their use has been mainly oriented to the retention of cationic pesticides, such as paraquat (Sánchez-Jiménez et al., 2012; Sidhoum et al.,

2013). For non-ionic pesticides some clay modifications have been proposed to increase pesticide retention, such as treatments with organic cations to obtain a hydrophobic coverage (Lagaly, 2001; El-Nahhal et al., 2001; Sánchez-Martín et al., 2006). However, the adsorption capacity of clays for non-ionic pesticides, though lower than for charged pesticides, could be sufficient for pesticide retention and release in seed coating strategies.

Therefore, the aim of this work was the selection of natural clay materials, with abundant deposits in the Andalusian region (south of Spain), for delivering pesticides in seed coatings. Two fungicides have been selected, the relatively polar metalaxyl and the hydrophobic fludioxonil, widely used for the control of different plant diseases. The clay mineralogy, chemistry, and properties of the clay materials were identified with different instrumental techniques and related with the retention of both fungicides.

2. Materials and methods

2.1. Clay materials

Potential clay deposits were localized in Southeastern Spain, in the provinces of Granada (Las Gabias and Padul) and Jaén (Bailén). The selected deposits respond to different types and geological context resulting in a wide array of textures and properties. Clays (between 5 and 10 kg each) were collected from nine sampling points.

2.2. Pesticides

Standards of the fungicides metalaxyl (methyl N-(methoxyacetyl)-N-(2,6-xylyl)-DL-alanine) and fludioxonil (4-(2,2-difluoro-1,3-benzodioxol-4-yl)pyrrole-3-carbonitrile) (99% purity, Dr. Ehrenstorfer, Germany), whose structure is shown in Fig. 1, were used without further purification. Fludioxonil is intended for seed treatment in the control of a range of diseases including *Fusarium*, *Rhizoctonia*, and *Alternaria*. It is a non-systemic fungicide with long residual activity, which acts by inhibiting transport-associated phosphorilation of glucose, thus reducing mycelial growth. Likewise, metalaxyl is a fungicide used to control diseases caused by air- and soil-borne *Peronosporales*. This systemic fungicide, with curative and protective action, acts by suppressing sporangial formation, mycelial growth, and the establishment of new infections. It disrupts fungal nucleic acid synthesis—RNA polymerase 1 (Pesticide Properties Database). Some formulations, such as Maxim35 from Syngenta, combine both fungicides because they complement each other.

The octanol/water partition coefficients (log K_{ow}) of metalaxyl and fludioxonil are 1.75 and 4.12, and their solubility in water 8400 and 1.8 mg L^{-1}, respectively (Tomlin, 2003). Stock standard solutions of both fungicides were prepared at 1 g L^{-1} in acetonitrile and diluted with high purity MilliQ water (Millipore, Bedford, MA).
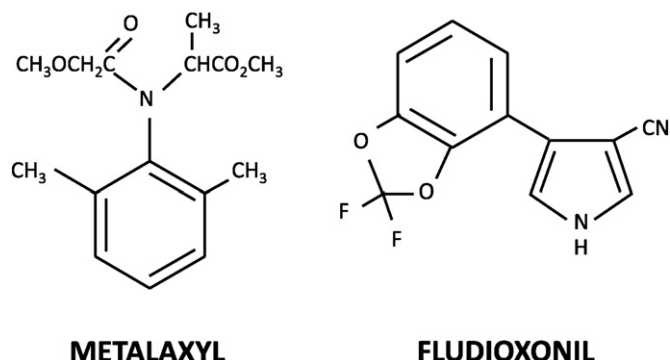

Fig. 1. Chemical structure of the fungicides metalaxyl and fludioxonil.

In the case of fludioxonil, an emulsifiable concentrate (EC) formulation (39.7% a.i.) was also used for the adsorption experiments.

2.3. Clay characterization

The mineralogical composition was determined by X-Ray Powder Diffraction (XRPD) using a Panalytical X-Pert Pro diffractometer with Cu Kα radiation (45 kV, 40 mA), Ni filter, RTMS X'Celerator detector, 4°–69.9928° scan range, 0.0084° step size, for a total of 7898 points and 11 min/sample. Random powder diffraction and oriented-aggregates (normal, ethylene glycol, and heated to 550 °C) were prepared on glass slides. The diffraction data were analyzed using the XPOWDER® software (Martín-Ramos, 2004). Semi-quantitative analysis was performed following Moore and Reynolds (1989), and the final contents of the different mineral phases were calculated by combining XRD and chemical analytical data, following Torres-Ruiz et al. (1994) and López-Galindo et al. (1996).

Microanalysis on individual particles of smectite and illite was performed on samples R5, R6, R7, and R8. The chemical composition of particles was quantitatively determined using a FEI Titan G2 60-300 electron microscope operated at 300 kV and equipped with a SUPER-X silicon-drift window-less EDX detector. The spectra were collected in STEM (Scaning Transmission Electron Microscopy) mode using a HAADF (High Angle Annular Dark Field) detector. EDX data were corrected by the thin-film method. The K-factors were determined using mineral standards. Atomic concentration ratios were converted into formulae according to stoichiometry (number of O atoms in theoretical formulae).

Chemical composition was determined using a commercial wavelength dispersive X-ray fluorescence instrument (BRUKER S4 Pioneer) equipped with an Rh anode X-ray tube (60 KV, 150 mA); three analyzer crystals (OVO-55, LiF 200, and PET) and a flow proportional counter for light element detection, and a scintillation counter for heavy elements. Quantification was made by the fundamental parameters method using the software linked to the equipment (SpectraPlus). Five grams of each powdered sample was mixed with 0.5 g of a binder (Hoechst wax C micropowder) and homogenized in an agate mortar. To obtain a XRF-pellet, a small metallic sample holder made of aluminum with a diameter of about 4 cm was used. The pellets were pressed at 90 bars in a Nannetti hydraulic press for 30 s. To determine loss on ignition (LOI), samples were heated to 900 °C for 1 h.

