



## Research paper

# Adsorption of phenols from olive oil waste waters on layered double hydroxide, hydroxyaluminium–iron-co-precipitate and hydroxyaluminium–iron–montmorillonite complex



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## ABSTRACT

Olive mill waste water (OMWW) contains high concentrations of phenols that are responsible for the high toxicity of the effluent. A one step precipitation process of OMWW by cold methanol yielded a polymeric organic precipitate (polymerin) and a supernatant (OMWW-S) rich in phenols, which were further concentrated in an ethyl acetate extract (OMWW-E). This extract was comparatively adsorbed on: i) a layered double hydroxide of magnesium and aluminium (LDH); ii) the LDH after calcination at 450 °C (LDH-450); iii) a hydroxyaluminium–iron-co-precipitate (HyAlFe); and iv) a hydroxy-aluminium–iron–montmorillonite complex (HyAlFe-Mt). Adsorption behaviour and kinetics of phenols with these materials were investigated. The Langmuir model better described adsorption ( $R^2 > 0.97$ ) in comparison to the Freundlich model ( $R^2 > 0.89$ ). Phenols were sorbed according to the following order: LDH-450 > LDH > HyAlFe > HyAlFe-Mt. Phenol adsorption on LDH matrices was strong, since desorption with simulated soil solution under dynamic conditions never exceeded 20%. Cyclic adsorption conducted with LDH-450 removed most phenols, ~94% from OMWW-E, consequently reducing its phytotoxicity. The study evidenced that calcined LDH represented an effective remediation process for OMWW.

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## 1. Introduction

The olive oil industry generates large quantities of a deleterious by-product known as olive mill waste water (OMWW) which is rich in inorganic and organic constituents with a COD (chemical oxygen demand) level from 70 to 150 g L<sup>-1</sup> (Santi et al., 2008). Even though OMWW contains a substantial quantity of plant nutrients (N, P, K, Ca, Mg, Fe) useful for agriculture, uncontrolled application of this effluent to soil can accumulate salts and phytotoxic compounds, change pH and leach chemicals that could contaminate ground water (Garcia Gomez et al., 2003). The phytotoxic activity of OMWW is mainly attributable to monomeric phenols (Fiorentino et al., 2003), which inhibit seed germination and seedling development (Krogmeier and Bremner, 1989).

Various methods have been proposed for the treatment of OMWW. These processes are based on the principles of precipitation and coagulation, chemical oxidation, sedimentation, filtration, osmosis, ion exchange, adsorption, etc. Among them, physical adsorption methods are the most frequently used for the removal of phenolic compounds as they are both effective and inexpensive.

In recent years, attention has been focused on various adsorbents that have suitable adsorption capacities and are able to remove unwanted phenols at low-cost. Polymeric hydroxyiron (HyFe) and hydroxyaluminium (HyAl) may serve as an important sink for phenols in OMWW due to high surface reactivity and high positive surface charge. Their surface properties and stability have been extensively studied (Ramesh et al., 2007). HyAl and HyFe ions are adsorbed on expandable phyllosilicate clays with high cation exchange capacity, particularly on montmorillonite (Mt), forming HyAl-Mt, HyFe-Mt or HyAlFe-Mt. Since their introduction in the late 1970s, metal oxide pillared clays have been prepared by preintercalation of 2:1 layered silicates with robust polycations. Previous research has shown that chemically modified clay minerals represent an innovative and promising class of sorbent materials (Zhao and Vance, 1998). Since the negatively charged and hydrophilic surfaces of the mineral render it to be less efficient in adsorption, the modified forms of clay are generally used in the removal of phenols. Evidence has shown that polymeric HyAl and HyFe cations are intercalated in interlayer spaces of clays and also adsorbed on the external planar surfaces and/or at the edges, and that the fixed cations are nonexchangeable. Polymeric HyAl and HyFe cations in the interlayer exhibit much greater surface area than free amorphous Fe and Al hydroxides formed via direct precipitation (Janssen et al.,

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2007). Adsorption/desorption of a wide variety of pollutants on HyAl or HyFe, HyAlFe, HyAl-Mt or HyFe-Mt complexes have been extensively investigated (Kasama et al., 2004; Masue et al., 2007). However, studies on phenol adsorption onto such matrices are to date very limited. Only a few papers exist on the direct use of clay minerals in the treatment of OMWW (Beccari et al., 2002).

Another group of compounds that is gaining increasing interest for contaminant removal is represented by layered double hydroxides (LDHs), or the so-called anionic clays, which belong to a family of layered materials. These matrices have received considerable attention due to their unique layered structure and high anion exchange capacities (Crepaldi et al., 2000; Youwen et al., 2001). LDHs are less frequent or rare in nature compared to cationic clays, but relatively inexpensive and simple to synthesize. In fact, they can be readily synthesized by co-precipitation methods under laboratory conditions (Rhee et al., 1997). LDHs are composed of octahedral brucite layers in which divalent metal cations are partially replaced by trivalent metal cations to generate positive charges. The positive charge of the layer is neutralized by the interlayer anions that govern the distance between the layers. LDH adsorption efficiency can be significantly increased by the calcination process (Goswamee et al., 1998; Hermosin et al., 1996). In fact, calcination at 450–800 °C converts the LDHs into mixed oxides which can be rehydrated to give the original LDH structure through the interaction of anions from aqueous solution. The use of LDHs for the purification of water is well understood and progress today is mainly in improving the efficiency and specificity of the LDHs and their applications. In fact, LDHs have widely been examined as potential adsorbents for environmental protection due to their capacity to remove from water organic compounds such as surfactants (Pavlovic et al., 1997), phenolic compounds (Barriga et al., 2002), inorganic oxyanions (Bakhti and Ouali, 2006) and pesticides (Cardoso et al., 2006).

This study reports on our recent investigations onto the sorptive properties of LDH materials (De Martino et al., 2011). Here we evaluate the efficiency of HyAlFe, HyAlFe-Mt, LDH and LDH calcined at 450 °C (LDH-450) to adsorb phenols from OMWW. X-ray diffraction (XRD), diffuse reflectance infrared Fourier transform spectroscopy (DRIFT), specific surface area (SSA) and point of zero charge (PZC) techniques were used to study the changes in the structure and surface properties of the prepared minerals. The optimal sorbent was tested in cyclic decontamination of an ethyl acetate extract at pH 2.0 from OMWW, whereas the phytotoxicity of the sorbent and of the treated water was assessed to verify the possibility of direct land application of the effluent.

