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## Environmental hazard of different clays as consequence of global change: a case study on *Aliivibrio fischeri*.

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### Supplementary materials

#### Materials and Methods Details

##### *1.1. Determination of physical-chemical properties of tested clays*

**Color.** The L\*a\*b\* color space (also known as CIEL\*a\*b\*) is currently one of the most widely used methods to objectively measure the color of solid materials and it is used in all of fields, including the characterization of the sediments color (Pranzini, 2008). It is one of the uniform color spaces defined by CIE (Commission Internationale de l'Eclairage) in 1976, which made it possible to bypass the previously used Munsell chromaticity diagram (Pranzini, 2008). The CIEL\*a\*b\* method aimed to reduce one of the main problems of the original XYZ color space (CIE, 1932). Namely, the equal distances in the XY chromaticity diagram did not correspond to the color differences perceived as equal. In this color space, L\* denotes lightness, while a\* and b\* refer to color coordinates. These analyses were performed using Eoptis' CLM-194, a digital tristimulus colorimeter. The colorimetric distance difference ( $\Delta E^*_{ab}$ ) of colors between pairs of samples was also calculated.

**Particle size, morphology, and elemental analysis.** Particle size distribution of clays in ASW water was measured by laser diffraction using Malvern Mastersizer 2000 (Malvern Instruments Ltd, Worcestershire, UK) equipped with the Hydro S Sampler (wet instrument), which allows measurement of particle size in the range of 0.01-10,000  $\mu\text{m}$ . Mie scattering theory was applied to convert the recorded signal into 62 particle size classes of the dimensions of interest from 0.01-2,000  $\mu\text{m}$ , as the samples were cut off at 1,700  $\mu\text{m}$ . The instrument was equipped with an ultrasonic sampler to pre-treat the samples before analysis (2 min; 500 Hz); measurements were performed 1 min after the end of sonication. Since the TOC contents in the analyzed samples were less than 1%, no H<sub>2</sub>O<sub>2</sub> pretreatment was performed, as described in the literature (Callesen et al., 2018). The sample was read in five runs to calculate the average particle size dimensions (PDS). The PDS was expressed in volume percent, which is considered equivalent to weight percent assuming uniform specific gravity of the particles across all particle sizes. For this reason, only the unit % is used in this work. The refractive index of the particles was 1.555 and the absorption index of the particles was 0.1; the refractive index of the dispersant (artificial seawater, ASW) was 1.55.

Morphological and elemental analyzes on clay samples were performed using a high-resolution scanning electron microscope (FESEM, Zeiss, mod. Merlin- II) equipped with a combined EDS /WDS microanalyzer optimized for quantitative and qualitative elemental analyzes with high energy resolution and equipped with secondary and backscatter electron detectors for image analysis down to subnanometer resolution (inLens detectors) and a charge compensator for the analysis of nonconforming samples, such as conductive and unprepared samples. With this microanalytical platform, it was possible to characterize both the morphology and the elemental composition of the studied clays. In addition, specific morphological aspects of interest were recorded and 30 particles per clay were randomly measured using FESEM microscopy to evaluate the differences in particle size distribution of the dusts compared to the water dispersions measured using the laser diffraction method in marine water. This analysis also included the presence of particles in nanoform to also relate ecotoxicity to the possible presence of nanoparticles.

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**Mineralogical composition.** The clays were analyzed through X-ray diffraction (XRPD), the most widely used technique in mineralogy and crystallography using an X-Ray diffractometer (Rigaku, Smartlab). XRPD allows to identify the crystal structure using an X-ray beam whose wavelength is of the same order of magnitude of the distance between the crystal lattice plane. When the X-Ray beam hits a crystal lattice plane, it is diffracted in one direction; to collect all the crystal lattice interplanar distance needed for the phase identification, powder sample were analyzed.

**Trace elements, REE, and macronutrient content.** The clays (C1-C10) were used to determine total metals (Cd, Co, Cr, Cr- VI, Cu, Hg, Ni, Pb, Sr, Zn), metalloids (As), rare earths (Ce, Dy, Er, Eu, Gd, Ho, La, Lu, Nd, Pr, Sm, Sc, Tb, Th, Tm, Yb, Y), and macronutrients (total organic carbon TOC; Total carbon, TC; total nitrogen, TN; total phosphorus, TP) analyzed. The methods, limits of quantification (LOQ), and list of abbreviations used in this work for the chemical analyzes performed on clays are given in **Table S1**.

**Table S1. Trace elements, rare earths (REE), and carbon content on clays.** Abbreviation, methods, limits of quantification (LOQ), and Units of measurement (UM) relating to chemical analyses carried out on clays are reported.

Element	Abbreviation	Method	LOQ	UM
Total Carbon	TC	DIN 19539:2016 Annex B	0.1	%
Total Organic Carbon	TOC	DIN 19539:2016 Annex B	0.1	%
Total nitrogen	TN	DM 13/09/1990 SO 185 Met. XIV2+XIV3	0.01	%
Phosphorus	TP	EPA 3050B 1996 + EPA 6020B 2014	0.05	%
Arsenic	As	EPA 3050B 1996 + EPA 6020B 2014	1	mg/kg
Cadmium	Cd	EPA 3050B 1996 + EPA 6020B 2014	0.1	mg/kg
Cobalt	Co	EPA 3050B 1996 + EPA 6020B 2014	1	mg/kg
Chromium	Cr	EPA 3050B 1996 + EPA 6020B 2014	0.1	mg/kg
Chromium VI	CrVI	CNR IRSA 16 Q 64 Vol 3 1986	0.1	mg/kg
Copper	Cu	EPA 3050B 1996 + EPA 6020B 2014	1	mg/kg
Lead	Pb	EPA 3050B 1996 + EPA 6020B 2014	1	mg/kg
Mercury	Hg	EPA 3050B 1996 + EPA 6020B 2014	0.1	mg/kg
Nickel	Ni	EPA 3050B 1996 + EPA 6020B 2014	1	mg/kg
Strontium	Sr	EPA 3050B 1996 + EPA 6020B 2014	100	mg/kg
Zinc	Zn	EPA 3050B 1996 + EPA 6020B 2014	5	mg/kg
Cerium	Ce	EPA 3050B 1996 + EPA 6020B 2014	10	mg/kg
Dysprosium	Dy	EPA 3050B 1996 + EPA 6020B 2014	1	mg/kg
Erbium	Er	EPA 3050B 1996 + EPA 6020B 2014	0.5	mg/kg
Europium	Eu	EPA 3050B 1996 + EPA 6020B 2014	0.1	mg/kg
Gadolinium	Gd	EPA 3050B 1996 + EPA 6020B 2014	1	mg/kg
Holmium	Ho	EPA 3050B 1996 + EPA 6020B 2014	0.1	mg/kg
Lanthanum	La	EPA 3050B 1996 + EPA 6020B 2014	1	mg/kg
Lutetium	Lu	EPA 3050B 1996 + EPA 6020B 2014	0.1	mg/kg
Neodymium	Nd	EPA 3050B 1996 + EPA 6020B 2014	1	mg/kg
Praseodymium	Pr	EPA 3050B 1996 + EPA 6020B 2014	1	mg/kg
Samarium	Sm	EPA 3050B 1996 + EPA 6020B 2014	1	mg/kg
Scandium	Sc	EPA 3050B 1996 + EPA 6020B 2014	0.1	mg/kg
Terbium	Tb	EPA 3050B 1996 + EPA 6020B 2014	0.1	mg/kg
Thorium	Th	EPA 3050B 1996 + EPA 6020B 2014	0.1	mg/kg
Thulium	Tm	EPA 3050B 1996 + EPA 6020B 2014	0.1	mg/kg
Ytterbium	Yb	EPA 3050B 1996 + EPA 6020B 2014	0.1	mg/kg
Yttrium	Y	EPA 3050B 1996 + EPA 6020B 2014	1	mg/kg

