C : N : P ratios and stable C isotopic ratios as indicators of sources of organic matter in the Gulf of Trieste (Northern Adriatic)

Jadran FAGANELI [a], Alenka MALEJ [a], Jože PEZDIČ [b], Vlado MALAČIČ [a]
[a] Marine Research Centre, JLA65, 66330 Piran, Yugoslavia.
[b] Jožef Stefan Institute, Jamova 39, 61000 Ljubljana, Yugoslavia.

ABSTRACT

Measurement of the C:N ratios and the isotopic composition of C of various classes of autochtonous and allochtonous organic matter (sewage and riverine POM, benthic and pelagic autotrophs, net-zooplankton, jellyfish, marine POM, surficial marine sediment) in the Gulf of Trieste was used to elucidate the sources of marine POM and sedimentary organic matter. The mixing models, constructed with $\delta^{13}C$ values and C:N ratios, indicate that the POM in the Gulf of Trieste is prevalently autochtonous marine in origin, while sedimentary organic matter comprises a mixture of marine POM and benthic producers in the ratio 2:1.

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RÉSUMÉ

Rapports C/N/P et rapports isotopiques du carbone stable comme indicateurs des sources de matière organique dans le golfe de Trieste

Les sources de matière organique particulaire marine et de matière organique sédimentaire dans le golfe de Trieste ont été identifiées à partir du rapport carbone/azote et de la composition isotopique du carbone dans plusieurs classes de matière organique autochtone et allochtone: matière organique particulaire des rejets, des rivières ou de la mer, autotrophes benthiques et pélagiques, zooplancton net, méduses, sédiment marin superficiel. Les modèles de mélange, établis avec les valeurs $\delta^{13}C$ et les rapports C/N indiquent que, dans le golfe de Trieste, la matière organique particulaire dominante est d'origine marine autochtone, tandis que la matière organique sédimentaire est formée d'un mélange de matière organique particulaire marine et de producteurs benthiques, dans un rapport de 2/1.

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INTRODUCTION

The composition of particulate and sedimentary organic matter in our study area is presumably determined by the intermixing of sources of marine, riverine, and sewage origin, subject in addition to chemical and biological modifications. For estuarine and near-shore systems, chemical analyses are required to provide data to help in resolving questions of the origin and sinks of organic matter. Terrigenous organic matter can be identified in marine sediments by its high organic carbon content and correspondingly high carbon:nitrogen (C:N) ratio. In addition, the presence of lignin is considered to be an unequivocal indicator of

terrigenous addition to marine sediments (Pocklington, 1976). Sewage in the marine environment has been traced using the contents of volatile solids, organic carbon and nitrogen (Sweeney, Kaplan, 1980). However, the extent of removal of land-derived organic matter at the river-sea interface, and the degree of mixing with marine organic matter in coastal sediments, are unknown.

Stable carbon isotope ratios have been used widely in geochemical, paleoenvironmental and plant studies (Craig, 1953; Park, Epstein, 1960; Degens, 1969; Schwarcz, 1969; Deuser, 1970). More recently, the measurement of stable C isotope ratios has been shown to be a useful tool for studying organic matter flow in

marine and freshwater environments, in general (Fry, Sherr, 1984), and especially in food webs (McConnaughey, McRoy, 1979; Hughes, Sherr, 1983; Fry *et al.*, 1983; Simenstad, Wissmar, 1985). It has also been demonstrated that the carbon isotope ratio provides an opportunity for elucidating the sources and fate of organic C in estuaries and near-shore marine waters, since riverine inputs produce ^{13}C depleted values in contrast to ^{13}C enriched autochtonous marine organic matter (Sackett *et al.*, 1965; Smith, Epstein, 1970). Shifts in stable isotope ratios can also provide evidence of the transfer of sewage-derived organic matter to marine organisms (Sweeney *et al.*, 1980; Burnett, Shaeffer, 1980; Rau *et al.*, 1981).

It was our intention in measuring the elemental composition of C, N and P, and the isotopic composition of C of organic matter, in the Gulf of Trieste to elucidate the sources of organic matter and also to trace the organic matter flow in this marine environment.

