Estimation of Similarity in the Qualitative Composition of Humic Substance in Marine Sediments By Means of an Uv-Spectroscopic Library

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ESTIMATION OF SIMILARITY IN THE QUALITATIVE COMPOSITION OF HUMIC SUBSTANCE IN MARINE SEDIMENTS BY MEANS OF AN UV-SPECTROSCOPIC LIBRARY

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In this paper a method for the study of the qualitative similarity of extractable humic substance (EHS) in marine sediments based on the realisation of UV spectral library of EHS is described. The spectral library was built including the digitised spectra of humic substance extracted from several marine sediments (46 samples) and two samples of standard materials (lignin sulphonate and humic substance) in a Statistica data file. The data matrix was elaborated by means of different multivariate statistic techniques such as Cluster Analysis (CA), Principal Components Analysis (PCA) and the univariate technique Spectral Correlation Analysis (SCA). This approach allows us to show the contribution of different organic compounds (aromatic, lipid and acid) to the composition of EHS and moreover it evidences also the structural characteristics of EHS in terms of similarities in the qualitative composition of samples such as presence of terrestrial and/or vegetal contributions. The UV library is an objective tool to study the qualitative composition of EHS and it represents a complementary approach to the most common methods based on the application of spectroscopic (IR, $^{13}CNMR$), potentiometric and chromatographic techniques.

Keywords: Marine sediments; Humic substances; UV spectroscopy; Spectroscopic libraries; Multivariate analysis; Univariate analysis

