



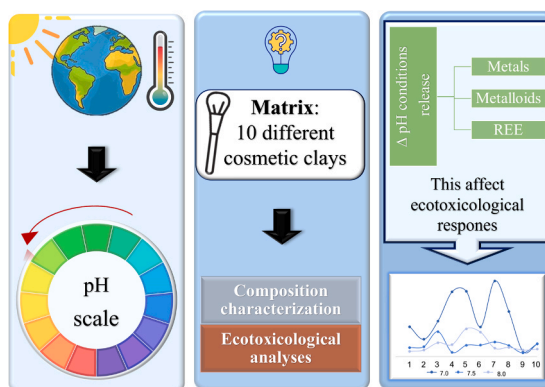
Research Paper

Impact of global change on environmental hazards of different clays: A case study on *Aliivibrio fischeri*Serena Anselmi^{a,d}, Andrea Cavallo^b, Luca Del Rio^b, Monia Renzi^{c,*}^a Bioscience Research Center, via Aurelia Vecchia, 32, 58015 Orbetello, GR, Italy^b CERTEMA Scrl, S.P. del Cipressino km 10, 58044, Cinigiano, Italy^c Dipartimento di Scienze della Vita, University of Trieste, 34127 Trieste, Italy^d CoNISMa, Piazzale Flaminio, 9, 00196 Rome, Italy

HIGHLIGHTS

- Impurities in natural clays (i.e., REE and nano-TiO₂) were detected and could affect ecotoxicity.
- Global change effects could act changing ecotoxicity of natural clays.
- *A. fischeri* was impacted by almost all tested clays at pH 7.00.
- Clays resulted able to absorb living cells of bacteria on their particles surfaces.
- In some types of clay absorption increases with the decrease of pH.

GRAPHICAL ABSTRACT



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ABSTRACT

The effects of global change in marine ecosystems are expected to lower pH from the current 8.1–7.5–7.0, which will have significant impacts on marine species. The purpose of this study is to investigate whether the ecotoxicity of ten different natural clays change significantly in response to the acidification process and what factors are associated with the observed changes. In this study, the ecotoxicological response of a bacterium (*Aliivibrio fischeri*) was tested under current (pH= 8.1) and acidified (pH 7.5 and 7.0) conditions. The ecotoxicity detected in the solid phase test (SPT protocol) and in the contact water was affected by the pH, which increased the ecotoxicity from 2/10 clays (pH 8.10) to 7/10 clays (pH 7.00), also shifting the detected effects from low to high toxicity values. The analyses performed on the natural clays studied show that pH can affect the release of metals, metalloids and rare earths from the clays into the contact water phase, affecting the toxicity observed. This phenomenon depends on the type of clay and is closely related to its mineralogical composition. As consequence, in a globally changing scenario, ecotoxicity, even of natural materials such as clay, cannot be considered stable, but must be accurately reevaluated depending on the mineralogical and chemical composition

Abbreviation list: STI, Standard toxicity Index; SPT, Solid Phase Test; Contact water, means the ASW water used to perform the SPT with *A. fischeri* and put in contact with tested clays for 20 min before being filtered to perform the assay.

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of the clay. Moreover, the mineralogical composition of clays showed different efficiency in absorbing bacteria on the surface of clay particles. It was found that live bacterial cells were absorbed on the clay surface in numbers that were dependent on both clay types and pH levels.

1. Introduction

One of the consequences of global climate change is acidification of pH in seawater due to temperature rise. Recent prediction models predict a decrease in pH levels in the marine environment from current levels (pH 8.20÷7.91) to pH 7.90÷7.61 in the near future and to pH 7.60÷7.21 in the far future to extreme levels (pH \leq 7.20; [14]). Acidification is expected to have significant direct and indirect impacts on marine species [36]; moreover, acidification is also responsible for changes in ecotoxicological responses of marine species as pH decreases, which is well documented in the literature [20,40,44]. However, this process is still poorly understood. In addition, decreasing seawater pH could lead to the release of chemicals from sediments into the water column, altering ecotoxicological impacts on marine species and trophic webs.

The marine bacterium *Aliivibrio fischeri* is one of the best species to study the above aspects, as it allows the evaluation and research of ecotoxicological impacts on marine species under standardized protocols both in sediment (solid phase test, SPT, UNI EN ISO 11348-1 + [30] S11, App.2; [1]) and in the water column (liquid phase test, UNI EN ISO 11348-1) according to a changing environment. *A. fischeri* has been used in ecotoxicological studies worldwide in both marine and freshwater ecosystems [1]. This species is a Gram-negative marine bacterium that exhibits natural bioluminescence that is directly proportional to the metabolic activity of the bacterial population. Therefore, any inhibition of enzymatic activity due to toxicity or cell death results in a corresponding decrease in bioluminescence produced by the tested population. Due to this phenomenon, this type of analysis provides a direct measure of ecotoxicity related to the inhibition of bioluminescence [22]. In fact, this analysis is used in several countries to monitor water quality (U.S. Environmental Protection Agency, European Union) because it provides a quantitative response to pollution and the methods used to determine the responses are well standardized.

Recent research suggests that lowering pH could have significant impacts on marine species, both directly and indirectly [36], and some recent studies have reported impacts on the ecotoxicity of chemicals, nanoparticles, and plastics tested under different pH conditions using assays that target the aquatic matrix [20,40,44]. However, the effects on sediments are also associated with acidification, and in this case the ecotoxicological tests developed for the solid matrix should be applied.

The natural sediment consists of a heterogeneous matrix composed of various proportions of gravel (particle size $>1000\ \mu\text{m}$), sand (particle size $1000\div 63\ \mu\text{m}$) and silt, composed of particles $< 63\ \mu\text{m}$. The latter is constituted by mud ($63\div 4\ \mu\text{m}$ of particle size) and clay (particle size $< 4\ \mu\text{m}$) in different percentages. The mineralogical composition of mud and clays also varies widely worldwide depending on latitude and local conditions [62]. It is known that clay minerals have high absorption efficiency of trace elements such as heavy metals in their ionic forms; sorption efficiency is also related to mineral composition of clay [55].

The SPT for *A. fischeri* is applicable to all types of sediments, from sands to clay without any difference concerning the mineralogical composition of the particles in the treatment of the sediment samples tested. To improve the precision and accuracy of the results, the tests should only be performed when the sand content of the total sediment sample is less than 90% of the total weight [37]. In the literature, for silty ones a natural toxicity of sediments is reported and interpreted as a direct effect of the matrix on the tested species and not as a result of chemical pollution [7]. According to the literature, the higher natural toxicity of silt is due to the clay content. When sediment is 100% clay, it has toxicity values of 273 TU [37]. The very high toxicity values of clays

are mainly due to their porous structure with small particle size, which allows the charges to bind to the structural units of silica and alumina through ionic bonding and ion exchange, resulting in unsaturated charges ([11]; Uddin, 2022). In fact, clay is the best fraction in natural sediments to explore the relationships among ecotoxicological effects of clay exposure and key physicochemical clay properties such as mineralogical composition, water content, levels of total organic carbon bound to porous clay particles, and chemicals released in water [2].

Although clay is a natural component of marine sediments, in certain cases it can become harmful to the environment. When sediments are dredged in harbors, rivers, lagoons, and estuaries, the clay particles are forced to resuspend in the water column, creating a deep contact between clay particles and the water matrix. In this case, when chemical pollutants are adsorbed onto clay particles, ionic forms can efficiently go in solution, which is also favored by changes in redox potential due to oxidation. Although metals and metalloids are commonly determined in sediments and clays, some aspects related to their mineralogical composition are not well understood, such as rare earth element content (REE), particle size, behavior, and morphological structure of minerals. In addition, clays may contain nanoparticles of natural origin and some oxides such as TiO_2 (Uddin, 2022) that could be significantly toxic for aquatic species (Dubourguier et al., 2008; [46]).

A. fischeri proves to be a particularly sensitive species to metals and metalloids [48]. This behavior underscores the importance of our decision to study the effects of clay, pH, and mineralogical composition on this species, as it can intercept responses that may not be clear when studying other species of ecotoxicological interest. To our knowledge, the literature has reported on the interaction between the solid sediment matrix and *A. fischeri* without considering the complex effects that occur between bacteria and the clay fraction as a function of variation in clay mineralogical composition and seawater pH.

Instead, only the natural water content and natural toxicity of the silt fraction in the sediment studied is used to scale the measured toxicity with respect to these factors (using the correction factor of 273 TU according to the M.D. n. 173, [37]). This represents a significant underestimation of the natural variability of sediments in aquatic ecosystems, which may be composed of different minerals depending on the varying geological composition of different coastal areas [62]. On the contrary, the variability of sediment composition, associated with the variability of pH values, does not prove that the ecotoxicological responses are representative of current or future scenarios, making it difficult to objectively evaluate the real toxicity of the matrix in geographical areas with high clay content in the sediments. Moreover, according to Italian legislation, for marine sediments with a sand content higher than 90%, the SPT can be replaced by liquid phase analyses. In this case, the effect of acidified water (pH = 7.0; 7.5) on the extraction of mineral constituents from clays with different mineralogical composition is an important aspect that should be evaluated.

The aim of this study is to determine the ecotoxicity of ten different natural clays for the species *Aliivibrio fischeri* under current pH conditions (8.10). We selected natural clays with different mineralogical compositions that could be assumed not to be polluted by human activities. The ecotoxicological results are related to the main physicochemical, morphological and mineralogical characteristics of the studied clays in order to evaluate the relationships between them and the detected ecotoxicity. The content of metals, metalloids, rare earths (REE) and nanoparticles is determined to evaluate the different risks related to the variability of the composition of natural clays, an aspect that is underestimated in the literature. The bacterium *A. fischeri* was chosen as the study species because of the existence of well-standardized

monitoring methods worldwide and because it is very sensitive to metals, metalloids and REE.

Experiments are also be conducted under acidic conditions to determine if the recorded responses change with decreasing pH (7.50 and 7.00). These results will allow us to better understand whether the effects of global change could significantly affect the ecotoxicity of natural, unpolluted materials.

To better understand the dynamics of clay ecotoxicity, we also investigated any factors that might affect the experiments acting on ecotoxicological responses and/or clay toxicity (e.g., chemicals released upon contact with water, precipitation of chemicals during acidification, matrix size due to false positive effects from absorption of bacteria on clays, and test interference from turbidity) with the aim of clarify the contribute of interferences on ecotoxicological results under different pH conditions.

2. Materials and methods

2.1. Logic model and aim of the study

Ten different clays (designated C1-C10, $n = 10$) were tested in this study to account for the greater mineralogical variability of natural clays. The collected C1-C10 clays were characterized physically (morphology, color, and mean particle size of dusts and water dispersions), mineralogically, and chemically (content of metals, metalloids, REE, macronutrients; $n = 10$). Ecotoxicological effects on *Aliivibrio fischeri* (bacteria) were measured under actual marine conditions (pH = 8.10) and under two different pH scenarios associated with global change impacts on seawater. The two pH values chosen were within the tolerance of the species tested but are representative of the future evolution of marine pH scenarios: pH 7.50 (conservative scenarios) and pH 7.00 (worst case scenarios) according to the literature [14]. The tests were performed in the solid phase under three different pH conditions of particular interest (10 clays, 3 pH conditions; $n = 30$ per replicate).