In our study we investigated: 1) the natural abundances of ^{13}C and elemental C, N, P composition of various classes of coastal organic material — *Gyrodinium* bloom as a representative of pelagic primary producers, marine particulate organic matter (POM), net zooplankton, and the jellyfish *Pelagia noctiluca* as a pelagic consumer, benthic macrophytes *Fucus virsoides* and *Ulva rigida* and surficial marine sediments; 2) isotopic C and elemental C, N, P composition of allochtonous organic matter — riverine POM and sewage. Finally, our objective was to construct a mixing model with the aim of assessing the importance of autochtonous and allochtonous inputs to POM and sedimentary organic matter in the Gulf of Trieste.

MATERIAL AND METHODS

Study area and sampling design

The Gulf of Trieste is the northernmost and the shallowest part of the Adriatic Sea, and is approximately bounded by the line connecting Savudrija in Yugoslavia and Grado in Italy (Fig. 1). Its depth does not exceed 30 m and about one fifth of its area has a depth of less than 10 m. The freshwater inputs along the Yugoslav coast are small, approximative rates of flow (yearly averages) being 4-5 $m^3 . s^{-1}$ for the Rižana river and 1-2 $m^3 . s^{-1}$ for the Dragonja. The waters of the gulf experience considerable temperature (7-26°C) and salinity (32.0-38.5) variations. In deeper waters, typical stratification develops during the warmer part of the year, while in other seasons the water column is well mixed. Our study area was located in the central and SE part of the Gulf of Trieste.

Samples for POM, net-zooplankton, jellyfish *Pelagia*, benthic macrophytes *Fucus* and *Ulva* and surficial sedimentary organic matter were collected periodically during 1980-85. A dinoflagellate bloom of *Gyrodinium* sp. was sampled in September 1984 in the Bay of Koper, and riverine POM in the Rižana river in 1985. Sewage samples were collected in the Koper and Piran sewage systems.

Marine POM was sampled at sea surface using Niskin bottles and in cylindrical sediment traps (i.d. 4 cm) located approx. 1 m above the bottom. POM and *Gyrodinium* samples were filtered through previously ignited (4 hours at 400°C) Whatman GF/C glass-fibre filters. Zooplantkon samples were collected by vertical tows from above bottom to surface by IO 250 μm mesh size. Macrophytes *Fucus* and *Ulva* were sampled by hand in the medio-littoral and surficial sediment samples (0-4 cm) taken with a Meischner-Rumohr (1974) core sampler at depths of 15-20 m.

Analyses

Samples for C, N and P elemental analyses of POM, plankton and macrophytes were freeze-dried without pretreatment. Samples for organic analyses of marine sediments were first acid-treated with 1 N HCl and subsequently freeze-dried. Samples for $\delta^{13}C$ analyses were acid-washed with 3 N HCl and then freeze-dried.

C and N contents in the samples were determined with a commercial C, H, N analyser (Coleman), calibrated with acetanilide. Total and inorganic P were measured using a modification of an acid leach technique (Aspila *et al.*, 1976). Organic phosphorus was taken as the difference between total and inorganic P.

For isotopic C analyses, samples were ignited to CO_2 in a recirculating stream of oxygen and the isotopic C composition determined using a Varian MAT 250 mass spectrometer. The results are reported relative to the international Pee Dee Belemnite (PDB) standard as:

$$\delta^{13}C = (R_{sample}/R_{stand.} - 1) . 10^3,$$

where R is $^{13}C/^{12}C$ content. The analytical precision of ^{13}C measurements was $\pm 0.2\,^o/_{oo}$.
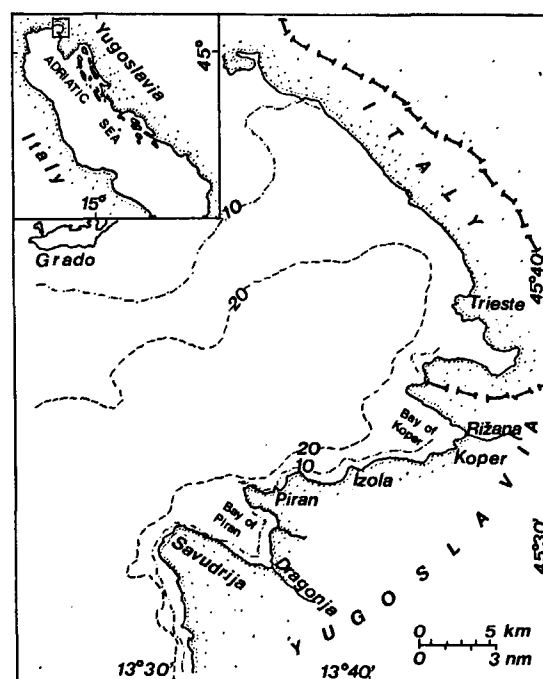

Figure 1
Sketch map of the study area in the Gulf of Trieste (Northern Adriatic).

