This is a post-peer-review, pre-copyedit version of an article published in *Environmental Science and Pollution Research*.

This version of the article has been accepted for publication, after peer review (when applicable) and is subject to Springer Nature's AM terms of use, but is not the Version of Record and does not reflect post-acceptance improvements, or any corrections. The Version of Record is available online at: http://dx.doi.org/10.1007/s11356-021-13876-9.

MODIFIED-ZEOLITE-SUPPORTED BIOFILM IN SERVICE OF PESTICIDE BIODEGRADATION

Nataliia Gorodylova[1,2,5], Caroline Michel[1], Alain Seron[1], Catherine Joulian[1], Fabian Delorme[2], Sophie Bresch[3], Catherine Garreau[4], Fabien Giovannelli[2], Karine Michel[1]

[1]BRGM, the French Geological Survey, Division of Water, Environment, Process and Analyses (DEPA), 3 Avenue Claude Guillemin, 45100 Orléans, France
[2]University of Tours, CNRS, INSA CVL, GREMAN UMR 7347, IUT de Blois, GREMAN, 15 rue de la chocolaterie, CS 2903, 41029 Blois, France
[3]CDHR Centre-Val-de-Loire, 620 Rue de Cornay, 45590 Saint-Cyr-en-Val, France
[4]Traitagri Centre, 28 Avenue des Pierrelets, 45380 Chaingy, France
[5]Corresponding author, tel. +420 466 037 182; e-mail natasha.gorod@gmail.com; present address: University of Pardubice, Studentska 95, 53210 Pardubice

1. Abstract

The development of biofilms on modified natural zeolites was investigated with purpose to obtain biocomposites with biodegradation activity towards pesticides MCPA (2-methyl-4-chlorophenoxyacetic acid) and glyphosate (*N*-(phosphonomethyl)glycine) for potential application in bioaugmentation of polluted agricultural soils. Microbial communities were selected from agricultural pesticide-contaminated soil/water samples and enriched on basis of their ability to biodegrade the pesticides. In order to enhance affinity of microbial communities to the support material, the natural mineral zeolite was modified by nontoxic environmentally friendly cations (Li^+, Na^+, K^+, NH_4^+, H^+, Mg^{2+}, Ca^{2+}, Fe^{3+}) by methods preserving its structure and characterised using powder XRD, surface area measurement and chemical composition analysis. Kinetics of pesticide degradation by the biocomposites was studied in liquid media. Results showed that according to zeolite modifications, the microbial activity and biodiversity changed. The best biodegradation rate of MCPA and glyphosate reached 0.12-0.13 mg/h with half life of 16-18 h, which is considerably quicker than observed in natural environment. However, in some cases, biodegradation activity towards pesticides was lost which was connected to unfavourable zeolite modification and accumulation of toxic metabolites. High-throughput sequencing on the 16S rRNA genes of the biofilm communities highlighted the selection of bacteria genera known to metabolise MCPA (*Aminobacter, Cupriavidus*, *Novosphingobium, Pseudomonas, Rhodococcus, Sphingobium* and *Sphingopyxis*) and glyphosate (*Pseudomonas*). Altogether, results suggested that zeolite do not only have a passive role of biofilm support, but also have protective and nutrient-supportive functions that consequently increase biodiversity of the pesticide degraders growing in the biofilm and influence the pesticide biodegradation rate.

Key words: biodegradation, biofilm, microbial community, zeolite, MCPA, glyphosate

2. Introduction

Pesticides are one of the major environmental pollutants linked not only to environmental issues but also to human health problems (World health organization 1990; Pimentel 2005). Most of the pesticides do not degrade completely in the natural environment or kinetics of their degradation is very slow, resulting in pesticides accumulation in soil and surface waters or their migration through groundwater. Consequences are disturbances in soil and water ecosystems and impacts on human health, as groundwater is one of the main sources of drinking water (World health organization 1990; Pimentel 2005). Therefore, development of effective approaches to pesticide remediation is one of the most urgent and important tasks for researches working in this field.

A number of remediation methods have been already developed and introduced, however most of them have significant drawbacks. For example, implementation of the classical methods of remediation (land filling, utilization of soil pits, open burning, incineration) leads to leaching and toxic emissions (Al Hattab and Ghaly 2012); ozonation usually involves high energy consumption and expensive equipment (Al Hattab and Ghaly 2012); use of adsorbents involves high costs and have very limited capacity (Al Hattab and Ghaly 2012); chemical treatments with alkalis, potassium polyethylene glycoiate, fenton oxidation have very limited efficiency in soil (Al Hattab and Ghaly 2012) while potential of safer methods such as photolysis is very limited due to poor penetration of radiation into the soil and strong inhibitory effects of soil particles that hamper photochemical degradation (Reddy and Kim 2015). In this view, our attention has been focused on bioaugmentation, which nowadays can be considered as one of the most promising methods for pesticide remediation (Al Hattab and Ghaly 2012; Cycoń et al. 2017).

Bioaugmentation is a green technology that consists in the improvement of biodegradative capacity of the polluted medium by introducing specific microorganisms able to breakdown and use hazardous molecules as nutrients for their growth. Nowadays, efficient biodegradation abilities towards pesticides of numerous microorganisms have already been reported (Bælum et al. 2006; Singh and Walker 2006; Ditterich et al. 2013; Önneby 2013; Önneby

et al. 2014; Helbling 2015; Gupta et al. 2016; Cycoń et al. 2017). However, one of the strongest limitation of bioaugmentation is its unpredictable outcome, which depends not only on environmental conditions (temperature, pH, humidity, type of soil, etc.), but also on biotic factors, in particular interactions between autochthonous and inoculated microorganisms (Van Veen et al. 1997; Cycoń et al. 2017). According to recent reports (Plangklang and Reungsang 2009; Stelting et al. 2012; Saez et al. 2012; Cycoń et al. 2017), immobilisation of microorganisms on a support material protects them against unfavourable physico-chemical conditions, enhances biological stability, and thus increases survival and prolongs activity of the microorganisms in the natural environment. However, practical implementation of such bioaugmentation approach requires more studies in this area and, in the first place, development of safe and efficient biocomposites.

The main demand to the support material, besides chemical stability and safety toward environment, is its capability to host and support steady microbial community on its surface. Initially, formation of biofilm on the support material is determined by interaction between microorganisms and material, which should lead to microbial adhesion and subsequently to colonisation of the material surface (Mills et al. 1994; Yee et al. 2000; Deo et al. 2001; Zheng et al. 2001; Jiang et al. 2007). Microbial adhesion is mainly governed by hydrophobic and electrostatic interactions which depend on the surface properties of both, the cells and the material, as well as on experimental conditions (pH, temperature, C/N ratio, agitation speed, ionic strength and composition of the medium) (Mills et al. 1994; Jucker et al. 1997; Yee et al. 2000; Deo et al. 2001; Zheng et al. 2001; Jiang et al. 2007). In this view, the following characteristics of the material should be considered: acid-base character, specific surface area, surface morphology/topology/roughness, hydrophobicity, chemical composition and toxicity (Mills et al. 1994; Deo et al. 2001; Zheng et al. 2001; Moreno-Castilla et al. 2003; Bautista-Toledo et al. 2015; Belaabed et al. 2016). Previously, biofilm formation has been achieved not only on natural materials (corncob, bagasse (Plangklang and Reungsang 2009), agar (Saez et al. 2012), wood particles (Yamashita et al. 2011)) but also on various minerals (silica (Mills et al. 1994; Yee et al. 2000; Deo et al. 2001; Bautista-Toledo et al. 2015), alumina (Yee et al. 2000; Deo et al. 2001; Bautista-Toledo et al. 2015), titania (Bautista-Toledo et al. 2015; Wang et al. 2021), zeolites (Stelting et al. 2012; Bautista-Toledo et al. 2015; Belaabed et al. 2016), iron oxide (Deo et al. 2001; Jiang et al. 2007), clay minerals (Jiang et al. 2007), apatite (Zheng et al. 2001), dolomite (Zheng et al. 2001), activated carbon (Moreno-Castilla et al. 2003; Mercier et al. 2014)) and synthetic materials (silicon wafer (He et al. 2016), biodegradable polymers PCL (Chu and Wang 2013), silicon tubes (Saez et al. 2012)).

One of the potentially attractive support material for biofilm development is natural aluminosilicate mineral zeolite (Stelting et al. 2012; Bautista-Toledo et al. 2015; Belaabed et al. 2016). Natural zeolite is a commonly occurring sedimentary deposit and its clinoptilolite type has been broadly accepted for usage in agriculture, soil amendment and feed additives (Jha and Singh 2016). In general, natural zeolites are crystalline, microporous, non-toxic, easily modifiable, readily available and inexpensive. The general formula of zeolite is $M_{2/n}O \cdot Al_2O_3 \cdot xSiO_2 \cdot yH_2O$, where M is any alkali or alkaline earth cation with the charge n, x varying from 2 to 10, and y varying from 2 to 7 (Jha and Singh 2016). Zeolite structure is based on negatively charged porous three-dimensional honeycomb-like network of silica/aluminum-oxygen tetrahedra, the negative charges are balanced with exchangeable alkali and alkaline earth cations such as calcium, magnesium, potassium or sodium. Therefore, zeolite properties can be tuned by simple chemical manipulations such as ion exchange, acid/base or thermal treatment (Townsend and Coker 2001; Kurama et al. 2002; Reeve and Fallowfield 2018). Modification of natural zeolite by cation exchange influences besides chemical composition also its surface properties, such as microporosity, specific surface area, point of zero charge, acidity (the number of accessible acid sites and ratio of Lewis to Brønsted acid sites), etc (Townsend and Coker 2001; Abbo and Titinchi 2009; Wu and Weitz 2014; Kennedy and Tezel 2018; Moradi et al. 2018). As it was already mentioned, these parameters influence microbial adhesion to material surface. More complex modifications, for example deposition of Fe_2O_3 coating on zeolite surface, may also enhance bacterial adhesion on natural zeolite. According to previous studies, various bacteria species have high affinity to iron(III) mineral surfaces (goethite (Jiang et al. 2007), hematite (Deo et al. 2001)) and Fe-oxyhydroxide-coatings also significantly enhances the adsorption of bacteria on silicate mineral (Mills et al. 1994; Ams et al. 2004).

The aim of this study is to evaluate the ability of zeolite-supported biofilms to biodegrade pesticides. In order to influence microbial adhesion and biofilm formation on the natural zeolite, it was modified by ionic exchange and Fe_2O_3 deposition. Two chemically unrelated pesticides - MCPA (2-methyl-4-chlorophenoxyacetic acid) and glyphosate (*N*-(phosphonomethyl)glycine) – are targeted. These pesticides are intensively used worldwide and can be biodegraded in soils by microorganisms: the half-life of MCPA is greater than 100 days (Institut national de l'environnement industriel et des risques 2013) in a natural water-sediment system vs 21-24 days (AGRITOX 2010) in aerobic laboratory conditions; the half-life of glyphosate is between 11 and 30 days in the natural environment (field), 4-180 days (AGRITOX 2018) in aerobic laboratory conditions. Application of these pesticides affects microbial community structure and activity in soil leading to the increase of abundance of pesticide degrading communities (Bælum et al. 2008; Lancaster et al. 2010; Jacobsen and Hjelmsø 2014). Accordingly, soil collected at contaminated sites are good sources of microorganisms able to degrade pesticides and can be used to obtain enriched and selected pesticides-degrading microbial communities.