## 2. Materials and methods

### 2.1. Preparation and characterization of OMWW fractions

OMWW was obtained from a centrifuge processing plant located in Monopoli (Italy). Samples were refrigerated at  $-20^{\circ}\text{C}$  in tightly closed PVC vessels and used when required. Aliquots of 1 L of OMWW were centrifuged at 10,000 rpm for 30 min and filtered to obtain a clear and dark solution, which was then concentrated to 0.5 L under reduced pressure. This solution was then treated with 1.5 L of cold methanol ( $-20^{\circ}\text{C}$ ), and after 4 h a dark precipitate, named polymerin (Capasso et al., 2002) was obtained. This was removed by centrifugation at 10,000 rpm for 30 min. The supernatant, named OMWW-S, was treated under reduced pressure at  $30^{\circ}\text{C}$  to remove all the methanol and reconstituted to a final volume of 1 L. Aliquots of 200 mL of OMWW-S, adjusted to pH 2.0 with  $\text{H}_2\text{SO}_4$ , were extracted with ethyl acetate by refluxing for 24 h using a Friedrich condenser. The organic extract, OMWW-E, was dried with sodium sulphate, filtered and concentrated under reduced pressure at  $30^{\circ}\text{C}$  to a greasy residue.

Electrical conductivity (EC;  $\text{dS m}^{-1}$ ) was measured using a conductivitymeter (Hanna Instruments, HI 8733). COD was determined by potassium dichromate in sulfuric acid at reflux temperature with

silver sulfate to remove  $\text{Cl}^{-}$  interference. Excess dichromate was titrated with  $\text{Fe}^{2+}$  using phenanthroline as indicator (AOAC, 1975). The fractions were analysed for total phenols by the Folin–Ciocalteu method (Jenning, 1981) using gallic acid as the reference standard and reading the absorbance at 760 nm; total biophenols were also measured directly at 280 nm using gallic acid as standard; hydroxycinnamic acid derivatives at 320 nm using caffeic acid as standard, flavonols at 360 nm using quercetin as standard, anthocyanins at 520 nm using cyanidin chloride as standard and *o*-diphenols with sodium molybdate at 370 nm using caffeic acid as standard (Obied et al., 2005).

The results of total phenols and different phenolic classes were expressed as gallic acid equivalents (GAE), hydroxycinnamic acid derivatives and *o*-diphenols as caffeic acid equivalents (CAE), flavonols as quercetin equivalents (QE) and anthocyanins as cyanidin chloride equivalents (CCE). Spectrophotometric measurements were performed with a Cary 50 UV/visible spectrophotometer, using Cary WinUV “version 3” software (Varian, Australia).

### 2.2. Preparation and characterization of sorbents

A LDH of magnesium and aluminium (LDH) with a molar ratio  $\text{M}^{\text{II}}/\text{M}^{\text{III}}$  of 2 and the anion  $\text{NO}_3^{-}$  in the interlayer was prepared at room temperature by a co-precipitation method. Briefly, 200 mL of a solution containing 0.50 M  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  and 0.25 M  $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  was added via dropwise addition to 200 mL of 1 M  $\text{NaNO}_3$  solution, under vigorous agitation. The pH was maintained at 10.0 by addition of a  $2 \text{ mol L}^{-1}$  NaOH solution. No effort was made to minimize contact with the atmosphere during the preparation in order to simplify the preparation method. The final suspension was maintained at  $20^{\circ}\text{C}$  for 20 h, centrifuged at 7000 rpm for 30 min, washed five times with deionized water and then dialyzed with Spectra/por membranes of 12,000–14,000 Da (Spectrum Medical Industry, Houston, TX). Finally, the sorbent was freeze dried and lightly ground to pass through a 0.315 mm sieve. A subsample of the resulting material was calcined in an oven for 4 h at  $450^{\circ}\text{C}$  yielding LDH-450 (Iorio et al., 2010).

A polymeric HyAlFe was prepared by mixing 0.25 mol of  $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  and 0.25 mol of  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  in 1 L solution, which was potentiometrically titrated (TIM 856 Titration Manager automatic titrator, Analytical De Mori, Milano, Italy) to pH 6.0 with 2 M NaOH at a feed rate of  $0.5 \text{ mL min}^{-1}$ . The precipitate was kept in a propylene container and maintained at  $20^{\circ}\text{C}$  for 24 h. The sample was then centrifuged, washed five times with water, dialyzed, freeze-dried, ground and sieved, as above (Violante et al., 2009).

HyAlFe-Mt was prepared by treating 40 g of Na-montmorillonite (Mt; Wyoming, montmorillonite; Clay Mineral Society Source Clays Repository, Columbia, MO) with 0.120 mol  $\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  and 0.120 mol of  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  in 1 L solution under the same experimental conditions described for HyAlFe (Violante et al., 1998).

Sorbents were characterised for specific surface area (SSA) by nitrogen adsorption isotherms at 77 K using the Brunauer–Emmett–Teller (BET) method with a Sorptometer 1042 (Costech, Italy), and for point of zero charge (PZC) in according to the method described by Mustafa et al., 2004 using the titrator described above. The sorbents were also characterised by XRD using a Rigaku Geigerflex D/Max IIIC diffractometer and by DRIFT spectroscopy using a Perkin-Elmer Spectrum One FT-IR. All determinations were carried out in duplicate.

### 2.3. Adsorption kinetics

Samples of 450 mg of LDH, LDH-450, HyAlFe, HyAlFe-Mt were stirred in separate batches with 360 mL of a solution containing  $25.0 \text{ mg L}^{-1}$  of total phenols from OMWW-E in lined glass bottles. Samples were taken at 0.5, 1, 2, 4, 8, 24 and 72 h. At each time point the suspension was left to settle for 10 min and 1 mL of supernatant was taken and analysed for total phenols. The periodic removal of a

relatively small amount of supernatant was assumed to have no influence on subsequent adsorption capacity. In order to scale up the adsorption methodology to a field setting with limited handling operations, adsorption was performed in the absence of any electrolytic background and at the natural pH of the sorbent/sorbate system. Supernatants were analysed for total phenols by flow injection analysis (FIA) according to a modified method of Moreno et al. (2004). Briefly, 10  $\mu\text{L}$  of sample was mixed, using an Ismatec SA (Extech, Australia) peristaltic pump, with commercial Folin–Ciocalteu reagent (diluted 1:10 in  $\text{H}_2\text{O}$ ) in a 75 cm mixing coil. This was joined by 5% sodium carbonate solution and reacted at 35  $^\circ\text{C}$  in a 12 m reaction coil. The absorbance was measured by a Varian PROSTAR 320 detector set at 699 nm, the maximum wavelength possible for this detector. Data were collected and processed by Varian STAR chromatography software. The method was validated against a standard spectrophotometric method where the total phenols were measured at 760 nm. The flow injection method had a run time of 4 min per sample and the working range was 0–2000 mg/L gallic acid. Results were expressed as mg gallic acid equivalents (GAE). The amount of sorbed phenols was calculated by the difference between the initial quantity of phenols added and that present in the equilibrium solution. The experiments were conducted in duplicate.