A granulometric analysis of the samples was carried out by using a laser diffraction particle size analyzer in a range between 0.02 and 1500 μm. The equipment (Mastersizer 2000LF, Malvern Instruments) consists of a wet sample dispersion unit (Hydromu, Malvern) and a microvolume wet sampler (Hydro 200Up, Malvern).

pH (Eutech Instruments Cyberscan pH 2100, Singapore) and electrical conductivity (EC) (XS Instruments COND 510, Italy) were measured in sample/deionized water suspensions (0.1/20, w/v). Cation exchange capacity (CEC) was determined using the method based on the triethylenetetramine–Cu complex (Meier and Kahr, 1999). Organic C (OC) was determined by a modified Walkley and Black method (Mingorance et al., 2007). Specific surface area was determined from N_2 adsorption isotherms at 77 K using the BET equation in the 0.05–0.2 p/p₀ range. Previously, the clay samples were evacuated of all water or CO_2 molecules which may be deposited on the sample surface, by degassing at 137 °C for 2 h.

The clays were also treated to remove organic matter. The treatment consisted of using hydrogen peroxide at 6% (heating in an oven at 40 °C for 1 h) following the USGS protocol.

2.4. Adsorption experiments

2.4.1. Effect of initial clay mass

Pesticide adsorption was evaluated using the batch equilibration system. Clay samples (0.05–0.2 g) were weighed per duplicate into

30-mL Pyrex centrifuge tubes to which was added 20 mL of aqueous pesticide solutions at concentrations of 2 mg L^{-1} for metalaxyl and 1 mg L^{-1} for fludioxonil. The mixture was shaken, in an end-over-end shaker (20 °C and natural adsorbent pH), for 24 h. Samples were centrifuged at 2800 rpm for 15 min and an aliquot of the corresponding supernatants was analyzed for pesticide concentration.

2.4.2. Adsorption kinetics

The batch equilibration method was also used to assess pesticide adsorption kinetics, by mixing 0.1 g adsorbent with 20 mL of aqueous pesticide solutions at a constant concentration (2 mg L^{-1} for metalaxyl and 1 mg L^{-1} for fludioxonil) from 5 min to 24 h. Samples were then treated as described above.

The fitting of the experimental data was addressed by pseudo-first- and pseudo-second-order kinetic models, whose equations can be found in the literature (Ouali et al., 2015; Azarkan et al., 2016).

2.4.3. Effect of initial pesticide concentration

Clay samples (0.1 g) were weighed per duplicate into 30-mL Pyrex centrifuge tubes to which was added 20 mL of aqueous pesticide solutions at concentrations ranging from 1 to 10 mg L^{-1} (metalaxyl) or from 0.4 to 2 mg L^{-1} (fludioxonil). After shaking in an end-over-end shaker (20 ± 1 °C) for 24 h, a sufficient time according to the preliminary kinetic studies, the samples were treated as described above. In all cases, a blank without adsorbent was run to rule out possible pesticide losses due to degradation, volatilization, or adsorption on tube walls.

For fludioxonil, the assay was also carried out under the same conditions with a formulation, to establish if the presence of adjuvants could affect pesticide adsorption.

2.4.4. Fitting to different mathematical models

To describe the adsorption equilibria, two commonly used mathematical expressions were applied to the experimental data, Langmuir and Freundlich isotherm models (Langmuir, 1918; Freundlich, 1906). The Langmuir model was originally developed to explain chemisorption on well-defined localized adsorption sites with identical adsorption energies. On the contrary the Freundlich model considers either monolayer or multilayer adsorption, irrespective of the adsorbate concentration, and assumes that the adsorbent surface is energetically heterogeneous.

2.5. Pesticide desorption

To evaluate the ability of the different clays to desorb pesticides, making them available for the pillored seeds, clays (10 g) were fortified with 0.5 mL of metalaxyl at 1 g L^{-1} in acetone, to give a nominal initial pesticide concentration of 50 μg g^{-1}. After solvent evaporation in a fume hood, fortified clay samples were transferred to glass bottles and homogenized by shaking overnight (16 h) in the dark and then stored in the dark at 10 or 20 °C for subsequent analysis (Rodríguez-Liébana et al., 2014).

Desorption was assessed by batch tests (n = 3) during 24 weeks. Fortified clay samples (0.2 g) were shaken with 5 mL MilliQ water for 24 h at 20 °C and the supernatants were treated as described for the kinetic experiments.

2.6. Phytotoxicity test

The possible phytotoxic effect of metalaxyl and fludioxonil on the development of onion seeds (*Allium cepa* L., Ramiro Arnedo, Spain) was assessed with pillored seeds without pesticides (control) or containing metalaxyl or fludioxonil. Two concentration levels were assayed, a low concentration (167 and 250 mg metalaxyl and fludioxonil per kg of seed, respectively) and a high concentration, doubling the preceding one.

Pillored seeds (n = 48) were germinated in an incubator at 25 °C for 5 days in pots (ca. 50 cm^3) containing approximately 18 g of peat covered with vermiculite, then moved to a greenhouse under natural lighting and irrigated three times a week with nutrient solution (Mingorance et al., 2012) to maintain substrate humidity. At the end of the growing period (32 days), different plant endpoints were measured: fresh biomass (shoot, root, and bulb), length of shoots and roots, and bulb diameter.

2.7. Pesticide analysis

The concentration of metalaxyl and fludioxonil in the supernatants was determined by HPLC-DAD (LaChrom Ultra, Hitachi). A 10-μl sample, after filtration by PVDF filters (0.45 μm), was injected into a Zorbax Eclipse XDB-C18 column (Agilent, 2.1 × 150 mm, 5 μm), protected by a precolumn (Vici, 0.115″ diam. × 0.028″, 0.5 μm) at a flow rate 0.25 mL min^{-1}. The mobile phase consisted of a 50:50 (v:v) mixture of acetonitrile/MilliQ water and wavelength detection at 200 nm for metalaxyl. For fludioxonil, a 60:40 acetonitrile/MilliQ water mixture and detection at 205 nm was used. Calibration was performed by triplicate injection of standard pesticide solutions between 0.1 and 10 mg L^{-1} ($R^2 = 1$) for metalaxyl, and between 0.1 and 2 mg L^{-1} ($R^2 = 0.997$) for fludioxonil. Under these conditions, the retention times were 3.6 min and 3.5 min for metalaxyl and fludioxonil, respectively.