121 In this paper, the results related to zeolite modifications, selection and enrichment of pesticide-degrading microbial
122 community from a contaminated soil, biofilm formation on zeolite and its characterisation, kinetics of
123 biodegradation of pesticides in liquid phase by the obtained biocomposites are presented and discussed. The final
124 goal of our study is to select biocomposites suitable for bioaugmentation application in contaminated agricultural
125 soils with purpose to increase pesticides biodegradation and thus limit pesticides migration through groundwater.
126
127 **3. Materials and methods**
128
129 *3.1. Chemicals*
130 All inorganic compounds used for modification of zeolite and in growth medium are minimum of 99.8% purity
131 and were purchased from Sigma Aldrich (USA), Merck (DE), Acros Organics (Thermo Fischer Scientific, USA)
132 or Prolabo (France). The pesticide solutions were prepared from analytical grade pesticide powders purchased
133 from Cluzeau Info Lab C.I.L (France).
134 The solvents used for the HPLC-MS/MS analysis were purchased from Fischer Scientific (USA) and analytical
135 standards (glyphosate, glyphosate-C13-N15, AMPA and AMPA-C13-N15) were purchased from Dr Ehrenstorfer
136 GMBH (CIL Cluzeau). FMOC-chloride (9-fluorenylmethyl-chloroformate) (purity ≥99 %) and borate buffer
137 sodium tetraborate decahydrate) (purity ≥99.5 %) were obtained from Sigma-Aldrich, and EDTA
138 (diaminoethanetetra-acetic acid disodium salt) from Fischer Scientific.
139 The solvents used for the HPLC–HRMS analysis (UPLC/MS grade) are purchased from Biosolve (Dieuze,
140 France), and formic acid (99%, LC/MS grade) purchased from Avantor (Deventer, the Netherlands). 29 isotope
141 labelled internal standards (ILIS) for HRMS analysis are used: benzotriazole-D4, carbamazepine-13C6,
142 clarithromycin-13C-D3, diclofenac-13C6, erythromycin-13C-2H3, MCPA-13C6, metoprolol-D7 (purchased from
143 Alsachim, France), atrazine-D5, diuron-d6, fénofibric acid-d6, furosemide-D5, gemfibrozil-D6, imidaclopride-
144 D4, mecoprop-D3, norfloxacin-D5, simazine-D10, sulfadimethoxine-D6 (purchased from CDN Isotopes, Canada),
145 Methylparaben-6C13, NBBS-D9, sotalol-D7 (purchased from Cluzeau Info Labo, France), DEA-D6, diazinon-
146 D10 (purchased from Dr Ehrenstorfer), acetochlore ESA-D5, alachlore OXA-D3, beflubutamid-D7, metsulfuron
147 methyl-D3 (purchased from HPC standards, DE), atenolol-D7, oxazepam-D5 (purchased from Sigma Aldrich,
148 USA) and DMST-D3, sucralose-D6 (purchased from TRC, Canada).
149
150 *3.2. Selection, cultivation and characterisation of microbial communities degrading pesticides*
151 Selection, enrichment and cultivation of microorganisms capable of degrading MCPA or glyphosate was
152 performed by classical microbiological approaches (subculturing in the presence of the target pesticide). As
153 inoculum, soil (top 15 cm) and stock water (phytosanitary effluents coming from the surplus of phytosanitary
154 solutions prepared for field applications, and stored in a OSMOFILM® system for their subsequent treatment)
155 samples were collected from an agricultural field with a history of pesticide usage in February 2017 (Comité de
156 Développement Horticole de la Région Centre, Saint Cyr en Val, France). The pesticides used on this agricultural
157 site are the following: glyphosate, flonicamid, λ-cyhalothrin, meptyldinocap, acetamiprid, metalaxyl-M, pyrethrins
158 and others. For enrichment, 15 g of soil sample and 15 ml of stock water were suspended in 135 ml of mineral salt
159 medium (MSM, 0.15 % wt. K_2HPO_4, 0.05 % wt. KH_2PO_4, 0.02 % wt. $MgSO_4$, 0.1 % wt. NaCl; pH 7.2). At this
160 stage, experiments with three different concentrations of the pesticides (glyphosate or MCPA, 1, 5 and 10 mg/L)
161 were performed. This concentration range corresponds to the pesticide concentration that reaches the soil during
162 the treatment according to agricultural company (Comité de Développement Horticole de la Région Centre, Saint
163 Cyr en Val, France). In order to compensate absence of nitrogen in MCPA molecule, NH_4NO_3 solution was added
164 to MSM solution in amount corresponding to molar equivalent of nitrogen element in glyphosate molecule at the
165 same glyphosate concentration (absence of phosphorus in MCPA molecule is compensated by phosphorus
166 presence in MSM). The cultures were incubated in dark at 20ºC under shaking (60 rpm) in aerobic conditions.
167 Incubation time was determined as the time required for complete degradation of the pesticide in the solution. Four
168 successive subculturing steps were conducted in the same conditions with 10 % of previous subculture as
169 inoculum. Similar experiments in abiotic conditions (non-inoculated MSM solution containing pesticides) and
170 biotic control (positive control, planktonic culture enriched using the same inoculant under the same conditions
171 but without addition of pesticides) were also performed in the same conditions and with the same duration.
172 The microbial structure of each subculture was analysed using CE-SSCP fingerprinting technique (Capillary
173 Electrophoresis Single Strand Conformational Polymorphism; ABI Prism 310 Genetic Analyser, Applied
174 Biosystems, Thermo Fischer Scientific, USA). For this, microbial samples were taken at the end of the growth of
175 each subculture, filtered or centrifuged to collect microbial cells and stored at -20ºC until analysis. DNA extraction
176 was carried out on frozen pellets using the FastDNA™ Spin Kit for soil (MP Biomedicals, USA). For CE-SSCP
177 community monitoring, the V3 region of 16S rRNA genes of *Bacteria* domain (about 200 bp) was amplified from
178 DNA extracts with the forward primer w49 (5'-ACGGTCCAGACTCCTACGGG-3'; *E. coli* position, 331) and
179 the reverse primer w34 (5'-TTACCGCGGCTGCTGGCAC-3'; *E. coli* position, 533) 5' end-labelled with the
180 fluorescent dye VIC (Applied Biosystems); 25 cycles, hybridization at 61°C, and 30 s elongation at 72°C were

used. Profile alignment with the internal standard (GeneScan 600LIZ, Applied Biosystems, Thermo Fischer Scientific, USA) and band matching were performed with GeneScan (Applied Biosystems, Thermo Fischer Scientific, USA) and BioNumerics (Applied Maths, Belgium) software.

3.3. Modification and characterisation of the support for biofilm growth (zeolite)

Natural mineral zeolite purchased from Saint Malo, France (z-SM), was used in this study. The sample was ground using a hummer crusher and its particle size was adjusted in the range 0.2-1.25 mm using laboratory sieves (diameter: 20 cm; openings: 0.2, 0.315, 0.4, 0.5, 0.63, 0.8, 1 and 1.25 mm). In order to remove dust, wood and plastic contaminants, the sample was washed by floatation in deionized water, filtered and dried at 60ºC during 24 h.

Modifications of the natural zeolite were performed by Li^+, Na^+, K^+, NH_4^+, Ca^{2+}, Mg^{2+}, H^+ ion exchange and by deposition of Fe_2O_3 coating (corresponding samples marked: z-Li, z-Na, z-K, z-NH_4, z-Ca, z-Mg, z-H and z-Fe). For ion exchange, 50 g of z-SM were treated with 400 ml of 0.1 N nitrate solutions of Li^+, Na^+, K^+, NH_4^+, Ca^{2+} or Mg^{2+} in 500-ml Erlenmeyer flasks placed into water-bath-shaker at 30ºC during 24-72 h. Then, the solution was removed and the corresponding nitrate solution was added again. The procedure was repeated 7 times. Afterwards, ion-exchanged samples were washed 3 times in 400 ml of deionized water during 2h and dried at 60 ºC during 24 h. In order to obtain H^+-exchanged zeolite, the NH_4^+-exchanged sample was treated at 500 ºC during 24 h; at this temperature, NH_3 molecules are eliminated from the structure (Kurama et al. 2002). In order to obtain Fe_2O_3-layer on zeolite, first, the sample was treated with $Fe(NO_3)_3$ in a similar way as in ion exchange procedure. In such way, FeO(OH) was deposited on the zeolite surface which was transformed into Fe_2O_3 by heat treatment at 500ºC during 24 h.

Phase composition of the samples was determined by powder X-Ray diffraction study. The measurements were performed at room temperature using a BRUKER D8 Advance θ/2θ diffractometer equipped with a Lynxeye energy-dispersive one-dimensional detector (Cu-Kα radiation, 40 kV and 40 mA; 2θ from 10° to 80° with a step of 0.02° and counting time of 0.5 s per step).

The specific surface area and pore size distribution of the modified samples were measured using nitrogen adsorption/desorption technique (Micromeritics BET ASAP 2050, Belgium, sample weight ≈ 2.5 g).

Surface texture and morphology as well as chemical composition analysis of the zeolite samples were performed by scanning electron microscopy (FE-SEM, Tescan MIRA3, Czech Republic) coupled with a backscattered electron detector (BSD) and an Energy-dispersive X-ray spectroscopy (EDX, Oxford INCA X-act). SEM observations were performed at 5-15 kV, BI = 8-12, WD = 2.8-5 using SE, InBeam and BSD detectors, samples were sputter coated with gold prior observation. EDX measurements were performed at 20 kV, BI = 17-18, WD = 15. Chemical composition was calculated as average of at least 10 measurements of different zeolite particles (analysed area 10000-15000 μm^2).

Amount of the cations incorporated into the zeolite structure for ion-exchanged samples was also evaluated. To this purpose, 1 g of each zeolite sample was ion exchanged in 50 ml of 1 M $NaNO_3$ or NH_4NO_3 solution under shaking at 25ºC during 15 days. Concentration of the released Li^+ was measured using ICP-MS analysis (Agilent 7800, Agilent Technologies, USA), Na, K, Mg, and Ca were measured by ICP-AES analysis (Ultima 2, Horiba Jobin Yvon, Japan) and NH_4 by colorimetric analysis (Photometer NOVA 60 Spectroquant, Merck, Germany). The quantitative analysis was performed in three replicates.

Amount of Fe_2O_3 deposited on zeolite surface was quantified using colorimetric analysis. First, Fe^{3+} was transferred into solution by treatment of 1 g of zeolite sample with 1 N HNO_3. Then, concentration of Fe^{3+} was estimated using spectrophotometry kit and photometer (Spectroquant NOVA 60, Merck, Germany). The analysis was performed in three replicates.

3.4. Biofilm growth and characterisation

Formation of biofilm on zeolite was carried out as follows. The experiments were performed in 250-ml Erlenmeyer flasks containing 50 g of zeolite, 135 ml of MSM solution, 15 ml of inoculum and appropriate amount of pesticide solution (MCPA or glyphosate, added in amount required to reach the concentration of 5 mg/L in liquid medium). As inoculum, a mixture of the three planktonic communities grown at three different pesticide concentrations (1, 5 and 10 mg/L, fourth subculture) was used. The experiment was performed at 22ºC in an incubator without shaking. When the added pesticide was degraded, the liquid phase containing planktonic microorganisms was replaced with fresh sterile MSM and the same amount of pesticide (amount required to reach the concentration of 5 mg/L in liquid medium) was added. During the first weeks of experiment the liquid medium was replaced only partially in order to keep some planktonic microorganisms to allow them to get attached to the zeolite surface and colonise it. After 10 weeks of biofilm growth, steady degradation of the pesticides was achieved. At this point, the biofilm abundancy was also proved by SEM (see below). Next, the zeolite/biofilm composites were filtered, washed 3 times in 50 ml of MSM and subjected to the pesticide degradation kinetic studies. The kinetic study was performed in triplicate in 250-ml Erlenmeyer flasks with 10 % wt. of biocomposite (15 g of biocomposite, 150 ml of MSM, initial concentration of the pesticide C_0 = 5 mg/L). For comparison, abiotic experiments with sterile