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1. INTRODUCTION

Humic substance (HS) represents the major constituent of organic matter in marine sediments and, due to its heterogeneous and poly-electrolyte nature, it can form stable complexes with most inorganic and organic compounds that are utilised by benthic organism, thus influencing their bio-availability (Stackhouse and Benson, 1989; Kopinke et al., 1997; Freidig et al., 1998). Based on its solubility, HS is divided into the three main fractions which are fulvic acids soluble at any pH, humic acids soluble at pH > 2 and humin insoluble at any pH. The composition of HS is highly heterogeneous because it depends on several anthropic and natural factors (Chester, 1996); so, the qualitative identification of chemical compounds present in sediments (proteins, phenols, carbohydrates, lipids) becomes a fundamental task to determine the factors which influence the characteristic of areas submitted to ecological studies. The characterisation of HS is generally performed by different instrumental techniques such as UV-VIS, NMR and IR spectroscopy, thermogravimetry and chromatography (Schulten et al., 1995; Bonavita et al., 1996; Conte et al., 1994; Zhang et al., 1999). UV-VIS spectroscopy is utilised to evaluate the degree of condensation of aromatic groups by measuring the absorbance ratio at 465 and 665 nm (i.e., E_4/E_6 ratio) (Senesi et al., 1989; Power et al., 1988) and to determine anionic functional groups which react with cationic surfactants (Hanschmann, 1991). However, the use of UV-VIS technique is not emphasized or recommended because of the characteristics of spectra of the extractable humic substance (EHS) which consist of strong overlapped bands of different functional groups (—C = O, —COOH, —C = C and aromatic groups). Anyway the interpretation of the qualitative information in UV-VIS spectroscopy is highly enhanced by using the so-called spectroscopic libraries where digitised spectra are elaborated and compared by means of the multivariate statistic techniques such as Principal Component Analysis (PCA) and Cluster Analysis (CA) and univariate statistic technique such as Spectral Correlation Analysis (SCA) (Clark, 1992; Martens and Naes, 1993). The aim of this study was to create an UV spectral library of EHS in order to develop a suitable tool to evidence similarities in the qualitative composition of EHS in a samples set of Italian marine sediments (Fig. 1). The use of
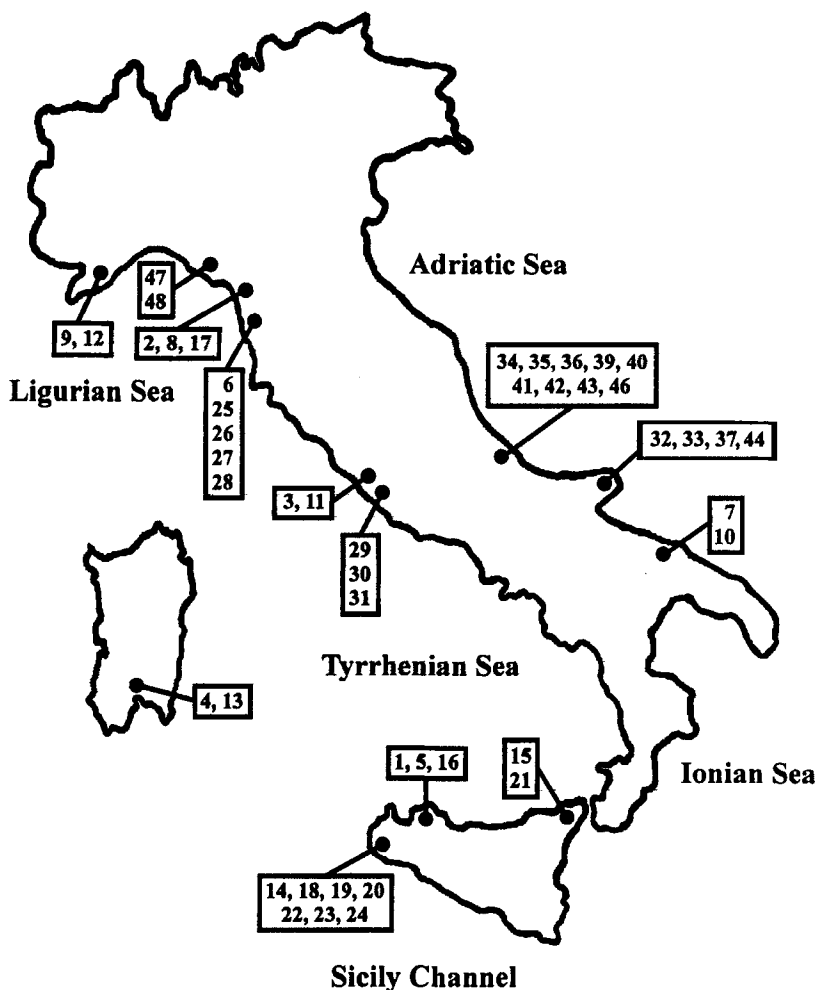
FIGURE 1 Sampling stations along the Italian coasts. Bari: 7-10; Cagliari: 4-13; Fiumicino 29-30-31; Imperia 9-12; La Spezia 47-48; Livorno 6-25-26-27-28; Mazara del Vallo: 14-18-19-20-22-23-24; Milazzo: 15-21; Palermo: 1-5-16; Pomezia: 3-11; Termoli (Molise): 34-35-36-39-40-41-42-43-46; Viareggio: 2-8-17; Vieste (Gargano): 32-33-37-44.

the UV spectral library allows accurate identification of the contributions of the principal compounds to the chemical composition of EHS, also the showing the vegetal contribution in EHS. Moreover, some information related to the re-distribution of EHS which is due to the dredging activity, can be provided.

2. MATERIALS AND METHODS

2.1. Sampling of Sediments and Station Locations

Sediments (46) were sampled by a Van Veen grab; 35 sediments were sampled in different Italian harbours and nine along the coast of Molise, South East Italy (Fig. 1). They were stored at −20°C until the analysis was carried out.