#### 2.4. Adsorption isotherms

The experiments were carried out by a batch equilibration technique at  $25 \pm 1$   $^\circ\text{C}$  using LDH, LDH-450, HyAlFe, and HyAlFe-Mt as sorbents and OMWW-E as the matrix containing the phenols. Adsorption isotherms were determined over the range 5–200 mg  $\text{L}^{-1}$  of phenols from OMWW-E, with 10 mg of matrix in 10 mL screw cap vials at a sorbent/liquid ratio of 1.25. After adsorption, samples were centrifuged at 7000 rpm for 30 min and the supernatants analysed for total phenols as described in Section 2.3.

#### 2.5. Desorption kinetics

Desorption was performed by first sorbing phenols for 24 h as described in Section 2.3 and then desorbing under static and flow conditions as follows: the supernatant was removed and replaced with 360 mL of desorbing solutions, which were distilled water or an ionic solution of 0.25 mM KCl, 1 mM  $\text{MgCl}_2$  and 5 mM  $\text{CaCl}_2$  (Modabber et al., 2008). One mL of desorbed solution was taken at 1, 2, 4, 8, 24, 48, 72 and 144 h and analysed as above. For the flow method, the flow rate was 2.5  $\text{mL h}^{-1}$ . The experiments were conducted in duplicate.

#### 2.6. Cyclic decontamination

One hundred mL of OMWW-E containing 1.02 g  $\text{L}^{-1}$  of phenols were placed in contact with 1.5 g of LDH-450, which was found to be the most adsorptive sorbent. After 24 h of stirring, the suspension was centrifuged at 7000 rpm for 30 min and the precipitate removed and lyophilized. This precipitate, named solid sorbed phenol (SSP), together with 7 mL of OMWW-E were used for phytotoxicity bioassay while the remaining solution was re-treated with fresh LDH-450 at the same solid/liquid ratio used in the first step. This procedure was repeated three times giving rise to OMWW-E-1 $^\circ$ , 2 $^\circ$ , 3 $^\circ$  cycle of adsorption. The removal of phenols in each fraction was evaluated by FIA and high performance liquid chromatography–mass spectroscopy (HPLC–MS). In this latter case samples were analysed on an Agilent 1200 series liquid chromatograph (Agilent technologies, Germany) by gradient elution on a 250 mm  $\times$  4.6 mm i.d., 5  $\mu\text{m}$ , Gemini C-18 column (Phenomenex, Australia). The flow rate was 0.7 mL/min and the injection volume was 10  $\mu\text{L}$ . Mobile phase used was a gradient of solvent A (0.1% formic acid in 10 mM ammonium acetate) and solvent B (0.1% formic acid in acetonitrile). The system was allowed to equilibrate under initial solvent composition 5% solvent B for 15 min between runs. The total

run time was 60 min. A five-step linear gradient was conducted as follows: from 5% solvent B to 20% solvent B in 10 min; isocratic for 5 min; then to 40% solvent B in 10 min; to 60% B in 15 min; isocratic for 10 min; and back to initial composition 5% B in 5 min. The diode array detector (DAD) was set to record chromatograms at 235, 245, 280 and 325 nm. The effluent from the DAD was directed to a 6410 triple quad LC–MS (Agilent Technologies, USA) equipped with an electrospray ionization (ESI) interface. MS analysis was performed in the negative ion mode ( $m/z$  100–1200) under the following conditions: nitrogen gas; gas temperature 350  $^\circ\text{C}$  and gas flow rate 9 L/min; the nebulizer pressure was 40 psi; capillary voltage was 4 kV; and cone voltage 100 V. Data analysis was performed using Agilent MassHunter workstation version B.01.04 2008 (Agilent Technologies, Germany).

#### 2.7. Phytotoxicity bioassay

Triplicate samples of two mL of OMWW-E-1 $^\circ$ , 2 $^\circ$ , 3 $^\circ$  cycle and 15 mg of SSP dispersed in 2 mL of distilled water were placed in 9-cm diameter glass Petri dishes lined with glass microfibre filters (GF/A; Whatman) and containing twenty seeds of *Lepidium sativum*. Germination was conducted over 5 days, at 25  $^\circ\text{C}$ . The number of germinated seeds was counted and radicle growth measured. As parameters of toxicity both root length (cm) and seed germination rate (%) were measured. The germination index (GI) was calculated according to the formula  $G_i = (G/G_0) \times (L/L_0) \times 100$ , where  $G$  and  $G_0$  were the number of germinated seeds in the sample and control respectively, and  $L$  and  $L_0$  the average sum of root lengths in sample and control, respectively. The data were submitted to analysis of variance (ANOVA) and the separation of means was performed using LSD test at  $p < 0.05$  level of significance.

#### 2.8. Data analysis

The amount of phenols sorbed ( $S$ ; mg  $\text{kg}^{-1}$ ) was calculated using the mass balance equation:

$$S = V(C_i - C_e)/m$$

where  $V$  is the solution volume (L),  $m$  is the amount of sorbent (kg), and  $C_i$  and  $C_e$  (mg  $\text{L}^{-1}$ ) are the initial and equilibrium phenol concentrations, respectively.

Two models were tested for their ability to describe the adsorption isotherm data, namely the Langmuir isotherm and Freundlich isotherm.

The Langmuir model is given by the equation:

$$S = S_m K C_e / (1 + K C_e)$$

where  $S$  (g  $\text{kg}^{-1}$ ) is the amount of phenols sorbed at equilibrium,  $K$  is the Langmuir constant related to the binding energy,  $S_m$  (g  $\text{kg}^{-1}$ ) the maximum amount of phenols sorbed and  $C_e$  (mg  $\text{L}^{-1}$ ) the equilibrium concentration of phenols.

