Jaques and Holcová *Heritage Science* *(2023) 11:140*
https://doi.org/10.1186/s40494-023-00974-9

Heritage Science

RESEARCH **Open Access**

Calcareous nannofossil assemblage in paintings chalk ground for provenance analysis: three original paintings compared to european source materials

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Abstract

Chalk has been used since Antiquity for various purposes, and since Gothic for preparatory layers of painted cultural heritage objects. Several materials are called chalk in Cultural Heritage, but this work especially focuses on chalk composed of calcareous nannofossils (up to 98%). These are fossil remains of photoautotrophic algae generally smaller than 30 µm. They are mainly visible as platelets of various shapes under a cross-polarised or scanning electron microscope. The provenance of chalk can be determined using calcareous nannofossils due to their well-known paleobiogeographic localities. They are already used as proxies since the 90s in Cultural Heritage, but rarely for paintings. In this work, 6 chalk historical mining areas were chosen: Germany (Ruegen), France (Champagne, Meudon), Belgium (Mons), England (Norfolk) and Italy (Bologna). Natural and processed chalk were used as reference materials and compared to 3 original paintings. The difference between the chalks calcareous nannofossil assemblages was shown using multivariate statistical analysis based on species relative abundance. Marker nannofossil species were defined for each chalk locality. One painting material could not be originated due to the preservation of its nannofossils assemblage, but the origins of the rock chalk material from the two other paintings could be geographically located in France.

Keywords Nannofossils, Painting, Provenance, Biogeography, Europe

Introduction

A painted artefact (easel, panel, statues) can be investigated for different reasons: origin verification [1, 2]; restoration [3]; conservation. Provenance analysis of the materials composing a painting can be crucial for identifying the region where the artwork was created in relation to trade roads [4] or the money available to the artist (patron). But it can also be used for restoration. The provenance of a painting and its components can be assessed by their specific physical and chemical properties, e.g. trace elements, mineralogy, wood essence, palynology and organic matter. Various analysing methods can be used to find it out e.g. Fourier Transformed Infra-Red (FTIR) [5–8], Raman [9–12], X-ray fluorescence spectroscopy (XRF) [13, 14], UV Visible Reflectance (UV-VIS) [15–17], X-ray diffraction (XRD) [18].

This study focuses on the source location of the materials of the ground layers: A painting relies on the mixing and layering of various materials. A simple painting generally consists of a support (canvas, wood, rock/wall), a preparatory layer, usually made of clay

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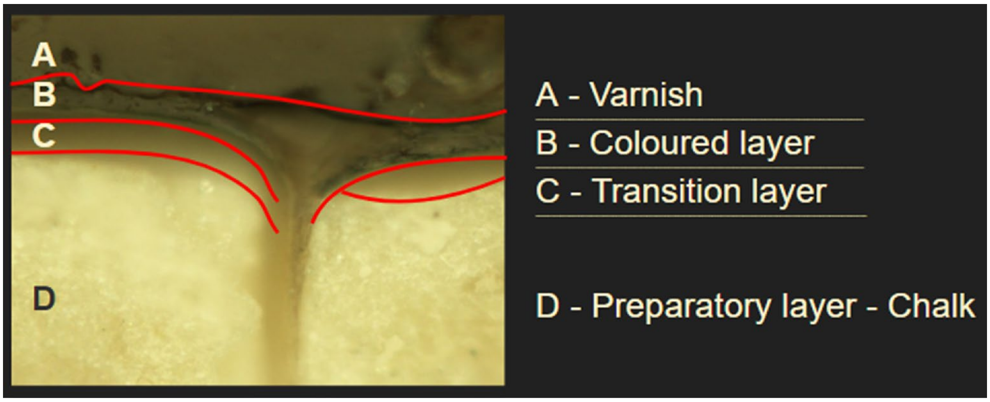

Fig. 1 Cross-section of an easel painting (FN).

or chalk, the coloured pictural element, and finally a protective varnish (optional; Fig. 1).

While pigments are already extensively studied, the provenance of fillers and materials used for the preparatory layers, such as chalk, much less. Preparatory layers are meant to flatten and smooth the canvas before painting it, sometimes to increase the visual depth when adding colours to it [5–7]. Depending on the artist, workshop or creation place, different chalks were used between medieval and new-age periods in Europe, such as Champagne chalk, considered the whitest and purest among all, but also Belgium and Bologna chalk [8, 9].

Chalk

Chalk is strongly exploited since ancient Egypt for various purposes, including paint, even though rare traces of its use appear already during prehistory around 10000 BC [10]. In Europe, chalk was used for priming in painted cultural heritage from the Gothic period [11] to the eighteenth century. It is a Cretaceous fine-grained, powdery, soft material, built of biogenic micritic calcium carbonates by variegated calcifiers belonging mainly to protists [12]. Chalk can be found all around the globe, hence Europe (Fig. 2), and its biggest geological formation was built in the Late Cretaceous (100.5–66 Ma) [12, 13]. In Europe, chalk was deposited since Cenomanian (100.5–93.9 Ma) to Masstrichtian (72.1–66 Ma). Four accumulation basins are known: Paris–London, North Sea, Westphalia–Lower Saxony and North Germany–Poland [4]. These relatively northern areas can be referred to as a boreal realm.