Table

Mean values ± SD (n) of organic carbon, nitrogen and phosphorus contents and C: N and C: P ratios (atomic) of various organic material from the Gulf of Trieste.

	% C	% N	% P	C: N	C: P
Fucus virsoides	31.4 ± 5.1 (18)	2.6 ±0.7 (18)	0.15±0.1 (18)	15.4± 5.7 (18)	804±386 (18)
Ulva rigida	26.5 ± 7.1 (19)	3.0 ±0.7 (19)	0.15±0.08 (19)	11.1± 4.8 (19)	579±265 (19)
POM (marine)	13.5 ±10.8 (31)	2.3 ±1.7 (31)	0.8 ±0.8 (31)	8.5± 7.1 (31)	57±108 (31)
Gyrodinium	21.7	5.0		5.0	
Net zooplankton	20.3 ± 7.5 (58)	4.4 ±1.5 (58)	0.3 ±0.2 (58)	4.2± 1.3 (58)	180±101 (58)
Pelagia noctiluca	8.6 ± 0.8 (5)	2.8 ±0.2 (5)	0.2 ±0.05 (5)	4.0± 0.2 (5)	99.1±1.9 (5)
Sedimented POM	2.5 ± 1.0 (16)	0.4 ±0.3 (16)	0.029±0.005 (16)	8.1± 4.2 (16)	148± 89 (16)
Sedimentary OM	1.38± 0.5 (34)	0.18±0.13 (34)	0.008±0.004 (34)	13.1±10.7 (34)	563±555 (34)
Sewage POM	26.9 ±23.7 (10)	11.0 ±7.3 (10)	13.3 ±9.9 (10)	4.7± 0.9 (10)	7± 3 (10)

RESULTS AND DISCUSSION

C:N:P ratios

The Table lists the mean C, N, and P contents and C:N and C:P ratios (atomic) of a variety of organic material from the Gulf of Trieste. The C:N ratio of organic matter in surficial marine sediments has been frequently used as an indicator of terrigenous addition to marine sediments (Trask, 1955; Pocklington, Leonard, 1979). The rationale was based on observed enrichment of organic C over N in land-derived organic matter (C:N atomic ratio ≥ 12, Kukal, 1971), as compared to autochtonous marine C:N (≤ 10, Parsons, 1975). Factors contributing to such high C:N ratios are especially forest humus and terrestrial plants and wood debris containing lignin (Pocklington, Leonard, 1979). Our mean C:N ratio of sedimentary organic matter (13.1) and that of Ri̮zana riverine POM (15.2) would indicate that surficial marine sediments were affected by terrigenous organic matter. On the other hand, high C:N and C:P ratios could also be derived from benthic macrophytes.

Another allochtonous source of organic material considered was sewage. The low C:N and C:P ratios of organic particulates from untreated sewage indicate the presence of a rather high content of proteinaceous matter and organic phosphorus, with some dissolved inorganic phosphorus adsorbed on particulate matter. Enhanced breakdown of organic N and P relative to organic C during decompositon in the marine environment leads to organic matter with higher C:N:P ratios, probably similar to partially digested organic matter in a treatment plant. We found, for example, the C:N:P ratio of the primary treated sewage in the Piran treatment plant to be 101:16:1. Thus, the C:N:P of organic effluent does not behave as a conservative component in the marine environment, as was also reported by Sweeney and Kaplan (1980).