The Freundlich model is given by the equation:

$$S = K_F C_e^{1/N}$$

where  $S$  and  $C_e$  have the same meaning in the equation reported above,  $K_F$  and  $N$  are constants that give estimates of the sorptive capacity and intensity, respectively.

The percentage of sorbed phenols was calculated using the following equation:

$$\text{Adsorption (\%)} = (C_i - C_e)/C_i \times 100.$$

where  $C_i$  is the initial concentration of phenols and  $C_e$  the equilibrium concentration (mg  $\text{L}^{-1}$ ).



**Table 1**  
Chemical properties of OMWW and OMWW-S.

|        | Phenols<br>(g L <sup>-1</sup> ) | EC<br>(dS m <sup>-1</sup> ) | COD<br>(g L <sup>-1</sup> ) | Dry residue<br>(g L <sup>-1</sup> ) |
|--------|---------------------------------|-----------------------------|-----------------------------|-------------------------------------|
| OMWW   | 2.14 ± 0.17                     | 13.2 ± 0.2                  | 62.2 ± 3.1                  | 42.4 ± 1.4                          |
| OMWW-S | 1.20 ± 0.06                     | 9.8 ± 0.1                   | 40.2 ± 2.4                  | 31.5 ± 1.6                          |

**Table 2**  
Total phenols and different phenolic classes of OMWW-E.

|  | Concentration (g L <sup>-1</sup> ) |
|--|------------------------------------|
| Total phenols <sup>a</sup>                     | 1.02 ± 0.15                        |
| Biophenols <sup>a</sup>                        | 0.52 ± 0.04                        |
| Hydroxycinnamic acids derivatives <sup>b</sup> | 0.073 ± 0.01                       |
| Flavonol (360 nm) <sup>c</sup>                 | 0.21 ± 0.012                       |
| Anthocyanins <sup>d</sup>                      | absent                             |
| <i>o</i> -diphenols <sup>b</sup>               | 0.074 ± 0.003                      |

Expressed as:

<sup>a</sup> gallic acid equivalents (GAE),

<sup>b</sup> caffeic acid equivalents (CAE),

<sup>c</sup> quercetin equivalents (QE), and

<sup>d</sup> cyanidin chloride equivalents (CCE).

Desorption percentages were determined by the difference between the concentration of phenols sorbed and the amount desorbed at each time:

$$\text{Desorption (\%)} = 100 - (C_s - C_d / C_s) \times 100$$

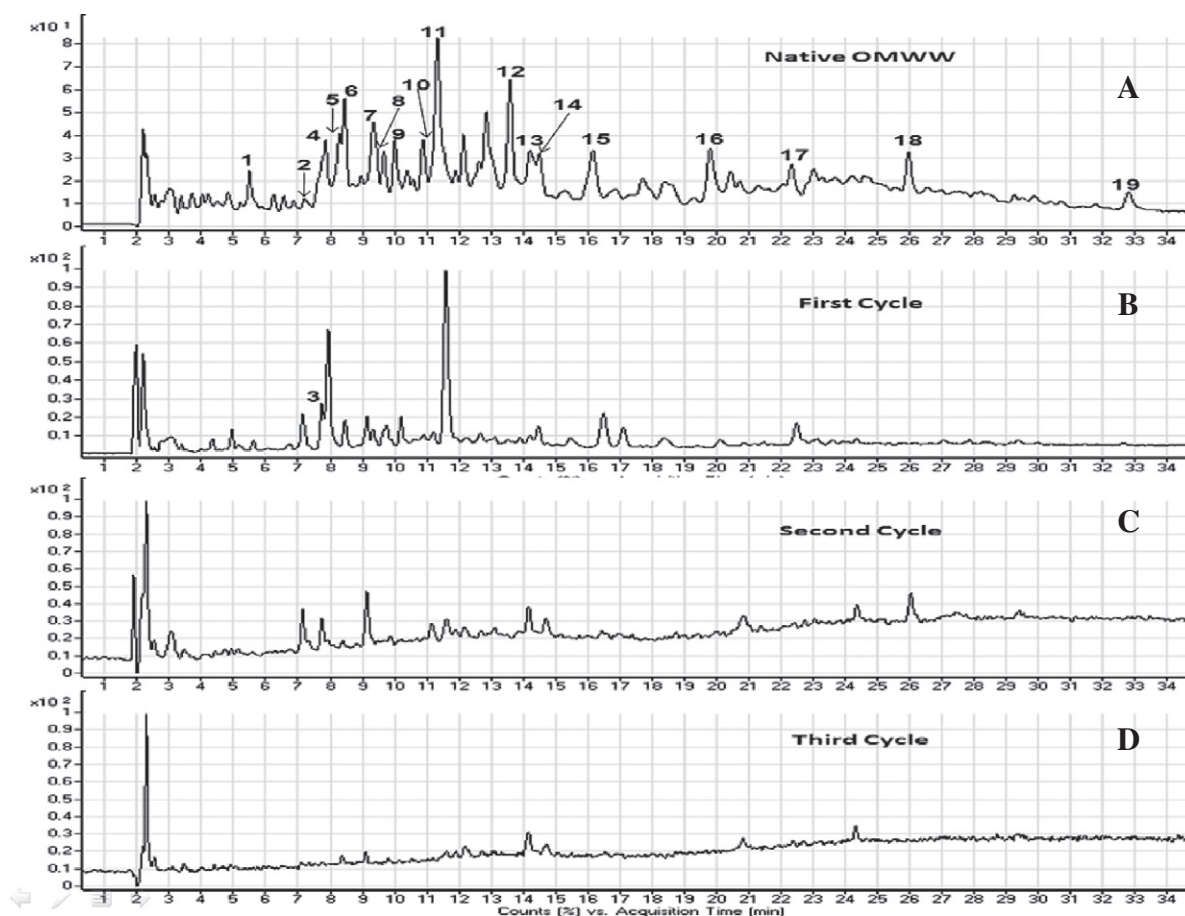
where  $C_s$  is the concentration (mg L<sup>-1</sup>) of phenol sorbed at 24 h and  $C_d$  is the concentration (mg L<sup>-1</sup>) desorbed at different times.

