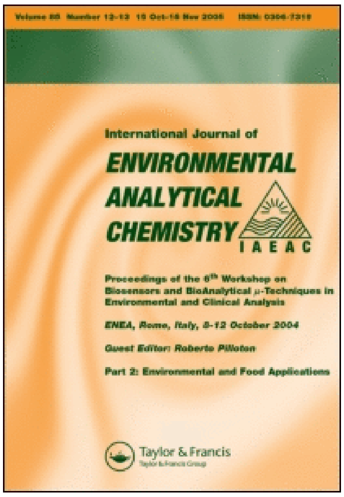

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Effect of the modification of natural clay minerals with hexadecylpyridinium cation on the adsorption-desorption of fungicides

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To cite this Article Andrades, M. Soledad , Rodríguez-Cruz, M. Sonia , Sánchez-Martín, M. Jesús and Sánchez-Camazano, Maria(2004) 'Effect of the modification of natural clay minerals with hexadecylpyridinium cation on the adsorption-desorption of fungicides', International Journal of Environmental Analytical Chemistry, 84: 1, 133 — 141

To link to this Article: DOI: 10.1080/03067310310001593701

URL: http://dx.doi.org/10.1080/03067310310001593701

Intern. J. Environ. Anal. Chem.
Vol. 84, No. 1–3, pp. 133–141, January–March 2004

EFFECT OF THE MODIFICATION OF NATURAL CLAY MINERALS WITH HEXADECYLPYRIDINIUM CATION ON THE ADSORPTION–DESORPTION OF FUNGICIDES

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(Received 14 November 2002; In final form 6 May 2003)

Clay minerals, montmorillonite (M), illite (I), kaolinite (K), muscovite (Mu), sepiolite (S) and atapulgite (A), modified with the organic cation hexadecylpyridinium (HDPY) were studied as sorbents of two fungicides of different hydrophobicity, penconazole and metalaxyl, using adsorption–desorption isotherms. The Freundlich constant values were low for the adsorption of the fungicides by natural clays and increased when the minerals were saturated with the organic cation. In HDPY-M, the K_f constant increased 190-fold for the adsorption of penconazole and 67-fold for the adsorption of metalaxyl. Adsorption of the fungicides by the organo-clays at an initial concentration of 25 μg/mL was related to their organic matter content, the highest adsorption values being found in HDPY-M, HDPY-I and HDPY-A, and the increase in adsorption was greatest for the more hydrophobic fungicide penconazole. Desorption of fungicides from modified clays pointed to a high degree of stability for penconazole adsorbed by HDPY-M. In the remaining samples, the stability of this pesticide was low and the stability of metalaxyl adsorbed by all the organo-clays was also low. The results obtained point to the potential value of clay minerals modified with HDPY for immobilising pesticides with different degrees of hydrophobicity. Of special interest is the HDPY-M sample, which could be considered for use in clay barriers aimed at protection of soil and water pollution by hydrophobic pesticides.

Keywords: Organo-clays; Adsorption; Desorption; Penconazole; Metalaxyl; Clay minerals; Hexadecylpyridinium

INTRODUCTION

The increasing presence in ground waters of pesticides used in agriculture is generating important environmental concern [1, 2]. This presence has generally been attributed to the diffuse contamination of soils arising from the use of pesticides. However, studies

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ISSN 0306-7319 print: ISSN 1029-0397 online
DOI: 10.1080/03067310310001593701

carried out in the past few years [3, 4] have indicated that soil pollution from point sources, characterised by involving significant amounts of pesticide and being highly localised in space and time, may be more important than diffuse contamination.

Most agricultural pesticides are hydrophobic organic compounds and their adsorption and mobility in soil depends on the soil organic matter content and the water solubility of the pesticide in question [5–7]. Natural clay minerals are not good adsorbents of these compounds but their adsorption capacities can be enhanced by replacing the exchangeable inorganic cations by cationic surfactants, because this changes the clay surface from hydrophilic to hydrophobic [8, 9]. Having this organic phase bound to the surface of these minerals means that organo-clays could be used as barriers for immobilizing pesticides deriving from point sources, thereby preventing the pollution from spreading through the soil and later contaminating ground waters. Organo-clays, which are generally prepared from the clay mineral montmorillonite, have usually been considered as good adsorbents of non-ionic organic contaminants from industrial wastes such as phenols, chlorophenols or benzene derivatives [10–13]. However, there are few reports of the adsorption of pesticides by organo-clays [14–17] and very few instances dealing with organo-clays prepared from minerals other than montmorillonite [11,18].