In spite of the considerable variability in the C:N and C:P ratios of suspended POM, sediment POM and sedimentary OM (Tab. 1), a distinct trend in these ratios can be ascertained, with higher values in the sedimentary OM. These results indicate the preferential degradation of N and especially P, relative to organic C during sedimentation. Thus, the C:N and especially C:P ratios alone could not provide an answer to the question of the origin of sedimentary organic matter in the Gulf of Trieste, especially with respect to alloch-
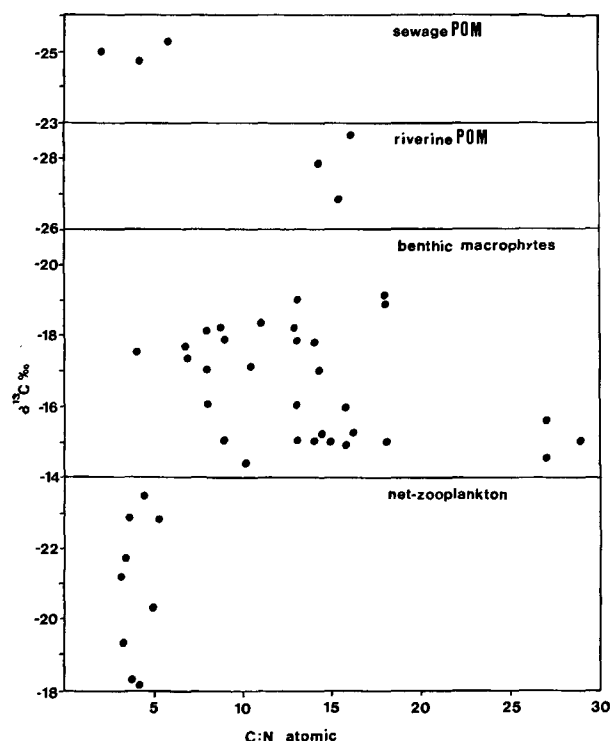

Figure 2

δ¹³C *values compared with C:N ratios (atomic) of different classes of individual end-members.*

tonous sources (sewage organic particulates and terrigenous organic matter).

Carbon isotope ratios

δ¹³C values for individual classes of the sources of organic matter examined in relation to their C:N ratios are presented in Figure 2. Sewage (-25.0 ± 0.05‰, $n=3$) and riverine (-27.9 ± 1.0‰, $n=3$) POM had the most negative δ¹³C values. Phytoplankton δ¹³C values and also marine POM δ¹³C values (-23.6 ± 1.3‰, $n=11$) were higher and presumably dependent on growth conditions (Wong, Sackett, 1978), and species composition, as illustrated by δ¹³C differences observed between cultured phytoplankton species isolated from the Gulf of Trieste (Faganeli et al., 1985). δ¹³C value of the *Gyrodinium* sp. bloom (-21.5‰) was assumed to be a value typical for phytoplanktonic POM. Benthic macrophytes exhibited the highest measured δ¹³C values (-17.0 ± 1.0‰, $n=33$) for organic matter in the Gulf of Trieste. δ¹³C values of primary

producers are influenced by the isotopic composition of the source inorganic carbon (Stiller, Nissenbaum, 1980), which showed systematic enrichment in the heavy isotope along a gradient from riverine ($-12.6\,°/_{oo}$) and brackish water ($-10.3\,°/_{oo}$) towards the near-shore marine environment of the Gulf of Trieste ($-5.9\,°/_{oo}$; Dolenec et al., 1987). An average value of $0\,°/_{oo}$ is usually assumed for oceanic waters (Tan, Strain, 1983) and ^{13}C depleted dissolved inorganic carbon in the neritic environment of our study area indicates some influence of fresh water inflow.

$\delta^{13}C$ values of net-zooplankton ($-20.9 \pm 1.9\,°/_{oo}$, $n=9$) and Pelagia noctiluca ($-18.4 \pm 0.8\,°/_{oo}$, $n=8$) were both less negative than phytoplanktonic POM but some overlap between isotope values of different pelagic organic material exists (Fig. 3). Rather close isotopic similarities between phytoplanktonic POM and net-zooplankton, and net-zooplankton and Pelagia noctiluca indicated that phytoplanktonic POM is the most important food source for the net-zooplankton and the latter for jellyfish. The ^{13}C enrichment in the food web is thus about $1.5\,°/_{oo}$ between phytoplanktonic POM and net-zooplankton and somewhat larger, about $2\,°/_{oo}$, between net-zooplankton and the jellyfish Pelagia noctiluca.

$\delta^{13}C$ values obtained for surficial sediments lay within a rather narrow range ($-21.2 \pm 1.1\,°/_{oo}$, $n=8$), and were enriched in ^{13}C with respect to POM but depleted in comparison to benthic macrophytes (Fig. 3).

In order to determine the source of POM and sedimentary organic matter we used mixing models based on $\delta^{13}C$ values; moreover for POM, which is thought to be composed of more than two sources, the $C:N$ ratio was applied as an additional tracer.