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### 1.2. Determination of physical-chemical properties of contact water

**Physicochemical determinations.** Salinity (S, g/L), pH, and temperature were determined in negative controls (artificial water used for elutriates, solid phase dilution, and contact water) and in tested samples. The method APAT CNR IRSA 2070 Man 29/03 for salinity (Hedge, Hanna Instruments, mod. HI763100) was used with a standard reference solution (Hanna Instruments, LAT standard reference solution n. S264170821CON128801223, at 12880  $\mu$ S/cm) was used. The pH was measured according to UNI EN ISO 10523:2012 using a Hedge instrument (Hanna Instruments, mod. HI10480) calibrated with a buffer reference solution (Hanna Instruments, LAT standard reference solution n. S310021121PH071223 at 7.01; n. S284220921PH101223 at 10.01). The accuracy of salinity and pH measurements was  $\pm 0.5$  g/L and  $\pm 0.2$  pH, respectively.

**Trace elements, REE, and carbon content.** Analyses of metals (Ba, Bo, Cd, Cr, Cr- VI, Cu, Fe, Mn, Hg, Ni, Pb, Sn, Zn), metalloids (Al, As, Se), rare earth metals (Ce, Dy, Er, Eu, Gd, Ho, La, Lu, Nd, Pr, Sm, Tb, Th, Tm, Yb, Y) and carbon content (TOC) in the studied waters are listed in **Table S2** with the methods, limits of determination (LOQ) and the list of abbreviations used in this work.

**Table S2. Trace elements, rare earths (REE) on water.** Abbreviation, methods, limits of quantification (LOQ), and Units of measurement (UM) relating to chemical analyses carried out on elutriates of clays are reported.

Element	Abbreviation	Method	LOQ	UM
Aluminum	Al	UNI EN ISO 17294-2:2016	0.001	mg/L
Arsenic	As	UNI EN ISO 17294-2:2016	0.001	mg/L
Barium	Ba	UNI EN ISO 17294-2:2016	0.001	mg/L
Boron	Bo	UNI EN ISO 17294-2:2016	0.050	mg/L
Cadmium	Cd	UNI EN ISO 17294-2:2016	0.001	mg/L
Chromium	Cr	UNI EN ISO 17294-2:2016	0.001	mg/L
Chromium VI	CrVI	APAT CNR IRSA 3150 C Man 29 2003	0.001	mg/L
Copper	Cu	UNI EN ISO 17294-2:2016	0.01	mg/L
Iron	Fe	UNI EN ISO 17294-2:2016	0.01	mg/L
Lead	Pb	UNI EN ISO 17294-2:2016	0.01	mg/L
Manganese	Mn	UNI EN ISO 17294-2:2016	0.005	mg/L
Mercury	Hg	UNI EN ISO 17294-2:2016	0.0001	mg/L
Nickel	Ni	UNI EN ISO 17294-2:2016	0.01	mg/L
Selenium	Se	UNI EN ISO 17294-2:2016	0.01	mg/L
Tin	Sn	UNI EN ISO 17294-2:2016	0.01	mg/L
Zinc	Zn	UNI EN ISO 17294-2:2016	0.01	mg/L
Cerium	Ce	EPA 6020B 2014	1	$\mu$ g/L
Dysprosium	Dy	EPA 6020B 2014	1	$\mu$ g/L
Erbium	Er	EPA 6020B 2014	1	$\mu$ g/L
Europium	Eu	EPA 6020B 2014	1	$\mu$ g/L
Gadolinium	Gd	EPA 6020B 2014	1	$\mu$ g/L
Holmium	Ho	EPA 6020B 2014	1	$\mu$ g/L
Lanthanum	La	EPA 6020B 2014	1	$\mu$ g/L
Lutetium	Lu	EPA 6020B 2014	1	$\mu$ g/L
Neodymium	Nd	EPA 6020B 2014	1	$\mu$ g/L
Praseodymium	Pr	EPA 6020B 2014	1	$\mu$ g/L
Samarium	Sm	EPA 6020B 2014	1	$\mu$ g/L
Terbium	Tb	EPA 6020B 2014	1	$\mu$ g/L
Thorium	Th	EPA 6020B 2014	1	$\mu$ g/L
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Ytterbium	Yb	EPA 6020B 2014	1	µg/L
Yttrium	Y	EPA 6020B 2014	1	µg/L

### 1.3. Ecotoxicological analyses on *Aliivibrio fischeri*

Solid phase analyses. The solid phase test with *A. fischeri* is certainly one of the fastest ecotoxicological assay among applicable tests to solid phases such as sediments of marine or freshwater environments, and soils. This assay has the advantage of allowing the direct contact between single organisms and the solid part of the matrix to be tested. The clay fraction of sediments tends to absorb bacteria by adhesion to particles (Ringwood et al., 1997) introducing a “natural toxicity” effect. It is already commonly known that the standard for the toxicity assay on the species *Aliivibrio fischeri* in solid phase only normalized obtained responses on the percentage of the sample with the particle-size <63 µm (ICRAM, 2001, S11 App.2). However, applied protocols for such normalization is referred only to the grain-size heterogeneity of tested sample (Onorati et al., 1998; Volpi Ghirardini et al., 2009). Consequently, in this approach is considered the conversion table developed by cited methods for marine sediments that correlates the granulometry with the measured toxicity resulting in the Natural Toxicity Threshold (NTT), Toxicity Units (TU), and the Sediment Toxicity Index (S.T.I.). Equation that are used to perform this correction are reported below (Eq. 1-4) according to ICRAM, 2001.

**Equation n.1. Threshold of natural toxicity:**  $NTT = 0.285 + 2.728 * \% Silt$

**Equation n. 2. Sediment Toxicity Units:**  $TU = 1/EC_{50} * 100$

**Equation n. 3. Sediment toxicity index, S.T.I. (ICRAM, 2001):**

$$S. T. I. = \frac{TU \text{ s. s. measured}}{\text{upper limit of the confidence range NTT}}$$

**Equation n. 4. Normalization accounting the percentage of silt (ICRAM, 2001):**

$$\% Silt = \frac{\% Silt}{(\% sand < 1mm + \% silt)} * 100$$

The procedure involves contact between the solid matrix and the extraction water in a 1:5 ratio of solid to liquid for 20 minutes with constant stirring by magnetic holders. The water used for extraction is artificial seawater (ASW, UNI EN ISO 11348-1: 2019). Then, scalar dilutions (1:2) are prepared starting from a concentration of 19.737% using the same extraction water (ASW). The prepared dilutions are thermostatic conditioned in test tubes at 15 °C for 10 minutes. Then the dilutions are inoculated with 20 µl of bacteria, which are in contact with the sediment matrix dilution for 20 minutes at the same temperature. After the contact time, the bacteria-containing matrix mixture is filtered using a column filter (Test-Tube and Sera-Separa, Bio2011, Ecotox), and 500 µL of the filtrate is placed in glass cuvettes and placed in the climate chamber of the luminometer for 10 minutes at 15 °C. At the end of this period, the dilutions are read to measure the bioluminescence relative to the bioluminescence of the controls. To perform the test, the ISPRA protocol was used to check the change between the measured toxicity, the STI and the natural toxicity threshold. The salinity of the extraction water was 35 g/L, and the pH was 7.50 ±0.2.