2.2. Extraction Method

EHS was extracted according to a previously described procedure which allows us to obtain qualitative homogeneous extracts of EHS (Mecozzi et al., 1998). Sediment samples (3 g) were transferred into an Erlenmayer flask and 25 ml of 8 M hydrochloric acid (Baker) were added. The flask was shaken for 24 h at room temperature. After centrifugation, the sediment was washed with deionized water until pH $\approx$ 5−6. The sediment was transferred into a polyethylene bottle and 30 ml of a 1:1 3 M hydrochloric/hydrofluoride acid (Merck) were added to the sample. The sample was shaken again for 24 h at room temperature. After centrifugation, the sediment was washed until pH $\approx$ 5−6. EHS were extracted with 30 ml of 0.5 M sodium hydroxide (Carlo Erba) for 24 h at room temperature under continuous shaking. After centrifugation, the caustic soda treatment was repeated twice and the three supernatants were pooled together and used for recording the UV spectra. Only MilliQ water was used for the treatments of extraction and dilution.

2.3. UV Spectroscopic Measurements

All the measurements were performed by using a Varian DMS 200 double-beam UV-VIS spectrophotometer (0.1 nm resolution) connected to a Pentium Texas Instruments Extensa 600 PC operating in Windows 95 for recording the spectra as ASCII files. The spectra were recorded by 10 mm quartz cells in the 350−200 nm range against a spectrophotometric blank consisting of 0.5 M caustic soda. According to the spectrophotometric error theory (Napoli, 1982), dilutions in the range 1:1÷1:5 by 0.5 M caustic soda were sometimes necessary for

UV spectra having peak of absorption higher than 2.0 absorbance. The digitised spectra were smoothed by a personal Microsoft BASIC software according to a polynomial smoothing filter (Gorry, 1995). Standard humic substance with terrestrial origin provided by Fluka, and lignin sulphonate provided by Carlo Erba Reagents, were introduced into the spectral library as comparison spectra. Each standards (0.5 g) were dissolved in 100 ml caustic soda 0.5 M without any preliminary pre-treatment and their spectra were collected as other samples.

2.4. Realisation of the UV Spectral Library

All the ASCII files were exported in a single file for Statistica software package, ver. 5.0 for Windows 95 by Statsoft Inc., before they were shown in to the statistic treatments. The data matrix was autoscaled preliminary to reduce differences related to the absolute intensity of absorption and to reduce the effect of noise (Clark *et al.*, 1992) then the matrix was examined by means of CA (according to the Euclidean distance), PCA and SCA. All the treatments, autoscaling, CA, PCA and SCA were performed by Statistica software.

3. RESULTS AND DISCUSSION

UV spectroscopy is generally considered a futureless technique which cannot give much support to the study of EHS (Kim *et al.*, 1992; Choudry *et al.*, 1992). This opinion arises from the characteristics of the UV spectra which present strong overlapped bands and only one peak of absorption between 215 and 230 nm is generally evident (Fig. 2). These spectra result from the absorptions of different functional groups which are simultaneously present in EHS: aromatic groups within 270–300 nm (Peschel *et al.*, 1986; Hawarl *et al.*, 1992; Chin *et al.*, 1994), —COOH and —C = O within 230–215 nm, C = C and C = O groups bonded with unsaturated heteroaromatic compounds typical of quinonoid and semiquinonoid groups in the range 260–280 nm (Choudry, 1992; Silverstain *et al.*, 1981). As a consequence, though UV spectra contain much information about the composition of EHS, the conventional interpretation based on the
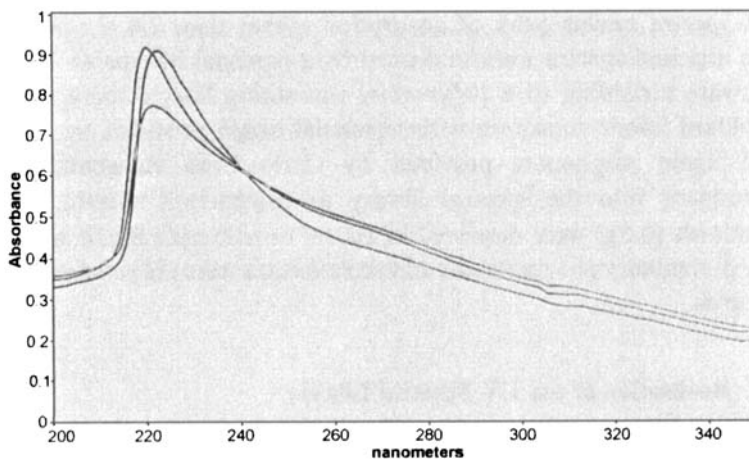
FIGURE 2   UV spectra of EHS from different sediment samples.