In the light of the above, this report addresses the adsorption–desorption of two fungicides of different hydrophobic character, penconazole and metalaxyl, from water by natural clay minerals of different structures (montmorillonite, illite, kaolinite, muscovite, sepiolite and atapulgite) and by these minerals following modification with the cationic surfactant hexadecylpyridinium bromide (HDPY). The aim of this study is to assess the possible use of these materials as barriers for the immobilisation of pesticides in soils and to prevent the pollution of soils and waters by these compounds.

EXPERIMENTAL

A series of clay minerals ($<0.1\,\mathrm{mm}$) with different structures was used: Tidinit montmorillonite (M) (Morocco), Cuenca kaolinite (K) (Spain), Fithian illite (I) (Illinois), Peñausende muscovite (Mu) (Spain), Vallecas sepiolite (S) (Spain) and Bercimuel atapulgite (A) (Spain), both in natural form and saturated with HDPY. The cation exchange capacity (CEC) of natural clays as determined by the ammonium acetate method [19] is indicated in Table I.

Unlabelled and labelled ^{14}C-penconazole (specific activity 1.02 MBq/mg) and ^{14}C-metalaxyl (specific activity 1.37 MBq/mg) were supplied by Novartis Crop Protection AG (Basle, Switzerland). Penconazole is a solid hydrophobic compound with water solubility 73 µg/mL and $\log K_{\mathrm{ow}}$ of 3.72 and metalaxyl is a solid compound with water solubility 8.4 mg/mL and $\log K_{\mathrm{ow}}$ of 1.75 (20).

Natural clays were saturated with the organic cation HDPY, using 100 mg of clay and 50 mL of an aqueous solution of HDPY bromide containing an amount of organic cation equivalent to 1.25 times the clay CEC. The samples were washed with water, freeze-dried and kept in an atmosphere with a relative humidity of 35% for later use. The carbon content of natural and saturated clays was determined using an automatic carbon analyser. Table I shows the carbon and organic matter (OM) contents and the *d*(001) basal spacings as determined by X-ray diffraction in the natural and saturated samples. The *d*(001) basal spacings of natural samples and organo-clays indicated

TABLE I Characteristics of natural clays and organo-clays studied

Samples	*CEC* (cmol/kg)	*OC* (%)	*OM* (%)	*CEC* (*Saturation* %)	d_{001} (Å)
Montmorillonite (M)	82	0.06	0.10	–	13.38
Kaolinite (K)	6.1	0.10	0.17	–	7.16
Illite (I)	15	1.74	2.99	–	10.04
Muscovite (Mu)	21	0.10	0.17	–	10.04 (12.62)[a]
Sepiolite (S)	5.0	0.08	0.13	–	12.26
Atapulgite (A)	27	0.96	0.79	–	10.64
HDPY-M	–	17.2	20.8	82	21.5
HDPY-K	–	1.60	1.94	97	7.16
HDPY-I	–	5.42	6.56[b]	96	10.04
HDPY-Mu	–	4.87	5.89[c]	90	10.04 (21.5)[a]
HDPY-S	–	1.19	1.44	88	12.26
HDPY-A	–	8.00	9.68	110	10.64

[a]Montmorillonite impurities; [b]total OM content from natural clay and from organic cation; [c]corresponding to saturation of Mu and M present as impurities with organic cation.

the presence of montmorillonite impurities in the Mu sample and an increase in the *d*(001) basal spacing of the M sample (21.5 Å) and in the *d*(001) basal spacing of M present in the Mu sample when saturated with HDPY cation. These increases point to the intercalation of this cation in the interlayer space of montmorillonites. The HDPY cation did not alter the X-ray diffraction patterns of other non-expansible minerals.