Economically speaking, chalk was not advantageous to transport too far from its source, and the first written source about the sale of chalk in Europe dates back only to the fifteenth century in Champagne, France [10]. It was officially exploited since the eighteenth century until 1920. Meudon chalk, referred to as Meudon white, is a pure soft stone with layers of black flint (SiO_2). Its deposition took place in relatively shallow marine (50–200 m depth) warm (20–25 °C) surface waters [4]. Both Champagne and Meudon chalk were mostly quarried in underground galleries. Belgium chalk, which is close to the Champagne region, was already exploited during the Neolithic for the flint layers in the Mons region in wells of 10 to 20 m depth ("Cayaux camp" in Robaszynsky [4]).

Like Champagne chalk, Sassnitz used to quarry chalk industrially officially since only 1840 in open pits. Although the use of Ruegen chalk in Bohemia and Slovakia in art during Gothic [11] testifies of earlier trade. It can therefore be assumed that the chalk was imported from the nearest source. Ruegen chalk is part of the Jasmund National Park, a UNESCO World Heritage Site since 1990. The chalk is covered by Quaternary glacial sediments [14] and is visible through the cliff side of the Jasmund Peninsula. It is composed of white lime and marls (carbonates, soft limestones) with horizons of flint deposited 70 Ma ago in a cold shallow sea [15]. It underwent only a very low diagenetic compaction.

More southern in the Northern region of Italy, Bologna chalk (Messinian gypsum $CaSO_4{\cdot}2H_2O$), Sarti chalk ($CaCO_3 + CaSO_4$) and white earth (Mesozoic $CaCO_3$ + bentonite) from the Vicenza area are also known to have been historically used in artwork. Although, they are part of different much younger, Cenozoic geological formations, which are only called chalk because of the physical characteristics and colour, but are not, geologically speaking, chalk.

Calcareous Nanofossils

The microfossils present in chalk (and in calcareous clays [17]) are dominated by foraminifera and calcareous nannofossils. These groups are broadly used as biostratigraphical and paleoenvironmental markers, which is also