visual examination of the spectra is generally difficult. An alternative and more suitable approach is the examination of the digitised UV spectra of EHS by CA, PCA and SCA because these statistic techniques improve the interpretation of spectra having strong overlapped absorptions (Martens *et al.*, 1993; Clark *et al.*, 1992).

3.1. Cluster Analysis (CA)

The simplest way of comparing a set of UV spectra consists of measuring the multi-dimensional distance among them. Here, each spectrum is considered as a sample described by its wavelengths of absorption (Massart and Kaufmann, 1989; Clark *et al.*, 1992). CA gives a general description of the data set as results from the dendrogram shown in Figure 3. A large fraction of samples is included in a single cluster while other samples are well separated. In this large cluster, the standard humic sample (n. 45) having terrestrial origin is included. This means that the real samples included in the same cluster could have terrestrial contributions to the EHS composition. This hypothesis will be discussed in detail by means of SCA.

On the contrary the standard sample of lignin (n. 38) is present in a clustered alone. Lignin is the main precursor of phenolic compounds
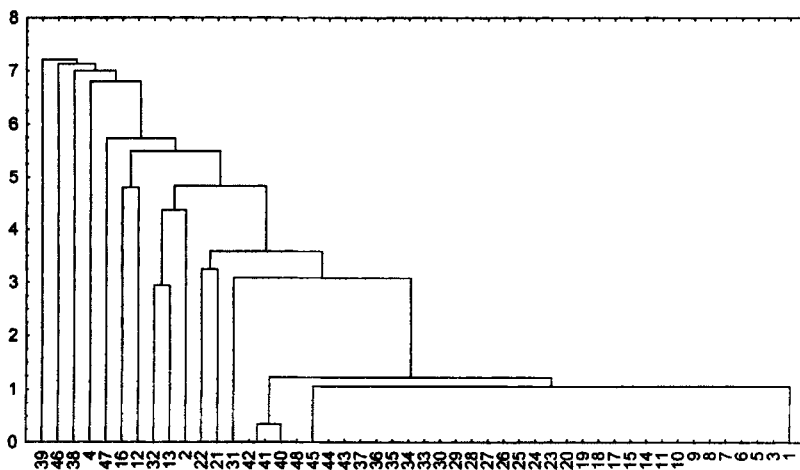
FIGURE 3 Dendrogram of CA according to the Euclidean distance and the unweighted pair groups algorithms.

in humic substance (Ishiwatari, 1992) but it can also indicate presence of wastes of the pulp and paper industry (Grasshoff et al., 1983). The absence of similarity between the standard of lignin sulphonate and other samples can depend on different reasons and one of this is that lignin can be produced also by brown algae (Grasshoff et al., 1983). For this reason diversity observed between lignin and other samples can show the poor relevance of vegetal contribution to the composition of EHS.

A second cause of diversity can arise from the characteristics of lignin which is involved into the degradation and re-polymerization reactions to form humic substance (Ishiwatari, 1992; Schulten et al., 1995). So, relevant differences between lignin and other sample of the data set can also depend on differences between initial and advanced process of humification. These evaluations can be extended to all the samples present as a single cluster such as samples n. 2, 4, 12, 13, 16, 31, 32, 39, 46 and 47. Detailed evaluations regarding the characteristics of these samples will be performed by PCA and SCA. In any case it is important to emphasise the screening power of CA in the evaluation of the general characteristics of a spectroscopic data set.

