Limnetica, 28 (2): 243-252 (2009). DOI: 10.23818/limn.28.20
 ISSN: 0213-8409

Lutein and the C/N as tracers of organic matter in the Palmones River estuary

Mª Angeles Arrojo[1,*], Carlos. Jiménez[1], Lourdes Rubio[2] and F. Xavier Niell[1]

[1] Department of Ecology. University of Málaga. Campus de Teatinos s/n 29071. Málaga. Spain.
[2] Department of Plant Biology. University of Málaga. Campus de Teatinos s/n 29071. Málaga. Spain.

[*] Corresponding author: maarrojo@uma.es

Received: 19/11/08 Accepted: 24/3/09

ABSTRACT

Lutein and the C/N as tracers of organic matter in the Palmones River estuary

Plant pigments have been used as biomarkers of the presence of phototrophic organisms in rivers, estuaries and sea sediments in present and in paleolimnological studies. Chlorophyll *a* and lutein concentration, C/N ratio and organic matter content have been studied in the sediment of the Palmones River estuary (Algeciras Bay, Southern Spain). Using the concentration of these two pigments at different depths, as well as the sedimentation rate (determined by Rubio *et al.* in 2003 by means of the ^{210}Pb method), lutein and chlorophyll *a* degradation rate has been determined, in the saltmarsh. Lutein persistence in the sediment was higher than the persistence of chlorophyll *a*. According to these results, it was possible to discriminate the organic matter sources.

Key words: Sediment, lutein, chlorophyll *a*, estuary, organic matter.

RESUMEN

La luteína y el C/N como trazadores de materia orgánica en el estuario del río Palmones

Los pigmentos vegetales han sido usados como bioindicadores de la presencia de organismos fototrópicos en ríos, estuarios y sedimentos marinos actuales y en estudios paleolimnológicos. En el sedimento del estuario río Palmones (Bahía de Algeciras, Sur de España) se ha estudiado las concentraciones de clorofila a *y luteína, el índice C/N y el contenido en materia orgánica. Utilizando la concentración de estos dos pigmentos en diferentes profundidades así como el índice de sedimentación (determinado por Rubio* et al. *En 2003 por el método del ^{210}Pb), se ha determinado el índice de degradación de la luteína y clorofila* a *en la marisma. La permanencia de la luteína en el sedimento es mayor que la de la clorofila* a*. Según estos resultados se ha podido discriminar las fuentes de materia orgánica.*

Palabras clave: *Sedimento, luteína, clorofila* a*, estuario, material orgánica.*

INTRODUCTION

Hydrodynamic factors (e.g. tidal movement, waves, river flow, winds) are the most important factors in estuaries formation. The intertidal zone accumulates and transforms the organic matter of marine and terrestrial origin (Mayer *et al.*, 1988; Cifuentes, 1991), including anthropogenic origin (Requejo *et al.*, 1986; Billen *et al.*, 1991).

Agricultural exploitations and industrial activities around the estuary can induce changes the transfer of organic and inorganic matter from the con-

tinent to the sea. Industrialization and deforestation increase the soil loss, with concomitant increased sedimentation in the coastal regions. In addition, dam construction also affects river contribution, drastically reducing the contribution with particulate material to the coasts. Thus, these changes in the material contribution also affects to the carbon, nitrogen and phosphorus biogeochemical cycles in coastal areas (Berner, 1980).

Increase in the nutrient input to the rivers (mainly nitrate and phosphate mobilized through human activity), and the transport of carbon from the rivers to the ocean, can force primary production in the shoreline. Coastal and estuarine sediments contain organic matter both of terrestrial and aquatic origin (Ertel and Hedger, 1985; Hedger *et al.*, 1988). In the sediments, plant pigments can be used to reflect the different sources of that organic matter (Watts *et al.*, 1975; Bianchi and Findlay, 1990). The usefulness of the pigments as tracers of organic matter sources can depend on the magnitude of the input, their decomposition rates and the specificity of the source of the organic matter (Repeta, 1989; Bianchi and Findlay, 1991; Sun *et al.*, 1991).

Lutein (3*R*, 3*R′* −*β*, *β*-caroten-3, 3′-diol) is an exclusive pigment of vascular plants, chlorophytes and rhodophytes, being the most abundant carotenoid in the photosynthetic tissues (van den Hoek *et al.*, 1995), so, it is being used as an indicator of this particular origin of the organic matter. C/N ratio is also being used as indicator of the organic matter source, due to its different range of variation depending on the origin of the organic matter. These main organic matter sources in estuarine regions are: the marsh, planktonic organisms, estuarine macroalgae, terrestrial vegetation of the basin and the anthropogenic material.

The first objective of this study is to use the two main photosynthetic pigments (e.g. lutein and chlorophyll *a*) and the C/N ratio in the sediment to discriminate the plant organic matter origin accumulated in Palmones estuary sediment (and then to be able to have an initial estimation of the importance of the organic matter natural sources to the estuary). The second objective is to determine the average rate of lutein, chlorophyll *a* and organic matter degradation in this marsh.