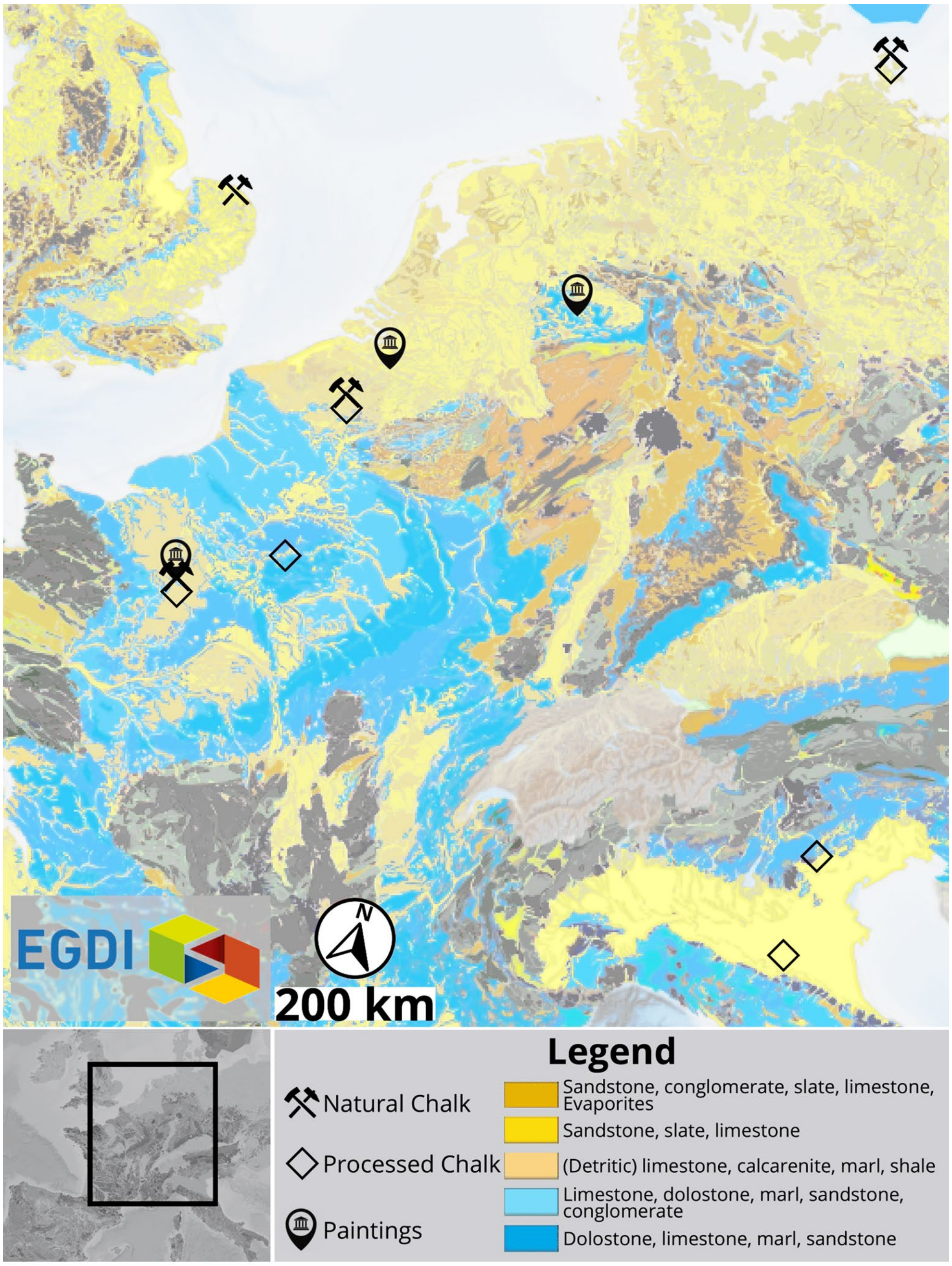

Fig. 2 Geological map (EGDI 1:1 Million pan-European Surface Geology, harvested from INSPIRE conformant National WFS services on GeologicUnit) of carbonates deposited from Permian (Paleozoic) onwards in Central Western Europe. Natural and processed chalks used for this study and region of the painting micro-samples [16]. The grey areas on the map are other rock types unrelated to this study

sometimes applied to cultural heritage and forensic provenance analysis.

The relative abundance of a taxa correlated with the presence/absence of a biogeographical marker can be related to the latitudinal distribution of water masses [18, 19]. The distribution and abundance of calcareous nannofossils is assumed to be related to nutrient concentration/availability, water temperature (Table 1), detrital input and surface-water salinity, according to Mutterlose [20]. Certain species are more sensitive than others, and their presence/abundance will record smaller changes in nutrient and temperatures [19]. Other variables are impacting nannofossil growth, such as sea water chemistry [21]. Also, each species fills an

Table 1 Palaeoecological preferences of selected Upper Cretaceous nannofossil species. Modified after Table 2 from Sheldon et al. [53]. The specimens were counted in the smear slides. The specimens without numbers were not determined in the assemblage. Rich = at least one specimen every 2 fields of view. Rare = at least 1 specimen determined by scanning electron or cross-polarised microscope. *Micula staurophora* can correspond to *M. decussata* according to literature

[%]	Water temperature		Productivity		Reference samples					Art sample		
Species	**Cold**	**Warm**	**High**	**Low**	**Rue/D**	**Nor/UK**	**Belg**	**Cha/F**	**Meu/F**	**FN**	**SM**	**NGL-Li**
Kamptnerius magnificus	X				Rich							
Nephrolithus frequens	X				< 3	Rare			Rare			
Micula decussata / staurophora	X	X		X	< 17	< 18	< 12	< 6	< 2	< 6	< 10	
Arkhangelskiella cymbiformis	X				2–41	< 3	< 7		< 3			
Watznaueria barnesiae		X		X	< 5	20–42	20–78	46–74	28–71	68–77	46–75	50
Prediscosphaera cretacea				X	< 6		< 1	< 2	< 1	< 1	12	
Eiffelithus turriseiffelii				X	< 8		< 8	< 1	< 5	< 16	< 3	
Discorhabdus ignotus			X								< 3	
Biscutum spp.			X		< 11		< 8					
Zeugrhabdotus spp.			X		< 3	< 9		< 4	< 5	< 3	< 3	

ecological niche, which should be taken into account in the assemblage variability analyses. In biogeography, the possibility of using calcareous nannofossil was first robustly demonstrated by McIntyre and Bé [18]. They studied coccolithophoridae assemblages of the Atlantic Ocean and distinguished 5 climatic groups, namely, tropical, subtropical, transitional, subarctic, and subantarctic, based on the temperature range for each calcareous nannofossil species [22–24].

Calcareous nannofossils are especially good markers for their quick evolution, since Late Triassic and broad geographic distribution, as well as small size and common occurrence already in small rock pieces. Biostratigraphical data were intensively collected, refined and improved for several decades [25–27]. Today, the key synthesis is available in the Nannotax database [28]. Nannofossils can be easily analysed in art primings, being the main component of chalk (up to 98%) and due to their minute size < 30 µm. Even though the study of provenance through micropaleontological studies has a great potential, it is still rarely used [11], [17–24].

The material in a natural rock undergoes diagenesis in different stages [4], [29–31]. The compaction and chemical recrystallisation that occurs during diagenesis [32] leads to the breakage and recrystallisation of the composing particles of the sedimentological layer, such as calcareous nannofossils. The possibility of determining fossils in chalk decreases the stronger the diagenesis is, also modifying the properties of the chalk [12, 33]. The number of determinable nannofossils (unbroken, not recrystallised) in the natural rock is therefore already an important factor for further analyses. When mined, chalk is often regarded as one homogeneous thick layer, even though there are fossils and events boundaries corresponding to different timeframes [4]. Some nannofossils contamination from other geological time frames can therefore occur.

When extracted from its outcrop (mining sites; [34]), chalk is milled and washed according to its usage, impurities and texture. The processes probably broke the most fragile microfossils, thus changing the ratio between the species from the natural outcrop. The processed material can then be sold or used. Also, depending on how the company treats the material (where they process it, where they pack it), contamination from other rock can occur and lead to ashtray the assemblage analyses.