The retention of fludioxonil on the filters used in the filtration step was assessed using different types (all 0.45 μm): Hydrophilic polypropylene (GHP, Pall Corporation), Nylon (Scharlau), regenerated cellulose (RC, Teknokroma), and polyvinylidene difluoride (PVDF, Sharlau) filters.

2.8. Data analysis

The equation that best fitted the experimental adsorption data was obtained by using non-linear regression with the Marquardt algorithm, which minimizes the sum of the squared differences between the observed and predicted values. The values of the determination coefficient (R^2) and of the standard error of the estimate were used to establish the goodness of fit. A hierarchical cluster analysis was used to arrange clay properties and adsorption parameters into groups using the Ward's method as linking algorithm and the square Euclidean distance as similarity measurement. The relationships between variables were assessed by regression analysis. For the phytotoxicity test, exploratory analysis was performed to check normality and outliers of the data sets. Data were analyzed with factor ANOVA, using the Tukey test for post-hoc comparisons. SPSS 17.0 software package was used.

3. Results and discussion

3.1. Properties of the clay samples

Samples were mainly made up of phyllosilicates (40–70%), quartz (13–25%), and calcite (up to 34%). Lesser amounts of dolomite, plagioclases, K-feldspar (Bailén samples), gypsum (Padul), and pyrite (only two samples from Bailén) were also detected (Table 1). The XRD profiles of samples R2 and R6, as representative from las Gabias and Bailén, respectively, are presented in Fig. 1S in supplementary material.

Dioctahedral mica (muscovite and/or illite) is the predominant phyllosilicate in all samples, except in R3, R5, and R6 whose content in smectite is very similar. The smectite was essentially beidellite, with varying amounts of Mg and Fe in the octahedral sheet (Table 2). Kaolinite content normally did not exceed 5% (except in samples R7 and R9, where this mineral accounted for around 10%). Samples taken in Granada province also included minor amounts of chlorite and paragonite, as they are derived from the weathering of metamorphic schists (Table 1).

Table 1
Mineralogical composition of the clay samples calculated from XRD analysis.

Location	Sample	Sm (%)	Mica	Kaol	Chl	Pg	Qtz	Ab	Or	Cal	Dol	Gy	Py
Las Gabias	R1	–	38	<5	5	5	23	6	–	14	6	–	–
Las Gabias	R2	–	40	<5	5	5	20	6	–	15	6	–	–
Bailén	R3	15	22	<5	–	–	25	<5	<5	27	<5	–	–
Bailén	R4	<5	64	<5	–	–	15	<5	<5	<5	9	–	–
Bailén	R5	17	22	<5	–	–	15	<5	<5	32	4	–	–
Bailén	R6	21	23	<5	–	–	13	<5	–	34	<5	–	–
Bailén	R7	<5	52	9	5	–	13	<5	<5	13	<5	–	<5
Bailén	R8	12	29	5	<5	–	16	<5	<5	25	6	–	<5
Padul	R9	–	25	10	10	5	23	–	–	15	10	<5	–

Sm, smectite; Kaol, kaolinite; Chl, chlorite; Pg, paragonite; Qtz, quartz; Ab, albite; Or, ortose; Cal, calcite; Dol, dolomite; Gy, gypsum; Py, pyrite.

The chemical composition of clay samples is shown in Table 3. Predominance of Si and Al was the general feature of the samples considered, with lower content of Mg oxides and negligible content of Mn oxides. All of them, except R4, presented high Ca content (≥7.61% CaO), with the highest values corresponding to R5 and R6, due to their carbonate content. Clay R4 exhibited high Fe (8.25% Fe_2O_3) and K contents (6.11% K_2O). The amount of S was higher for R7 and R8, the two clay samples with higher OC content, due to the presence of pyrite.

All samples were alkaline, and the OC content was in general ≤0.4% (except for samples R7 and R8, with an OC content of 0.81 and 0.66%, respectively, Table 4). The values of CEC were in general low and significantly related ($p < 0.05$) with those of BET through the equation $clays_{BET} = 3.61 + 2.97 \times clays_{CEC}$ ($R^2 = 0.602$). The specific surface area, ranging from 14.3 to 60.1 $m^2\ g^{-1}$, was also significantly related ($p < 0.05$) with the concentration of smectite, higher for R5 and R6. The distribution of particle sizes varied also among the samples, coarser for R8 and finer for R4.

3.2. Pesticide analysis

For the relatively hydrophobic fludioxonil, the importance of the filtration step in its analysis should be highlighted. The possible retention of this fungicide on the filters was suspected because of literature reports concerning some hydrophobic fungicides (Pose-Juan et al., 2010). Therefore, the decrease in fludioxonil concentration in the supernatants was evaluated by using different filters and by comparing the fungicide concentration in filtered and non-filtered samples.

The results show (Table 1S, supplementary material) that all kinds of filters considered retained, to a lower or greater extent, the fungicide in the first washing. However, for PVDF filters, pesticide loss was negligible already in the second washing, so they were selected after discarding the first two washings as a precaution.

3.3. Pesticide adsorption

3.3.1. Adsorbent mass

Adsorbent dosage allows establishing the adsorption capacity of the different clays for a given initial concentration and shaking time. The adsorption of both fungicides on the nine different adsorbents was higher with 200 mg clay (Fig. 2), above which an adsorption plateau seemed to be reached. However, with a view to reducing the mass of clay to be employed in pillored seeds and because the errors associated to weighing a clay mass of 50 mg were too big, the adsorbent mass employed in the rest of experiments was 100 mg.

Table 2
Average structural formulae of smectite and illite in selected samples.

	Tetrahedral sheet		Octahedral sheet			Interlayer space			
	Si	Al	Al	Mg	Fe^{3+}	K	Ca	Na	Mg
Smectite									
R5	3.73	0.27	1.40	0.39	0.32	0.18	0.07	0.00	0.02
R6	3.72	0.28	1.26	0.41	0.44	0.21	0.07	0.03	0.00
R7	3.70	0.30	1.38	0.39	0.30	0.35	0.06	0.00	0.00
R8	3.69	0.31	1.27	0.42	0.37	0.41	0.06	0.01	0.00
Illite									
R5	3.35	0.65	1.53	0.37	0.22	0.59	0.03	0.02	0.00
R6	3.34	0.66	1.54	0.34	0.21	0.57	0.00	0.13	0.00
R7	3.36	0.64	1.64	0.29	0.15	0.54	0.02	0.09	0.00
R8	3.38	0.62	1.58	0.29	0.19	0.55	0.05	0.09	0.00

Table 3
X-ray fluorescence analysis of oxide content (%) for the clay samples.