Adsorption isotherms of both fungicides by natural and organo-clays were obtained in aqueous medium using the batch equilibrium technique. Duplicate 50-mg samples were treated with 10 mL of aqueous solution of C^{14}-penconazole or C^{14}-metalaxyl at concentrations 5, 10, 15, 20 and 25 μg/mL and activity 100 Bq/mL. These solutions were obtained from 1000-μg/mL fungicide stock solutions prepared by dissolving the solid product in methanol. The suspensions were shaken intermittently at 20°C for 24 h in a thermostatted chamber (2 h every 4 h). Preliminary experiments revealed contact for 24 h to be long enough for equilibrium to be reached. Subsequently, the suspensions were centrifuged at 5000 *g* for 15 min. To determine the pesticide concentration at equilibrium, a 1.0-mL aliquot of supernatant solution was mixed with 4 mL of scintillation liquid and its activity was measured in disintegrations per minute (dpm) on a Beckman LS 1800 liquid scintillation counter. The dpm value recorded for the supernatant aliquot was related to the dpm obtained for the aliquots of the respective standards of fungicide solutions and the penconazole or metalaxyl equilibrium concentration was determined. The amount of fungicide adsorbed was considered to be the difference between that initially present in solution and that remaining after equilibration with the soil.

Desorption of penconazole and metalaxyl was studied in clay samples initially treated with 25-μg/mL fungicide solution during the adsorption study. After adsorption equilibrium had been reached, 5 mL were removed from the solution and immediately replaced by 5 mL of water. The resuspended samples were shaken for 24 h at 20°C, after which the suspensions were centrifuged and the desorbed fungicide was measured as reported above. This desorption procedure was repeated four times for each soil. The amount of compound adsorbed by the clay at each desorption stage was calculated as the difference between the initial amount adsorbed and the amount desorbed. All determinations were carried out in duplicate.

RESULTS AND DISCUSSION

Figures 1 and 2 show the adsorption–desorption isotherms of penconazole and metalaxyl by natural clays and organo-clays. The shape of the isotherms shows that the adsorption capacity of all the organo-clays is greater than that of the natural samples, the increase being higher in the samples with higher organic matter contents. Additionally, for all the adsorbents the adsorption of penconazole is greater than that of metalaxyl. According to the classification of Giles *et al.* [21] the isotherms for natural clays are L or S type and the isotherms for the organo-clays are L or C type, indicating the good affinity of the organic adsorbents for both fungicides.

The adsorption data were fitted to the linearized form of the Freundlich adsorption equation

$$\log C_s = \log K_f + n_f \log C_e$$

with r values ≥ 0.97 where C_s (μg/g) is the amount of adsorbed fungicide, C_e (μg/mL) is the equilibrium concentration of fungicide in solution, and K_f (μg^{1-n_f} mL^{n_f} g^{-1}) and n_f are two characteristic constants of fungicide adsorption. The K_f and n_f constants obtained from this equation together with the K_{om} values ($K_{om} = K_f \times 100/\%OM$) are shown in Table II for penconazole and metalaxyl.

K_f values for penconazole adsorption by natural clays varied between 8.11 (A) and 40.2 (M). These values increased in all the samples saturated with the organic cation, the greatest increase (190-fold) corresponding to the HDPY-M sample (Table II). The n_f values ranged between 0.53 and 1.12 in natural clays and in general they decreased in all the organo-clays (0.65–0.88). Adsorption constants normalised with respect to 100% of OM ($\log K_{om}$) are very close (4.11–4.56) for all the organo-clays, indicating adsorption of the fungicide by the hydrophobic surface created by saturation of the clay minerals with the organic cation. These $\log K_{om}$ values are higher than both the $\log K_{ow}$ value of penconazole (3.72) and the $\log K_{om}$ values found for the adsorption of penconazole by soils of different characteristics (3.01–3.68) [22], indicating that the HDPY-derived organic phase in different clays is more efficient than octanol and the organic matter of soils in removing penconazole from water.

To compare the adsorptive capacity of the organo-clays, an efficiency coefficient for fungicide adsorption, E_{ads}, was calculated from the relationship between the amounts of fungicide adsorbed by the HDPY samples and by the natural samples. Table III shows the adsorption percentages of penconazole (Ads %) by all the samples obtained for an initial fungicide concentration of 25 μg/mL. Adsorption by the natural clays is higher in I and S than in the rest of samples. In the organo-clays, adsorption of the fungicide was related to the organic matter content, the highest adsorption values being found in the organo-clays corresponding to samples with higher cation exchange capacities (HDPY-M, HDPY-I and HDPY-A) [11]. The efficiency coefficients for penconazole adsorption, E_{ads}, were in the following order: HDPY-A > HDPY-M > HDPY-K > HDPY-Mu > HDPY-I > HDPY-S in the modified clays. High efficiency coefficients were observed in the samples with very low adsorption by natural samples.