### 3. Results and discussion

#### 3.1. Properties of LDH, LDH-450, HyAlFe and HyAlFe-Mt

Properties of OMWW and OMWW-S are summarised in Table 1. Total phenols in OMWW were 2.14 g L<sup>-1</sup>, exceeding levels allowed in wastewater by the European Union (<1 mg L<sup>-1</sup>, Urban Water Directive 91/271/EC). After cold methanol addition, phenols were divided between OMWW-S and polymerin (1.20 and 0.80 g L<sup>-1</sup>, respectively). Total phenol concentration remained high in polymerin due to the catecholmelaninic nature of this moiety (Capasso et al., 2002). The values of EC and COD of OMWW were high (Casa et al., 2003; 13.2 dS m<sup>-1</sup> and 62.2 g L<sup>-1</sup>) and after methanol treatment decreased for OMWW-S to 9.8 dS m<sup>-1</sup> and 40.2 g L<sup>-1</sup>, respectively. Arienzo and Capasso (2000) reported that most of the cations, anions and low molecular weight compounds of OMWW concentrate mainly in OMWW-S.

Total phenol concentration of the organic extract, OMWW-E, was almost the same as that of the parent material, 1.02 g L<sup>-1</sup> cf. 1.20 g L<sup>-1</sup>, respectively (Tables 1 and 2). The gross characterization of the phenolic composition of OMWW-E is provided in Table 2. The OMWW-E had high concentrations of total phenols, as measured by the Folin–Ciocalteu assay and direct spectrophotometric measurement at 280 nm. The levels of individual phenolic classes in the extract were low and in most cases indistinguishable with the exception of



**Fig. 1.** Total ion chromatogram phenolic profile of OMWW-E. (1) unknown phenol with mass of 472 amu; (2) unknown phenol hexoside with mass of 350 amu; (3 and 4) unknown molecular masses of 188 and 408 amu; (5) hydroxytyrosol glucoside; (6) hydroxytyrosol; (7) tetrahydroxymandelic acid; (8) oleoside; (9) secologanoside; (10) tyrosol; (11) dihydroxymandelic acid; (12) elenolic acid; (13–19) unknown peaks with masses of 482, 226, 198, 244, 228, 356 and 314 amu (A). Total ion chromatogram phenolic profile of OMWW-E after three sorption cycles, OMWW-E-1° cycle (B), OMWW-E-2° cycle (C) and OMWW-E-3° cycle (D).

**Table 3**

X-ray basal distance, point of zero charge (PZC) and specific surface area (SSA) of sorbents in comparison with montmorillonite (Mt).

|           | Basal spacing (Å) | PZC  | SSA (m <sup>2</sup> g <sup>−1</sup> ) |
|-----------|-------------------|------|---------------------------------------|
| LDH       | 7.64              | 10.4 | 414                                   |
| LDH-450   | –                 | 10.4 | 481                                   |
| HyAlFe    | –                 | 7.3  | 243                                   |
| HyAlFe-Mt | 19.0              | 5.8  | 290                                   |
| Mt        | 12.6              | 2.8  | 23.8                                  |

flavonols, 0.21 g L<sup>−1</sup>. Pigments, presumably anthocyanins, absorbing at 520 nm were absent. HPLC with detection by DAD and ESI-MS provides valuable information on the phenolic composition of OMWW-E (Fig. 1A). Identification of phenols was performed by comparing retention times and UV spectra of standards in HPLC-DAD and confirmed by relevant molecular mass data from LC-MS. Where an authentic standard was not available, as in the case of hydroxytyrosol glucoside, relative retention time and molecular mass from LC-MS provided tentative identification. Simple phenols and hydroxybenzoic acids seemed to be prevalent with major peaks for dihydroxymandelic acid, tetrahydroxymandelic acid, hydroxytyrosol, hydroxytyrosol glucoside and tyrosol. Oleuropein was not detected yet its hydrolysis products were prominent, hydroxytyrosol and elenolic acid. Secoiridoid derivatives such as oleoside and secologanoside were also found, however, verbascoside and related compounds were absent. This can be attributed to varietal and environmental effects. The possibility of

verbascoside hydrolysis is ruled out due to the absence of caffeic acid as a major peak. Although there is a spate of phytochemical studies extensively investigating the phenolic composition of OMWW, the large number of unidentified compounds in OMWW-E warrant further studies.

Basal spacings of LDH, Mt and HyAlFe-Mt determined by XRD are given in Table 3. Diffractograms (not reported) for LDH showed sharp and symmetrical peaks with a  $d_{003}$  of 7.64 typical of an LDH of magnesium and aluminium and comparable to that reported in the literature (Iorio et al., 2010). Diffractograms of LDH-450 reflected the disappearance of the typical peaks of hydrotalcite, due to the destruction of the layer structure, and the appearance of broad peaks attributable to the formation of Mg and Al oxides. The increased SSA of LDH-450 (481 m<sup>2</sup> g<sup>−1</sup>; Table 3) compared to LDH (414 m<sup>2</sup> g<sup>−1</sup>) was due to thermal treatment that promoted the formation of channels and a particularly porous and reactive material (Pavlovic et al., 2005).

Mt showed a basal distance ( $d_{001}$ ) of 12.6 Å, which in the presence of aluminium and iron increased to 19.0 Å, indicating a high degree of interlayering of these metals (Jiang et al., 2002; Torres Sánchez et al., 2011). Moreover, the intercalations also caused significant changes in surface properties of Mt (Table 3). In fact, SSA increased from 23.8 m<sup>2</sup> g<sup>−1</sup> for Mt to 290 m<sup>2</sup> g<sup>−1</sup> in HyAlFe-Mt, while PZC increased from 2.8 to 5.8.