Sample	SiO$_2$ (%)	SO$_3$ (%)	Al$_2$O$_3$ (%)	Fe$_2$O$_3$ (%)	MnO (%)	MgO (%)	CaO (%)	Na$_2$O (%)	K$_2$O (%)	TiO$_2$ (%)	P$_2$O$_5$ (%)	LOI (%)	TOTAL
R1	50.16	0.72	15.80	5.13	0.06	3.03	9.23	0.88	3.04	0.51	0.13	11.41	99.82
R2	44.56	0.24	17.49	5.94	0.07	3.02	9.94	0.99	3.35	0.51	0.12	13.66	99.65
R3	52.78	0.15	10.91	4.16	0.03	1.43	13.06	0.52	2.51	0.44	0.11	13.75	99.58
R4	50.57	0.04	19.42	8.25	0.12	3.05	2.77	0.23	6.11	0.59	0.15	8.47	99.56
R5	46.15	0.18	8.20	3.18	0.04	1.81	18.27	0.20	1.53	0.28	0.10	20.02	99.84
R6	42.80	0.21	8.43	3.05	0.06	1.80	19.79	0.25	1.56	0.28	0.11	21.51	99.58
R7	52.84	2.22	14.53	5.31	0.03	2.16	7.61	0.48	3.27	0.51	0.09	11.31	99.62
R8	48.93	1.66	8.16	3.39	0.03	2.59	15.90	0.39	2.09	0.35	0.10	16.66	99.65
R9	59.72	0.95	14.97	5.42	0.08	3.64	10.47	1.00	3.12	0.52	0.11	12.25	99.62

Table 4
Some clay properties.

	pH	EC (dS m⁻¹)	OC (%)	CEC (cmol₊ kg⁻¹)	BET (m² g⁻¹)	Granulometry		
						< 10 (µm)	10–20 (µm)	20–70 (µm)
R1	9.23	97.1	0.42	5.5	14.3	38.1	17.6	26.1
R2	9.57	71.4	0.33	7.4	18.1	53.3	19.5	22.2
R3	9.57	37.5	0.18	9.2	41.5	41.1	15.7	33.2
R4	9.31	23.9	0.18	8.0	50.2	62.3	20.6	15.3
R5	9.44	42.7	0.24	16.9	60.1	46.1	17.4	32.4
R6	9.43	41.9	0.25	17.9	55.4	50.3	19.6	28.6
R7	9.34	53.1	0.81	13.8	29.6	43.1	19.9	34.5
R8	9.29	75.9	0.66	6.7	17.4	32.9	13.9	42.2
R9	9.22	137.2	0.23	6.6	19.2	48.3	18.1	25.3
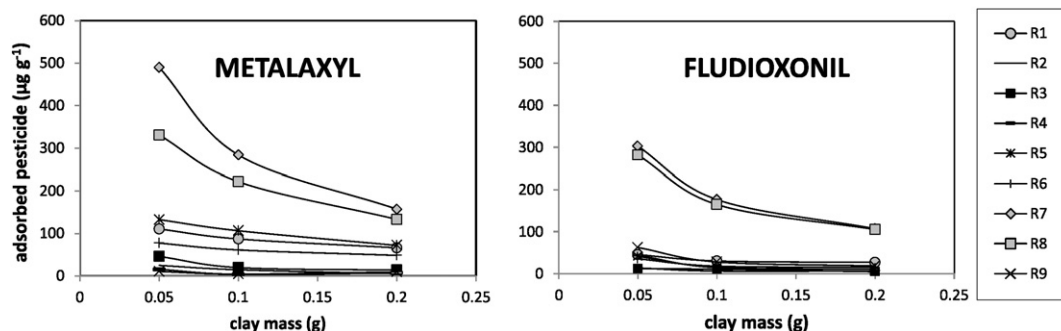
Fig. 2. Influence of clay mass on the adsorption of metalaxyl and fludioxonil.

3.3.2. Adsorption kinetics

Adsorption kinetics was evaluated for metalaxyl with four clays (R1, R5, R7, and R8), and only with two clays (R7 and R8) for fludioxonil (Fig. 3), because this pesticide showed less affinity for the adsorbents, according to the previous experiments with different adsorbent/solution ratios (Fig. 2).

The pseudo-first-order model only fitted weakly the experimental metalaxyl data, with low R^2 values from 0.58 to 0.77 and the calculated X_{max} values greatly underestimated experimental data (Table 5). For fludioxonil, the fitting was improved ($R^2 > 0.93$), though the X_{max} values were still far away from the experimental ones. The kinetic rates for fludioxonil were lower, between one half and one third, than for metalaxyl, which showed more rapid adsorption rates.

On the contrary, the pseudo-second-order model adequately explained the experimental data of metalaxyl and fludioxonil on the different adsorbents with $R^2 > 0.99$ in all cases (Table 5), and was able to estimate with higher confidence the maximum adsorbed values (differences between 2 and 3.5%). Therefore, this model, which considers rapid and slow binding phases, better conformed the adsorption of both fungicides on the different adsorbents, in agreement with other studies concerning adsorption of pesticides on clays or different

adsorbents (Ipek, 2014; Long et al., 2015; Ouali et al., 2015: Azarkan et al., 2016). For metalaxyl, the adsorption rates were ranged as R1 > R8 > R5 > R7. Kinetic rates for fludioxonil were similar on R7 and R8 but lower than those calculated for metalaxyl, as occurred with the rates calculated using the pseudo-first-order kinetic model. Finally, no significant relationship was found for metalaxyl between kinetic rates and maximum pesticide adsorbed, in contrast with previous reports (Ouali et al., 2015).