In addition, to test the relationship between the ecotoxicity observed in the solid phase and the release of chemicals from the clay into the contact water as a function of water pH, supernatant contact water obtained after 20 min of clay/water exposure at the three different pH values (pH = 7.00; 7.50; 8.10; $n = 30$ per replicate) was collected and filtered at 0.45 μm to perform a screening test in the liquid phase (90% screening test of the sample).

Chemical analysis (metals, metalloids, REE) were performed on the contact water of clay C7 and C10 and on the negative control (ASW) to evaluate ($n = 5$) the release of chemicals of potential interest into the water column as a function of water pH. In addition, NaOH was added to the contact water at pH 7.00–8.10 to determine changes in ecotoxicity due to precipitation of dissolved metals as pH conditions changed. This part of the experiment allowed us to clarify whether changes in the pH of the water could determine the precipitation of dissolved chemicals in the water and thus alter the observed ecotoxicity.

Finally, a special part of the experiment was performed to evaluate possible interferences on the ecotoxicological effects recorded. Some interferences could occur and affect the recorded bioluminescence of bacteria, and pH could be an important factor promoting their occurrence.

We aimed to find out if different clays had different efficiency in absorbing bacteria on their surfaces depending on their physicochemical and mineralogical composition. To this end, we counted the live bacterial cells present in the clay after 20 min of contact with the water and after filtration, which occurred while the SPT was being performed. To better understand whether the counted bacteria were bound to clay particles or whether they were free to move within the sediment matrix, we performed the counts twice: on wet, filtered clay and on filtered clay lightly rinsed with ASW water. We also investigated whether changes in pH could affect absorption counts under different pH conditions ($n = 30$ per replicate).

Filtered contact water for solid-phase tests usually exhibits high turbidity because the clay can penetrate the pore size of the filter. Turbidity shows peaks at the highest concentrations tested and decreases scalarly with dilution. To evaluate this possible degradation of the recorded bioluminescence, the bioluminescence measurement was correlated with the turbidity measurement performed on contact water from the ten clays tested. Measurements were also made at different pH values to determine a correlation between the occurrence of turbidity and the pH of the water. This part of the experiments allows us to better understand the occurrence of false positives related to the properties of the clay and the pH of the water, and to better evaluate the impact of global change.

All experiments were conducted in a series of random statistical replicates as described in the following methods.

2.1.1. Selection of tested clays

The natural clays (C1-C10) used in this study were selected to evaluate the effects of different mineralogical and chemical compositions of natural clays on the ecotoxicity of *A. fischeri* under different pH conditions and to reduce other possible factors for the variability of the observed results. Clays were purchased from different manufacturers; they were selected among those designated as "cosmetic grade" to ensure that they were natural clays originating from geographical areas not polluted by human activities, as they are considered "safe for human use" according to the legislation (CE 1223/2009). Commercial clays were selected for several reasons:

- i) Natural marine sediments are a mixture of clay, sand, silt and gravel with different weight percentages. This study focuses only on the fraction with a particle diameter $< 63 \mu\text{m}$, on clay, and the commercial products represent a good selection of the fraction of particular interest.
- ii) The use of commercial clays makes it possible to obtain natural clays from different geographical areas (which also have different geological origins and corresponding mineralogical compositions).
- iii) Natural marine sediments are a mixture of minerals of natural origin and chemical pollution from human activities; it is not possible to isolate these variables, either when using bulk sediments or when using natural sediments grinded $< 63 \mu\text{m}$, since pollution is also present in non-polluted marine areas. Natural clays allowed us to exclude incidental contamination and focus only on effects related to differences in minerals and chemical composition of the different clays.
- iv) The natural origin of commercial clays did not affect the ability to perform *A. fischeri* SPT, which are suitable for both marine and freshwater sediments and are also applicable to dusts and powders of different origins.

2.1.2. Determination of physical-chemical properties of tested clays

The physical and chemical properties of the studied clays were determined by standardized and normalized methods. Further details of the methodological approach used in this study can be found in the [supplementary materials](#) (Materials and Methods section), including method performances, LOQ, and instrument details.

Physical features of tested clays.

Color was measured using the uniform color spaces defined by CIE (Commission Internationale de l'Éclairage; CIE L*a*b*) in 1976. This method aimed to reduce one of the main problems of the original XYZ color space [13] and allowed to bypass the previously used Munsell color chart [42] and represents the most solid approach used for sediments and incoherent materials [10].

The particle size distribution was measured using two different approaches: A first measurement was made in ASW water with laser diffraction (0.01–10,000 μm ; Malvern, Multisizer 2000) using the instrument settings reported in the literature for sediment particles. The

second approach was to use morphological microscopy (FESEM electron microscope, Zeiss, mod. Merlin- II) to perform measurements on dried dust particles ($n = 30$; randomly selected). These results were used to compare the different behaviour of the tested clays when dispersed in seawater. It was also possible to determine particles in nanoform that could exert significant ecotoxicity on the species tested.

Chemical features of tested clays.

The mineralogical composition of the studied clays was analyzed using an X-ray diffractometer (XRD, Rigaku, Smartlab). Elemental analyses were also performed on clay samples using a high-resolution scanning electron microscope (FESEM, Merlin II, Zeiss) to determine the elemental composition from the micro- to nanoscale. In addition, classical scale chemical determinations focusing on the presence of chemicals at concentrations of $\mu\text{g}/\text{kg}$ and mg/kg were performed on the studied clays to evaluate the trace element content. In the clays (C1-C10), the content of metals (Cd, Co, Cr, Cr- VI, Cu, Hg, Ni, Pb, Sr, Zn), metalloids (As), rare earths (REE; 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). The methods, limits of quantification (LOQ), and list of abbreviations used in this work for the chemical analysis performed on clays are given in the [Supplementary Material \(Table S1\)](#).

2.1.3. Determination of physical-chemical properties of contact water

To better clarify the role of the mineralogical composition of the tested clays under different pH conditions on the properties of the contact water used to perform the ecotoxicological tests, salinity (APAT CNR IRSA 2070 Man 29/03), pH (UNI EN ISO 10523:2012), and temperature were determined using calibrated probes (Hedge, Hanna Instruments) on negative controls and tested water samples. The accuracy of salinity and pH measurements was ± 0.5 g/L and ± 0.2 pH, respectively. Chemical content of trace elements, REE, and carbon content were determined in contact water. Details on methods, limits of quantification (LOQ), and the list of abbreviations used in this work are given in the [supplemental materials \(Table S2\)](#).

2.2. Ecotoxicological analyses on *Aliivibrio fischeri*

Ecotoxicological tests using *A. fischeri* are the most standardized and globally applied approach to assess the ecotoxicity of natural samples, chemical substances, and industrial effluents and allow obtaining robust and meaningful results in both liquid and solid matrices [1,20,40].

Solid phase test. In this study, SPT were performed using artificial seawater (ASW, [54]) as contact water with the tested clays, starting with a concentration of 19.7%. The method for performing the tests was standardized ([30], S11 App 2). With a contact time between ASW and clay of 20 min, 20 μL of bacteria was added and incubated for 10 min at 15 °C in a scalar dilution of the tested clays. At the end of the contact time, a column filter (Test-Tube and Sera-Separa, Bio2011, Ecotox) was used to obtain a solution of the contact water with exposed bacteria on which the biological effects were tested in terms of reduction of natural bioluminescence. A reduction of bacteria is predicted by the standardized method due to the filtration process, and for this reason the bioluminescence of the samples is compared with that of the negative controls obtained with the same methods without the addition of clay (but only of bacteria) to the contact water. The test with *Aliivibrio fischeri* in solid phase was performed at three different pH values: 7.00, 7.50 and 8.10. The pH corrections were performed adding to ASW HCl (1 M) in opportune drops till the desired value occurred before the contact with clays. Results were calculated using equation reported in [Supplementary materials \[30\]](#) and expressed in different scales (natural toxicity, measured toxicity, sample toxicity index) on the wet weight (natural toxicity) and after correction for silt content (measured toxicity, toxicity index) of the samples.

Liquid phase analysis. The test method used for the liquid phase test [54] 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 10^6 cells of Gram-negative marine bacteria 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 μL 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. After 20 min of contact with tested clays, contact water were centrifuged (15 °C, 18,000 rpm for 10 min) and analysed by the *Aliivibrio fischeri* assay in liquid phase, with a screening assay (90% maximum concentration tested) under different pH conditions ($n = 30$).

pH correction. To the solid phase contact water with extraction water at pH 7.00, 1 M NaOH was added to shift the pH to 8.10 to test if toxicity changes in terms of bioluminescence inhibition percentage, which could be due to chemical precipitation of dissolved ions due to pH correction [48].

2.3. Determination of experimental interferences

Possible impairments of the recorded ecotoxicity in solid and liquid phase tests were investigated to evaluate the relative weight of interfering factors during the tests on the obtained results leading to false positives. Furthermore, we also wanted to evaluate the absorption potential of the tested clays on bacterial cells. For this reason, we measured the relationship between the measured bioluminescence and turbidity in the contact water after filtration. In the wet clay, which is waste after filtration during the SPT, we measured the number of live bacteria present, and the percentage of live cells bound to the clay particles relative to the total amount lost during filtration.

Effect of turbidity of filtered contact water on measurements. Turbidity of the contact water was measured in conjunction with bioluminescence at each dilution of the SPT tested. Turbidity was determined by method APAT CNR IRSA 2110 using a spectrophotometer (PEAK Instrument, C-7100S). The results are expressed in formazin units (FTU). Also in this case, the measurements were performed under different pH conditions to evaluate the effects of acidification on this type of interference.

Loss of bacteria during filtration. The fluorescence optical microscope (Nikon; Eclipse, Ci-E M569 E; FITC philtre) was used to observe the filtered wet clays after contact with the bacteria and to determine the number of *Aliivibrio fischeri* that remained attached to the studied clays, based on the fluorescence phenomena induced in the sample. Live cell counts (fluorescent bacteria) were performed with a Burkert's counting chamber using the diagonal counting method with three replicates per sample. Measurements were made for all pH values tested to evaluate the effects of acidification on bacterial binding.

Absorption potential of the tested clays. To determine the number of bacteria that were absorbed onto the clay particles during the contact period, as opposed to bacteria that were trapped in the wet sediment but not absorbed, the wet clay was carefully rinsed with ASW water after the contact period, and then live cells were counted as previously described. Measurements were made for all pH values tested to evaluate the effects of acidification on bacterial binding.

