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Short communication

Evaluating and comparing geochemical sampling protocols in dinosaur eggshells: refining Cretaceous ecosystem research

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The geochemical signatures of dinosaur eggshells represent well-established proxies in paleoenvironmental and paleobiological research. The variable sampling procedures reported in the literature, however, deserve attention. In order to evaluate the impact of different sampling methodologies on carbon and oxygen isotope and elemental concentrations, grinding was contrasted with drilling to extract powder samples from eggshell fragments collected at several locations. Eggshell data were further contrasted with surface materials, encasing matrix and compared with independent proxies using petrographic and elemental techniques. Iron and manganese elemental concentrations revealed an enrichment sequence depending on the sampling strategy for the same eggshell fragment. This pattern can be mistaken for a variable state of preservation. In contrast, carbon and oxygen isotope values exhibited only subtle differences and lacked clear trends. This suggests that isotope data are less susceptible to different methodological approaches. It is shown that drilling offers a wider range of possibilities compared to grinding (e.g., faster and less destructive). Additionally, drilled powder samples can confidently be used for elemental and isotope analysis, excluding contamination, thus providing a more accurate set of proxy data from eggshell archives.

1. Introduction

Fossils of mineralized amniote eggshells have been recovered worldwide, from Lower Jurassic to Holocene strata, but are significantly more abundant in Cretaceous geological archives (Hirsch, 1994; Pol et al., 2021). This bias in the record has been discussed in detail in the literature, and its generally attributed to the late acquisition of the hard-mineralized eggshell in most dinosaur lineages (Stein et al., 2019; Norell et al., 2020; Choi et al., 2022). The mineral composition of the mineralized amniote eggshell — calcium carbonate in the form of aragonite (Testudines) or calcite (all other amniotes with mineralized eggshells) — makes them ideal archives for carbon and oxygen isotope analysis (Erben et al., 1979).

Geochemical analysis of eggshells has long been a powerful tool to evaluate their degree of preservation and their potential for paleoenvironmental and paleobiological reconstructions (Erben et al., 1979; Folinsbee et al., 1970; Yang et al., 1996; Cojan et al., 2003; Montanari et al., 2013; Riera et al., 2013; Amiot et al., 2017; Graf et al., 2018; Dawson et al., 2020; Leuzinger et al., 2021). Specifically, isotope signatures ($\delta^{13}C$ and $\delta^{18}O$) and trace elemental concentrations (Mg, Sr, Fe, and Mn) from dinosaur eggshell archives provide insight into dinosaur behavior and related paleoecological

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context. They provide evidence for ingested organic materials (e.g., main vegetation cover, dietary preferences), drinking water (water sources, temperature, salinity), palaeohydrology or atmospheric carbon dioxide content (Zhao and Yan, 2000; Cojan et al., 2003; Kim et al., 2009; Montanari et al., 2013; Riera et al., 2013; Amiot et al., 2017; Leuzinger et al., 2021).

Among the previous geochemical case studies, variable sampling procedures were described to obtain powder from eggshells (grinding and drilling). There is a clear preference for grinding (i.e., the process of crushing eggshell fragments to form a powder) in the literature. Here, the question is raised if geochemical proxies respond to differences in sampling strategy. The literature is replete with data sets displaying carbon and oxygen isotope values (see Montanari, 2018 for summary, critique, and references cited). In contrast, the potential of trace elemental concentrations as paleoecological proxies and/or screening tools for the degree of shell preservation is underexplored (Erben et al., 1979; Cojan et al., 2003; Eagle et al., 2015; He et al., 2019; Kim et al., 2019; Leuzinger et al., 2021). Hence, records of dinosaur eggshell elemental composition are scarce and even more so for related abiogenic materials (host sediment, carbonate nodules, diagenetic/phases, among others). Additionally, data normalization/corrections limitations render the comparison of one data set with such from previous work difficult.

The present paper aims to (i) test the two different sampling strategies (drilling *versus* grinding) applied to the same eggshell fragments; (ii) to compare the outcome of geochemical analyses (C and O isotope values and elemental concentrations), to (iii) contrast these findings with data from the same fossil sampling sites and the arguably best-preserved coeval case example documented in the literature, and to (iv) validate these findings with independent proxies. The broader goal is to establish a sampling protocol that best eliminates the contribution of non-carbonate-bound minerals and/or diagenetic products. If successful, deciphering depositional and biological archive data in dinosaur eggshells (and related materials) will be more successful.

2. Geological and paleoecological context of selected materials

The studied material comes from Mesozoic fossil localities situated in the Lusitanian Basin (western Portugal), the South-Pyrenean basins (northern Spain) and the Iberian Basin (northeastern Spain). A detailed description of geological aspects and general characterization of paleoenvironmental conditions are presented as Supplementary Material (Fig. S1).

3. Materials and methods

3.1. Materials

We selected eggshells from ornithopod, sauropod, and theropod dinosaurs from five Mesozoic localities of the Iberian Peninsula in order to cover a wide range of taxa and geological ages. Theropod eggshell samples from the Upper Jurassic Caniçal locality and sauropod Upper Cretaceous from the Santa Marina 1 locality were collected from whole eggs obtained in the nesting sites, thus, can be considered autochthonous fossils. In contrast, all Lower Cretaceous specimens were collected by screen washing of bulk rock. Specimens sampled at the Collado del Cuchillo and Escarpe Pelejón localities are part of vertebrate microfossil assemblages, thus should be regarded as allochthonous. Specimens from the type locality of *Guegoolithus turolensis* (i.e., Cuesta Corrales 2, Moreno-Azanza et al., 2014) were collected around main eggshell fragment accumulations identified as a dismantled clutch and thus can be considered parautochthonous.

3.2. Laboratory pre-treatment and sampling

To compare different sampling methodologies, eggshells of a range of ootaxa from localities of different ages, as well eggshells from the same ootaxon collected at different coeval localities were used in order to ensure wider applicability of the results obtained (Table 1 and Figs. S1 and S2 in Supplementary file). Selected samples comprise largely Cretaceous eggshell fragments ($N = 26$) and additional Jurassic eggshell fragments ($N = 3$) used for independent validation. Before sampling, all eggshell fragments were cleaned using a variable-intensity ultrasound bath to eliminate surface matrix residues without damaging the eggshells.