After the processing, a preparatory layer made of a mixture of chalk and binders, such as animal glues or vegetal oils, is applied. Again, mechanical destruction can occur at this stage.

For material analysis of arts, scientists need samples. Sampling is generally done when the necessity to restore arises (using cracks and loose material), and if in-situ non-destructive measurements are not sufficient. The investigation of an artwork begins therefore before and continues during the restoration process [35].

In this study, we compared the assemblages of calcareous nannofossils from different localities in Europe, known for their historical mining and use for painting (Ruegen, Germany; Meudon, France; Champagne, France; Bologna, Italy; Mons, Belgium; Norfolk, England). We determined and quantified the index nannofossils in each material. Also, we compared natural (directly from outcrop) and processed (milled, washed) chalk to verify if the assemblage ratio between the species was similar. Finally, we analysed the nannofossil assemblages of three original paintings and compared them to our previous results.

Materials and methods

Kremer Pigmente GmbH & Co. KG (Aichstetten, Germany) [36] is a renowned company for their historical fabrication of pigments and art supplies. They offer an assortment of processed chalks from historical localities, such as Belgium, Champagne, Bologna, Sarti, and Ruegen chalk. They process the chalk after extraction, as well as pack the largest quantities directly on-site, reducing contamination across different sites. Though, smaller quantities can be packed at other locations.

The material used here are chalks from 4 regional field outcrops (Natural; France: ICr - Meudon; Belgium: BCr—Mons, Germany: RCr—Sassnitz; England: ECr—Norfolk), chalk prepared as powder from 7 Kremer GmbH & Co. KG quarries (58xxx), 1 bought in an art shop in Paris (Processed; France: MSC—Meudon, CC—Champagne 58000; BC—Belgium 58158, Germany: RC—Ruegen 58010; Italy: BC—Bologna chalk 58100, SC—Sarti chalk greyish 58190, VWE—Vicenza natural white earth 58180; Unknown: STC—Stone chalk white 58162) and 3 micro-samples from original paintings of different regions (SM—Belgium, FN—France, NGL-Li—Germany) with chalk preparatory layer(s) (Table 2).

Bologna chalk is mined in the region of Bologna in Italy, and more precisely from Veina del Gesso [37], which is a sulphur-rich Messinian-Tortonian formation. Bologna "chalk" is generally composed of gypsum and marls (Additional file 1: Appendix A3). This material is called "chalk" because of its macroscopic resemblance to geological chalk.

SM micro-sample is an oil painting on a wood panel, from Flemisch origin in the Brabant region. It dates to the last quarter of the sixteenth century. The painting is conserved in Seine-Maritime, France.

FN micro-sample is an oil painting on a fibre canvas (probably jute) from the early beginning of the nineteenth century [38]. This easel painting is conserved in the region of the Hauts-de-Seine, France.

NGL-Li micro-sample comes from a panel painting of "St.-Margaret" by the circle of the Master of Liesborn (oil paint on oak wood support; 80.7 × 47.9 cm). The painting was originally in a chapel in Lippstadt (Westphalia, Germany), and is now in the National Gallery of London's collection (not on display) [39]. It is a fragment from an altarpiece of the Virgin and Saints painted by an anonymous painter, referred to as the circle of the Master of Liesborn (named after the altarpiece he painted in the Benedictine Abbey at Liesborn). The painting dates to the second half of the fifteenth century.

Nannofossils slides from natural and processed chalk were prepared by conventional decantation method [40]. Painting micro-samples were disaggregated according to a custom procedure optimised by Jaques et al. [41], due to their particular composition, using heat, mechanical and chemical disaggregation processes.

The disaggregated material in solution was left to settle for 10 to 30 s, depending on the amount of material. Then a drop of the solution was applied on a glass slide for the calcareous fraction to settle and the liquid to evaporate. Finally, a cover slip #0 was fixed with Canada Balsam on

Table 2 Natural, processed and artwork samples classified by regions, prepared, analysed, and compared. The age refers to the geological timescale (G) for natural and processed samples, and the creation period (C) for artworks

Locality	Region	Age (G/C)	Sample	Sample
France [4]	Champagne	Late Cretaceous (G)	Processed	CC
	Meudon	Late Cretaceous (G)	Natural	ICr
			Processed	IC
	Paris, Ile-de-France	Early nineteenth century (C)	Easel painting, Hauts-de-Seine, France	FN
Germany [15, 37]	Sassnitz, Ruegen	Late Cretaceous(G)	Natural	RCr
			Processed	RC
	Lippstadt, Westphalia	fifteenth century (C)	"St.-Margaret" from the Master of Liesborn, National Gallery of London	NGL-Li
Belgium [38, 39, 41]	Mons	Late Cretaceous (G)	Natural	BCr
			Processed	BC
	Flemisch area, Brabant	Last quarter of sixteenth century (C)	Oil painting on wood panel, Seine-Maritime, France	SM
England [42, 43]	Cromer Beach, Norfolk	Late Cretaceous (G)	Natural	ECr
Italy	Bologna [44], [45]	Messinian-Tortonian (G)	Processed	IBC
	Italy	Unknown	Sarti Chalk; Processed	SC
	Vicenza [46], [47]	Cenozoic-Mesozoic	Processed	VWE

top. All disaggregated material was prepared in a minimum of 2 smear slides [42].