The DRIFT spectra of sorbents are shown in Fig. 2. LDH showed a peak in the hydroxyl stretching region at 3451 cm<sup>−1</sup> attributed to –OH groups attached to Al and Mg and peaks at 947, 783, 551 cm<sup>−1</sup>,

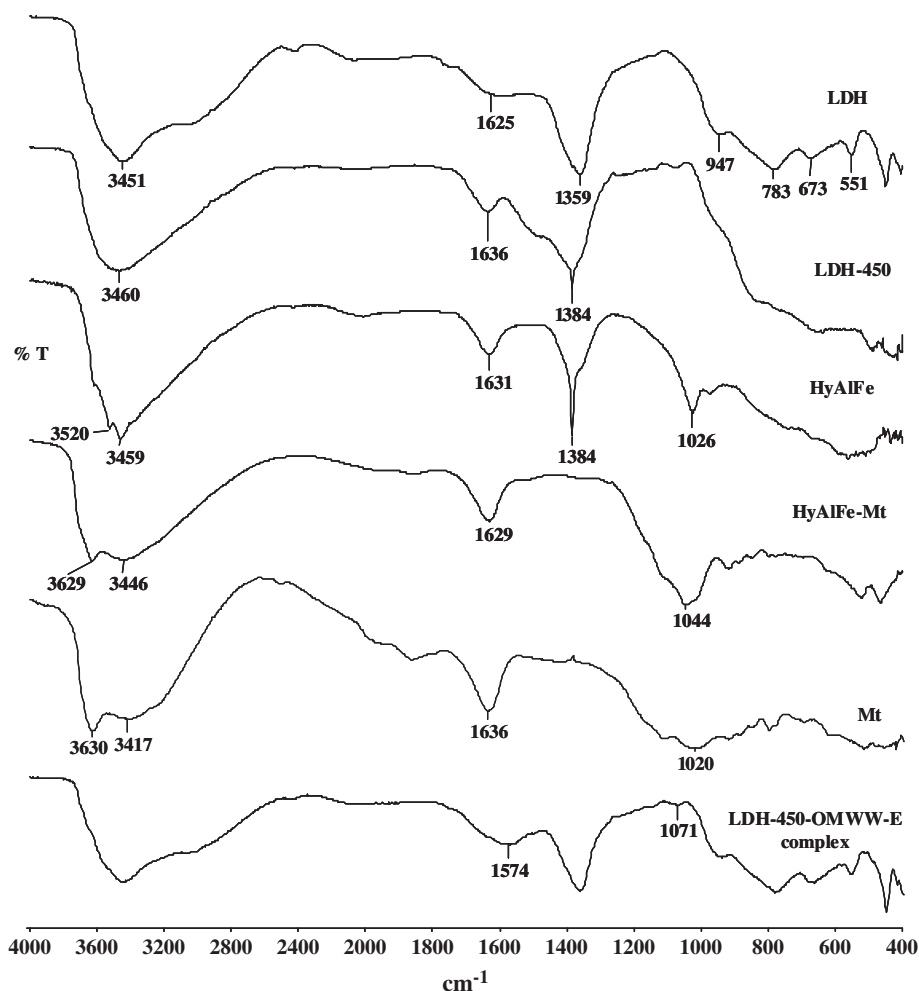


Fig. 2. DRIFT spectra of LDH, LDH-450, HyAlFe and HyAlFe-Mt sorbents, and of complex LDH-450-OMWW-E.

**Table 4**

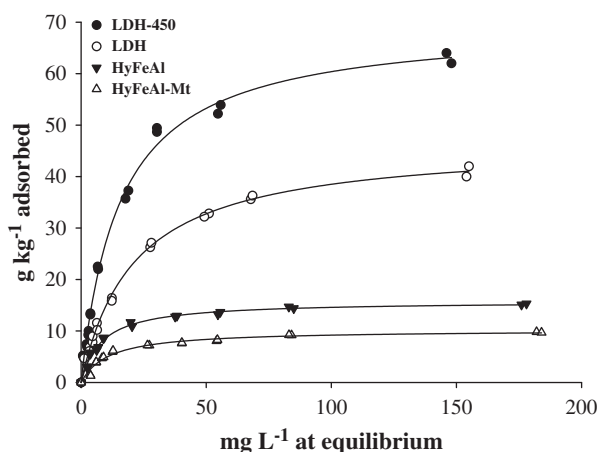
Langmuir and Freundlich parameters, and percentages of phenols removed from OMWW-E on different sorbents in the range of concentration used ( $5\text{--}200\text{ mg l}^{-1}$ ).

| Matrix    | Langmuir parameters |      |       | Freundlich parameters |      |       | % adsorbed |
|-----------|---------------------|------|-------|-----------------------|------|-------|------------|
|           | $S_m$               | K    | $R^2$ | $K_f$                 | 1/N  | $R^2$ |            |
| LDH       | 46.7                | 0.05 | 0.99  | 5.99                  | 0.40 | 0.95  | 75–44      |
| LDH-450   | 69.7                | 0.06 | 0.99  | 10.4                  | 0.38 | 0.92  | 83–65      |
| HyAlFe    | 15.7                | 0.12 | 0.99  | 4.41                  | 0.26 | 0.91  | 51–22      |
| HyAlFe-Mt | 10.2                | 0.09 | 0.97  | 2.6                   | 0.28 | 0.89  | 47–13      |

which can be ascribed to metal-oxygen stretching modes. Signals at  $1625\text{ cm}^{-1}$  can be attributed to the bending vibration of the interlayer water, while peaks at  $1359$  and  $673\text{ cm}^{-1}$  to interlayer nitrate. The DRIFT spectra of the LDH-450 showed a sharp decrease in the intensity of most of the above-mentioned peaks confirming the destruction of the layer structure and the conversion of the metal hydroxide to the metal oxide (Iorio et al., 2010; Silverstein et al., 1991). DRIFT spectra of HyAlFe coprecipitate showed the signal of  $\text{--OH}$  groups attached to Fe and Al at  $3520$  and  $3459\text{ cm}^{-1}$ ; signals at  $1631$  and  $1384\text{ cm}^{-1}$  were due to water molecules and nitrate ions respectively, and a signal at  $1026\text{ cm}^{-1}$  corresponding to metal-oxygen stretching modes. As expected, the DRIFT spectrum of HyAlFe-Mt was very similar to that of Mt except for the strong intensity of the signal at  $1044\text{ cm}^{-1}$  attributable to Al–O and Fe–O stretching modes.