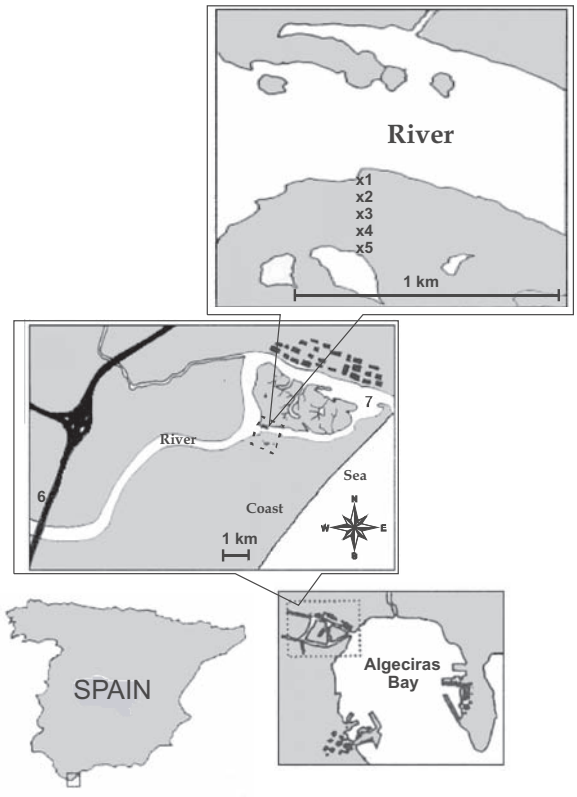

Figure 1. Sampling stations in Palmones estuary. *Estaciones de muestreo en el estuario de Palmones.*

MATERIAL AND METHODS

Study area

Palmones River estuary is the westernmost estuary in the Mediterranean (from 36°16′55.40″N; 5°35′06.39″W to 36°16′19.59″N; 5°15′35.69″W). It is located in Algeciras Bay (Southern Spain) at the end of a small basin (302 km^2), being representative of the small estuaries along the Western Mediterranean coast. Palmones estuary is a well-mixed, shallow estuary with a maximal depth of 2.5 m and a range of salinity from 29 to 35 (Clavero *et al.*, 1997a). Tidal movement in the area has a maximal amplitude of 2 m, so an extensive area of mud and sand emerge daily at low tide (Clavero *et al.*, 1997a).

Seven sampling stations have been selected in the estuary in order to obtain a spatial and depth

Table 1. Characteristics of the sampling stations. *Características de las estaciones de muestreo.*

Station name	High tidal level (cm)	Substrate	UTM Coordinates X	UTM Coordinates Y	Vegetation
1	0	Mud	0280581.09	4005767.33	No vegetation
2	45	Mud	0280582.28	4005764.17	*Halimione portulacoides* *Sarcocornia fruticosa*
3	60	Mud	0280583.47	4005759.81	*Sarcocornia fruticosa*
4	50	Mud	0280585.06	4005755.05	*Sarcocornia fruticosa*
5	45	Mud	0280586.64	4005750.07	*Sarcocornia fruticosa* *Sarcocornia perennis*
6	0	Mud	0279445.07	4006436.06	*Juncus maritimus*
7	0	Sand	0281306.08	4005951.15	No vegetation

profile of lutein, chlorophyll *a* and organic matter distribution in the sediment. Five sampling stations were located in the middle zone according with the gradient of emersion (from 1 to 5; Fig. 1), while station 6 was placed in the upper zone of the estuary. Finally, station 7 was in the lower part of the tidal level. Six of them were located in the right margin of the river (1, 2, 3, 4, 5, 7) and one in the left margin (station 6). The characteristics of the sampling stations are summarized in Table 1.

Cores of 4.5 cm of diameter and 20 cm long were inserted by hand in the sediment and removed. Then they were transported to the laboratory in cold and dark conditions. In the laboratory they were kept at −20 °C until further processing. The cores were cut in slices of 1 cm thick from the surface to 5 cm deep. Then, 2 g of each slice were submerged in acetone (8 ml), and extracted in continuous shaking for 48 hours at 4 °C in dark, and then centrifuged at 5 000 rpm. Successive extractions were made until colourless supernatant (near zero absorbance) were obtained. A mixture of diethyl-ether (2 ml) and water (2 ml) was added to every 4 ml of extract. Phase separation was achieved after centrifugation, and the diethyl-ether phase containing the pigments was extracted and concentrated under N_2 flow. Extracts were kept at −20 °C until HPLC analysis.

A Waters 600 (Waters Cromatografía S.A., Barcelona, Spain) system provided with a reverse-phase VYDAC201TP54 C-18 column (25 cm × 4.6 mm, 5 μm particle size) with a precolumn VYDAC201TPC18301A was used for pigment separation. Elution was performed in a two-solvent gradient system: Solvent A, acetonitrile (75 %), methanol (15 %) and tetrahydrofurane (10 %); and Solvent B, 100 % bi-distilled water.