241 zeolite were performed in the same conditions (without microbial inoculation) and duration of time in order to
242 verify possible adsorption of the pesticides onto zeolite.
243 Structure of the microbial community attached to zeolite and the planktonic one was regularly monitored using
244 CE-SSCP as described in part 2.2. DNA extraction was performed from zeolite particles (after washing 3 times
245 using MSM) and planktonic community (by filtration at 0.2 µm). SSCP data were submitted to Principal
246 Conponent Analysis (PCA) using XLSTAT (Pearson correlation matrix, distance biplot). In addition, some
247 samples were characterized by high-throughput sequencing using an Illumina MiSeq sequencer (INRA Transfert
248 Environnement, Narbonne, France). In particular, biocomposites showing different SSCP profiles and with
249 different pesticides degradation activity (comparatively high activity – 'MCPA z-Fe/biofilm', 'glyphosate z-
250 SM/biofilm' - and comparatively low - 'MCPA z-SM/biofilm', 'Glyphosate z-NH_4/biofilm'); in addition, MCPA
251 planktonic culture (which remained active in opposite to glyphosate planktonic culture which biodegradation
252 activity stopped in time) and positive control where chosen for comparison. For Illumina sequencing, a portion of
253 the 16S rRNA gene was amplified using the barcoded, universal primer set 515WF/918WR (Wang et al. 2009).
254 PCR reactions were performed using AccuStart II PCR ToughMix kit and cleaned (HighPrep PCR beads,
255 Mokascience). Pools were submitted for sequencing on Illumina MiSeq instrument at GeT-PlaGe (Auzeville,
256 France). Sequences were processed using Mothur (version 1.36.1) according to MiSeq SOP pipeline (Schloss et
257 al. 2009). Barcodes, primers, and sequences showing homopolymers of more than 8bp have been discarded.
258 Sequences showing 100% homology were grouped in unique sequences, then in OTUs (operational taxonomic
259 unit), based on 97% homology. Sequences were then assigned to match to a sequence in Greengenes for taxonomic
260 identification. The data for this study have been deposited in the European Nucleotide Archive (ENA) at EMBL-
261 EBI under accession numbers PRJEB36612 (16S rRNA genes) and PRJEB36626 (18S rRNA genes).
262 The biofilm was also characterised by SEM in similar conditions as those used for characterisation of zeolite
263 particles. Prior to the SEM observations, microorganisms of biocomposites were fixed using 2.5 % wt.
264 glutaraldehyde in phosphate buffer during 1 h, rinsed with phosphate buffer and ethanol, dried and sputter coated
265 with gold.
266
267 *3.5. Pesticide analysis*
268 Degradation of pesticides was monitored during all stages of the experiments. Concentration of MCPA was
269 evaluated using UV-spectroscopy (Sirotiak et al. 2015) (Spectrophotometer Cary 100 UV-visible, 200-800 nm,
270 Agilent Technologies, USA) and ELYSA kit for 2,4-D (ref 154003A, Abraxis, USA). Concentration of glyphosate
271 was evaluated using ELYSA kit for glyphosate (ref 1500086, Abraxis, USA).
272 The degradation pathway of MCPA and glyphosate by microorganisms is presented in Online Resource 1 and
273 Online Resource 2, respectively. In order to confirm biodegradation of pesticides, formation of glyphosate and
274 MCPA metabolites was analysed by two different liquid chromatography (LC) coupled to mass spectrometry
275 analysis. The first one is a Acquity ultra-performance liquid chromatography system (UPLC™, Waters) interfaced
276 to a triple quadrupole mass spectrometer (Quattro Premier XE, Waters) was used to analyse glyphosate and AMPA
277 in biocomposites and planktonic experiments at different points of time to confirm elimination of glyphosate and
278 the formation of glyphosate metabolite AMPA. Briefly, a derivatization step with FMOC-chloride in the presence
279 of a borate buffer is required prior to analysis and ILIS are added to samples. 1.5 mL of derivatized sample is
280 extract online with an SPE cartridge (Oasis HLB 25 µm 2.1×20 mm) before separation in an Acquity UPLC HSS
281 column (T3 1.8 µm× 2.1 mm×100 mm). The mobile phase was composed of solvent A (5 mM ammonium acetate
282 in water) and solvent B (acetonitrile) at a constant flow rate of 0.4 mL/min. The gradient was programmed to begin
283 at 90 % of A for 2 min, at which time the amount of B was increased from 10 to 50 % in 5 min, and 50 to 100%
284 in 0.25 min with stabilization for 3 min, before returning to the initial conditions in 0.25 min for 2 min. The column
285 temperature was 30 °C. Mass spectrometry involved a triple quadrupole fitted with an electrospray (electrospray
286 ionization (ESI)) interface (positive mode) and controlled by MassLynx software. Cone gas and desolvation
287 (drying gas) used is N_2 and Ar as the collision gas at a pressure of $3.7 \cdot 10^{-3}$ mbar. Multiple reaction monitoring
288 (MRM) transitions were selected and adjusted individually for each analyte. Calibration curves were derivatized
289 as samples and control quality were Volvic® water spiked with analytes. The quantification limit is 30 ng/L.
290 The second one is a Waters Acquity UPLC I-Class system (Waters, Guyancourt, France) interfaced to high
291 resolution mass spectrometer (hybrid quadrupole time-of-flight mass spectrometer, XEVO G2S QTOF, Waters,
292 Manchester, United Kingdom), LC-QTOF, using an electrospray ionization interface (ESI), positive and negative
293 mode. This technique was previously described in detail by (Soulier et al. 2016) and was used for identification of
294 MCPA and its metabolites after 30 h of MCPA degradation. Briefly, a volume of 100 µl of samples was injected
295 by direct injection on 150 mm × 2.1 mm ACQUITY BEH C18 1.7 µm column (Waters). Mass spectrometry data
296 were acquired in centroid, resolution and MS^E modes over a m/z range of 50–1200. The resolution of QTOF-MS
297 was between 24,000 and 30,000 in resolution mode at m/z 556.2766 (positive ionization) and 524.262 (negative
298 ionization). The potential mass deviation was corrected automatically during the injection with a solution Leucine
299 Enkephalin (LeuEnk, 1 ng/µL). For assurance and quality control, 29 ILIS were added in water (UPLC/MS grade)

300 and in all samples to control the injection; and a solution of 73 no-labelled standards and blanks are analyses
301 analogously to the samples.
302 For statistical analysis of the biodegradation results, boxplots were calculated from triplicate flasks using R4.0.1
303 and RStudio (The R Development Core Team, 2009; http://www.R-project.org). Data (n=3) were analyzed using
304 the non-parametric Kruskal-Wallis test. Difference was considered significant at p-value < 0.05.
305
306 ## 4. Results
307
308 ### *4.1. Preparation and characterisation of microbial communities and the support material*
309
310 #### *4.1.1. Selection and enrichment of microbial communities able to biodegrade MCPA and glyphosate*
311 First of all, microbial communities able to use MCPA or glyphosate as nutrient were selected and enriched. An
312 agricultural pesticide-contaminated soil/water sample was used as source of pesticides degrading microorganisms
313 and as inoculum in mineral growth medium. After three subcultures, kinetics of MCPA degradation was very rapid
314 (less than 1 week whatever the initial concentration of MCPA), while complete degradation of glyphosate took
315 more than 20 days. Similar kinetics of degradation were obtained for the 4th subculture. No pesticide degradation
316 was observed in the absence of microorganisms (abiotic tests).
317 Evolution of the bacterial community structure during the selection steps was assessed by CE-SSCP. This method
318 was used as a rapid and simple tool to obtain a general view of the evolution of the communities and thus of the
319 selection approach applied in this study. According to the results, the structure of the community of the 4th
320 subcultures, either with or without (positive control) pesticide addition, changed drastically when compared to that
321 of the initial inoculum (Online Resource 3). The result suggests that selection procedure has significantly reduced
322 the number of dominant taxa in the samples. However, the samples were still characterised by wide biodiversity
323 with profiles varying according to the presence of pesticide, its type and concentration.
324 Four months and four subcultures were necessary to select microbial communities efficiently degrading MCPA
325 and glyphosate. Because the pesticide concentration has high influence on biodiversity of planktonic community
326 (Online Resource 3), for each pesticide, the subcultures obtained at various pesticide concentration (1, 5 and 10
327 mg/L) were mixed and used as inoculum for biofilm growth (see section 3.4).
328
329 #### *4.1.2. Preparation and characterisation of the support materials for biofilm growth*
330 As support material, nine different zeolite samples were prepared. The powder XRD patterns of the unmodified
331 and some of the modified samples are presented in Fig. 1. The unmodified zeolite (z-SM) consists mainly of
332 clinoptilolite and heulandite types (PDF card # 01-080-0464 and 01-082-1228), which are one of the most common
333 naturally occurring zeolite species (Smith 1963; Filippousi et al. 2015; Jha and Singh 2016). The main crystalline
334 impurity phases that were identified in the phase composition of the sample are: illite (PDF card # 00-058-2014),
335 quartz (PDF card # 01-070-7344) and feldspar (PDF card # 01-072-1114). The identified phases are commonly
336 found in natural zeolites (Ates and Hardacre 2012). The surface area of the unmodified zeolite sample (z-SM) was
337 determined as 15.7 m^2/g (BET) and total pore volume is 0.072 cm^3/g (pore size < 2526.6 Å at the point P/P_0 =
338 0.9925).
339 Concerning the modified zeolite samples, in general, the XRD analysis showed essentially similar patterns (Fig.
340 1). Whatever the treatment performed on raw zeolite, the clinoptilolite phase remained well preserved after
341 modification, even if a slight change in the intensity of the peaks was detected (Fig. 1). These observations indicate
342 that the clinoptilolite framework has not undergone any significant structural changes during incorporation of the
343 metal cations. However, some of the reflections characteristic of clinoptilolite, especially those at 2 theta 22-23°,
344 are less resolved for the samples heat-treated at 500 °C (Fig 1, samples z-Fe and z-H). This likely indicates lower
345 crystallinity due to the structural disorder and defects induced by thermal treatment. All other impurity phases
346 (illite, quartz, feldspar) have been still identified in the modified samples. At the same time, surface area of the
347 samples has not been significantly affected by modification, with exception of sample z-Fe. Surface area and pore
348 volume of this sample were respectively increased up to 31.4 m^2/g and 0.09 cm^3/g.
349 SEM images of zeolite samples are shown in Fig. 2. Images a-b show typical microporous structure of the
350 unmodified zeolite surface (z-SM). In some places, crystal inclusions/deposits within the zeolite grains or on its
351 surface can be observed (Fig. 2c and 2d). EDX analysis of this inclusions showed high content of Ca and P in
352 atomic ratio 1:1 that may correspond to dicalcium phosphate. In general, texture and appearance of most of the
353 modified zeolite samples look very similar to those of z-SM. Differences in surface appearance were only observed
354 for two samples: z-Ca and z-Fe. For both samples, a thin layer (≈ 200 nm thick) of deposits of irregular shape have
355 been observed on the surface of the particles. Typical appearances of the deposits are shown in Fig. 2e and 3f for
356 z-Ca and z-Fe respectively. According to EDX, both deposits are enriched in content of the corresponding added
357 element. However, while surface of z-Fe was almost completely covered with such deposits (more than 90 % of
358 the surface), for the sample z-Ca it was observed only in some places (less than 10 % of the surface). Composition
359 of the deposition for z-Fe should correspond to Fe_2O_3, while for z-Ca it may correspond to some calcium salt.