Calcareous nannofossil can be recognised in smear slides with a cross-polarised microscope (x1000 magnification). The quantification and determination of the micropaleontological assemblages of each material was done by using a Reichert and a Nikon polarising cross-polarised microscope in transmission mode on the smear slides. For statistical comparison of the nannofossil assemblages from reference material and grounds, the multivariate statistic techniques of the PAST software were used [43]. The specification of methods is given in Results.

We took high-resolution images of specimens using a MIRA 3XMU (TESCAN) scanning electron microscope in SE mode. We used a gold-coated smear stub preparation. Similarly to the smear slide, disaggregated material in solution was left to settle for 10 to 30 s depending on the amount of material. A drop of the solution was applied on a metallic stub and left to dry. The stub was then coated with an approximately 15 nm layer of gold.

Results

Characteristics of the calcareous nannofossil assemblages from natural and processed chalk

In natural and processed chalks from France (Meudon, Champagne), Belgium (Mons), Germany (Ruegen) and England (Norfolk), calcareous nannofossil were common to abundant. In the other materials (Sarti chalk greyish, Vicenza natural white earth (58,180; Italy), Stone chalk white) no nannofossils were found.

Bologna chalk was also analysed, and surprisingly in one batch of chalk, nannofossils occurred. The assemblage has a low diversity, with a significant dominance of *Watznaueria*.

The nannofossil assemblages from all localities show different preservation degrees (Additional file 1: Appendix A1/2/3/4). The France assemblages (ICr, IC, CC) (Additional file 1: Appendix A1) have a high percentage of broken particles from shells and destroyed coccoliths with moderate preservation. IC shows some etching and secondary overgrowth, but it remains relatively rare and negligible. Several nannofossils are perfectly preserved. Broken fragments are also more recognisable as broken nannofossils and shells than in the other assemblages. ICr shows more signs of etching, recrystallisation and overgrowth than IC and CC.

The nannofossil assemblage from Belgium chalk (BCr; BC) shows a high percentage of broken particles, mostly destroyed nannofossils (Additional file 1: Appendix A1). Secondary overgrowth appears on some specimens, but it is not constant. The dissolution of most taxa is negligible, with only minor etching signs. Nannofossils and recognisable fragments are relatively well preserved. Mechanical fragmentation is the main factor here.

England chalk (ECr) contains a high amount of fragments from shells and broken nannofossils. The ECr nannofossils assemblage shows strong signs of secondary overgrowth (Additional file 1: Appendix A2). Central areas are still visible for the thicker/stronger species, such as *Watznaueria* spp.

Nannofossils of the Rügen assemblages (RCr; RC), similarly to the other chalk assemblages, show different preservation degrees (Additional file 1: Appendix A2). RC shows a high dissolution–diffusion–re-precipitation of calcite, with the fusion of the nannofossils structural elements, and with secondary overgrowth on all specimens. Signs of etching appear on the best preserved nannofossils. RCr also shows signs of secondary overgrowth and dissolution–diffusion–re-precipitation of calcite, but specimens show different degrees of calcite accretion, not as strong as the RC assemblage.

The species composition of the nannofossil assemblages from France, Belgium, Germany and England, natural and processed chalk, are comparable. However, the relative abundance, especially of two taxa present in the assemblages, differs according to the origin of the chalk, allowing their discrimination (Fig. 3).

Ruegen chalk has a significantly high abundance of *Arkhangelskiella* spp. (20–60%; Fig. 3), which is a typical cold water taxa, along *Kamptnerius magnificus* and *Nephrolitus frequens*.

Watznaueria spp. are present in all samples, but their abundance is drastically different between Ruegen (0 – 12%) and the other chalks (19–74% for the reference chalks; Fig. 3). The percentage of Watzenaueria spp. is generally lower (max. 48%) in the England assemblage than in France and Belgium (max. 71–78%).

Non-parametric multivariate statistical method (non-metric Multidimensional Scaling: n-MMDS, Euclidean distance) was used to compare the nannofossil assemblages (Fig. 4).

Firstly, all the assemblages were included in the statistical analysis (Fig. 4A). We observed a clear distinction between Ruegen and Norfolk chalk from the others. Therefore, we only selected the chalks that looked more like a cluster to determine if smaller differences could be visible (Fig. 4B). We observed that the Belgium chalk has some small differences and a higher species variance than France chalks, but there is an unclear distinction between them. Meudon and Champagne chalk cannot be distinguished through the plots.

Both plots show statistically significant results with a very low stress (< 0.1; Sheppard plots in Fig. 4).