An initial adsorption rate was calculated as $h_0 = k_2 \times X_{max}^2$ (Ho, 2006) to establish the contribution of the initial mass transport to the whole process (Table 5). For the different clays, the values of h_0 were ranged as R7 ≈ R8 > R1 > R5, showing that the initial metalaxyl adsorption was faster in the first two than in the latter two clays, between 4- and 6-fold higher. For fludioxonil, the initial contribution was similar for R7 and R8 and about 4-times lower than in the same clays when using metalaxyl, reinforcing the abovementioned idea that metalaxyl adsorption proceeded more rapidly than that of fludioxonil.

3.3.3. Adsorption isotherms on untreated clays

In general, adsorption of non-ionic compounds by clays is higher when both the charge density of clays and the hydration energy of the
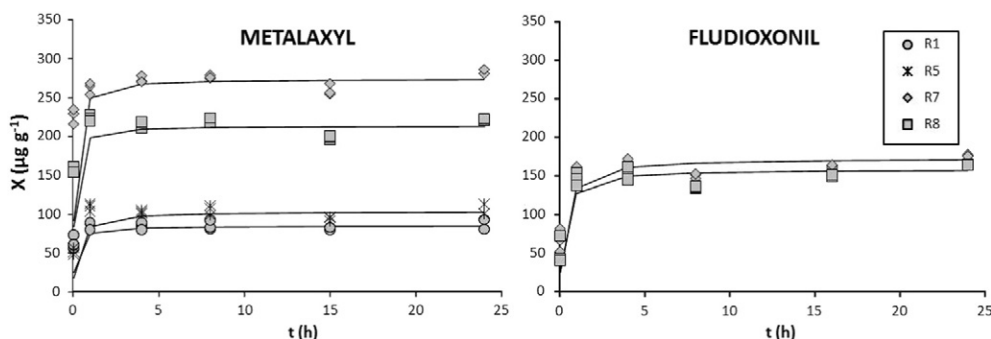
Fig. 3. Kinetics of metalaxyl and fludioxonil adsorption on selected clay samples. Experimental data (symbols) are fitted to the pseudo-second order kinetic equation (lines).

Table 5
Parameters of the fitting to the pseudo-first- and to the pseudo-second-order kinetic models for metalaxyl and fludioxonil on different Spanish clays.

	Metalaxyl				Fludioxonil			
	Pseudo first order kinetic model							
	$X_{max-exp.}$ (µg g^{-1})	$X_{max-calc}$ (µg g^{-1})	k_1 (h^{-1})	R^2	$X_{max-exp.}$ (µg g^{-1})	$X_{max-calc}$ (µg g^{-1})	k_1 (h^{-1})	R^2
R1	87 ± 9	27 ± 2	0.24 ± 0.09	0.582				
R5	107 ± 9	45 ± 4	0.41 ± 0.12	0.627				
R7	284 ± 4	45 ± 3	0.26 ± 0.05	0.755	176 ± 1	96 ± 3	0.13 ± 0.01	0.934
R8	221 ± 1	49 ± 4	0.37 ± 0.07	0.770	164 ± 0.3	100 ± 2	0.13 ± 0.01	0.950
	Pseudo-second-order kinetic model							
	$X_{max-exp.}$ (µg g^{-1})	$X_{max-calc}$ (µg g^{-1})	k_2 (g µg^{-1} h^{-1})	h_0 (µg g^{-1} h^{-1})	R^2	$X_{max-exp.}$ (µg g^{-1})	$X_{max-calc}$ (µg g^{-1})	k_2 (g µg^{-1} h^{-1}) h_0 (µg g^{-1} h^{-1}) R^2
R1	87 ± 9	85 ± 2	0.092 ± 0.024	671	0.991			
R5	107 ± 9	103 ± 3	0.042 ± 0.018	445	0.988			
R7	284 ± 4	275 ± 5	0.037 ± 0.009	2774	0.995	176 ± 1	172 ± 4	0.020 ± 0.004 595 0.992
R8	221 ± 1	214 ± 4	0.061 ± 0.012	2784	0.994	164 ± 0.3	159 ± 4	0.026 ± 0.004 649 0.991

exchangeable cation are low; highly polar, non-ionic pesticides can interact with interlayer cations either directly or through water bridges (Boyd et al., 2011). The adsorption capacity of clays for non-ionic and highly hydrophobic compounds is often considered to be very limited due to the very hydrophilic character of the clay surfaces (Mortland, 1970; Jaynes and Vance, 1996). The adsorption of this type of pesticides by clays has been related to the presence of hydrophobic microsites on the clay mineral surface. In these cases, the organic compound can effectively compete with water molecules for such relatively hydrophobic regions (Laird, 1996; Hundal et al., 2001).

3.3.3.1. Metalaxyl. The adsorption of metalaxyl was ranged as R7 > R8 > R5 > R1 (Fig. 4), following the X_{max} values calculated in the previous kinetic studies.

The fitting to the Langmuir equation was only found to be appropriate for R1 and R8, with determination coefficients ranging from 0.949 to 0.967 (Table 6), with similar K_L values for both clays. For the other two clays this model was not acceptable because it provided negative confidence intervals of X_{max} and K_L, a fact that has been reported in the adsorption of other pesticides on a variety of adsorbents (Rojas et al., 2013; Ouali et al., 2015).

On the contrary, the Freundlich equation was able to fit the adsorption data of metalaxyl on the four clays with R^2 values ≥ 0.970, providing similar (R8) or lower (R1) standard errors of the estimate when compared with the fitting to Langmuir (Table 6). The values of 1/n, lower than 1 for R1 and R8, classified both isotherms as L-type (Giles et al., 1960) which indicates a decrease in specific adsorption sites with concentration as the adsorptive sites become occupied. On the contrary,

clays R5 and R7 present values of 1/n > 1, that is, an S-type isotherm, implying that adsorption increases as the pesticide concentration increases. This result suggests a cooperative adsorption and the formation of multilayers and may explain the lack of fit of the adsorption data from these two clays to the Langmuir equation, since this model assumes monolayer adsorption. According to the Freundlich K_f constants, metalaxyl adsorption was ranged as R7 > R8 > R5 ≈ R1.