360 However due to low content and crystallinity, the phases corresponding to the deposits have not been revealed by
361 XRD analysis.
362 Chemical composition of the samples according to EDX analyses is presented in Tables 1 and 2. For unmodified
363 zeolite z-SM, besides Si^{4+} and Al^{3+} related to zeolite crystalline framework, other cations involved in charge
364 compensations, such as K^+, Ca^{2+}, Mg^{2+} and Na^+ were detected. The atomic content of the light cations such as H^+,
365 Li^+ and NH_4^+ has not been quantified using EDX due to limited detector sensitivity. Consequently, excluding light
366 cations, charge compensation seems to be mainly obtained by Ca (0.99 at.% corresponding to 997 meq/kg), K
367 (1.35 at.% corresponding to 682 meq/kg) and Mg (0.69 at.% corresponding to 697 meq/kg) cations, for a total
368 CEC at 2461 meq/kg calculated considering K, Ca, Mg and Na content. Traces of other elements, such as In, Sn,
369 Zn, Cl, Ti, Lu, and Cu have been also detected. In modified zeolites, concentration of the introduced cation is, in
370 general, increased. In particular, there is 13 fold increase of Na at. % for z-Na; 2.4 fold increase of K at. % for z-
371 K; very moderate increase of Mg in z-Mg; 5 fold increase of Fe at. % for z-Fe; while concentration of Ca remains
372 almost the same. The later may be explained with the fact that the Ca-containing deposits were observed by SEM
373 only in some places covering less than 10 % of the surface and unevenly distributed through the sample. Therefore,
374 due to limitation of scanned area by EDX, the areas rich in Ca were not always considered.
375 In order to evaluate the amount of the modifying elements introduced into the zeolite structure, an additional
376 investigation of the chemical composition of the samples was performed using alternative techniques. To this
377 purpose, modifying elements have been leached into solution (by ion exchange or acid treatment) and analysed by
378 ICP-AES (Na, K, Mg, Ca), ICP-MS (Li) or colorimetric analysis (NH_4, Fe). The results were compared to those
379 obtained by EDX and are presented in Table 2. The results of EDX measurements showed, in general, higher
380 content of the modifying elements.
381
382 *4.2. Formation and characterisation of biocomposites*
383
384 *4.2.1. Formation of biofilm able to biodegrade MCPA and glyphosate*
385 Activity of the biocomposites towards degradation of pesticides was tested throughout biofilm development. At
386 the beginning of the experiments, degradation of MCPA by biocomposites took up to one week while up to 5
387 weeks were necessary for glyphosate degradation. After 2-3 month, the time required of glyphosate degradation
388 in most cases was gradually reduced to a couple of days while in some cases (z-Fe and z-H) the ability was lost
389 (Fig 3). For z-SM and z-Na, degradation time of 99 % of glyphosate (C_0 = 5 mg/L) was reduced from 35 days
390 (Fig. 3, batch 5) to 2-4 days (Fig. 3, batch 9). However, for the samples z-Fe and z-H, glyphosate biodegradation
391 was lost after the 6th subculture: 22 and 50 % of glyphosate was degraded directly after addition of z-H and z-Fe,
392 respectively, within the tested period of 35 days (Fig. 3, batch 5); for the next subculture no degradation at all was
393 achieved within the 35 days (Fig. 3, batch 6). Similar situation was observed for the planktonic community for
394 which glyphosate biodegradation was also lost after 6th subculture (not shown in Fig. 3), although ability to degrade
395 glyphosate was confirmed for its earlier subcultures: 99 % of glyphosate was degraded within 19 days (Fig 3,
396 planktonic community, batch 4).
397
398 *4.2.2. Observation of biofilm*
399 According to SEM observations, the microorganisms have heterogeneously colonised the surface of each zeolite
400 grain and were spotted on different parts of the surface (rough areas, holes, smooth areas). As example,
401 micrographs for the sample biofilm/z-Fe (MCPA) are presented in Fig 6, which shows microorganisms of different
402 shape and size attached to the surface of the sample. Presence of extracellular polymeric substances that can be
403 clearly observed on some images (appearing as wires between individuals on Fig. 6c and 6d) indicates that
404 microorganisms are not only attached to the surface but do colonise it and form biofilm (Mills et al. 1994; Yee et
405 al. 2000; Deo et al. 2001; Zheng et al. 2001; Jiang et al. 2007). On basis of SEM images it is difficult to compare
406 the biofilm abundance on the different zeolite types because surface colonisation was not uniform. However, the
407 sample biofilm/z-Fe (MCPA) showed the most abundantly colonised surface testifying in favour of enhances
408 biofilm formation on Fe-modified zeolite. However, due to characteristic texture of the Fe-oxide coating it was
409 easier to spot the attached microorganisms and capture SEM micrographs for this sample.
410 Attempt to quantify the biofilm abundance was also performed using quantification of extracted DNAs from the
411 zeolite/biofilm samples and total organic carbon (TOC) analysis. However, amount of extracted DNA varied
412 according to grains' sampling (biofilm's and zeolite's surface heterogeneity, water content), while TOC analysis
413 showed too high variation in carbon content in a reference sample (original natural zeolite). Thus, no reliable
414 results on biofilm quantification were obtained.
415
416 *4.2.3. Pesticide degradation activity of biocomposites*
417 Biodegradation kinetics study was performed after a period of 10 weeks which was considered to be sufficient to
418 achieve the formation of biofilms in the selected conditions. Biodegradation kinetics are shown in Fig. 4a and 4b
419 respectively for MCPA and glyphosate. Separate experiments with sterilised zeolite confirmed that neither MCPA

420 nor glyphosate are adsorbed on the zeolite and thus, decline in the pesticide concentration can be ascribed in full
421 to biodegradation. The abiotic tests with zeolite were performed in parallel with the experiments with
422 biocomposites under the same conditions and duration of time. The experiments did not show any reduction of
423 concentration of pesticide, thus no significant adsorption of the pesticides can be ascribed to zeolite at the studied
424 conditions. Moreover, some of the studied biocomposites stopped to degrade glyphosate with time and the
425 glyphosate concentration remained constant over the long period of time. This result suggests that no significant
426 adsorption of pesticide occurred on biomass or zeolite (even if there was some insignificant adsorption at the first
427 stages of the experiments, the amount of the adsorption sites should be very low and gets saturated very quickly).
428 Based on these results, we could ascribe the decline in the pesticide concentration in full to biodegradation.
429 The MCPA-grown biocomposites were able to degrade MCPA within 30-96 h, with degradation rates of 0.05-013
430 mg/h (Fig. 4a). Whatever the biocomposite, no lag phase was observed in degradation of MCPA, while with
431 planktonic community MCPA degradation was delayed. The samples z-Li/biofilm, z-Na/biofilm, z-K/biofilm, z-
432 Fe/biofilm and z-Ca/biofilm showed the best results with degradation rate up to 0.13 mg/h. Planktonic culture, z-
433 SM/biofilm, z-Mg/biofilm, z-NH_4/biofilm and z-H/biofilm showed significantly lower MCPA biodegradation
434 activities. In all cases, it was impossible to detect any MCPA metabolites by UV-VIS spectroscopy analysis as no
435 new peaks appeared on the UV-VIS spectra. Typically, concentration of the main metabolite MCP do not reaches
436 more than 5% of the initial MCPA content (Danish Environmental Protection Agency 1998), which is below the
437 detection limit of UV-VIS spectroscopy. Therefore, in order to get information on the potential pathway of MCPA
438 degradation that occurred during our experiments, high resolution mass spectrometry analysis was performed at
439 the middle point (after 30 h) of the kinetics. Results showed presence of main metabolites of MCPA – 4-chloro-o-
440 cresol (MCP) and 5-chloro-3-methylcatechol – as well as other potential MCPA metabolites (Online Resource 4)
441 which suggests classical way of MCPA bacterial degradation schematically shown in Online Resource 1 (Danish
442 Environmental Protection Agency 1998; Roberts et al. 2007; Paszko et al. 2016).
443 Considering glyphosate (Fig. 5b), in several cases (planktonic community, z-H/biofilm and z-Fe/biofilm), ability
444 to degrade the pesticide was lost. For all other biocomposites, glyphosate biodegradation took between 40 and 72
445 h, its half-life ranging from 18 up to 40 h. Four samples (z-SM/biofilm, z-Li/biofilm, z-Ca/biofilm and z-
446 Mg/biofilm) showed high degradation activity (degradation rate of 0.12 mg/h and total degradation within 40 h)
447 compared to the others (z-Na/biofilm, z-K/biofilm and z-NH_4/biofilm) for which biodegradation activity was
448 significantly lower (degradation rate 0.08 mg/h and total degradation time about 70 h). Biodegradation of
449 glyphosate by microorganisms is schematically shown in Online Resource 1 (Institut national de l'environnement
450 industriel et des risques 2014; Sviridov et al. 2015). In presence of phosphate in the medium (like in our case) it
451 leads to the formation of another toxic compound, AMPA. Another biodegradation route of glyphosate with the
452 formation of less toxic compounds (sarcosine pathway) has been described (Sviridov et al. 2015; Zhan et al. 2018),
453 however it has been detected only in laboratory conditions when the experiments are performed in phosphorus
454 deficient environment. Therefore, in our experiment that were performed in phosphorus-rich environment, the
455 main metabolite of glyphosate that could be expected is AMPA. Formation and accumulation of AMPA in our
456 experiments were confirmed by mass spectrometry. It has not been degraded by any of the biocomposites during
457 the experiment (more than 2 months).
458 Additional experiment has been performed in order to verify the ability of biofilms to degrade other pesticides.
459 The results showed that MCPA-degrading biofilms were unable to degrade glyphosate, and glyphosate-degrading
460 biofilms unable to degrade MCPA. However, MCPA-degrading biofilms were able to degrade the MCPA-related
461 pesticide 2,4-D.
462
463 *4.2.4. Structure and diversity of the microbial communities of the biocomposites*
464 Structure and diversity of the microbial communities of the biocomposites with biodegradation activity towards
465 the pesticides were studied after 10 weeks of biofilm's development. CE-SSCP fingerprints and their statistical
466 analysis (PCA analysis) showed that the structure of the bacterial community is influenced the most by the
467 pesticide nature and less by the zeolite type (Online Resource 5). In particular, the PCA analysis showed that
468 biocomposites grown in the presence of glyphosate (whatever the zeolites, blue circle) are separated from those
469 grown in the presence of MCPA according to F1 and F2 axes, suggesting that pesticides influence bacterial
470 diversity the most. Concerning evolution of the community structure of biofilms over time, statistical analysis has
471 shown that for glyphosate-grown biofilms there is no significant evolution. Thus, the community structure of the
472 glyphosate-grown biofilms does not depend much either on zeolite type or growth time. In the other hand, for
473 MCPA the result revealed significant differences between young biofilms developed within 2-6 weeks and the old
474 biofilms developed for more than 2 months suggesting an evolution of biofilm community structure along time.
475 Thus, the community structure of the MCPA-grown biofilms similar to glyphosate grown biofilms is not
476 influenced much by the zeolite type, but, in opposite to glyphosate grown biofilms, it significantly depends on the
477 biofilm age.
478 Prokaryotic and eukaryotic microorganisms' taxa that prevail in 10 weeks grown biofilms were identified for
479 selected samples by high-throughput gene sequencing. The results are summarised in Table 3 for biodiversity

480 indexes, Fig. 7 shows classification of identified bacterial genera and Online Resource 6 contains detailed
481 information on relative abundance of the major taxa identified at the genus level in each sample (for OTUs
482 accounting for >1 % of sequences in at least one of the samples). Classification of the identified genera was
483 performed with focus on their relation to mineralisation of MCPA and glyphosate or other pesticides already
484 demonstrated in the literature. Accordingly, the genera were classified into the following groups: 1) the group of
485 the confirmed degraders that consists of the bacteria genera which have been reported to degrade MCPA or/and
486 glyphosate either in liquid media, soil or sand; 2) the group of potential degraders - genera found in communities
487 degrading the target pesticide or genetically related to confirmed degraders; 3) genera related to other pesticides -
488 confirmed degraders/found in presence of structurally unrelated pesticides; 4) genera involved in nitrogen redox
489 cycle in soil; 5) genera with no known connection to any pesticide or nitrogen cycle.
490 All analysed samples contain high variety of bacterial and eukaryote taxa. *Proteobacteria* is the dominant bacterial
491 phylum which accounts for 66-92% of the bacterial sequences in each sample. For MCPA-grown communities,
492 proportion of confirmed MCPA degraders is the highest in the planktonic culture (85.1 %), while they account for
493 32.9-40.4 % in zeolite/biofilm samples, which is close to 41.4 % retrieved in the positive control (planktonic
494 culture enriched using the same inoculant under the same conditions but without addition of pesticide). At the
495 same time, 'MCPA planktonic culture' has the lowest biodiversity with the smallest number of OTUs for bacteria
496 and the lowest Simpson's and Shannon's indexes in comparison to biofilms and positive control (Table 3). Thus,
497 addition of MCPA significantly reduces number of OTU's and bacterial diversity of planktonic community, which
498 confirms directional selection towards MCPA degraders. For the planktonic culture, the genus *Sphingobium,*
499 comprising confirmed MCPA bacterial degraders, accounts for 71.5%, while 5 other identified MCPA degraders
500 account only from 1 to 5 % each. For MCPA-grown biocomposites, seven confirmed (*Aminobacter* (Gözdereliler
501 et al. 2013), *Cupriavidus* (Önneby 2013), *Novosphingobium* (Önneby 2013), *Pseudomonas* (Smejkal et al. 2003)
502 (Evangelista et al. 2010), *Rhodococcus* (Evangelista et al. 2010), *Sphingobium* (Önneby 2013) and *Sphingopyxis*
503 (Önneby 2013)) and three potential (*Methylibium* (Stibal et al. 2012), *Oceanibaculum* (Góngora-Echeverría et al.
504 2018), *Planctomyces* (Liu et al. 2011)) MCPA-degrading bacterial genera were identified. Although the genus
505 *Sphingobium* is one of the dominating that accounts for 12-13 % of the sequences for the biocomposites, however
506 the proportion between the different MCPA degraders is more homogeneous which also reflects in higher
507 Simpson's and Shannon's indexes of the biocomposites in comparison to the planktonic culture.
508 Concerning samples with glyphosate ('glyphosate z-SM/biofilm' and 'glyphosate z-NH_4/biofilm'), biodiversity
509 indexes (Table 3) suggest a lower biodiversity for the 'z-NH_4/biofilm' sample, both for bacteria and eukaryotic
510 cells. Only one confirmed glyphosate degrader – bacterial genus *Pseudomonas* (Jacob et al. 1988; Zhao et al. 2015)
511 – is present in the samples (accounting for 4-6 %); six other genera (*Aminobacter* (Firdous et al. 2020),
512 *Chitinophagaceae* (Muturi et al. 2017), *Fluviicola* (Schlatter et al. 2017), *Oceanibaculum* (Góngora-Echeverría et
513 al. 2018), *Planctomyces* (Wang et al. 2017) and *Pseudoxanthomonas* (Newman et al. 2016)) were identified as
514 potential degraders (accounting for 25-35 %). The most dominant genus in the samples is *Aminobacter* (10-25%);
515 93 % of the *phnJ* gene sequence of *Aminobacter aminovorans* strain KCTC 2477 (CP015005.1) is identical to the
516 gene involved in glyphosate degradation by the known glyphosate-degrading strain *Comamonas odontotermitis*
517 *P2* (Firdous et al. 2020), so very probably *Aminobacter* includes glyphosate degrading members as well.
518 Considering identified eukaryotes, our attention has been focused mainly on Fungi (including yeasts) because
519 some of their representatives are known to degrade pesticides including MCPA and/or glyphosate (Ditterich et al.
520 2013). However, to our knowledge fungi genera found within our samples (*Simplicillium, Phialocephala,*
521 *Cryptococcus*) do not have any known connection either to MCPA or glyphosate or any other pesticide.
522
523 **5. Discussion**
524 The results concerning zeolite modification and its impact on physical-chemical properties of the support material
525 are discussed in section *5.1. Impact of modification on the physical-chemical properties of the support material*.
526 The results regarding biodegradation activity and biodiversity of biocomposites are discussed in section *5.2.*
527 *Impact of modified zeolites on biodegradation activity and biodiversity of biofilms*
528
529 *5.1. Impact of modification on the physical-chemical properties of the support material*
530 Modifications of natural zeolite, chosen as support material, were undertaken to favour microbial affinity to its
531 surface to promote biofilm growth. In general, the modifications did not lead to substantial changes of the phase
532 composition of surface area, but had significant impact on its chemical profile. In general, the obtained values for
533 surface area (15.7 m^2/g) and porosity (0.072 cm^3/g) of not-modified zeolite (z-SM) are much lower than those
534 typical of zeolites: surface area between 300 and 700 m^2/g and porosity between 0.1 and 0.35 cm^3/g (Reeve and
535 Fallowfield 2018). Such a difference can be explained by high content of impurity phases of low porosity arising
536 due to natural origin of our zeolite sample. Increase of the surface area and pore volume of the sample z-Fe sample
537 can be explained by the ultra-divided structure of the deposited Fe oxide layer on the surface of zeolite which was
538 observed by SEM analysis (Fig. 2f).