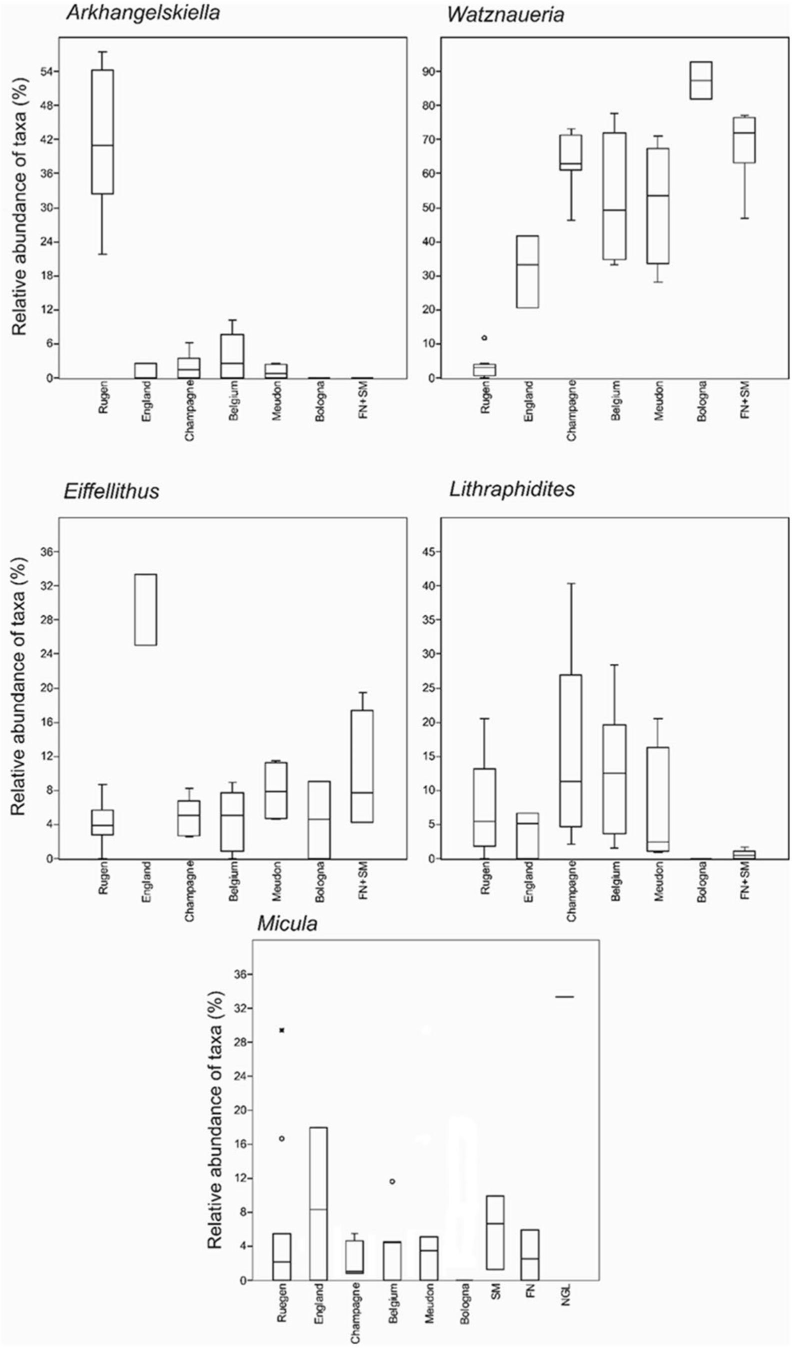

Fig. 3 Differences in relative abundances of the most common taxa in individual natural and processed chalks and art FN and SM.

Calcareous nannofossils in original paintings

Three micro-samples from paintings were analysed. FN and SM samples showed a high abundance of nannofossil (Additional file 1: Appendix A4), while NGL-Li only contained rare to very rare nannofossil (Additional file 1: Appendix A6).

FN and SM nannofossil preservation is excellent, with minor recrystallisation traces (Additional file 1: Appendix A4). Perfectly preserved specimens and low signs of etching and secondary overgrowth are comparable to the MSC assemblage preservation. More broken samples (central part and rims) appeared compared to processed and natural chalk. NGL-Li nannofossils observed