Liquid phase analysis. The test method used for the liquid phase test (UNI EN ISO 11348-1: 2019) allows the evaluation of acute toxicity of contact water samples, using as target response the inhibition of bioluminescence naturally emitted by a monospecific population of 10<sup>6</sup> cells of Gram-negative marine bacteria

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of the species *A. fischeri* (strain NRRL-B-11177). After thawing, the bacteria were transferred to the cuvette. The assays were performed after checking the initial bioluminescence by contacting 100  $\mu$ l of the bacteria with the sample and recording the inhibition of bioluminescence after 15 and 30 min of exposure. The assay includes the negative control with the ASW water and the positive control with 4.5 mg/L 3,5-dichlorophenol (3,5-DF; Sigma Aldrich, No. LRAC5200). The first replicate of the negative controls was used to determine the baseline level of natural bioluminescence of the bacteria tested. Contact waters at three different pH levels in contact with the ten clays (contact time 20 min) were centrifuged (15 °C, 18,000 rpm for 10 min) and analyzed by the *Aliivibrio fischeri* assay in liquid phase, with a screening assay in which all samples were analyzed at 90% of concentration (maximum concentration tested) under different pH conditions (n = 30).

## 2. Results

### 2.1. General features of tested clays: supplementary details.

**Color.** Additional data concerning morphological and mineralogical results are reported. In **Table S3** Color analysis (CIE L\*a\*b\*) and evidence of the color measured in tested clays are reported.

**Table S3. Color measured on clays.** CIE L\*a\*b\* results and associated color are reported.

Clay	CIE L*a*b*			Color
	L*	a*	b*	
C1	81.28	-0.52	9.47	
C2	69.64	3.51	12.10	
C3	89.66	4.38	8.02	
C4	74.64	3.53	26.76	
C5	73.02	0.11	13.86	
C6	67.88	12.59	23.24	
C7	99.07	-0.53	3.24	
C8	78.62	0.80	14.98	
C9	87.77	2.16	15.45	
C10	41.65	-1.09	1.31	

The colorimetric distance difference ( $\Delta E^*_{ab}$ ) of colors between couple of samples were, also calculated. In general, almost all of the widest distances were with clay C10 (black clay) except C2 (brown, maximum  $\Delta E^*_{ab}$  with C7, white), and C6 (reddish brown, maximum  $\Delta E^*_{ab}$  with C7, white). On the contrary, the minimum colorimetric distances are variable depending on the clay tested; in fact, the sample C1 (light green) is more similar to clay C8 (light brown, 6.3  $\Delta E^*_{ab}$ ), C2 with C5 (green, 5.1  $\Delta E^*_{ab}$ ), C3 (light pink) with C9 (light grey, 8.0  $\Delta E^*_{ab}$ ), C4 (yellow) with C6 (11.8  $\Delta E^*_{ab}$ ), C7 with C3 (11.6  $\Delta E^*_{ab}$ ), C8 with C5 (5.8  $\Delta E^*_{ab}$ ), and C10 with C2 (30.4  $\Delta E^*_{ab}$ ). The minimum value of colorimetric distance is that between C2 and C5 (5.1  $\Delta E^*_{ab}$ ).

**Mineralogical composition.** The mineralogical composition of tested clays is summarized in **Table S4**. In this table are highlighted differences related to declared mineralogical composition and effective composition recorded by XRD analyses. Furthermore, minerals detected by the analyses but not declared are reported in **Table S4b,c**.

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**Table S4.** Differences between the components found with the X-ray diffractometric analysis (green), the composition declarations on the label but not recovered (zebra); declarations verified (dark green). The table reports the complete list of minerals that was declared in labels (Tab 4a), determined for tested samples (Tab 4b); and those that are not properly associated to clays but that are present as associated mineralization (Tab. 4c). HE = high expandability; NE = no expandability (Warr, 2022). In red are indicated minerals recorded only in one clay type.

Tab4.a Declared	Features	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
Bentonite	2:1 HE										██████████
Biotite	2:1 NE										
Clinochlore	2:1:1 NE										
Hectorite	2:1 HE		██████████								
Illite	2:1 NE	██████████			██████████	██████████			██████████		
Kaolinite	1:1 NE			██████████				██████████		██████████	
Montmorillonite	2:1 HE			██████████			██████████				
Muscovite	2:1 NE										
Vermiculite	2:1 NE										

Tab4.b Verified	Features	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
Bentonite	2:1 HE										
Biotite	2:1 NE									██████████	
Clinochlore	2:1:1 NE					██████████					
Hectorite	2:1 HE										
Illite	2:1 NE					██████████					██████████
Kaolinite	1:1 NE	██████████	██████████		██████████						██████████
Montmorillonite	2:1 HE	██████████									██████████
Muscovite	2:1 NE	██████████									
Vermiculite	2:1 NE		██████████	██████████		██████████	██████████		██████████		██████████

Tab4.c – Present but not declared	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
Albite										██████████
Anatase			██████████				██████████			
Anorthite								██████████		
Apatite-(CaF)	██████████									
Augite						██████████				
Barite			██████████							
Calcite	██████████	██████████			██████████	██████████			██████████	██████████
Clinoptilolite-Like			██████████							
Dolomite		██████████							██████████	
Fluoro-edenite		██████████								
Graphite										██████████
Gypsum		██████████							██████████	
Magnetite				██████████		██████████				
Mullite			██████████				██████████			
Pargasite								██████████		
Phlogopite				██████████						
Pyrite	██████████									
Quartz	██████████	██████████	██████████	██████████	██████████	██████████			██████████	██████████
Rutile				██████████	██████████	██████████				██████████
Sulphur-beta									██████████	
Tremolite								██████████		

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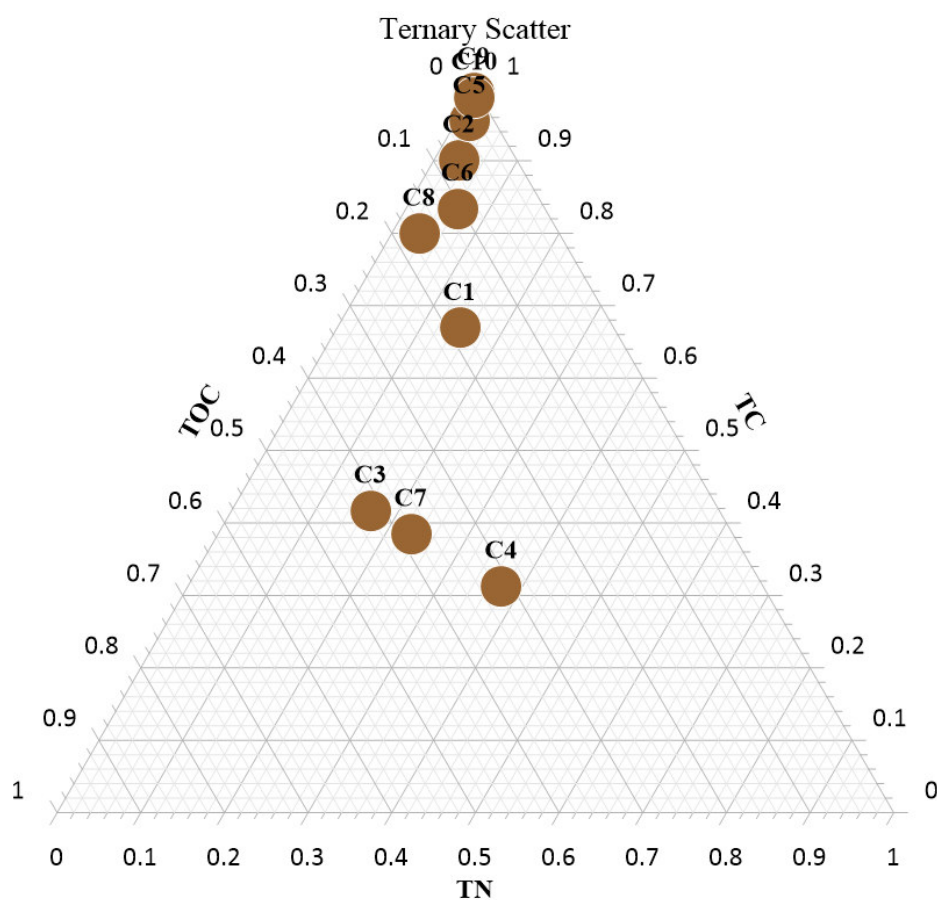
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Chemical composition. In **Figure 1S** ternary diagrams concerning levels of TN, TOC, TC in tested clays is represented.