Considering chemical composition, first of all we can notice that when a new cation is introduced into zeolite structure, the concentration of other exchangeable cations such as Na, K, Mg and especially Ca is reduced confirming exchange phenomena (Table 1). At the same time, content of Si and Al has also decreased due to increase of the content of other elements in the structure. However, more indicative is the value of Si/Al, which in our case is decreased for all modifications, indicating some fundamental changes in the zeolite structure. Normally, Si/Al remains stable with ion exchange or slightly increases due to dealumination (Kurama et al. 2002; Kennedy and Tezel 2018). In particular, dealumination is typical for Fe^{3+} modification of zeolites (Kennedy and Tezel 2018). However in our case, z-Fe sample showed slight Si/Al decrease that may lay within the measurement uncertainty which implies that Fe did not substitute Al, but was mainly deposited on surface as oxyhydroxide. For other samples, the Si/Al ratio substantially declines (as much as by 10 %) implying that the amount of silicate is reduced that is a very untypical situation. The evaluated Si/Al values of the zeolite samples are 4.6-5.2, which is in the range typical for clinoptilolite (4-6 (Reeve and Fallowfield 2018) or 1-5 (Jha and Singh 2016)). In general, the zeolites with the intermediate Si/Al values (between 2 and 5) tend to be the most stable species characterised with heterogeneous hydrophilic surface (Ramesh and Reddy 2011). As the ratio of Si/Al increases, so does the hydrothermal stability and hydrophobicity of the zeolite framework (Reeve and Fallowfield 2018). However, the zeolites with Si/Al < 10 are still hydrophilic (Jha and Singh 2016), which should also correspond to the surface properties of our samples. Considering acidity, it positively correlates with Si/Al but the number of the acid sites usually increases in the same order as Brønsted acidity of the introduced cations (Wu and Weitz 2014), which in our case is $Mg^{2+} > Ca^{2+} > Li^{+} > Na^{+} > NH_4^{+} = K^{+}$ (Kolthoff and Willman 1934). As discussed earlier, the mentioned surface parameters may greatly influence the microbial affinity to the material surface and thus be important for the initial stage of the biofilm formation.

The results of EDX measurements showed, in general, higher content of the modifying elements that corresponds to their total content (originally present in the natural zeolite and the introduced one) in comparison to the results obtained by leaching (Table 2). The difference can be also explained by the fact that particles measured by EDX were definitely zeolite grains, while the leaching was performed for whole sample that contains naturally occurring impurities contributing to the total weight of the sample. However, for the samples z-K and z-Mg the difference is especially high. In case of K, its underestimated amount can be explained with the preparation of the samples, in particular utilisation of the concentrated NH_4NO_3 solution for leaching. According to the literature (Wang and Peng 2010), ion exchange selectivity of clinoptilolite follows the row $K^{+} > NH_4^{+} > Na^{+} > Ca^{2+} > Fe^{3+} > Mg^{2+} > Li^{+}$. Therefore, in case of K, even prolonged treatment with a very concentrated NH_4^{+} solution may not be able to replace all K^{+} in the structure. In case of Mg, such difference can be explained by the formation of the insoluble Mg-deposits that enabled its leaching into solution.

5.2. Impact of modified zeolites on biodegradation activity and biodiversity of biofilms

Concerning biodegradation activity, our results demonstrated that degradation rate of pesticide was considerably accelerated after addition of zeolite for most of the samples. Full degradation of MCPA with a 10 weeks grown biocomposite in the selected conditions took place within 30-96 h, with a half-life in those conditions of 16-33 h, which is considerably lower than in the natural environment. Indeed, half-life of MCPA in natural water-sediment system is greater than 2400 h (Institut national de l'environnement industriel et des risques 2013) and between 504-576 h in aerobic laboratory conditions (AGRITOX 2010)). Similarly to MCPA, in the natural environment half-life of glyphosate is considerably longer, between 264 and 720 h in the field and between 96 and 4320 h in aerobic laboratory conditions (AGRITOX 2018), whereas in our experiments, glyphosate biodegradation took between 40 and 72 h, its half-life ranging from 18 up to 40 h. Ability of the MCPA-degrading biofilms to degrade the MCPA-related pesticide 2,4-D was also confirmed. Similar observations has been already reported in the literature: commonly, MCPA-degrading microorganisms possess all of the structural and regulatory genes needed to convert phenoxy herbicides including MCPA and 2,4-D to CO_2 and chloride (Bælum et al. 2006).

Considering the possible dissipation of the pesticides due to adsorption, in our experiments no adsorption of MCPA or glyphosate was detected either on the zeolites and biomass or on the pots. Although the zeolites have high adsorption potential towards the positively charged species (Kassa 2019), to make zeolite adsorb negatively charged or neutral species (such as MCPA and glyphosate in water solution) may require modification, for example by surfactants or CeO_2 deposition (Rathi et al. 2019; Jevremoví et al. 2020). Moreover, the medium in which where conducted the experiments contained large amounts of inorganic ions, therefore if there were some active sites on the surface of zeolite to adsorb negatively charged or neutral species, they would by fully blocked by inorganic ions, which concentration in the medium was significantly higher than the concentration of pesticides. Therefore, reduction of pesticides' concentration in our experiments was fully ascribed to biodegradation.

According to our results, introduction of zeolite as support material allows to keep higher biodiversity of confirmed MCPA degraders and other genera. Positive effect of zeolite on the survival and growth of wide variety of bacterial genera in the presence of the pesticide could be explained by the fact that the natural zeolite provides an alternative source of nutrients (organic carbon impurities) and could support microorganisms growth as structured biofilms that are known to protect cells from environmental stress such as the presence of toxic compounds (Saez et al.

599 2012; Haque et al. 2020). In general, modification of zeolite as well as its thermal treatment has limited influence
600 on the bacterial community structure grown in presence of MCPA. Two confirmed MCPA degraders, *Sphingobium*
601 and *Sphingopyxis* prevail in both biocomposites ('MCPA z-SM/biofilm' and 'MCPA z-Fe/biofilm') accounting
602 for more than half of the confirmed MCPA degraders (Online Resource 6).There are also clear similarities in
603 bacterial community structure of these biocomposites. In particular, the ratio between 6 classified groups of
604 bacterial genera (Fig 7) as well as proportion of some identified genera within each group in these samples are
605 alike. Moreover, proportion between the genera within the group of confirmed MCPA degraders in these samples
606 is particularly close to each other (Online Resource 6). Considering the relations between the community structure
607 and the kinetics of MCPA degradation, the sample 'MCPA z-Fe/biofilm', which degrades MCPA quicker, contains
608 higher percent of the confirmed MCPA degraders (40.4 against 32.9 % in 'MCPA z-SM/biofilm'). Thus, better
609 performance of 'MCPA z-Fe/biofilm' biocomposite positively correlates with proportion of identified confirmed
610 MCPA degraders. Better performance of z-Fe/biofilm and z-Ca/biofilm can probably be explained by enhanced
611 adhesion of microorganisms to the corresponding zeolite samples due to the developed surface texture (generally,
612 surface irregularities foster bacterial adhesion whereas smooth surfaces reduce adhesion (Sauer-Budge et al.
613 2013)). At last, Fe modification was reported earlier as an efficient way to increase microbial adhesion to the
614 surface (Mills et al. 1994; Deo et al. 2001; Ams et al. 2004; Jiang et al. 2007). Moreover, z-Fe/biofilm (MCPA)
615 showed the most abundantly colonised surface testifying in favour of enhances biofilm formation on Fe-modified
616 zeolite which correlates with the previous reports (Mills et al. 1994; Ams et al. 2004) and its enhanced degradation
617 activity towards MCPA.
618 As it was mentioned earlier, no lag phase was observed in degradation of MCPA, while with planktonic community
619 MCPA degradation was delayed (Fig. 4a). This result can be explained by a difference in active biomass content
620 between planktonic and sessile cultures, and/or by a difference in the state, metabolically active or not, of the cells
621 when the kinetic experiments were started, and/or a different biodiversity leading to a difference in microbial
622 activity.
623 Bacterial community structure of glyphosate-grown biofilms with two different zeolites are more different from
624 each other than the communities grown with MCPA (Fig. 7). The sample 'Glyphosate z-SM/biofilm' which
625 quicker degrades glyphosate, contains approximately the same proportion of the confirmed glyphosate degraders
626 (4.4 against 6.2% in 'Glyphosate z-NH_4/biofilm'), but significantly higher amount of potential glyphosate
627 degraders (35.4 against 24.7% in 'Glyphosate z-NH_4/biofilm') and is more diverse (Table 3). Thus, performance
628 of the glyphosate-degrading biocomposites positively correlates with proportion of the glyphosate-related bacteria
629 genera. Almost 40 % of 'Glyphosate z-NH_4/biofilm' bacteria are involved in nitrogen redox cycle that can be
630 explained by modification of this zeolite sample with ammonia. When comparing MCPA- and glyphosate-grown
631 biocomposites, the former can be characterised by higher biodiversity and considerably higher amount of
632 confirmed degraders.
633 The loss of activity towards glyphosate degradation of some of the samples (z-Fe/biofilm and z-H/biofilm) can be
634 explained with extinction of the glyphosate degrading community. Both zeolite samples were treatment at 500 °C
635 that has eliminated the organic part (residue of dead plants and microorganisms) normally providing alternative
636 nutrient essential for the growth glyphosate degrading community. Therefore, the extinction of the glyphosate
637 degrading community can be explained due to the leak of an alternative nutrient essential for their growth.
638 Moreover, the sample performance correlates well with the acidity of zeolite surface connected with the modifying
639 cation nature and Si/Al (Wu and Weitz 2014). In particular, Brønsted acidity of Mg^{2+}, Ca^{2+} and Li^{+} is higher that
640 of Na^{+}, NH_4^{+} and K^{+} (Kolthoff and Willman 1934); while Si/Al is the highest for the unmodified z-SM. Thus, it
641 seems that the affinity of the glyphosate-grown community to zeolite surface positively correlates with the number
642 of the acid sites. In addition, the positive effect of Mg^{2+} and Ca^{2+} on biofilm growth has been reported earlier (Das
643 et al. 2014; He et al. 2016). However, it depends in first turn on the microorganism species. Thus, in our case both
644 elements had positive effect on glyphosate-grown biofilms, while for MCPA the sample z-Mg/biofilm showed one
645 of the worst performances. In general, when looking at the performance of the MCPA- and glyphosate-grown
646 biocomposites regarding the type of zeolite, it appears that the preferable type of zeolite differs dependent on the
647 pesticide. Two samples (z-SM and z-Mg) that provided the worst results for MCPA, appear to be the best for
648 glyphosate. From the other side, two samples z-Ca and z-Li showed very good result for both pesticides.
649 Considering environmental abundance of the MCPA and Glyphosate degraders identified in our study, similar
650 bacterial genera have been previously enriched from other pesticides-polluted environmental sites. Moreover,
651 some of these genera were also identified within the same communities. For example, *Pseudomonas*, *Sphingobium*,
652 *Sphingopyxis* and *Novosphingobium* genera were detected in soil substrates originating from Mexico that were
653 effectively used in dissipation of a wide range of pesticides (including MCPA-related 2,4-D and glyphosate) in
654 lab-scale experiments (Góngora-Echeverría et al. 2018). Another bacterial community that includes *Rhodococcus*,
655 *Aminobacter*, *Cupriavidus* and *Pseudomonas* was previously enriched in a sediment from an aquifer, Denmark,
656 by addition of MCPA as the sole carbon source (Gözdereliler et al. 2013). In this study the authors confirmed
657 mineralisation of ^{14}C-labeled MCPA to $^{14}CO_2$ due to metabolic activity of the community towards MCPA.
658 According to other studies, *Sphingobium* genus prevail in wastewater from insecticide factories in China,