The desorption isotherms of the fungicides were fitted to the linearized form of the Freundlich equation:

$$\log C_s = \log K_{fd} + n_{fd} \log C_e,$$

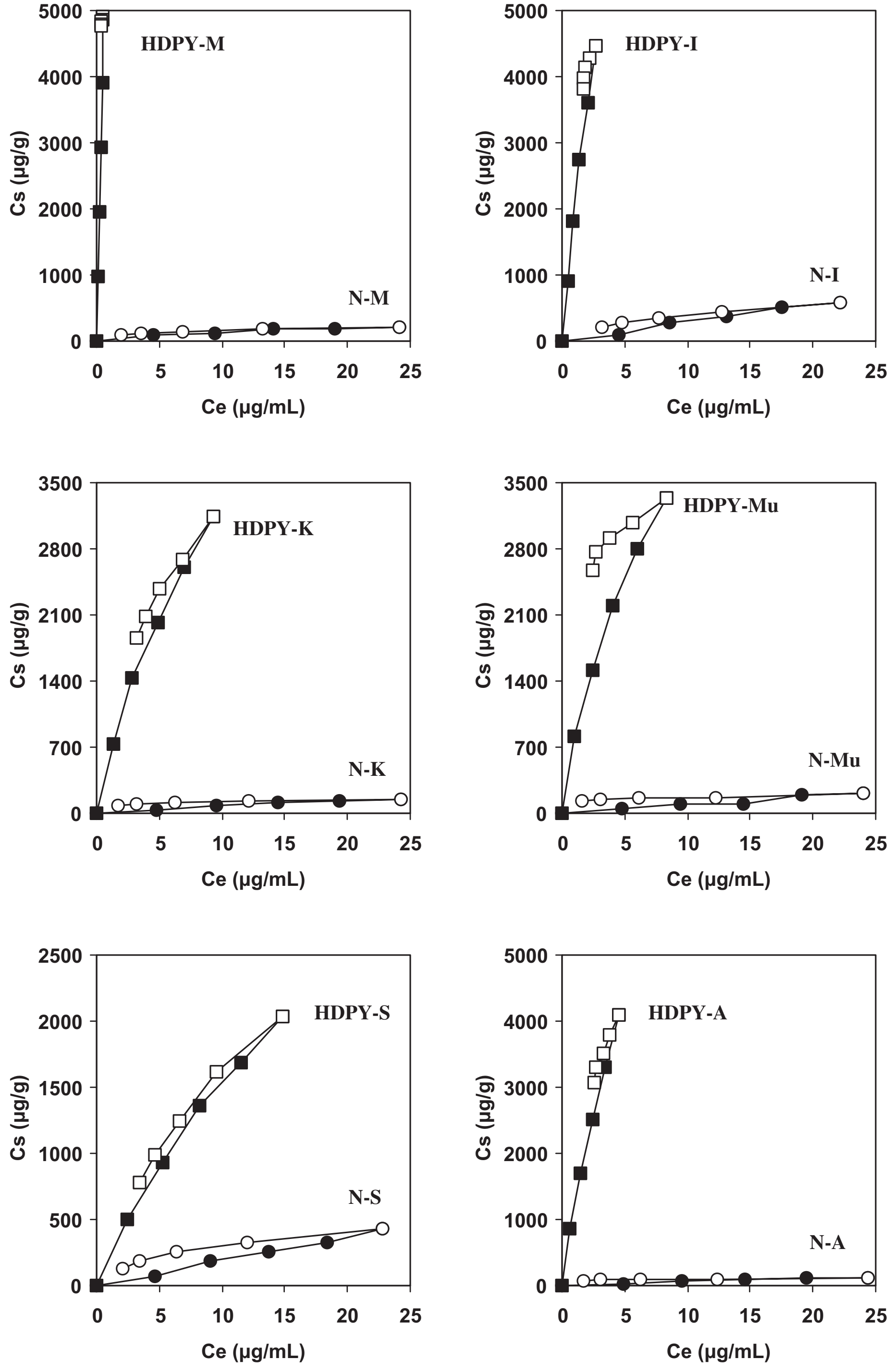

FIGURE 1 Adsorption isotherms (full symbols) and desorption isotherms (open symbols) of penconazole by natural clays and HDPY-clays.

