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If speed is of the essence: rapid analysis of ambergris by APCI compact mass spectrometry

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Chemical analysis of flotsam ambergris

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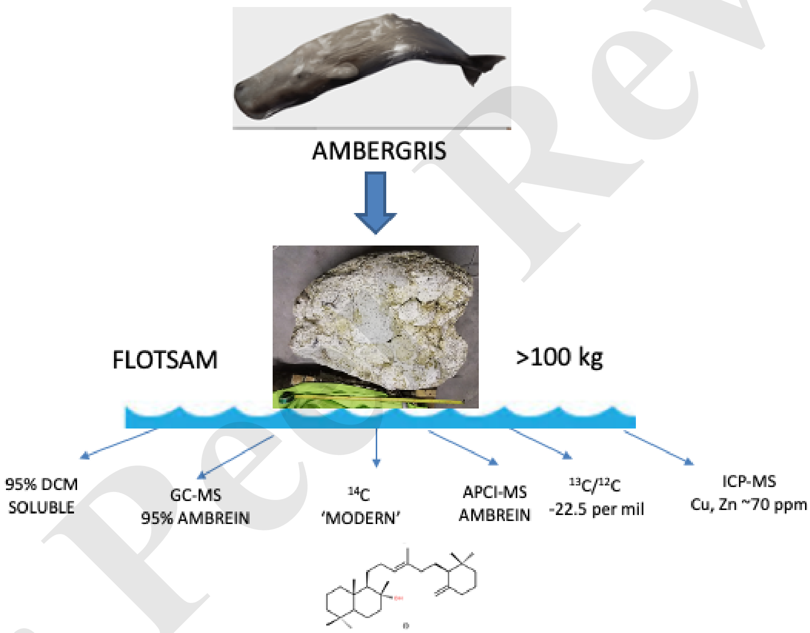
AMBERGRIS
FLOTSAM
>100 kg
95% DCM SOLUBLE
GC-MS 95% AMBREIN
^{14}C 'MODERN'
APCI-MS AMBREIN
^{13}C/^{12}C -22.5 per mil
ICP-MS Cu, Zn ~70 ppm

To be submitted to *Natural Product Research* as a Short Communication

Running Head: Analysis of flotsam ambergris

Chemical analysis of flotsam ambergris

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Abstract

The natural product ambergris is only rarely found on beaches as jetsam. Even more scarce, or even absent, are accounts of flotsam ambergris.

Here, we report the chemical analysis of a rare, large piece (>100kg) of flotsam found in the Atlantic in 2019.

About 95% of subsamples from the outside of the coprolith was soluble in dichloromethane; of this, FTIR spectroscopy, APCI-MS and GC-MS indicated the presence of ambrein. Radiocarbon dating indicated that the sample was post 1950s in age. The $^{13}C/^{12}C$ isotope ratio (-22.5 ‰) was typical of those reported to date for whale 'body' ambergris.

Metals of ambergris have hardly been reported previously. The distribution found here for the flotsam, was dominated by copper and zinc, which is similar to that of several squid species. This is also consistent with the presence of squid beaks in the coprolith. Squid are a major prey species of sperm whales.

Word count 149

Keywords: Ambergris, flotsam, sperm whale, *Physeter macrocephalus*, Atlantic, Madeira, ambrein, metals, squid, copper, zinc.

1. Introduction

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In 1954, in the journal *Nature*, Clarke reported a "huge haul of ambergris" (Clarke 1954), which is a rare natural product of sperm whales *Physeter macrocephalus* (Cornon 1955, Clarke 2006, Mikhalev 2014). This 'body' ambergris was found inside a male whale in the Southern Ocean (Clarke 1954). Ambergris has also been rarely but consistently found as jetsam on beaches around the globe (Brito et al. 2016; Rowland et al. 2018, 2021; Wilde et al. 2020). Since it is found as jetsam, ambergris must logically also occur as flotsam, but so far as we are aware, no scientific reports of flotsam ambergris have been published.

Here, we studied the chemical composition of a floating boulder of ambergris (flotsam) retrieved from the Atlantic in 2019, which weighed over 100 kg (Figure S1). Flotsam ambergris is very rare, and this is an unusually large piece.