Samples were treated in a 0-30 min linear gradient in 80 % A + 20 % B followed by a 10 min of isocratic gradient to 100 % A; then passed through a 10 min linear gradient to newly reach 80 % A + 20 % B, and finally 10 more min through isocratic 80 % A + 20 % B for column stabilization between consecutive injections (Carnicas *et al.*, 1999).

A programmable photodiode array detector (Waters 996) was used for pigment detection between 400 and 750 nm. Data were collected and analyzed using the computer programme Millennium™. Peaks were identified both by retention time and their absorption spectra. Standards of chlorophyll *a* and *b* (Sigma) and lutein (Fluka) were

Table 2. Retention times and absorption maxima of commercial standards of lutein, chlorophyll *a* and *b* (values in brackets correspond to shoulders in the spectra). *Tiempo de retención y máximos de absorción de los estádares comenrciales de luteína,clorofila* a y *b* (los valores entre paréntesis corresponden a hombros del espectro).

Pigment	Peaks of absorption I	II	III	Retention time
Lutein	429	448.8	476.6	22.726-26.257
Chlorophyll *a*	431.9	662.5	—	31.120-35.794
Chlorophyll *b*	462	647.9	—	26.257- 30.739

used for this propose (Table 2). Results of previous studies have also been taken into account for peak identification (Brotas *et al.*, 1995; Bianchi *et al.*, 1993; Cartaxana and Brotas, 2003) (Table 2). Pigment concentrations were calculated applying an extension of the Lambert-Beer equation through the area of the peaks in the chromatogram, and expressed in μg of pigment g^{-1} of sediment.

Organic matter and C/N determination

Organic matter (o.m.) was estimated as percentage of weight loss by at 550 °C (3 hours). Before incineration, sediment was dried at 110 °C (24 h) and grinded in a mortar to a particle size below 125 μm. (Boyle, 2004).

Total particulate carbon and nitrogen in the sediment were determined in the surface and deep layers using a CHN 2400C Perkin Elmer Elemental Analyser, following the method of Kristiensen and Andersen (1987).

RESULTS

Pigment analyses

Two characteristic chromatograms are presented in Fig 2. They correspond to the surface of station 2 (Fig. 2a) and to its homologous extract obtained at 4 cm deep (Fig. 2b). Clear differences can be detected between samples, evidencing the fast di-

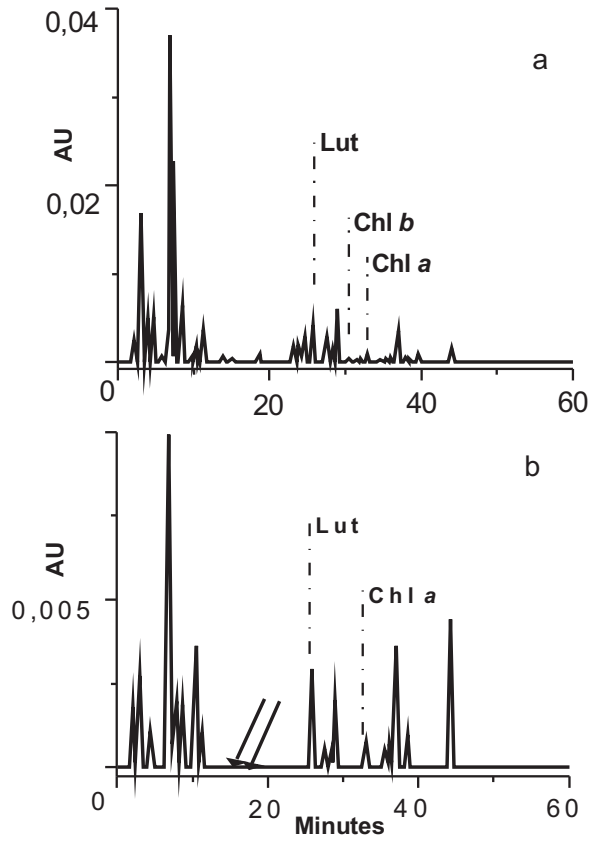

Figure 2. Chromatogram for the first cm of Station 2 (a) and between 3 and 4 cm of depth (b). The area of the lutein, chlorophyll a and chlorophyll b diminishes. Notice the lack of peaks between 12 and 25 minutes of retention time in figure 2b. *Cromatograma del primer centímetro de la estación 2 (a) y entre 3 y 4 cm de profundidad (b). El área de la luteína, clorofila a y b dismunuye. Nótese la falta de picos entre los 12 y 25 minutos de retención en la figura 2b.*

Table 3. Lutein (top table) and chlorophyll a (bottom table) average concentration expressed in ppm with respect to the C content in the sediment (n.d.-not detected). *Concentración media de luteína (tabla superior) y clorofila a (tabla inferior) expresada en ppm respecto al contenido en C en el sedimento (n.d.: no detectado).*