2.4. Statistical analyses

Univariate statistics were performed using Excel® routines or Primer® v. 7.0 software (University of Plymouth UK). Multivariate statistics were performed to determine the significance of observed variability with respect to various factors of interest, such as pH (three levels, fixed). Principal component analyses, multidimensional scaling (*nm*-MDS), one-way Anosim tests (9999 permutations, Kruskal stress

formula) were performed on the Euclidean distance matrix calculated with pretreated (fourth root) and normalised data. Graphical representations were created using the 2D and 3D graphics software Grapher® from Golden Software, LLC.

3. Results

3.1. General features of tested clays

In this paragraph declarations reported in labels by sellers concerning geographical origin of natural clays and mineralogical composition are reported. Furthermore, this chapter summarizes results concerning physical, chemical, and morphological analyses performed on tested clays to define principal characteristics of tested materials. Some more details are reported in [Supplementary materials](#) (par. 2.1. of the supplementary).

3.1.1. Declarations and label composition

The mineralogical composition of the ten clays tested (C1-C10) was determined and compared with the composition indicated on the label of the commercial product. The clays have different compositions and come from three different countries. Seven of the tested clays were collected in Italy (C1–3, C5–8), two in Ukraine (C9–10) and one in France (C4). The studied clays have the following composition: illite (4/10; C1, C4–5, C8), kaolinite (2/10; C7, C9), hectorite (1/10; C2), montmorillonite (1/10; C6), bentonite (1/10; C10), and a mixture of kaolinite and montmorillonite (1/10; C3). An overview of these aspects is shown in [Fig. 1](#).

3.1.2. Morphological and mineralogical results

Color. The results of the colour determination in the investigated clays (CIE L*a*b*) are listed in the [supplementary material](#) ([Table S3](#)). The determined colours are heterogeneous and reflect the different mineralogical composition. The largest distances were observed for clay C10 (black clay), for brown (C2) and reddish (C6) colours, and the maximum distance was measured for both with C7 (white). In contrast, the smallest value of colorimetric distance is that between C2 and C5 (5.1 ΔE^*ab).

X-ray powder diffraction (XRPD). Compared to label claims, X-ray diffraction analyses show the presence of many more minerals. Despite the five minerals indicated for the ten clays studied, the total number of minerals detected by XRD analyses was higher (at least 28). Based on the

analyses performed ([Table S4a,b,c](#) and XRD results in the supplementary), none of the clays studied were found to have the pure composition (one mineral represented at 100%) indicated on the labels. Clay C7 had the lowest number of minerals (two), while C10 the highest (nine). Interestingly, a significant difference was found between the minerals declared by the producer and those identified by XRD: C7 not containing even one of the declared minerals. Some minerals were detected in only one clay type (red characters, [table 4c supplementary material](#)). Quartz, on the other hand, is the most abundant mineral, detected in 8 of the 10 clays (it was not found only in C7–8). TiO₂ in the anatase isoform was detected in C3 and C7, while C4–5 and C6 contain the rutile isoform. Carbon black was contained in C10.

Morphological and elemental analysis. The occurrence of elements varies greatly depending on the clay studied. The studied clays contained an average of 12 ± 3 elements per sample. A total of 17 elements were identified, of which two (Al and Si) were present in all the clays studied. C1 was the clay with the highest number of elements detected. Na, Mn, K, Ca, and Fe were detected in 9 of the 10 clays studied. P (except C3, C7, C10) and Cl (except C7, C9–10) were present in 7/10 of the clays studied. Other elements are occasionally present, such as: Ti ($2.1 \pm 2.0\%$, C3–7, C10); Te (C1); Fl (C1–2); Sr (C1–2 and C9); Ba (C1, C3–5, C7 and C9); S had the lowest relative content (0.003%, C6). With the exception of C6 and C9, the clays contain the same amount of Si, while Fe (46%) is most abundant in sample C6 and Ca (62.4%) in sample C9. C7 was the only clay in which a large proportion of the above elements were not detected, and the clay with the lowest number of elements detected.

The main morphological aspects of C1-C10 clay dusts observed under the FESEM microscope are shown in [Fig. 2 \(C1-C10\)](#). [Fig. 3](#) shows some specific details at higher magnification (C7 and C10 clays). In particular, in C7, the presence of TiO₂ nanoparticles forming needle-shaped lamellae a few nanometers thick was observed. In contrast, the carbon particles in C10 were much larger and of natural origin.

3.1.3. Chemical composition

The results of chemical analyses of clays to determine the content of macronutrients (TC, TOC, TN, TP), metals and metalloids (As, Cd, Co, Cr, Cr VI, Cu, Hg, Ni, Pb, Sr, Zn), and rare earth elements (REE; Gd, Yb, Y, La, Lu, Dy, Er, Eu, Pr, Ho, Nd, Ce, Sm, Th, Te) are given in [Table 1](#). Some important results are summarised here; for a more detailed presentation of the results of the univariate and multivariate statistical studies on these aspects, the presentation in the [Supplementary Material](#)

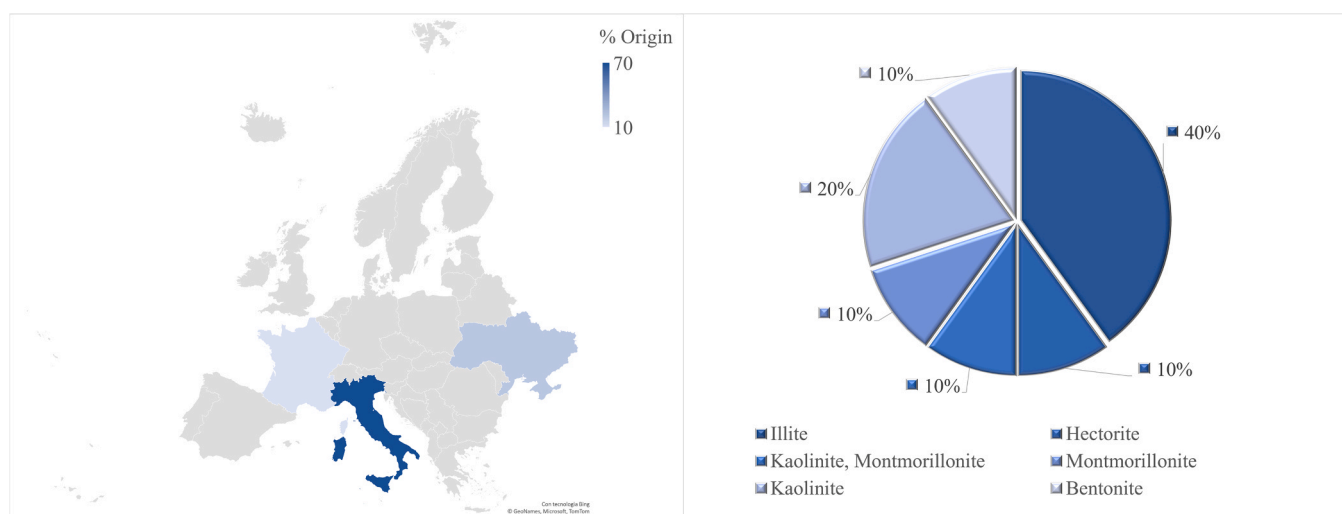


Fig. 1. Geographic origin of the studied clays and indicated mineralogical composition. The origin is given as the percentage of clays originating from the respective country out of the total number of clays studied ($n = 10$). The mineralogical composition of the studied clays indicated on the product labels is given as a percentage of the clays consisting of the indicated mineral.

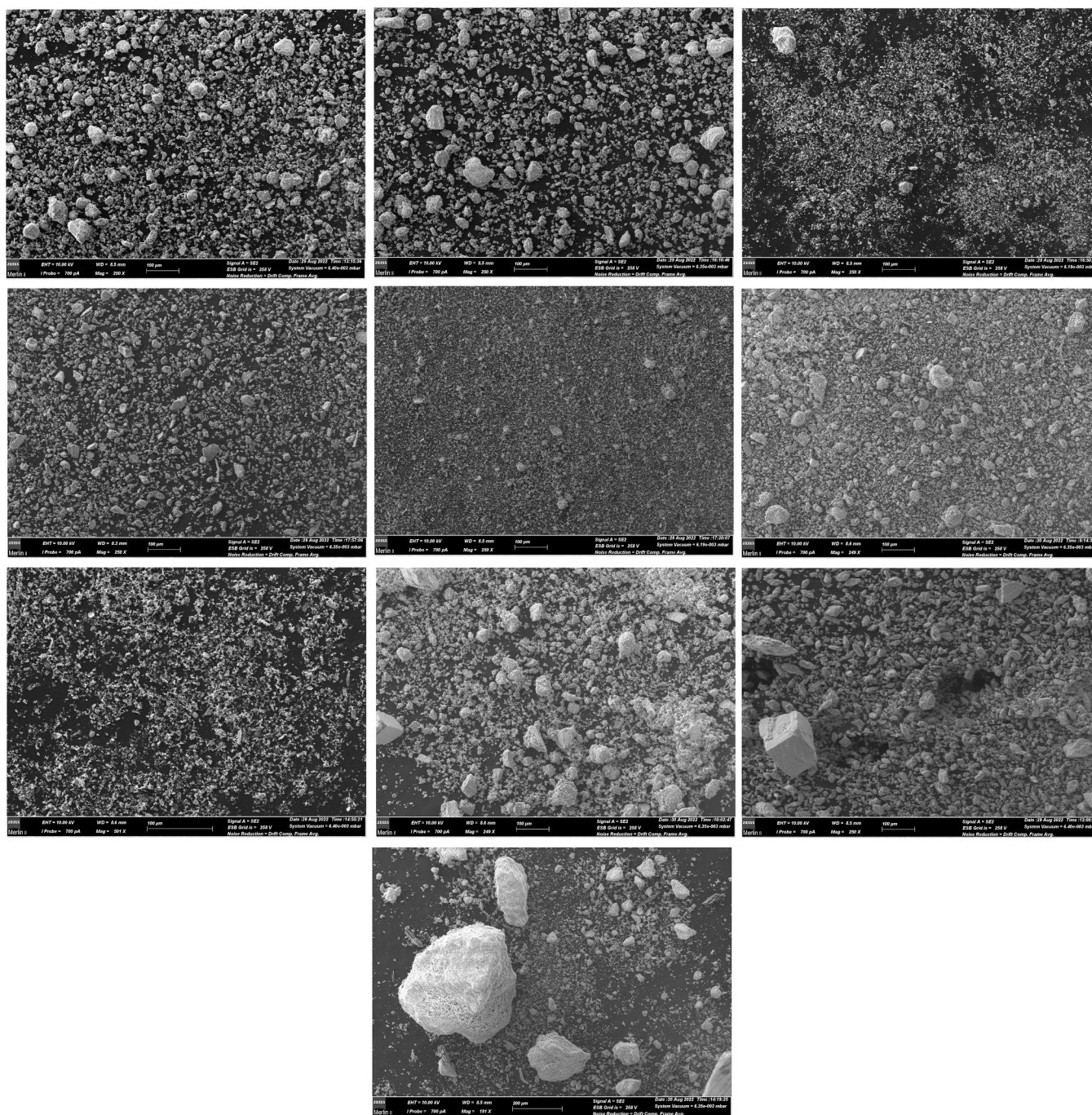


Fig. 2. Morphological structure of the studied clays. FESEM scanning electron micrographs of the studied clays are shown at a similar scale (100 μm for all; 200 μm for C10) to highlight the morphological and ultrastructural features of the studied dried dusts of the considered clays. Figures are reported in a lecture order from C1 (top left) to C10 (down center).

section is recommended.