Though relatively high, the adsorption capacity of R7 clay is approximately 10 times lower than that of a natural stevensite from North of Morocco (Azarkan et al., 2016). However the proposed clay would be appropriate since minute amounts of fungicide need to be released in the microenvironment surrounding a seed.

3.3.3.2. Fludioxonil. The adsorption of fludioxonil was evaluated on R7 and R8, the two clays with higher adsorption capacity for this fungicide.

The adsorption experimental data were fitted to the Langmuir equation with R^2 > 0.91 and with K_L values higher than those corresponding to the adsorption of metalaxyl (Table 6).

The fitting to the Freundlich equation was appropriate, but R^2 values (>0.97) and standard errors of the estimate were roughly similar to the ones provided by the fitting to the Langmuir model. Therefore, both models could adequately explain the behavior of this fungicide. The 1/n values were lower than 1 (L-type), indicating a decrease in adsorption with pesticide concentration increase, which suggests a clay surface coverage, reaching a plateau beyond which no adsorption occurs.

Because comparison of K_f values is not appropriate when 1/n values are different (Delgado-Moreno et al., 2010), a distribution coefficient (K_d) was calculated for a C_e = 1.5 mg L^{-1}, a concentration value
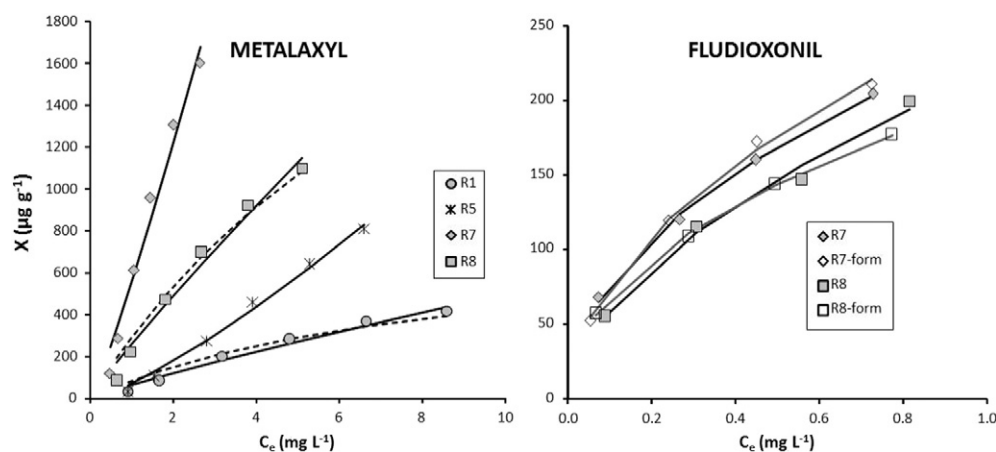
Fig. 4. Isotherms of the adsorption of metalaxyl and fludioxonil on selected Spanish clays. The experimental values (symbols) are fitted to the Langmuir (dotted lines) and to the Freundlich (straight lines) equations. In the case of fludioxonil, isotherms were performed with the fungicide standard (grey symbols) and with an EC formulation (open symbols).

Table 6
Parameters of the fitting to the Langmuir and Freundlich equations for the adsorption of metalaxyl and fludioxonil o on natural Spanish clays.

Metalaxyl									
	Freundlich					Langmuir			
	K_f (μg^{1-n} mL^n g^{-1})	$1/n$	K_d (mL g^{-1})[a]	SEE[b]	R^2	K_L (L mg^{-1})	X_{max} (μg g^{-1})	SEE	R^2
R1	68 ± 7	0.87 ± 0.05	65	24.76	0.971	0.12 ± 0.04	772 ± 114	32.56	0.949
R5	75 ± 6	1.28 ± 0.04	84	26.56	0.991	Not fitted			
R7	562 ± 29	1.12 ± 0.06	590	94.25	0.970	Not fitted			
R8	263 ± 17	0.90 ± 0.05	253	58.03	0.976	0.10 ± 0.03	3300 ± 600	57.25	0.976
Fludioxonil									
	Freundlich					Langmuir			
	K_f (μg^{1-n} mL^n g^{-1})	$1/n$	K_d (mL g^{-1})	SEE	R^2	K_L (L mg^{-1})	X_{max} (μg g^{-1})	SEE	R^2
R7	238 ± 3	0.49 ± 0.02	194	15.65	0.993	1.62 ± 0.48	389 ± 69	13.35	0.911
R8	217 ± 6	0.56 ± 0.04	182	9.12	0.972	2.02 ± 0.50	304 ± 36	12.76	0.945
R7-formulation	253 ± 5	0.52 ± 0.02	208	6.51	0.989	Not fitted			
R8-formulation	199 ± 3	0.47 ± 0.02	161	4.30	0.991	Not fitted			

[a] K_d, distribution coefficient for a C_e value 1.5 mg L^{-1}.
[b] SEE, standard error of the estimate.

included in the isotherms of both fungicides (Table 6). The values of K_d for R7 and R8 indicated that adsorption was higher for metalaxyl, a more polar fungicide, than for fludioxonil (Fig. 4), a fact also confirmed by the maximum adsorption capacities (X_{max}) calculated by the Langmuir equation. This result suggests that polar interactions were the driving forces of the adsorption of metalaxyl on the clays. Azarkan et al. (2016), who studied the adsorption of two fungicides on natural Moroccan clays, attributed the strong retention of metalaxyl to the presence of functional groups, in particular carbonyl groups (Fig. 1), which could interact with other functional groups in the clays though hydrogen bonds and electrostatic interactions.

Additionally, in the case of fludioxonil, its adsorption was evaluated not only using the pesticide standard but also a formulation, since it has been established that the adjuvants included in the pesticide formulation may affect pesticide adsorption. The literature has reported controversial results showing either no effect (Fernández-Bayo et al.,

2007) or a strong influence, due to a solubilization increase in the presence of adjuvants (Pose-Juan et al., 2011). In our case a slight or negligible effect on fludioxonil retention was found (Fig. 4).