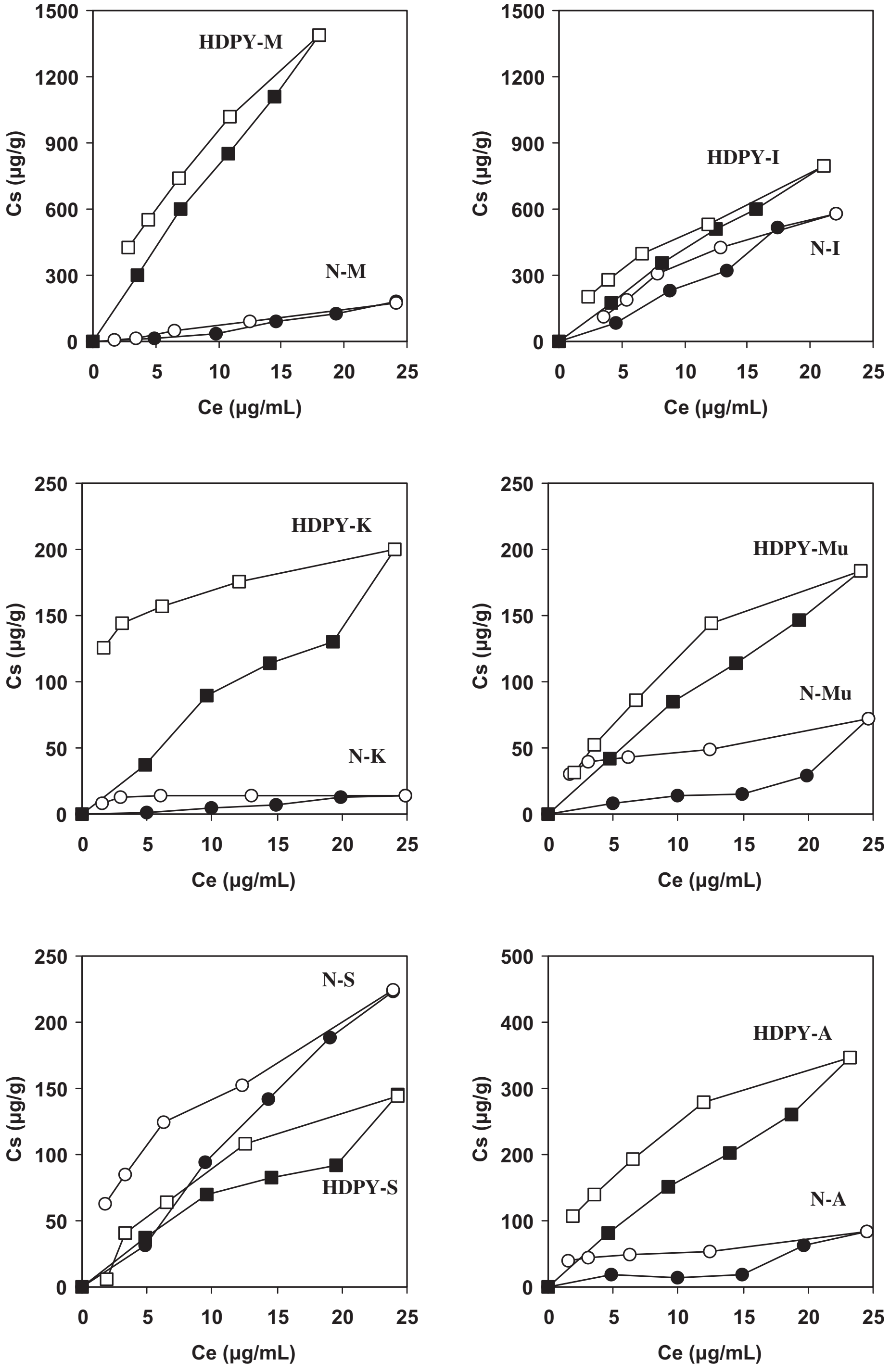

FIGURE 2 Adsorption isotherms (full symbols) and desorption isotherms (open symbols) of metalaxyl by natural clays and HDPY-clays.