Macronutrients. TN ($0.04 \pm 0.02\%$), TC ($2.31 \pm 3.46\%$), and TOC ($0.06 \pm 0.03\%$) were measurable in all clays studied, while TP was lower than LOQ (0.05%). TN varied slightly among samples ($\pm 0.02\%$; minimum of 0.01% in C8–9; maximum of 0.06% in C2, C4–5). Small differences between clays were also observed in TOC ($\pm 0.03\%$; minimum of 0.05% in C1, C3–4, C6–10; maximum 0.14% in C2). Otherwise, TC showed the greatest variability ($\pm 3.46\%$; minimum of 0.05% in C3–4, C7; maximum of 9.87% in C9). Fig. S1 shows the ternary plot of the macronutrient composition of the studied clays. The highest TC contents were found in samples C9–10: 9.87% (C9) and 6.90% (C10). The contents of TN were similar in these clays (0.05%). Atomic ratios

and Pearson correlation matrix were calculated to evaluate the origin of organic matter and correlations between macronutrient pairs (Table S5a). The mean atomic TC / TN ratio was 2.72 ± 1.74 (0.97, C4; -5.83, C8–9). TOC was positively correlated with TN (0.637), while correlations between TC and TN were not reported because TP was always lower than LOQ and correlations could not be calculated.

Metals and metalloids. Two elements (Cr VI, and Hg) were always below the limit of quantification. In C7, the lowest concentrations were found for almost all measured elements. In C2, the highest concentrations of the elements studied were found for Cr (30.9 mg/kg), Cu (21.7 mg/kg), and Sr (4856 mg/kg). Cd showed the least variability (± 0.06 mg/kg, $n = 10$); in contrast, Sr varied greatly (± 2175.1 mg/

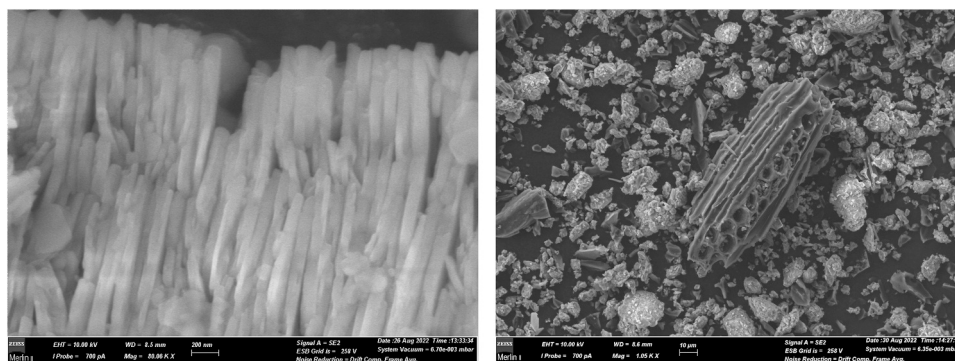


Fig. 3. Morphological details of C7-C10 clays. FESEM scanning electron micrographs of C7 (left) and C10 (right). The high-resolution image shows anatase formation and its crystal structure in C7, while a carbon structure of biological origin is seen in C10.

Table 1

Dimensional size class in ASW. Data are expressed in micrometers and reported as minimum, maximum, mean and standard deviation measured in water dispersions.

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
Min	0.01	0.02	0.01	0.11	0.01	0.02	0.10	0.02	0.09	0.03
Max	27.54	23.48	15.11	20.14	20.90	24.58	15.10	24.32	20.91	21.65
Mean	4.17	4.00	4.17	3.45	4.17	4.00	3.70	4.00	3.85	3.57
DS	6.01	5.21	5.04	4.38	4.96	5.27	4.48	5.18	4.43	4.67

kg, with maximum values in C2 and lowest values in the other 6/10 clays). The element with the highest concentration in almost 60% of the studied clays was Zn. The atomic Cr/Ni ratio had a mean value of 1.72 ± 1.02 and ranged from a minimum value of 0.48 (C4) to a maximum value of 4.04 (C8). The measured metals and metalloids were not strictly correlated with each other or with macronutrients (> 0.900) (Table S5a). Nevertheless, lower significant correlations were found

between the following pairs with TN: -Cd (0.834), -Cr (0.705), -Ni (0.807), -Zn (0.899). TOC was positively correlated with Cd (0.833), Cr (0.747), Cu (0.735), Sr (0.791). As was positively correlated with Co (0.824), Cr (0.736), Ni (0.810); while Cd was correlated with Ni (0.772), Zn (0.802). Co correlated with Cr (0.718), Ni (0.815), Zn (0.717); Cr correlated with Ni (0.853), Cu (0.803); while Ni was correlated with Zn (0.788).

Table 2

Chemical analyses performed on tested clays. Data are reported per each tested chemicals as levels measured in tested clays, minimum, maximum, mean values and standard deviation (SD); nc = not calculable.

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	Min	Max	Mean	SD	
TC	%	0.18	1.77	0.05	0.05	3.61	0.40	0.05	0.24	9.87	6.90	0.05	9.87	1.8	3.25
TOC	%	0.05	0.14	0.05	0.05	0.11	0.05	0.05	0.05	0.05	0.05	0.14	0.07	0.07	0.03
TN	%	0.04	0.06	0.02	0.06	0.06	0.03	0.03	0.01	0.01	0.04	0.01	0.06	0.04	0.02
TP	%	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	Nc
As	mg/kg	1.50	5.38	1.12	6.30	3.75	0.50	1.18	0.50	4.23	5.71	0.50	6.30	3.02	2.30
Cd	mg/kg	0.05	0.16	0.05	0.14	0.17	0.05	0.05	0.05	0.05	0.05	0.05	0.17	0.08	0.05
Co	mg/kg	3.20	8.09	2.31	13.00	5.91	4.03	0.50	1.24	2.18	15.50	0.50	15.50	5.60	5.11
Cr	mg/kg	2.79	30.90	4.70	12.10	25.10	7.05	1.02	1.79	6.09	27.50	1.02	30.90	11.90	11.51
CrVI	mg/kg	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	Nc
Cu	mg/kg	12.70	21.70	11.90	11.00	20.10	16.70	5.61	11.20	6.21	16.80	5.61	21.70	13.39	5.39
Hg	mg/kg	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	Nc
Ni	mg/kg	2.99	21.40	2.48	28.50	35.10	3.60	0.50	0.50	4.57	28.90	0.50	35.10	12.85	13.88
Pb	mg/kg	14.70	11.90	2.80	4.26	7.19	3.17	1.55	3.44	1.60	20.30	1.55	20.30	7.09	6.42
Sr	mg/kg	< 0.5	4856.0	< 0.5	< 0.5	135.1	< 0.5	< 0.5	< 0.5	421.3	115.7	< 0.5	4856.0	553.1	1517.7
Zn	mg/kg	39.20	61.40	20.40	80.70	54.10	28.40	9.07	17.50	11.30	37.70	9.07	80.70	35.98	23.48
Ce	mg/kg	68.8	8.2	67.9	63.5	25.9	20.1	87.6	46.7	3.5	61.6	3.5	87.6	45.4	29.0
Dy	mg/kg	3.0	0.5	2.5	40.3	3.7	1.9	2.7	4.2	0.3	2.6	0.3	40.3	6.2	12.1
Er	mg/kg	1.4	0.3	0.7	25.5	2.1	1.1	0.6	2.3	0.2	1.3	0.2	25.5	3.6	7.7
Eu	mg/kg	1.2	0.2	1.6	5.9	0.8	0.6	1.7	0.9	0.1	1.1	0.1	5.9	1.4	1.7
Gd	mg/kg	4.5	0.8	5.2	33.8	4.1	2.2	6.6	5.1	0.4	4.2	0.4	33.8	6.7	9.7
Ho	mg/kg	0.5	0.1	0.3	8.7	0.7	0.4	0.3	0.8	0.1	0.5	0.1	8.7	1.2	2.6
La	mg/kg	35.3	3.9	32.4	16.2	12.2	9.0	42.2	22.3	1.9	25.9	1.9	42.2	20.1	13.8
Lu	mg/kg	0.2	0.1	0.1	2.8	0.2	0.2	0.1	0.3	0.1	0.1	0.1	2.8	0.4	0.9
Nd	mg/kg	27.7	4.0	30.6	53.6	14.4	10.2	38.5	22.7	2.0	23.8	2.0	53.6	22.8	16.0
Pr	mg/kg	7.5	1.0	7.7	9.0	3.3	2.4	9.9	5.5	0.5	6.2	0.5	9.9	5.3	3.3
Sm	mg/kg	4.9	0.8	6.0	20.0	3.4	2.2	7.5	4.8	0.4	4.5	0.4	20.0	5.5	5.6
Sc	mg/kg	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	nc
Tb	mg/kg	0.6	0.1	0.6	6.0	0.6	0.3	0.7	0.7	0.1	0.5	0.1	6.0	1.0	1.8
Th	mg/kg	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	nc
Tm	mg/kg	0.2	0.1	0.1	3.3	0.3	0.2	0.1	0.3	0.1	0.2	0.1	3.3	0.5	1.0
Yb	mg/kg	1.2	0.2	0.4	19.5	1.6	1.0	0.2	2.0	0.1	1.0	0.1	19.5	2.7	5.9
Y	mg/kg	12.8	2.4	6.4	419.8	28.7	10.3	6.7	21.4	1.6	11.0	1.6	419.8	52.1	129.5

Rare earths elements (REE). Sc and Th were never detected in the clays studied. Lu had the lowest values with a mean of 0.41 ± 0.85 mg/kg (min. 0.05 mg/kg, C2, C7, C9; max. 2.80 mg/kg, C4). Ce was measured in the range of minimum values of 3.50–87.60 mg/kg in all the clays studied, with the highest values recorded in C7 and the lowest in C9. Clay C7 had the highest levels of La, Pr, and Ce, while almost all other REE were highest in C4. In contrast, almost all REE had the lowest values in C9. Yttrium content was high in clay C4, reaching the absolute maximum (419.80 mg/kg). Levels of REE (Gd, Yb, Y, Lu, Dy, Er, Eu, Ho, Sm, Th, Te) showed a strong positive correlation ranging from 0.90 to 1.00 (Table S5b). La was correlated only with Ce (0.94), there were no other correlations. Pr was strongly correlated (> 0.94) with Ne and Ce and less strongly with Eu (0.65) and Sm (0.72). Ne was positively correlated with all other REE, but with lower significant levels ranging between 0.65 (La) and 0.94 (Pr). Ce correlated only with La, Pr, and Ne.