2. Results and Discussion

Jetsam ambergris sometimes contains whole or fragments of squid beaks, which were abundant in the present flotsam sample (Figure S1). Scientific methods for the assignment of ambergris on the basis of ambrein (I) content, have been described in detail previously, including the use of radiocarbon dating (Rowland et al. 2019), Fourier transform infra-red spectroscopy (FTIR), gas chromatography-mass spectrometry (GC-MS) with and without derivatisation (Governo et al. 1977; Rowland and Sutton 2017), ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy (Rowland et al. 2018), isotope ratio GC-MS (Rowland et al. 2021), atmospheric pressure chemical ionisation-MS (APCI-MS; Rowland et al 2024) and DNA profiling (Macleod et al. 2020). In the present study of flotsam, several of the above methods were used to assign the material as ambergris.

For instance, radiocarbon dating (Table S1) indicated an age post-1950s (i.e., 'modern') with an F14C value (1.0566) consistent with this (Rowland et al. 2019).

About 95 $\pm$ 3% (n=3) of each of three 40-60 mg subsamples was soluble in dichloromethane (DCM). This is typical of many samples of jetsam ambergris examined previously, which also had high contents of organic-soluble material (Rowland et al. 2019). The FTIR spectra of DCM extracts contained the typical features of spectra of ambergris solutions, dominated by absorptions due to the functional groups of ambrein (I). Thus, a broad transmittance at 3372 cm^{-1} was attributed to H-bonded hydroxyl O-H stretching. A weak transmittance at ~ 3067 cm^{-1} was indicative of unsaturation and attributed to C-H stretch in an alkene. Transmittances at 2925 and 2863 cm^{-1} were attributed to C-H stretching in methyl and methylene groups and those at 1461 and 1382 cm^{-1} to the corresponding bending vibrations. Transmittances at 1644 cm^{-1} were attributed to C=C stretch and at 887 cm^{-1} to the =C-H out of plane bend (cf Governo et al. 1977; Rowland and Sutton 2017; Rowland et al. 2018).

Similarly, the ^{1}H and ^{13}C NMR spectra (Figure S2) comprised resonances indicative of the distinctive features of ambrein, including those assigned to the alkenic H and C (cf Rowland et al. 2018, 2018a). For example, resonances at 4 to about 5 ppm (Figure S2a) in the ^{1}H NMR spectrum were assigned to alkenic protons. The two broad singlets occurring at 4.5 and 4.7 ppm were assigned to the two methylenic protons (Rowland et al. 2018). Similarly, the triplet at about 5.2 ppm was assigned to the vinylic proton at 3' (Figure S2a). These characteristic resonances allow the presence of ambrein (and identity of the origin of the sample as ambergris) to be assigned. In addition, the resonances due to two methyl groups of ambrein were also assigned (Figure S2a). The presence of minor unknown constituents was indicated by other minor resonances in the region between 0.5 and ~2.2 ppm. These underlay the characteristic resonances of pure ambrein (Rowland et al. 2018). The ^{13}C NMR spectrum of the whole dichloromethane extract of the flotsam ambergris re-dissolved in deuterated chloroform, is shown in Figure S2b. Resonances at ~100-150 ppm were assigned to alkenic carbon atoms by comparison with published spectra (Rowland et al. 2018). The resonances occurring in this region were each assigned to C6", 4', 3' and the methylenic C6"=CH_2. In addition, the resonance due to C2 was also assigned (Figure S2b).

GC-MS indicated an average composition of 95 ± 0.5% (n=3; Table S2) ambrein, measured and identified as the TMS ether (Figure S3; cf Rowland and Sutton 2017; Rowland et al. 2018, 2018a). Thus, a small ion *m*/*z* 485 was assigned to the $M^{+\cdot}$-CH_3 ion of the TMS ether (Rowland and Sutton 2017), whilst a significant ion at *m*/z 143 was attributed to a mono-unsaturated, C_4H_6-OTMS moiety (Rowland and Sutton 2017).

Positive ion APCI-MS (Figure S4; cf Rowland et al. 2024) also confirmed the presence of ambrein. The spectrum was characterised by a base peak ion, *m*/*z* 411, shown previously by high resolution accurate mass APCI-MS to originate from the protonated molecular ion (*m*/*z* 429; $[MH]^+$) of the hindered alcohol ambrein (I) by water loss (Rowland et al. 2024).

The approximate $^{13}C/^{12}C$ isotope ratio was -22.5 ‰ (Table S1). (Repeat analyses established previously that the reproducibility of the latter method was ± 0.4 ‰; Rowland et al. 2019). For comparison, the stable isotopic compositions of the carbon in jetsam ambergris from both hemispheres examined previously had a mean value of -21.6 ± 1.9 ‰; n=26: Rowland et al. 2019). The stable isotope ratios of blubber, skin, liver and muscle from seven northern hemisphere sperm whales from the Adriatic coast of Southern Italy was −18.5 ±1.2 ‰ (Mazzariol et al. 2011).