Eggshell powder samples were drilled (hand-held device) using a 1 mm diamond drill-bit (Fig. 1A), a conventional sampling method in carbonate geochemical research. Medical grade hardened stainless steel alloys prevent contamination during mechanical abrasion. The surface material not eliminated during ultrasound bath was removed using the drill (abrading the surface), and only clean surfaces were sampled. However, the discarded powder of three eggshells was also considered for geochemical analysis, as these are representative of surrounding matrix when eggshell samples are no longer encased on their original matrix (Fig. 1B). When available, the matrix encasing the eggshells was sampled for comparison ($N = 4$; Table 1). For contrast comparison with the grinding method, four eggshells were ground (i.e., crushed into a fine powder using a manual agate mortar and pestle). Surface abrasion/scratching was not applied. In this way, frictional heat of the drill and related temperature increase is eliminated as a factor (Fig. 1C). Additionally, three Jurassic eggshell samples were used for contrast validation via petrographic inspection and independent elemental analysis (Fig. 1D and Table 1; see below). Eggshell samples from this locality have their external surfacers covered by a thin diagenetic epitaxial overgrowth of calcite, formed during the early stages of fossilization, here referred as the diagenetic layer. This layer has a more or less constant thickness of up to 100 microns across an eggshell but varies in thickness in different eggs of the assemblage, being absent in some of them. In thin section, it is easily distinguished from the true eggshell due to being devoid of organic matter and being completely transparent. Its identification in SEM is somewhat more difficult, but in general it comprises more euhedral crystals and sharper cleavage patterns than the true eggshell. Furthermore, the ultrasound-residue of these samples was recovered (Fig. 1D) by drying at room-temperature (*ca.* 20 °C) and used for further analysis (see below). These procedures were conducted at the facilities of the Geosciences Department, University of Aveiro (Portugal).

3.3. Geochemical analysis

Powder samples were analyzed for their major and trace elemental concentrations (Ca, Mg, Sr, Fe, Mn) using an inductively coupled plasma-atomic emission spectrometer (ICP-AES) at the facilities of the Institute for Geology, Mineralogy and Geophysics (Ruhr University Bochum, Germany). Dissolution of 1.5 mg of powdered sample in 1 ml of 3 M HNO_3 (over 12 h) was followed by further dilution with 2 ml of distilled water and filtering. The use of absolute elemental data (in ppm) instead of calcium-corrected abundance (element/Ca) is here preferred to allow direct comparisons with literature (see Supplementary file, Fig. S3). The maximum elemental scatter (external reproducibility) for duplicate samples was approximately 5% for all elements. Internal calibration was controlled by using blanks (HNO_3 3.5%) and reference materials (CRM-512 and CRM-513), with a maximum relative standard deviation of 3%.

Table 1
Summary of used materials and performed analytical work. Note the variety of different locations and eggshell fragments used in this study. For details on used methodologies see main text.

Ootaxa/Related taxa	Location	Horizon	Distinctive features	Eggshell fragments	Eggshell powder samples	Encasing matrix (powder samples)	Analytical work
Megaloolithidae/ Sauropods	Santa Marina 1	Upper Cretaceous	Thick eggshells (2–3 mm), ornamented, high porosity	6	8	–	Geochemistry
Spheroolithidae/ Ornithopods	Collado del Cuchillo	Lower Cretaceous	Medium eggshells (1 mm), ornamented, low porosity	3	6	–	Geochemistry
Spheroolithidae/ Ornithopods	Cuesta Corrales 2 Perfil Norte y Sur	Lower Cretaceous	Medium eggshells (0.5–1.3 mm) ornamented, low porosity	17	19	4	Geochemistry
Prismatoolithidae/ Theropods	Caniçal	Upper Jurassic	Thin eggshells (0.5–0.8 mm), flat outer surface, low porosity	3	–	–	SEM-EDS