3.1.4. Particles size dimensions

Determination in ASW. The results are given in Table 2 as mean, minimum, maximum, and standard deviation (the ternary distribution is shown in Fig. S2 in the supplementary material). In the ASW dispersions, the mean value of the studied clays is $4 \mu\text{m}$, which is the correct limit for the dimensions of the clays. C4 showed the smallest mean value and C1, C3, C5 the largest. The maximum values were in the silt range ($4\text{--}63 \mu\text{m}$), but always below $30 \mu\text{m}$. A large part of the dimensional curve was in the nanodimensional range with minimum values less than 100 nm and in some cases up to 10 nm for almost all the clays studied.

Particle sizes in dried dusts. The mean overall size of the dried dusts was larger ($5.06 \pm 1.21 \mu\text{m}$) than that of the dispersions in ASW. The size ranged from an absolute minimum of 0.58 (C10) to a maximum of $24.67 \mu\text{m}$ (C9). The percentages of each size class are shown in the supplementary (Fig. S3) with a specific description of the observed deviations. Regarding the particle size recorded in the dried dusts, a slightly significant correlation was observed between the particle size distribution and the presence of anatase (TiO_2) in the studied clays (Anosim test one-sided, 9999 permutations, sample statistic (R): 0.276; significance level of sample statistic: 13.3%; number of permuted statistics greater than or equal to R: 6).

3.2. Ecotoxicological effects

Ecotoxicological results of tests with *Aliivibrio fischeri* (SPT, liquid phase test) conducted on clays under standard (8.1) and acidified pH conditions (7.50, 7.00) are reported. Further data can be found in the Supplementary, Section 2., par. 2.2.

Solid phase test. In the SPT, the natural toxicity of clays is associated with any variation in pH and moisture conditions (i.e., dry weight or wet weight), and the value uniformly applicable to all clays tested was 273.09 TU (from 100% silt in Equation 1, Supplementary Material). The results of TU, measured at different pH values tested, are shown in Fig. 4 (a,b,c) and compared with the natural baseline of toxicity.

Considering the mean toxicity measured on the tested clays ($n = 10$), the results are as follows (TU, expressed in dry weight): 537.2 ± 338.2 (pH 7.00); 161.9 ± 82.6 (pH 7.50), and 173.6 ± 110.0 (pH 8.10). Lower and significantly different values were found when calculating wet weight (see Appendix, Section 2.2.).

Significant differences were found when comparing toxicity expressed as dry weight and wet weight at different pH values ($p = 0.001$ for pH 7.00; 8.10; and $p = 0.0001$ for pH 7.50). The absolute lowest value of 11.3 TU (range 6.2–20.5) was obtained for C9 (wet weight and pH = 7.50), while the highest value of 1064.5 TU (range 821.8–1377.1) was obtained for C7 (dry weight and pH = 7.00).

When testing clays at 8.10 (standard conditions, dry weight), the measured toxicity showed a maximum value of 392.5 TU (C5; range 264.5–582.6 TU). The minimum value of measured toxicity was 76.1 TU (C1; range 60.5–95.7 TU), as shown in Fig. 4a. Under these conditions, the measured TU value of C5 and C6 was above the natural threshold for

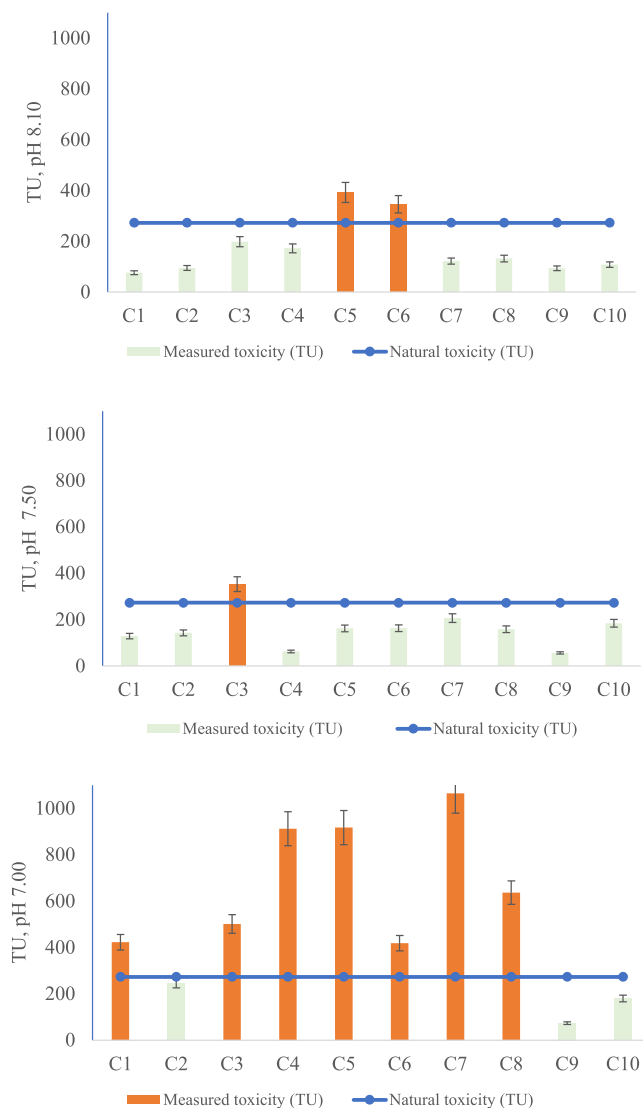


Fig. 4. Toxic unit (TU) measured in the tested C1-C10 clays under different pH conditions. In particular, a) pH = 8.10; b) pH = 7.50; c) pH = 7.00. The blue line represents the natural toxicity threshold (NTT) determined by the applied method for a sediment composed of 100% silt. Error bars are expressed as percent deviation from TU between two replicates analysed in the context of repeatability.

toxicity of 100% silt sediments, while all other tested clays were less toxic, indicating a significant importance of the water content of the samples for the recorded toxicity data.

Testing of clays under different pH conditions (Fig. 4b,c) showed that:

1. at pH 7.50, the maximum value was 353.1 TU (C3, range 190.8–653.4 TU). The minimum value was 56.3 TU (C9, range 30.9–102.5 TU). Interestingly, C5 and C6 TU were below the natural toxicity threshold at this pH, while C3 increased significantly.
2. at pH 7.00, the greatest variability between samples was observed (± 338.2 TU) with a maximum of 1064.5 TU (C7; range 821.8–1377.1 TU). The minimum value was 73.7 TU (C9; range 57.9–93.7 TU). C7 clay had a maximum value of 212.9 TU (range 164.4–275.4 TU), while the minimum value was 14.7 TU (C9; range 11.6–18.7 TU). Lower pH values resulted in a significant increase in clay toxicity; almost all clays tested were more toxic than the natural threshold for sludge toxicity, with C7 reaching the highest value of 1000 TU. C4 and C5 again become toxic, and only a few exceptions

reported in Fig. 6. In all clays, at both pH, living bioluminescent emitting bacteria were found. Results showed a marked both clay type and pH dependency. In fact, the highest cell counts at pH 7.00 were $1.66 \cdot 10^4 \pm 3.54 \cdot 10^2$ cells/7 g (C5) and $1.89 \cdot 10^4 \pm 3.11 \cdot 10^2$ cells/7 g (C6). At pH 8.10, maxima levels of bacteria recovered were $1.14\text{--}1.70 \cdot 10^4 \pm 1.13 \cdot 10^3$ cells/7 g (C4–6). The minimum value, however, at both pH, was found in sample C10 ($4.95 \cdot 10^3 \pm 5.89 \cdot 10^2$ cells/7 g at pH 7.00, and $6.57 \cdot 10^3 \pm 9.43 \cdot 10^1$ cells/7 g at pH 8.10). The pH dependent behavior resulted clay type dependent. In general, samples C1–8 showed a decrease in the number of cells trapped in the solid phase of tested clays at pH 8.10 compared to pH 7.00. Clays C3–C7 remained similar while, C9–10, showed an opposite behavior, decreasing the trapped cells in clays at pH 7.00 compared to the number

of cells trapped at pH 8.10. Statistically significant differences (T-test, $p < 0.05$) between pH 7.00 and pH 8.10 are reported for the clay type tested.

Absorption potential of the tested clays. Fig. 6 shows the living enclosed cells on clays compared to the total number of cells. As can be seen from the figure, the percentage of cells bounded by clays is almost a half less than the total number of cells counted (respectively on average 1.3 times at pH 7.00 and 1.5 times at pH 8.10); nevertheless, the trends observed for the total number of live cells remain supporting that absorption by clay is occurring and determining the recorded behaviour on the whole amount.

