

Identification of A Liverwort Fossil on Pottery from A Church Excavation in Northern Sudan: Evidence for Moist Paleoenvironment

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ABSTRACT:

This paper aims to document the first record of coalified compression fossil of a liverwort on pottery from a church excavation in the desert, northern Sudan. The outline or surface feature of the fossil is clearly observed. Morphological distortion is minimal while the internal anatomy is not preserved. The shapes of the archaeological specimens are closely similar to the morphology of a *Riccia* sp collected from the Red Sea state and the Blue Nile bank in Khartoum state. Energy-Dispersive X-ray fluorescence (EDXRF), Scanning Electron Microscopy with Energy (SEM/EDS), and Gas Chromatography and Mass Spectroscopy (GC-Mass) analytical techniques were used to determine the chemical composition of the fossil materials. Higher concentrations were reported for Carbon and Oxygen indicating the organic nature of the materials and also excluded the presence of manganese dentrites which perfectly imitate liverwort fossils. Also n-hexadecanoic acid, dodecanoic acid, tetradecanoic acid, hexadecanoic acid, methyl ester, cis-9-hexadecenoic acid, 9, 12-octadecadienoic acid (z,z)-, methyl ester, tert-butyl dimethylsilyl decanoic acid were recorded for the fossil materials. This study also provides additions to the chemical profile for the fresh material from the same genus which provides important data or monitors for the assessment of the chemical or thermal alteration in the corresponding coalified compression fossil.

KEYWORDS: Liverwort; Fossil; Paleoenvironment; Desert; Northern Sudan.

1. INTRODUCTION

Liverworts are small flowerless green plants with leaf-like stems or lobed leaves, occurring in moist habitats. They belong to the bryophytes which include also mosses and hornworts. Several studies about taphonomic effects on bryophytes have shown that moss and liverwort gametophytes and sporophytes material has a high potential for fossil preservation (Kroken et al., 1996; Kodner and Graham, 2001; Hemsley, 2001; Graham et al., 2004). Bryophytic impressions are relatively rare, but in some can provide taphonomic information, such as by revealing mass accumulations (mats) of Marchantiophyta thalli in a parautochthonous assemblage. Charcoalified bryophyte occurrences are rare: only two moss taxa have been formally described from the Upper Cretaceous (Santonian and Campanian, c. 85e75 Ma) of Georgia (USA) by Konopka *et al.* (1997, 1998). About one-third of 449 fossil bryophyte records (172; 38%) come from amber of Cretaceous or Cenozoic age (Alexandru *et al.*, 2018: 381). This paper aims to document a record of coalified compressions of a liverwort on pottery from a church excavation in the desert, northern Sudan. Morphological and chemical analysis methods were adopted to identify and confirm the nature of fossil materials.

2. MATERIALS AND METHODS

2.1. Materials

In Ga'ab El Lagia oasis (19°28'.356 N / 030°16'.266 E) which is the biggest villages in El Ga'ab depression (Northern Sudan) (Map 1), an ancient church which is probably abandoned since sixteenth has been excavated by El Ga'ab Archaeological Project in season 2017-2018 (Tahir, 2018) Figs.(1 and 2). 16 layers of 10 cm width were excavated. From all layers, a large number of pots and pottery sherds which show a similar- structured, black compressed materials on their surface were recovered.

2.2.1. Methods

The following Standard morphological and chemical analysis methods were adopted in this study to identify the nature of the compressed material on the sherds:

I. Morphological identification method:

Morphological identification of the archaeological specimen was done by comparing the outline of the surface feature of the black compressed materials with a closely similar morphology of two species of the plant genus *Riccia* collected from Erkowit in the Red sea state and the Blue Nile bank in Khartoum state. Delta Optical Smart PRO Microscope with a 5MP sensor was used to obtain magnified images for the examined materials.

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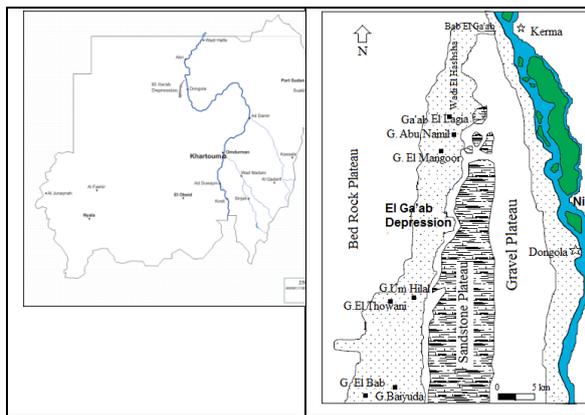