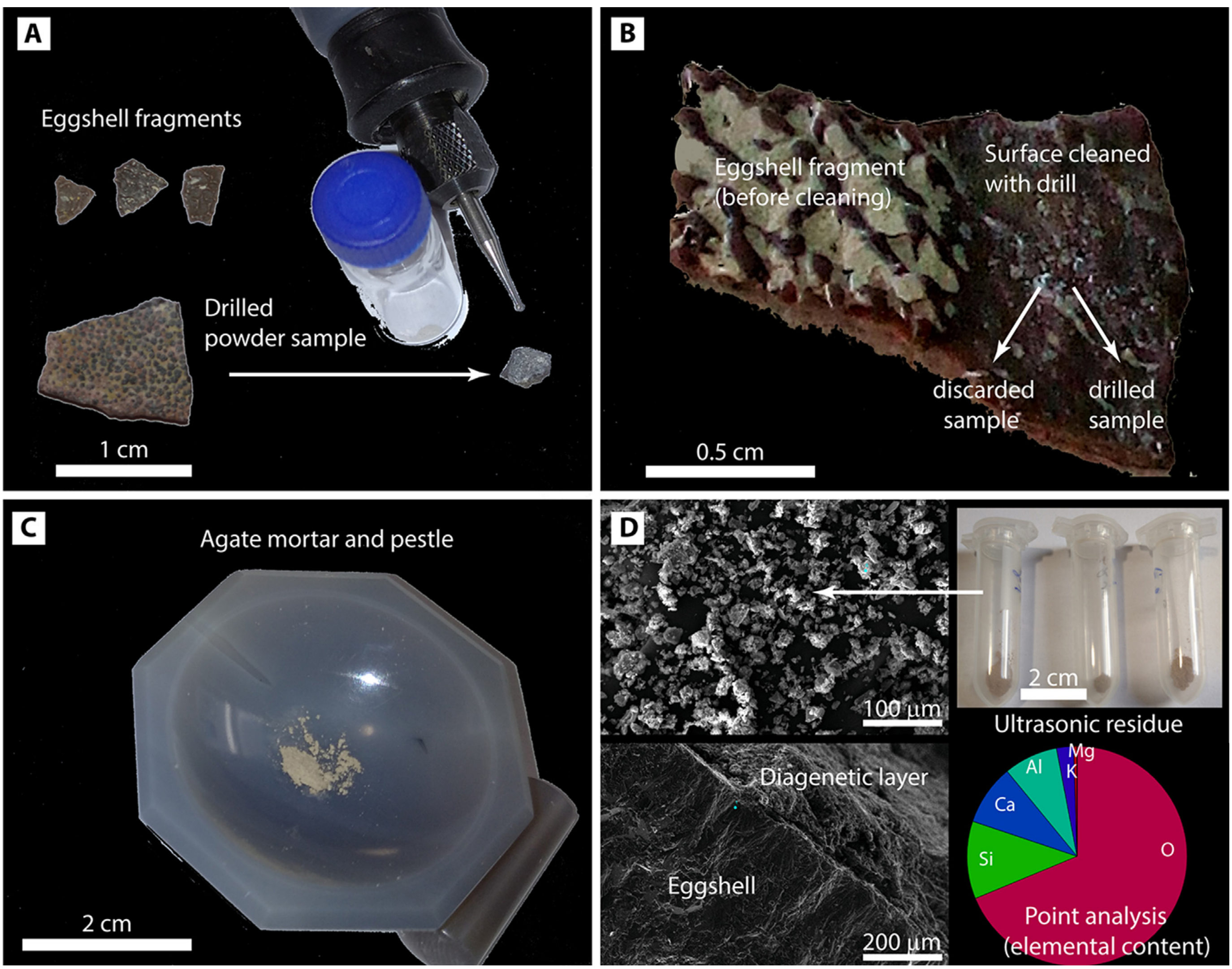

Fig. 1. Overview of the different approaches used in this study and resulting sample types. A) Drilled powder samples, obtained after discarding the most external layer. B) Example of an eggshell before and after cleaning the surface material with the drill. The discarded powder was in some cases analyzed for comparison. Note that this shell was previously cleaned using ultrasounds, yet the adherent matrix is visible. C) Powder sample ground in an agate mortar. D) Independent validation by using alternative proxies on eggshell, diagenetic layer, and ultrasound residue. These include ultrastructure analysis (SEM) and elemental point analysis for contrasting geochemical composition.

The same powder samples were also analyzed for their carbon and oxygen isotope composition using a Thermo Fisher Scientific Gasbench II carbonate device connected to a ThermoFinnigan MAT 253 Mass Spectrometer. Analytical precision ($\pm 1\sigma$) for carbon and oxygen-isotope data, controlled by NBS19 and internal standards, was better than ±0.08 and ±0.07‰ for $\delta^{13}C$ and $\delta^{18}O$, respectively. Duplicate samples presented a maximum deviation of ±0.06‰ for $\delta^{13}C$ and ±0.05‰ for $\delta^{18}O$. Isotopic values are reported in the standard δ-notation in permil (‰) relative to V-PDB.

3.4. Scanning Electron Microscope-Energy Dispersive Spectroscopy (SEM-EDS)

Petrographic elemental proxies were used on three Jurassic eggshell samples to obtain independent validation. Carbon-coated shell fragments and respective ultrasonic residue were analyzed for their ultra-structure and nano-particle geochemical characterization (Fig. 1D) using a Scanning Electron Microscope-Energy Dispersive Spectroscopy (SEM-EDS). An ultra-high resolution analytical scanning electron microscope Tescan VEGA was used (Department of Geosciences, University of Aveiro, Portugal), operating at 20 keV and variable magnification adapted to each sample. This technique combines ultra-structural and elemental evidence, used to perform an analysis on selected points (elemental results in weight fraction %). Due to carbon coating, this element is not included in the composition of inspected materials.

All materials were collected under the local and national legislation for palaeontological heritage of Portugal and Spain (Exp. 128-10/2011). Portuguese specimens and additional unused samples are housed at Museo da Lourinhã, Portugal. Spanish specimens and additional unused samples are housed at Museo de Ciencias Naturales de la Universidad de Zaragoza, Zaragoza, Spain. The exact location of the fossil localities is not here disclosed in order to protect this paleontological heritage but is publicly available upon justified request at the housing institution in Portugal, and in the Dirección General de Patrimonio Cultural of the Gobierno de Aragón in Spain.

4. Results

4.1. Application of different methods to selected samples

When comparing geochemical results of samples extracted by different methods, clear differences were depicted (Fig. 2). With respect to elemental concentrations, Fe and Mn will be highlighted as these are elements commonly used to establish the degree of preservation of ancient carbonates (including matrix micrite from different settings, late cement phases and biominerals, including eggshells) via elemental screening and to detect terrigenous/continental contributions (Erben et al., 1979; Brand and Veizer, 1980; Cojan et al., 2003; Vincent et al., 2006; Coimbra et al., 2015; Eagle et al., 2015; Coimbra et al., 2017, 2018; He et al., 2019; Coimbra et al., 2020; Dawson et al., 2020). These elements also show a higher offset when comparing drilling versus grinding protocols (see Supplementary file, Fig. S4). Accordingly, differences in Fe and Mn concentrations were conspicuous throughout all studied locations (Fig. 2A-C). All drilled samples provided the lowest Fe and Mn concentration, ranging from 30 to 375 ppm and 30 to 90 ppm, respectively. In contrast, ground samples and surface materials persistently showed considerably higher Fe and Mn concentrations through all studied locations (up to 700 and 800 ppm; Fig. 2A-C).

Regarding C and O-isotope values, obtained differences are more subtle in terms of their absolute values and do not follow a clear pattern (Fig. 2D-F). When compared to drilled samples, ground samples show moderately lower carbon isotope values (maximum offset of 0.4‰; Fig. 2D and E). But their respective surface materials can either be higher or lower than their respective drilled samples, depending on the location (Fig. 2E and F). Concerning oxygen isotope values, drilled samples show persistently higher values than their ground counterparts (Fig. 2D and E). Surface samples always denote lower $\delta^{18}O$ (Fig. 2E and F).