TABLE II Adsorption and other constants for penconazole and metalaxyl in natural clays and organo-clays

Sample	*Penconazole*						*Metalaxyl*					
	K_f	n_f	K_{om}	K_{fd}	n_{fd}	H[a]	K_f	n_f	K_{om}	K_{fd}	n_{fd}	H[a]
M	40.2	0.53	–	79.9	0.31	1.72	1.27	1.54	–	3.17	1.31	1.17
K	11.3	0.83	–	78.2	0.20	4.25	0.05	1.84	–	8.93	0.17	10.8
I	24.1	1.06	–	127	0.49	2.17	14.1	1.23	–	39.0	0.91	1.34
Mu	9.39	0.98	–	121	0.15	6.71	1.08	1.15	–	26.5	0.29	4.04
S	13.4	1.12	–	97.5	0.49	2.30	5.02	1.22	–	48.0	0.48	2.56
A	8.11	0.88	–	57.2	0.21	4.08	2.49	0.98	–	33.9	0.24	4.09
HDPY-M	7956	0.86	4.56	–	–	–	96.6	0.92	2.67	215	0.65	1.43
HDPY-K	627	0.73	4.51	1067	0.48	1.52	8.82	0.96	2.66	116	0.17	5.68
HDPY-I	1958	0.88	4.47	3321	0.31	2.83	50.2	0.91	2.88	122	0.61	1.50
HDPY-Mu	858	0.65	4.16	2245	0.19	3.50	10.8	0.89	2.26	20.5	0.73	1.23
HDPY-S	244	0.79	4.23	347	0.67	1.18	11.9	0.74	2.92	5.75	1.13	0.66
HDPY-A	1238	0.79	4.11	2002	0.48	1.66	21.4	0.87	2.34	83.7	0.49	1.76

[a] Hysteresis coefficient.

TABLE III Amount of penconazole and metalaxyl adsorbed (Ads) and desorbed (Des) in natural clays and organo-clays, and efficiency coefficients for the adsorption (Eads)

Sample	*Penconazole*			*Metalaxyl*		
	Ads[a] (%)	*Eads*[b]	*Des*[c] (%)	*Ads*[a] (%)	*Eads*[b]	*Des*[c] (%)
M	4.03	–	53.4	3.58	–	96.7
K	2.98	–	40.9	0.29	–	44.4
I	11.5	–	61.9	11.5	–	91.9
Mu	4.14	–	37.8	1.44	–	66.5
S	8.64	–	60.0	4.46	–	73.3
A	2.32	–	30.5	1.69	–	41.3
HDPY-M	98.1	24.3	2.99	27.8	7.76	69.5
HDPY-K	62.7	21.0	41.1	4.00	13.9	37.4
HDPY-I	89.3	7.78	14.6	15.9	1.38	74.3
HDPY-Mu	66.7	16.1	22.8	3.66	2.55	82.9
HDPY-S	40.7	4.72	62.7	2.89	0.65	95.8
HDPY-A	81.7	35.2	25.4	6.90	4.07	69.1

[a] Expressed as percentage of initial amount of fungicide in solution ($C_i = 25\,\mu g/mL$); [b] relationship between amounts of fungicide adsorbed by the organo clay and by the respective natural clay; [c] expressed as percentage of initial amount of fungicide adsorbed.

with r values ≥ 0.96, where C_s (μg/g) is the amount of fungicide still adsorbed; C_e (μg/mL) is the equilibrium concentration of fungicide in solution after desorption, and K_{fd} ($\mu g^{1-n_{fd}}$ mL$^{n_{fd}}$ g^{-1}) and n_{fd} are two characteristic constants of fungicide desorption. The values of the K_{fd} and n_{fd} constants for penconazole and metalaxyl are shown in Table II.