Depth (cm)	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7
1	0.01	0.14	0.78	0.14	0.06	0.62	1.04
2	n.d.	0.18	0.02	0.04	n.d.	0.04	1.09
3	n.d.	0.04	0.08	0.03	n.d.	0.28	0.60
4	n.d.	0.04	n.m.	n.d.	n.d.	n.d.	0.25
5	n.d.	0.03	n.d.	n.d.	0.03	n.d.	0.21
Depth (cm)	**Station 1**	**Station 2**	**Station 3**	**Station 4**	**Station 5**	**Station 6**	**Station 7**
1	0.39	1.20	7.37	2.77	0.71	5.08	1.92
2	n.d.	0.20	0.28	0.21	1.05	0.16	2.25
3	n.d.	0.81	0.48	0.17	n.d.	1.01	0.71
4	n.d.	0.63	0.57	0.05	0.85	n.d.	0.19
5	n.d.	0.14	n.d.	n.d.	0.44	n.d.	0.14

sappearance of some pigments in the deep layers. Among them, it is worth noting the disappearance of some products (xanthophylls, between 12 and 24 min), and lutein. Lutein and chlorophyll *a* concentrations in all sampling stations are summarized in Table 3.

Figure 3 shows the average profile of lutein and chlorophyll *a* in all stations, referred as percentage of the pigment concentration found in the surface. Chlorophyll *a* decreased quickly in the first 2 cm (at 2 cm it remained just 20 % of the chlorophyll *a* found in the surface of the sediment). From this depth, disappearance of this pigment was gradual. Lutein decrease was slower, and more gradual than that of chlorophyll *a*; thus, nearly 40 % of the lutein concentration present in the surface was still found at 4 cm deep.

Organic matter

Organic matter content in the sediment ranged from 0.96 to 25.36 % of the dry weight. Data are summarized in the Table 4. Lowest average value was found in station 7, that is, in the river mouth (less than 2 %, thus it can be assumed to be a non-polluted sediment). Values in the mid and high marsh reflected the general eutrophization of the estuary. The most distant location from the river mouth (station 6) and the one in the lowest tidal level (station 1, that shows the highest renewal rate) averaged values of organic matter between 2.16 and 8.41 %. Largest values of o.m. were found in the highest stations above the tidal level (7.21 % to 25.36 % in stations 5 and 3), the differences being statistically signi-

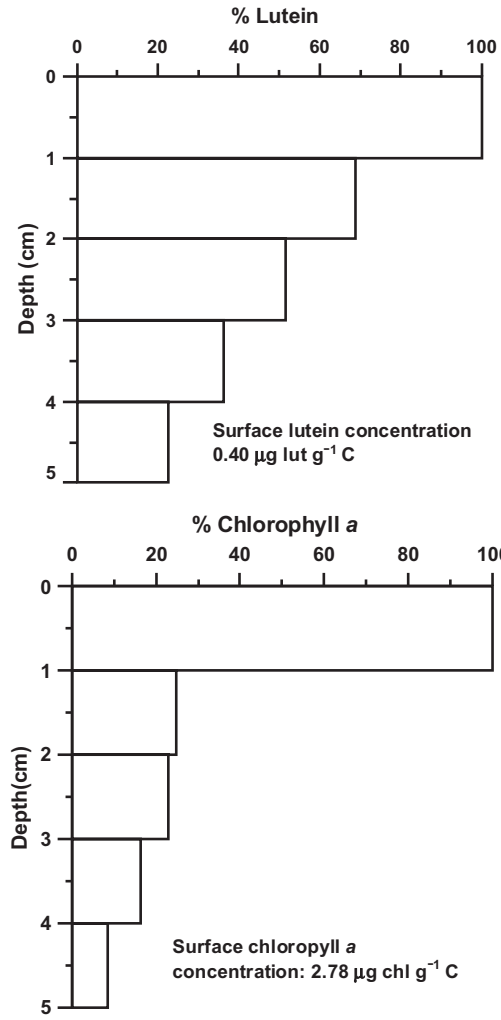

Figure 3. Mean profiles for lutein and chlorophyll a in the first 5 cm, expressed as percentage with respect to the surface concentration. *Perfiles medios de luteína y clorofila a en los 5 cm superficiales expresados en porcentaje respecto a la concentración en superficie.*

Table 4. Average organic content matter in the sediment, expressed in % over its dry weight. *Media del contenido de materia orgánica en el sedimento, expresada en % del peso seco.*

Depth	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7
1	5.72	15.35	24.98	16.27	7.21	5.13	1.26
2	7.95	14.69	13.72	12.37	7.31	2.50	0.96
3	7.61	12.90	11.27	12.55	7.52	2.16	1.35
4	8.41	8.91	12.11	17.29	13.11	2.40	1.52
5	8.13	10.62	25.36	17.42	13.95	2.91	1.96
Mean value	7.56	12.49	17.49	15.18	9.82	3.02	1.41
S.D.	1.07	2.72	7.07	2.52	3.40	1.21	0.37