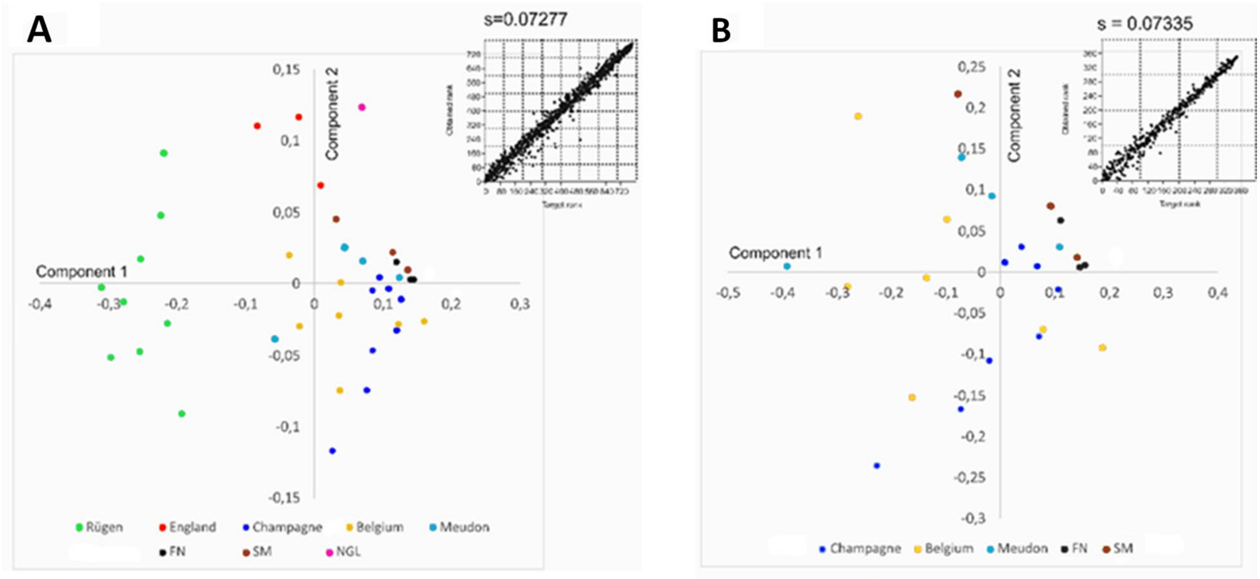

Fig. 4 Statistical classification of the calcareous nannofossil assemblages using non-metric multidimensional scaling, Euclidean distance. **A** Comparison off all samples; **B** Recalculation omitting the outliers chalk assemblages from Rugen, England, and the strongly damaged assemblage from NGL-Li. The Sheppard plots show high statistical reliability of the results

by a cross-polarised light microscope were heavily recrystallised. It was not possible to observe them by SEM.

Watznaueria spp. (68–77%) dominated the assemblages, although SM_0 has the lowest content among the samples (42% ; Additional file 1: Appendix A6).

NGL-Li was characterised by a very low diversity (3 species) and the dominance of *Watznaueria* spp. and *Micula* spp.

Discussion

Biostratigraphy

Lithraphidites quadratus was detected in all samples, except for NGL-Li and Bologna chalk. It is the best correlative biostratigraphical marker between all samples, confirming Late Maastrichitian geological deposition age of all materials [27, 28], [40, 54–56].

The *Arkhangelskiella* spp. found in the Ruegen assemblage are about 10 µm length (largest specimens), which would correspond to Maastrichtian period as well, according to Thibault [57] and Linnert and Mutterlose [21].

According to Thibault et al. [58], that studied the Equatorial Atlantic, the dominant taxa of the Maastrichtian assemblage were *Micula decussata* (5–49%), *Watznaueria barnesiae* (16–45%), *Cribrosphaerella ehrenbergii* (2–13%), *Prediscosphaera cretacea* (4–16%) and *Retecapsa* spp. (2–20%). All of them were found except for *Retecapsa* spp. Other common taxa were considered *Arkhangelskiella cymbiformis*, *Ahmuellerella regularis*, *Chiastozygus* spp., *Eiffelithus* spp., *Microrhabdulus* spp., *Prediscosphaera stoveri*, *Tetrapodorhabdus decorus*, *Thoracosphaera operculata* and *Zeugrhabdotus spiralis*. They reported this assemblage and considered it similar to low and mid-latitudinal sites. We found these genera, except for *Tetrapodorhabdus* spp. and *Thoracosphaera* spp.

Most of the determined species are present throughout the Late Cretaceous. The occurrence of *Staurolithites integer* and *Eiffellithus parallelus* in all samples except NGL-Li especially constricts the stratigraphical position of the material to Upper Cretaceous.

The taxa with the Lower Cretaceous stratigraphical range are considered reworked. *Micrantholithus* spp. range is Lower Cretaceous, and is only present in two smear slides from Kremer processed Belgium chalk. *G. obliquum* (Coniancian - Cenomanian) is only present in one smear slide from Kremer processed Champagne chalk. These two species are supposed to be reworked or contaminations during technical processes.

Concerning NGL-Li, the nannofossils assemblage only restricted the geological deposition age of the material to Albian-Maastrichtian. The absence of a better index taxa does not allow for a preciser geological deposition age.

Provenance analysis

The provenance of the artwork chalk from FN and SM was interpreted from the results of the nannofossil assemblages given by nMMDS (Fig. 4).

The nannofossil assemblage from the artwork NGL-Li probably represents the relict of the original assemblage, consisting of massive and dissolution-resistant taxa [59]. Although Švábenická (2007) [60] considers the presence of *M. staurophora* an indication of the boreal chalk (Ruegen), *M. staurophora* occurs in all studied chalks of this study. The highest percentage is at the highest latitude (Ruegen) and it decreases with the lowering of the latitude (Table 1; Fig. 4). Granchovsky [61] found a positive correlation between *M. staurophora* and *Arkhangelskiella* spp. and a negative one with *W. barnesia*, which our results do not fully confirm, but go into the same direction. Due to the strong secondary alteration of the nannofossil assemblage from the NGL-Li artwork, it is impossible to determine the origin of this chalk from calcareous nannofossil.

FN and SM art micro-samples were rich in calcareous nannofossil and allowed good comparison with the reference samples analysed (natural and processed chalk).