659 *Pseudomonas* genus was found in soil sample collected around a herbicide manufacturing plants (Smejkal et al.
660 2003; Zhao et al. 2015), while *Cupriavidus, Pseudomonas, Rhodococcus* and *Novosphingobium* genera are
661 typically found in pesticides contaminated agricultural sites all over the world (Pandey et al. 2009; Gupta et al.
662 2016; Cycoń et al. 2017; Wu et al. 2017). In this view, the results of our study agree well with many previous
663 reports showing typical abundance and possibility being enriched of these genera from polluted environmental
664 sites as well as metabolic activity of such communities towards MCPA and/or glyphosate. What concerns biofilms,
665 there are numerous reports focused on immobilisation of pure strains (including *Pseudomonas*) cultivated in
666 laboratory conditions (Kesseru et al. 2002; Moreno-Castilla et al. 2003; Quintelas et al. 2008; Plangklang and
667 Reungsang 2009; Stelting et al. 2012; Wu et al. 2014). Although some of the reports deal with immobilisation of
668 microbial consortium (Saez et al. 2012; Rivelli et al. 2013; Bouteh et al. 2021; Wang et al. 2021) including in some
669 cases *Pseudomonas* strain (Shabani et al. 2021) (Haque et al. 2020) (Ławniczak et al. 2011), none of them deals
670 with such broad community (in terms of represented genera) like in our study.
671 It can be summarised that introduction of zeolite as support material leads to several positive effects. The first one
672 is that it considerably increases biodiversity of pesticide degraders (MCPA-degraders of biofilms in comparison
673 with planktonic culture). This is expected to positively impact the adaptation and thus survival of the bacteria
674 bearing the pesticide-degrading activity once introduced in the natural environment. Thus, introduction of
675 biocomposite biofilm/zeolite into soils may have advantages in comparison to planktonic community: more
676 pesticides-degrading species (higher biodiversity) are introduced, and thus more chances of pesticide
677 biodegradation activity to be expressed. The second positive effect of zeolite introduction is that as it allows
678 microorganisms to grow as biofilms, it probably enables their protection from toxic conditions such as the
679 accumulation of toxic metabolites. This is illustrated, for glyphosate, by the fact that biodegradation activity of
680 planktonic cells was lost after several weeks of contact with glyphosate, whereas the activity of most biocomposites
681 was kept. In our opinion, accumulation of glyphosate toxic metabolite AMPA may be responsible for the loss of
682 glyphosate-degrading activity of planktonic community. It is well known that sessile cells attached to a surface are
683 more resistant to toxic compounds than planktonic ones, which could also explain why the planktonic microbial
684 community was affected by repeated addition of glyphosate. The consequence of this protective effect of zeolite
685 on microbial community is also illustrated by the higher microbial diversity of biocomposites compared to
686 planktonic culture in the case of MCPA. At last, the third main positive effect of zeolite addition is that it may
687 provide an additional source of organic nutrient, which in the case of glyphosate seems to be essential for survival
688 of the glyphosate-degrading community. Indeed, glyphosate biodegradation activity was lost with z-Fe and z-H
689 zeolites. Both samples have been treated at 500ºC, therefore, such behaviour of the microbial community may be
690 due to the fact that not calcined zeolites provide additional source of organic nutrient (confirmed by total organic
691 carbon analysis of zeolite samples) that is essential for survival of the glyphosate-degrading community. The
692 absence of additional organic nutrient in the planktonic culture and in two calcined samples (due to the elimination
693 of organic matter during thermal treatment) may explain the loss of glyphosate biodegradation activity. In this
694 view, our study provided a solution to solve biodegradation limitation under nutrient-deficient environmental
695 pressure.
696 This work thus clearly showed that microbial communities have good ability to degrade the targeted pesticides
697 (MCPA and glyphosate) after a selection step. Moreover, it was demonstrated that according to the microbial
698 community (here MCPA- or glyphosate-degrading community), best results in terms of biodegradation activity
699 are obtained with differently modified zeolites. This suggests that zeolite is an efficient growth support for
700 microbial communities that degrade pesticides, and modification of zeolite is a promising approach to enhance
701 biocomposite performance in terms of pesticide biodegradation rate.
702
703 ## 6. Conclusions
704 In the frame of the development of green and cost effective technologies for the treatment of pesticides polluted
705 agricultural sites, bioaugmentation involving pesticides-degrading microorganisms is an effective approach
706 allowing treating polluted soils on site i.e. without excavation. Microorganisms introduced as biofilms are known
707 to have higher survival rate and persistent activity in comparison to planktonic cells. Our study shows another
708 advantage of the use of biofilm as it allows to preserve a higher pesticides degraders' biodiversity, which is
709 expected to improve the adaptation and thus survival of the pesticide-degrading bacteria once introduced in the
710 polluted environment to be treated. This work also demonstrates that the nature of support material for biofilm
711 growth can greatly influence the community structure and thus activity of the selected microbial biofilms. In
712 particular, natural zeolite, which is environmental-friendly and cost effective material, is confirmed to be an
713 efficient growth support for microbial communities with pesticides-degrading activity. But modifications of zeolite
714 surface are shown to be a promising approach to enhance biocomposite performance in terms of pesticide
715 biodegradation rate that can also be a way to improve the efficiency of bioaugmentation approaches. In addition,
716 such introduction in agricultural soils in the frame of bioaugmentation purposes could potentially enhance - as
717 positive "side-effect" - the physical/chemical properties of some specific soils such as chalky soils (that lack of
718 clay involved in fertilizers retention for instance) or loamy soils (by protecting soil from slaking crust). At last, in

719 view of microorganims spreading on agricultural soils for regenerative agriculture via bioaugmentation, the zeolite
720 grains size used in this study is compatible with existing agricultural practices and devices. The application of such
721 biocomposites could thus be coupled to other existing agricultural practices (such as fertilisation) without
722 additional costs for farmers (no new equipment, no new technical operations etc.). In future, microcosm studies in
723 soil will help to confirm the concept of high effectiveness of the elaborated biocomposites for bioaugmentation
724 and pesticide biodegradation in agricultural sites, in view to continue its development up to agricultural use.
725
726 **7. Acknowledgment**

727 The authors would like to thank Cindy GASSAUT (BRGM) for molecular analysis, Pascal AUGER (BRGM) for
728 surface area and pore size analysis of zeolites and Bruno Pignon (GREMAN lab of the IUT de Blois, University
729 of Tours) for the technical assistances in SEM observation and imaging.
730
731 **8. Ethics approval and consent to participate**

732 Not applicable.
733
734 **9. Consent for publication**

735 Not applicable.
736
737 **10. Authors Contributions**

738 CM, AS, FD, SB, CG, FG and KM conceived and designed research. NG conducted experiments. CM was actively
739 involved in the microbiological part of the experiments. AS and KM were involved in experiments on modification
740 and characterisation of zeolites. FD and FG were involved in characterisation of the samples using XRD analysis
741 and SEM. CJ was actively involved in analysis of the microbial community structure of biofilms. CS performed
742 mass-spectroscopy analysis and analysed its results. NG wrote the manuscript, CM and AS actively involved in
743 its modification. All authors read, corrected and approved the manuscript.
744
745 **11. Funding**

746 This study was funded by the French Region Centre Val de Loire (APR 2016 BIOPEPS) and BRGM.
747
748 **12. Competing Interests**

749 The authors declare that they have no competing interests.
750
751 **13. Availability of data and materials**

752 The datasets used and/or analysed during the current study are available from the corresponding author on
753 reasonable request.
754
755 **14. References**

756 Abbo HS, Titinchi SJJ (2009) Di-, tri- and tetra-valent ion-exchanged NaY zeolite: Active heterogeneous
757 catalysts for hydroxylation of benzene and phenol. Appl Catal A Gen 356:167–171.
758 https://doi.org/10.1016/j.apcata.2009.01.003
759 AGRITOX (2010) MCPA. Règlement n° 1107/2009
760 AGRITOX (2018) Glyphosate. Règlement n° 1107/2009
761 Al Hattab TM, Ghaly EA (2012) Disposal and treatment methods for pesticide containing wastewaters: Critical
762 review and comparative analysis. J Environ Prot (Irvine, Calif) 3:431–453.
763 https://doi.org/10.4236/jep.2012.35054
764 Ams DA, Fein JB, Dong H, Maurice PA (2004) Experimental measurements of the adsorption of *Bacillus*
765 *subtilis* and *Pseudomonas mendocina* onto Fe-oxyhydroxide-coated and uncoated quartz grains.
766 Geomicrobiol J 21:511–519. https://doi.org/10.1080/01490450490888172
767 Ates A, Hardacre C (2012) The effect of various treatment conditions on natural zeolites: Ion exchange, acidic,
768 thermal and steam treatments. J Colloid Interface Sci 372:130–140.
769 https://doi.org/10.1016/j.jcis.2012.01.017
770 Bælum J, Henriksen T, Hansen HCB, Jacobsen GS (2006) Degradation of 4-chloro-2-methylphenoxyacetic acid
771 in top- and subsoil is quantitatively linked to the class III *tfdA* gene. Appl Environ Microbiol 72:1476–
772 1786. https://doi.org/10.1128/AEM.72.2.1476-1486.2006
773 Bælum J, Nicolaisen MH, Holben WE, et al (2008) Direct analysis of *tfdA* gene expression by indigenous
774 bacteria in phenoxy acid amended agricultural soil. ISME J 2:677–687.
775 https://doi.org/10.1038/ismej.2008.21
776 Bautista-Toledo MI, Espinosa-Iglesias D, Carrasco-Marín F, et al (2015) Influence of the physicochemical
777 properties of inorganic supports on the activity of immobilized bacteria for water denitrification. J Environ
778 Manage 156:81–88. https://doi.org/10.1016/j.jenvman.2015.03.031