Table 5. Average C/N ratio at different depths in all sampling stations. *Media del índice C/N a diferentes profundidades en todas las estaciones de muestreo.*

Depth	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7
1	9.60	11.92	11.57	11.24	9.78	13.04	12.73
2	11.93	11.69	11.13	10.31	10.79	25.14	9.85
3	10.92	11.06	11.25	10.33	10.17	29.84	10.67
4	11.83	10.15	11.59	11.82	10.43	29.70	12.05
5	10.77	11.98	13.80	10.59	10.87	23.79	16.65
Mean value	11.01	11.36	11.87	10.86	10.41	24.30	12.39
S.D.	0.95	0.77	1.10	0.66	0.45	6.85	2.64

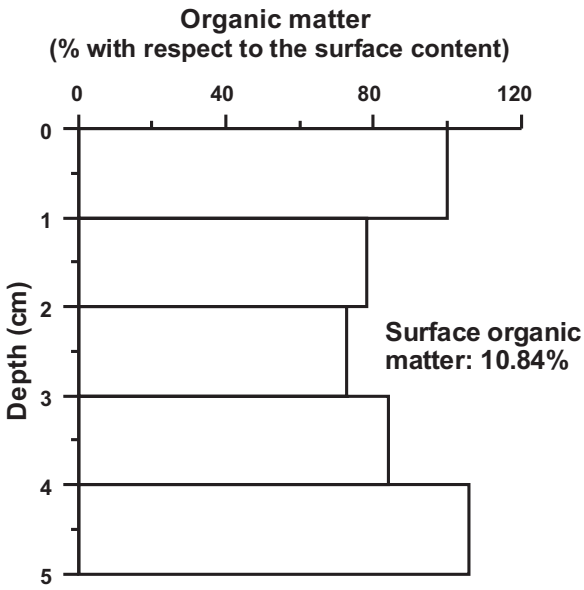

Figure 4. Organic matter content in the first 5 cm, expressed as % with respect to the surface content. *Contenido de materia orgánica en los 5 cm superficiales expresados como % respecto al contenido superficial.*

ficant (ANOVA, $p < 0.005$). Figure 4 shows the average profile of organic matter in all stations, as referred to the surface of the sediment.

C/N

Carbon content in the sediment ranged between 1.75 mmol g^{-1} DW and 7.55 mmol g^{-1} DW, whereas Nitrogen values were between 0.14 mmol g^{-1} DW and 0.47 mmol g^{1} DW. C/N ratio (Table 5) was around 11 in all transect stations (1 to 5), and in station 7, whereas in station 6 average value was 24.3. These differences in the C/N ratio were statistically significant (ANOVA, $p < 0.001$), with the highest values in station 6 and the lowest in the marsh stations.

DISCUSSION

Organic matter content in the sediment of Palmones river estuary found in this work agrees with previous reports of Clavero *et al.* (1996, 1999), whereas C/N in the area was around 11. These values are also in agreement with the expected ones for estuarine sediments (Moreno and Niell, 2003). However, in station 6, close to the dam, the accumulated organic matter is more resilient, and its degradation much more difficult (Moreno and Niell, 2003), with C/N values around 24.

According to Rubio *et al.* (2003), present sedimentation rate in the zone is 0.9 cm $year^{-1}$, in agreement with previous observations of direct deposition (Clavero *et al.*, 1999; Rubio, 2000)). Then, the sediment column studied in this work includes a period of 5.5 years.

Both the average profile of organic matter (Fig. 4) and the organic matter profiles in the different stations (Fig. 5) show the existence of two differentiated processes: one in the surface of the sediment, where o.m degradation seems to be the prevalent process, and a second process, occurring from 2-3 cm of depth, where accumulation, compaction and mineralization of the organic matter coexist, in other words, early diagenesis takes place. Depending on which of these three processes prevails in these deeper layers,