**Figure S1. Ternary scatter of macronutrients recorded in tested clays.** Concerning levels, TOC measured in C1, C3-4, C6-10 resulted similar (0.05%) TN levels were scaled as follow: 0.06% (C4) > 0.04% (C1, C10) > 0.03% (C6-7) > 0.02% (C3) > 0.01% (C8-9). Much wider variability was found for TC ( $\pm 3.89\%$ ) ranging between 0.401% (C6), 0.239% (C8), 0.182% (C1), and 0.05% (C3-4, C7).





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Pearsons' correlations among measured chemicals are reported in **Table S5**. In particular, in **Table S5a** correlation are reported among macronutrients and metals or metalloids; while in Table S5b, are reported correlations among REE detected in tested clays. Significant correlations are highlighted using colors to underline levels of significant ( $p=1.000$  in green;  $p>0.900$  in yellow;  $0.600<p<0.900$  in pink).

**Table S5. Pearson's correlation matrix between measured macronutrients, metals, metalloids, rare earths (REE).** Only measurable (values > LOQ) macronutrients, metals, metalloids and REE are reported in these tables.

a)	TN	TC	TOC	As	Cd	Co	Cr	Ni	Pb	Cu	Zn	Sr
TN		-0.177	0.637	0.614	0.834	0.634	0.705	0.807	0.439	0.641	0.899	0.409
TC	-0.177		0.035	0.495	-0.056	0.239	0.347	0.267	0.220	-0.050	-0.175	0.026
TOC	0.637	0.035		0.378	0.833	0.160	0.747	0.536	0.228	0.735	0.494	0.791
As	0.614	0.495	0.378		0.583	0.824	0.736	0.810	0.428	0.291	0.689	0.395
Cd	0.834	-0.056	0.833	0.583		0.393	0.691	0.772	0.098	0.607	0.802	0.521
Co	0.634	0.239	0.160	0.824	0.393		0.718	0.815	0.632	0.460	0.717	0.170
Cr	0.705	0.347	0.747	0.736	0.691	0.718		0.853	0.613	0.803	0.617	0.594
Ni	0.807	0.267	0.536	0.810	0.772	0.815	0.853		0.468	0.611	0.788	0.226
Pb	0.439	0.220	0.228	0.428	0.098	0.632	0.613	0.468		0.549	0.363	0.257
Cu	0.641	-0.050	0.735	0.291	0.607	0.460	0.803	0.611	0.549		0.574	0.524
Zn	0.899	-0.175	0.494	0.689	0.802	0.717	0.617	0.788	0.363	0.574		0.361
Sr	0.409	0.026	0.791	0.395	0.521	0.170	0.594	0.226	0.257	0.524	0.361	

b)	Gd	Yb	Y	La	Lu	Dy	Er	Eu	Pr	Ho	Nd	Ce	Sm	Th	Te
Gd		0.98	0.98	0.09	0.98	0.99	0.98	0.99	0.56	0.99	0.81	0.40	0.98	0.98	1.00
Yb	0.98		1.00	-0.09	1.00	1.00	1.00	0.95	0.40	1.00	0.68	0.23	0.92	1.00	0.99
Y	0.98	1.00		-0.10	1.00	1.00	1.00	0.95	0.40	1.00	0.68	0.22	0.92	1.00	0.99
La	0.09	-0.09	-0.10		-0.09	-0.04	-0.09	0.21	0.87	-0.08	0.65	0.94	0.29	-0.10	0.01
Lu	0.98	1.00	1.00	-0.09		1.00	1.00	0.95	0.39	1.00	0.68	0.22	0.92	1.00	0.99
Dy	0.99	1.00	1.00	-0.04	1.00		1.00	0.96	0.44	1.00	0.72	0.28	0.94	1.00	1.00
Er	0.98	1.00	1.00	-0.09	1.00	1.00		0.95	0.40	1.00	0.69	0.23	0.92	1.00	1.00
Eu	0.99	0.95	0.95	0.21	0.95	0.96	0.95		0.65	0.95	0.87	0.51	0.99	0.95	0.98
Pr	0.56	0.40	0.40	0.87	0.39	0.44	0.40	0.65		0.41	0.94	0.98	0.72	0.39	0.49
Ho	0.99	1.00	1.00	-0.08	1.00	1.00	1.00	0.95	0.41		0.70	0.24	0.93	1.00	1.00
Nd	0.81	0.68	0.68	0.65	0.68	0.72	0.69	0.87	0.94	0.70		0.86	0.91	0.68	0.76
Ce	0.40	0.23	0.22	0.94	0.22	0.28	0.23	0.51	0.98	0.24	0.86		0.58	0.22	0.32
Sm	0.98	0.92	0.92	0.29	0.92	0.94	0.92	0.99	0.72	0.93	0.91	0.58		0.92	0.96
Th	0.98	1.00	1.00	-0.10	1.00	1.00	1.00	0.95	0.39	1.00	0.68	0.22	0.92		0.99
Te	1.00	0.99	0.99	0.01	0.99	1.00	1.00	0.98	0.49	1.00	0.76	0.32	0.96	0.99	



**Environmental hazard of different clays as consequence of global change: a case study on *Aliivibrio fischeri*.**

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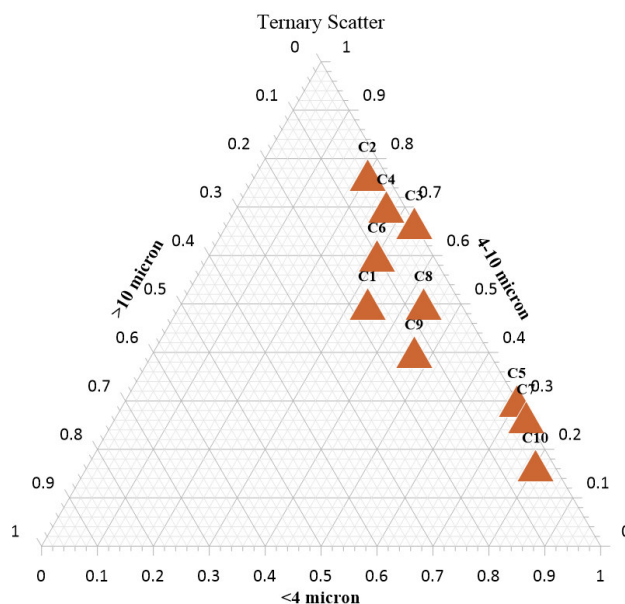
<sup>3</sup> Dipartimento di Scienze della Vita, University of Trieste, 34127 Trieste, Italy