4.2. Geochemical data in a wider context

To evaluate the significance of observed geochemical differences derived from different sampling methods (Figs. 3 and 4; Table 1), obtained results were contextualized within a larger dataset ($N = 37$), including other drilled eggshells and matrix samples

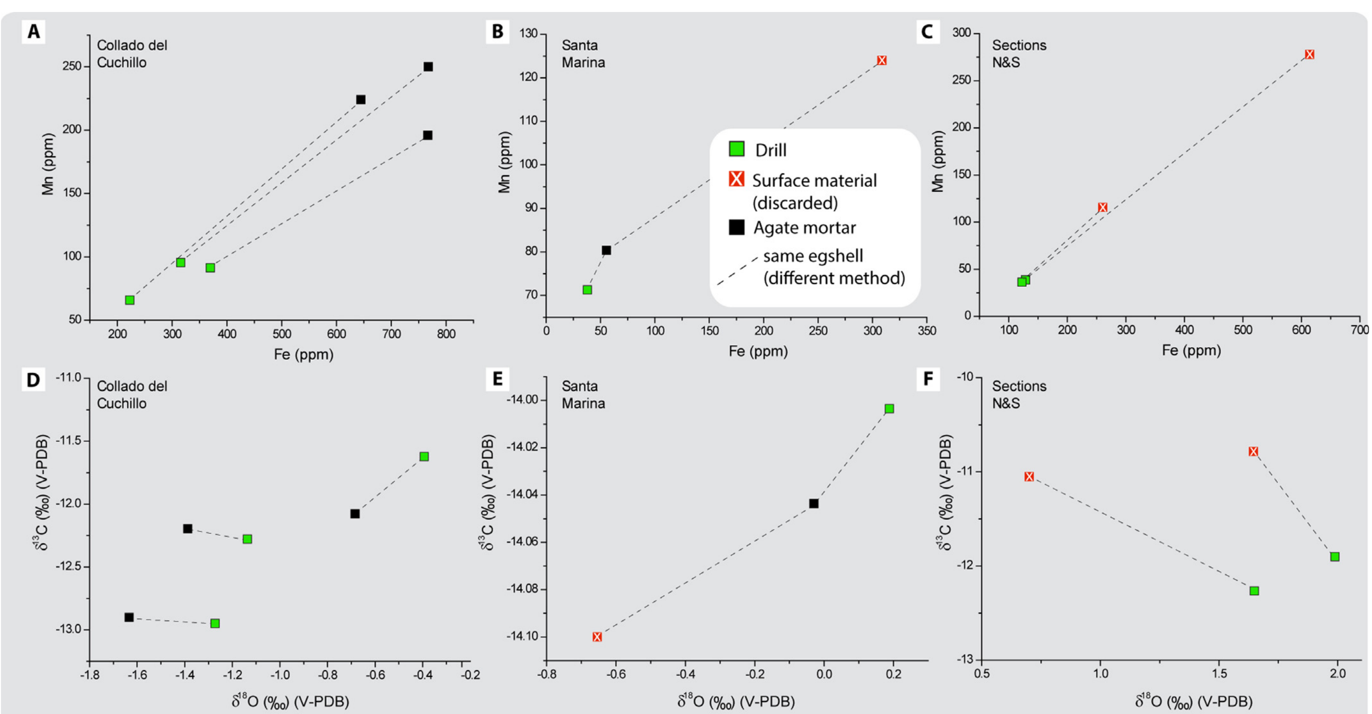

Fig. 2. Geochemical results of different approaches used on the same samples. A—C) Variability on elements of interest Fe and Mn in samples from the three selected locations. D—F) Carbon and oxygen-isotope values obtained using different methodologies in samples from the three selected locations. Dash line connects data points from the exact same eggshell. Note that results from the same shell do not overlap.

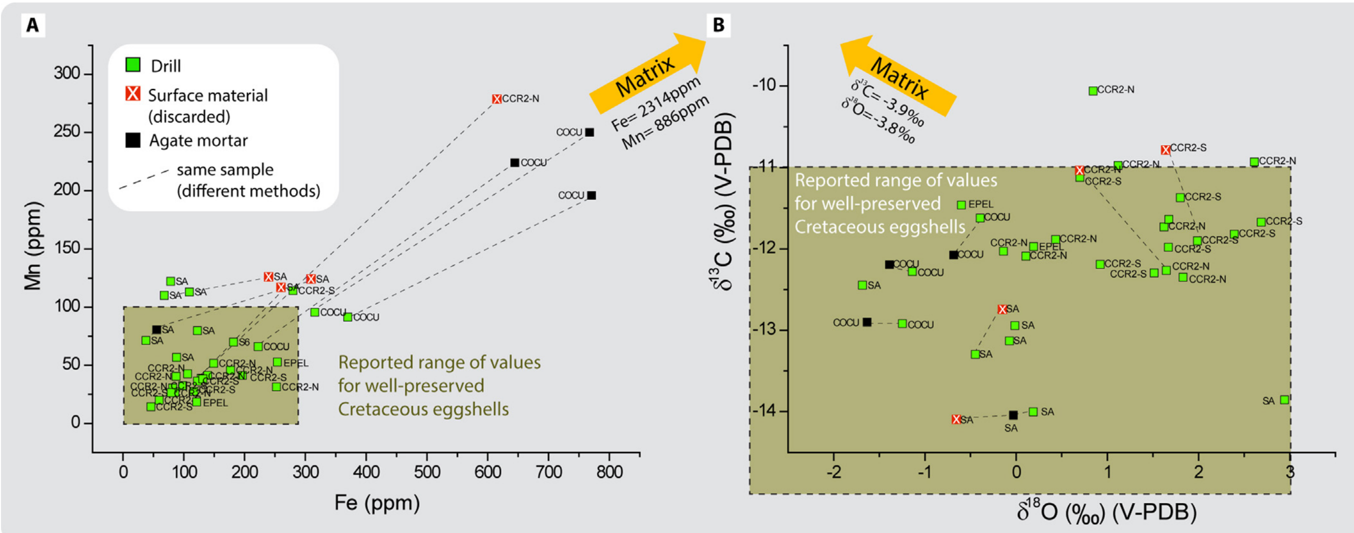

Fig. 3. Geochemical results for all Cretaceous samples used in this study (see also Table 1), including all sample types and tested methods (drilled eggshells, surface materials, ground eggshells, and encasing matrix). A) Elemental data for Fe plotted against Mn, evident deviation of different sample types follows a clear trend line. B) Isotope crossplot showing most of the samples within the range of expected values, as well as no clear trend lines. Dash line connects results from the same sample; dash rectangle delimits the range of values reported in the literature (Erben et al., 1979; Riera et al., 2013; Eagle et al., 2015; Leuzinger et al., 2021). Abbreviations used for sampled locations according to Fig. S1.

(where available) from the same locations. These data were also compared to what are considered well-preserved Cretaceous dinosaur eggshells (Fig. 3).

Differences in Fe and Mn concentrations derived from sample processing are still considerable even when included within a wider set of samples from all studied localities (Fig. 3A). Accordingly, 75% of drilled samples fall within the expected range of well-preserved coeval samples and the remaining 25% deviate more than only 50–100 ppm (Fig. 3A). Samples produced via grinding and surface material samples show intermediate Fe and Mn values, in between drilled and matrix samples. The latter are highly enriched in both elements of interest (average values of 2314 and 886 ppm, respectively; Fig. 3A). Varying methods in one single sample results in a range of Fe and Mn values equal or even higher than the full range of variation of all the samples used in this study (Fig. 4A).