3.3.4. Adsorption isotherms on treated clays

An exploratory cluster analysis, undertaken to assess the variables which affected to a greater extent the adsorption of both pesticides, showed that it was related with the OC content of the adsorbate and the particle size distribution, rather than mineralogy (Fig. 2S, supplementary material). This would explain the greater fungicide adsorption for R7 with the highest OC content and high proportion of particles included in the 20–70 μm range, while R8, with relatively high OC content, exhibited the lowest proportion of finest particles (<20 μm). To confirm the effect of OC, clays were treated with H_2O_2 to partially remove clay OC and new adsorption experiments were undertaken with the modified clays.
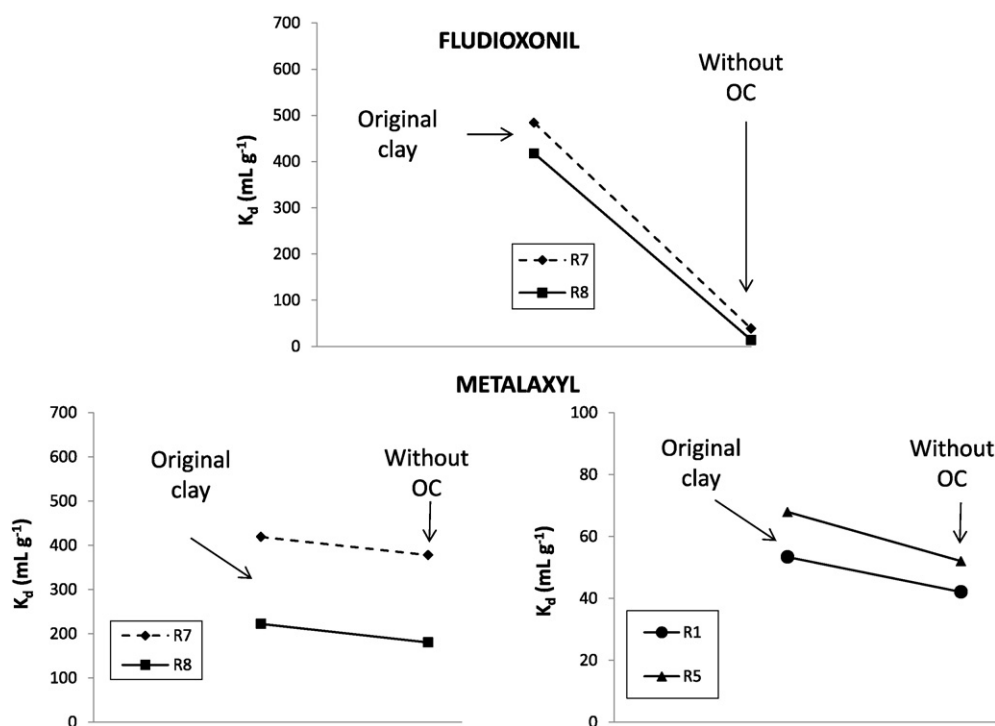
Fig. 5. Reduction of the distribution adsorption coefficients (K_d) for fludioxonil and metalaxyl on selected clays after treatment with H_2O_2.

Table 7
Some properties of the clays after treatment with H_2O_2.

	pH	EC (dS m^{-1})	OC (%)
R1	9.38	47.65	0.29
R5	9.32	43.35	0.09
R7	9.32	46.75	0.77
R8	9.26	45.15	0.45

As can be seen in Fig. 5, the partial elimination of clay OC resulted in a reduction of adsorption extent in all cases. However, this reduction was lower for metalaxyl (between 10 and 23%) than for fludioxonil (92% in R7 and 97% in R8), highlighting that OC was more relevant for this hydrophobic compound than for the more polar metalaxyl. To our knowledge, no previous studies concerning fludioxonil adsorption on clays have been reported. However, the relationship between OC content in soils and fludioxonil adsorption has been shown previously (Pose-Juan et al., 2011; Fenoll et al., 2011).

If the values of K_{OC} are calculated, a parameter that takes into account the adsorption relative to the OC content, fludioxonil K_{OC} values decreased after treatment with H_2O_2, that is, the remaining OC was either less available or less effective in the retention of this fungicide (Fig. 3S, supplementary material). On the contrary for metalaxyl the partial elimination of OC led to an increase of K_{OC} values in R7 and R8, a fact that suggests that either the remaining OC in both clays was able to selectively enhance the retention of this fungicide or else that the treatment with H_2O_2 liberated adsorption sites specific for metalaxyl adsorption because of the destruction of the organic molecules retained on the adsorption sites or due to a modification of the clay structure as a consequence of the treatment with the oxidant. However, the examination of the clays by X-ray analysis after treatment with H_2O_2 confirmed the absence of clay modification. Other reports have indicated that, apart from reducing the OC content, treatment with hydrogen peroxide may modify other sample properties (Fernandes et al., 2003). In our case, treatment with H_2O_2 led to a slight pH decrease and to a general reduction of EC, more marked for R1 and R8 (Table 7). Elimination of organic matter associated to the clay surfaces has been claimed to increase the surface available for metalaxyl adsorption (Fernandes et al., 2003), in agreement with our own results, though no report on the influence on fludioxonil adsorption has been found. As can be seen, pesticide adsorption behavior depends greatly on pesticide polarity and on the specific interactions between the adsorbent and the adsorbate.

3.4. Desorption experiments and stability in storage

During storage, fungi may accelerate the deterioration of seeds, resulting in reduced germination and vigor (Nisar et al., 2009). Therefore, the stability of metalaxyl release for prolonged periods was evaluated at two temperatures (10 and 20 °C), conditions normally used in

seed storage. This fungicide was selected as the best candidate because it is further retained by the clays (Table 6).

The results show that metalaxyl desorption remained stable along the storage period and that, in general, desorption from the four clays was inversely related with pesticide adsorption (Fig. 6). However, desorption from R1 was initially lower than from R5, contrary to what was observed in adsorption experiments, but after approximately a month, desorption increased and remained stable for the rest of the evaluated period. Both temperatures (10 and 20 °C) preserved similarly the retained pesticide for the period evaluated. Previous reports on the stability of various pesticides in pellet formulations have shown losses up to 90% of the initial pesticide concentration depending on pellet type, storage time, and pesticide nature (Huijbregts et al., 1995).