<sup>4</sup> CoNISMa, Piazzale Flaminio, 9, 00196, Roma

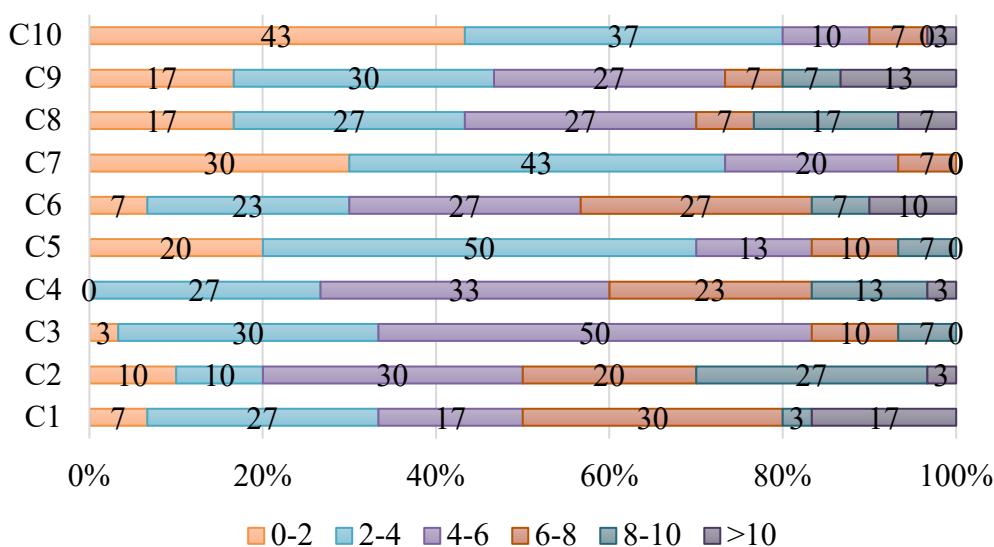
\*Corresponding author e-mail: mrenzi@units.it

Particle size dimension. In **Figure S2** particle-size distributions obtained by Multiple-light scattering in AWS dispersions is reported. On the contrary, **Figure S3** represents the particle-size distribution obtained by measurements performed by FESEM technology on dried dusts.

**Figure S2. Ternary scatter of particle-size recorded in ASW dispersions.**



**Figure S3. Percentage of particle-size recorded in dry dusts of tested clays by FESEM technology.** Colours represent different size-classes in microns; numbers on bars represent percentages recorded for each size-class (n=30 counts per clay).



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As regards as counts performed by FESEM on dried dusts, even if the statistical representativeness of obtained results is not comparable to those obtained by the Multisizer technique, some differences were observed:

- Minimum values between 1.5-2.0  $\mu\text{m}$  were measured in six samples (1.67  $\mu\text{m}$  in C1; 1.61  $\mu\text{m}$  in C2; 1.64  $\mu\text{m}$  in C3; and C8, 1.67  $\mu\text{m}$  in C5, 1.72  $\mu\text{m}$  in C6), and between 1.0-1.5  $\mu\text{m}$  in two samples (1.29  $\mu\text{m}$  in C7 and 1.49  $\mu\text{m}$  in C9).
- Maximum values ranged from 7.36  $\mu\text{m}$  (C7) to 24.67  $\mu\text{m}$  (C9).
- The intermediate size classes were defined as 0-2  $\mu\text{m}$  (C10), 2-4  $\mu\text{m}$  (C5, C7 and C9), 4-6  $\mu\text{m}$  (C2-4), 6-8  $\mu\text{m}$  (C1). Samples C6-7 had the same percentage (27%) in the different size classes (4-6  $\mu\text{m}$  and 6-8  $\mu\text{m}$  for C6, while 2-4  $\mu\text{m}$  and 4-6  $\mu\text{m}$  for C7).
- The average values of the measured particles in clay dust were above 4  $\mu\text{m}$ , which is considered as the upper limit for clay (6.8  $\mu\text{m}$  C1, 6.2  $\mu\text{m}$  C2, 5.0  $\mu\text{m}$  C3, 5.7  $\mu\text{m}$  C4, 6.2  $\mu\text{m}$  C6, 5.3  $\mu\text{m}$  C8, 5.7  $\mu\text{m}$  C9). For C1-4, C6, C8-9, the percentage of particles larger than 4  $\mu\text{m}$  exceeded 50% of the total particles measured in the dusts.
- In contrast, for C5, C7, and C10, the particles measured in the dusts with a size of 0-4  $\mu\text{m}$  predominated.

### 2.2. Ecotoxicological measurements

#### Solid phase test (TU wet weight).

Results of toxicity recorded on solid phase tests expressed as TU (wet weight) were:

- $107.4 \pm 67.6$  (pH 7.00),  $32.4 \pm 16.5$  (pH 7.50), and  $34.7 \pm 22.0$  (pH 8.10), wet weight.

Significant differences were reported comparing toxicity expressed as dry weight and wet weight measured at different pH ( $p=0.001$  for pH 7.00; 8.10; and  $p=0.0001$  for pH 7.50). Absolute minimum value found 11.3 TU (range 6.2-20.5) is C9 (wet weight and pH= 7.50) while the maximum value 1064.5 TU (range 821.8-1377.1) was recorded for C7 (dry weight and pH =7.00). Testing clays at 8.10 (standard conditions), the toxicity measured showed a maximum value of 78.5 TU (C5; wet weight, range 52.9-116.5 TU). The minimum value of measured toxicity was 15.2 TU (C1; wet weight, 12.1-19.1 TU).

Testing clays under different pH conditions showed that:

- at pH 7.50, maxima were 70.6 TU (C3, wet weight, range 38.2-130.7 TU). The minima value was 11.3 TU (C9, wet weight, range 6.2-20.5 TU).
- at pH 7.00, a highest variability between samples. Minimum and maximum toxicity wet weight were recorded respectively on samples showing the least and the most toxic value dry weight.

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<sup>4</sup> CoNISMa, Piazzale Flaminio, 9, 00196, Roma

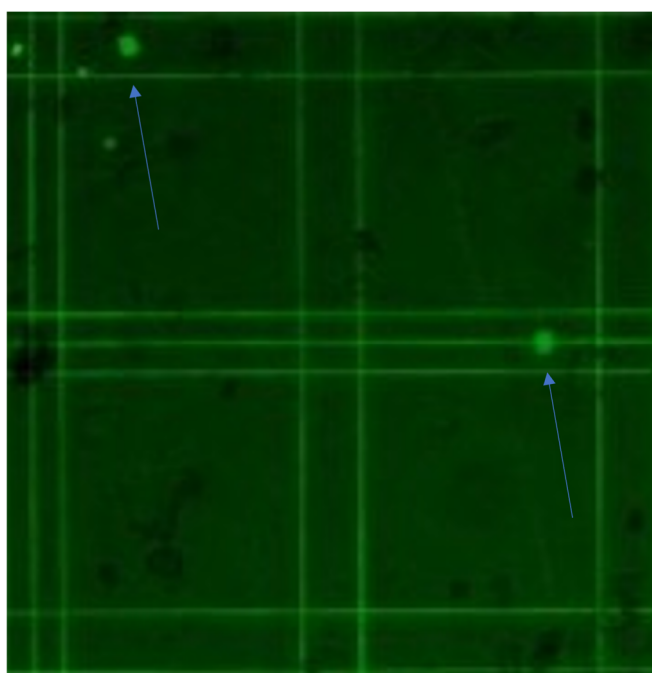
\*Corresponding author e-mail: mrenzi@units.it

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### Interferences.