Considering carbon and oxygen isotope values, differences derived from sampling strategies are less evident and largely mitigated within the full range of variation of the complete set of drilled samples (Fig. 3B). Most samples (92%) fall within the expected range of values for Cretaceous eggshells. Comparatively, matrix samples show significantly ^{13}C-enriched carbon isotope and ^{18}O-depleted isotope values. However, none of the remaining samples follow this trend (Fig. 3B). In agreement, the range of C and O isotope data obtained via different sampling strategies shows minimal dispersal when compared to the full range of variation depicted at each location (0.4 and 0.5‰; Fig. 4B).

4.3. Validation by independent proxies (SEM-EDS)

A set of Jurassic samples ($N = 3$) was tested by means of different analytical techniques to provide complementary petrographic and geochemical evidence for the composition of eggshells, respective diagenetic layer, and attached particles (residue after ultrasonic bath). Representative results obtained for the same shell are shown in Figs. 5 and 6. Eggshell ultra-structure imaging showed no significant evidence for alteration other than the presence of the external diagenetic layer, which is distinct (Fig. 5A). The selected analytical points provided very consistent results (Fig. 5B): eggshell composition corresponds to 100% Ca and O (C omitted; minor elements without significant signal along the spectrum); the diagenetic layer is composed of 83–99% Ca and O (C omitted) with a minor contribution of Si, Al, P (and others). Comparatively, residue particles (Fig. 6A) attached to the eggshells—taken as representatives of the surrounding matrix—show Ca as only a minor component (very scarce to 20%; Fig. 6B).

5. Interpretation

5.1. Different sampling protocols induce differences in geochemical data

When using the same eggshell fragment, drilled samples consistently provided the lowest Fe and Mn values, host sediment materials yielded the highest Fe and Mn concentrations, while ground samples were intermediate (Fig. 2A-C). This sequence of increasing elemental enrichment suggests that variable amounts of surface material add a significant bias to the elemental concentrations of ground samples. The implication is a value that is intermediate between the eggshell value, the value of the diagenetic layer and the host sediment that might be still attached to the shells surface following cleaning in the ultrasonic bath. A high sensitivity to terrigenous components during elemental analysis is linked to the fact that both carbonate-bound and detrital fraction elements are leaching during acid attack.

If changes in temperature during drilling were to be of concern for interpreting oxygen isotope data, other variables would remain unchanged. This is clearly not the case regarding elemental composition and independent geochemical analysis via SEM, hence, this effect can be discarded. For the case of carbon and oxygen isotope values, subtle differences between ground and drilled samples and the lack of a clear variation pattern due to changing sampling method indicates that these proxies are less sensitive to different sampling protocols (Fig. 2D-F). This fact is most probably due to the specifications of the analytical procedure, depending largely on the carbonate fraction, hence minimizing the effects of terrigenous components. Still, some common traits merit attention: (i) carbon-isotope values of surface materials fluctuate without