All desorption isotherms displayed positive hysteresis, the K_{fd} values being higher than the K_f values in all the samples [23]. The hysteresis coefficients H, determined from n_f/n_{fd} ratio [24] indicate the greater or lesser irreversibility of adsorption in all the samples, the highest values corresponding to the natural samples for which the lowest adsorption constants were obtained (K, Mu and A) and to the HDPY-M saturated sample for which the highest adsorption constant was obtained.

The desorption percentages of penconazole from all the samples after four desorptions (Des %) are indicated in Table III. In the HDPY-M sample, the desorption percentage was 17-fold lower than the desorption percentage of the fungicide from the natural M sample. A decrease, although less marked, was also observed in the HDPY-I sample, while in the rest of the samples the desorption percentages from the natural samples and from the modified samples were similar. These findings could be explained because in HDPY-M the whole surface must be completely covered by the organic cation while in the other samples saturation with organic cation would be expected to result in small and isolated zones of adsorbed HDPY [11].

The Freundlich K_f constant values for metalaxyl adsorption by natural clays varied between 0.05 (K) and 14.1 (I) (Table II). In the organo-clays, metalaxyl adsorption increased in all samples, although to a lower extent than penconazole adsorption, and adsorption was also seen to be related to the organic matter content of the samples. The n_f values were higher than 1 in natural clays and they decreased in organo-clays ranging within a tight range (0.74–0.96). The $\log K_{om}$ values are also very similar (2.26–2.92) for all the organo-clays, and higher than the $\log K_{ow}$ of metalaxyl (1.75) and the $\log K_{om}$ for the adsorption of metalaxyl by soils (1.21–1.83) [25]. As in the case of penconazole, the efficiency of the HDPY-derived organic phase in different clays was also greater than the octanol and organic matter of soils in removing metalaxyl from water. However, the K_{om} values are lower than for penconazole owing to the less hydrophobic nature of metalaxyl.

The adsorption percentages of metalaxyl for all the samples obtained for an initial fungicide concentration of 25 μg/mL (Table III) point to the higher adsorption by the natural samples of M, I and S. In the modified samples, the highest adsorption percentages were obtained for HDPY-M, HDPY-I and HDPY-A. The efficiency coefficients, E_{ads}, for metalaxyl adsorption by the organo-clays followed the order HDPY-K > HDPY-M > HDPY-A > HDPY-Mu > HDPY-I > HDPY-S.

Desorption isotherms of metalaxyl displayed positive hysteresis, the K_{fd} values being in general higher than those of K_f. The hysteresis coefficients, H, determined from the desorption isotherms also point to the greater irreversibility of metalaxyl adsorption in the natural K, Mu and A samples, with the lowest adsorption constants. These coefficients increased slightly in the HDPY-M and HDPY-I modified samples and decreased in the rest of the samples. The desorption percentages of metalaxyl from the organo-clays with respect to the natural clays were only lower in the HDPY-M, HDPY-I and HDPY-K samples.

CONCLUSIONS

The results obtained point to the low adsorption capacity for both fungicides of the natural clays studied. The adsorption of both compounds increases when the clays are modified with the HDPY cation. This increase is small for metalaxyl adsorption (less hydrophobic). However, clay mineral modification leads to a dramatic increase in penconazole adsorption by all the minerals; in HDPY-M, the adsorption percentage is 24-fold higher than for the natural M sample. Desorption studies point to the great stability of penconazole when adsorbed by HDPY-M. In the rest of the samples, the stability of this pesticide was low and the stability of metalaxyl adsorbed by all the HDPY clays was also low. In view of the efficiency of HDPY-M in the adsorption of penconazole, together with the stability of the compound against washing with water, M saturated with this organic cation could be considered in technologies based on the use of clay barriers aimed at the prevention of soil and water pollution by hydrophobic pesticides.

Acknowledgements

This work was financially supported by the "Ministerio de Ciencia y Tecnología" as part of Project REN2000-0884/TECNO. The authors thank L.F. Lorenzo, J.M. Ordax, and A. Nuñez for technical assistance, and Novartis Crop Protection AG for kindly supplying the labeled and unlabeled fungicides used in this work.