3.2. Principal Component Analysis (PCA)

If CA shows general similarities and differences between samples, the application of PCA gives information on structural characteristics which cause similarities and differences because PCA identifies the most significant variables, able to characterise the samples in the data matrix (Meglen, 1992). The results of PCA are reported in Table I. Seven factors account for 80% of the total variance of the data set and the first two factors accounting for 35.6 and 17.5% of total variance respectively, are the most significant. Because the total variance represents the quantitative information included in the data set (Meglen, 1992), the first two factors are more significant than the other ones and for this reason the study can be restricted to the examination of the first two factors.

PCA decomposes the original data matrix into the matrix of the new variables called 'scores' (reported in Tab. II) and into the matrix of statistical weight of the variables called "loadings" (reported in Tab. III). Some samples in Table II showed high negative value scores in the first factor (between − 3.5 and − 1.33), while most samples have low positive or negative scores (close to 0) in the same first factor. The samples having high negative scores (n. 2, 4, 12, 13, 16, 31, 32, 38, 39, 46 and 47) are the same samples shown as unique cluster by CA. PCA confirms the general trend of similarity obtained by CA but gives additional information related to the variables which cause similarity among samples.

High values of loadings in the first factor identify the most significant variables which give the highest contribution to the absorption spectra. These significant variables are evidenced in Table III.

TABLE I   Results of PCA: eigenvalues extraction and explained variance by each factor

	Eigenvalue	% total variance	Cumul. eigenvalue	Cumul. %
1	17.77717	35.55435	17.77717	35.55435
2	8.73668	17.47336	26.51385	53.02771
3	4.19795	8.39590	30.71181	61.42361
4	3.15246	6.30491	33.86426	67.72852
5	2.37890	4.75780	36.24316	72.48632
6	2.00169	4.00338	38.24485	76.48970
7	1.82468	3.64936	40.06953	80.13906

TABLE II Results of PCA: scores of the seven factors

Sample no.	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
1	-0.01985	-0.10952	-0.36022	0.552969	0.448957	-0.09684	-0.33532
2	-0.70614	2.545439	3.704436	1.860603	-0.06688	1.157376	1.465071
3	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
4	-2.38189	1.393059	-2.72593	-3.11759	0.367336	3.854386	-0.91128
5	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
6	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
7	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
8	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
9	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
10	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
11	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
12	-0.52523	-1.47867	1.200248	-0.96609	-1.57016	-0.84564	-4.02015
13	-0.80717	2.413828	1.995612	0.882376	1.471067	-0.06506	-2.11227
14	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
15	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
16	-1.56248	-2.70286	1.066042	1.696296	-1.44218	2.497992	-2.53335
17	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
18	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
19	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
20	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
21	-0.95505	-2.41469	1.443981	-0.2017	-1.80837	-0.39226	0.745263
22	-0.3705	-0.6256	0.474702	1.302424	-1.24718	-2.26159	0.782469
23	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
24	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
25	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
26	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
27	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
28	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
29	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
30	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838

TABLE II (Continued)

Sample no.	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
31	-1.51262	0.669174	0.530073	-0.13566	-0.54291	0.448684	1.954372
32	-1.34303	2.940304	2.046932	-1.09006	0.117399	-0.07231	-0.57079
33	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
34	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
35	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
36	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
37	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
38	-3.47806	1.817632	-2.84409	0.63124	-3.18862	-2.89566	0.614036
39	-2.4974	-1.181	-1.95096	4.026496	4.070782	0.003379	-0.18767
40	-0.31345	-0.26159	0.234283	0.076307	-0.45331	0.361229	-0.33213
41	-0.22854	-0.18887	0.115344	0.184072	-0.49823	0.152016	-0.15802
42	-0.22854	-0.18887	0.115344	0.184072	-0.49823	0.152016	-0.15802
43	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
44	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
45	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838
46	-2.10708	-1.70537	1.523319	-3.63107	3.36793	-3.08252	0.139443
47	-1.33655	-2.30033	1.071096	-0.84046	-0.2814	2.117128	3.867701
48	0.624307	0.047479	-0.23502	-0.04843	0.06825	-0.03589	0.057838