Figure 1. location of El Ga'ab Depression



Figure 2. Image showing Ga'ab El Lagia Church Excavation - Northern Sudan

2.2.2. Chemical Identification method:

2.2.2.1. Gas Chromatography and Mass Spectroscopy (GC-Mass):

Three fractions of methanolic extracts were obtained from powdered samples of the archaeological materials, the fresh reference sample obtained from the Blue Nile bank, and a control sample from the surface of the pottery that free of the fossil. They were investigated using Gas Chromatography and Mass Spectroscopy for the determination of bioactive volatile compounds. GC-MS analysis of the samples was carried out using Shimadzu Make QP-2010 with a non-polar 60 M RTX 5MS Column. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 40°C and held for 3 min and the final temperature of the oven was 480°C with the rate at 100C [min.sup.1]. 2-µL samples were injected with splitless mode. Mass spectra were recorded over 35 - 650 amu range with electron impact ionization energy 70 eV. The chemical components from the methanolic extracts were identified by comparing the retention times of chromatographic peaks using Quadra pole detector with NIST Library to relative retention indices. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS.

2.2.2.2. X-ray fluorescence (XRF) and scanning electron microscopy with energy dispersive X-Ray spectroscopy (SEM/EDS):

These standard techniques were used to characterize the elemental composition of the archaeological materials.

3. RESULTS

3.1. Identification of the fossil based on the morphological features:

The materials are two-dimensional remains that have been compressed on the surface of pottery from a liverwort *Riccia* sp. The morphology of the margin and the outline of the surface feature are clearly shown. Morphological distortion is minimal while the internal anatomy is not preserved. The shapes of the archaeological specimens were observed to be closely similar to the morphology of a specimen from the Red Sea state (Fig. 3) and another one from the Blue Nile bank in Khartoum state (Fig. 4).

3.2. Identification of chemical constituents of the fossil:

Chemical components separated by the GC-Mass technique in both archaeological and its reference samples proved the presence of different forms of decanoic acid and its derivatives (salts and esters). Undecane and 7, 9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-dien are separated from both samples. Archaeological sample was dominated by 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien (12.14%) while the reference sample is dominated by e 9,12-Octadecadienoic acid, methyl ester (19.13%). Fractionations reported for the archaeological samples, the recent fresh reference sample, and a control sample are presented in tables (1, 2, and 3). (XRF) and (SEM/EDS) analysis showed that the highest concentration is reported for carbon and oxygen using indicating the organic nature of the materials. EDS mapping for both Carbon and manganese excludes the presence of the manganese dendrites which perfectly imitate liverwort fossils Figs. (5,6, and 7)



Figure 3. (a) Reference specimen from the Red sea state, (b) and (c) Coalified compression of *Riccia* sp. from the archaeological site.

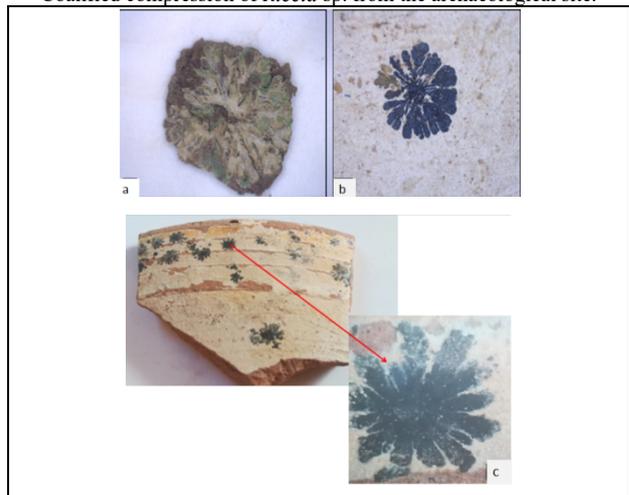


Figure 4. (a) Reference specimen from the Blue Nile bank in Khartoum state (b and c) coalified compressions of *Riccia* sp. from the archaeological site.

Table 1. Gas Chromatography- Mass Spectrometry characterization of methanol extract of the archaeological material

S.No.	Retention time (Sec.)	Name of compound	Area%
1	4.029	Undecane	3.21
2	9.467	Cyclooctasiloxane, hexadecamethyl-	6.33
3	11.036	Heptasiloxane, hexadecamethyl-	6.39
4	12.277	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien	12.14
5	12.447	n-Hexadecanoic acid	4.15
6	12.575	Heptasiloxane, hexadecamethyl-	7.78
7	14.048	Heptasiloxane, tetradecamethyl- (Mark-V)	6.76
8	15.400	Heptasiloxane, hexadecamethyl-	6.15
9	16.701	Azacyclotridecan-2-one	7.32
10	18.090	Heptasiloxane, hexadecamethyl-	5.93
11	18.242	Cyclooctasiloxane, hexadecamethyl-	6.54
12	19.359	Diioctyl phthalate	5.53
13	20.757	Trans- Traumatic acid	7.32
14	21.899	9,12-Tetradecadien-1-ol, acetate (Z,E)-	7.10
15	16.701	3,4-Dihydroxymandelic acid, 4TMS derivative	6.96