Therefore, the clays proved to retain in a stable way the pesticide both at 10 and 20 °C and for long periods of time (for at least 6 months), indicating the feasibility of using them as adsorbents to retain pesticides.

3.5. Evaluation of phytotoxicity

Phytotoxicity is defined as a delay in seed germination, inhibition of plant growth or any adverse effect on plants caused by specific substances or growing conditions. The ability of a particular compound to diffuse through the seed coat is related to the physico-chemical properties of the compound applied (Briggs et al., 1982; Peña et al., 2014) and to the chemical nature of the seed-covering tissues, which have been classified as permeable, non-permeable, or selectively permeable (Taylor and Salanenka, 2012). Therefore, seed treatments may be phytotoxic to one plant family and not injurious to another one (Ma et al., 2015).

Onions were selected because this crop represents the third largest fresh vegetable industry in the U.S., with a per capita consumption of about 20 lb. per year (National Onion Association) and because their seeds belong to the selectively permeable group, which means permeability to non-ionic, moderately lipophilic compounds (Beresniewicz et al., 1995). Additionally, this plant species has been previously used in studies of different plant stresses (Whalley et al., 1999, 2001).

Results showed that the type of the pesticide in the pills did not have a significant effect ($P > 0.05$) on the plant endpoints. Therefore, the relatively polar metalaxyl (log K_{ow} 1.75) and the more hydrophobic fludioxonil (log K_{ow} 4.12) similarly affected onion growth. On the contrary, pesticide concentration led, in general, to a significant decrease in the various plant endpoints measured, except in bulb diameter for which no significant differences were observed or in root elongation (Table 8). Root elongation increased as the pesticide concentration increased, contrary to expectations. However, roots from these treatments were fragile and brittle and root elongation did not result in a concomitant increase in root biomass (Table 8), indicating that the root system was less dense or branched. This behavior may be an adaptation to cope with the stress produced by the pesticides provided.

In this preliminary assay, some phytotoxicity was observed in onion seeds pillored with metalaxyl and fludioxonil, an effect also described
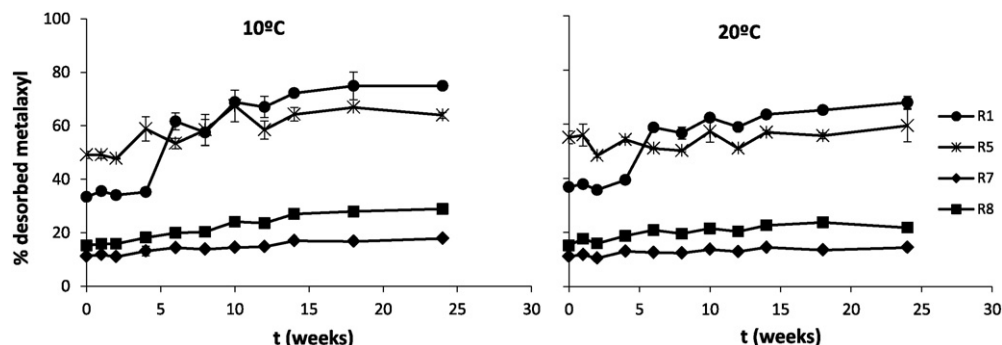
Fig. 6. Stability of metalaxyl desorption from fortified clay samples stored at two temperatures, 10 and 20 °C. Vertical bars indicate standard deviation (n = 3).

Table 8
Phytotoxicity test with *Allium cepa* L. pillored seeds exposed to different pesticide concentrations. Numbers indicate average plant endpoints ($\pm$ standard deviation).

Pesticide	Concentration	Shoot length (mm)	Root length (mm)	Bulb Ø (cm)	Shoot biomass (g)	Root biomass (g)	Bulb biomass (g)
Control		161.7 ± 18.1	37.2 ± 11.6	2.42 ± 0.31	0.23 ± 0.06	0.029 ± 0.016	0.023 ± 0.006
Metalaxyl	Low[a]	145.3 ± 23.9	36.4 ± 10.2	2.29 ± 0.27	0.16 ± 0.05	0.017 ± 0.009	0.018 ± 0.006
	High[b]	127.4 ± 47.1	50.6 ± 23.6	2.25 ± 0.50	0.18 ± 0.12	0.023 ± 0.015	0.021 ± 0.009
Fludioxonil	Low	141.0 ± 28.4	35.9 ± 8.7	2.15 ± 0.56	0.19 ± 0.09	0.026 ± 0.015	0.018 ± 0.008
	High	141.8 ± 40.6	42.9 ± 9.7	2.34 ± 0.43	0.20 ± 0.08	0.016 ± 0.007	0.020 ± 0.007

[a] Low concentration, 167/250 mg metalaxyl/fludioxonil per kg of seed.
[b] High concentration, 334/500 mg metalaxyl/fludioxonil per kg of seed.

for cyromazine in treated onion seeds (Taylor et al., 2001) or for di-n butylphtalate (Ma et al., 2015). Improvement of the seed coating process to alleviate phytotoxicity, knowledge of the behavior of other plant species, and evaluation of effective pesticide concentrations without phytotoxic effects are aspects which deserve further detailed investigation.

4. Conclusions

Natural clays, abundant and therefore cheap materials, could be used for the retention of organic pollutants, such as pesticides, which are widely spread in the environment due to agricultural activities. In addition, clays could be used as carriers in seed coatings for the delivery of pesticides to protect seeds from different pests and diseases in early development stages. In the evaluated clays, the adsorption of metalaxyl, a polar fungicide, was more rapid and higher than that of fludioxonil, a more hydrophobic compound, probably because electrostatic interactions or hydrogen bonds were the mechanisms involved. The clay OC content and the granulometry seemed to play a fundamental role in the retention of metalaxyl and fludioxonil. The partial removal of OC, by treating the clay with an oxidant, led to a higher reduction of fludioxonil retention, leaving unaffected other clay properties, such as pH, EC, or crystalline structure.

Finally, if clays are envisaged for seed treatments, the retention of metalaxyl has proven to be stable at two temperatures and for long periods of time, indicating that this strategy could be safely used. Future research will be needed to assess the antifungal activity of the proposed coating using biocidal assays in different plant species.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.clay.2016.07.006.

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