TABLE III Results of PCA: loadings of wave-
lengths having significant values in the first or in the
second factor. Level of significance 95%. Significant
values are in bold text

nm	Factor 1	Factor 2
341	− .70910	.449086
338	− .82268	.336385
335	− .81994	.340236
332	− .70318	.478059
326	− .80104	.185979
323	− .76723	− .176544
320	− .81260	− .024635
317	− .62789	.193180
311	− .76633	− .365164
308	− .76196	− .365822
305	− .75717	− .366332
302	− .75196	− .366685
299	− .74630	− .366872
271	.07382	− .874669
268	.08911	.873422
255	.20073	− .855833
252	.21565	− .849471
230	.80958	.117687
227	.75999	.305903
224	.78952	.378683
221	.82822	.294159
219	.82973	.293668
216	.79558	.375821
213	.75514	.384590
209	.79989	.374836

The highest loadings include wavelengths between 295 and 340 nm
which are typical of many natural phenolic compounds (Ryan et al.,
1998) and wavelengths of —C = O and —COOH groups between 210
and 230 nm. On the contrary, the wavelengths of quinonoid and
semiquinonoid groups which have typical bands between 250 and
280 nm are not significant because of their low loadings. In fact the
absorption of quinonoid and semiquinonoid groups have significant
values of loadings only in the second factor between 265 and 272 nm.
So, we can suggest that samples no. 2, 4, 12, 13, 16, 31, 32, 38 (lignin
sulphonate), 39, 46 and 47 are different with respect to other samples
of the data are set due to their relevant aromatic and carboxylic
content. This result is confirmed by the spectrum of Cagliari Harbour,
no. 4, which is reported in Figure 4. This is a peculiar sample because
it is the only one with a such high aromatic content that it presents two
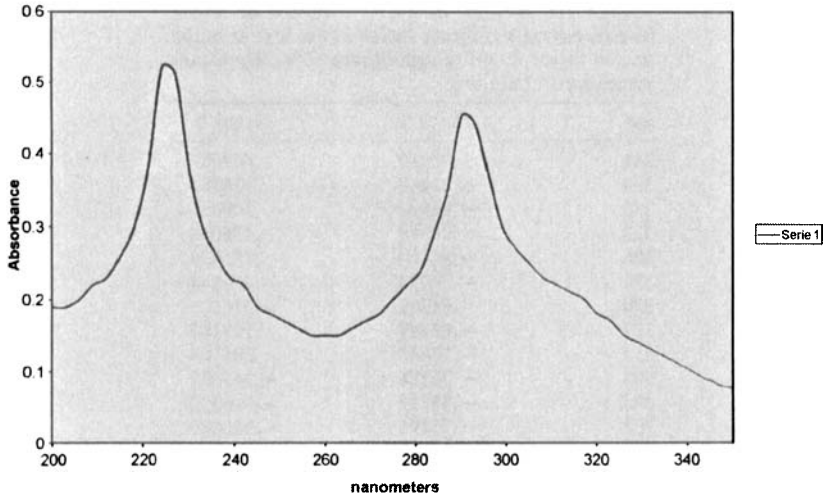separated peaks of absorption related to the carboxylic and ketonic

FIGURE 4   Spectrum of Cagliari sample; this is the only spectrum in the whole data with two well separated peaks of aromatic and carboxylic groups.

(223 nm) and aromatic groups (295 nm). At last, because the composition of EHS depends on its origin (Klavins *et al.*, 1997), a high aromatic content is a marker of a relevant vegetal contribution to the EHS composition.