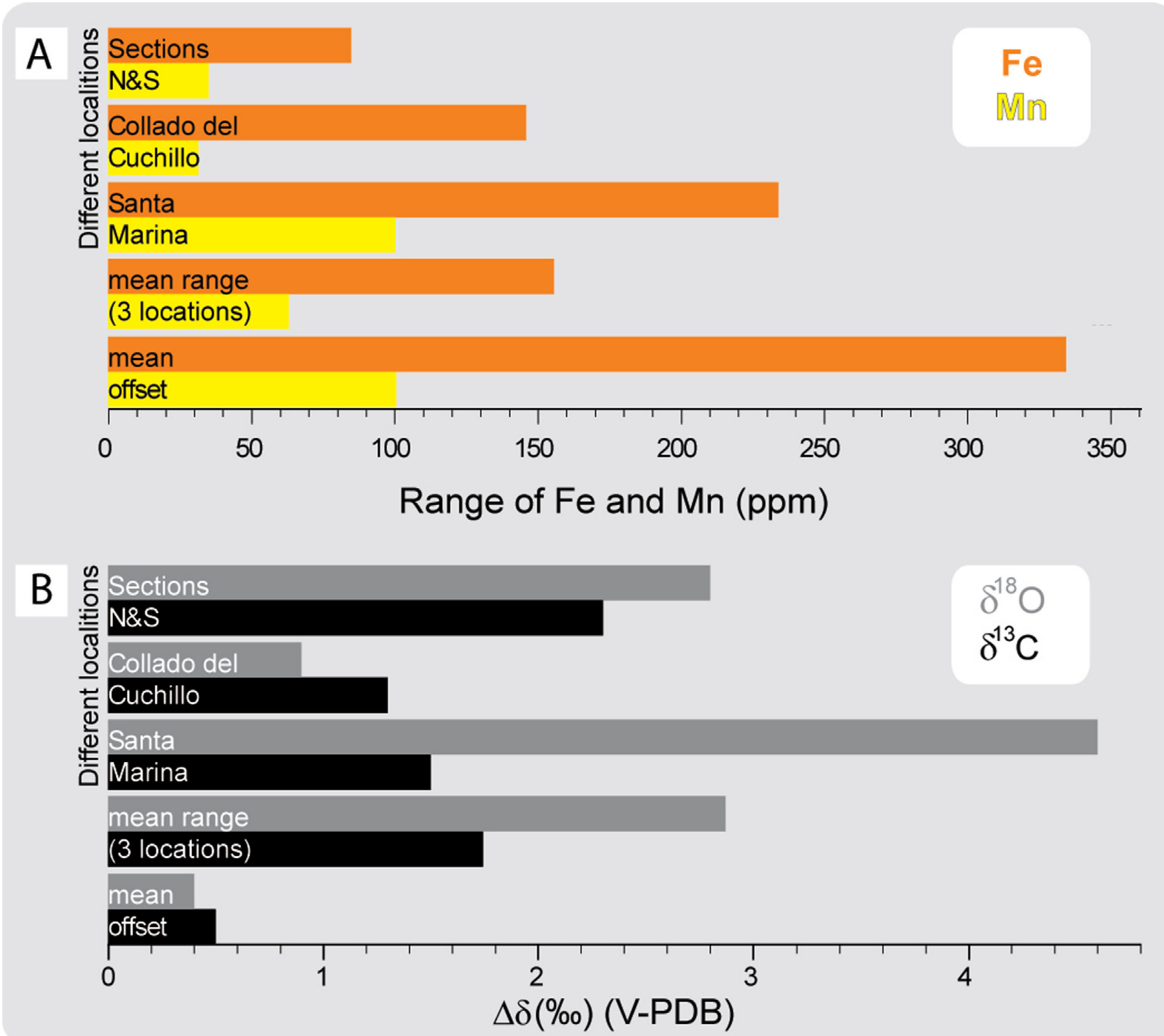

Fig. 4. Geochemical range of values (max.−min.) obtained at the different locations compared with their respective mean and the deviation generated by using different method (mean offset). A) Range of Fe and Mn values, note considerable range of the mean offset when compared to the range of variations in any site. B) Range of variation of C and O-isotope values (min. −max.), note minimal mean offset comparing to the range obtained for all the locations.

obvious pattern when comparing different locations, suggesting that local sedimentary contributions can exert control on $\delta^{13}C$ values; (ii) oxygen-isotope values follow a tenuous trend towards progressively lower values in the order of: (i) drilled powder samples, (ii) ground samples and finally (iii) surface materials. This is taken evidence that the diagenetic layer or remnants of host sediment attached to the shell induce a trend to ^{18}O depleted values.

5.2. Comparison with local and published data

Differences induced by the methodological approach were placed in context with a wider set of data from the same location, other locations (reported in the literature), and other materials (e.g., host matrix sediment). This provides a clear overview of the differential impact of sampling strategies (Figs. 3 and 4) and can evaluate differences in absolute value plotted against the natural variability of Cretaceous eggshells.

At present, it is obvious that Fe and Mn concentrations are biased by the different sampling protocols, with ground sample data deviating significantly from their corresponding drilled sample data. Moreover, drilled sample data even plot out of the range of values for coeval eggshells. Specifically, matrix samples represent an end-member value for high Fe and Mn concentrations (Fig. 3A). The implication is that drilled samples provide values more representative of the eggshell iron and manganese values. Furthermore, comparing the range of Fe and Mn data obtained at the different locations highlights the impact of the offset generated by different sampling strategies (mean offset; Fig. 4A).

Concerning C and O isotopes, changes induced by the sampling protocol have only a minimal impact compared to the background fluctuation found in published Cretaceous eggshell data (Figs. 3 and 4). Most samples fall within the range of well-preserved eggshells (Fig. 3B). Additionally, the homogenous carbon and oxygen isotope values of surface materials indicate that the diagenetic layer was replaced during an early diagenetic stage.

5.3. Constraints on the nature of the sampled materials

Independent petrographic and geochemical proxies provided further arguments for a better understanding of the three different components involved during eggshell sampling (Figs. 5 and 6): (i)

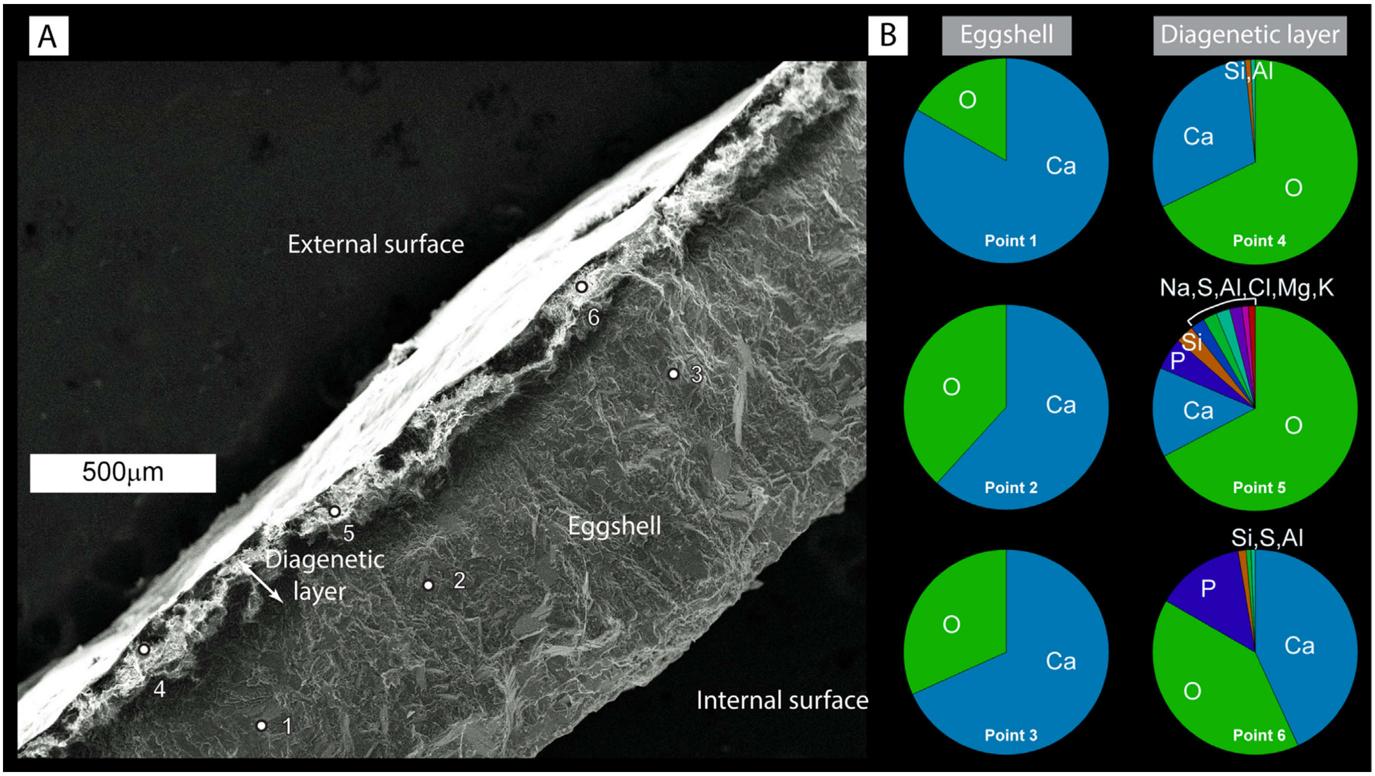

Fig. 5. Representative example of ultrastructure inspection and elemental point analysis of Jurassic eggshells. A) Detail of eggshell structure with clear identification of the limits of the diagenetic layer. Numerals indicate the exact points chosen to perform elemental analysis. B) Point analysis results for eggshell (points 1 to 3) and adjacent diagenetic layer (points 4 to 6). Note a minor contribution of non-carbonate components (Si, Al, P, and others) throughout the diagenetic layer (see text for details).