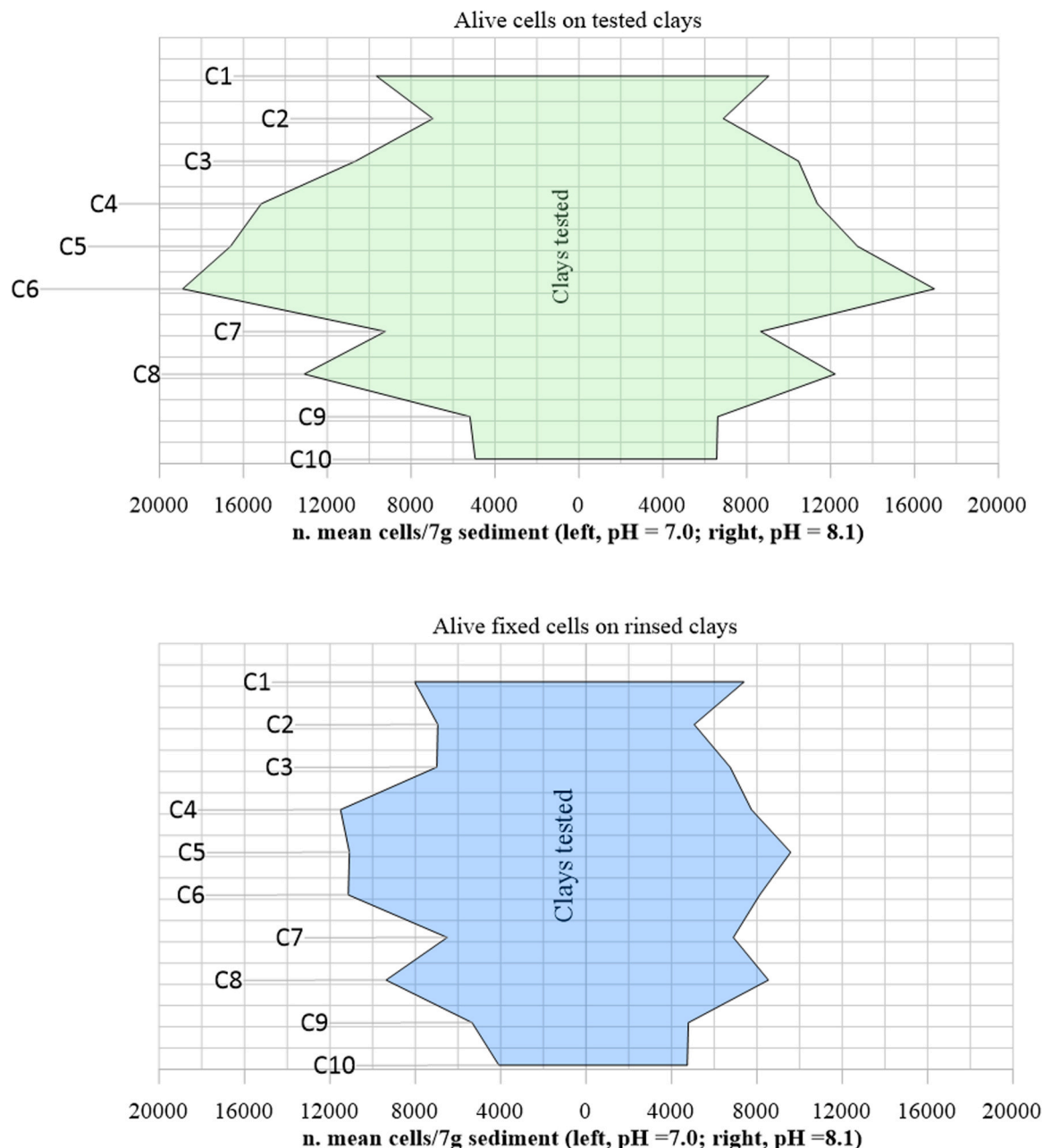


Fig. 6. Absorption of living cells on the clay surface. These graphs summarise the average number of cells/7 g of wet clay recorded in the solid phase test after filtration. The cells counted were alive (fluorescent), indicating a false positive effect due to matrix absorption of bacteria under the two extreme pH conditions tested: pH 7.00 (left side of graph) and pH 8.10 (right side of graph). The counts are given separately for all C1–C10 clays tested. The upper part of the graph (green) shows the mean cell count on filtered and wet analysed clays (total live bacteria both fixed to the matrix and unfixed inside the clay). The lower part of the graph (blue) shows the mean cell count on filtered and gently ASW-rinsed clays (representing living bacteria that are fixed to the clay surface).

4. Discussion

4.1. Species sensitivity

Aliivibrio fischeri is a well-standardized species commonly used for ecotoxicological studies in marine and freshwater ecosystems, both in water (UNI EN ISO 11348-3/1) and in the solid phase of sediments [30]. The widespread use of this species is well documented in the literature due to its versatility in application in different matrices, ease of implementation, and cost-effectiveness of this technique [1]. Studies have reported that ecotoxicity is mainly due to enzymatic changes leading to a decrease in natural bioluminescence in a kind of "enzymatic shutdown" rather than cell death [59]. As a result, cells are alive 15–30 min after exposure, but bioluminescence is not detected. The species tested is very sensitive to contamination by metals and metalloids [24,38,59]. With respect to trace elements such as metals and metalloids, recent studies have shown that *A. fischeri* is variably sensitive to the species tested, with decreasing sensitivity reported: Mercury > Lead > Cadmium > Arsenic [31]. In addition, the presence of other cofactors such as humic acids may increase the toxicity of some elements (e.g., lead) and decrease that of others (e.g., copper; [52]). The organic carbon (TOC) detected in this study is very low and comparable to that of C1–10 clay, and the organic content of the clays studied could affect the measured toxicity.

4.2. Physical, chemical, and mineralogical features affecting ecotoxicity

Color. Sediment color is of great ecological importance in marine ecosystems because it affects the thermal properties of sediments [27] and impacts the reproductive success of some intertidal species, such as horseshoe crabs [4], sea turtle nests [27], and the sex ratio of sea turtles breeding near shore [17]. In this study, the clays examined differed significantly in color, ranging from white (C7) to dark gray (C10). It has been reported that watercolor can negatively affect ecotoxicological results of *A. fischeri* liquid tests due to methodological interference between color and bioluminescence citations (UNI EN ISO 11348-1). However, no correlation between solid phase color and ecotoxicological effects is reported in the literature [30], and this factor is not considered. However, the correlations between the colors of the clays and the observed ecotoxicity are also related to the different mineralogical composition of the studied clays and, therefore, could be indirectly related to ecotoxicity. White colors may be associated with the presence of gypsum or TiO₂, while black color may be due to the presence of iron oxide, coal, or sulfur-containing minerals such as hematite and pyrite, or to the presence of phyllosilicates, while green colors are usually associated with chlorites.

Mineralogical composition. The mineralogical composition indicated by the clay seller on the product labels consisted in almost all cases of single natural minerals (illite C1, C4–5, C8, kaolinite C3, C7, C9, hectorite C2, montmorillonite C6, bentonite C10), except for C3, which was indicated as a mixture of kaolinite and montmorillonite. However, the actual mineralogical composition of the investigated clays differs considerably from the suppliers' data. The mineralogical composition determined by XRD analyses revealed 28 different minerals compared to the 5 indicated by the supplier. This aspect was relevant to the ecotoxicological effects of the clays studied, since illite, which was indicated by the suppliers in 4/10 of the clays studied, was detected in relevant concentrations in only one of these clays (C5) and in one clay (C10) in which it was not indicated. In C10, the bentonite indicated was not detected, but illite, kaolinite, montmorillonite, and vermiculite were determined. The hectorite indicated in C2 was not detected, but kaolinite and vermiculite were detected. The kaolinite indicated in C3, C7, and C9 was not detected, but was detected in C1–2, C4, and C10. The montmorillonite indicated in C3 and C6 was detected only in C1 and C10. It is interesting to note that no clayey minerals were detected in C7, and the indicated kaolinite was not present.

These results indicate that more detailed analyses should be performed (and shall be required by the law) prior to marketing to ensure consistency between the declared minerals and those present in commercial products, and to avoid possible effects on consumers due to the presence of large amounts of undeclared constituents (e.g., anatase).

Silicates are the major constituents of the Earth's crust [32,41]. Al-Si combines with oxygen to form a variety of aluminosilicate minerals such as kaolinite, muscovite, amphibole, pyrophyllite, mullite, zeolites, and feldspars [41]. Quartz, on the other hand, is the most abundant mineral and was detected in eight of the ten clay samples examined. It should be noted that quartz was not found in C7–8. Clinocllore (C5) is a silicate that belongs to the chlorite group and contains Mg and Fe in ionic form in its structure. Albite (C10) is a feldspar mineral, a tectosilicate of Al and Na (NaAlSi₃O₈); apatite Ca₅(PO₄)₃[F, OH, Cl] (C1) is a Ca-based phosphate mineral, while graphite (C10) is a carbon-based mineral.

Clay minerals can have amorphous or crystalline forms. According to Uddin [55], who summarized Grim's 1962 classification, crystalline clays can be categorized based on disposition of the tetrahedral (T) and octahedral (O) sheet in the kaolinite like structure (1:1 of tetrahedral and octahedral layers), smectite group (2:1 of tetrahedral and octahedral layers) and chlorite (2:1:1 of tetrahedral-octahedral and octahedral layers). Moreover, these different kinds of clay layers should be alternate with each other generating mixed-layer clay minerals. Vermiculite and smectite (smectite group) have the highest capacity of cation exchange. Indeed, it is commonly observed the substitution of the Si⁴⁺ ions by Al³⁺ ions in vermiculite, with the exchangeable cations that compensate the negative charge excess.

Crystalline structure and diagenetic origin. Clays composition differ worldwide according to their origin: volcanic originated clays include alteration haloes consisting of mixtures of muscovite (-K-bearing mica), biotite (Fe, Mg bearing mica) pyrophyllite and chlorite (Mg, Fe, Mn, Ni bearing phyllosilicates). Also, quartz, pyrite, albite, and epidote are associated to this type of clays [62]. The presence of TiO₂ at relevant percentages was documented in montmorillonite (0.52%; [15]), kaolinite clay (0.10%, [33]), illitic clay (0.82%, [26]), dolomite (0.02%, [25]), while vermiculite [39], kaolin [12], hectorite [45], resulted absent. It is interesting to underline that the surface charges of clay minerals are dependent on the pH of external conditions. Alternating Tetrahedral and octahedral sheets forming planar structures show different behavior when exposed to water; in particular, the structure characterized by a T:O ratio of 1:1 has zero equilibrium between negative and positive charges [8]. In the space between repeating T:O:T structures there are charge compensating cation like Na, K other than water. In the chlorite group, water layers are larger and the presence of octahedral metals (Al³⁺, Mg²⁺, Fe²⁺, Fe³⁺) is common and affects both mineral density and weight H₂O + OH⁻ [62]. The different mineralogical composition of the studied clays and the different behavior in terms of ion/layer exchange between clays and contact water under standard pH conditions could be a crucial aspect to better understand the results of this study. It is interesting to observe that C7 is characterized by the presence of 1:1 no expandable kaolinite; according to the literature [62], kaolinite is originated in early part of the diagenetic process under low temperature (about 0–100 °C); on the contrary chlorite is originated in the epizone (over than 300 °C). For the reasons presented, different origins of clays could lead to different behavior in the uptake and subsequent release of ions and chemicals. Therefore, a better understanding of these aspects could be the key to a better understanding of the factors affecting the ecotoxicity of clays and the changes in ecotoxicity under different environmental conditions.

Expandability. Water content and the capability of structural minerals to associate water and expand their crystal structure, could be associated to different ecotoxicological behavior. The layer type of tested minerals and associated no, low, or high expandability could explain the water amount linked to the dry weight unit of tested clays. This means a significant difference concerning the number of exchangeable ions from the mineralization towards the water column as well as kaolinite is reported

to have no or low expandability, its polytypes, the halloysite is reported to occur in its hydrated form in various sediments [62]. Among minerals, 2:1 phyllosilicate (i.e., illite, muscovite, biotite, illite-smectite, and mixed mineral layers), are characterized by high densities (2.6–2.9 g/cm³) and the lowest hydrous content [62]. Different clay/water interactions when testing ecotoxicity of clays in water environments could produce increases or decreased of measured ecotoxicity as consequence of this type of interaction.

Grain-size. Particle size significantly affects ecotoxicity for several reasons. First, it affects the surface area exposed to water exchange per gram of clay particle. Anselmi and colleagues (2023) reported a strong correlation between the levels of metals, metalloids, and organic pollutants measured in natural, unpolluted sediments and their particle size. The higher the silt content, the more chemicals were absorbed by sediment particles. Kahle et al., [34] showed that the number of cations bound to siliceous clay minerals and the surface area size of the siliceous minerals were important for macronutrient storage potential. In addition, nanoscale materials exhibited different physical-chemical and mechanical behavior than bulk materials, and particle size and crystal shape were found to be important cofactors that can affect the dissolution of nanoparticles in water and their ecotoxicity [46] as well as the behavior of nano-TiO₂ [5]. The clays investigated in this study showed significantly different particle sizes when particle size was measured in dried dusts or in ASW. Dusts have a larger particle size than particles dispersed in water, and clays dispersed in ASW were on average smaller than 4 µm in almost all C1–10 samples studied (C1–3 was an exception, C5–6 yielded the size limit of 4 µm). Only two of the measured dusts could be correctly classified as clays by any type of measurement (C7 and C10). The presence of nanoparticles was detected in a measurable amount. This aspect suggests that a water dispersion might have a different mechanical behavior than dried dusts.