3.3. Spectral Correlation Analysis (SCA)

SCA is a tool for comparing couple of UV spectra. According to the theory of spectral correlation, spectra with high qualitative and quantitative similarity have a correlation coefficient very close to 1, slope and intercept very close to 1 and 0 respectively. A good correlation with a non-unity slope indicates qualitative similarity but quantitative difference between spectra (Clark *et al.*, 1992). As already reported in 2.1 section, most sediments were sampled in harbours where an intense dredging activity is present. Harbour dredging activities cause a redistribution of organic and inorganic materials so that neighbouring samples can have relevant differences in concentration of pollutants and organic matter (Ausili *et al.*, 1998). A first example of the dredging effect, evidenced by SCA, is shown in the sediments sampled in the Livorno harbour. Samples no. 27 and 28 are very similar (r = 0.999, equation y = 0.044 + 1.00 x) while the sample 27

has lower similarity with 26 (r = 0.636) and no similarity with the sample 25 because the correlation is not significant. Also samples coming from Fiumicino harbour, where an intense dredging activity is present, show a wide qualitative and quantitative variability of EHS composition, as observed above. Samples 29 and 31 are identical (r = 0.999, equation y = 0.080 + 1.00 x) while sample 30 bears no relation to both 29 and 31. For the three samples of Viareggio (8, 2 and 17), the correlation between neighbouring samples is not significant. As far as Mazzara Harbour is concerned, an excellent correlation was found between 22 and 23 (r = 0.999, equation y = 0.035 + 1.01 x) but the similarity tends to diminish with the increasing of the distance as shown by the correlation between the samples 22 and 18 (r = 0.634) and sample 22 with 14 (r = 0.684). In the same way, the correlation between the samples 22 and 24 is not significant so as the correlation for the samples collected in the Cagliari Harbour (4 and 13). Therefore SCA is a tool to evidence an effect of dredging activity in neighbouring samples but other applications in the study of EHS are possible. In fact, if we consider the sample no. 43, which was sampled at the mouth of Fossombrone river (Molise samples), we can suggest the presence of a terrestrial contribution to the chemical composition of EHS; a confirmation of this hypothesis arises from the high correlation (r = 0.877, equation y = 0.06 + 0.932 x) with the standard sample Fluka having a terrestrial origin. Because the sample 43 is highly correlated with no. 46 (r = 0.999, equation y = 0.006 + 0.961 x) we can suppose that also in this sample there is the presence of a terrestrial contribution. When the distance with the river mouth increases (samples no. 34, 35, 36, 39 and 40) the chemical composition varies naturally and the correlations become lower.

Other interesting results arise from the specific application of SCA to spectra of EHS which show similarity between samples of different places. Some typical examples are sample no. 1 (Palermo, South Italy) and sample no. 17 (Viareggio, Central Italy) with r = 0.638 having evident similar composition; even a case of identical composition is present for sample no. 14 (Mazzara, South Sicily) and no. 15 (Milazzo, Northern Sicily) with r = 0.999 and equation y = 0.04 + 1.00 x. High similarity for different zones can depend either by common origins of chemical composition and/or by an advanced state of humification process (Ishiwatari, 1992; Schulten et al., 1995). A re-examination of

PCA results can give a reasonable explanation of the results of SCA about the similarity of the unneighbouring couples no. 14 no. 15 and no. 1 no. 17. Scores of these samples (Tab. II) have values close to zero which is the main characteristic of low aromatic content, the marker of a low vegetal contribution to the EHS composition (Grasshoff *et al.*, 1983). So the joint examination of UV spectra by means of PCA and SCA reveals this qualitative similarity in EHS composition.

4. CONCLUSION

In this paper the application of an UV spectroscopic library to study the chemical similarity of EHS in marine sediments has been described. The univariate and multivariate examination of UV spectra allows us to establish several characteristics of EHS such as general similarity of composition, presence of terrestrial contributions, estimation of relative high content of aromatic fractions, effect of re-distribution of organic matter due to dredging activity. All these characteristics are relevant in ecological studies and, though a UV library cannot be an unique tool to examine EHS in marine sediments, its use must be valued because it represents a complementary tool in the study of EHS performed by spectroscopic and chromatographic techniques.

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