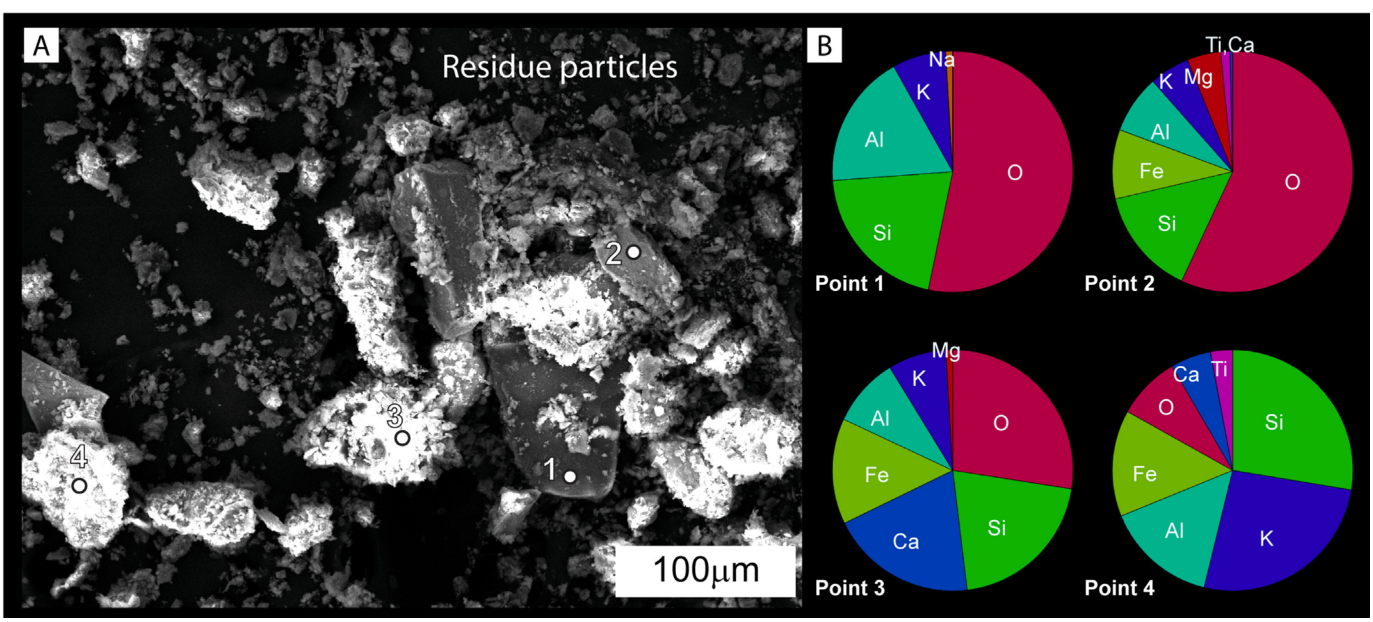

Fig. 6. Representative example of ultrastructure inspection and elemental point analysis of ultrasound residue particles obtained from the same eggshell fragment represented in Fig. 5. A) Detail of different textures and grain shapes detected along residue particles. Numerals indicate the exact points chosen to perform elemental analysis. B) Point analysis results covering the range of different aspects in grain shape and texture. Note the scarce presence of Ca in all cases, along the dominance of Si, Al, Fe, K, and others (see text for details).

the eggshell, composed of calcium carbonate (100% $CaCO_3$); (ii) the adjacent diagenetic layer primarily composed by calcium carbonate (>80%; potentially slight overestimation due to partial allocation of Ca in aluminosilicate species) and a minor fraction of aluminosilicate minerals; (iii) and the host matrix sediment largely composed by aluminosilicate minerals with a small contribution of calcite (wt% Ca <20%, probably shared with other minerals).

The fact that the diagenetic layer provides minimal elemental contribution other than Ca (Fig. 5) suggests that the main source of elemental enrichment in dinosaur eggshell powder are remnants of the sedimentary host matrix attached to the outside of the eggshell or occluding pores of the shell. This interpretation agrees with the fact that ground samples largely plot outside of the range of well-preserved Fe and Mn concentrations, as well as the thicker, more porous eggshells collected at Santa Marina 1 (Fig. 3A). Thicker-shelled eggs with larger pores (compared to thinner shells), can thus account for the higher contribution of sedimentary Fe and Mn.

6. Discussion and recommended protocol for eggshell sampling

Powdered rock samples are used in various analytical techniques (mineralogy and geochemistry). Powdered samples offer a large reactive surface and can be weighted accurately. Choosing the ideal strategy is pivotal to increasing the reliability of obtained results and corresponding interpretations. Nevertheless, this choice is not necessarily universal, and available materials, methods and study aims must be balanced to make most of the material under consideration.

6.1. Drilling versus grinding: advantages and limitations

In order to evaluate which analytical protocol is most commonly applied to dinosaur eggshells analysis, evidence from a total of 13 scholarly papers was compiled (Follinsbee et al., 1970; Yang et al., 1996; Zhao and Yan, 2000; Cojan et al., 2003; Kim et al., 2009; Montanari et al., 2013; Riera et al., 2013; Eagle et al., 2015; Amiot et al., 2017; Graf et al., 2018; He et al., 2019; Dawson et al., 2020; Leuzinger et al., 2021).

In these papers, a wide range of sampling protocols was applied: (i) grinding with previous ultrasonic bath and/or mechanical/manual abrasion (n = 4); (ii) grinding with previously drilling surface material to eliminate a contribution of material other than the eggshell (n = 2); (iii) drilling without any further analytical steps (using carbide-tip and micro-drilling; n = 2); (iv) five papers refer to “drilled material” but lack any details of the analytical techniques used. Drilling is thus the less preferred option.