Mineralization types. As reported in this study, a large proportion of natural clays contain a relevant amount of TiO₂; however, C7 and C3 have been shown to contain a highly toxic and potent form of TiO₂ as well as anatase, while C4–6 and C10 contain the less toxic form (rutile). Titanium dioxide is used as a food additive (E171) and has been shown to be toxic to both isoforms [21]. Among TiO₂ crystals, the rutile isoform (C4–6) is considered by some research less toxic than the anatase one (C3, C7). Nevertheless, the ecotoxicity of nanoparticle isoforms of the TiO₂ throughout in vitro and in vivo in gastrointestinal experiments, are reported by the literature [6]. Nanoparticle toxicity was explored by the literature and some research reported contrasting data on toxicity on nano-TiO₂ on *A. fischeri* [3,28]. This behavior was also supported by a recent review [46] that highlighted as many factors can affect nanoparticles toxicity on aquatic species such as particle size, pretreatments, water features such as salinity and the content of humic acids or TOC. A recent paper of Tsidis et al. [53] showed that nanoparticles are toxic but the hydrophilic and hydrophobic conditions significantly affect results. The Anatase crystals of TiO₂ observed in this study are structurally like flat crystals that flake off forming needle-like structures as reported in Fig. 5. This occurrence reported for C7 clay could explain the major ecotoxicity observed in this study as toxic needle-shaped particles could exert both chemical and physical effects on exposed cells. On the contrary, biobased carbon was recorded in C10 (Fig. 5) that is the only one among tested clays that contains graphite.

Metals, metalloids and REE content. The origin of the studied clays, the mineralization process and the mineral production could significantly influence the impurities and the proportion of other minerals associated with the clays. As highlighted in this study, the composition of the investigated C1–10 clays differs greatly from the information on the clay labels. This aspect is important in terms of their ecotoxicity. Depending on the various impurities associated with the clay, metals, metalloids, trace metals, and rare earths could be present in relevant concentrations and in a chemical form that could be soluble and bioavailable to marine animals depending on the pH of the water. Chemical analyses of clay dusts revealed the presence of large amounts

of elements (17; average 12 ± 3 elements); Al and Si are the most frequently recovered elements, which is due to the presence of Al silicates in clays. The detected toxicity does not correlate with the number of elements detected in clay dusts, since C7, which is the most toxic in terms of absolute TU values, has the lowest number of trace elements. The presence of macronutrients (i.e., TOC) is associated with natural clays and is positively correlated with metals and organic pollutants ([2] and [supplementary materials](#)). In addition, TOC may contain humic and fulvic acids, which may affect the ecotoxicological response of *A. fischeri* [52]. In clay C1–10, the values of TN and TOC were low and similar, while the values of TC were more widely separated, indicating a significant difference in inorganic carbonate content, with C7 having the lowest value. In contrast, C10 had the higher TC content (6.90%) associated with the lowest toxicity compared to the other clays and C7 (TU), indicating that the carbonate and carbon content in this case "dilutes" the total amount of chemical pollution detected per gramme of clay by the presence of the highest amount of an ecotoxicologically inert mineral (i.e., carbonate) per unit weight of clay. As for the measured contents of metals and metalloids, the results in the studied clays are considered low and below the threshold of environmental risk according to the Italian law on the risks related to the dredging of marine sediments [2,37]. Relative maximum values were found in C10 (40% of the measured elements gave the maximum value), C2 (30%), C4 and C5 (20%). Some metals were below the detection limit in all the clays studied (Cr- VI; Hg).

Recent research has shown that 2:1 clay mineral such as montmorillonite and illite also efficiently lead to the absorption of REE; the absorption performance of halloysite and kaolinite has also been reported [58]. Kaolinite and illite can trap all REEs investigated in this study at pH between 4 and 7 in their structure; nevertheless, Yang et al. [58] reported that the sorption capacity of REEs in both clays increases with pH (3.0–7.5) and the associated increasing ability of clay surfaces to bind REEs. Almost all the clays examined in this study contained illite and kaolinite and had high REE contents, although only C1, C4, and C10 contained both mineral types. The high REE content in C4 could be related to the highest concentration of these specific minerals. Some REEs had high concentrations in C7 (Ce, La, Nd, Pr), while high Sr contents were recorded in C2 (4856 mg/kg). The observed strong correlation between REEs is related to the kaolinite and illite content of the studied clays; however, some elements are not correlated with others, such as Ce, La and Pr, which are correlated only with themselves.

For the reasons presented herein, and based on the results of this study, the morphological, mineralogical, and chemical properties of the tested clays, as determined by the microanalyses performed in this study, may affect the ecotoxicological response during the monitoring study and should be carefully considered in environmental monitoring and the use of *A. fischeri* to evaluate the ecotoxicity of natural sediment samples to properly weight their effects on the responses obtained.

4.3. Ecotoxicity: standard and acidified conditions, solid-phase test

Standard pH conditions. The results of the samples tested were always within the acceptance range for the species used in this experiment (*A. fischeri*, UNI EN ISO 11348–1). Under standard marine pH conditions (8,10), the results of this study showed that only C5–6 clays exhibited toxicity above the natural limits proposed by ISPRA [30]. Based on the S.T.I. approach and the determined TU values, C5–6 were classified in the "low toxicity" category. The clays that showed lower toxicity compared to the others showed the presence of rutile and vermiculite as mineralogical formations. The mineralogical and chemical analyses performed on the clays are not statistically related to the toxicity observed.

Acidified conditions. On average, acidification has significant effects on observed ecotoxicity ($p < 0.01$, T-test). The relationship between the values of TU and pH depends on the clay type. At a pH of 7.50, C3 becomes more toxic than under standard pH conditions, reaching levels above the natural thresholds defined by ISPRA [30], while all other clays

tested were non-toxic, including C5–6, for which TU were higher under standard pH conditions. At pH 7.00, almost all the clays tested (except C2, C9–10) proved effective, and TU exceeded the thresholds defined for natural conditions [30]. In addition, clay C7 proved to be the most toxic of all the clays tested under these conditions. The observed differences were statistically significant ($p < 0.01$, t-test). Interestingly, the toxicity of C2 increased with decreasing pH, resulting always below the natural thresholds under all conditions tested. C9–10, on the other hand, remained stable, and the values of TU did not vary with decreasing pH. This behavior could be due to the presence of a large amount and variety of minerals associated with clay in C10, which ensure a limited release of absorbed elements of ecotoxicological interest. In addition, the presence of very high values of TC is associated with the presence of inorganic carbon (carbonates, C9–10) and the presence of minerals such as biotite (C9). In addition, the presence of calcite (C2, C9–10), dolomite (C2, C9), fluorendenite (C2), and graphite minerals in C10, as also demonstrated by FESEM analyzes, could be responsible for the lower toxicity observed under both natural and acidified conditions. These minerals act as diluents for the total amount of impurities, and it is known in the literature that some of them, such as carbon (C10), can absorb metals and metalloids with a strong chelating effect [56]. Otherwise, the increasing toxicity observed for C3 and C7 could be due to the reduced absorption activity of these clays for contaminants at acidified pH conditions, which is related to the absence of clay-associated minerals that have high surface absorption activity for cations (i.e., illite, bentonite, kaolinite, montmorillonite). In addition, recorded behavior, may be related to the presence of nanoparticles of TiO_2 in anatase form, which is reported to be the most toxic form of this mineral [6]. Literature reports that anatase can cause oxidative stress, NP degradation, and toxic effects at lower pH values [16]. C3 and C7 clays are characterized by the presence of mullite. A recent study shows that mullite eluates are cytotoxic and genotoxic to mouse fibroblast cells depending on particle size, concentration, and composition of the mineralization [50]. Studies on the ecotoxicity of fly ash show that mullite is associated with ecotoxicity to *A. fischeri* in the samples studied when contact water is used at pH 7.5 [51]. The higher toxicity was observed for C7 at pH 7.00, resulting in a STI > 3.00 classified as "toxic" [30]. This could also be exacerbated by the low quartz content in addition to the factors mentioned above. Quartz is an ecotoxicologically inert mineral that in practice acts by diluting (by weight) the total amount of minerals that could release toxic elements under lower pH conditions.

4.4. Ecotoxicity: standard and acidified conditions, contact water

Effects of pH on the release of pollutants from clays. In this study, the release of metals, metalloids, and REE from the studied clays towards the contact water was documented. Of all the elements studied, some metals such as Al, Ba (0.247 mg/L) were released from C7 under the pH conditions 7.00. Furthermore, considering REE, in C7, the release of europium (1.05 $\mu\text{g/L}$, pH 7.00) and cerium (1.43 $\mu\text{g/L}$, pH 7.00; and 1.68 $\mu\text{g/L}$ pH 8.10) was detected. Kurvet et al. [35] showed that REEs are toxic to *A. fischeri* due to cell membrane integrity disruption. The concentration that caused an effect in 50% of the tested samples (EC_{50}) was 3.5–21 mg/L for the tested REE (La, Ce, Pr, Nd, Gd). The results of this study show that the ecotoxicity of the contact water correlates only with the presence of some metals, metalloids, and REE in the tested clays. The results found in the literature suggest that these elements could be responsible for the observed toxicity. Indeed, toxicity of some of these elements has been reported in the literature [1], although in some cases at higher doses than those found in the contact water. However, some elements and REEs (e.g., Ba, Eu) have not been tested on *A. fischeri* and no reference data are available. In addition, the combined ecotoxicological effects on *A. vibrio* of released in contact water of metals, metalloids, and REEs are not described in the literature and should be better investigated in future research. In fact, mixtures are reported to be much more toxic than single chemicals and could produce

higher effects than those reported by literature.

Effects on speciation of pollutants. Abbas et al. [1] reported some studies on the ecotoxicity of metals and metalloids under different pH conditions and showed a clear effect related to lowering pH. Although this study shows a clear inverse relationship between the contact water pH and the observed ecotoxicological effect, further studies are needed to better clarify the chemical speciation of the detected elements in contact water as a function of pH. Indeed, changes in pH may affect the ecotoxicity of the species tested, since the speciation of some metals and metalloids depends on the pH of the water [23,24,29,60]. In addition, seawater is a complex matrix consisting of many salts in varying concentrations that can interact differently with the released elements depending on the pH. In this study, changing the pH of the water from 7.00 to 8.10 after contact with the clays ceased resulted in little change in the observed toxicity, indicating that salt complexation and chemical species change were not effective under the experimental conditions. However, Tsiridis et al. [51] reported that the leachability was related to the initial pH of the leaching medium, which significantly affected the transfer of chemical elements from clays to the liquid phase.