In **Figure S4** a picture of living bacteria (light green dots) in a Burker's chamber field used to count alive bacteria fixed on clays is reported as example of obtained results. Image was performed under fluorescent microscopy.

**Figure S4. Living bacteria in clay sample (an example).**



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## clay1-Evaluation report (clay1)

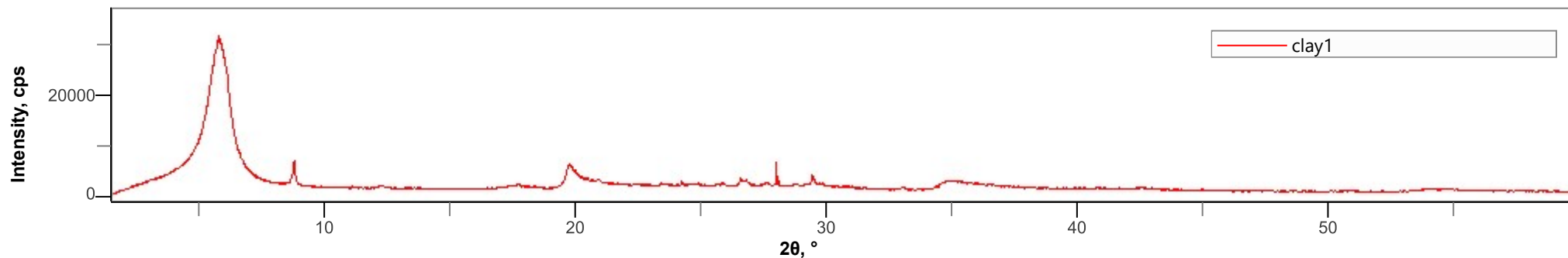
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Analyst	Default	Operator	Default
Sample name	argilla campione1	Comment	
Measured data name	C:\Users\acava\OneDrive\Documenti\UserData\BsRC\clay1.ra...	Memo	

### Measurement Conditions

Name	Value	Name	Value
X-Ray generator	40 kV, 40 mA	Scan mode	1D(scan)
Incident primary	Standard	Scan speed/Duration time	1.50 °/min
Goniometer	Standard Goniometer	Step width	0.008 °
Attachment	Standard attachment head	Scan axis	$\theta/2\theta$
Filter	K $\beta$ filter for Cu	Scan range	1.5 ~ 60 °
Selection slit	BB	Incident slit box	1/5deg
Diffracted beam mono	None	Length-limiting slit	10 mm
Detector	D/teX Ultra 250	Receiving slit box #1	10.000mm
Optics attribute	BB	Receiving slit box #2	Open

### Measured profile view

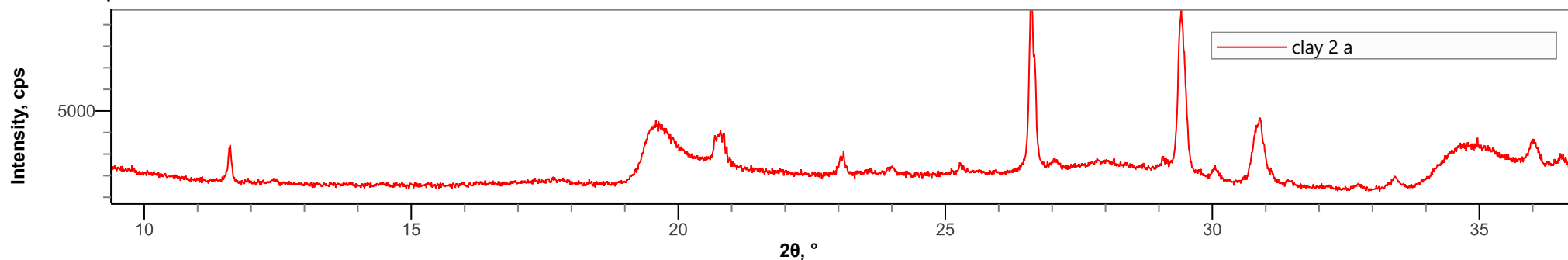


**clay 2 a-Evaluation report (clay 2 a)****General information**

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Analyst	Default	Operator	Default
Sample name	hectorite	Comment	
Measured data name	C:\Users\acava\OneDrive\Documenti\UserData\BsRC\clay 2 a...	Memo	

**Measurement Conditions**

Name	Value	Name	Value
X-Ray generator	40 kV, 40 mA	Scan mode	1D(scan)
Incident primary	Standard	Scan speed/Duration time	1.50 °/min
Goniometer	Standard Goniometer	Step width	0.008 °
Attachment	Standard attachment head	Scan axis	$\theta/2\theta$
Filter	K $\beta$ filter for Cu	Scan range	2 ~ 45 °
Selection slit	BB	Incident slit box	1/5deg
Diffracted beam mono	None	Length-limiting slit	10 mm
Detector	D/teX Ultra 250	Receiving slit box #1	Open
Optics attribute	BB	Receiving slit box #2	Open

**Measured profile view**

## clay 3 a-Evaluation report (clay 3 a)

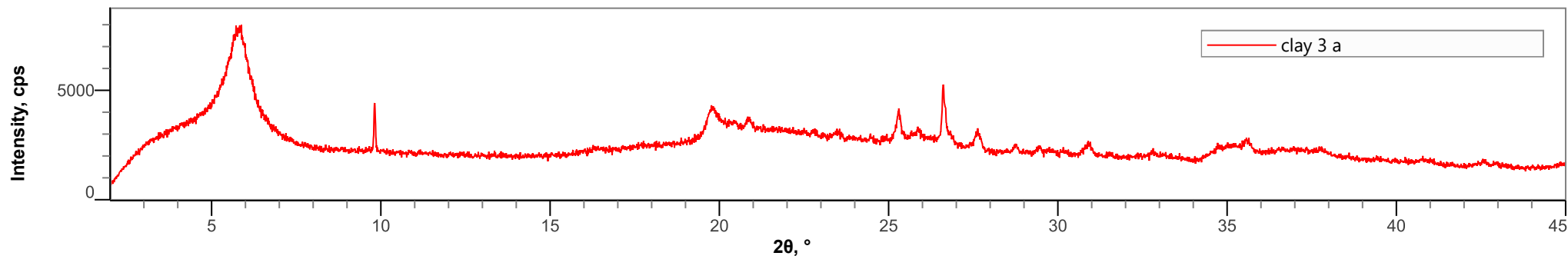
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Analyst	Default	Operator	Default
Sample name	Kaoline / montmorillonite	Comment	
Measured data name	C:\Users\acava\OneDrive\Documenti\UserData\BsRC\clay 3 a...	Memo	

### Measurement Conditions

Name	Value	Name	Value
X-Ray generator	40 kV, 40 mA	Scan mode	1D(scan)
Incident primary	Standard	Scan speed/Duration time	1.50 °/min
Goniometer	Standard Goniometer	Step width	0.008 °
Attachment	Standard attachment head	Scan axis	$\theta/2\theta$
Filter	K $\beta$ filter for Cu	Scan range	2 ~ 45 °
Selection slit	BB	Incident slit box	1/5deg
Diffracted beam mono	None	Length-limiting slit	10 mm
Detector	D/teX Ultra 250	Receiving slit box #1	Open
Optics attribute	BB	Receiving slit box #2	Open