Mixing models

Each of the organic matter classes we examined was statistically unique (t-test between means) in either elemental or isotopic composition; exceptions were net-zooplankton and the jellyfish Pelagia noctiluca which had similar elemental and isotopic compositions (Fig. 3). Sewage organic matter and riverine POM were both depleted in ^{13}C, but sewage was also distinguished by low $C:N$ and $C:P$ ratios. Marine POM showed ^{13}C enrichment versus allochtonous POM (sewage and riverine), but was more ^{13}C depleted than other classes of marine organic matter. Thus, marine POM is presumably influenced by the terrestrial organic carbon contributions. Macrophytic algae had a fairly narrow range of carbon isotopic composition with an overall average about $5\,°/_{oo}$ higher than the surficial sedimentary organic matter. Therefore, a mixing between marine POM and contributions of benthic producers was suspected, supposing that our macrophytic $\delta^{13}C$ values are reasonably representative of other benthic producers (Fry, Sherr, 1984). Also, macrophytes are characterized by higher $C:N$ and $C:P$ ratios than pelagic primary producers but similar to sedimentary organic matter.

On the basis of our $\delta^{13}C$ data and $C:N$ ratios in different classes of organic matter and using the method of linear programming (Dantzig, 1963), we tried first
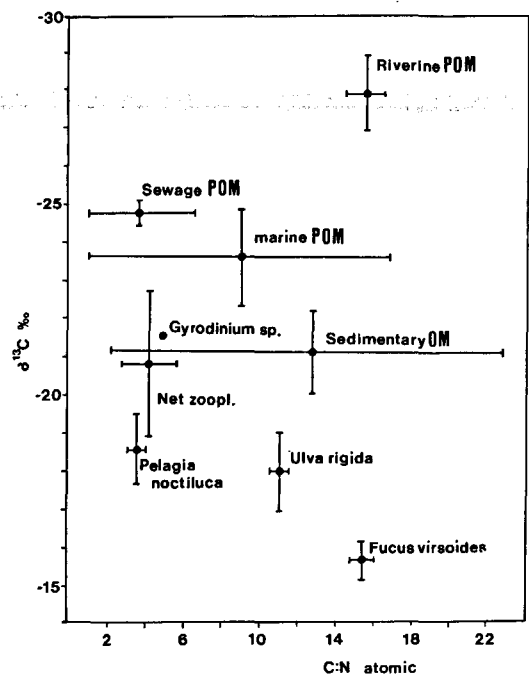

Figure 3
Mean $\delta^{13}C$ values compared with mean $C:N$ ratios (atomic) of different classes of organic matter in the Gulf of Trieste. The important organic matter sources are underlined.

to quantify the contributions of allochtonous (riverine and sewage) and phytoplanktonic organic matter on the POM in the Gulf of Trieste. For the solution of an n-component mixing system we combined the equations:

$$\sum_{i=0}^{n} \alpha_i = 1 \tag{1}$$

$$\sum_{i=0}^{n} \alpha_i = X_T \tag{2}$$

where n is the number of constituents and α_i denotes the fraction of the individual i-th component and X_i is the $\delta^{13}C$ value or $C:N$ ratio of the individual i-th component in the mixture X_T. For a three component mixing system the elimination of α_3 fraction in the system of two equations with three variables gives the equation of a line in the plane $\alpha_1 \alpha_2$:

$$\left(\frac{X_1 X_3}{X_T - X_3}\right)\alpha_1 + \left(\frac{X_2 X_3}{X_T - X_3}\right)\alpha_2 = 1 \tag{3}$$

where α_i lies between $0-1$ and $i=1, 2, 3$ (Fig. 4). Considering the mixing of three $\delta^{13}C$ end-members, i.e. sewage ($X_1 = -25.0\,°/_{oo}$), riverine POM ($X_2 = -27.9\,°/_{oo}$) and phytoplanktonic organic carbon ($X_3 = -21.5\,°/_{oo}$), we obtained the intervals of solution for fractions of three end-members in the mixture in POM ($X_T = -23.6\,°/_{oo}$). The contributions of riverine POC range between 0-33%, sewage POC between 0-60% and phytoplanktonic carbon between 40-67%. A similar mixing model constructed with $C:N$ ratios (atomic) as end-members, i.e. sewage POM ($X_1 = 4.7$), riverine POM ($X_2 = 15.2$), and phytoplanktonic organic matter ($X_3 = 5.0$), mixed to form