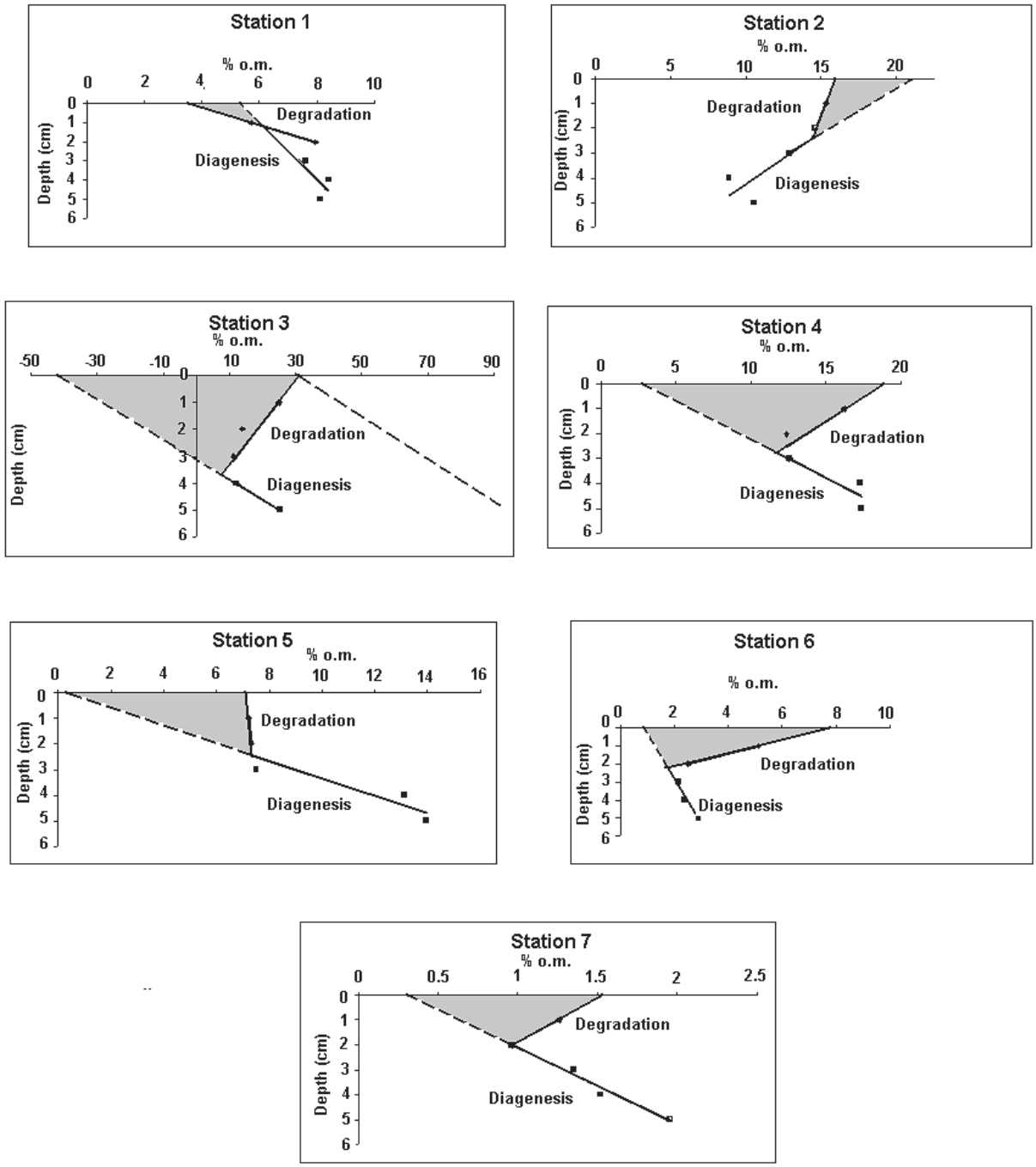

Figure 5. Organic matter profiles in the different sampling stations. *Perfiles de materia orgánica en las distintas estaciones de muestreo.*

organic matter may either increase (accumulation and compaction) or decrease (mineralization). In all cases the degradation-diagenetic model takes different organic matter profiles (Fig. 5).

From the organic matter profiles (Table 4), we can see that degradation is the main process that takes place in the surface, except in station 1. In this station the sediment becomes anoxic, with

negative redox potential, in the first millimetres of depth, thus organic matter degradation is drastically reduced, thus explaining organic matter accumulation in this sediment. From 2-3 cm of depth, station 2 is the only one in which the organic matter content decreases (mineralization prevails in an early diagenetic process) (Fig. 5).

Pigment content in the sediment clearly follows the degradation model. Using lutein and chlorophyll *a* profiles, together with the sedimentation rate of organic matter in the zone, it is possible to calculate pigment degradation rate. Average lutein degradation rate in the first 5 cm of the sediment column is around 17 % year^{-1}, while chlorophyll *a* is around 83 % in the first centimetre and around 4.6 % year^{-1} from 2 to 5 cm deep, with an average chlorophyll *a* degradation rate in the first five centimetres of 20 % year $^{-1}$.

Abele (1991) obtained much smaller pigment degradation rates in the sediment of Kiel Bight (Baltic Sea); however, in his system the water column above the sediment was larger, and with frequent anoxic periods. In these conditions, approximately 70 % of the lutein present in the surface was still detected at 5 cm of depth in the sediment, while chlorophyll *a* still accounted for nearly 20 %. Sedimentation rate in the zone was 0.14 cm year $^{-1}$, thus the annual degradation rate was 0.17 % for lutein and 0.45 % for chlorophyll *a*. Event though these data differ from those obtained in Palmones by two orders of magnitude for lutein and one for chlorophyll *a*, lutein and chlorophyll *a* profiles in both cases are similar. In Abele study both pigments decrease with a slope of −13.6 for the lutein in the 5 cm studied and, for the chlorophyll *a*, −80 in the first centimeter and −5 between 2 and 5 studied cm. In Palmones River they are −22.13 for the lutein and −75 and −5.5 for the chlorophyll *a* both depths.