ICP-MS revealed the relative and absolute concentrations of metals in the flotsam (Figure S5). Lederer (1950) postulated that the metals in ambergris might reflect the metal composition of squid, particularly the haemolymph, in which copper complexed as haemocyanin, would be the major metal. Ohloff et al. (1977) suggested that copper porphyrins in ambergris would act as catalysts for photo- and auto-oxidation of ambrein; photo-oxidation experiments with 5, 10, 15, 20-tetraphenyl-21H, 23H-porphine copper (II), supported this (Rowland et al. 2018a). Few data for metal contents of ambergris have been published to date.

Copper was the major element detected in the flotsam ambergris studied herein (~70 µg g^{-1} dried weight in the solid; Figure S5), but concentrations of zinc were similar (~50 µg g^{-1}), whilst iron and cadmium were present at appreciable, but somewhat lower, concentrations (~20 µg g^{-1}). Although ambergris is heterogenous (Baynes-Cope 1962), the metal contents of triplicate samples were quite reproducible herein (Figure S5). This metal profile suggests to us that the metal content of the flotsam reflects that of the liver, mantle, eyes and haemolymph of the squid in the sperm whale diet, rather than that of squid haemolymph alone (cf Lederer 1950). Examination of a wider collection of samples (e.g., those studied by Rowland et al. 2019), will now allow this theory to be tested further.

3. Experimental

The experimental details are provided in the Supplementary Material.

4. Conclusions

Analysis by multiple methods of a large piece of rare flotsam ambergris found in the Atlantic in 2019 produced data consistent with a post 1950s origin from a sperm whale. The metals profile was similar to that of the 'whole' body (e.g., liver, mantle, eyes and haemolymph) of the squid in the sperm whale diet, rather than that of squid haemolymph alone.

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Running Head: Analysis of flotsam ambergris

SUPPLEMENTARY MATERIAL

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Keywords: Ambergris, flotsam, sperm whale, *Physeter macrocephalus*, Madeira, Atlantic, ambrein, metals, squid, copper, zinc.

Experimental

A somewhat elliptical boulder of grey ambergris was recovered by boat (Figure S1a) from the ocean off Madeira Island (Eastern Mid Atlantic) in September 2019. It weighed over 100 kg and had approximate dimensions 80 x 50 x 50 cm (Figure S1). The boulder had greenish algal growths and goose barnacles on the outside and obvious pieces of squid beaks imbedded in the mass.

Figure S1. Flotsam ambergris (ca 100 kg) recovered off Madeira, September 2019. (Scale: 1m). **Insert.** Subsample of flotsam ambergris. Probable squid beaks are evident (bottom, dark features). (Scale ~ 13 cm).

Main photo: A. Berenguer. Insert: S. Rowland.

A piece weighing about 43 g (Figure S1, insert), free of obvious outgrowths, was removed from the outside for analysis of small aliquots by published methods including ^{14}C radiocarbon dating by accelerator MS (Table S1: Rowland et al. 2019), NMR spectroscopy of a $CDCl_3$ extract (Figure S1: cf Rowland et al. 2018), FTIR spectroscopy of a dichloromethane (DCM) extract (cf Rowland and Sutton 2017; Rowland et al. 2018), GC-MS analysis of a silylated DCM extract (Figure S3, Table S2: cf Rowland and Sutton 2017) and direct atmospheric pressure chemical ionisation (APCI) MS (Figure S4: cf Rowland et al. 2024). Solvent and procedural blanks were also examined.

Table S1. Data for ^{14}C radiocarbon dating, ^{13}C isotopic ratio ('Delta C13') and elemental (CN) composition of flotsam.

BRAMS S#	User Label	C14 Age	C14 Sig.	Delta C13	F14C	F14C Sigma	Material	C%	N%
3558	ACL 834	-442.3	13.5	-22.5	1.0566	0.00177	organic matter	89.3359	0.33277

Table S2. Compositional data (%) for blank-subtracted GC-MS detectable organic components of triplicate samples (a-c) of trimethylsilylated DCM extracts of flotsam.