Table 2. Gas Chromatography- Mass Spectrometry characterization of methanol extract of the reference material

S.No	Retention time (Sec.)	Name of compound	Area%
1	3.525	3-Butyn-1-ol	1.36
2	4.205	Undecane	12.37
3	9.021	1,2-Benzenedicarboxylic acid,2-ethoxy-2-oxo	2.74
4	12.258	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien	3.50
5	13.833	9,11-Octadecadienoic acid, methyl ester	12.81
6	14.033	2-Methyl-6-(1- propenyl) piperidine	13.16
7	14.117	Glycerol 1- palmitate	15.64
8	14.187	9,12-Octadecadienoic acid, methyl ester	19.13
9	17.439	9,12- Octadecadienoyl chloride,(Z,Z)-	14.68
10	17.806	Allylnonanoate	4.62

Table 3. Gas Chromatography- Mass Spectrometry characterization of methanol extract of the pottery surface free of archaeological material

S.No	Retention time(Sec.)	Name of compound	Area%
1	6.401	Cyclohexasiloxane, dodecamethyl-	6.50
2	7.884	5-tetradecene, (E)-	5.85
3	8.005	Cycloheptasiloxane, tetradecamethyl-	6.62
4	8.317	Dodecanoic acid,methyle ester	2.56
5	8.622	Dodecanoic acid	7.46
6	9.032	Diethyl phthalate	18.07
7	9.334	Benzene (1-butylloctyl)-	1.95
8	9.433	Benzene (1-propylnonyl)-	2.04
9	9.467	3,4-Dihydroxyphenylglycol, 4TMS derivative	3.67
10	9.636	Benzene,(1-ethyldecyl)-	3.46
11	10.002	Benzene,(1-methylundecyl)-	2.23
12	10.082	Ethanol, 2-(dodecyloxy)-	5.96
13	10.236	Benzene, (1-pentylheptyl)-	3.09
14	10.287	Benzene,(butylloctyl)-	3.06
15	10.415	Benzene, (1- propylnonyl)-	2.45
16	10.644	Benzene,(1-ethyldecyl)-	1.74
17	11.035	Hippuric acid, 2TMS derivative	4.25
18	12.135	Heptacosanoic acid, methyl ester	2.80
19	12.575	Heptasiloxane, hexadecamethyl-	1.73
20	14.049	2,6-Dihydroxybenzoic acid, 3TMS derivative	1.75
21	15.401	Heptasiloxane. Hexadecamethyl-	1.86

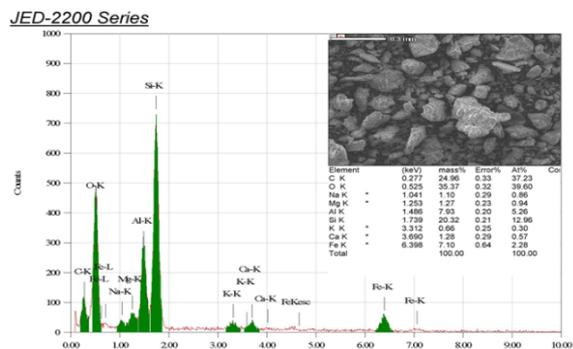


Figure 5. SEM-EDS analysis of a homogenized sample of the archaeological material

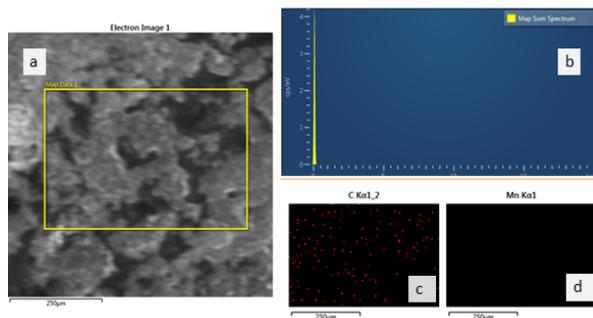


Figure 6. Elemental analysis for the selected area on the surface of the archaeological material: (a) SEM image, (b) EDS spectra, and (c) EDS mapping taken from carbon, (d) EDS mapping taken from manganese