### 3.2. Adsorption and desorption

Adsorption data and isotherms, analysed using Langmuir and Freundlich equations, are shown in Table 4 and Fig. 3. The Langmuir adsorption model is based on the assumption that maximum adsorption corresponds to a saturated monolayer of solute molecules on the adsorbent surface, with no lateral interaction between the sorbed molecules. The Freundlich isotherm is an empirical equation employed to describe heterogeneous systems. Examination of  $R^2$  values showed that data were better fitted to the Langmuir model. The adsorption isotherms were of L-type (Giles et al., 1974). This type of isotherm is typical of systems where monofunctional molecules are strongly attracted by the sorbent, reaching the saturation value at the isotherm 'plateau'. This 'plateau' corresponds to the formation of a monolayer of the solute on the solid surface, which gives the maximum adsorption value. Based on  $S_m$  values, the more efficient sorbents of phenols were the LDH based matrices with  $S_m$  values of  $46.7\text{ g kg}^{-1}$  (LDH) and  $69.7\text{ g kg}^{-1}$  (LDH-450), whereas for HyFeAl and HyFeAl-Mt the  $S_m$  values were much lower,  $15.7$  and  $10.2\text{ g kg}^{-1}$  respectively.



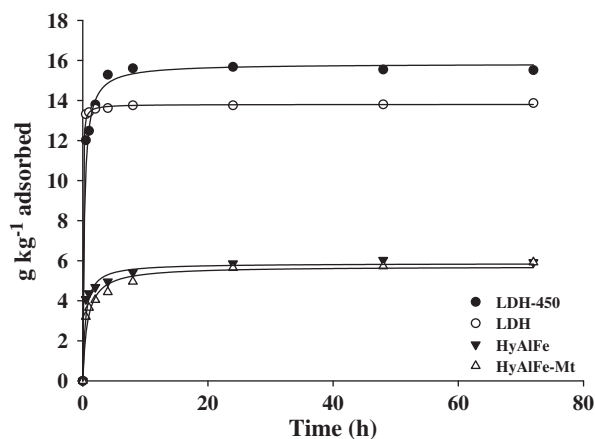
**Fig. 3.** Sorption isotherms of phenols from OMWW-E on LDH, LDH-450, HyAlFe and HyAlFe-Mt.

LDHs are positively charged (see PZC reported in Table 3) and consequently can sorb anionic compounds. Considering that in our experimental conditions the pH of solutions were greater than 9.0 the  $\text{--COOH}$  and  $\text{--OH}$  groups of phenols present in anionic form can be adsorbed by electrostatic interaction with the positive charged of brucite-like layers or by ligand exchange by displacing of  $\text{--OH}$  and/or  $\text{H}_2\text{O}$  from the surface of LDH (Gao et al., 2005). The higher adsorption capacity of LDH-450 (Table 4) was due to the effect of thermal treatment, which generated a metal oxide with the highest surface area and porosity (Lazaradis, 2003), and which upon rehydration gave a native LDH with increased adsorption capacity (Hermosin et al., 1996). In addition, transport from the bulk solution to the external surface of the LDHs, solute diffusion and adsorption on the inner surfaces of the pores and capillary spaces can further increase the removal of phenols from solution (Ahmaruzzaman, 2008). The observed limited adsorption capacity of HyAlFe and HyAlFe-Mt with respect to LDH based materials is linked to the lower PZC and surface area of these matrices compared to LDH based materials (Table 3).

Adsorption of phenols from OMWW-E on LDH-450 was supported by the DRIFT spectrum (Fig. 2). In fact, the LDH-450-OMWW-E complex showed the appearance of two new signals at  $1574$  and  $1071\text{ cm}^{-1}$  attributable to  $\text{C}=\text{C}$  of aromatic rings and  $\text{--OH}$  groups of phenols, respectively (Silverstein et al., 1991). XRD (not reported) of LDH and LDH-450 after adsorption of OMWW-E revealed basal spacings of  $7.68$  and  $7.72\text{ \AA}$ , respectively, which were slightly changed compared to those of native LDH ( $7.64\text{ \AA}$ ). This result can be attributed to the fact that phenols are trapped in the porous network of LDH or that the LDH structure is too stiff to expand.

Fig. 4 shows the results of batch kinetic experiments conducted to determine the equilibrium time required for the uptake of phenols on the various sorbents. The equilibrium time was 8 h for LDH based materials and 24 h for HyAlFe and HyAlFe-Mt.

Desorption isotherms exhibited strong hysteresis due to strong adsorption of the phenols on all adsorbents. This is consistent with a high host-guest affinity resulting from strong electrostatic interactions. Desorption from all the matrices was slightly higher in simulated soil solution (Fig. 5B and D, Table 5) than in water (Fig. 5A and C, Table 5). This could be due to the occurrence in simulated soil solution of diffusion processes and of ion exchange processes between the sorbed phenols and  $\text{Cl}^-$  from the salts employed. In pure distilled water only diffusion might have occurred. Release in dynamic conditions was higher than that in static conditions, but proceeded more slowly. Observing the curves of Fig. 5, it was clear that all matrices reached a desorption plateau with limited desorption still occurring after 6 days (144 h). Desorption values were quite low (14–22%) and comparable to our previous study (De Martino et al., 2011) where desorption of phenols from a calcined LDH of zinc and aluminium was