References

[1] T.B. Moorman, D.B. Jaynes, C.A. Cambardella, J.J. Hatfield, R.L. Pfeiffer and A.J. Morrow, *J. Environ. Qual.*, **28**, 35–45 (1999).
[2] D.W. Kolpin, J.E. Barbash and R.J. Gillion, *Ground Water*, **32**, 835–863 (2000).
[3] O.S. Jacobsen, W. Brusch, T. Laier, S.U. Hansen, N.H. Spliid, A. Helweg, In: A.A.M. Del Re, C. Brown, E. Capri, G. Errera and M. Trevisan (Eds.), *Proceedings of the XI Symposium on Pesticide Chemistry*, pp. 355–362. La Goliardica Pavese, Pavia (1999).
[4] P.J. Mason, I.D. Foster, A.D. Carter, A. Walker, S. Higginbotham, R.L. Jones and A.J. Hardy, In: A.A.M. Del Re, C. Brown, E. Capri, G. Errera and M. Trevisan (Eds.), *Proceedings of the XI Symposium on Pesticide Chemistry*, pp. 405–412. La Goliardica Pavese, Pavia (1999).
[5] M. Arienzo, T. Crisanto, M.J. Sánchez-Martín and M. Sánchez-Camazano, *J. Agric. Food Chem.*, **42**, 1803–1808 (1994).
[6] J. Kozak, In: A. Piccolo (Ed.), *Humic Substances in Terrestrial Ecosystem*, pp. 625–664. Elsevier, Amsterdam (1996).
[7] M. Sánchez-Camazano, M.J.Sánchez-Martín and R. Delgado-Pascual, *J. Agric. Food Chem.*, **48**, 3018–3026 (2000).
[8] M.M. Mortland, S. Shaobai and S.A. Boyd, *Clays Clay Miner*, **34**, 581–585 (1986).
[9] M. Sánchez-Camazano and M.J. Sánchez-Martín, *Toxicol. Environ. Chem.*, **56**, 11–21 (1996).
[10] J-F. Lee, M.M. Mortland, C.T. Chiou, D.E. Kile and S.A. Boyd, *Clays Clay Miner*, **38**, 113–120 (1990).
[11] W.F. Jaynes and S.A. Boyd, *Soil. Sci. Soc. Am. J.*, **55**, 43–48 (1991).
[12] L. Zhu, X. Ren and S. Yu, *Environ. Sci. Technol.*, **32**, 3374–3378 (1998).
[13] W.F. Jaynes and G.F. Vance, *Clays Clay Miner*, **47**, 358–365 (1999).
[14] M. Sánchez-Camazano and M.J. Sánchez-Martín, *Water Air Soil Pollut.*, **74**, 19–28 (1994).
[15] M.J. Carrizosa, M.J. Calderón, M.C. Hermosín and J. Cornejo, *Sci. Total Environ.*, **247**, 285–293 (2000).
[16] T. Undabeitya, S. Nir and B. Rubin, *J. Agric. Food Chem.*, **48**, 4767–4773 (2000).
[17] R. Celis, M.C. Hermosin, L. Cornejo, M.J. Carrizosa and J. Cornejo, *Int. J. Environ. Anal. Chem.*, **82**, 503–517 (2002).
[18] B. Casal, J. Merino, J.M. Serratosa and E. Ruiz-Hitzky, *Appl. Clay Sci.*, **18**, 245–254 (2001).
[19] M.L. Jackson, *Soil Chemical Analysis*, 2nd Edn., University of Wisconsin, Madison, WI (1975).
[20] C. Tomlin, *The Pesticide Manual.*, 10th Edn., 1341pp. British Crop Protection Council, Cambridge (1995).
[21] C.H. Giles, T.H. MacEwan, S.N. Nakhwa and D. Smith, *J. Chem. Soc.*, **111**, 3973–3993 (1960).
[22] M.J. Sánchez-Martín, M.S. Andrades and M. Sánchez-Camazano, *Soil Sci.*, **165**, 951–960 (2000).
[23] E. Barriuso, D.A. Laird, W.C. Koskinen and R.H. Dowdy, *Soil. Sci. Soc. Am. J.*, **58**, 1632–1638 (1994).
[24] P. Jamet, *Int. J. Food Pharm. Cosmet. Agric. Adv. Technol.*, **May/June**, 19–21 (1993).
[25] M.S. Andrades, M.J. Sánchez-Martín and M. Sánchez-Camazano, *J. Agric. Food Chem.*, **49**, 2363–2369 (2001).