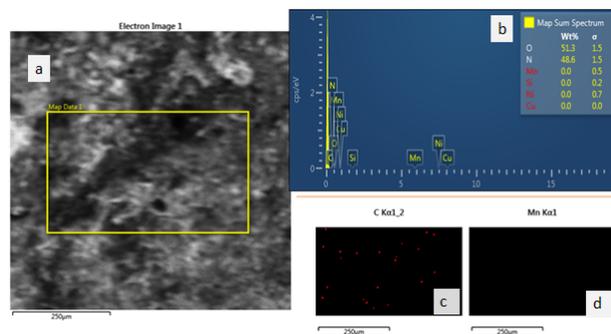


Figure 7. Elemental analysis for the selected area on a surface free of the archaeological material: (a) SEM image, (b) EDS spectra, and (c) EDS mapping taken from carbon, (d) EDS mapping taken from manganese

4. DISCUSSION

The possible route of entry of this plant in the studied area is completely natural and it differs from that of other archaeological plant remains. Remains such as residues of crop processing like cereal leaves, pollen and stems fragments are usually encountered at most settlements, either because the processing of the crop took place in this site or brought intentionally as fodder, fuel, or building material. In this case, the place in which the identified liverwort is found is probably the place in which it was naturally growing. Recording of many separated individuals of this plant in the same place is another evidence of a natural habitat in which the plant was successfully multiplied and spread around the area. Bryophytes tolerate high temperatures (in some species up to 80-100 °C) when dry and stay dormant and survive in the very hot desert. The presence of liverwort preserved materials may not be available in all archaeological sites reported in the desert of northern Sudan. The Underground water oasis phenomenon in El Ga'ab as a depression is available at a higher level of 1.8-4 m (Tahir, 2012: 102), some intervals after abandoning, making damp habitat where such lower plant propagates. The materials are recovered near the walls of the church where shade, water vapor, and air moving very slowly close to the ground are good humid microclimate required for the plant growth. Rough Surface of the pottery which contains alkaline soil trap and hold the water for a long time providing good germination growth, and sexual reproduction condition for liverwort. Liverwort grows on soil that accumulates in the pottery pits. After the establishment of the first colony, the colony may spread to the surrounding area. (XRF) and (SEM/EDS) analysis results excluded the possibility that the studied remains are

manganese dendrites which are inorganic crystals (minerals) that grow on porous materials such as sandstone, slate and ceramics and perfectly imitate liverwort namely *Riccia* sp.. Recording the presence of decanoic acid derivatives in the Riccian fossil is in harmony with the GC-MS analysis results reported by different authors. Hexadecanoic acid or palmitic acid and pentadecanoic acid in liverwort were reported by Abay et al. (2013), Hexadecanoic acid is also reported by Wankhedet and Manik (2015) n-hexadecanoic acid, dodecanoic acid, tetradecanoic acid, hexadecanoic acid, methyl ester, cis-9-hexadecenoic acid, 9,12-octadecadienoic acid (z,z)-, methyl ester, tert-butyl dimethylsilyl ester, and hexadecanoic acid were reported by Negi (2015).

From this research, identification of this bryophytic fossil will be added to the preservation potentiality of such structurally delicate organisms. Also, characterization of some of the chemical constituents of the studied material is a new data added to the diagnostic paleobiochemical profile of the fossil of this taxon which needs analysis of more specimens from different strata of the genus *Riccia* fossil. The use of living material from the same genus which is not been subjected to fossilization also provides important data or monitors for the assessment of the chemical or thermal alteration in the corresponding coalified compression fossil. Paleo-chemical data also help in assessing the physical labile constituents used to infer the maximum temperature experienced by the fossil. Polymerization reactions are degassing resulting from dehydration or decarboxylation indicated by color change of the organic compound. Results of this research also considers the pottery surface in the desert oasis as a new suitable place to find this type of plant fossil and could be added to the rocks in river deltas, lagoons, along rivers, and in ponds, as reported by Taylor and Taylor, (1993). The organic material compressed on the pottery surface and leaked into pottery surface could be extracted and used for Carbon 14 dating. So dating and knowing the level at which the pottery is found will provide the underground water levels during the obtained date. It provides archeologists the date of burial of the sherds. This is a new method for environmental archaeologists to adopt in paleomicrohabitat reconstruction studies.

5. CONCLUSION

The liverwort fossil is preserved on pottery surface under certain circumference as bio-archaeological materials. The fossil is a valuable evidence which can be used in many aspects of the archaeological sciences specially environmental archaeology. The organic material compressed on the pottery surface and leaked into the pottery could be extracted and used for Carbon 14 dating. This is a new method for environmental archaeologists to adopt in paleomicrohabitat reconstruction studies.

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