Bianchi *et al.* (1993) obtained a degradation rate of chlorophyll *a* in the River Hudson of 78 % in the first cm of the sediment. From this depth the rate decreased to 4.6 %. These values are similar to the ones obtained in Palmones. In addition, it is worth noting that chlorophyll *a* profiles in the sediment of both rivers are similar. However, lutein profile is absolutely different in both rivers. While in Palmones lutein concentration decreases with depth, in the River Hudson it increases with depth. These authors concluded that this increase of lutein with depth was due the large amount of vascular terrestrial plants remains, of very difficult degradation, that are continuously buried in the sediment. Then, lutein concentration increases with depth due to the very low degradation rates. Sedimentation rate in the Hudson River ranges from 0.38 to 1.20 cm year^{-1}, making it vary difficult to obtain a reliable average annual degradation rate.

In general, chlorophyll *a* degradation rate is larger than that of the lutein (Abele, 1991; Bianchi and Findlay, 1991; Bianchi *et al.*, 1993; this work) though other authors indicate that both degradation rates might be similar (Brotas and Plante-Cuny, 2003).

Using the average values of C/N, o.m. content in the sediment and pigment concentration in the sampled stations, it is possible to deduce the origin of the plant organic matter accumulated in every sampling point. C/N values lower than 10, absence of lutein and low amount of chlorophyll *a* indicate that the main origin of the plant organic matter of the zone is the phytoplankton. C/N values slightly higher than 10, with significant amounts of lutein and chlorophyll *a* in the sediment, will indicate that the origin of the organic matter are red and/or green macrophytes. C/N values between 11-12 and presence of lutein indicate organic matter from marsh plants (these plants have a C/N value of 30-32, being able to come even to 60 in the most woody part of the plants; Palomo, 2004). C/N values higher than 20 and presence of lutein show that the origin of the organic matter is in the catchment basin vascular terrestrial plants.

Thus, organic matter of plant origin accumulated in station 6 corresponds to allocthonous plants remains of difficult degradation. In station 1, the low concentration of pigments and the C/N value make us to think that the organic matter accumulated seems to be of allocthonous origin, and possibly of antropogenic origin. The quantity of pigments in the sediment in stations 2, 3, 4 and 5 is high, which together with C/N values indicate that the contribution of plant matter is higher in the stations placed at the higher intertidal level. Highest pigment concentration in the sediment has been found in station 7, with C/N

around 11, thus the organic matter accumulated would come mainly from the remains green algae of the zone, due to the fact that the quantity of red algae is minimal with regard to the green algae, then nearly no contribution of red algae is expected to the organic matter of the sediment.

CONCLUSIONS

Plant pigment concentrations in the sediment of all sampled stations were very low, thus it is possible to deduce that the contribution of plant matter to the pool of organic matter accumulated in the sediment is also very low. Then, we assume that the major contribution corresponds to organic matter of anthropogenic origin. This is supported by C/N values; our values of C/N are low enough to help us to discard an important contribution of vascular plants remains, and high enough to discard that the main origin is the phytoplankton.

Lutein degradation rate averaged for the first 5 cm of the sediment was 17.2 % year^{-1}. Chlorophyll *a* degradation rate was 83.4 % year^{-1} in the surface of the sediment, averaging 4.6 % year^{-1} from the first centimetre. Then, average chlorophyll degradation ratio for first 5 cm of the sediment was 20 % year^{-1}.

The organic matter averaged degradation rate for the first three centimetres of the sediment was 10 % year^{-1}. From that depth, diagenetic accumulation rate accounted for 16.7 % year^{-1}.

ACKNOWLEDGEMENTS

This work has been supported by projects P06-RNM-01892 of the Junta de Andalucía and CTM2005-05011/MAR of the Ministerio de Educación y Ciencia, Spain.

REFERENCES

ABELE, D. 1991. Potential of some carotenoids in 2 recent sediments of Kiel Bight as biogenic indicators of phytodetritus. *Mar. Ecol. Progr. Ser.,* 70: 83-92.

BERNER, R. 1980. *Early diagenesis: A theoretical approach*. Princeton University Press, New York, 421 pp.

BIANCHI, T. S. & S. FINDLAY. 1990. Plant pigments as tracers of emergent macrophytes from Hudson River. *Canad. J. Fish. Aquat. Sci*., 47: 492-494.

BIANCHI, T. S. & S. FINDLAY. 1991. Decomposition of Hudson Estuary macrophytes: Photosynthetic pigment transformations and decay constants. *Estuaries*, 14: 65-73.

BIANCHI, T. S., J. E. DIBB & S. FINDLAY. 1993. Early diagenesis of plant pigments in Hudson River sediments. *Estuar. Coast. Shelf Sci*., 36: 517-527.