Retention Time (min)	Sample a	Sample b	Sample c
29.68	0.3	0.1	0.2
30.43	0.2	0.1	0.2
31.00	0.8	0.9	1.2
31.48	0.3	0.4	0.4
31.84	0.6	0.7	0.4
31.95	2.4	3.2	2.8
32.56	95.4	94.5	94.9

Figure S2 (a). ^{1}H NMR spectrum of flotsam $CDCl_3$ extract.

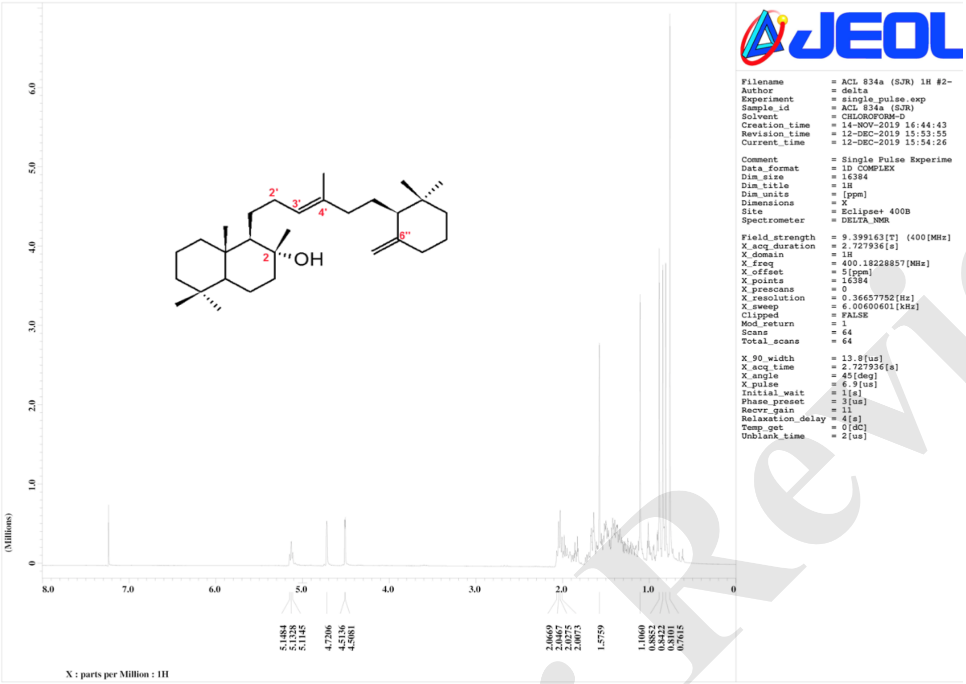


Figure S2(b). ^{13}C NMR spectrum of flotsam $CDCl_3$ extract.

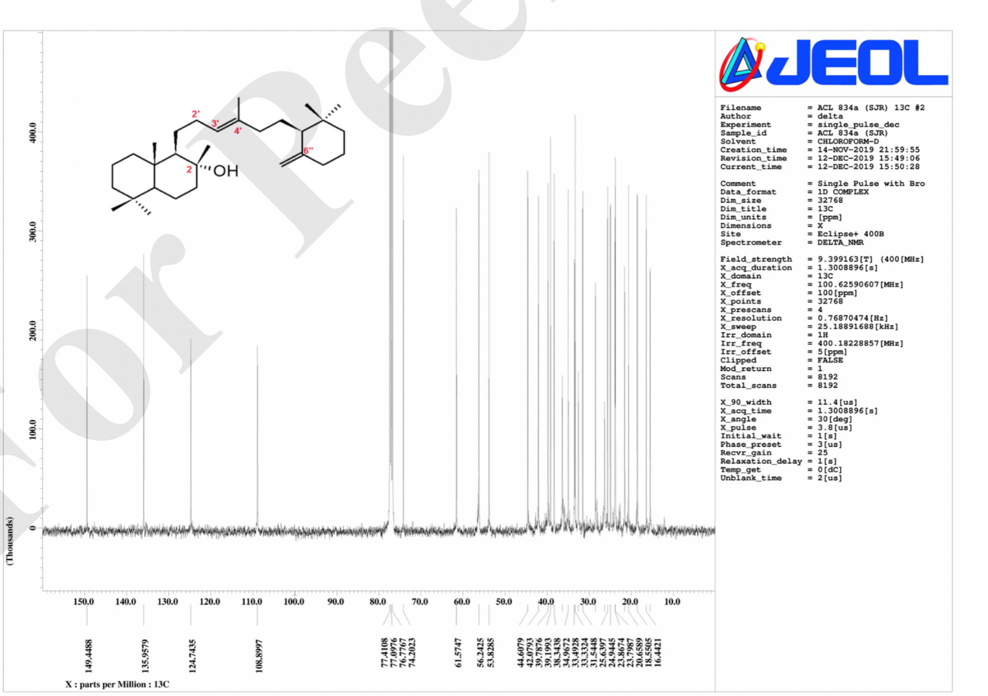


Figure S3 (a). GC-MS total ion current chromatogram of trimethylsilylated extract of flotsam. Component with retention time 32.56 min, assigned as due to ambrein-TMS ether.