779 Belaabed R, Elabed S, Addaou A, et al (2016) Synthesis of LTA zeolite for bacterial adhesion. Bol la Soc Esp
780 Ceram y Vidr 55:152–158. https://doi.org/10.1016/j.bsecv.2016.05.001
781 Bouteh E, Ahmadi N, Abbasi M, et al (2021) Biodegradation of organophosphorus pesticides in moving bed
782 biofilm reactors: Analysis of microbial community and biodegradation pathways. J Hazard Mater
783 408:124950. https://doi.org/10.1016/j.jhazmat.2020.124950
784 Chu L, Wang J (2013) Denitrification performance and biofilm characteristics using biodegradable polymers
785 PCL as carriers and carbon source. Chemosphere 91:1310–1316.
786 https://doi.org/10.1016/j.chemosphere.2013.02.064
787 Cycoń M, Mrozik A, Piotrowska-Seget Z (2017) Bioaugmentation as a strategy for the remediation of pesticide-
788 polluted soil: A review. Chemosphere 172:52–71. https://doi.org/10.1016/j.chemosphere.2016.12.129
789 Danish Environmental Protection Agency (1998) Risk assessment 4-chloro-2-methylphenol CAS N°: 1570-64-5.
790 Copenhagen
791 Das T, Sehar S, Koop L, et al (2014) Influence of calcium in extracellular DNA mediated bacterial aggregation
792 and biofilm formation. PLoS One 9:1–11. https://doi.org/10.1371/journal.pone.0091935
793 Deo N, Natarajan KA, Somasundaran P (2001) Mechanisms of adhesion of *Paenibacillus polymyxa* onto
794 hematite, corundum and quartz. Int J Miner Process 62:27–39. https://doi.org/10.1016/S0301-
795 7516(00)00042-9
796 Ditterich F, Poll C, Pagel H, et al (2013) Succession of bacterial and fungal 4-chloro-2-methylphenoxyacetic
797 acid degraders at the soil-litter interface. FEMS Microbiol Ecol 86:85–100. https://doi.org/10.1111/1574-
798 6941.12131
799 Evangelista S, Cooper DG, Yargeau V (2010) The effect of structure and a secondary carbon source on the
800 microbial degradation of chlorophenoxy acids. Chemosphere 79:1084–1088.
801 https://doi.org/10.1016/j.chemosphere.2010.03.018
802 Filippousi M, Turner S, Katsikini M, et al (2015) Direct observation and structural characterization of natural
803 and metal ion-exchanged HEU-type zeolites. Microporous Mesoporous Mater 210:185–193.
804 https://doi.org/10.1016/j.micromeso.2015.01.043
805 Firdous S, Iqbal S, Anwar S (2020) Optimization and modeling of glyphosate biodegradation by a novel
806 *Comamonas odontotermitis* P2 through response surface methodology. Pedosphere 30:618–627.
807 https://doi.org/10.1016/s1002-0160(17)60381-3
808 Góngora-Echeverría VR, Quintal-Franco C, Arena-Ortiz ML, et al (2018) Identification of microbial species
809 present in a pesticide dissipation process in biobed systems using typical substrates from southeastern
810 Mexico as a biomixture at a laboratory scale. Sci Total Environ 628–629:528–538.
811 https://doi.org/10.1016/j.scitotenv.2018.02.082
812 Gözdereliler E, Boon N, Aamand J, et al (2013) Comparing metabolic functionalities, community structures, and
813 dynamics of herbicide-degrading communities cultivated with different substrate concentrations. Appl
814 Environ Microbiol 79:367–375. https://doi.org/10.1128/AEM.02536-12
815 Gupta M, Mathur S, Sharma TK, et al (2016) A study on metabolic prowess of *Pseudomonas* sp. RPT 52 to
816 degrade imidacloprid, endosulfan and coragen. J Hazard Mater 301:250–258.
817 https://doi.org/10.1016/j.jhazmat.2015.08.055
818 Haque MM, Haque MA, Mosharaf MK, Marcus PK (2020) Novel bacterial biofilm consortia that degrade and
819 detoxify the carcinogenic diazo dye Congo red. Arch Microbiol. https://doi.org/10.1007/s00203-020-
820 02044-1
821 He X, Wang J, Abdoli L, Li H (2016) Mg^{2+}/Ca^{2+} promotes the adhesion of marine bacteria and algae and
822 enhances following biofilm formation in artificial seawater. Colloids Surfaces B Biointerfaces 146:289–
823 295. https://doi.org/10.1016/j.colsurfb.2016.06.029
824 Helbling DE (2015) Bioremediation of pesticide-contaminated water resources: The challenge of low
825 concentrations. Curr Opin Biotechnol 33:142–148. https://doi.org/10.1016/j.copbio.2015.02.012
826 Institut national de l'environnement industriel et des risques (2013) Valeur Guide Environnementale. MCPA –
827 n° CAS : 94-74-6. France
828 Institut national de l'environnement industriel et des risques (2014) Valeur Guide Environnementale. Glyphosate
829 – n° CAS : 1071-83-6. France
830 Jacob GS, Garbow JR, Hallas LE, et al (1988) Metabolism of glyphosate in *Pseudomonas* sp. strain LBr. Appl
831 Environ Microbiol 54:2953–2958
832 Jacobsen CS, Hjelmsø MH (2014) Agricultural soils, pesticides and microbial diversity. Curr Opin Biotechnol
833 27:15–20. https://doi.org/10.1016/j.copbio.2013.09.003
834 Jevremoví A, Božinovíbožinoví N, Arsenijeví D, et al (2020) Modulation of cytotoxicity by consecutive
835 adsorption of tannic acid and pesticides on surfactant functionalized zeolites. Environ Sci Process Impacts
836 22:2199–2211. https://doi.org/10.1039/d0em00251h
837 Jha B, Singh DN (2016) Basics of zeolites. In: Fly ash zeolites. Innovations, applications and directions.
838 Springer Science+Business Media Singapore, Singapore, pp 5–31

Jiang D, Huang Q, Cai P, et al (2007) Adsorption of *Pseudomonas putida* on clay minerals and iron oxide. Colloids Surfaces B Biointerfaces 54:217–221. https://doi.org/10.1016/j.colsurfb.2006.10.030

Jucker BA, Harms H, Hug SJ, Zehnder AJB (1997) Adsorption of bacterial surface polysaccharides on mineral oxides is mediated by hydrogen bonds. Colloids Surfaces B Biointerfaces 9:331–343. https://doi.org/10.1016/S0927-7765(97)00038-6

Kassa MG (2019) Promising application of natural zeolites: for waste water treatment - a review. Int Res J Environ Sci Sci 8(4):69–74

Kennedy DA, Tezel FH (2018) Cation exchange modification of clinoptilolite – Screening analysis for potential equilibrium and kinetic adsorption separations involving methane, nitrogen, and carbon dioxide. Microporous Mesoporous Mater 262:235–250. https://doi.org/10.1016/j.micromeso.2017.11.054

Kesseru P, Kiss I, Bihari Z, Polyák B (2002) Investigation of the denitrification activity of immobilized *Pseudomonas butanovora* cells in the presence of different organic substrates. Water Res 36:1565–1571. https://doi.org/10.1016/S0043-1354(01)00364-5

Kolthoff IM, Willman A (1934) The acid strength of inorganic cations and the basic strength of inorganic acetates in acetic acid. II. J Am Chem Soc 56:1014–1016. https://doi.org/10.1021/ja01320a003

Kurama H, Zimmer A, Reschetilowski W (2002) Chemical modification effect on the sorption capacities of natural clinoptilolite. Chem Eng Technol 25:301–305. https://doi.org/10.1002/1521-4125(200203)25:3<301::AID-CEAT301>3.0.CO;2-#

Lancaster SH, Hollister EB, Senseman SA, Gentry TJ (2010) Effects of repeated glyphosate applications on soil microbial community composition and the mineralization of glyphosate. Pest Manag Sci 66:59–64. https://doi.org/10.1002/ps.1831

Ławniczak Ł, Kaczorek E, Olszanowski A (2011) The influence of cell immobilization by biofilm forming on the biodegradation capabilities of bacterial consortia. World J Microbiol Biotechnol 27:1183–1188. https://doi.org/10.1007/s11274-010-0566-5

Liu YJ, Liu SJ, Drake HL, Horn MA (2011) *Alphaproteobacteria* dominate active 2-methyl-4-chlorophenoxyacetic acid herbicide degraders in agricultural soil and drilosphere. Environ Microbiol 13:991–1009. https://doi.org/10.1111/j.1462-2920.2010.02405.x

Mercier A, Joulian C, Michel C, et al (2014) Evaluation of three activated carbons for combined adsorption and biodegradation of PCBs in aquatic sediment. Water Res 59:304–315. https://doi.org/10.1016/j.watres.2014.04.021

Mills AL, Herman JS, Hornberger GM, DeJesus TH (1994) Effect of solution ionic strength and iron coatings on mineral grains on the sorption of bacterial cells to quartz sand. Appl Environ Microbiol 60:3300–3306

Moradi M, Karimzadeh R, Moosavi ES (2018) Modified and ion exchanged clinoptilolite for the adsorptive removal of sulfur compounds in a model fuel: New adsorbents for desulfurization. Fuel 217:467–477. https://doi.org/10.1016/j.fuel.2017.12.095

Moreno-Castilla C, Bautista-Toledo I, Ferro-García MA, Rivera-Utrilla J (2003) Influence of support surface properties on activity of bacteria immobilised on activated carbons for water denitrification. Carbon N Y 41:1743–1749. https://doi.org/10.1016/S0008-6223(03)00123-4

Muturi EJ, Donthu RK, Fields CJ, et al (2017) Effect of pesticides on microbial communities in container aquatic habitats. Sci Rep 7:44565:1–5. https://doi.org/10.1038/srep44565

Newman MM, Hoilett N, Lorenz N, et al (2016) Glyphosate effects on soil rhizosphere-associated bacterial communities. Sci Total Environ 543:155–160. https://doi.org/10.1016/j.scitotenv.2015.11.008

Önneby K (2013) Bioaugmentation for reduction of diffuse pesticide contamination. A bioprophylactic concept. SLU Service/Repro, Uppsala

Önneby K, Håkansson S, Pizzul L, Stenström J (2014) Reduced leaching of the herbicide MCPA after bioaugmentation with a formulated and stored *Sphingobium* sp. Biodegradation 25:291–300. https://doi.org/10.1007/s10532-013-9660-3

Pandey G, Dorrian SJ, Russell RJ, Oakeshott JG (2009) Biotransformation of the neonicotinoid insecticides imidacloprid and thiamethoxam by *Pseudomonas* sp. 1G. Biochem Biophys Res Commun 380:710–714. https://doi.org/10.1016/j.bbrc.2009.01.156

Paszko T, Muszyński P, Materska M, et al (2016) Adsorption and degradation of phenoxyalkanoic acid herbicides in soils: A review. Environ Toxicol Chem 35:271–286. https://doi.org/10.1002/etc.3212

Pimentel D (2005) Environmental and economic costs of the application of pesticides primarily in the United States. Environ Dev Sustain 7:229–252. https://doi.org/10.1007/s10668-005-7314-2

Plangklang P, Reungsang A (2009) Bioaugmentation of carbofuran residues in soil using *Burkholderia cepacia* PCL3 adsorbed on agricultural residues. Int Biodeterior Biodegrad 63:515–522. https://doi.org/10.1016/j.ibiod.2009.02.003

Quintelas C, Fernandes B, Castro J, et al (2008) Biosorption of Cr(VI) by a *Bacillus coagulans* biofilm supported on granular activated carbon (GAC). Chem Eng J 136:195–203. https://doi.org/10.1016/j.cej.2007.03.082