POM ($X_T = 8.5$) would yield the contribution of each end-member in the ranges between 0-64%, 34-36% and 0-66%, respectively. Constructing this model it was assumed that the compositional alteration of this organic material in the sea water column is of minor importance, especially in the period of intense vertical mixing or above the pycnocline. Since we used only two tracers, i.e. $\delta^{13}C$ values and C : N ratios, it was not possible to obtain a unique solution for contribution of each source of organic matter to marine POM. In order to resolve uniquely these equations it would be necessary to introduce additional tracers, such as $\delta^{15}N$, $\delta^{34}S$ and lignin, or to perform a sampling design containing river-sea water transects (Fontugne, Jouanneau, 1987). Comparison of the two mixing models showed that the contribution of sewage-born POM to marine POM is the most variable, depending on the distance of the pollution sources. Excluding this source of organic matter, the contribution of riverine POM amounts to

about one third of the total POM in the Gulf of Trieste.

The wide range of $\delta^{13}C$ values observed in marine organic matter is in general retained in marine sediments, since the isotopic changes during burial and diagenesis of organic matter are low (Schidlowski, 1986). The application of the two-source mixing model of $\delta^{13}C$ to the sedimentary organic matter ($X_T = -21.2°/_{oo}$) consisting of a marine POM fraction ($X_1 = -23.6°/_{oo}$) and a macrophytic fraction ($X_2 = -17.0°/_{oo}$), and supposing that the latter could be a reasonable value for the whole benthos, gave the contribution of POM in the mixture forming sedimentary organic matter of about 64%.
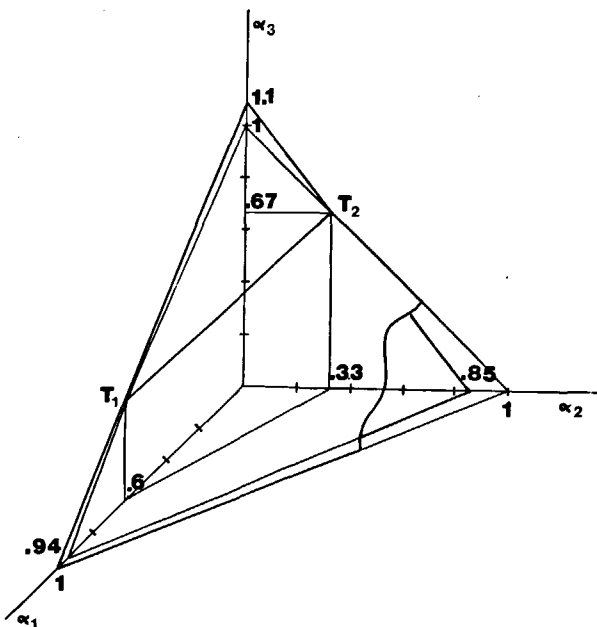

Figure 4
The intersection of equations (1) and (2) of two planes in the space with axes $\alpha_1 \alpha_2 \alpha_3$. The solution of the three end-members system lies on the line segment between points T_1 (0.60, 0, 0.40) and T_2 (0, 0.33, 0.67). The equation (3) is represented as a projection of this line on the $\alpha_1 \alpha_2$ plane.

CONCLUSIONS

Quite clear distinctions could be made among the different classes of neritic organic matter and allochtonous inputs in the Gulf of Trieste considering both C : N ratios and C isotopic composition. The application of the mixing models using C : N ratios and $\delta^{13}C$ values indicated that marine POM is a mixture of 1/3 of riverine POM and 2/3 of phytoplanktonic organic matter in the absence of the input of sewage POM. The contribution of the latter is the most variable and dependent on the pollution sources. The two source $\delta^{13}C$ mixing model indicated the contribution of the marine POM to sedimentary organic matter to be about 2/3. The $\delta^{13}C$ values and C : N ratios as dual-tracers appear to be a useful approach in studying the origins of marine POM and sedimentary OM in coastal environment when the sewage is absent.

Food web analysis showed that the food for net-zooplankton is primarily phytoplankton rather than ^{13}C depleted detritus of riverine or sewage origin which is probably deposited on the surficial sediment. The apparent ^{13}C enrichment in the pelagic food web of the Gulf of Trieste is considerable, amounting to about 1.5-2 °/$_{oo}$ per trophic level.

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