BILLEN, G. F., C. LANCELOT & M. MEYBECK. 1991. N, P, and Si retention along the aquatic continuum from land to ocean . In: *Ocean Margin Processes in Global Change.* R. F. C. Mantoura, J. M. Martin, R. Wollast. (eds.): 19-44. Wiley, New York.

BOYLE, J. 2004. A comparison of two methods for estimating the organic matter content of sediments. *J. Paleolimnol.*, 31: 125-127.

BROTAS, V. & M. R. PLANTE-CUNY. 2003. The use of HPLC analysis to study microphytobenthos communities. *Oceanol. Acta*, 24: 109-115.

CARNICAS, E., C. JIMÉNEZ & F. X. NIELL. 1999. Effects of changes of irradiance on the pigment composition of *Gracilaria tenuistipitata* var. *liui* Zhang et Xia. J. *Photochem. Photobiol.,* 50: 149-158.

CIFUENTES, L. A. 1991. Spatial and temporal variations in terrestrial derived organic matter from sediments of the Delaware Estuary. *Estuaries,* 14: 414-429.

CLAVERO, V., P. GARCÍA-VALLEJO, C. JIMÉNEZ, J. A. FERNÁNDEZ & F. X. NIELL. 1996. Eutrofización del sedimento del estuario del río Palmones debido a inundaciones. *I Seminario de Química Marina,* 115-122.

CLAVERO, V., J. A. FERNÁNDEZ & F. X. NIELL. 1997a. The influence of tidal regime on phosphate accumulation in a shallow small estuary (Palmones River, Southern Spain). *Sci. Mar*., 6: 59-65.

CLAVERO, V., J. J. IZQUIERDO, L. PALOMO, J. A. FERNÁNDEZ & F. X. NIELL. 1999 b. Water management and climate change increases the phosphorus accumulation in the small shallow estuary of Palmones River (Southern Spain). *Sci.Total Environ.,* 228: 193-202.

ERTEL, E. R. & J. I. HEDGES. 1985. Sources of sedimentary humic substances: structure, genesis and properties. *Rev. Aquatic Sci.*, 49: 2097-2107.

HEDGER, J. I., W. A. CLARK & G. I. COWIE. 1988 Organic matter sources to the water column and surficial sediments of a marine bay. *Limnol. Oceanogr*., 35: 1116-1136.

KRISTENSEN, E. & F. O. ANDERSEN. 1987. Determination of organic carbon in marine sediments: A comparison of two CHN-analyzer methods. *J. Exp. Mar. Biol. Ecol*., 109: 15-23.

MAYER, L. M., S. A. MACKO & L. CAMMEN. 1988. Provenance, concentrations and nature of sedimentary organic nitrogen in the Gulf of Maine. *Mar. Chem.,* 25: 291-304.

MORENO, S. & F. X. NIELL. 2004. Scales of variability in the sediment chlorophyll content of the shallow Palmones River estuary, Spain. *Estuar. Coast. Shelf Sci.,* 60: 49-57.

PALOMO, L. 2004. *La influencia de Sarcocornia perennis alpini en el flujo se nutrientes del estuario del río Palmones*. Tesis doctoral. Universidad de Málaga. 225 pp.

REPETA, D. 1989. Early diagenesis of carotenoids in recent marine sediments. II. Degradation of fucoxanthin to loliolides. *Geochim. Cosmochim. Acta,* 53: 699-707.

REQUEJO, A. G., J. S. BROWN & P. D. BOEHM. 1986. Lignin geochemistry of sediments from the Narragansett Bay estuary. *Geochim. Cosmochim. Acta,* 50: 2702-2717.

RUBIO, L. 2000. *Distribución vertical del* ^{137}*Cs y cronología del sedimento en el estuario del río Palmones. Tesis de Licenciatura.* Universidad de Málaga. 130 pp.

RUBIO, L., A. LINARES-RUEDA, C. DUEÑAS, M. C. FERNÁNDEZ , V. CLAVERO, F. X. NIELL & J. A. FERNÁNDEZ. 2003. Sediment accumulaion rate and radiological characterisation of the sediment of Palmones River estuary (Southern of Spain). *J. Environ. Radioactiv.*, 65: 267-280.

SUN, M., R. C. ALLER & C. LEE. 1991. Early diagenesis of chlorophyll *a* in Long Island Sound sediments: A measure of carbon flux and particle reworking. *J. Mar. Res.*, 49: 379-401.

VAN DEN HOEK, C., D. G. MANN & H. M. JAHNS. 1995. *An introduction to phycology.* Cambridge University Press. Cambridge. 623 pp.

WATTS, C. D., J. R. MAXWELL & H. KJOSEN. 1977. The potential of carotenoids as environmental indicators. *Adv. Org. Geochim.,* 391-413. Proc. 7th. Int. Meeting Org. Geochem. Madrid 1975. Pergamon.