**Fig. 4.** Sorption kinetics of phenols from OMWW-E on LDH, LDH-450, HyAlFe and HyAlFe-Mt.

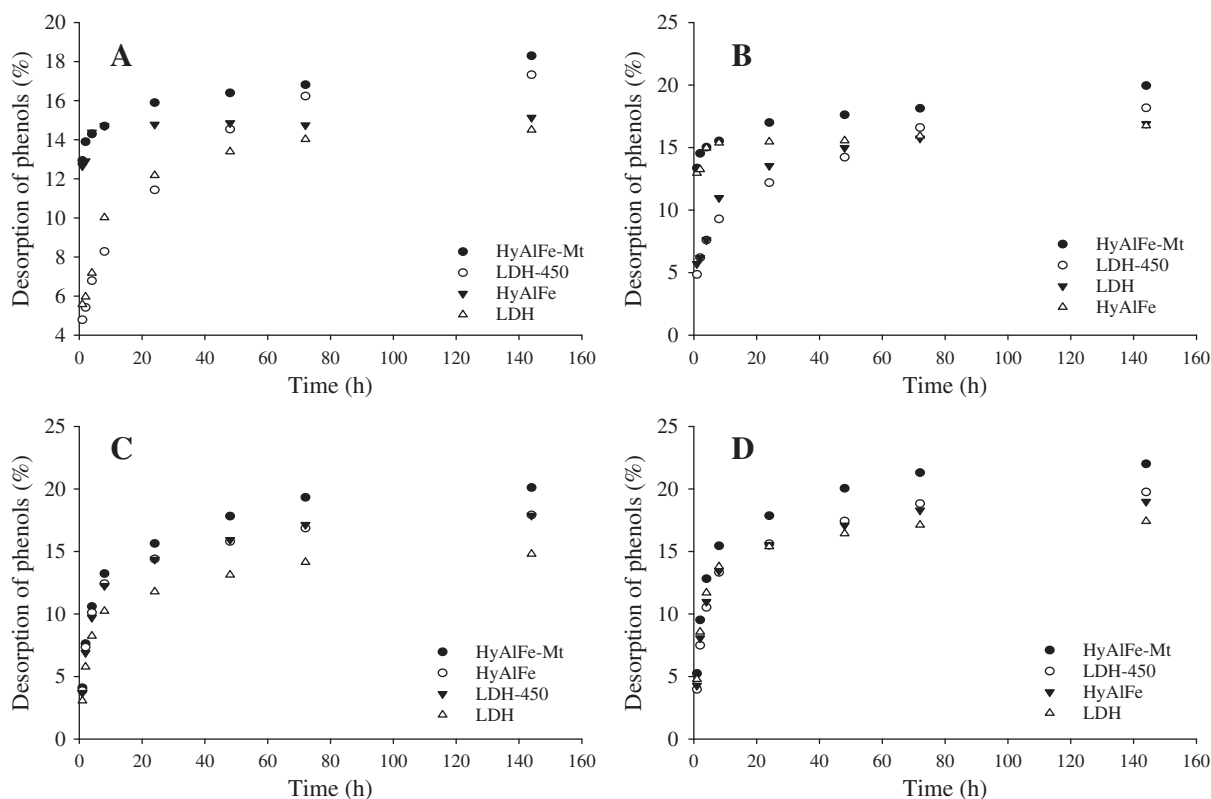


Fig. 5. Desorption of phenols from different sorbents in static water (A), static soil solution (B), dynamic water (C) and dynamic soil solution (D).

about 15%. Similarly, Anirudhan and Suchithra (2008) showed that tannin adsorption on calcined LDH was not completely reversible and the binding between the adsorbent particle and adsorbed tannin was quite strong.

### 3.3. Cyclic removal and phytotoxic bioassay

After three cycles of phenol removal from OMWW-E by LDH-450, the amount of phenols decreased by 94%, from 1.02 to 0.06 g L<sup>-1</sup> (Table 6). Phytotoxic bioassay data indicated that OMWW-E-1°cycle completely

inhibited the germinability ( $G_1 = 0$ ), even though phenol load decreased by about 37%.  $G_1$  increased to 92% with OMWW-E-3°cycle, with a significant phenolic content decrease from 0.64 g L<sup>-1</sup> of the first decontamination cycle to 0.06 g L<sup>-1</sup> of the last cycle. This decrease was supported by LC–MS analysis (Fig. 5B–D), which showed a marked decrease in signal intensity after every decontamination step. Our bioassay data matched with the findings of Aranda et al. (2007) who showed that phenols from OMWW were the main determinants of germinability suppression in *L. sativum*.

The solid sorbed phenol, SSP, also did not show any phytotoxic activity (Table 6). In fact, the values of  $G_1$  were very close to the control after every decontamination cycle. Many studies have reported that toxicity of nanomaterials can be due to two different mechanisms: 1) chemical toxicity based on the release of toxic ions, or 2) stress caused by the surface, size and/or shape of the particles (Brunner et al., 2006). Our preliminary phytotoxicity data seem to exclude any phytotoxicity mechanism coming from SSP physical and chemical interferences with root growth. This means that LDH sorbents have a significant potential for pollutant removal from OMWW, and in the meantime the spent SSP material has a low disposal risk in terms of toxicity for humans and possible introduction in the environment, during their handling, transport and recycling. This behaviour makes this material a potential suitable adsorbent for real applications. However, the need exists to understand the eventual toxicity

Table 5  
Percentage of desorption of phenols in water and simulated soil solution under static and dynamic conditions from different sorbents.

| Matrix    | Desorption in static conditions (%) |                         | Desorption in dynamic conditions (%) |                         |
|-----------|-------------------------------------|-------------------------|--------------------------------------|-------------------------|
|           | H <sub>2</sub> O                    | Simulated soil solution | H <sub>2</sub> O                     | Simulated soil solution |
| LDH       | 14.4 ± 1.2                          | 16.9 ± 0.6              | 14.8 ± 2.1                           | 17.4 ± 1.4              |
| LDH-450   | 17.3 ± 1.4                          | 18.2 ± 1.4              | 17.9 ± 0.8                           | 19.7 ± 1.9              |
| HyAlFe    | 15.1 ± 0.9                          | 16.7 ± 2.2              | 17.9 ± 1.1                           | 18.9 ± 3.4              |
| HyAlFe-Mt | 18.3 ± 1.1                          | 19.9 ± 1.8              | 20.1 ± 0.5                           | 22.1 ± 2.5              |

Table 6  
Phenol concentrations and germination index from phytotoxic bioassay with *Lepidium sativum* of OMWW-E and corresponding complex LDH-450-OMWW-E (SSP) after three decontamination cycles.

|                              | Controls         |         |        | 1° cycle |        | 2° cycle |        | 3° cycle |        |
|------------------------------|------------------|---------|--------|----------|--------|----------|--------|----------|--------|
|                              | H <sub>2</sub> O | LDH-450 | OMWW-E | SSP      | OMWW-E | SSP      | OMWW-E | SSP      | OMWW-E |
| Phenols (g L <sup>-1</sup> ) | –                | –       | 1.02   | –        | 0.64   | –        | 0.29   | –        | 0.06   |
| Germination index (%)        | 100 a            | 98 a    | 0 b    | 113 a    | 0 b    | 106 a    | 67 c   | 112 a    | 92 a   |

Row values followed by the same letter were not significantly different by ANOVA test



of this material under more extreme experimental conditions before their effective employment.

#### 4. Conclusions

The combination of the results of this study with the previous one (De Martino et al., 2011) indicates that calcined LDH could be effectively used for the treatment of the supernatant derived from a simple one step processing phase of OMWW by cold methanol precipitation. Moreover, and at least in the short term, the solid sorbed phenol phase was shown to be quite stable with only limited release of phenols in simulated soil solution and with no phytotoxic activity. Thus, both by-products of the chemical precipitation, polymerin and detoxified OMWW-S, could be used in a land disposal scenario reclaiming soil fertility and preserving water in soils of poor quality.

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