An important distinction between the reference samples was the abundance difference between *Arkhangelskiella* spp. and *Watznaueria* spp., mainly concerning Ruegen chalk. The biogeography of both species was described by Huber [62], and the absence of *W. barnesiae* in boreal (Ruegen) chalk by Švábenická [60]. *Watznaueria barnesiae* is an especially dissolution-resistant taxa common during the Cretaceous and generally associated to warm waters [53]. In both art assemblages, the absence of *Arkhangelskiella* spp. discriminated against Ruegen for the provenance of their material. Concerning Norfolk chalk, its assemblage was different in terms of abundance, particularly with the high number of *Cyclagelosphaera reinhardtii* and the increasing abundance of *Eiffelithus* spp., which distinguished it from the artworks' assemblages.

Nannofossils were found in one Bologna chalk sample, which is similar to the French and Belgium cluster, due to the high abundance of *W. barnesiae*. This low diversified assemblage of dissolution-resistant coccolith is considered reworked, or their could have been a mixing during chalk processing. These coccoliths are relics of a Cretaceous assemblage. The high resistance to dissolution of *Watznaueria* spp. is well known (e.g. Thierstein [59]). Bologna chalk clearly differs in terms of structural and chemical composition (gypsum) from the other chalk. There is therefore no reason to assume that the chalk from the artworks comes from the Bologna region.

Finally, based on the nMMDS (Fig. 4), the FN and SM assemblages are closer to the French chalk (Champagne, Meudon) cluster than the Belgium cluster (Fig. 4B). The assemblage preservation of FN shows also more similarity to Meudon chalk, which matches with FN painted in a workshop in Paris. For SM, which is a Flemish painting, we would have assumed the chalk would have been from Belgium, but Champagne chalk was considered the purest and whitest, and it was already traded, at least, since the fifteenth century. The SM nannofossil assemblage' preservation is not as good as the FN assemblage, with higher etching, recrystallisation and overgrowth. This would tend more to the similar preservation of Champagne chalk. Chalk being relatively cheap and Champagne being relatively close to Belgium, it is not surprising that the painter could have brought back French chalk or ordered it for a cheap price.

Conclusion

We tested the applicability of the calcareous nannofossils assemblages for determining the provenance of chalk from original painting preparatory layers. We compared the composition of the nannofossils assemblages from six Western to Southern European localities (Rügen, Norfolk, Mons, Champagne, Meudon, Bologna) to three chalk preparatory layers of original paintings dated between fifteenth to nineteenth Centuries.

According to multidimensional statistics (non-metric multidimensional scaling), Ruegen and England are different from France and Belgium's nannofossils assemblages, mainly in their ratio between *Arkhangelskiella* spp. and *Watznaueria* spp. France and Belgium chalks are rich in *Watznaueria* spp., while *Arkhangelskiella* spp. are nearly absent. On the contrary, Rügen chalk contains common to abundant *Arkhangelskiella* spp.

Multidimensional statistics (non-metric Multidimensional Scaling) showed similarity between the diversified nannofossil assemblages from two paintings (FN and SM) and the nannofossil assemblages from Champagne and Meudon. Although we cannot completely rule out Belgium as origin, the species present, and their ratios remaining very similar. The distinction between the France and Belgium nannofossil assemblages is complex and remains therefore unclear.

The third painting had a very poor and low-diversified assemblage, which did not enable its correlation to a specific chalk type.

Surprisingly, Mesosoic nannofossils (*Watznaueria* spp.) were recorded in the Bologna chalk.

This dissolution-resistant genus was naturally reworked during its deposition (Neogene), or the original gypsum material was mixed with a Mesosoic one during chalk processing.

Although the precision and complexity of this type of analysis on paintings can be discussed, calcareous nannoplankton are promising provenance markers for chalk grounds. We hope this study will ease the provenance verification of chalk-related artworks and engage further studies to expand the nannofossils assemblages referencing and comparison.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40494-023-00974-9.

Additional file 1. Figure A1 SEM images of nannofossils present in the assemblage from France (F) and Belgium (B). | Meudon Nannoflora | Champagne Nannoflora | Belgium nannoflora | **Figure A2** SEM images of nannofossils present in the nannofossils assemblage from Ruegen (R) and Norfolk (N). | Ruegen Nannoflora | **A2.1** England Cromer beach Norfolk Nannoflora | **Figure A3** SEM images of nannofossils present in the nannofossils assemblage from processed chalk of Bologna area, Italy. | Nannoflora | **Figure A4** SEM images of nannofossils present in the nannofossils assemblage from original paintings. | FN Nannoflora | SM Nannoflora | NGL-Li - Nannoflora | **A5** Lithostratigraphy |**A6** Table with species quantification.

Acknowledgements

CzechNanoLab project LM2018110 funded by MEYS CR is gratefully acknowledged for the financial support of the measurements at CEITEC Nano Research Infrastructure. The authors are grateful for the access to painting micro-samples facilitated by Quentin Arguillère and Marika Spring, and to Kremer GmbH & Co. KG for the processed chalks.

Author contributions

VAJJ wrote the main manuscript, prepared Figs. 1 and 2, the tables and the Appendix. KH determined and quantified the species, prepared the figure statistics 3 and 4. Both authors reviewed the manuscript.

Funding

This research was carried out under the COOPERATIO project.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors declare there is no conflict of interest regarding the publication of this article, and that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Received: 30 January 2023 Accepted: 10 June 2023

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