4.5. Exploring for test interferences

Effects on bacteria absorption onto clay particles. In the tests carried out under different acidified pH, a difference was found with the ecotoxicological results under standard conditions (pH 8,10). Any difference in the physical, mineralogical, and microchemical composition of clays may affect the bacterial absorption on the clay surface during SPT at the three different pH values tested. This could be a major reason for the ecotoxicity observed in *A. fischeri*. The results of this study show that a certain portion of the exposed bacteria is actively entrapped by the clay particles. Specifically, the lowest value at both pH 7.00 and pH 8.10 was measured in C10. C5 trapped bacteria better under pH 8.10 conditions than under more acidic conditions, suggesting that this phenomenon contributes to the observed "low toxicity" under standard pH conditions. Lower pH is associated with higher absorption efficiency, reflected in greater numbers of trapped bacteria. A large proportion of the live bacteria trapped in SPT were absorbed by the clay particles. The difference in bacterial entrapment efficiency is due to the complex charge interaction between the bacterial wall and the clay particles. The results of this study show that different minerals which have different crystalline extent, structure, and surface charge distribution [62], produce different results probably due to different degrees of interaction with the bacterial charges. It is important to note that the entrapment behavior depends on the type of clay; C2, for example, showed an equal number of absorbed bacteria under all pH conditions tested. As the entrapped bacteria are absorbed, it is likely that differences in the mineral composition of the clay may lead to differences in the efficiency of charge-surface interactions. The clay fraction of sediments tends to absorb bacteria by adhering to the particles [47], resulting in "natural toxicity" This behavior is at odds with the actual "ecotoxicity" found when chemicals are released in contact with water, as shown by C3 and C7. For these clays, the increasing toxicity observed could be due to a decreased absorption activity of these clays for pollutants at acidified pH conditions, related to the absence of clay-associated minerals that exhibit high surface absorption activity for cations (i.e., illite, bentonite, kaolinite, montmorillonite). This is also confirmed by our results, as the number of live bacteria trapped on C3 and C7 did not increase with increasing acidification, suggesting the absence of minerals with ion trapping activity (illite, bentonite, kaolinite, and montmorillonite).

Effects on bioluminescence lectures. The results reported in this study show that the recorded turbidity does not correlate with the measured bioluminescence at any of the pH conditions tested. Turbidity at the highest concentration tested (19.7%) did not appear to have a significant effect on the measured toxicity values of the clays tested under standardized, controlled conditions. These results indicate that the scope of the tested methods is suitable for 100% pure clay samples with

mineralogical and granulometric compositions corresponding to the tested C1-C10 clays.

4.6. Environmental implications

Field of application of tested method. The natural clays C1-C10 studied are assimilable to any natural clay in marine environments [62]. For this reason, conclusions can be drawn from many of the results of this study for the use of this test to monitor the environmental quality of natural sediments. It is known that the standard for solid phase toxicity tests for the species *Aliivibrio fischeri* normalises the responses obtained only to the percentage of the sample with the particle size $< 63 \mu\text{m}$ ([30], S11 App.2). However, the protocols used for such normalisation refer only to the heterogeneity of the particle size of the studied sample (Onorati et al., 1998; [57]). Consequently, this approach considers the conversion table developed by the cited methods for marine sediments, which correlates granulometry with measured toxicity, resulting in the natural toxicity threshold (NTT), toxicity units (TU) and sediment toxicity index (S.T.I.). The main disturbance observed in this study was related to clay absorption of live bacteria on the surface of clays. This occurrence depends on the type of clay and could lead to "false positives" in environments that are naturally rich in effective minerals that trap bacteria. In addition, the pH of the contact water had a critical influence on this behaviour and contributed to "false positive" results in tests conducted at lower pH values near 7.00, such as SPT on natural freshwater sediments. For clays with mineralogical compositions similar to those of the samples tested, turbidity could not affect the results of this test. For these reasons, the use of this method to determine the ecotoxicity of sediments must be supported by mineralogical analysis and strict consideration of this aspect to avoid the possibility that something natural and ecologically safe could lead to misclassification and expansive management actions for classified sediments.

Relations with global changes. In the first place, our results show that the progressive acidification of the natural environment could have significant effects on the ecotoxicity of the studied clays. The toxicity of natural sediments changes with acidification due to the release of metals, metalloids, and REE into the contact water. This behaviour depends on the type of clay, and studying the mineralogical composition of the silt fraction of natural sediments could be crucial for a better understanding of environmental monitoring results in a future global change scenario.

4.7. Principal goals of the research and future perspectives

This study achieved several important goals in the field of ecotoxicological studies, starting with a closer look at the method itself, which may have some significant biases when applied to clay. First, although it is known that SPT is affected by a kind of "matrix effect" that leads to false-positive results because there are no living cells in the wet sediment matrix after filtration, our study showed that this behaviour depends on the type of clay, since different clays trap living cells better than others. The entrapped cells are bound to the clay as they cannot be removed even after careful rinsing with ASW. In addition, the behaviour seems to change with pH as a function of clay type. These results should be further explored in further studies, and the mechanism underlying the interactions between bacteria and clay should be better understood. Nevertheless, caution should be exercised when reporting the toxicity of natural clays that have similar mineralogical compositions to those reported to effectively entrap living cells to avoid false positive results.

Moreover, from an environmental application point of view, the results suggest that in such cases (high clay contents of natural origin), a detailed analysis of the minerals and mineralogical formations is required to improve the knowledge of the matrix and to better understand the results.

Our results showed that turbidity had no effect on bioluminescence measurements under the conditions tested, either at the standard natural

marine pH of the contact water or at an acidic pH. We were surprised to find that the clays tested in this study differed significantly in mineralogical and chemical composition from label claims and exhibited relevant toxicity in 2/10 cases under standard pH conditions. Since these clays are natural substances obtained from natural deposits and sold as uncontaminated and "cosmetically safe", more caution is needed to protect the environment during the extraction, production and final disposal of such clays and also to evaluate the aspects related to human health, as explained in a special section. The presence of anatase in nanoforms suggests more caution, as does the presence of high amounts of some REE in the clays studied.

Acidification represents a critical point in the ecotoxicity of the studied materials, since for almost all the clays studied (7/10), toxicity increased to a high degree with the decrease in the pH of the contact water. In contrast, filtered and successively acidified contact water had no significant effect on the observed ecotoxicity. This result is important because it shows that acidification during the clay-water contact phase causes a broader extraction of the effective contaminants. Otherwise, changing the pH after the contact between water and clay ends is inefficient.

Future studies will aim to better understand how minerals present in clay interact with bacteria under different pH conditions in order to develop a correction factor that could allow ecotoxicity effects due to the mineralogical composition of clays and natural sediments tested under different pH conditions to be properly accounted for. The goal is to make more accurate corrections to the results to provide a better and truer picture of environmental quality.

4.8. Focusing to human health

The objective of this study was not focused on human health, but only on environmental aspects. Nevertheless, some important findings will be discussed. First of all, the natural clays studied showed significant differences between the composition indicated on the labels and the proven composition. This could have significant implications for commercial aspects and health impacts. Clays are used directly and indirectly in various cosmetic formulations to improve organoleptic and physicochemical properties and to increase stability [61]. Discrepancies between the stated and actual composition can have a decisive impact on the final use, e.g., due to the presence of impurities. Studies on pharmaceutical and cosmetic clays found natural radionuclide activity ranging from 0.71 to 1459 Bq/kg for the various radionuclides tested, with oral intake higher than annual dietary intake for some of these radionuclides [49]. These results must draw attention to the need to strictly control the chemical and mineralogical composition of natural clays to adequately label the data and establish procedural strategies aimed at reducing potential risk factors for users.

In this study, we measured the grain size of clays twice under dry dust conditions and under wet dispersion on ASW water. The reason for these measurements was to understand if there were some differences between the presence of aggregates in dry form, which could potentially affect the dispersion of clays in air, which is of interest to human health, and the particle size when clays are dispersed in water with implications for ecotoxicity and water-clay interactions and chemical release.

Results of particle size in dried dusts showed the production of aggregates that could significantly affect air dispersion and inhalation risks associated to the use of clays as dusts. The presence of TiO_2 in asbestos form in some of the clays tested, as detected by XRD analyses, could pose a significant health risk to humans, and more in-depth studies on this issue should be conducted to rule out the possibility that the application and cutaneous absorption, dust inhalation, and/or ingestion of commercial clays could pose a hazardous exposure to humans. In addition, some of the clays tested contain asbestos in needle-like nanoforms, as shown by FESEM analyses, which could be extremely hazardous to health. Asbestos has caused asbestosis, carcinomas, mesotheliomas, and pleural pathologies; several studies conducted in the 1970 s and 1980 s

have shown that its effectiveness is due to the formation of fibres that can effectively deposit in the lungs and cause oxidative stress and inflammation [18].

Although the literature is not yet exhaustive, metals, metalloids, and rare earths (REE) could affect human health and have been associated with various diseases such as anaemia and endomyocardial fibrosis, and some of them, such as Gd, accumulated in human tissues and caused kidney disease and/or fibromyalgia [9]. The reported presence of high amounts of REE, as evidenced by chemical analyses, needs to be carefully evaluated because we found that these elements have different solubilities depending on the pH studied, ranging from 7.0 to 8.1. The solubility of REE at pH 4.1–5.8, the physiological pH of the stratum corneum [43], and at pH 1.7–6.1, the mean gastric and duodenal pH of healthy young people, respectively [19], needs to be evaluated to better assess potential human exposure and associated health risks.

5. Conclusions

Results obtained in this study highlight a significant effect of mineralogical, and chemical composition of tested clays on toxicity recorded testing *A. fischeri* with SPT protocols. These results suggest improving the diagnostic of these aspects when testing natural clayey sediments to avoid positive responses that are due more to minerals content of clays rather than a real ecotoxicity of the sediment coming from pollution. Under standard pH conditions, different minerals showed a different efficiency to trap bacteria onto clays surfaces producing a different matrix-effect that shall be accurately evaluated. Changing pH conditions are resulted to significantly affect clays ecotoxicity and trapping efficiency of bacteria on clays surfaces. pH conditions resulted able to significantly affect ecotoxicity of SPT and release of metals, metalloids and REE from clays towards the contact water also affecting ecotoxicological responses. This behavior was clay-type dependent. Global change effects could produce significant variations of natural toxicity of clays recorded under standard conditions producing effects on risk evaluation that shall be considered accurately. Caution when apply such tests on clay enriched natural matrices such as harbor and fluvial sediments and to better improve analyses and control points in these cases to understand and correctly weight obtained results.

CRedit authorship contribution statement

Serena Anselmi: Conceptualization, Methodology, Resources, Investigation, Data curation, Writing – original draft. **Andrea Cavallo:** Methodology, Data curation, Funding acquisition, Writing - original draft. **Luca Del Rio:** Formal analysis, Data curation, Writing – original draft. **Monia Renzi:** Project administration, Supervision, Methodology, Validation, Conceptualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.131806.

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