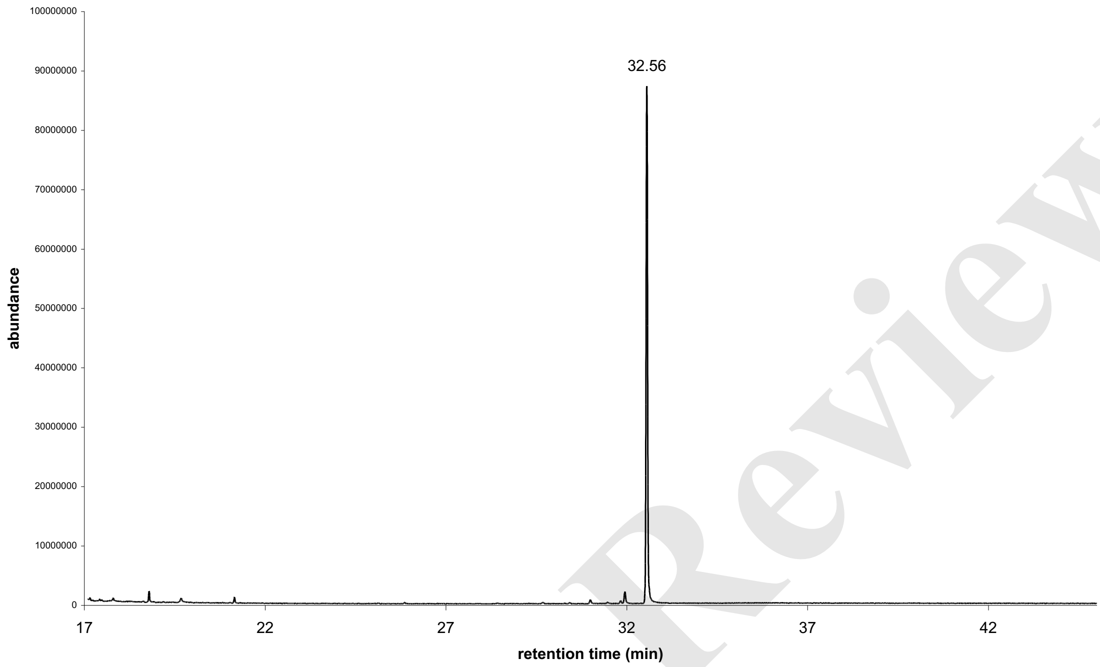


Figure S3 (b). GC-MS mass spectrum of major component in trimethylsilylated extract of flotsam, assigned as ambrein TMS ether (cf Rowland and Sutton 2017; TMS derivatising group not shown on structure I).