### Measured profile view



## clay 4 a-Evaluation report (clay 4 a)

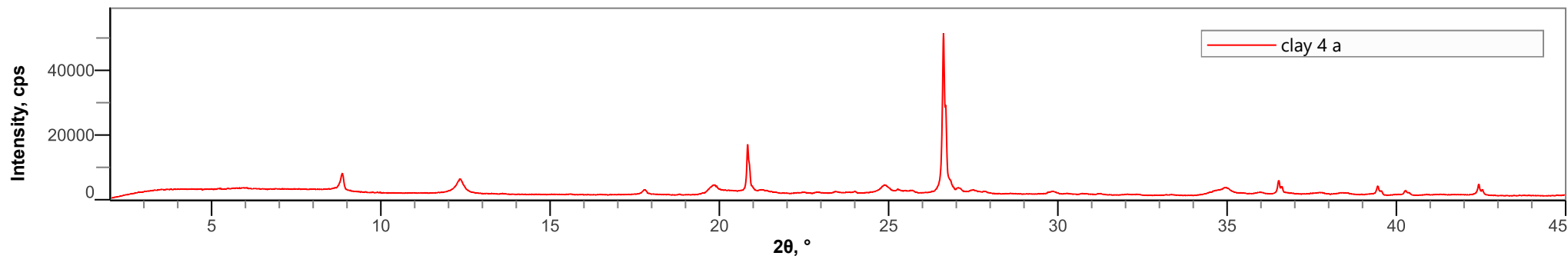
### General information

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Analyst	Default	Operator	Default
Sample name	illite	Comment	
Measured data name	C:\Users\acava\OneDrive\Documenti\UserData\BsRC\clay 4 a...	Memo	

### Measurement Conditions

Name	Value	Name	Value
X-Ray generator	40 kV, 40 mA	Scan mode	1D(scan)
Incident primary	Standard	Scan speed/Duration time	1.50 °/min
Goniometer	Standard Goniometer	Step width	0.008 °
Attachment	Standard attachment head	Scan axis	$\theta/2\theta$
Filter	K $\beta$ filter for Cu	Scan range	2 ~ 45 °
Selection slit	BB	Incident slit box	1/5deg
Diffracted beam mono	None	Length-limiting slit	10 mm
Detector	D/teX Ultra 250	Receiving slit box #1	Open
Optics attribute	BB	Receiving slit box #2	Open

### Measured profile view





## clay 5 a-Evaluation report (clay 5 a)

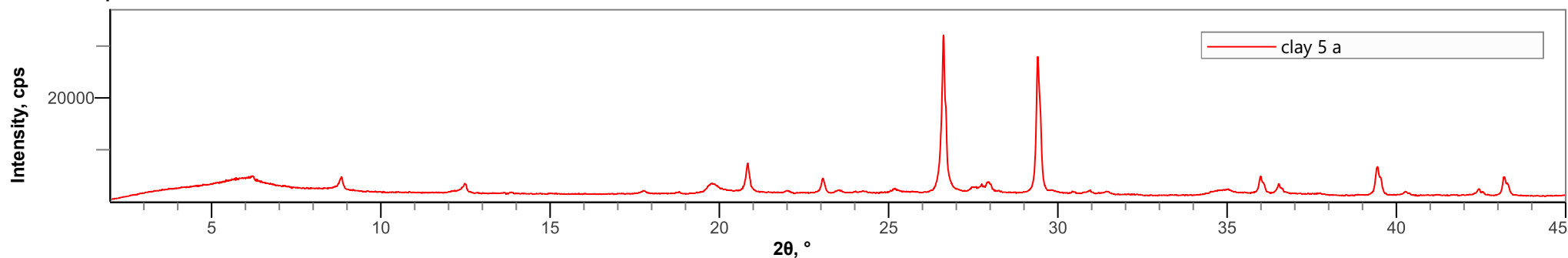
### General information

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Analyst	Default	Operator	Default
Sample name	illite	Comment	
Measured data name	C:\Users\acava\OneDrive\Documenti\UserData\BsRC\clay 5 a...	Memo	

### Measurement Conditions

Name	Value	Name	Value
X-Ray generator	40 kV, 40 mA	Scan mode	1D(scan)
Incident primary	Standard	Scan speed/Duration time	1.50 °/min
Goniometer	Standard Goniometer	Step width	0.008 °
Attachment	Standard attachment head	Scan axis	$\theta/2\theta$
Filter	K $\beta$ filter for Cu	Scan range	2 ~ 45 °
Selection slit	BB	Incident slit box	1/5deg
Diffracted beam mono	None	Length-limiting slit	10 mm
Detector	D/teX Ultra 250	Receiving slit box #1	Open
Optics attribute	BB	Receiving slit box #2	Open

### Measured profile view



## clay 6 a-Evaluation report (clay 6 a)

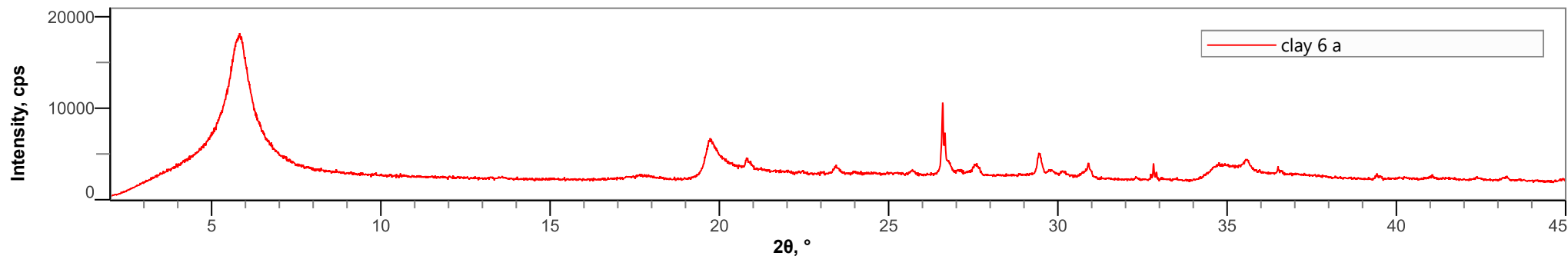
### General information

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Analyst	Default	Operator	Default
Sample name	illite	Comment	
Measured data name	C:\Users\acava\OneDrive\Documenti\UserData\BsRC\clay 6 a...	Memo	

### Measurement Conditions

Name	Value	Name	Value
X-Ray generator	40 kV, 40 mA	Scan mode	1D(scan)
Incident primary	Standard	Scan speed/Duration time	1.50 °/min
Goniometer	Standard Goniometer	Step width	0.008 °
Attachment	Standard attachment head	Scan axis	$\theta/2\theta$
Filter	K $\beta$ filter for Cu	Scan range	2 ~ 45 °
Selection slit	BB	Incident slit box	1/5deg
Diffracted beam mono	None	Length-limiting slit	10 mm
Detector	D/teX Ultra 250	Receiving slit box #1	Open
Optics attribute	BB	Receiving slit box #2	Open