899 Ramesh K, Reddy DD (2011) Zeolites and their potential uses in agriculture. Adv Agron 113:219–241.
900 https://doi.org/10.1016/B978-0-12-386473-4.00004-X
901 Rathi A, Basu S, Barman S (2019) Adsorptive removal of fipronil from its aqueous solution by modified zeolite
902 HZSM-5: Equilibrium, kinetic and thermodynamic study. J Mol Liq 283:867–878.
903 https://doi.org/10.1016/j.molliq.2019.02.140
904 Reddy PVL, Kim K-H (2015) A review of photochemical approaches for the treatment of a wide range of
905 pesticides. J Hazard Mater 285:325–335. https://doi.org/10.1016/J.JHAZMAT.2014.11.036
906 Reeve PJ, Fallowfield HJ (2018) Natural and surfactant modified zeolites: A review of their applications for
907 water remediation with a focus on surfactant desorption and toxicity towards microorganisms. J Environ
908 Manage 205:253–261. https://doi.org/10.1016/j.jenvman.2017.09.077
909 Rivelli V, Franzetti A, Gandolfi I, et al (2013) Persistence and degrading activity of free and immobilised
910 allochthonous bacteria during bioremediation of hydrocarbon-contaminated soils. Biodegradation 24:1–11.
911 https://doi.org/10.1007/s10532-012-9553-x
912 Roberts TR, Hutson DH, Lee PW, et al (2007) Metabolic pathways of agrochemicals. Royal Society of
913 Chemistry, Cambridge
914 Saez JM, Benimeli CS, Amoroso MJ (2012) Lindane removal by pure and mixed cultures of immobilized
915 actinobacteria. Chemosphere 89:982–987. https://doi.org/10.1016/j.chemosphere.2012.06.057
916 Sauer-Budge AF, Boardman AK, Allison S, et al (2013) Materials and surface properties optimization to prevent
917 biofouling of a novel bacterial concentrator. Procedia CIRP 5:185–188.
918 https://doi.org/10.1016/J.PROCIR.2013.01.037
919 Schlatter DC, Yin C, Hulbert S, et al (2017) Impacts of repeated glyphosate use on wheat-associated bacteria are
920 small and depend on gyphosate use history. Appl Environ Microbiol 83:1–10.
921 https://doi.org/10.1128/aem.01354-17
922 Schloss PD, Westcott SL, Ryabin T, et al (2009) Introducing mothur: Open-source, platform-independent,
923 community-supported software for describing and comparing microbial communities. Appl Environ
924 Microbiol 75:7537–7541. https://doi.org/10.1128/AEM.01541-09
925 Shabani M, Pontié M, Younesi H, et al (2021) Biodegradation of acetaminophen and its main by-product 4-
926 aminophenol by *Trichoderma harzianum* versus mixed biofilm of *Trichoderma harzianum/Pseudomonas*
927 *fluorescens* in a fungal microbial fuel cell. J Appl Electrochem. https://doi.org/10.1007/s10800-020-01518-
928 w
929 Singh BK, Walker A (2006) Microbial degradation of organophosphorus compounds. FEMS Microbiol Rev
930 30:428–471. https://doi.org/10.1111/j.1574-6976.2006.00018.x
931 Sirotiak M, Bartošová A, Lipovský M (2015) Spectrometric determination of selected herbicides in modified
932 aqueous solutions. J Environ Prot Safety, Educ Manag 3:77–85
933 Smejkal CW, Seymour FA, Burton SK, Lappin-Scott HM (2003) Characterisation of bacterial cultures enriched
934 on the chlorophenoxyalkanoic acid herbicides 4-(2,4-dichlorophenoxy) butyric acid and 4-(4-chloro-2-
935 methylphenoxy) butyric acid. J Ind Microbiol Biotechnol 30:561–567. https://doi.org/10.1007/s10295-003-
936 0086-5
937 Smith J V (1963) Structural classification of zeolites. Mineral Soc Am Spec Pap 1:281–290
938 Soulier C, Coureau C, Togola A (2016) Environmental forensics in groundwater coupling passive sampling and
939 high resolution mass spectrometry for screening. Sci Total Environ 563–564:845–854.
940 https://doi.org/10.1016/j.scitotenv.2016.01.056
941 Stelting S, Burns RG, Sunna A, et al (2012) Immobilization of *Pseudomonas* sp. strain ADP: A stable inoculant
942 for the bioremediation of atrazine. Appl Clay Sci 64:90–93. https://doi.org/10.1016/j.clay.2011.12.006
943 Stibal M, Baelum J, Holben WE, et al (2012) Microbial degradation of 2,4-dichlorophenoxyacetic acid on the
944 Greenland ice sheet. Appl Environ Microbiol 78:5070–5076. https://doi.org/10.1128/AEM.00400-12
945 Sviridov A V., Shushkova T V., Ermakova IT, et al (2015) Microbial degradation of glyphosate herbicides
946 (Review). Appl Biochem Microbiol 51:188–195. https://doi.org/10.7868/s0555109915020221
947 Townsend RP, Coker EN (2001) Chapter 11 Ion exchange in zeolites. Stud Surf Sci Catal 137:467–524.
948 https://doi.org/10.1016/S0167-2991(01)80253-6
949 Van Veen JA, Van Overbeek LS, Van Elsas JD (1997) Fate and activity of microorganisms introduced into soil.
950 Microbiol Mol Biol Rev 61:121–35
951 Wang C, Lin X, Li L, et al (2017) Glyphosate shapes a dinoflagellate-associated bacterial community while
952 supporting algal growth as sole phosphorus source. Front Microbiol 8:2530.
953 https://doi.org/10.3389/fmicb.2017.02530
954 Wang S, Peng Y (2010) Natural zeolites as effective adsorbents in water and wastewater treatment. Chem Eng J
955 156:11–24. https://doi.org/10.1016/j.cej.2009.10.029
956 Wang Y, Fan H, Keung Wong P, et al (2021) Biodegradation of tetracycline using hybrid material (UCPs-TiO_2)
957 coupled with biofilms under visible light. Bioresour Technol 323:124638.
958 https://doi.org/10.1016/j.biortech.2020.124638

959 Wang Z, Tollervey J, Briese M, et al (2009) CLIP: Construction of cDNA libraries for high-throughput
960 sequencing from RNAs cross-linked to proteins *in vivo*. Methods 48:287–293.
961 https://doi.org/10.1016/j.ymeth.2009.02.021
962 World health organization (1990) Public health impact of pesticides used in agriculture. Geneva
963 Wu H, Chen W, Rong X, et al (2014) Adhesion of *Pseudomonas putida* onto kaolinite at different growth
964 phases. Chem Geol 390:1–8. https://doi.org/10.1016/j.chemgeo.2014.10.008
965 Wu W, Weitz E (2014) Modification of acid sites in ZSM-5 by ion-exchange: An in-situ FTIR study. Appl Surf
966 Sci 316:405–415. https://doi.org/10.1016/j.apsusc.2014.07.194
967 Wu X, Wang W, Liu J, et al (2017) Rapid biodegradation of the herbicide 2,4-dichlorophenoxyacetic acid by
968 *Cupriavidus gilardii* T-1. J Agric Food Chem 65:3711–3720. https://doi.org/10.1021/acs.jafc.7b00544
969 Yamashita T, Yamamoto-Ikemoto R, Zhu J (2011) Sulfate-reducing bacteria in a denitrification reactor packed
970 with wood as a carbon source. Bioresour Technol 102:2235–2241.
971 https://doi.org/10.1016/j.biortech.2010.10.015
972 Yee N, Fein JB, Daughney CJ (2000) Experimental study of the pH, ionic strength, and reversibility behavior of
973 bacteria-mineral adsorption. Geochim Cosmochim Acta 64:609–617. https://doi.org/10.1016/S0016-
974 7037(99)00342-7
975 Zhan H, Feng Y, Fan X, Chen S (2018) Recent advances in glyphosate biodegradation. Appl Microbiol
976 Biotechnol 102:5033–5043. https://doi.org/10.1007/s00253-018-9035-0
977 Zhao H, Tao K, Zhu J, et al (2015) Bioremediation potential of glyphosate-degrading *Pseudomonas* spp. strains
978 isolated from contaminated soil. J Gen Appl Microbiol 61:165–170. https://doi.org/10.2323/jgam.61.165
979 Zheng X, Arps PJ, Smith RW (2001) Adhesion of two bacteria onto dolomite and apatite: Their effect on
980 dolomite depression in anionic flotation. Int J Miner Process 62:159–172. https://doi.org/10.1016/S0301-
981 7516(00)00050-8
982

Fig. 1. XRD patterns of the unmodified zeolite (z-SM) and modified samples before (z-Fe(before heating) and z-NH_4) and after (z-Fe and z-H) thermal treatment.

Fig. 2. SEM images of the zeolite samples: a-d – z-SM; e – z-Ca; f – z-Fe.

Fig. 3. Effect of addition of different zeolite types on glyphosate degradation.

Fig. 4. MCPA (a) and glyphosate (b) degradation kinetics by biocomposites and planktonic community; C_0 – initial MCPA concentration, 5mg/L; C – MCPA concentration of the sample at particular moment of time, h. Sensitivity limit: 0.5% for MCPA and 0.0015 % for glyphosate.

Fig. 5. MCPA (a) and glyphosate (b) biodegradation rates for the planktonic culture and various biocomposites in batch experiments (initial pesticide concentration: 5mg/L). Significant differences between conditions were searched applying the Krustal-Wallis non-parametric test and were mentioned as a, b and c letters.

Fig. 6. SEM images of the sample biofilm/z-Fe (MCPA).

Fig. 7. Bacterial community structure of the analysed biocomposites and planktonic cultures. Genera with abundance >1% where classified into 6 groups: 1) confirmed degraders of the target pesticide (there is a confirmation in bibliography that some members of the identified genus degrade the target pesticide); 2) potential degraders of the target pesticide (genera found in communities degrading the target pesticide or genetically related to confirmed degraders); 3) genera related to other pesticides (found in presence or are confirmed degraders of structurally unrelated pesticides); 4) genera involved in nitrogen cycle in soil; 5) genera with no known connection to any pesticide or nitrogen cycle; 6) sum of other genera accounting <1% each.

Online Resource 1. Pathway of degradation of MCPA by microorganisms.

Online Resource 2. Pathways of degradation of glyphosate by microorganisms in normal and phosphor-deficient conditions.

Online Resource 3. Bacterial community structure: CE-SSCP fingerprints of the inoculum and the 4th subcultures grown without (control) and with addition of MCPA or Glyphosate (1, 5 and 10 mg/L).

Online Resource 4. Identification of MCPA metabolites using LC-HRMS.

Online Resource 5. Principal component analysis (PCA) of bacterial diversity profiles (SSCP data) of the biocomposites obtained with different zeolites in the presence of glyphosate (blue) or MCPA (green).

Online Resource 6. Relative abundance (%) of bacterial OTU identified at genus or family level (OTU accounting for >1 % of sequences in at least one of the samples were considered).

1025
1026 Table 1. Chemical composition of zeolite samples according to EDX analysis.

Element, at. %	z-SM	z-Li	z-Na	z-K	z-NH_4	z-Ca	z-Mg	z-H	z-Fe
Na	0.17	0.03	2.2	-	0.07	0.06	0.03	0.03	-
Mg	0.69	0.52	0.37	0.31	0.45	0.47	0.87	0.42	0.46
Al	4.11	3.87	3.90	3.83	4.28	3.81	3.6	3.85	3.21
Si	21.13	18.18	18.35	18.62	19.84	17.49	17.7	18.35	16.12
Si/Al	5.18	4.7	4.71	4.87	4.7	4.6	4.91	4.77	5.03
K	1.35	1.16	0.95	3.27	1.09	1.02	1.05	0.52	0.84
Ca	0.99	0.62	0.32	0.04	0.06	1.02	0.65	0.07	0.46
Fe	0.41	0.37	0.19	0.18	0.6	0.22	0.34	0.35	2.01

1027
1028
1029
1030

1031 Table 2. Concentration of cations in zeolite samples according to different evaluation methods: total content
1032 evaluated by EDX; content of leached ions evaluated on basis of ion exchange (1N $NaNO_3$ or NH_4NO_3) or acid
1033 treatment (1 N HNO_3) analysed in solution by ICP-AES (Na, K, Mg, Ca), ICP-MS (Li) or colorimetric analysis
1034 (NH_4, Fe).

Sample	Cation	Content, meq/kg	
		total	leached
z-Li	Li^+	-	397
z-Na	Na^+	1148	831
z-K	K^+	1674	746
z-NH_4	NH_4^+	-	546
z-Mg	Mg^{2+}	905	199
z-Ca	Ca^{2+}	1064	1211
z-Fe	Fe^{3+}	345	298

1035

1036

1037 Table 3. OTU's (operational taxonomic units) richness and diversity indexes calculated from the Illumina
1038 sequencing data of the 16S and 28S rRNA genes for prokaryotes and eukaryotes, respectively.

Sample	Number of sequences		Number of OTU's		Simpson's index 1/D[a]		Shannon's index[b]	
	bact.	eukar.	bact.	eukar.	bact.	eukar.	bact.	eukar.
MCPA planktonic culture	38857	36192	74	138	1.7	2.0	1.3	1.7
MCPA z-SM/biofilm	35322	9823	225	82	4.0	2.2	3.7	2.0
MCPA z-Fe/biofilm	28140	28162	200	104	3.7	2.4	3.4	1.9
Glyph. z-SM/biofilm	33179	15797	258	30	3.5	1.5	3.6	1.1
Glyph. z-NH_4/biofilm	33206	48996	158	66	2.8	1.3	2.8	0.7
Positive control[c]	2848	32890	184	201	3.1	3.0	2.9	2.5

1039 [a]The Simpson's index (D or 1/D in this paper) corresponds to the probability that two randomly selected reads will
1040 belong to the same OTU. Low 1/D values indicate that only few OTUs dominate the sample, while higher values
1041 indicate that the reads are distributed over many OTUs.
1042 [b]The Shannon's index quantifies the heterogeneity of the microbial community. High values indicate greater
1043 number of different individuals present in the community and also their more equitable distribution among
1044 different genera.
1045 [c]Planktonic culture enriched using the same inoculant under the same conditions but without addition of pesticide