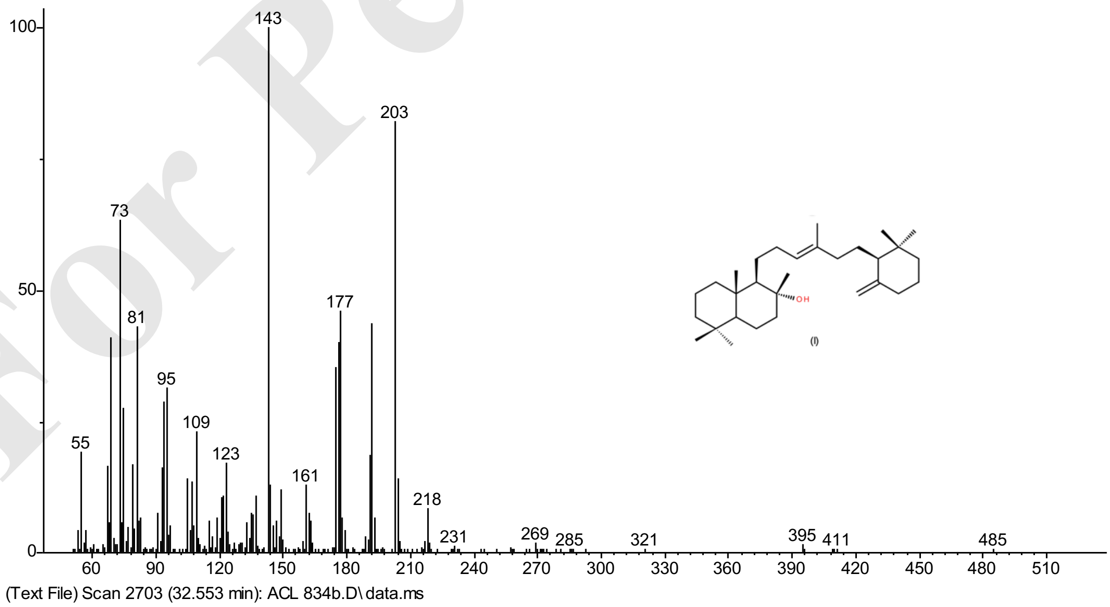
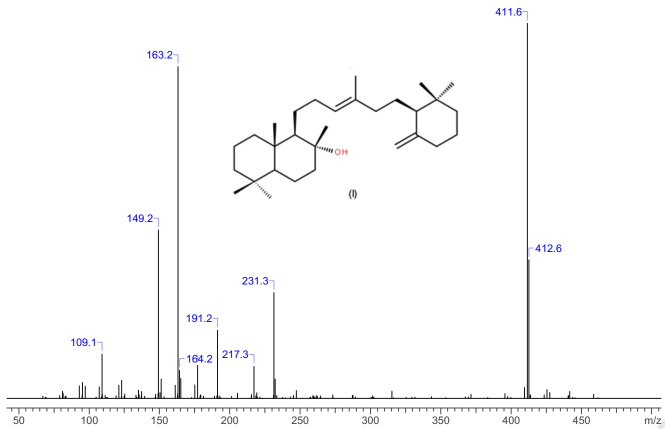
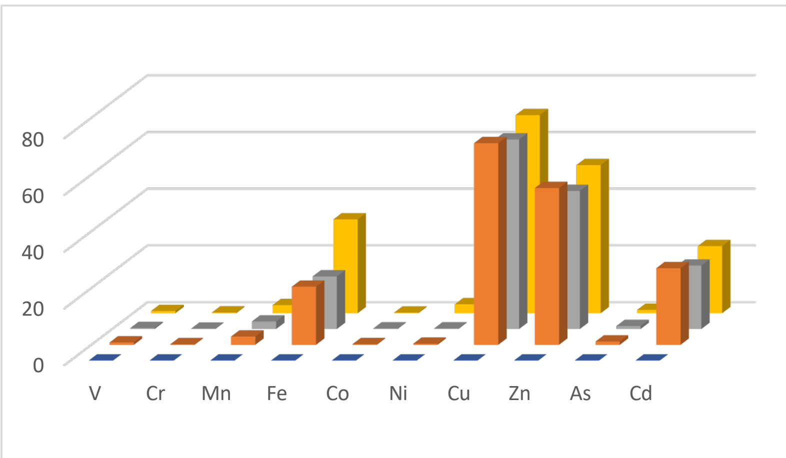


Figure S4. APCI positive ion mass spectrum of flotsam.



Acidic extracts were examined by inductively coupled plasma-mass spectrometry (ICP-MS). Metal contents were quantified using a Thermo (Hemel Hempstead, UK) iCAP RQ inductively coupled plasma- mass spectrometer operated in KED mode. Prior to analysis the instrument was tuned using a multi-element solution (Ba, Bi, Ce, Co, In, Li, and U at 1 μg L^{-1}) to ensure performance to manufacturer's installation specifications for stability and sensitivity and oxide (CeO/Ce) formation of <0.01%. A solution of In and Ir was added to all standards and samples to account for any instrumental drift and a Certified Reference Material, EP-L acidified water (SCP Science, Duisberg, Germany) was used for method validation purposes. On addition of concentrated nitric acid (0.6 mL) to about 70 mg of triplicate samples of the solid ambergris, solutions were left to stand for 24 hours, before dilution to 6 mL, followed by centrifugation. The supernatants were then analysed by ICP-MS (Figure S5).

Figure S5. Trace metals distributions of acid digests of triplicate samples of flotsam ambergris (μg g^{-1} air dried weight). Limits of quantitation (Fe 4, Cu 0.09, Zn 0.7, Cd <<0.01 μg g^{-1} air dried weight).



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