Hand-held drilling devices are easily accessible, small, easy to use, inexpensive and long-lasting tools with equally easy-to-acquire and affordable consumables (drill bits). The drilling procedure does not require a previous cleaning of the egg's surface (attached sediment and/or diagenetic layer), as this material can be eliminated prior to the sample acquisition. Drilling is a fast and comparably non-invasive process, i.e., the same shell fragment can be used for other complementary analyses (e.g., petrography; Fig. 5). Drilling can be performed on loose fragments and on eggshells encased in their original sedimentary matrix (e.g., nests), an attractive feature for exploring museum pieces or private collections with minimal impact. Adhesives, such as glue, cyanoacrylate, epoxy resins, and non-penetrative Paraloid B72 coatings can be eliminated prior to sample acquisition. It is worth noting that even untrained users can easily drill an eggshell without penetrating the shell. This is important because reaching the inner surface of the shell could result in contamination. Eggs with thin eggshells require more training when sampling (e.g., constant powder color signals a successful sample). An identified limitation of drilling is that eggshell pores are too small to be identified macro- or even mesoscopically. Sediment or products of diagenetic stages occluding pores may induce contamination and analytical bias (Cojan et al., 2003; Kim et al., 2019 for discussion and critique). Pore abundance and pore diameters show a high taxonomic variability across egg-laying vertebrates (Mikhailov, 1997; Kim et al., 2019). The size and number of pore channels, as well as the shell thickness, will control, to some degree, the type and amount of material that might occlude them (Moreno-Azanza et al., 2016; Kim et al., 2019). Moreover, nesting strategies (Vila et al., 2010), the host sediment in the nest's environment (Díaz-Molina et al., 2007; Tanaka et al., 2018), and the diagenetic pathways will be of significance too (Dauphin et al., 1998; Fiorelli et al., 2013). Concluding, the influence of non-eggshell material in pores ranges from negligible to significant, depending on the case study considered.

The alternative sampling approach involves grinding a shell fragment in an agate (or other types) mortar usually found in most sedimentology/geochemistry laboratories. Grinding eggshells requires that the fragment is previously cleaned (e.g., ultrasonic bath). This is time-consuming and has limited cleaning effectiveness when used at low frequency, as in the case of thinner fragile shells. This procedure is restricted to shards of the egg, which implies the destruction of the eggshell fragment and results in a powder sample that combines eggshell material combined with other components (diagenetic layer or surficial sedimentary material).

Concluding, drilling offers a wider range of possibilities when compared to grinding, but the selection of the sampling tool is dictated by the type and context of the available materials. Some of the factors to be considered are eggshell morphology (thick-*versus* thin-shelled; smooth *versus* ornamented surface; abundance and size of pore channels), possibility of (semi)conservative *versus* destructive sampling (e.g., museum specimens or holotypes), the abundance of material, among others.

6.2. Geochemical archive of eggshells: avoiding sampling bias

Geochemical analysis of dinosaur eggshells is a tool to evaluate the degree of preservation and potential of this specific archive type for paleoenvironmental reconstructions (Folisbee et al., 1979; Yang et al., 1996; Cojan et al., 2003; Montanari et al., 2013; Riera et al., 2013; Amiot et al., 2017; Graf et al., 2018; Dawson et al., 2020; Leuzinger et al., 2021). As documented here, however, the sampling protocol selection might affect the outcome of geochemical analysis.

Based on the tests performed on 26 eggshell fragments, the bias induced by the sampling protocol chosen (drilling *versus* grinding) ranged from minimal for carbon and oxygen isotope analysis to significant when referring to trace elemental concentrations (Figs. 3 and 4). Therefore, grinding poses no limitation for further interpretations based on C and O-isotope values, but independent information is necessary to confirm the pristine state of the eggshell fragments, as optical inspection and/or isotopic comparison with other materials as nodules (Cojan et al., 2003; Grellet-Tinner et al., 2010; Riera et al., 2013; Montanari, 2018; Leuzinger et al., 2021). Concerning trace elemental analysis, tests on eggshell preservation often rely on absolute elemental abundance (elemental screening, Eagle et al., 2015). Here, we demonstrate that the outcome of this screening approach might depend on the sampling technique (Fig. 2A-C and Fig. 3A).

Based on the outcome of our study, grinding is not recommended as the resulting data are most likely biased (Fig. 3A). This specifically refers to approaches aiming to establish the degree of preservation based on absolute elemental data abundances. Clearly, drilling is the more reliable approach.

In contrast, when aiming to interpret elemental patterns in the context of paleoenvironmental proxies, the focus can be on relative elemental differences (higher/lower), regardless of the absolute value. In this case, grinding can be used without major concerns when assuming that the degree of bias by contamination is near-homogenous throughout the material.

The increase in abundance of dinosaur eggs and eggshell fragments in the fossil record during the Late Cretaceous is observed globally. Their low-magnesium calcite composition, and quick deposition makes them close to ideal specimens for geochemical analysis, often unrivalled in continental deposits. This has translated in an abundance of works sampling both Oxygen and Carbon isotopes on dinosaur eggshells, which are in turn used to infer paleoclimate, paleogeography and palaeoecological structure of Cretaceous ecosystems. Our results highlight the necessity of standardized sampling methods in order to produce comparable results among different taxa, ecosystems, and time intervals.

Furthermore, we have demonstrated that less invasive sampling methods are more precise and reduce the risk of contamination of samples. This increases the number of potential sampled specimens, as it allows access to key valuable specimens that may not be available for more destructive sampling methods. Thus, we consider that standardization of a sampling methodology for dinosaur eggshells is pivotal to properly use this key paleoenvironmental proxy for Cretaceous continental ecosystems. Accordingly, the drilling of powder samples is proposed as the most convenient approach.

7. Conclusions

Various sampling methods focused on dinosaur eggshells are presented in the literature. Two approaches—grinding and drilling—were compared to evaluate a potential sampling bias on C and O isotope data and elemental concentration (particularly concerning Fe and Mn elemental abundances). Tests performed on 26 eggshell fragments from various localities and ages provided the following guidelines:

- Ground powder samples posed no limitations for C and O isotope data analysis. This sampling method is more prone to contamination as the powder represents the bulk shell potentially containing non-shell material. This affects iron and manganese elemental analysis when exploring the degree of preservation of the shell material (screening).
- Drilled powder samples lead to better results in all tested eggshells. Geochemical analyses display more consistent geochemical results and can be used for elemental and isotope analytical work. Drilling provided a more realistic record of eggshell composition by minimizing the contribution of non-shell material.

Concluding, drilling fossil eggshell powder samples is recommended. The best sampling strategy, however, depends on available materials and the aims of the research project.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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