### Measured profile view



## clay 7 a-Evaluation report (clay 7 a)

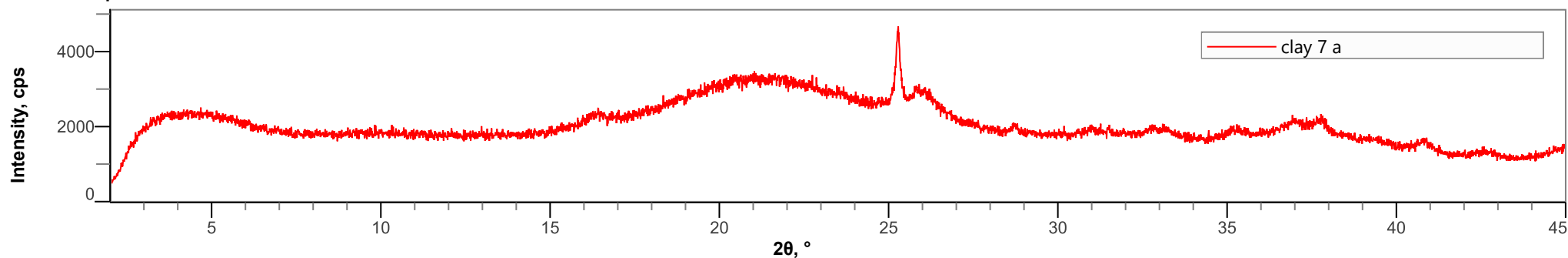
### General information

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Analyst	Default	Operator	Default
Sample name	kaoline	Comment	
Measured data name	C:\Users\acava\OneDrive\Documenti\UserData\BsRC\clay 7 a...	Memo	

### Measurement Conditions

Name	Value	Name	Value
X-Ray generator	40 kV, 40 mA	Scan mode	1D(scan)
Incident primary	Standard	Scan speed/Duration time	1.50 °/min
Goniometer	Standard Goniometer	Step width	0.008 °
Attachment	Standard attachment head	Scan axis	$\theta/2\theta$
Filter	K $\beta$ filter for Cu	Scan range	2 ~ 45 °
Selection slit	BB	Incident slit box	1/5deg
Diffracted beam mono	None	Length-limiting slit	10 mm
Detector	D/teX Ultra 250	Receiving slit box #1	Open
Optics attribute	BB	Receiving slit box #2	Open

### Measured profile view



## clay 8 a-Evaluation report (clay 8 a)

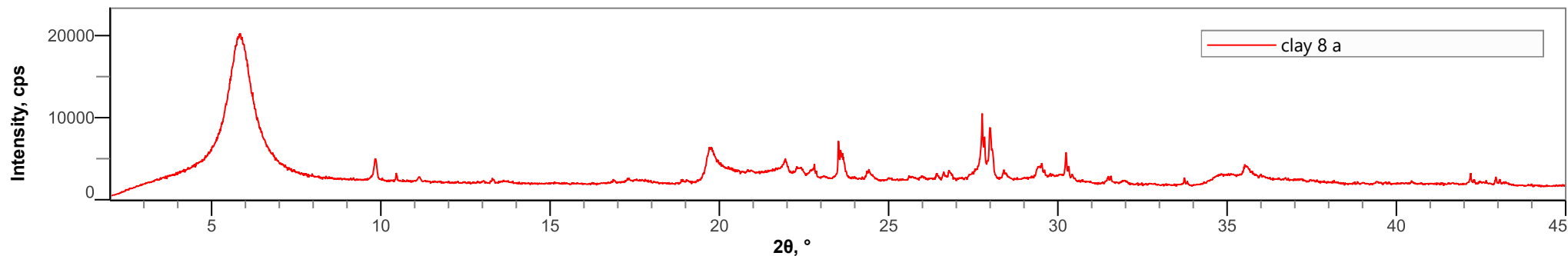
### General information

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Analyst	Default	Operator	Default
Sample name	illite	Comment	
Measured data name	C:\Users\acava\OneDrive\Documenti\UserData\BsRC\clay 8 a...	Memo	

### Measurement Conditions

Name	Value	Name	Value
X-Ray generator	40 kV, 40 mA	Scan mode	1D(scan)
Incident primary	Standard	Scan speed/Duration time	1.50 °/min
Goniometer	Standard Goniometer	Step width	0.008 °
Attachment	Standard attachment head	Scan axis	$\theta/2\theta$
Filter	K $\beta$ filter for Cu	Scan range	2 ~ 45 °
Selection slit	BB	Incident slit box	1/5deg
Diffracted beam mono	None	Length-limiting slit	10 mm
Detector	D/teX Ultra 250	Receiving slit box #1	Open
Optics attribute	BB	Receiving slit box #2	Open

### Measured profile view



## clay9 a-Evaluation report (clay9 a)

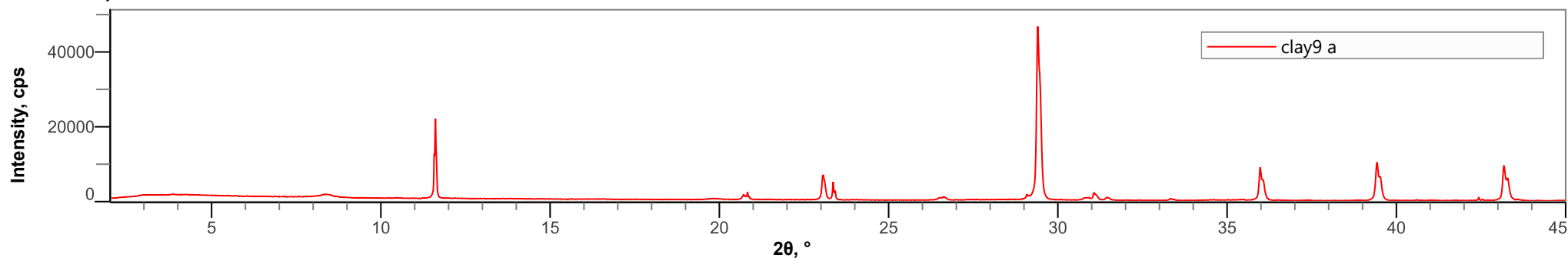
### General information

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Analyst	Default	Operator	Default
Sample name	kaolinite campione9	Comment	
Measured data name	C:\Users\acava\OneDrive\Documenti\UserData\BsRC\clay9 a...	Memo	

### Measurement Conditions

Name	Value	Name	Value
X-Ray generator	40 kV, 40 mA	Scan mode	1D(scan)
Incident primary	Standard	Scan speed/Duration time	1.50 °/min
Goniometer	Standard Goniometer	Step width	0.008 °
Attachment	Standard attachment head	Scan axis	$\theta/2\theta$
Filter	K $\beta$ filter for Cu	Scan range	2 ~ 45 °
Selection slit	BB	Incident slit box	1/5deg
Diffracted beam mono	None	Length-limiting slit	10 mm
Detector	D/teX Ultra 250	Receiving slit box #1	Open
Optics attribute	BB	Receiving slit box #2	Open

### Measured profile view



## clay10 a-Evaluation report (clay10 a)

### General information

Name	Value	Name	Value
Analysis date	2022-09-02 14:53:26	Measurement start time	2022-08-24 15:20:28
Analyst	Default	Operator	Default
Sample name	bentonite	Comment	
Measured data name	C:\Users\acava\OneDrive\Documenti\UserData\BsRC\clay10...	Memo	

### Measurement Conditions

Name	Value	Name	Value
X-Ray generator	40 kV, 40 mA	Scan mode	1D(scan)
Incident primary	Standard	Scan speed/Duration time	1.50 °/min
Goniometer	Standard Goniometer	Step width	0.008 °
Attachment	Standard attachment head	Scan axis	$\theta/2\theta$
Filter	K $\beta$ filter for Cu	Scan range	2 ~ 45 °
Selection slit	BB	Incident slit box	1/5deg
Diffracted beam mono	None	Length-limiting slit	10 mm
Detector	D/teX Ultra 250	Receiving slit box #1	Open
Optics attribute	BB	Receiving slit box #2	Open

### Measured profile view

